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Cross-talks between Transcription and Post-transcriptional Events within a 'mRNA Factory'

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Eukaryotic gene expression is controlled in multi steps. Pre-messenger RNAs (pre-mRNAs) transcribed by RNA polymerase II (RNAPII) are capped at 5′ end, spliced to remove introns and polyadenylated at 3′ end. These processes are followed by other post-transcriptional steps that include mRNA export, translation and mRNA degradation. Recent studies have shown that individual events occurring during eukaryotic gene expression are coupled together under more elaborate regulatory controls. In this review, the molecular and functional cross-talks between transcription and the post-transcriptional events are overviewed.

Key words: export, mRNA factory, RNA processing, splicing, transcription.

Abbreviations: CBC, cap-binding complex; CE, capping enzyme; ChIP, chromatin immunoprecipitation; CPSF, cleavage/polyadenylation-specific factor; CTD, the carboxyl terminal domain of the largest subunit of RNA polymerase II; EJC, exon-junction complex; MLP, adenovirus major late promoter; mRNP, mRNA-protein complex; NMD, nonsense-mediated mRNA decay; REF, RNA and export factor binding proteins; RNAPII, RNA polymerase II; TREX, transcription/export (complex).

The molecular cross-talk (or coupling) of multiple steps of gene expression have been suggested by co-purification and co-localization of regulatory factors of each step. In addition, functional cross-talk has been discussed with *in vitro* coupling assay and chromatin immunoprecipitation (ChIP) assay. The molecular cross-talk means that components are shared in more than two processes, and the functional cross-talk of two processes is defined as one process affecting the rate and/or efficiency of another (*1*). These definitions exclude the simple coupling that one process requires the product of another. Also excluded by the definitions are processed where the machinery of one associates with that of another, but neither process is affected by the association.

FUNCTIONAL COUPLING OF TRANSCRIPTION TO CAPPING

Pre-mRNAs processing appears to be physically and functionally coupled to RNAPII transcription (*2*, *3*) as shown in Fig. 1. At present, most is known about functional coupling of transcription to capping (*4–6*). Eukaryotic mRNAs are modified at the 5′ end by the addition of an m7GpppG cap by the sequential action of three enzymatic activities (*7*). Evidence is accumulating that capping is facilitated by an association of the capping enzymes (guanyltransferase and methyltansferase) with the RNAPII elongation complex through an interaction with the phosphorylated form of the carboxyl terminal domain (CTD) of the largest subunit of the polymerase (*2*, 8, 9). Ser5-, but not Ser2-phosphorylated CTD stimulates the GMP intermediate formation, and the stimulation is dependent on Cdk7 (*5*). Interestingly, capping enzymes stimulate early steps in RNAPII transcription including promoter clearance, and the functional coupling is thought to serve as an important timing mechanism, which is required to prevent degradation of the nascent transcript (*10*). Furthermore, the 5′ cap structure, a target for the cap-binding complex (CBC), stimulates pre-mRNA splicing (*11*, *12*) and polyadenylation (*13*) in the nucleus. A ChIP assay in yeast has shown that the CBC is necessary for the correct co-transcriptional assembly of the spliceosome (*14*) and that CBC depletion reduced the recognition of cap-proximal 5′ splice sites but did not affect that of cap-distal splice sites (*15*). These observations indicate that capping reaction is coupling to both transcription and post-transcriptional events.

REGULATION OF PRE-MRNA SPLICING BY RNAPII

The first demonstration of co-transcriptional splicing came from the direct visualization of Miller chromatin spreads from *Drosophila melanogaster* embryos (*16*). Studies examining the splicing rate of transcripts from the Balbiani ring 1 gene in *Chironomus tentans* and from the human dystrophin gene demonstrated that 5′ introns are mostly removed co-transcriptionally, while the 3′ introns of these long transcripts are generally removed post-transcriptional splicing (*17*, *18*). Recent studies have presented following evidence to support co-transcriptional splicing; (*1*) certain mutations in the CTD specifically affect splicing, but not

