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Review

The eukaryotic transcriptional machinery regulates mRNA translation and decay in the cytoplasm ☆

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ABSTRACT

In eukaryotes, nuclear mRNA synthesis is physically separated from its cytoplasmic translation and degradation. Recent unexpected findings have revealed that, despite this separation, the transcriptional machinery can remotely control the cytoplasmic stages. Key to this coupling is the capacity of the transcriptional machinery to "imprint" the transcript with factors that escort it to the cytoplasm and regulate its localization, translation and decay. Some of these factors are known transcriptional regulators that also function in mRNA decay and are hence named "synthegradases". Imprinting can be carried out and/or regulated by RNA polymerase II or by promoter *cis*- and *trans*-acting elements. This article is part of a Special Issue entitled: RNA polymerase II Transcript Elongation.

1. Introduction

The eukaryotic transcription apparatus, which utilizes *cis*-acting DNA elements and *trans*-acting factors, functions in the nucleus. Up until recently, it was believed that this machinery has no direct effect on the fate of the RNA in the cytoplasm. This common view is currently being challenged, at least in the case of RNAs that are synthesized by RNA polymerase II (Pol II). Pol II is responsible for the transcription of all mRNAs, many non-coding RNAs, all snoRNAs (except for snRN52), most of the snRNAs and the telomerase RNA. The ten-subunit structure of Pol II is capable of transcription *in vitro* and is considered to be the "core Pol II" [1–5]. Two other subunits, Rpb4p and Rpb7p, form a distinct substructure. These two subunits strongly interact with each other, forming the Rpb4/7 heterodimer [6]. The Rpb7p "tip" interacts with a small "pocket" in the Pol II core, composed mainly of a small region of Rpb1p, Rpb2p and Rpb6 [2,7]. The interface between Rpb7p and the core Pol II is small enough such that the two substructures readily dissociate *in vitro*. Indeed a single mutation in either Rpb1p or Rpb6p can destabilize and substantially weaken the interaction between the two substructures [2,7–9]. Rpb4p and Rpb7p are present in excess over the core subunits [10], raising the possibility that they also function outside the context of the polymerase [6]. Indeed, it was found that both subunits play roles in mRNA export [11], translation [12] and degradation [13–15]. Here we discuss the various roles of Rpb4/7, emphasizing its post-transcriptional functions. Remarkably, Pol II regulates these post-transcriptional stages by providing the correct context for Rpb4/7 to interact with the transcript, as it emerges from Pol II. In addition to Pol II, other elements of the transcription apparatus can control the fate of their transcripts; among them are promoter elements and *trans*-factors that bind these elements. The latter have the capacity to regulate both mRNA synthesis and decay. In this review we summarize the recent findings that assign additional roles to the transcription machinery in regulating the fate of its transcripts in the cytoplasm.

2. Structural and biochemical data indicate that Rpb4/7 binds the emerging transcript

X-ray crystallographic studies of Pol II have revealed structural elements with functional implications [3,4]. In a transcribing Pol II, nascent RNA moves from the active center to the exterior through an RNA exit tunnel [16–18]. Until recently, it was not clear which path the exiting RNA follows beyond the exit tunnel. Based on their positively charged surfaces, two prominent grooves on either side of the dock domain were proposed to further accommodate the exiting RNA [17–20]. Groove 1 winds along the base of the clamp towards the Rpb4/7 subcomplex, which can bind RNA via its ribonucleoprotein fold and/or oligonucleotide binding domain [5,7,21,22]. Groove 2 leads along Rpb11p towards Rpb8p, which also has a single-stranded nucleic acid-binding domain [23,24]. Two studies have provided evidence that exiting RNA follows the path along groove 1. The first study used cross-linkable nucleotides (located 3 nt from the RNA 5′ end) to characterize the interaction between nascent RNA and components of the transcription complex. They demonstrated that the 5′ end of the nascent RNA could be cross-linked to Rpb7p as soon as the RNA exited from the core Pol II, once the cross-linkable nucleotide was 23 nt from the 3′ end. When Pol II continued to transcribe and the cross-linkable nucleotide was 39 nt from the 3′ end, it could still crosslink, albeit less efficiently; no cross-linking could be detected when the Pol II moved

