

Assembly of Export-Competent mRNP: It's All about Being Connected

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In this issue of *Molecular Cell*, Johnson et al. (2009) reveal a physical link between the yeast Pcf11p and Yra1p proteins, providing insights into the coupling of mRNA 3' end formation and export.

Transcription of genes by RNA polymerase II (RNAPII) can be viewed as a molecular assembly line with mRNA nucleoprotein particles (mRNPs) as the output material. During transcription, 5' and 3' RNA ends are modified by protective cap- and poly(A)-tail moieties, and the nascent molecule is wrapped with proteins. These molecular transactions all contribute to maturation of the nascent mRNP, in preparation for its release from the chromatin template. A good decade of research has disclosed that individual steps in such cotranscriptional mRNP biogenesis are closely coupled so that one reaction normally exerts a positive impact on the next. However, unlike a simple sequential assembly process, a tight network of cross-stimulatory connections and physical interdependencies are in place. Such intimacy of reactions is believed to optimize gene expression, while at the same time allowing the functional status of the emerging mRNP to be "communicated" to nuclear quality control systems (Jensen et al., 2003).

To understand in detail how mRNPs are built, it is therefore pertinent to decipher this complicated web of interactions down to its individual molecular interactions. An important step forward is provided in the present issue by Johnson and colleagues, who reveal an unexpected physical link between the *S. cerevisiae* mRNA 3' end processing complex and the mRNP export adaptor protein Yra1p (Johnson et al., 2009). Yra1p is a central molecule as it bridges the interaction between the nascent mRNP and its essential export receptor, the Mex67p/Mtr2p heterodimer (Strässer and Hurt, 2000). Earlier reports suggested that cotranscriptional recruitment of

Yra1p takes place inside the biochemically stable TREX (*transcription/export*) complex, also containing the DECD-box RNA helicase Sub2p and the tetrameric THO complex (Strässer et al., 2002; Zenklusen et al., 2002). Yra1p, Sub2p, and THO interact stoichiometrically, yet due to its tighter interaction with THO, which in turn associates with the transcription machinery, it was proposed that Sub2p recruits Yra1p to transcriptionally active chromatin for incorporation into nascent mRNP (Strässer and Hurt, 2001; Strässer et al., 2002). However, direct proof for this idea has been lacking. In addition, whereas Sub2p chromatin immunoprecipitation (ChIP) signals are sensitive to RNase treatment, Yra1p ChIP signals are only partially sensitive, suggesting an alternative pathway for Yra1p recruitment (Abruzzi et al., 2004).

In their paper, Johnson et al. (2009) set out to directly test the requirement of Sub2p for Yra1p recruitment and find that, relative to the occupancy of RNAPII along several reporter genes, Yra1p levels remain unchanged or are even slightly higher in *sub2* mutants exhibiting dramatic decreases in Sub2p ChIP signals. Instead, loading of Yra1p to the chromatin template requires a functional 3' end-processing machinery, as mutation of *Rna14p*, *Rna15p*, or *Pcf11p*, which, together with *Clp1p*, constitute the 3' end cleavage factor IA (CFIA), all decrease Yra1p "ChIP-ability." This result appears to reflect a specific and direct interaction between Pcf11p and Yra1p, as (1) recombinant Yra1p binds Pcf11p, but not *Rna14p* or *Rna15p*, and (2) artificial tethering of Pcf11p onto RNA increases Yra1p association. To integrate their observations with previous data, Johnson et al. (2009) propose that Yra1p,

after initial recruitment by Pcf11p, is transferred to the nascent transcript in a Sub2p-dependent manner (Figure 1A).

