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Review

"Cotranscriptionality": The Transcription Elongation Complex as a Nexus for Nuclear Transactions

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Much of the complex process of RNP biogenesis takes place at the gene cotranscriptionally. The target for RNA binding and processing factors is, therefore, not a solitary RNA molecule but, rather, a transcription elongation complex (TEC) comprising the growing nascent RNA and RNA polymerase traversing a chromatin template with associated passenger proteins. RNA maturation factors are not the only nuclear machines whose work is organized cotranscriptionally around the TEC scaffold. Additionally, DNA repair, covalent chromatin modification, "gene gating" at the nuclear pore, Ig gene hypermutation, and sister chromosome cohesion have all been demonstrated or suggested to involve a cotranscriptional component. From this perspective, TECs can be viewed as potent "community organizers" within the nucleus.

What Does It Mean to Be Cotranscriptional?

The major steps in mRNA biogenesis—transcription, 5′ capping, splicing, and cleavage/polyadenylation—can be reconstituted in vitro entirely independently of one another, yet in the nucleus, they occur at the same time and place in intimate proximity (Bauren et al., 1998; Beyer and Osheim, 1988; Rasmussen and Lis, 1993). The substrate for pre-mRNA processing is actually a transcript that is being extruded through an exit channel in the RNA polymerase. In bacteria, translation and ribosome assembly both occur cotranscriptionally, suggesting that linking transcription to other steps in gene expression is an ancient invention. Cotranscriptional processes are often tailored to a specific RNA polymerase. Ribosome assembly in *E. coli* requires transcription by the cell's own polymerase and is impaired if the rDNA is transcribed by the bacteriophage T7 RNA polymerase (Lewicki et al., 1993). Some eukaryotic rRNA processing is also cotranscriptional, and in yeast, it can be disrupted by mutation of RNA polymerase I (Pol I) (Schneider et al., 2007). By the same token, pre-mRNA capping, splicing, and cleavage/polyadenylation require transcription by RNA Pol II (Sisodia et al., 1987). RNA polymerases, therefore, appear to have been selected for the ability to support cotranscriptional events. RNA Pol II, in particular, has acquired a unique C-terminal domain (CTD) comprising heptad repeats on its large subunit, which enables efficient mRNA processing (Meinhart et al., 2005; Phatnani and Greenleaf, 2006) (Figure 1A).

A number of general principles are emerging about the benefits of "cotranscriptionality" as a means of integrating diverse aspects of nuclear metabolism. In this review, we will attempt to highlight several of these principles and then discuss examples for which they apply, focusing on mRNP biogenesis and modifications of the chromatin template. A number of excellent recent reviews also discuss aspects of this wide field (Iglesias and Stutz, 2008; Kornblihtt et al., 2004; Li and Manley, 2006; Schmid and Jensen, 2008; Pandit et al., 2008).

Principles of "Cotranscriptionality"

1. Cotranscriptionality Permits "Coupling" between Different Steps in mRNP Biogenesis

When different steps in mRNA biogenesis occur at the same time and place, there will be opportunities for "coupling" or crosstalk that makes those steps interdependent, thereby enhancing efficiency or accuracy. On the other hand, distinguishing between events that are simply "concurrent" from those that are functionally coupled is not always trivial (Lazarev and Manley, 2007). Coupling is implied when mutation of a protein that carries out one step has an additional effect on a second step that occurs at the same time and place. For instance, coupling between Pol II transcription and pre-mRNA processing is suggested by the fact that CTD deletion impairs processing (McCracken et al., 1997) in most cases studied so far, but this effect can vary between genes (Ryman et al., 2007). The possibility of indirect secondary effects in genetic experiments must be kept in mind, however, and reconstituted in vitro systems can be helpful in ruling out such effects. Establishing functional coupling between transcription, processing, and packaging of RNA in vitro is a major technical challenge; however, some encouraging headway has been made (Das et al., 2007; Hicks et al., 2006; Rigo and Martinson, 2009).

Coupling can work in different ways to link transcription with mRNA biogenesis and chromatin modification. The simplest form is a mass action effect resulting from colocalization of factors at the TEC, thereby accelerating reactions that would otherwise be too slow. Localization is a major function of the Pol II CTD that acts as a "landing pad," binding directly to factors involved in pre-mRNA capping, 3′ end processing, transcription elongation, termination, and chromatin modification (Phatnani and Greenleaf, 2006) (Figures 1A and 2A). A similar function is fulfilled by the unrelated CTD of RNA Pol V, which binds an Argonaut protein that initiates gene silencing in plants (El-Shami et al., 2007). Direct interactions with the CTD have been characterized

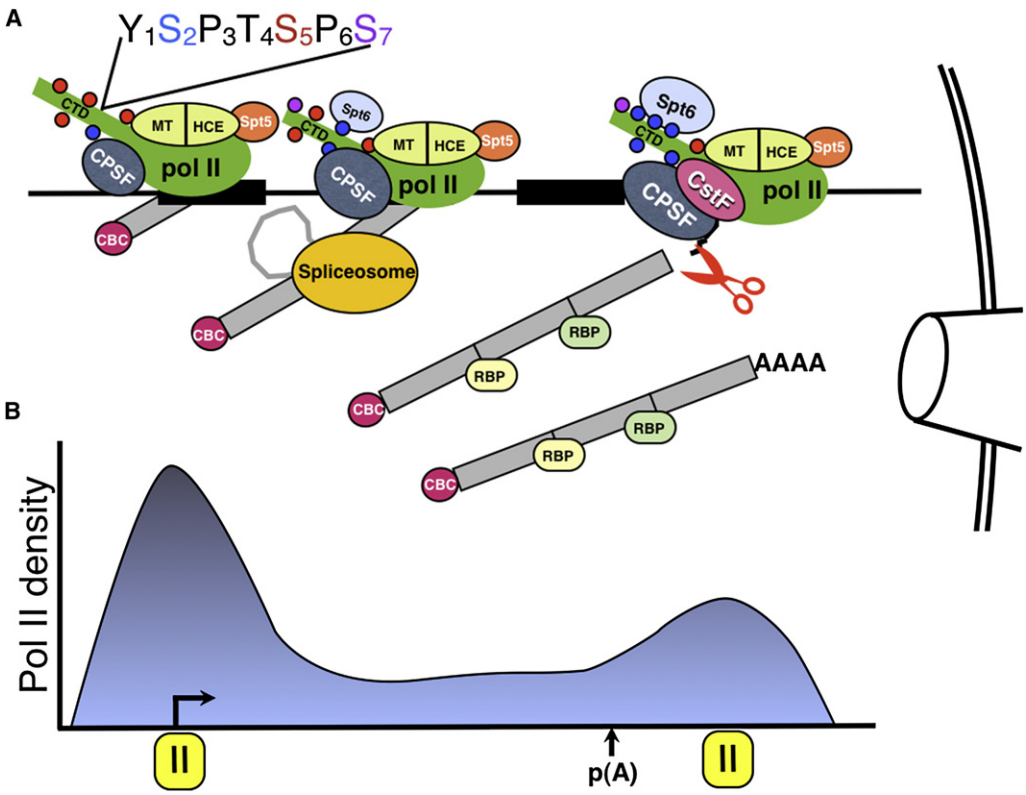

Figure 1. Processing and Elongation Factors Associated with the Human Pol II TEC

(A) The conserved heptad repeat sequence with serines 2, 5, and 7 that are differentially phosphorylated during the transcription cycle are highlighted. The 5′-3′ distributions of CTD phosphorylations, processing factors, and elongation factors are derived from ChIP studies (e.g., Glover-Cutter et al., 2008; Yoh et al., 2007). Human capping enzyme (HCE), cap methyltransferase (MT), and the cleavage polyadenylation factors CPSF and CstF are shown. Elongation factors Spt5, which binds HCE, and Spt6, which binds phospho-Ser2, are shown. Scissors depict RNA cleavage at the poly(A) site.

