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The Spanish Connection: Transcription and mRNA Processing Get Even Closer

Meeting Review

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The synthesis of mRNA by RNA polymerase II appears coupled to numerous RNA-processing events, based on physical or functional connections revealed by biochemical or genetic tests. New findings were presented at a recent meeting in Spain that begin to illuminate the mechanisms underlying the connections between mRNA processing and specific steps in transcription (initiation, elongation, and termination) as well as recombination.

The production of functional messenger RNA in eukaryotes requires not only transcript synthesis by RNA polymerase II (Pol II) but also multiple RNA-processing steps such as capping, splicing, polyadenylation, and RNA editing. Recent research has shown that many of these processing steps occur cotranscriptionally, revealing a close association (coupling) between transcription and mRNA processing (Proudfoot et al., 2002). At a recent meeting in Spain (November 7–10, 2004), new findings were presented that begin to clarify the connections between transcription (initiation, elongation, and termination) and messenger RNA processing and even recombination. The meeting, titled "Coupling between Transcription and RNA Processing" and organized by Miguel Beato and Juan Valcarcel, was hosted by the Universidad Internacional de Andalucia at the Sede Antonio Machado, a restored 17th century palace in Baeza, site of a Renaissance university in the south of Spain. The intimate setting and small size of the meeting successfully encouraged open discussion. A shared goal of the participants was to learn more about the molecular mechanisms coupling transcription and RNA processing. Such connections are already in part defined by protein-protein or protein-nucleic acid interaction data using either biochemical or genetic tests. However, the precise mechanisms by which coupling occurs remain largely mysterious.

Early Elongation Coupling

Once Pol II has escaped from a gene promoter into early elongation, a number of molecular events appear to take place before elongation can fully proceed (Figure 1A). At early elongation, the C-terminal domain (CTD) of the Pol II large subunit (Rpb1) becomes phosphorylated on serine 5 positions within its characteristic heptad repeats. Ser5 phosphorylation at CTD recruits the mRNA capping complex, which adds a cap to the initial nascent transcript. Ser5 phosphorylation is also associated with specific nucleosomal marks at the 5′ end of the gene, such as histone H3 lysine 4 trimethylation (Hampsey and Reinberg, 2003).

Several presentations dwelt on new aspects of coupling during early transcription elongation. John Lis (Cornell University, New York) described the presence of the elongation factor TFIIS on RNA polymerase II that is paused at the beginning of *Drosophila* heat shock genes prior to heat induction. During normal elongation, TFIIS is thought to promote cleavage of those transcripts whose 3′ ends are misaligned in the polymerase active site, thus allowing polymerase to escape transcript arrest (Fish and Kane, 2002). A similar scenario now appears to operate at the heat shock genes where TFIIS-stimulated transcript cleavage is required to allow the stalled Pol II to resume elongation following heat shock activation (Adelman et al., 2005). Danny Reinberg (University of Medicine and Dentistry of New Jersey, New Jersey) described biochemical analysis of a large complex containing both elongation and chromatin remodeling factors. Components of this complex specifically associate with Lys4 trimethylated histone H3, a well-known histone modification found at the transcription start site (Ng et al., 2003). Interestingly, this complex significantly stimulates in vitro splicing reactions, as shown by a collaborative experiment with the laboratory of Jim Manley (Columbia University, New York). These results raise the possibility that recruitment of this complex during early elongation may facilitate subsequent splicing reactions that occur cotranscriptionally.

Packaging mRNA into mRNPs during Elongation: The R Loop Story

It has long been known that pre-mRNA is packaged into protein-associated complexes (mRNPs, originally called hnRNPs) that contain both general nuclear proteins such as hnRNP A (and other family members) and more specific splicing/mRNA export-associated protein factors such as the THO/TREX complex (Jensen et al., 2003). While this packaging process is well known to be required for mRNA maturation, it also appears to be required for protection of the integrity of the DNA template itself. New findings were presented on the intriguing phenomenon of transcription-dependent hyperrecombination, which is stimulated by defects in elongation factors or in mRNA export. Recently published studies in yeast from Andres Aguilera's lab (University of Seville, Spain; Huertas and Aguilera, 2003) suggested a provocative model in which, if the nascent RNA emerging from Pol II is not immediately and properly coated by mRNP packaging proteins, then it will result in the formation of an R loop with underwound DNA located just behind

