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Connections between mRNA 3′ end processing and transcription termination

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Discoveries within the last few years have revealed that the multiple steps in gene expression are remarkably integrated. There have recently been several advances in deciphering how mRNA 3′ end processing is linked with transcription elongation and termination. It has been known for quite a long time that transcription termination is somehow intertwined with polyadenylation, but it is still unclear exactly how these two processes influence each other. Some recent reports are shedding light on these connections.

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Introduction

The connection between polyadenylation and transcription termination was established early on with the discovery that both processes were dependent upon the same DNA sequences at 3′ ends of genes [1,2]. This connection was further reinforced when it was found that at least some of the mRNA cleavage and polyadenylation factors were also required for termination [3–5]. Two general models were put forward to explain the linkage. The first, sometimes known as the 'anti-terminator' model (Figure 1), proposes that the emergence of the polyadenylation sequences on the RNA triggers a change in the factors associated with the polymerase [2]. For example, binding of the polyadenylation factors could displace a positive elongation factor or recruit a negative elongation factor. The consequently less processive RNA polymerase II (RNApII) would then terminate. In the second scenario, often called the 'torpedo' model (Figure 2), the cleavage event at the polyadenylation site generates a new 5′ end [1]. Unlike the capped 5′ end of the pre-mRNA, this end could act as an entry point for an activity (such as an exonuclease or helicase) that would track along the RNA and dissociate the polymerase. This type of mechanism resembles bacterial rho-dependent termination.

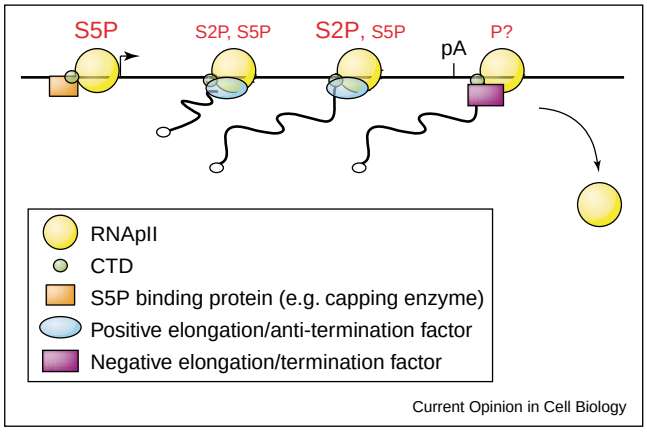
In addition to a requirement for polyadenylation sequences to trigger termination, several studies have shown that induced pausing of the polymerase downstream of the polyadenylation site encourages termination [6]. Pausing can be triggered by transcription of particular DNA/RNA sequences or by a sequence-specific DNA binding protein blocking forward movement of the RNApII (sometimes referred to as a 'roadblock'). Nevertheless, there are clearly no consensus sequences for the actual site of termination. Instead, termination apparently occurs stochastically within a window downstream of the polyadenylation site [7,8].

Although there have been many excellent recent reviews addressing how mRNA processing events are linked to transcription, this article will concentrate on recent advances in deciphering how mRNA 3′-end processing is linked to transcription elongation and termination. These recent discoveries provide support for both the 'anti-terminator' and 'torpedo' models.

The C-terminal domain and phosphorylation

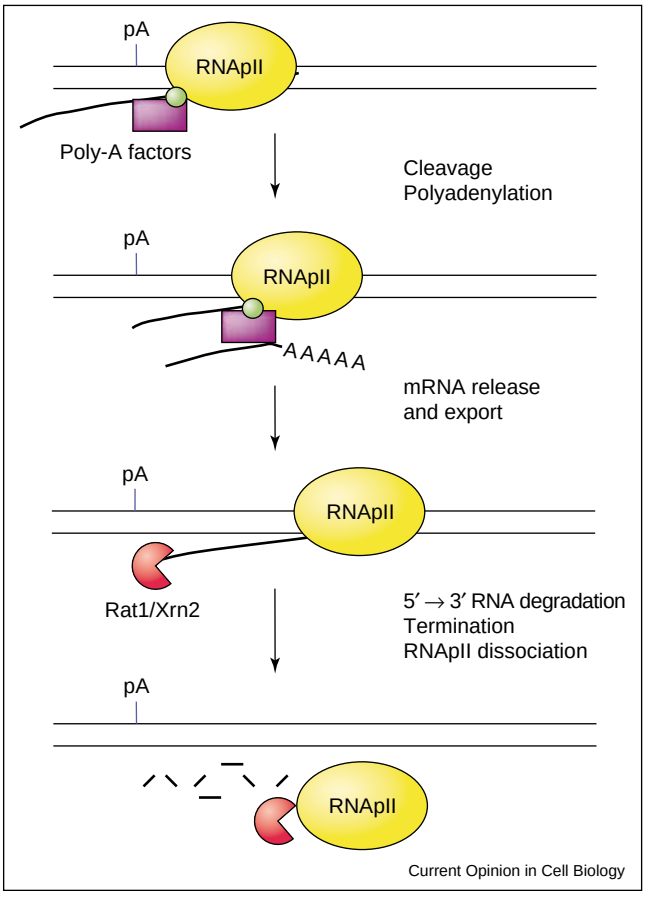
Like other mRNA processing events, coupling of 3′ end formation to transcription is mediated by the C-terminal domain (CTD) of the RNApII largest subunit. This domain consists of multiple (27–52) repeats of the heptamer sequence YSPTSPS. Serines 2 and 5 of this sequence are the major sites of CTD phosphorylation. The current paradigm is that different phosphorylation patterns predominate at different stages in the transcription cycle and that different proteins bind to specific phosphorylated forms of the CTD. An oversimplified incarnation of this model is biphasic, proposing that the basal transcription factor TFIIH phosphorylates CTD serine 5 at the promoter and that a second kinase (Ctk1 in yeast, Cdk9 in higher eukaryotes) phosphorylates CTD serine 2 during elongation phase. However, the available evidence suggests a more complex and subtle set of changes. As shown by chromatin immunoprecipitation (ChIP) experiments, serine 2 phosphorylation levels in yeast appear to increase during elongation, reaching a peak near the polyA site after which they drop again [9,10•,11]. Furthermore, at least some repeats probably remain unphosphorylated and serine 5 phosphorylation levels don't drop to zero during elongation, indicating that CTD phosphorylation is not the same at all repeats [10•,12,13]. The Ctk1 kinase can phosphorylate serine 5