(B) 5′-3′ distribution of RNA Pol II density on a typical human gene, showing pausing at the start site and downstream of the poly(A) site.

at the structural level for a capping enzyme, Cgt1; a cleavage/polyadenylation factor, Pcf11; a histone methyltransferase, Set2; and an RNA-binding termination factor, Nrd1 (Meinhart et al., 2005; Vasiljeva et al., 2008) (Figures 1A and 2A).

A second coupling mechanism afforded by the meeting of factors at the TEC is allostery. An example is activation of the capping enzyme's guanylyltransferase activity by the phosphorylated Pol II CTD (Ho and Shuman, 1999). It seems likely that protein:protein interactions that first evolved to colocalize mRNA processing and transcription may have subsequently acquired allosteric functions.

A third iteration is "kinetic coupling" that can facilitate mRNA biogenesis by optimizing the timing of sequential events in this process. "Kinetic coupling" between transcript elongation and spliceosome assembly very likely regulates alternative splicing decisions (Kornblihtt et al., 2004).

2. Cotranscriptionality Can Impose Order or Control over Assembly of mRNPs and Processing Machines

Juxtaposition of proteins that have congregated at the TEC may permit assembly reactions, competitive interactions, and handoffs that would not be possible posttranscriptionally without the TEC as a scaffold. Some protein complexes may be regulated by being assembled cotranscriptionally, rather than being loaded onto the TEC as fully preassembled units. A common relationship between TEC-associated factors is that of mutually exclusive protein:protein and protein:RNA interactions that replace one another in handoff reactions to establish an ordered sequence of events (Figure 4B). One example is handoff of the yeast RNA helicase Sub2 from the THO complex, an elongation factor that rides with the TEC (Strasser et al., 2002) to the nascent transcript, where it binds the mRNA export adaptor protein Yra1 (Iglesias and Stutz, 2008).

A second way that order is imposed on cotranscriptional events is through directions emanating from phosphorylation of the Pol II CTD heptad repeats (26 in yeast, 52 in mammals) with the consensus sequence $YS_2PTS_5PS_7$. Phosphorylation of Ser5 by the TFIIH-associated kinase Cdk7, or Kin 28 in yeast, occurs first at initiation, whereas Ser2 phosphorylation by Cdk9/PTEFb (positive transcription elongation factor b), or Ctk1 and Bur1 in yeast, occurs later during elongation (Komarnitsky et al., 2000; Phatnani and Greenleaf, 2006) (Figure 2B). In addition, dephosphorylation at Ser5 and Ser2 by the Rtr1 and Fcp1 phosphatases (Mosley et al., 2009; Phatnani and Greenleaf, 2006) helps to define how the CTD is decorated as Pol II transits from initiation to elongation and termination of transcription. The combined action of kinases and phosphatases results in a characteristic switch from higher to lower ratios of Ser5:Ser2 CTD phosphorylation as Pol II moves along a gene (Figure 2B)

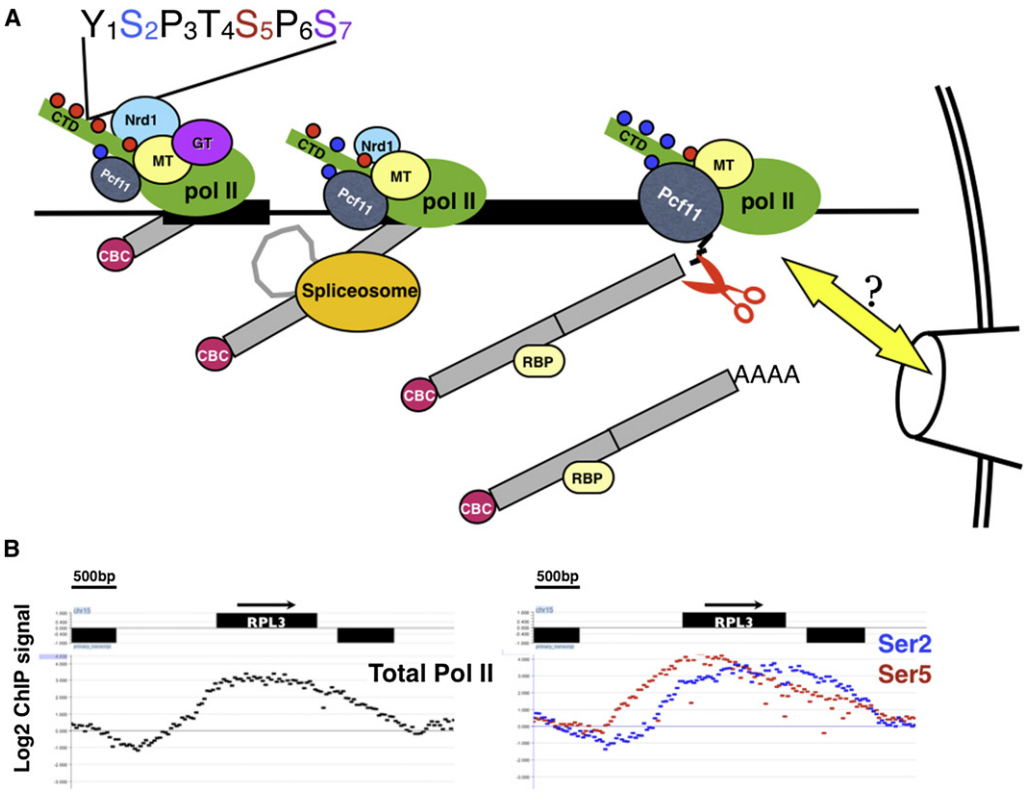

Figure 2. CTD Phosphorylation and Processing Factor Recruitment at the Budding Yeast Pol II TEC

(A) 5′-3′ distributions of factors are based on ChIP studies as in Figure 1A. Four factors that bind directly to the CTD are depicted: the cap methyltransferase (MT) and guanylyltransferase (GT), the termination factor Nrd1, and the cleavage/polyadenylation factor Pcf11. Gene gating by putative interaction between the nuclear pore and the TEC is depicted by a yellow arrow.

(B) ChIP-Chip analysis of the 5′-3′ distribution of total Pol II density (left) and Ser2 and Ser5 phosphorylated Pol II (right) on a typical highly transcribed yeast gene (RPL3). Results are from P. Megee and D.B., unpublished data.

(Komarnitsky et al., 2000). Ser7 residues of the CTD heptads are also phosphorylated within genes (Chapman et al., 2007) by Kin28/Cdk7 in yeast and mammalian cells (Akhtar et al., 2009). As a result of sequential phosphorylation, proteins that recognize Ser 5 phosphorylated heptads, such as the yeast Nrd1 protein (Vasiljeva et al., 2008), will be recruited to the TEC earlier than those that recognize Ser2 phosphorylated heptads such as the 3′ processing factor Pcf11 (Licatalosi et al., 2002).

3. The TEC Is a Locator for Nuclear Machines

The impact of cotranscriptionality is not limited to mRNA production. The TEC is used to localize protein machines that carry out DNA repair, covalent DNA modification, and gene silencing to the places in the genome where they are required. An example of this locator function is transcription-coupled DNA repair in which the nucleotide excision repair machine recognizes a stalled Pol II TEC and specifically removes DNA lesions on the template strand (Lindsey-Boltz and Sancar, 2007). The TEC may also be an active participant in relocating chromatin-associated proteins, including histones and cohesins (Lengronne et al., 2004; Workman, 2006) (Figures 4A and 4C).

