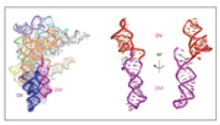
RNA Biology



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Alternative polyadenylation coupled to transcription initiation: Insights from ELAV-mediated 3′ UTR extension

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Transcription initiation and mRNA maturation were long considered co-occurring but separately regulated events of gene control. In the past decade, gene promoters, the platforms of transcription initiation, have been assigned additional functions such as the regulation of splicing and 3′ end processing. In a recent study, Oktaba and Zhang and al. reveal that neural 3′ UTR extension is dependent on promoter sequences. In Drosophila neurons, promoter regions of a subset of genes recruit the RNA-binding protein ELAV, which is required for subsequent ELAV-mediated alternative polyadenylation. Intriguingly, RNA Polymerase II pausing at promoters seems to facilitate ELAV recruitment. How transcription initiation and alternative polyadenylation, processes separated by an entire gene length, are functionally linked, remains unsolved. In this article, I summarize recent findings and discuss possible mechanisms.

Keywords: alternative polyadenylation, cleavage and polyadenylation, drosophila, ELAV, 3′ UTR extension, nervous system, pol II pausing, promoter, transcription initiation

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Introduction

Alternative cleavage and polyadenylation (APA) has emerged as a major means of co-transcriptional gene regulation. An estimated 50–75% of metazoan genes express distinct APA isoforms by using alternative polyadenylation signals (PASs). In the most common form of APA, UTR-APA, mRNAs are generated with identical coding sequences but 3′ UTRs of different lengths. UTR-APA often depends on cellular or developmental context. For example, short 3′ UTRs are typical for cell proliferation,[1] whereas usage of distal PASs correlates with cell differentiation and organism development.[2] The biological implications of this considerable 3′ UTR diversity have only been grazed to this day (for recent reviews, see[3,4]).

Neural-specific 3′ UTR extension represents the perhaps most dramatic form of UTR-APA. In the nervous system, hundreds of genes use increasingly distant PASs as development progresses, resulting in 3′ UTRs that can reach tens of kilobases in length. This phenomenon has been observed in Drosophila[5] as well as vertebrates such as zebrafish,[6] mouse and human,[7] and thus seems to be a conserved feature of neurogenesis. Specific RNA-binding proteins regulate APA by inhibiting cleavage and polyadenylation (CPA) at proximal sites. In flies, the pan-neuronal protein ELAV co-transcriptionally binds to the nascent mRNA in the vicinity of the proximal PASs, causing transcriptional elongation and 3′ UTR extension of hundreds of genes.[8,9] Although no global regulator of neural APA has yet been identified in mammals, ELAV homologues represent likely candidates. In human cells, the neuron-specific ELAV-like proteins HuB, HuC and HuD mediate the 3′ UTR extension of at least one gene, HuR.[10]

How regulators of APA are specifically directed to their target transcripts remains unclear. ELAV recruitment to a proximal PAS of the nascent mRNA must occur very efficiently in order to inhibit CPA and transcription termination at that site. However, no binding sites for ELAV have consistently been identified in the 3′ UTRs of ELAV targets.[8,11] This led to the question whether determinants other than mRNA sequence help recruit ELAV during transcription.

Promoter sequences regulate 3′ UTR extension in the nervous system

Recent studies uncovered an unexpected role for promoter sequences in APA, and suggest that APA regulators are recruited to their target genes during transcription initiation. First, ELAV recruitment is not directed solely by 3′ UTR sequences: reporter transgenes, expressed under the control of a strong synthetic promoter and carrying the full 3′ UTR of validated ELAV targets, did not exhibit ELAV-mediated APA in Drosophila neurons. Replacing the synthetic promoter with native promoters of genes that endogenously produce extensions (referred to as 'extended genes') led to expression of extended mRNAs. These unexpected results imply that ELAV-mediated APA depends on specific sequences that are found in the promoter of extended genes. Computational analyses aimed at finding such sequences revealed that the GAGA element is significantly enriched in the promoter regions of extended genes. In flies lacking the GAGA-binding factor (GAF), 3′ UTR extension was reduced, demonstrating physiological relevance of this motif in ELAV-mediated APA.[9]