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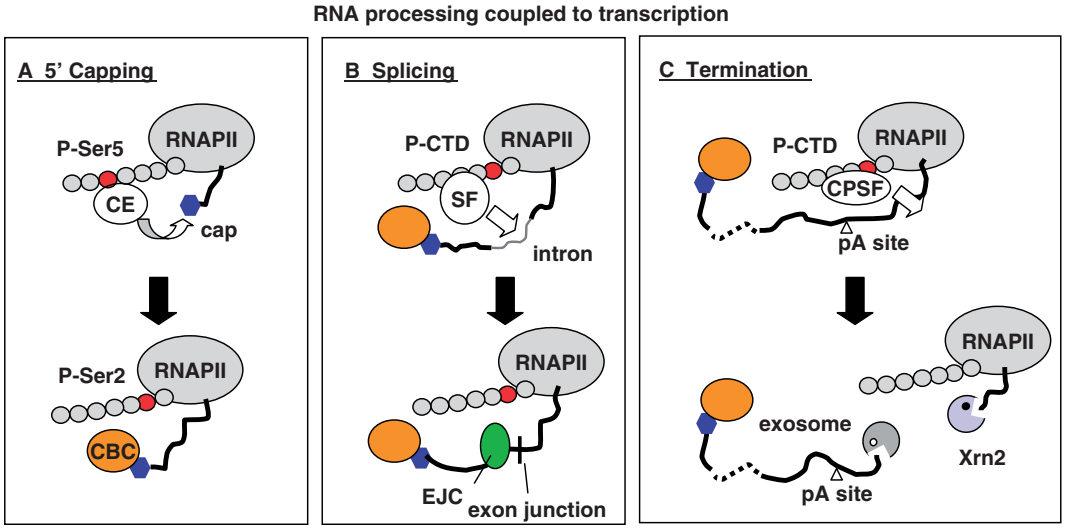

Fig. 1. **Cross-talk between transcription and post-transcriptional events.** (A) Capping enzymes (CE) are recruited on the CTD of RNAPII. (B) Splicing factors (SF) are also recruited on the CTD of RNAPII and export factors of EJC are bound on mRNA through splicing. (C) Cleavage/polyadenylation-specific factors (CPSF), an exosome and a 5′–3′ RNA exonuclease termed Rat1/Xrn2 are recruited on the CTD of RNAPII.

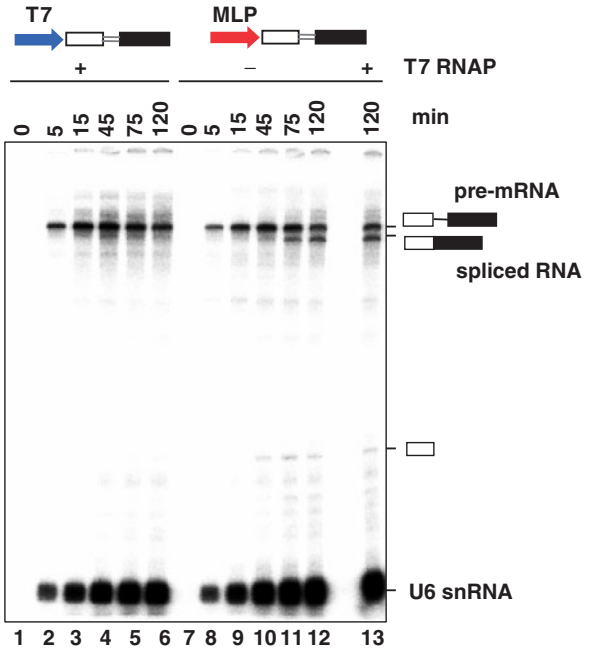

Fig. 2. **Coupling of transcription and splicing.** Human β-globin DNA driven by T7 (lanes 1–6) or MLP (lanes 7–13) was incubated with HeLa nuclear extract under transcription/splicing coupling condition. β-globin pre-mRNAs transcribed from MLP were spliced more efficiently than those transcribed from T7.