The Pol II CTD Organizes an "mRNA Factory"

The CTD functions as a flexible landing pad for Pol II-interacting proteins, including pre-mRNA-processing factors and chromatin modifiers (Phatnani and Greenleaf, 2006). The length of the fully extended CTD would be many times the diameter of core Pol II (Meinhart et al., 2005), providing ample space, in principle, for binding to multiple partners. The heptad repeats are phosphorylated and dephosphorylated by kinases and phosphatases at the S2, S5, and S7 positions in a manner that is coordinated with the initiation, elongation, and termination phases of the transcription cycle (reviewed in Meinhart et al., 2005; Phatnani and Greenleaf, 2006). CTD phosphorylation is potentially astronomically complex, and elucidating how it controls loading and unloading of Pol II passenger proteins is an important problem.

In vitro, the CTD enhances capping, splicing, and 3′ end formation independently of ongoing transcription, and CTD deletion disrupts these processing reactions in vivo (reviewed in Hirose and Manley, 2000). Although the CTD is necessary for efficient pre-mRNA processing, it is not sufficient. Simply pinning the CTD onto T7 RNA polymerase or Pol I does not permit efficient processing (Natalizio et al., 2009).

A dynamic "mRNA factory" complex of Pol II with associated proteins is probably responsible for simultaneous synthesis, processing, and packaging of the mRNP (Figure 1A). The polymerase with nascent RNA and numerous passenger proteins would almost certainly exert too much viscous drag to move at a high speed through nucleoplasm. The most likely solution to

this hydrodynamic problem is that the chromatin template is threaded through a stationary mRNA factory (Jackson and Cook, 1995).

First, Put Your Cap On

Pre-mRNAs are modified at their 5′ ends by addition of a 7-methyl G5′ppp5′N cap (reviewed in Shuman, 2001) when the transcript is only 25–50 bases long. Capping is a three-step process that does not require specific sequences in the RNA. RNA triphosphatase removes the γ-phosphate of the first nucleotide, and then GMP is added by RNA guanylyltransferase. Finally, the guanine is methylated at N7. In metazoans, the capping enzyme is a bifunctional polypeptide with triphosphatase and guanylyltransferase activities. The GMP transfer reaction is reversible, and capping is driven forward by the concerted action of both guanylyltransferase and methyltransferase, yet these two enzymes do not associate with one another (Shuman, 2001). The solution to this problem is that both the capping enzyme and the methyltransferase bind directly and specifically to the phosphorylated Pol II CTD (Shuman, 2001). When transcription initiates, phosphorylation of the CTD on Ser5 residues permits loading of capping enzyme onto the TEC and allosteric activation of the guanylyltransferase (Ho and Shuman, 1999). The overall capping reaction is, therefore, facilitated by both colocalization of the capping enzymes on the phosphorylated CTD and allosteric activation.

The relationship between capping enzymes and Pol II illustrates the two-way nature of communication between processing factors and the transcription machinery. Not only is capping enhanced by interaction of capping enzymes with the CTD, but capping enzymes can also stimulate or inhibit transcription initiation and/or early elongation (Mandal et al., 2004; Myers et al., 2002; Schroeder et al., 2004). Polymerase complexes paused at 5′ ends are found on many metazoan genes, and they are probably important for cotranscriptional capping. 5′ pausing is regulated by the Pol II-associated elongation factor Spt5, which also allosterically activates the cap guanylyltransferase (Wen and Shatkin, 1999). Another regulator of elongation, the HIV Tat protein, also activates guanylyltransferase and enhances capping of viral transcripts (Chiu et al., 2002). Capping could be coupled to escape of paused Pol II, thereby ensuring that polymerases that enter productive elongation will have an appropriately modified 5′ end. Conversely pausing could promote capping, perhaps by restricting the distance between the RNA 5′ end and capping enzymes sitting on the CTD. Capping is not usually regarded as a regulated step in gene expression; however, capping factor recruitment could, in principle, be regulated by Spt5 or CTD phosphorylation. A block to capping enzyme recruitment has been reported at yeast silent mating-type loci (Gao and Gross, 2008). In the future, it will be of interest to investigate whether or not capping is affected by polymerase pausing at the transcription start site.

The influence of capping enzymes on mRNA production may not be limited to 5′ ends. Human capping enzymes are found at 5′ ends and throughout genes, including 3′ flanking regions even more than a kilobase downstream of the poly(A) site (Glover-Cutter et al., 2008) (Figure 1B). Capping factors, therefore, appear to linger on the Pol II "landing pad" long after addition of the cap, and they could, therefore, potentially influence elongation, termination, and 3′ end processing like the vaccinia virus capping enzyme. They might also cap the pervasive noncoding short transcripts detected within genes and at 5′ and 3′ ends (Kapranov et al., 2007). In addition, capping enzyme has been suggested to promote R loop formation (see below) and could thereby affect transcription elongation (Kaneko et al., 2007).

Cotranscriptional Splicing…Some Assembly Required

Many, but not all, introns are removed cotranscriptionally rather than posttranscriptionally, as vividly shown by EM studies (Beyer and Osheim, 1988). Although not all splicing is completed cotranscriptionally, it is probable that assembly of most spliceosomes, including those at alternative splice sites (Pandya-Jones and Black, 2009), is initiated on the nascent transcript. The spliceosome is one of the most elegant examples of ordered self-assembly. In vitro, on a pre-made substrate without ongoing transcription, U1 snRNP first base pairs to the 5′ splice site, and U2AF binds the 3′ splice site, followed by U2 snRNP base pairing the branch point. The tri-snRNP U4-U6/U5 then engages, and the complex rearranges to assume the catalytically active conformation as U1 and U4 are discarded. ChIP analysis of cotranscriptional splicing on yeast genes supports a step-wise assembly process similar to that which occurs in vitro (Gornemann et al., 2005; Lacadie and Rosbash, 2005). On the other hand, the possibility that preassembled higher-order snRNP complexes are recruited cotranscriptionally cannot yet be excluded, especially in mammalian cells (Listerman et al., 2006). Little is known about spliceosome assembly at the TEC, but there are reasons for thinking that it might differ from assembly that is uncoupled from transcription. Splicing of synthetic pre-mRNAs in injected *Xenopus* oocytes is less efficient than splicing coupled to transcription in the same cells, consistent with stimulation of splicing in vitro by the phosphorylated CTD (Bird et al., 2004; Hirose and Manley, 2000). It has been suggested that cotranscriptional splicing might differ from splicing uncoupled from transcription because, in the former case, exons are held in place by tethering to the polymerase (Dye et al., 2006). The functional significance of exon tethering has recently been questioned, however (Fong et al., 2009). Unlike the other mRNA-processing steps, splicing is reiterated many times on most transcripts. How coupling with transcription might affect the recycling of spliceosome components for use on multiple introns within a transcript is an interesting open question.

A number of intriguing connections have been uncovered between the splicing machinery and TEC-associated proteins, although the extent to which splicing is facilitated by direct protein:protein interactions between spliceosomes and Pol II remains unresolved. The yeast U1 snRNP protein Prp40, which bridges the 5′ splice site and branch point, can bind directly to the phospho-CTD (Phatnani and Greenleaf, 2006), but the functional significance of this interaction remains to be established. Human U1snRNP, but not other snRNPs, also coimmunoprecipitates with Pol II (Das et al., 2007). Furthermore, U1snRNP at a 5′ splice site can activate recruitment of Pol II and general transcription factors to the promoter independently of splicing

(Damgaard et al., 2008). The U1 snRNP may, therefore, have a special relationship with Pol II TECs.