The GAGA motif is a typical landmark of promoters that contain paused RNA Polymerase II (Pol II).[12] At paused promoters, Pol II has initiated RNA synthesis, but transcription elongation is inhibited. The resulting accumulation of Pol II at the transcription start site (TSS) is thought to serve several possible functions: it might render, or maintain, chromatin accessible at the promoter, and prompt fast and synchronous activation of paused genes.[13] Pol II pausing might also act as a checkpoint, to ensure integration of different regulatory signals before productive transcription elongation takes place.[14] Consistent with the hypothesis that Pol II pausing might help recruit APA regulators such as ELAV, most extended genes are highly paused in Drosophila embryos. Moreover, a physical association of ELAV at the promoter region of its target genes was demonstrated using ELAV ChIP-Seq.[9]

These observations suggest a mechanism in which ELAV is selectively recruited to the promoter of its target genes, an association facilitated by Pol II pausing and the GAGA motif (**Fig. 1**). Upon transcription elongation, ELAV binds to proximal PASs, effectively preventing CPA at those sites, and fostering the formation of long 3′ UTRs.

This novel link between transcription initiation and alternative polyadenylation raises numerous questions: How is ELAV recruited to target promoters? Apart from GAGA, which is rather widespread, no single sequence motif is significantly and highly enriched in promoters of extended genes. Pausing alone is unlikely to be able to trigger 3′ UTR extension, since many highly paused genes do not undergo neural APA. In addition, how is ELAV guided from promoter sequences to newly transcribed PASs that usually reside many kilobases downstream?

How is ELAV recruited to proximal PASs?

Additional insights were obtained from ELAV ChIP-Seq experiments. ELAV broadly associates with promoter DNA and proximal PASs. In addition, ELAV was also found to bind intronic sequences, but was consistently and strikingly depleted from coding sequences[9] (**Fig. 2A**).

The observed association with these gene regions might simply reflect ELAV's binding to mRNA, the recovery of the corresponding chromatin being due to its association to the nascent RNA (**Fig. 2B**, 'RNA-mediated'). I consider this scenario unlikely because ELAV binding often occurs several hundred base pairs upstream of the TSS, where little or no transcription is expected to occur. Alternatively, ELAV might interact with promoter sequences through gene looping. In yeast, intragenic chromatin loops bring transcription initiation factors in physical contact with termination factors, thereby spatially connecting promoters and PASs.[15] Such a structural arrangement might represent a mechanism of crosstalk between Pol II pausing and ELAV-mediated APA (**Fig. 2B**, 'gene looping'). The