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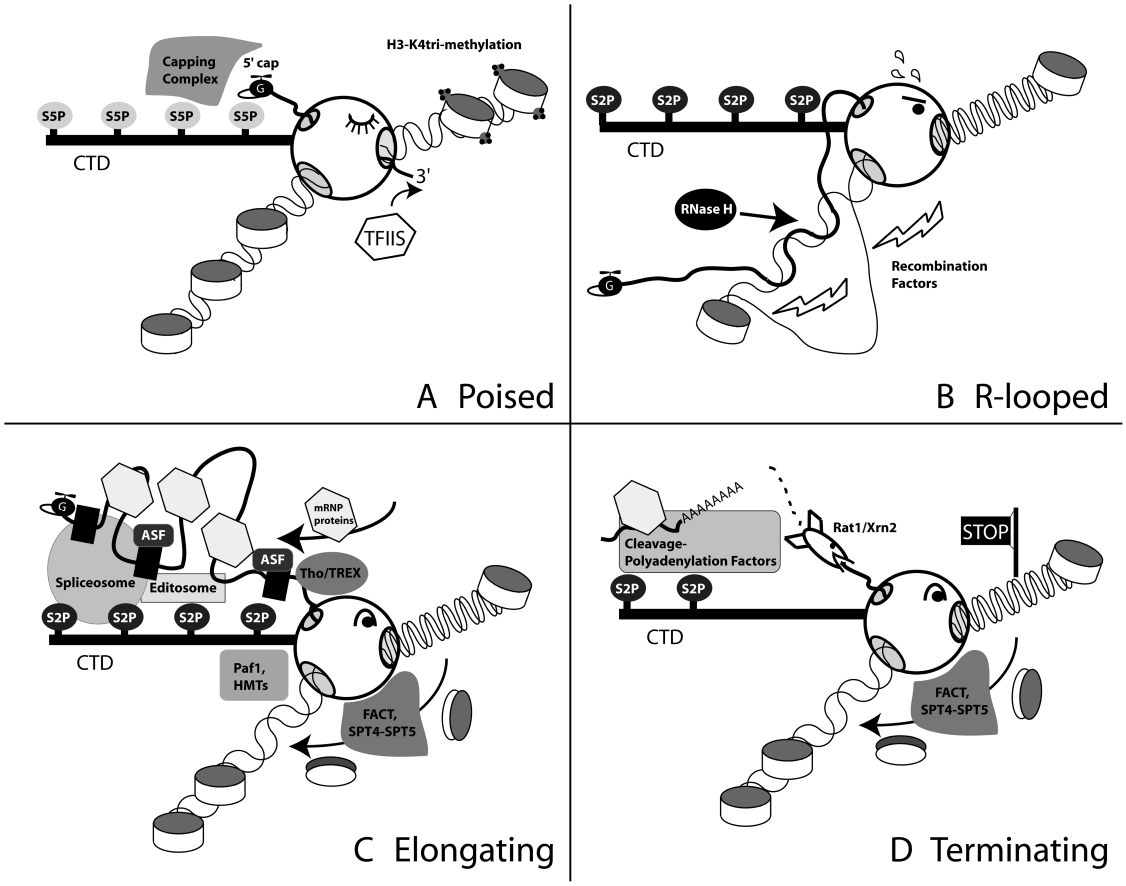

Figure 1. Cartoons Depicting Four Stages in the Coupling of Pol II Transcription with Pre-mRNA Processing

See text for full details.

(A) Elongating Pol II may be poised at the start of the gene with its CTD heptad repeats in a serine 5-phosphorylated state and the nucleosomes marked by histone H3 lysine 4 trimethylation (H3-K4 trimethylation). The involvement of TFIIS and the capping complex is indicated.

(B) Deficient pre-mRNA packaging may result in topological pausing of elongating Pol II through the formation of pre-mRNA:DNA R loops. The looped-out sense DNA strand is then susceptible to DNA damage by hyperrecombination. R loops may be relieved by RNase H activity.

(C) Pre-mRNA is cotranscriptionally packaged, spliced, and edited during elongation. Nucleosomes are also repositioned on the DNA chromatin template during elongation.