The SR family of splicing factors binds the nascent transcript at exonic splicing enhancer elements and regulates spliceosome assembly by contacting the U1 and U2 snRNPs (reviewed in Long and Caceres, 2009). SR proteins coimmunoprecipitate with Pol II, and the SC35 family member has been implicated in stimulating transcriptional elongation through its interaction with PTEFb (Lin et al., 2008). SR proteins other than SC35 appear to associate with TECs exclusively through the nascent RNA, rather than through protein:protein contacts with Pol II (Sapra et al., 2009). A functional link between SRs and transcription is suggested by the finding that SRp20 regulation of alternative splicing requires the Pol II CTD (de la Mata and Kornblihtt, 2006). SR proteins have also been implicated in coupling transcription with splicing in an in vitro system in which transcripts made by Pol II are selectively stabilized and spliced relative to T7 transcripts (Hicks et al., 2006). This channeling of Pol II transcripts into a productive splicing pathway is probably due to facilitated binding of the nascent transcripts to RNA-binding proteins (RBPs), including SR proteins that protect them from degradation and enhance spliceosome assembly (Das et al., 2007).

In summary, although copurification of splicing factors with Pol II complexes is consistent with coupling between splicing and transcription, there is at present no compelling example of a functionally important direct interaction between a splicing factor and RNA Pol II itself. Therefore, it remains possible that, in contrast to capping and 3′ end processing, all of the major signals for the loading of splicing factors onto the TEC lie in the nascent RNA, with the CTD and other transcriptional factors playing indirect roles. The extent to which cotranscriptional spliceosome assembly may vary between introns within a gene and between different genes remains an interesting open question.

Cotranscriptional Splicing, Elongation, and RNA Folding

RNA chain elongation is not a uniform monotonous process, but instead, it is interrupted by numerous pauses that are dictated by the local sequence environment. Pol II elongation in live cells occurred at an average rate of 1.9 kb/min in one study (Boireau et al., 2007) and at a maximum rate of 4.3 kb/min in a second case (Darzacq et al., 2007). In addition to the many extrinsic factors that influence elongation, the intrinsic rate of elongation can be limited by the diffusion of NTPs through the funnel domain to the active site. Conserved charged residues in the funnel hinder NTP diffusion to the active site, potentially limiting the rate of transcription (Batada et al., 2004). Selective pressure acting on the funnel may, therefore, have tuned the transcription rate of RNA polymerase II so that it is within an optimal range that is compatible with cotranscriptional mRNA processing and packaging. One reason why transcription by T7 RNA polymerase does not support coupled pre-mRNA processing (Hicks et al., 2006; Natalizio et al., 2009) may be that it elongates several times faster than RNA Pol II.

Nascent RNA is extruded through an exit channel in RNA polymerase that lies close to the attachment point of the CTD. Newly minted RNA sequences that exit the RNA polymerase are immediately available for interaction with RBPs and base pairing with upstream RNA sequences. The folding pathway of a growing RNA chain differs fundamentally from the folding of a premade full-length transcript such as synthetic substrate RNA added to a processing reaction. Moreover, the folding pathway adopted by a particular transcript can differ depending on its rate of growth and, in particular, on polymerase pausing (Pan and Sosnick, 2006) (Figures 3B and 3C). Transcription by T7 RNA polymerase, which is 5- to 10-fold faster than *E. coli* polymerase, impairs cotranscriptional ribosome assembly (Lewicki et al., 1993) and correct folding of structured RNAs in *E. coli* (Pan and Sosnick, 2006).

The formation of productive versus nonproductive processing complexes on nascent pre-mRNAs is probably influenced by sequential folding of the RNA that exposes or sequesters splice sites and splicing enhancer and silencer sequences. Although the general significance of cotranscriptional RNA folding for splicing is not yet established, a growing body of evidence suggests that it can have important effects on alternative splicing (Shepard and Hertel, 2008). The practical significance of cotranscriptional folding in splicing is shown by the fact that the efficacy of therapeutic antisense oligonucleotides that induce exon skipping in the dystrophin mRNA can be predicted by taking into account the accessibility of target sequences during cotranscriptional folding (Wee et al., 2008).

A "Window of Opportunity" for Alternative Splicing

Alternative splicing affects the expression of most human genes, and it is possible that the most important effect of transcription elongation on mammalian gene expression is mediated through effects on alternative splicing. The simplest way in which elongation rate can influence alternative splicing is by controlling the duration of the "window of opportunity" during which the upstream splice site can assemble a functional spliceosome before it has to compete with the downstream site (Figures 3B and 3C). Hence, slowing elongation can enhance the use of a poor upstream 3′ splice site relative to a better site downstream and, therefore, favors inclusion of an alternative exon (Kornblihtt et al., 2004). It remains to be tested whether accelerated rates of transcription have the opposite effect of decreasing exon inclusion, as predicted by the window of opportunity model.

Given the intimate relationship between elongation and splicing, it is perhaps not surprising that numerous factors implicated in control of Pol II pausing and processivity also affect constitutive or alternative splicing. These factors include the state of CTD phosphorylation, the elongation factor Spt5, promoter- and enhancer-associated transcription factors, and coactivators (reviewed in Kornblihtt et al., 2004), as well as covalent histone modifications (Kolasinska-Zwierz et al., 2009). The molecular basis for the connection between splicing and elongation is still unresolved; however, it is intriguing that the elongation factors PTEFb, CA150, and TAT-SF1 all associate directly or indirectly with spliceosomal U snRNPs (Fong and Zhou, 2001; Lin et al., 2008; Pandit et al., 2008). PTEFb also interacts with SKIP, which is both a transcriptional coactivator and a subunit of U5 snRNP (Bres et al., 2005). Another connection is suggested by interaction of the U2 snRNP component SF3a with the chromatin-remodeling ATPase Chd1 (Sims et al., 2007). It was recently discovered that elongation rates can be modulated by