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further away from this position [25]. This indicates that, *in vitro*, Rpb7 serves as a tunnel through which the RNA exits the 12 subunit Pol II. The second study employed fluorescence resonance energy transfer (FRET) analysis to map the path of nascent RNA on Pol II. They demonstrated that the growing RNA leads towards Rpb4/7 and, once it extends to 26 nt, its 5′ end forms contacts with the ribonucleoprotein-binding domain of Rpb7 [26]. The emerging transcript can take an alternative route *in vitro* by binding to the dock domain without contacting either of the grooves on the Pol II surface [16].The choice as to which route to take may be dependent on the conditions of the *in vitro* studies [26]. *In vivo* analysis supports binding of the emerging transcript with Rpb4/7, because Rpb4/7 binds Pol II transcripts co-transcriptionally [13]. It is possible that in the absence of Rpb4/7 the RNA exits invariably from groove 2. The picture that emerges from these studies is that Rpb4/7 serves as the initial contact for the newly emerging RNA just beyond the mouth of exit groove 1. The Rpb4/7 heterodimer contacts the main body of Pol II adjacent to the C-terminal domain (CTD) of Rpb1p [2,7]. The CTD is known to have a role in recruitment of factors involved in transcription regulation, RNA capping, splicing, polyadenylation and termination [27–29]. Thus, Rpb4/7 may play a significant role in directing the transcript to the appropriate processing factors recruited to the CTD.

3. Rpb4/7 regulates transcription initiation, elongation and polyadenylation

Rpb4/7 is considered to be a canonical Pol II subunit with a role in transcription. *In vitro* studies demonstrated that Pol II requires Rpb4/7 for promoter-dependent transcription initiation [5,30], and for elongation [10]. In archaea, the Rpb4/7 homologue, F/E, facilitates DNA melting in conjunction with the basal transcription factor, TFE, during initiation [31,32]. In yeast cells, Rbp4p and Rpb7p do not contribute equally to the function of the heterodimer. Rpb7p is an essential protein, whereas Rpb4p is dispensable under optimal growth conditions at moderate temperatures. However, as soon as the environmental conditions deviate from the optimum, most notably temperature extreme, starvation, and ethanol, Rpb4p becomes important [33–37]. We suspect that Rpb7p is the core subunit that binds both Pol II and the transcript whereas Rpb4p mediates some of the many interactions between Rpb4/7 and regulatory factors [6].

Analysis of the genome-wide occupancy of Rpb4p using chromatin immunoprecipitation revealed that Rpb4p is recruited to coding regions of most transcriptionally active genes, similar to the core Pol II subunit, Rpb3p, although to a lesser degree [38,39]. Interestingly, the extent of Rpb4p recruitment increases with increasing gene length, and Pol II lacking Rpb4p is defective in transcribing long, GC-rich transcription units, as it is sensitive to 6 aza-uracil [39]. Moreover, Rpb4 was found to be important for Pol II processivity [40]. Consistently, *in vitro* studies demonstrated that Rpb4/7 enhances Pol II elongation activity [10] and that F/E has a profound effect on the transcription elongation properties of Pol II by enhancing processivity [41]. Importantly, this function is attributed to the ability of F/E to interact with the RNA transcript [41]. Thus, the capacity of Rpb4/7 or its archaeal homolog to enhance transcription elongation might be related to its binding of the emerging transcript. It would be interesting to examine whether this binding has some effect on the capacity of Pol II to backtrack, thereby affecting processivity.

4. Rpb4/7 is recruited onto mRNAs co-transcriptionally and is directly involved in all major post-transcriptional stages of the mRNA lifecycle

Consistent with its interaction with the emerging transcript *in vitro* (see previous section), Rpb4/7 has been shown to interact with Pol II transcripts *in vivo* [12,13], probably at the 3′-untranslated region (Guterman and Choder, unpublished result). This interaction occurs only in the context of Pol II. Surprisingly, at some stage of the transcription process, Rpb4/7 dissociates from Pol II together with the transcript and remains bound to the transcript throughout its life. This “mRNA imprinting” has bearing on each and every stage of the mRNA lifecycle.