Figure 1



The 'anti-terminator' model for coupling polyadenylation and termination. In this model, the extrusion of the polyadenylation signal (pA) on the RNA results in a change in the factors associated with the polymerase. Positive elongation factors (light blue oval) may dissociate or termination factors (purple rectangle) may be recruited. Also shown are the changing CTD phosphorylation patterns at different stages of transcription. The larger text size indicates higher levels of phosphorylation.

Figure 2



The 'torpedo' model for transcription termination. In this model, cleavage of the transcript by the polyadenylation machinery (purple rectangle) generates a new, uncapped 5′-end that is a substrate for degradation by the Rat1/Xrn2 nuclease. This not only degrades the 'extra' part of the mRNA, it also somehow triggers transcription termination.

as well as serine 2 *in vitro*, and has a significantly higher activity on serine 2 if serine 5 is phosphorylated first [14•]. Therefore, it is likely that at least some repeats of the CTD are doubly phosphorylated during elongation, adding additional complexity to the potential information that can be encoded in the CTD [15].

A strong connection has been made between phosphorylation of the CTD by Ctk1/Cdk9 and recruitment of the polyadenylation factors. First, overexpression of the polyadenylation factor Pti1 suppresses mutant phenotypes caused by deleting the gene for Ctk1 [16]. Second, the robust crosslinking of polyadenylation factors normally seen at 3′ ends of genes is no longer observed in strains lacking Ctk1 [10•]. Third, *in vivo* inhibition of Cdk9 in *Drosophila* cells leads to defects in polyadenylation [17•]. Finally, *in vitro* binding of Pcf11 (a subunit of the yeast polyadenylation factor cleavage factor IA) to the CTD is dramatically increased by serine 2 phosphorylation [18]. A co-crystal structure of this interaction shows that the phosphorylated serine 2 does not make a direct contact with Pcf11. Instead it may stabilize a particular CTD conformation that fits the Pcf11 surface [19•]. The CTD interaction domain (CID) of Pcf11 has sequence similarity to two other yeast proteins that function at 3′ ends: Nrd1 [20] and Rtt103 (see below). This suggests that this particular protein fold serves as a module for targeting to the CTD of polymerases near the ends of genes.

ChIP experiments show a strong recruitment of polyA factors and a reduction in CTD phosphorylation just downstream of the polyA site. However, the resolution of this assay doesn't allow any conclusions to be made about whether one causes or even precedes the other. Interestingly, the polyadenylation factor Ssu72 has CTD phosphatase activity, although it (perhaps unexpectedly) seems to be specific for phosphorylated serine 5 [21••]. Ssu72 also appears to function at promoters [22], raising several possibilities. Ssu72 may load at the promoter for a function at 3′ ends. Alternatively, Ssu72 phosphatase may function independently at both 5′ and 3′ ends. Finally, Ssu72 may dephosphorylate the CTD at the 3′ ends of genes to facilitate recycling of the polymerase back to a form competent for initiation. In this respect, a recent report suggests that the 5′ and 3′ ends of genes may be brought into proximity with each by a mechanism that involves CTD serine 5 phosphorylation [23]. There is conflicting data as to whether Ssu72 is important for transcription termination [24–26].