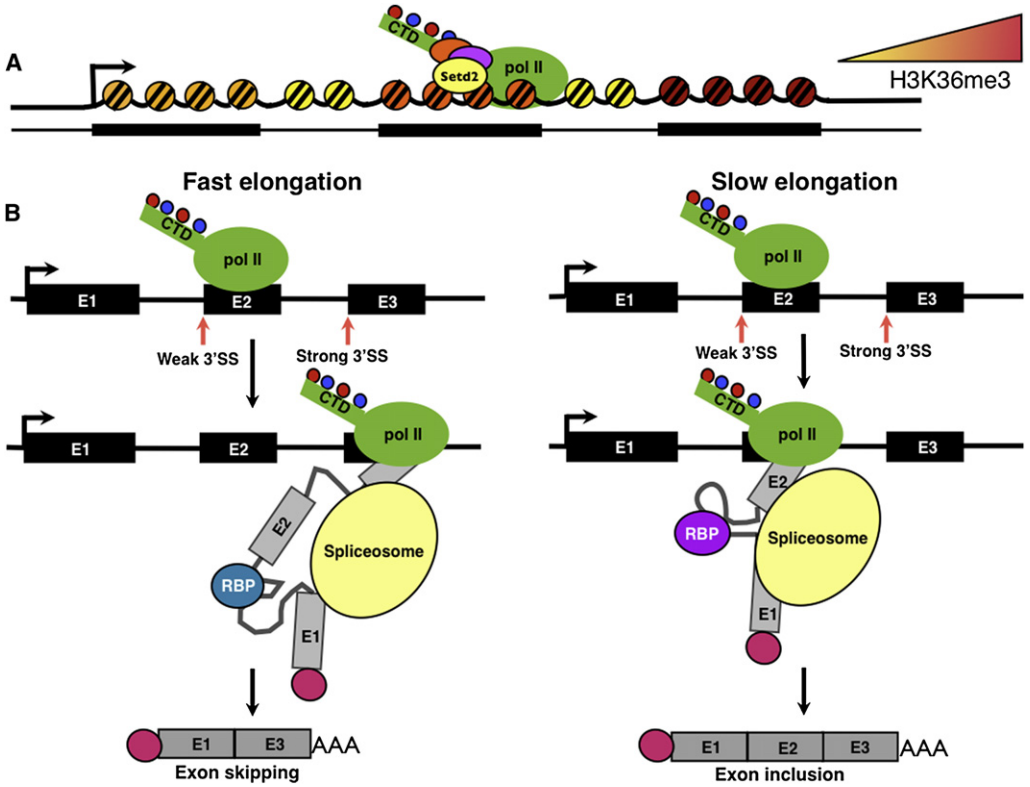

Figure 3. The Rate of Elongation Affects Splice Site Selection and Folding of the Nascent RNA

(A) Differential histone H3 K36 trimethylation (H3K36me3) of worm and mouse exons and introns (Kolasinska-Zwierz et al., 2009) by Setd2, which binds the CTD (Yoh et al., 2008), is indicated. H3K36me3 increases 5′-3′ on most genes.

(B) A fast elongation rate and short window of opportunity favor exon skipping, whereas slow elongation favors exon 2 (E2) inclusion (based on de la Mata et al., 2003). Elongation rate can also affect cotranscriptional RNA folding and potentially binding of different RNA-binding proteins (RBP).

a physiological stimulus, resulting in new alternative splice site choices. In response to UV-induced DNA damage, the CTD becomes hyperphosphorylated, transcription elongation slows down, and alternative splice choices are switched in favor of the proapoptotic isoforms of Bcl-x and caspase 9 (Munoz et al., 2009).

An intriguing connection with chromatin is suggested by the discovery that the SWI/SNF and Chd1 chromatin-remodeling ATPases influence splicing (Batsche et al., 2006; Sims et al., 2007). Batsche and colleagues suggested that alternative splicing decisions may be influenced by differences in elongation rates within constitutive versus alternatively spliced exons in a manner regulated by SWI/SNF and CTD phosphorylation (Batsche et al., 2006). Remarkably, exons have a chromatin signature characterized by a higher density of nucleosomes than adjacent introns. High nucleosome occupancy in exons occurs regardless of the level of transcription and is largely determined by their nucleotide composition. Of interest, nucleosome enrichment is most pronounced for exons with weak splice sites (Schwartz et al., 2009; Spies et al., 2009; Tilgner et al., 2009). Furthermore, exonic nucleosomes are enriched with specific covalent modifications on histone H3—notably, trimethylation of K36 and K79 and dimethylation of K27. These modifications could possibly mark nucleosomes for binding to RNA-processing factors. Changes in K36 trimethylation have been associated with different patterns of alternative splicing (Kolasinska-Zwierz et al., 2009; Schor et al., 2009) (Figure 3A) and with cotranscriptional loading of the mRNA export adaptor Aly (Yoh et al., 2008). Whether the chromatin signature of exons affects splicing or vice versa is still unclear, but a functional connection of some sort is strongly suggested by the fact that the accuracy of exon prediction can be improved by taking into account not only sequence motifs, but also chromatin structure (Spies et al., 2009). High nucleosome occupancy or a particular set of histone modifications in exons could spell lower elongation rates than in introns and in that way influence splicing and coupled export factor loading.

Cotranscriptional Assembly of the 3′ Processing Machinery

3′ end processing of most mRNAs is a two-step reaction comprising endonucleolytic cleavage shortly after the AAUAAA sequence, followed by polyadenylation of the exposed 3′ OH. Homologous multisubunit complexes, including cleavage stimulation factor (CstF) and cleavage polyadenylation specificity factor (CPSF) in mammals and cleavage factor 1A (CF1A) and cleavage polyadenylation factor (CPF) in yeast perform coupled cleavage and polyadenylation. Some components, including the

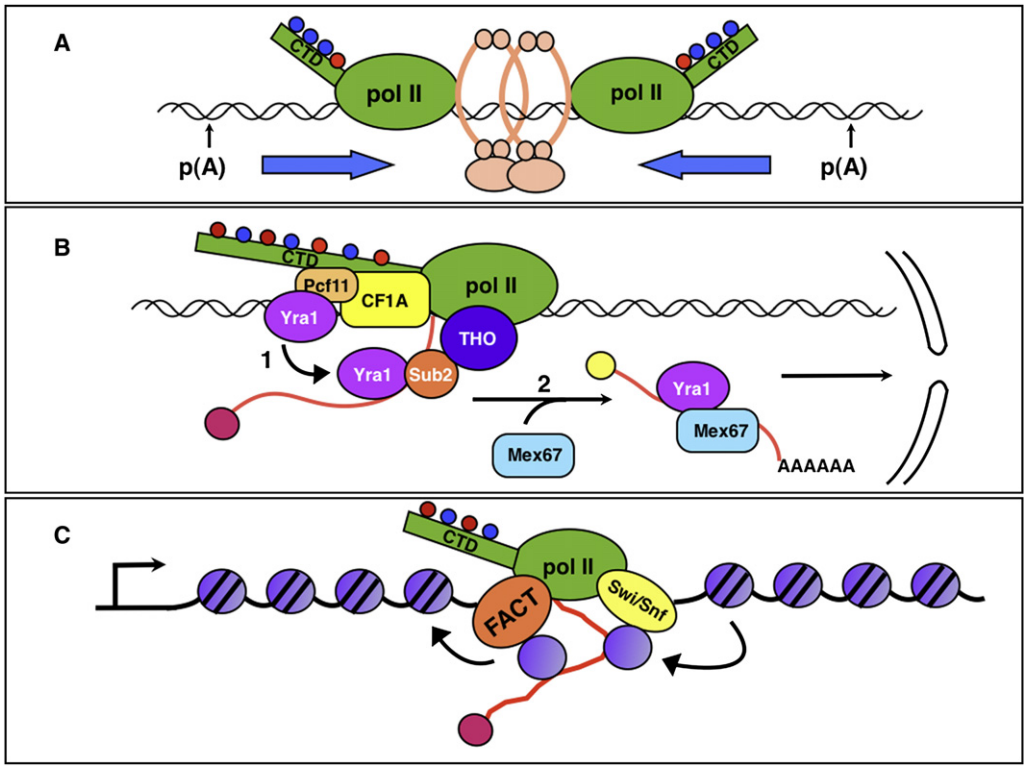

Figure 4. The RNA Polymerase II TEC as a Locator of Chromatin Proteins

(A) Possible mechanism of cohesin (pink rings) localization by convergent transcription in yeast (based on Lengronne et al., 2004).

(B) Mutually exclusive protein:protein and protein:RNA interactions and handoff reactions facilitate assembly of export-competent mRNPs. In reaction 1, the yeast export adaptor Yra1 is handed off from Pcf11 to the RNA helicase Sub2. In reaction 2, Yra1 is handed off to the export receptor Mex67/Mtr2.