Several studies have described the diverse roles of Rpb4/7 in post-transcriptional stages, linking its role in the nucleus to the cytoplasmic stages of gene expression. One such study demonstrated that Rpb4p is required for mRNA export under stress conditions. The roles of Rpb4p in transcription and in mRNA export can be uncoupled genetically by specific mutations in Rpb4p. Both activities are essential for survival under stress conditions [11].

Another study revealed a role for Rpb4/7 in translation [12]. This study demonstrated that the Rpb4/7 heterodimer interacts physically and functionally with components of the translation initiation factor 3 (eIF3), and is required for efficient translation initiation. This function is more apparent during starvation, suggesting that the role of Rpb4/7 in translation permits appropriate responses to environmental cues.

Two major mRNA decay pathways operate in the yeast cytoplasm. They both initiate by a shortening of the mRNA poly(A) tail; one culminates in exonucleolytic degradation of the mRNA from 5′ to 3′ by the Xrn1 exonuclease, and the other in 3′ to 5′ degradation by the exosome [42]. Rpb4/7 functions directly in shortening of the poly(A) tail and in these two degradation pathways [14,15]. Unlike Rpb7p whose role in mRNA decay is not specific [15], Rpb4p is involved in the degradation of a specific class of mRNAs encoding protein biosynthetic factors, including ribosomal proteins, translation initiation factors, aminoacyl tRNA synthetases and ribosomal biosynthetic proteins [14]. Overexpression of Rpb7p could not restore proper mRNA decay in *rpb4*Δ cells, suggesting that Rpb4p has a distinct role in the decay of these mRNAs. Both Rpb4p and Rpb7p interact with the basal decay factor Pat1p/Lsm1-7p via direct interactions with Pat1p and Lsm2p [14,15]. This interaction might be important for recruiting the Pat1/Lsm1-7 complex to the mRNP, or for stimulating Pat1/Lsm1-7 decapping activity. Consistent with their role in the major decay pathway, Rpb4p and Rpb7p are constituents of P bodies [14,15], where decapping and 5′ to 3′ degradation can occur [43].

Thus, by assuming various subcellular localizations and switching interacting partners, Rpb4/7 exerts its effect on different processes temporarily. Consistently, the Rpb4/7 heterodimer shuttles between the nucleus and the cytoplasm. Shuttling occurs via two distinct shuttling mechanisms (one dependent on transcription and the other not), depending on the environmental conditions [44].

5. mRNA imprinting

Several lines of evidence have started to reveal that nascent mRNAs emerge from the nucleus with “imprinted” information that serves to regulate post-transcriptional stages of gene expression [45]. As detailed above, Rpb4/7 represents a classic example of mRNA imprinting, whereby its co-transcriptional association with the nascent transcript affects all the major post-transcriptional stages that the mRNA undergoes [see Fig. 1].

In addition to Rpb4/7, She2p and Dbf2p have been shown to be loaded onto specific mRNAs during transcription, affecting their fate in the cytoplasm. She2p affects localization and translation of specific mRNAs [46,47] and Dbf2p binds *SWI5* and *CLB2* mRNAs and specifically affects their decay during mitosis [48]. Several other factors have been proposed to bind RNA in the nucleus and affect its fate in the cytoplasm: Exon-Junction Complex (EJC) components regulate localization and translation of *oskar* mRNA in *Drosophila* oocytes in a splicing-dependent manner [49]; Ssd1p affects mRNA localization [50]; ZBP1 affects localization and translatability of several different RNAs [51,52]; the TREX complex and Mex67p mediate mRNA export [53]; CPEB affects alternative splicing, cytoplasmic polyadenylation and translation [54]; Npl3p [55] and Sro9p [56] affect mRNA export