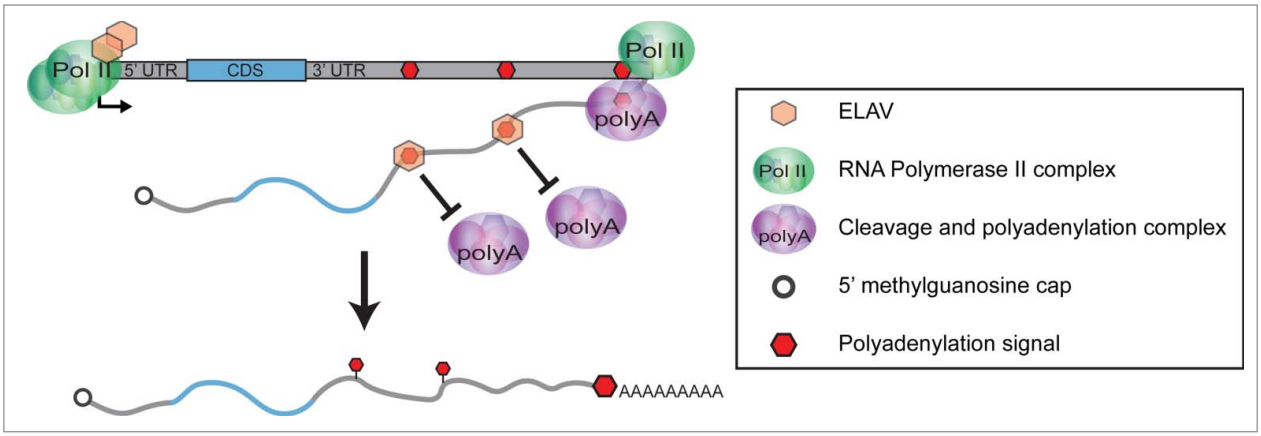

Figure 1. Model of ELAV-mediated 3′ UTR extension. In neurons, ELAV associates with the promoter region of its target genes, which usually contain paused Pol II. During transcription, ELAV binds to the nascent transcript in the vicinity of each proximal PAS. The inhibition of CPA at proximal sites causes transcriptional read-through and formation of an extended 3′ UTR.

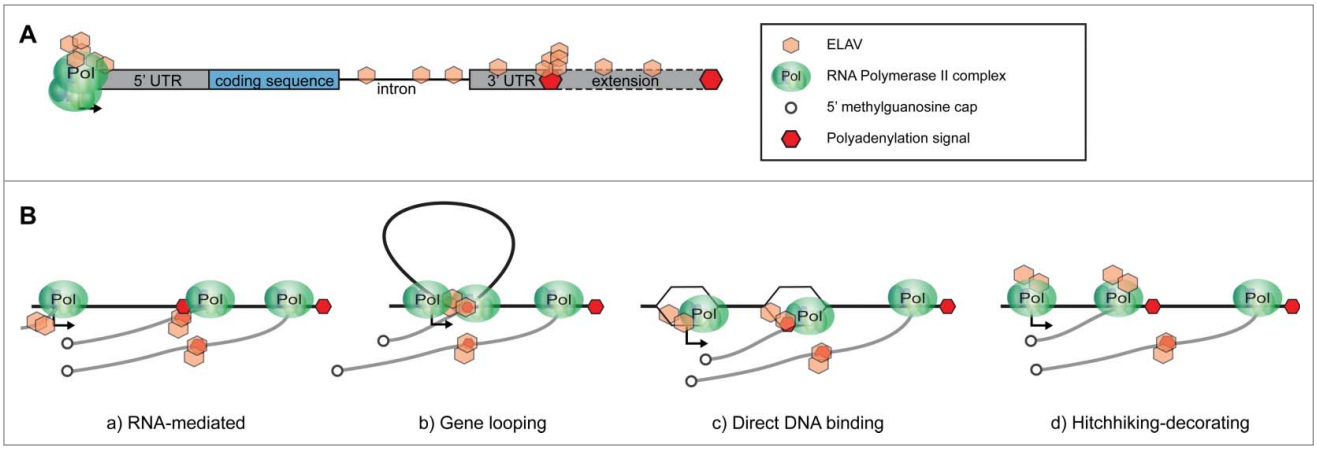

Figure 2. Possible mechanisms of promoter-regulated APA. (**A**) Distribution of Pol II and ELAV along the transcription unit of a typical extended gene. Pol II accumulation at the promoter is representative of gene pausing. By ChIP-Seq, ELAV was found to bind broadly around the transcription start site, but also in introns, 3′ UTRs, and around proximal PASs. (**B**) Four models of ELAV recruitment to proximal PASs. For simplicity, only one proximal PAS is depicted. (**A**) ELAV solely binds the nascent transcript, cotranscriptionally recognizing specific RNA sequences or structures. (**B**) A gene loop provides proximity between promoter sequences and the proximal PAS. (**C**) ELAV binds to specific accessible DNA sequences in the wake of Pol II. (**D**) ELAV associates with the paused, then transcribing Pol II, traveling along the transcription unit and decorating specific nascent RNA sequences.