(C) Mobilization of histones during Pol II transcription elongation. FACT and Swi/Snf travel with the TEC to facilitate disassembly and reassembly of nucleosomes. Displaced histones could be handed off to the nascent RNA before they are redeposited behind the polymerase.

endonuclease, CPSF73, are shared with the histone 3′ end-processing complex that makes nonadenylated ends (reviewed in Mandel et al., 2008). Cleavage and early polyadenylation can occur at the site of transcription (Bauren et al., 1998), consistent with the fact that cleavage/polyadenylation factors are found at transcribed genes (Ahn et al., 2004; Gall et al., 1999; Glover-Cutter et al., 2008; Licatalosi et al., 2002). It has also been reported that poly(A) site cleavage can occur posttranscriptionally following polymerase release from the template (West et al., 2008).

The Pol II CTD binds 3′ end-processing factors and stimulates cleavage/polyadenylation in vivo and in vitro (Hirose and Manley, 1998; McCracken et al., 1997). The 50 kD subunit of CstF, the Pcf11 subunit of CF1A, and the yeast termination factor Rtt103 all bind the CTD directly (Meinhart et al., 2005; Phatnani and Greenleaf, 2006; Kim et al., 2004). Ser2 phosphorylation of the CTD is of special significance for 3′ end processing, at least in part because the cleavage/polyadenylation factor Pcf11 preferentially binds to heptad repeats with this modification (Ahn et al., 2004; Licatalosi et al., 2002; Meinhart and Cramer, 2004). Modulation of CTD phosphorylation as polymerase traverses a gene therefore helps to coordinate the assembly of the 3′ end-processing machinery at the site of transcription.

Unexpectedly, 3′ end-processing factors are not confined to the 3′ ends of transcribed genes. In fact, human CPSF and yeast CF1A subunits (Dantonel et al., 1997; Glover-Cutter et al., 2008; Licatalosi et al., 2002) are found at 5′ ends, long before transcription of 3′ end-processing signals. These factors are, therefore, probably recruited to the TEC initially by protein:protein interaction and are subsequently handed off to the nascent RNA after the poly(A) site has been transcribed.

CPSF can bind both the body of Pol II and CstF in a mutually exclusive way (Nag et al., 2007), suggesting that formation of a functional CPSF/CstF complex may be controlled by handoff of CPSF from Pol II to CstF. A handoff reaction may also control assembly of the Pcf11 and Clp1 subunits of the yeast 3′ end-processing complex, CF1A (Figure 4B). Clp1 and the export adaptor Yra1 (Johnson et al., 2009) bind the same short region of Pcf11 and are thus likely to compete with one another. CF1A may, therefore, be recruited to the gene in a partially assembled form, with Yra1 occupying the place of Clp1. At the poly(A) site, Clp1 may displace Yra1, which is handed off to the RNA, thereby completing assembly of a functional 3′ processing complex (Saguez and Jensen, 2009).

Competition between mutually exclusive interactions at the TEC may be exploited for quality control of 3′ end processing. The yeast RBPs Npl3 and Rna15, a CF1A subunit, both load onto the TEC before it reaches the 3′ end of the gene. These two factors compete for binding to similar sites on the nascent RNA, and it has been suggested that this competition enhances

the accuracy of 3′ processing by preventing Rna15 from recognizing cryptic poly(A) sites within genes (Bucheli et al., 2007).

3′ End Processing and Transcription Elongation

Like exons, poly(A) sites are associated with their own chromatin signature: a localized nucleosome depletion over the AATAAA consensus sequence (Spies et al., 2009). The function of this chromatin feature is unknown, but it could cause a local increase in transcriptional elongation rate that might influence 3′ processing. Further downstream, Pol II pausing 1–2 kb past the poly(A) site is common to many genes (Boireau et al., 2007; Darzacq et al., 2007; Glover-Cutter et al., 2008) (Figure 1B). At this pause site, CTD Ser2 is highly phosphorylated (Figure 1), and maximal levels of cleavage/polyadenylation factors are associated with the TEC (Glover-Cutter et al., 2008). Although the precise relationship between poly(A) site processing and subsequent transcription termination is unclear, it seems likely that both events are coordinated with the downstream pause.

Alternative poly(A) site choice has recently been recognized as a widespread phenomenon that shapes the repertoire of 3′UTR sequences (Licatalosi et al., 2008; Sandberg et al., 2008) and is perturbed in cancer cells (Mayr and Bartel, 2009). It is possible that the rate of transcriptional elongation can affect the choice between alternative poly(A) sites, and if this were the case, then effects of chromatin structure on elongation at 3′ ends could influence these important RNA processing decisions. Although early recruitment of 3′ end-processing factors appears to be a general phenomenon, it remains to be determined whether RNA cleavage at the poly(A) site always precedes transcription termination or whether the timing of cleavage and termination differs between genes.

Poly(A) site cleavage at the 3′ end may not be sufficient to cut the mRNA loose from the TEC. In mammalian cells, an additional release step requires the CTD (Custodio et al., 2007) and completion of splicing (Rigo and Martinson, 2009). In yeast, the THO complex, the RNA helicase Sub2, the export factor Mex67, and the phosphatase Glc7 remodel the RNP at the 3′ end of the gene and pry it away from the cleavage/polyadenylation apparatus (Gilbert and Guthrie, 2004; Qu et al., 2009; Rougemaille et al., 2008).

Cotranscriptional Recruitment of Factors that Dismantle the TEC

A major advantage of cotranscriptional rather than posttranscriptional recognition of 3′ end-processing sites is that it permits the coupling of transcription termination with recognition of the poly(A) signal that marks the end of the message (Rosonina et al., 2006). There are two main models for how cleavage/polyadenylation factors stimulate dissociation of the extraordinarily stable TEC. The "allosteric" model invokes a poly(A) site-dependent conformational change in the TEC that reduces its processivity. The "torpedo" model, on the other hand, proposes that the cut site in the nascent RNA permits access to a 5′-3′ RNA exonuclease that degrades the nascent RNA tail and destabilizes the TEC. Evidence on these models is divided, but in both the allosteric and torpedo scenarios, it is clear that cotranscriptional loading of 3′ end-processing factors, including Pcf11 and the 5′-3′ RNA exonuclease Xrn2/Rat1, ultimately leads to dissociation of the TEC (reviewed in Rosonina et al., 2006).

A second mechanism of Pol II termination is employed in yeast at noncoding genes that lack poly(A) sites. At these genes, a complex comprising Nrd1, Nab3, and the RNA helicase Sen1 is recruited to the TEC and terminates transcription independently of a 5′-3′ RNA exonuclease (Steinmetz et al., 2001; Kim et al., 2006). The Nrd1 protein binds directly to Ser5-phosphorylated CTD heptads (Vasiljeva et al., 2008), as well as RNA. Position-specific CTD phosphorylation on Ser5 at 5′ ends of genes ensures that this mechanism of termination only operates at short distances from the transcription start site (Gudipati et al., 2008). In summary, Pol II TECs, in a carefully regulated way, recruit the factors that ultimately lead to their dissociation from the template.