Pcf11p plays a critical role in promoting association of CFIA with the transcription machinery through its interaction with the Serine-2 phosphorylated C-terminal domain (CTD) of RNAPII. In doing so, it not only sets the stage for mRNA 3' end formation, but also prepares for transcriptional termination. Hence, the findings of Johnson et al. (2009) are noteworthy as they pinpoint a functional connection between transcription termination, 3' end processing, and mRNA export, and, once again, underscore the importance of the phosphorylation status of the RNAPII CTD in coordinating these events. The new data also demonstrate that the Yra1p-binding site of Pcf11p encompasses its Clp1p interaction domain. In contrast to the other CFIA subunits that interact with each other, Clp1p only binds Pcf11p. Thus, although it remains undetermined whether the Yra1p/Pcf11p interaction functionally affects 3' end processing, it is an appealing possibility that during transcription elongation it may hinder full CFIA assembly, thereby preventing premature cleavage of the nascent mRNA (Figure 1B). Interestingly, Yra1p ChIP levels dramatically decrease when RNAPII reaches the poly(A) site where 3' end processing factors are robustly recruited (Kim et al., 2004). Thus, at this point in time, Clp1p might counter the Pcf11p/Yra1p interaction and trigger mRNA 3' end processing.

Equally interesting is the finding that Pcf11p-interaction domains on Yra1p are localized within the two areas of the protein onto which Sub2p and Mex67p also bind (Strässer and Hurt, 2001;

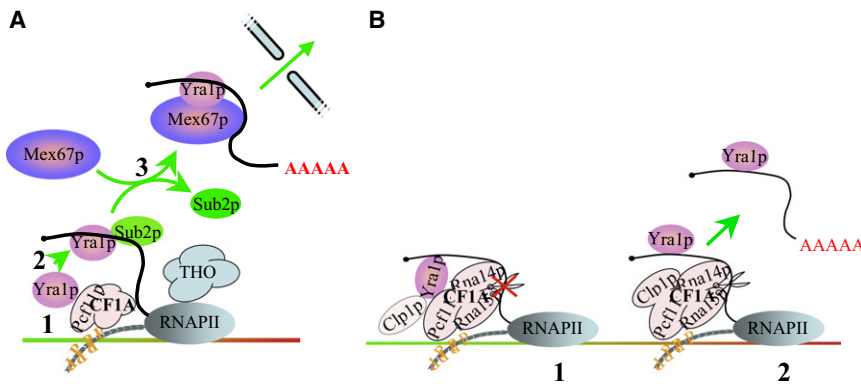


Figure 1. Models for Connecting mRNA 3' End Processing and Export

(A) Pcf11p-dependent recruitment of Yra1p to chromatin. (1) Yra1p binds the Clp1p interaction domain of Pcf11p. (2) Transfer of Yra1p to the nascent transcript in a Sub2p-facilitated manner. (3) Exchange of “early” (Sub2p) and “late” (Mex67p) mRNA export factors.

(B) An mRNP effect on 3' end processing? (1) Binding of Yra1p to the Clp1p interaction domain of Pcf11p might prevent premature assembly of an active 3' end cleavage complex. (2) Association of Clp1p might out-compete Yra1p/Pcf11p binding leading to both mRNA processing and mRNP release.

Strässer et al., 2002). The association of Yra1p with Sub2p and Mex67p is mutually exclusive, suggesting how exchange between “early” and “late” mRNA maturation factors may occur (Strässer and Hurt, 2001). Hence, in a similar fashion, Yra1p could swap partners from Pcf11p to Sub2p in a reaction prior to the Sub2p/Mex67p exchange. Although based on available data, this is certainly possible, other scenarios seem equally likely. This is because Mex67p is also cotranscriptionally recruited to chromatin (Dieppo et al., 2006). Moreover, THO/Sub2p-mediated mRNP biogenesis

appears to occur in close proximity with the nuclear pore complex (Rougemille et al., 2008). Thus, the division of mRNP maturation factors into “early” and “late” is blurred, and it is an open question how loading and exchange of these factors occur in their possible combinatorial competition for the same partners and binding sites.

In fine, the observations by Johnson et al. (2009) advance the field by suggesting a direct link by which the process of 3' end formation can enhance mRNP assembly and nuclear export. At the same time, the present study provides

a starting point to dissect the *in vivo* dynamics of TREX complex components around active chromatin templates and how these important factors partake in the mRNP assembly process. A continued combination of *in vitro* characterization of molecular interactions with *in vivo* functional analyses provides one productive way forward.

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