transcription (*19*); (*2*) different promoters, enhancers and transcription activators can affect the choice of splicing sites *in vivo* (*20*, *21*); (*3*) rate of transcriptional elongation affects the choice of alternative splicing sites (*22*) and (*4*) splicing factors are recruited from nuclear speckles, in which they are concentrated, to sites of transcription upon the transcriptional activation of a gene *in vivo* (*23*). Although it is not precisely understood how transcription exerts its effect on splicing, the CTD is supposed to serve as a scaffold for splicing factors. Various metazoan splicing-related factors, including p54^{nrb}, PTB-associated splicing factor (PSF) (*24*), CA150 (*25*), UsnRNPs and SR proteins (*26*), are found in large RNAPII complexes and/or directly bound to the CTD. Actually RNAPII transcription results in a dramatic increase in both the kinetics of splicing and overall yield of spliced mRNA relative to that observed for T7 transcription (Fig. 2). Thus RNAPII helps recruitment of splicing factors to nascent introns, which in turn may encourage further transcription. In addition, the presence of a promoter proximal intron increases the amount of nascent transcript, and the targeting of splicing factors to a gene resulted in the stimulation of transcription elongation. These observations suggest a reverse relationship between transcription and splicing. On the contrary to co-transcriptional splicing discussed earlier, the presence of functional spliceosomes and signal-dependent pre-mRNA splicing in the cytoplasm of platelet was recently reported (*27*). It is conceivable that, splicing is regulated by, at least, two types (co-transcriptional and transcription-independent) of mechanisms.

CO-TRANSCRIPTIONAL TERMINATION

Pre-mRNAs are processed at their 3′ ends by a two-step reaction; (*1*) the nascent pre-mRNA is cleaved 20–30 bases downstream of a conserved poly (A) site by cleavage/polyadenylation factors which consist of CstF, CPSF, CFIm and CFIIm in mammals; and (*2*) poly(A) polymerase (PAP) adds a poly(A) tail to the 3′ OH that is created on the upstream side of the cut (*28*). The recent development of *in vitro* assay systems for coupling of RNAPII transcription to 3′-end cleavage/polyadenylation

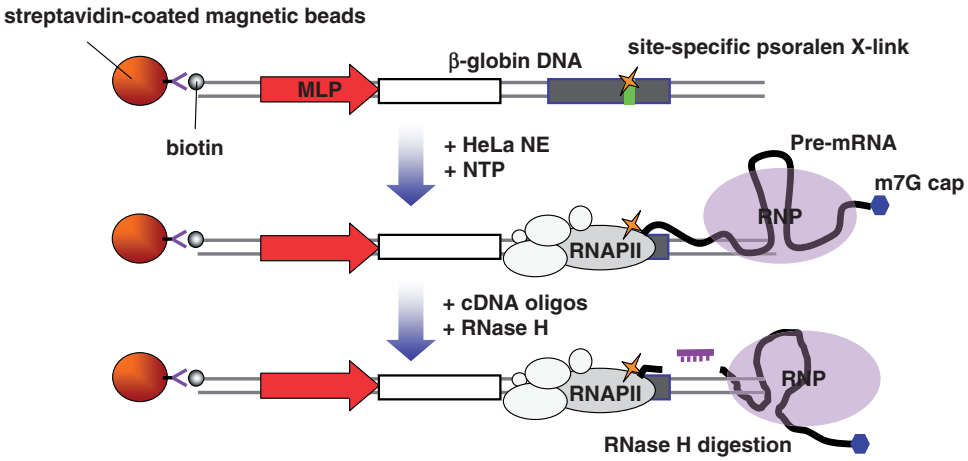

Fig. 3. **Novel method to isolate mRNPs recruited by RNAPII.** Using an immobilized template DNA with psoralen UV cross-linking, RNAPII transcription was performed. The RNPs recruited by RNAPII were precipitated with beads. After extensive washing, the RNPs were released by the cut with RNase H.

has also led to insights into the mechanisms involved in this coupling event; maturation of mRNA 3′ ends is initially coupled to termination of transcription, and a 5′–3′ RNA exonuclease termed Rat1/Xrn2, which binds to the CTD of RNAPII, is shown to play a key role in this reaction (*29*, *30*). ChIP experiments in yeast revealed that 3′ mRNA processing factors are recruited not only to the 3′ end and to the promoter and throughout the length of the gene (*31*), though they increase towards the 3′ end in other genes.