Specialized Cotranscriptional RNA Processing

In addition to capping, splicing, and cleavage/polyadenylation, some transcripts are processed cotranscriptionally by A-I editing or miRNA excision from introns. In the nascent GluR-B pre-mRNA, an ADAR (adenosine deaminase acting on RNA) recognizes RNA duplexes formed by intramolecular base pairing between exon 11 and intron 11 and converts an A to I, thereby switching a Q codon to R. Editing in this case must occur before splicing, and the Pol II CTD is implicated in imposing this sequence of events (Ryman et al., 2007).

miRNAs are released from Pol II-transcribed precursors by the microprocessor complex that includes the RNase III family member Drosha, which clips miRNA precursors at the base of a hairpin. Drosha is found at genes harboring intronic miRNAs (Morlando et al., 2008), strongly suggesting that it works cotranscriptionally. Furthermore, miRNA excision from introns is completely compatible with splicing (Kim and Kim, 2007). The microprocessor associates with spliceosomes (Kataoka et al., 2009), but it is not known whether, like RNA editing, the timing of miRNA excision relative to splicing is regulated by interactions with Pol II.

Packaging, Export, and the Determination of RNA Fate

As it is extruded from the polymerase, a nascent transcript encounters numerous RBPs, including cap binding complex (CBC) (Listerman et al., 2006; Visa et al., 1996), hnRNPs (Daneholt, 2001), SR proteins (Long and Caceres, 2009), the exon-junction complex (EJC) (Custodio et al., 2004), and zipcode-binding proteins (ZBP) (Pan et al., 2007), some of which remain attached for much of the transcript's lifetime. In this way, cotranscriptional RBP loading governs the transport, translation, cytoplasmic localization, and life span of the mRNA (Daneholt, 2001; Glisovic et al., 2008).

In addition to providing early protection from nucleases, cotranscriptionality may impose order on RBP association with the nascent transcript as the mature mRNP assembles. For example, CBC association with the 5′ cap early in transcript synthesis (Listerman et al., 2006; Visa et al., 1996) may direct subsequent interaction with the TREX complex (Cheng et al., 2006). TREX comprises a subcomplex called THO plus the export adaptor REF/Aly (Yra1 in yeast) and the RNA helicase UAP56 (Sub2 in yeast) (Figure 4B). TREX associates with the

TEC and enhances elongation and mRNA export (Strasser et al., 2002).

A particularly critical aspect of cotranscriptional mRNP assembly is preparation for export. Nascent transcripts are packaged for export by loading of adaptor proteins that allow the mature mRNP to engage the export receptors Mex67/Mtr2 in yeast and TAP/p15 in metazoans (Iglesias and Stutz, 2008). Recruitment of the export adaptors Yra1 and REF/Aly to the nascent transcript is carefully monitored so that only perfectly formed fully processed mRNPs become export competent (Lei et al., 2001; Schmid and Jensen, 2008). Yra1 binds the RNA helicase Sub2 that also associates with the THO complex on the TEC (Strasser et al., 2002). Yra1 recruitment requires interaction with a 3′ end-processing factor, Pcf11, that binds the phosphorylated CTD (Johnson et al., 2009) (Figure 4B). This mechanism, therefore, may ensure that export adaptor loading will only occur if the machinery is in place to properly process the mRNA 3′ end. The mechanism of Yra1 loading also illustrates how the order of assembly of mRNPs can be determined by the sequence of CTD phosphorylation. Ser2 phosphorylation specifically facilitates recruitment of Pcf11 and thereby indirectly specifies recruitment of the export factor Yra1 at later times in the transcription cycle. Following initial Yra1 loading by interaction with Pcf11, it is probably transferred to Sub2, whose helicase activity then facilitates a second transfer to the mRNA/Mex67 complex (Figure 4B). Both handoff reactions most likely occur at the TEC because all players, Pcf11, Yra1, Sub2, and Mex67, localize to sites of transcription (reviewed in Iglesias and Stutz, 2008).

In mammalian cells, the Yra1 homolog Aly is loaded cotranscriptionally by interaction with Iws1, a partner of the elongation factor Spt6 that, like Pcf11, binds CTD heptads phosphorylated on Ser2 (Yoh et al., 2007). In summary, the sequence of CTD phosphorylations that accompanies the transcription cycle helps to direct cotranscriptional mRNP assembly, as well as the processing of the nascent transcript.

Cotranscriptional Quality Control

Quality control of mRNPs is facilitated by their cotranscriptional assembly. This notion is suggested by the fact that slowing transcription elongation suppresses the growth defects of mutants that disrupt mRNP formation (Jensen et al., 2004). Yeast mRNPs that are deemed not to be of "export quality" because they have improperly formed 3′ ends are detained close to the site of transcription in a process that requires the exosome (Hilleren et al., 2001), a complex of 3′-5′ exonucleases that degrades defective RNAs. The exosome can be recruited to transcribed genes (Andrulis et al., 2002) and could, therefore, be positioned for degradation of defective mRNPs when they are released from the template with exposed 3′ ends. A possible mechanism of exosome loading in yeast is through Nrd1 and Npl3, which both bind the TEC and the exosome (Burkard and Butler, 2000; Vasiljeva and Buratowski, 2006).

Invasive Nascent Transcripts and R Loops

Another important reason for having RNPs assemble cotranscriptionally, rather than posttranscriptionally, is to protect the DNA template from invasion by naked RNA. When normal cotranscriptional handling of nascent RNA by RBPs is disrupted, R loops can form by reannealing of the transcript with DNA behind the polymerase. The displaced single-stranded nontemplate DNA strands are highly recombinogenic, causing genetic instability (Li and Manley, 2006). Similarly, in *E. coli*, disruption of cotranscriptional translation may induce nascent mRNA, no longer engaged with ribosomes, to form R loops (Gowrishankar and Harinarayanan, 2004). In yeast, R loops form in mutants of the TREX complex (Huertas and Aguilera, 2003). R loop formation and genomic instability also occur in mammalian cells when the SR proteins, ASF/SF2, or SC35 are depleted (Li and Manley, 2006). R loops can obstruct elongating RNA polymerases if they are not removed by RNaseH, and this mechanism may help to explain the pile-ups of Pol II within transcribed genes when SC35 is depleted (Lin et al., 2008).

Nuclear Pores and the TEC, a Role in "Gating"?

In addition to processing and export factors, the TEC can also be contacted by nuclear pore components, as predicted by the "gene gating" hypothesis, which proposes that export is facilitated by localizing active genes at the pore (Akhtar and Gasser, 2007) (Figure 2A). Coupling between transcription and mRNP exit from the nucleus is suggested by the observation that ongoing transcription facilitates interaction of Chironomus Balbiani Ring (BR) RNPs with nuclear pores (Kylberg et al., 2008). Specific promoters, transcriptional activators, the coactivator SAGA, 3′UTRs, and the exosome have all been implicated in "gene gating" (reviewed in Akhtar and Gasser, 2007). TEC interaction with the pore is supported by the fact that, when the THO complex is compromised, pore proteins accumulate at the 3′ ends of genes. This phenomenon may reflect a trapped intermediate in normal mRNP export (Rougemaille et al., 2008). TREX2 is a four-subunit complex that bridges the pore with the Mex67/Mtr2 and the THO complex (Fischer et al., 2002). Recent resolution of the TREX2 structure (Jani et al., 2009) gives cause for optimism that the connection between transcription and mRNP delivery to the nuclear pore will ultimately be elucidated at the atomic level.

Cotranscriptional Looping?