Linking termination to polyadenylation

Experimental results in the last few years provide evidence supporting both the 'anti-terminator' and the 'torpedo' models. It seems unlikely that there is a single uniform mechanism for RNApII termination. Instead, there are probably multiple processes that can contribute to termination, and different processes may predominate at different genes. Electron microscopy of *Drosophila* chromosomes shows different patterns at 3′ ends, with most genes showing abrupt termination without cleaved transcripts [7,27,28]. This pattern is most consistent with an 'anti-terminator' model. Other genes clearly show cleaved transcripts associated with elongating polymerases at 3′ ends of genes, which is more in line with the torpedo model.

Cleavage does not seem to be an obligatory precursor to termination [7,25,27,28]. Studies in mammalian cells indicate that termination can be separated into two steps: pausing and polymerase release. Interestingly, the first step is CTD-independent whereas the second is not [29]. An interesting possibility is that the pausing step reflects a processivity change in the elongation complex consistent with the anti-terminator model, while the CTD-dependent release step could be related to recruitment of the polyadenylation factors by CTD phosphorylation.

A pausing step could be triggered by changing properties of the elongation complex. Relevant to this, two putative elongation factors, the PAF and TREX complexes, crosslink throughout the transcribed regions of genes up to the polyadenylation site. Although the polymerase and several other elongation factors continue further, very little PAF and TREX crosslinking is seen 3′ to the polyA site. This pattern is independent of CTD phosphorylation by Ctk1 [10•,11]. Interestingly, these two factors, which were originally found to affect transcription, have also been implicated in mRNA processing. The TREX complex contains several RNA binding proteins that package the mRNA for proper splicing and transport [30]. The function of the PAF complex remains unclear, but recent experiments suggest that it may also affect polyadenylation [31]. If these two factors act as positive elongation factors, polymerases downstream of the polyadenylation site that are not associated with PAF and TREX may be more competent for termination. Although some crosslinking of polyadenylation factors is sometimes seen in transcribed regions [18], a very strong signal is seen at and 3′ to the polyadenylation site [10•,11]. One interesting possibility is that PAF, TREX or some other RNA binding protein prevents the polyadenylation machinery from accessing the RNA except when a clear polyA consensus sequence emerges.

Although it had fallen out of favor in recent years, the torpedo model of termination has been resurrected by recent findings. Affinity chromatography with serine 2 phosphorylated CTD resulted in purification of the Rtt103 protein, which contains a CID related to that of the polyA factor Pcf11 [32••]. Associated with the RNApII/Rtt103 complex is the Rat1/Rai1 nuclease complex, a 5′-to-3′ exonuclease that plays a role in trimming several ribosomal and small nucleolar RNAs. Rtt103, Rat1, and Rai1 crosslink at the polyadenylation site, suggesting that they play a role in 3′ end processing. It should be noted, however, that only Rtt103 crosslinking is dependent upon CTD phosphorylation by Ctk1. Although cleavage and polyadenylation are normal in strains mutated for these factors, a striking defect in termination is seen [32••]. The data strongly support a mechanism in which Rat1 attacks the new 5′ end generated by cleavage. As the polymerase continues transcribing, the excess transcript is degraded by Rat1, which eventually reaches the elongation complex and somehow induces termination.

The homolog of yeast Rat1 is in higher eukaryotes is Xrn2. An RNAi knockdown of Xrn2 also leads to termination defects in mammalian cells [33••], suggesting conservation of an exonuclease-mediated termination mechanism. One interesting variation on the standard model for this type of termination has been documented at the 3′ end of the human β-globin gene. In this case, the RNA downstream of the polyadenylation site can fold into a self-cleaving ribozyme structure [34•]. At this gene, the Xrn2/Rat1 exonuclease can enter at this co-transcriptional cleavage site rather than the polyA cleavage site. It remains to be seen whether this observation also applies to many other genes.

It should be noted that the interval for Rat1 to 'catch up' with RNApII could be reduced by the pausing sites sometimes seen in the 3′ regions of genes (see above). Although it remains unclear whether Rat1 can directly trigger transcription termination, *in vitro* studies have shown that elongation complexes with transcripts <50 nucleotides long are prone to pausing [35]. Contact between Rat1 and the paused RNApII may lead to release of the remaining transcript by a mechanism similar to that used by the bacterial rho factor, an RNA helicase. Alternatively, another enzyme may actually trigger transcript release. One interesting candidate is the Sen1 RNA helicase, which has already been implicated in 3′ end formation of snoRNAs [20]. Another is Ttf2/lodestar, a Snf/Swi-family factor that can displace RNApII from mitotic chromosomes [36].

Conclusions

Given the rapid pace of progress in the area of coupling between transcription and mRNA processing, it is likely that the next few years will provide further interesting surprises. There is still a great deal that remains unknown about how the polyadenylation factors work, and this will need to be worked out before we completely understand how termination is connected. In the meantime, more

factors connected to other steps in termination will probably be discovered. It will be important to determine whether these all work together or instead provide multiple independent mechanisms for dissociating transcription elongation complexes.

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