TRANSCRIPTION-DEPENDENT mRNA EXPORT PATHWAY

The transport of mRNA from the nucleus to the cytoplasm is linked to pre-mRNA splicing, especially in metazoans (*32*). Exon junction complexes (EJCs), which are deposited on mRNAs at specific sites relative to the exon junction as a consequence of splicing, form the basis of this connection (*33*). The EJC consists of multiple proteins including REF and UAP56. The recruitment of REF during mRNA biogenesis is thought to be responsible for the increased export of spliced mRNA (*34*). UAP56 is required for the recruitment of REF to mRNA (*35*). Subsequently, UAP56 is displaced from REF by the mRNA export factor TAP (also named NXF1) (*36*). TAP forms a heterodimer with p15 that then directly interacts with the nuclear pore to facilitate mRNP transport into the cytoplasm (*37*).

Although the above model explains the apparent link between splicing and RNA export in metazoans, the question of how intronless mRNAs, which lack EJC deposition, are exported to the cytoplasm naturally arises. Some intronless transcripts (e.g. histone H2A) have been reported to contain specific sequences that recruit export factors independently of splicing (*38*). SRp20 and 9G8, which belong to members of the evolutionarily conserved SR (serine/arginine-rich) protein family, specifically bind to a sequence in intronless mRNA and greatly facilitate the export of mRNA by recruiting TAP (*39*). However, the intronless mRNAs coding Ftz, DHFR and β-globin, which lack such *cis*-acting sequences, can be effectively exported regardless of whether splicing has occurred. The injection experiments of α-REF antibody into the nuclei of *Xenopus* oocytes indicated that REF stimulates directly the export of these intronless mRNAs (*40*). Mass spectrometry and Western blotting of purified spliceosomes revealed that REF is a component of H complex (*41*), a heterogeneous complex that forms on any largely single-stranded RNA, suggesting that REF can associate with mRNAs in a splicing-independent manner. *In situ* analysis of GFP-tagged REF showed its accumulation at sites of transcription (*42*), suggesting that REF binds to mRNA co-transcriptionally.

In yeast, the THO complex interacts genetically and physically with components of mRNA export machineries (*43*). Yra1p and Sub2p (REF and UAP56 in mammals, respectively) are stoichiometrically associated with the heterotetrameric THO complex, and is recruited to transcription-activated genes with or without introns. The complex is designated the transcription/export (TREX) complex (*44*) in mammals. Recently REF binds to CBC at 5′ cap site for mRNA export in mammals (*45*). These observations suggest that one of mRNA export pathways is cooperated with transcription, because capping occurs co-transcriptionally.

CROSS-TALKS OF TRANSCRIPTION, TRANSLATION AND NMD?

Introduction of an in-frame stop codon into an earlier exon often triggers destruction of the faulty transcript, and this interesting phenomenon was named as nonsense-mediated decay (NMD) (*46*). As ribosomes detect the termination codons, Iborra *et al*. hypothesized the presence of nuclear ribosomes and translation, if NMD occurs in the nucleus. They proposed the molecular crosstalk between the transcription, translation and NMD machineries in the nucleus (*47*). But their hypothesis is still under debate.

FUTURE PERSPECTIVES

As described earlier, mRNA processing, such as capping, splicing and termination, is clearly cooperated with RNAPII transcription in eukaryotic cells. There are still many questions left to be answered about mRNA processing *in vivo*. Especially, the mechanism of alternative mRNA splicing coupling to transcription is obscure. How are alternative splice sites selected during RNAPII transcription? Which factors or complexes are recruited to splice sites on nascent RNAs by RNAPII? The answers to these questions require a novel method to analyse the machinery. Recently, we developed an efficient *in vitro* splicing/transcription system (*45*), which could isolate mRNP complex recruited by RNAPII (Fig. 3). We expect that our *in vitro* system will contribute to clarify the coupling mechanisms of mRNA processing with transcription.

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