Transcribed genes can adopt a looped conformation that was first graphically revealed in the lampbrush chromosomes of amphibian oocytes. Pol II and mRNA-processing factors are distributed around lampbrush loops, and these structures critically depend on ongoing transcription (Gall et al., 1999). 3C studies, which detect the spatial proximity of noncontiguous DNA regions in the nucleus (Dekker et al., 2002), show that loops also form between 5′ and 3′ ends of transcribed genes in somatic cells (O'Sullivan et al., 2004). Insertion of the finger domain of promoter-bound TFIIB into the RNA exit channel of Pol II at both ends of the gene has been suggested as a mechanism for tying together the base of a loop (Singh and Hampsey, 2007). Cotranscriptional mRNA processing has also been proposed to promote looping of the HIV provirus (Perkins et al., 2008). Many questions remain about the role of cotranscriptional events in gene looping, including: Are loops a result of threading a gene through a stationary mRNA factory or a specific conformation designed to facilitate communication and polymerase recycling between the two ends of a gene? How often does

a transcribed gene adopt a looped conformation? One percent of the time? Fifty percent of the time? And can a loop withstand the torsional stress exerted by multiple polymerases on the same gene?

The TEC Is a Locator for Chromatin Modifiers and Silencers

The Pol II TEC is targeted by diverse proteins that modify the chromatin template. One of the first cotranscriptional processes identified in eukaryotes was transcription-coupled DNA repair in which the TEC stalled at a DNA lesion attracts the nucleotide excision repair machinery. Indeed, Pol II itself might be the "the most specific damage recognition protein" (Lindsey-Boltz and Sancar, 2007). Another DNA modification that is probably guided by the Pol II TEC is somatic hypermutation of immunoglobulin genes by deamination of Cs in the displaced nontemplate strand of transcribed DNA. This reaction is catalyzed by activation-induced deaminase (AID), which is probably associated with Pol II (Nambu et al., 2003).

Modification of histone N-terminal tails vastly enriches the chromatin landscape, and accurate deposition of these covalent marks is vital to many nuclear processes. The Pol II TEC serves an important auxiliary function in guiding the placement of covalent histone modifications at specific regions of the genome. Some chromatin modifiers, including the H3K4 and K36 methyltransferases Set1 and Set2, bind to the Pol II CTD phosphorylated on Ser5 and Ser2 residues, respectively (Phatnani and Greenleaf, 2006; Yoh et al., 2008). Transcription-dependent H3K36 methylation by Set2 in yeast recruits a histone deacetylase that helps to shut down cryptic promoters (Workman, 2006). Other chromatin modifiers can latch onto the RNA component of the TEC. A Chironomus histone acetyltransferase is targeted to actively transcribed chromatin by interaction with a complex of the RBP hrp65 and actin that binds to nascent RNA (Sjolinder et al., 2005).

As a result of modifiers piggy-backing on the Pol II TEC, the primary function of some transcription units is not to produce an RNA transcript, but to establish a chromatin domain that is marked by specific histone marks. In this way, one round of transcription can exert a profound influence on the future expression of that sequence. A recently identified antisense RNA of the yeast GAL10 gene is present in only 1 cell in 14; however, the transient presence of the Pol II TEC on the gene is sufficient to establish a stable domain of H3K36 trimethylation and H3 deacetylation (Houseley et al., 2008).

Paradoxically, transcription by RNA Pol II plays a central role in establishment of transcriptionally silent heterochromatin. This new paradigm is based on silencing of centromeric chromatin in fission yeast (reviewed in Moazed, 2009). Silencing is established by the RITS complex (RNA-induced initiation of transcriptional gene silencing) that recruits the methyltransferase CLRC, which deposits the signature mark of heterochromatin, methylated H3K9. RITS is targeted to the TEC by binding of its Argonaut 1 subunit to short RNA duplexes formed between siRNAs and the nascent chains made as Pol II transcribes the centromeric repeats. Transcription of the repeats is limited to a brief interval in S phase, but this is sufficient to establish a persistent heterochromatin state that persists throughout the cell cycle.

Silencing at fission yeast centromeres is specifically disrupted by mutations in the Rpb2 and Rpb7 subunits of Pol II (Djupedal et al., 2005; Kato et al., 2005). Remarkably, splicing factors, likely operating together with Pol II, help to maintain centromeric silencing (Bayne et al., 2008). In plants, a separate nonessential nuclear RNA polymerase, Pol V (IVb), forms the scaffold for cotranscriptional gene silencing. The CTD of the Pol V large subunit has GW/WG containing repeats unrelated to the Pol II CTD heptads, but like the Pol II CTD, it is a landing pad for other proteins, notably Argonaut 4 (El-Shami et al., 2007). Argonaut 4 situated on the CTD is thought to initiate silencing by binding siRNAs that are duplexed with nascent transcripts (Wierzbicki et al., 2008). In summary, like pre-mRNA processing, cotranscriptional gene silencing is specific to particular RNA polymerases.

The TEC as a Machine that Moves Chromatin-Associated Proteins

In addition to localizing histone modifiers, the Pol II TEC probably also positions other chromatin proteins along the chromosome, including histones. The act of transcription almost certainly influences nucleosome positioning by stimulating displacement in front of the TEC and replacement in its wake (Workman, 2006). This effect is mediated, in part, by remodelers and histone chaperones that travel with the TEC. In yeast, the remodeler SWI/SNF and the H2A/H2B histone chaperone FACT travel with Pol II (Workman, 2006) and facilitate transcription-coupled eviction and redeposition of histones (Figure 4C). Whether these factors bind directly to the TEC in addition to histones that are mobilized by passage of the TEC is not known. It is also possible that factor-independent effects of transcriptional elongation could influence the localization of chromatin-associated proteins as a result of torsional stress that accumulates within transcribed DNA. When a restrained template is transcribed, twin domains of supercoiling are established: positive in front and negative behind the polymerase. Positive supercoiling ejects H2A/H2B dimers from nucleosomes (Levchenko et al., 2005) in vitro, consistent with their cotranscriptional displacement in vivo (Workman, 2006). Histones displaced from chromatin by T7 RNA polymerase transcription in vitro bind more tightly to the RNA transcript than to competitor DNA (Peng and Jackson, 1997). Displacement of histones by Pol II passage could, therefore, precipitate their binding to the nascent transcript unless a mechanism is in place to avert this situation. Alternatively, transient interaction with nascent RNA could serve to localize a pool of histones at the site of transcription ready for redeposition (Figure 4C).

Another example of positioning by the Pol II TEC has been proposed for cohesins that are thought to encircle pairs of sister chromatids and maintain their cohesion during interphase. Cohesins show a remarkable tendency to congregate between convergent genes in yeast (Lengronne et al., 2004), where they may facilitate transcriptional termination (Gullerova and Proudfoot, 2008). Preventing transcription of one member of a pair of convergent genes can abolish the build-up of cohesin in the intergenic region, suggesting that the Pol II TEC pushes cohesins into place (Lengronne et al., 2004) (Figure 4A). It remains to be demonstrated directly, however, that the TEC can take on this "bulldozer"-like function.

Future Directions

A detailed understanding of the role of the TEC as an integrator and organizer of cotranscriptional activities will require answers to a number of important questions, including:

How do the right factors associate with the Pol II TEC at the right time on a particular gene? To what extent does a code of CTD phosphorylation provide the necessary information as opposed to specific signals in the sequence of the nascent transcript?

What is the relationship between cotranscriptional pre-mRNA processing and chromatin? How does chromatin structure affect cotranscriptional processing? Conversely, can processing, particularly splicing, feed back on chromatin modification?

How does the rate of growth of the RNA chain affect mRNP assembly and processing, particularly alternative splicing? How is spliceosome assembly affected by being coupled to transcription, and how does cotranscriptional spliceosome assembly differ between different constitutive and alternative introns.

What is the structural basis for targeting of the TEC by processing, packaging, and chromatin-modifying machines? In a few cases, structures of these complexes have been elucidated, but in many other cases, the nature of contacts with the TEC is still undefined.

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