(D) Termination of Pol II transcription. Cleavage and polyadenylation at the 3′ end of the pre-mRNA is mediated by cleavage/polyadenylation factors. The downstream transcript that remains attached to the elongating Pol II is then subjected to 5′-3′ exonucleolytic degradation, eventually leading to the release of Pol II from the DNA template.

the elongating polymerase (Figure 1B). This in turn leads to both impairment of transcription and a hyperrecombination phenotype restricted to the transcribed locus. Now Jim Manley's group reports the unexpected finding that genetic inactivation of the SR protein splicing factor ASF/SF2 in chicken DT40 cells (Wang et al., 1998) results in a similar phenotype. This new study highlights the critical importance of properly integrating transcription with the correct processing of the nascent transcript. Together, these findings bolster the hypothesis that defective packaging of the nascent transcript leads to R loop formation behind the polymerase, which stimulates recombination and may also drag the elongating polymerase to a halt.

The contribution of nascent RNA packaging to elongation was further illuminated by exciting data from Andres Aguilera and Torben Heick Jensen (Aarhus University, Denmark). They both described experiments illustrating the effect of THO complex mutations on transcription elongation. Jensen showed that mutations in elongation factors such as Rad3 (a subunit of TFIIH) and Dst1 (a homolog of mammalian TFIIS) mitigated the transcription elongation defect caused by mutations in the THO complex, suggesting that the inadequacy of mRNP packaging caused by lack of THO is more pronounced when transcription elongation proceeds at a higher rate (Jensen et al., 2004). On a related theme, he also showed that THO defects appear to adversely affect later elongation stages, causing apparent premature termination. Similar observations were also made in the Aguilera lab. Manny Ares (University of Santa Cruz, California) reported on experiments with Grant Hartzog's lab in which they found that yeast cells carrying mutations in genes for THO/TREX/, PAF, and FACT complex subunits share highly similar genome-wide reduction in pre-mRNA and mRNA levels. Both PAF and FACT are chromatin-associated elongation factors (Hampsey and Reinberg, 2003; Belotserkovskaya et al., 2003). This is consistent with

the notion that compromising the function of proteins involved in mRNA packaging and elongation leads to common downstream defects in gene expression (Burckin et al., 2005). All of these results suggest that, in the absence of efficient mRNP packaging, topological problems arise when the nascent RNA exits the elongating Pol II complex. First, R loops may form (as described above), and second, transcription elongation may be topologically restricted, a problem that could be exacerbated at the end of highly transcribed genes.

Coupling Splicing to Transcription Elongation

Although mRNA splicing can be shown to occur in test tubes on synthetic RNA substrates, it is likely that splicing in vivo is tightly regulated (Figure 1C). Indeed, it has been demonstrated over recent years that transcriptional elongation can directly influence splicing patterns across larger genes (de la Mata et al., 2003; Proudfoot, 2003). Alberto Kornblihtt's group (University of Buenos Aires, Argentina) presented evidence of a polar effect for alternative splicing. It appears that the inclusion of an upstream alternative exon enhances the subsequent inclusion of a downstream alternative exon in the same nascent transcript. This exciting development could open the way to understand how remote alternative splicing events might be coordinated within the same transcript, possibly through interactions that occur during elongation. In particular, Kornblihtt described experiments on the fibronectin gene with either of two alternative exons mutated in heteroallelic transgenic mice (made in Tito Baralle's laboratory at ICGEB, Trieste, Italy; Chauhan et al., 2004). He showed that the polar effect occurs in *cis* and that, although inclusion of the upstream exon enhances inclusion of the downstream exon, the inclusion of the downstream exon does not influence inclusion of the upstream exon. Both the polar effect and its allelic specificity strongly suggest that alternative splicing decisions in the upstream parts of a transcript can influence those downstream, possibly during transcription elongation.

More is sure to be learned about the molecular events connecting splicing to transcription now that a coupled in vitro system is under development. Barbara Natalizio of Mariano Garcia-Blanco's laboratory (Duke University, North Carolina) presented her work employing human cell extracts that could support both transcription and splicing. Comparison of splicing of exogenously added transcripts generated by T7 polymerase with those synthesized in situ by either added T7 polymerase or the endogenous RNA polymerase II indicates that in situ-synthesized Pol II transcripts are more rapidly spliced. Splicing of a two intron β-globin precursor is much more efficient in this system than observed previously (Ghosh and Garcia-Blanco, 2000). Several critical modifications in the composition of the system will be described in a forthcoming manuscript. Although it is still not clear how precisely "cotranscriptional" these splicing events are (the precursor accumulates rapidly, but splicing occurs quite slowly), the system described by Natalizio may present an attractive alternative to simply tossing in naked T7-derived transcripts for those of us who want to know how the machinery of transcription anticipates the need to splice.