ELAV protein contains 3 highly conserved RNA recognition motifs (RRMs) and directly binds to target mRNAs.[16] This does not exclude the possibility that ELAV binds to promoter sequences at the DNA level, for example within the transcription bubble (**Fig. 2B**, 'direct DNA binding'). One last hypothesis is that ELAV, associated with the transcription machinery at initiation, remains bound to Pol II complexes after the release of Pol II from the proximal promoter. ELAV travels along the transcription unit and, upon Pol II lingering at specific regions of the nascent mRNA, such as introns and proximal PASs, is directed to the nascent transcript. The striking depletion of ELAV in coding regions vs. introns (**Fig. 2A**) favors this 'hitchhiking-decorating' model. It also implies that yet unidentified signals, perhaps cryptic sequences or structure elements, attract ELAV to these specific locations.

Integration of multiple signals at transcriptional checkpoints

Multiple factors involved in mRNA processing, including the CPA machinery, interact with the Pol II C-terminal domain (CTD) at all stages of transcription.[17,18] It appears that neural-specific APA is similarly coupled to transcription. ELAV likely associates with Pol II at the promoter, either directly through the Pol II CTD, or indirectly via as yet unidentified factor(s). I propose that Pol II pausing at the promoters of extended genes acts as a checkpoint to ensure the integration of APA regulators such as ELAV into the transcription machinery.

Remarkably, in addition to promoter-proximal pausing, RNA Pol II can also exhibit delays in transcriptional elongation at locations of mRNA processing decisions, such as intron-exon junctions[19,20] and the end of the transcription unit.[21] These pausing events are thought to represent checkpoints to reinforce effective and accurate mRNA synthesis. A recent study reports an additional elongation checkpoint, where Pol II pauses just upstream of terminal PASs.[22] Since promoter-proximal Pol II pausing is not sufficient to cause 3′ UTR extension, an additional, downstream signal is likely necessary to promote functional ELAV recruitment. This signal could be delivered through Pol II elongation checkpoints, for example in the vicinity of the proximal PASs, where ELAV was shown to bind.[8,9] A strongly paused promoter would therefore be able to elicit APA only if 3′ UTR regions are favorable to ELAV binding. Downstream pausing of Pol II might help reinforce (or disable) the functional interaction of the transcription machinery with ELAV. I predict that a reporter construct carrying the promoter of a strongly paused gene, and the 3′ UTR of an endogenously extended gene, will be able to undergo 3′ UTR extension, because ELAV will be recruited at both transcription initiation and proximal PASs. By the same logic, a non-extended gene will not undergo UTR-APA even if its promoter were replaced with the promoter of an endogenously extended gene.

It seems that transcription factors and various mRNA processing factors involved in capping, splicing, termination, and polyadenylation, are intimately linked as early as transcription initiation, at least in part through the Pol II CTD. Consequently, regulation of alternative processing events (such as ELAV-mediated APA or alternative splicing) can also occur at that stage and become reinforced as transcription proceeds, possibly during further Pol II elongation checkpoints: downstream pausing might modulate mRNA processing "on the go."

Outlook

The exact mechanism of ELAV recruitment, the identity of specificity factors (if any), and whether gene (or mRNA) looping is involved in the cross-talk between Pol II pausing and APA is still to be resolved. Many mRNA processing events,

now including ELAV-mediated 3′ UTR extension, depend on transcription initiation. Therefore, it seems likely that other APA processes are similarly regulated through promoter sequences. Considering the high conservation of factors and processes involved in transcription from initiation to termination, mRNA maturation and 3′ processing, I expect this mechanism to be utilized widely among metazoans.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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