Attempts to capture the synchronicity of transcription and splicing events in vivo using chromatin crosslinking of splicing factors continue to move forward in yeast, following on the initial finding that the U1 snRNP is cotranscriptionally recruited to intron-containing genes only (Kotovic et al., 2003). That work showed that the U1 snRNP associates with chromatin via splice donor site sequences, rather than at promoters or downstream regions in general, indicating that its recruitment is specified by introns. New work from Karla Neugebauer's lab (University of Dresden, Germany) presented by Janina Gornemann extends this study by showing that additional components of the splicing machinery also accumulate on intron-containing genes in a temporal and spatial pattern consistent with nascent RNA-dependent recruitment or stabilization. Studies using yeast strains harboring deletions of conserved but nonessential splicing factors are beginning to provide intriguing information on how different factors influence the timing of snRNP association with the transcribed gene.

Coupling of Polyadenylation and Editing to Transcription

Connections between transcription and mRNA processing are also important at the end of genes. Indeed, mRNA 3′ end processing and polyadenylation have been known to be associated with Pol II termination for almost two decades. Now, independent studies in both yeast and mammalian systems provide new insights into this coupling process. Depletion of a nuclear 5′-3′ exonuclease (Rat1p in yeast or Xrn2 in human cells) causes a clear termination defect, and data presented by Steve Buratowski (Harvard Medical School, Boston) and Nick Proudfoot (University of Oxford, UK) showed that the exonuclease acts like a molecular torpedo by attaching to the 5′ end of the nascent RNA generated by cleavage either at the poly(A) signal or at cotranscriptional cleavage sites. In a model that is reminiscent of rho-dependent termination in bacteria, the 5′-3′ exonuclease torpedo is thought to track its way along the RNA chain to the elongating polymerase and trigger destabilization of the ternary complex (Kim et al., 2004; West et al., 2004; Teixeira et al., 2004) (Figure 1D).

A final example of coupling is the intriguing process of mRNA editing (Keegan et al., 2001), which is manifested by targeted deamination in mRNAs (cytosine to uracil and adenosine to inosine). In the later case, adenosine deaminases (ADARs) recognize specific RNA duplexes in pre-mRNA, resulting in conversion of selected adenosine to inosine. Inosine is recognized in the translation process as guanosine, hence changing the genetic code. Two presentations from Liam Keegan (MRC Human Genetics Unit, Edinburgh, United Kingdom) and Marie Ohman (Stockholm University, Sweden) reminded us that mRNA editing sites recognized by the enzyme ADAR are often partly intronic; therefore, in such cases, editing must occur prior to splicing. Furthermore, Marie Ohman described the requirement of the Pol II CTD for efficient editing at these intron-exon junction sites. Thus, akin to capping, splicing, and polyadenylation, the mRNA editing process also appears to be tightly linked to Pol II through the versatile CTD.

What Does Coupling Really Mean?

Toward the end of the meeting, a panel discussion was held to address the question, "What does coupling really mean?" While many are happy with the flexibility afforded by the ambiguity of the word "coupling," others felt that specific biochemical or molecular criteria ought to be applied. These criteria may vary and could be articulated by applying various adjectives, such as "kinetic coupling," "tight coupling," "physical coupling," or "informational coupling." However, no consensus could be reached before dinner. Everyone did agree that they heard new interpretations of what coupling might be and how it might be implemented during Pol II transcription. The notion that the CTD is central to the coupling process, while still not proven, was not significantly diminished, either. Regardless of the precise role of the CTD, the focus is clearly on the events that occur on the nascent transcript as it emerges from the polymerase and on how these molecular events are coordinately managed. The events that trail behind the elongating polymerase, including capping, mRNP assembly, chromatin reassembly, efficient splicing, alternative splicing, polyadenylation, editing, and termination, exert diverse impacts both on the transcript structure and on polymerase function. It is safe to anticipate that there will be new surprises in this area in the near future.

Acknowledgments

We are grateful to our colleagues for allowing their unpublished work to be described. We apologize to participants at the meeting whose work we were unable to include in this account of the Baeza meeting. Finally, we are indebted to the meeting organizers and the Universidad Internacional de Andalucia Sede Antonio Machado for a unique scientific and Spanish experience.

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