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U1 snRNA Rewrites the "Script"

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Abstract

Expression of eukaryotic mRNAs requires the collaboration of a host of RNA processing factors acting upon the transcript. Berg et al. describe how a pre-mRNA splicing factor modulates the activity of the polyadenylation machinery to regulate mRNA length, with important implications for isoform expression in activated neuronal and immune cells.

Formation of messenger RNAs (mRNAs) from pre-mRNA transcripts involves multiple processing steps, including 5′ end capping, intron removal via premRNA splicing, and 3′ end cleavage/polyadenylation. Although these processes have been studied as biochemically distinct reactions, they each appear to occur cotranscriptionally and in close spatial and temporal proximity to one another, raising important questions about how they may act coordinately to affect gene expression outcomes. One of these cotranscriptional processes, polyadenylation, can occur at multiple sites in more than half of mammalian transcripts (Tian et al., 2005), and genome-wide surveys of poly(A) RNA have recently demonstrated that 3′ cleavage and polyadenylation may occur throughout a premRNA (Kaida et al., 2010; Weill et al., 2012). In this issue, Berg and colleagues demonstrate that U1 small nuclear RNA (snRNA), an RNA typically associated with splicing, regulates transcript length through cotranscriptional recognition of cryptic polyadenylation signals (PASs) and inhibition of premature cleavage and polyadenylation (PCPA) at these sites (Figure 1) (Berg et al., 2012). This process, which the authors term "telescripting," appears to make functional contributions to transcriptional control of activated neuronal cells.

U1 snRNA is best characterized for its role in recognizing the 5′ splice site (5′ss) during removal of introns from premRNAs; however, it is present at levels far exceeding what is necessary for premRNA splicing in the cell (Baserga and Steitz, 1993), and it is the only snRNA that localizes to intronless genes (Brody et al., 2011). Additionally, the splicing-associated U1 small nuclear ribonucleoprotein (snRNP) particle inhibits pre-mRNA polyadenylation through its interaction with poly(A) polymerase (Gunderson et al., 1994, 1998). U1 snRNA has also been implicated in a splicing-independent role in transcriptional activation (Kwek et al., 2002), and the Dreyfuss lab previously described a role for U1 in protecting pre-mRNAs from cleavage and polyadenylation (Kaida et al., 2010). Although

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integral to pre-mRNA splicing, these observations point to nonsplicing functions for the U1 snRNA and perhaps its associated proteins.

The Kaida et al. (2010) study described above found that U1 snRNA bound to cryptic 5′ss within the mRNA protected internal PASs from premature processing. To look more closely at how U1 influences pre-mRNA cleavage/polyadenylation, Berg et al. (2012) now use antisense morpholino oligonucleotides (AMO) to knock down U1 snRNA expression and genome-wide high-throughput sequencing of differentially expressed transcripts (HIDE-seq) to identify global poly(A) transcripts. Looking at expression patterns in a number of organisms, this analysis confirms that U1 snRNA protects transcripts from premature cleavage and polyadenylation.

To then address whether a 5′ss sequence is necessary and sufficient for PCPA, the authors utilize minigenes with “actionable” polyadenylation sequences and find that PCPA occurs at a proximal PAS when the 5′ss has been mutated— that is, when U1 cannot bind. However, if the 5′ss-proximal PAS is also mutated, a PAS sequence further downstream from the 5′ss is used. Intriguingly, even in this context of a mutated 5′ss that is unable to support U1 snRNA binding, U1 snRNA knockdown exacerbates use of the downstream PAS, indicating that PCPA suppression is conferred by U1 interactions outside the 5′ss and suggesting a 5′ to 3′ directionality to U1 protection from PCPA. The authors see similar directional effects and shortened mRNAs when they titrate the amount of AMO to progressively inhibit U1 snRNA, which is consistent with U1 “telescripting” acting in a 5′ to 3′ direction, mirroring RNA synthesis. Berg and colleagues also observe that changes in U1 can alter alternative splicing by an unexpected mechanism; namely, PCPA-dependent changes in poly(A) sites trigger 3′ exon switching. This suggests that there may be a splicing-independent role for U1 in regulation of alternative splicing.

An important question raised by these studies is that, as U1 snRNA levels are high and remain relatively constant in the cell, how can they change enough under physiological conditions to modulate PCPA? The authors propose a model whereby cells undergoing rapid activation, e.g., activated neuronal and immune cells, experience a transient but dramatic increase in the transcript to U1 snRNA ratio due to an increase in transcription. In this case, the comparative decrease in the available U1 would be predicted to lead to PCPA and mRNA shortening. Indeed, the authors see a 40%–50% increase in nascent pre-mRNA transcripts in activated neurons and show that shorter mRNAs result. Importantly, they demonstrate that shortening of specific transcripts can be overcome by U1 over-expression. These changes to the mRNAs can result in shifts in protein isoform abundance (for instance, Homer-1 long and short isoforms) that may serve distinct functional roles in activated neurons.

In addition to creating different protein isoforms, changes to the 3′ end of transcripts by alternative polyadenylation site usage have the potential to alter cellular amounts of these transcripts. “Telescripting” may lead to a change in transcript levels due to loss of binding sites for miRNAs (or other RNA stability elements) in the 3′ untranslated region (UTR). The extent to which the available 3′ UTR regulatory sequences are altered by PCPA remains to be explored.

It appears that signals outside of known 5′ss contribute to U1 suppression of PCPA. Most simply, U1 binding to unannotated cryptic 5′ss may be involved. However, it is also possible that signals in chromatin, such as histone modifications or nucleosome occupancy, or changes in the elongation properties of polymerase may facilitate U1 snRNA localization to the nascent transcript. Several recent studies from yeast to mammals support a role for chromatin in cotranscriptional splicing factor localization and rearrangements (see, for example, Gunderson et al., 2011). Identification of genome-wide binding sites for the U1 snRNP by using high-throughput analyses will be extremely informative for identifying these signals, particularly under conditions in which U1 snRNA becomes limiting, such as in neuronal activation, and will ultimately help elucidate the mechanism by which the U1 snRNP influences transcript length.

Finally, what is the outcome of transcript length changes in response to PCPA? One might imagine that, under conditions in which rapid proteome expansion is necessary, such as during immune activation or perhaps during development, U1 snRNA-modulated PCPA might produce new transcripts with new biological functions, thus increasing proteome diversity upon translation. Conversely, in diseases of transcriptional dysregulation, such as many types of cancers, it will be important to determine whether increased mRNA transcription leads to truncated transcripts generated by PCPA and concomitant production of truncated proteins with roles in pathogenesis. The use of powerful, validated high-throughput tools, such as HIDE-seq, to help enrich the discovery and identification of these transcripts and their respective proteins will aid in our understanding of how cotranscriptional crosstalk between RNA processing factors leads to coordinated regulation of gene expression.

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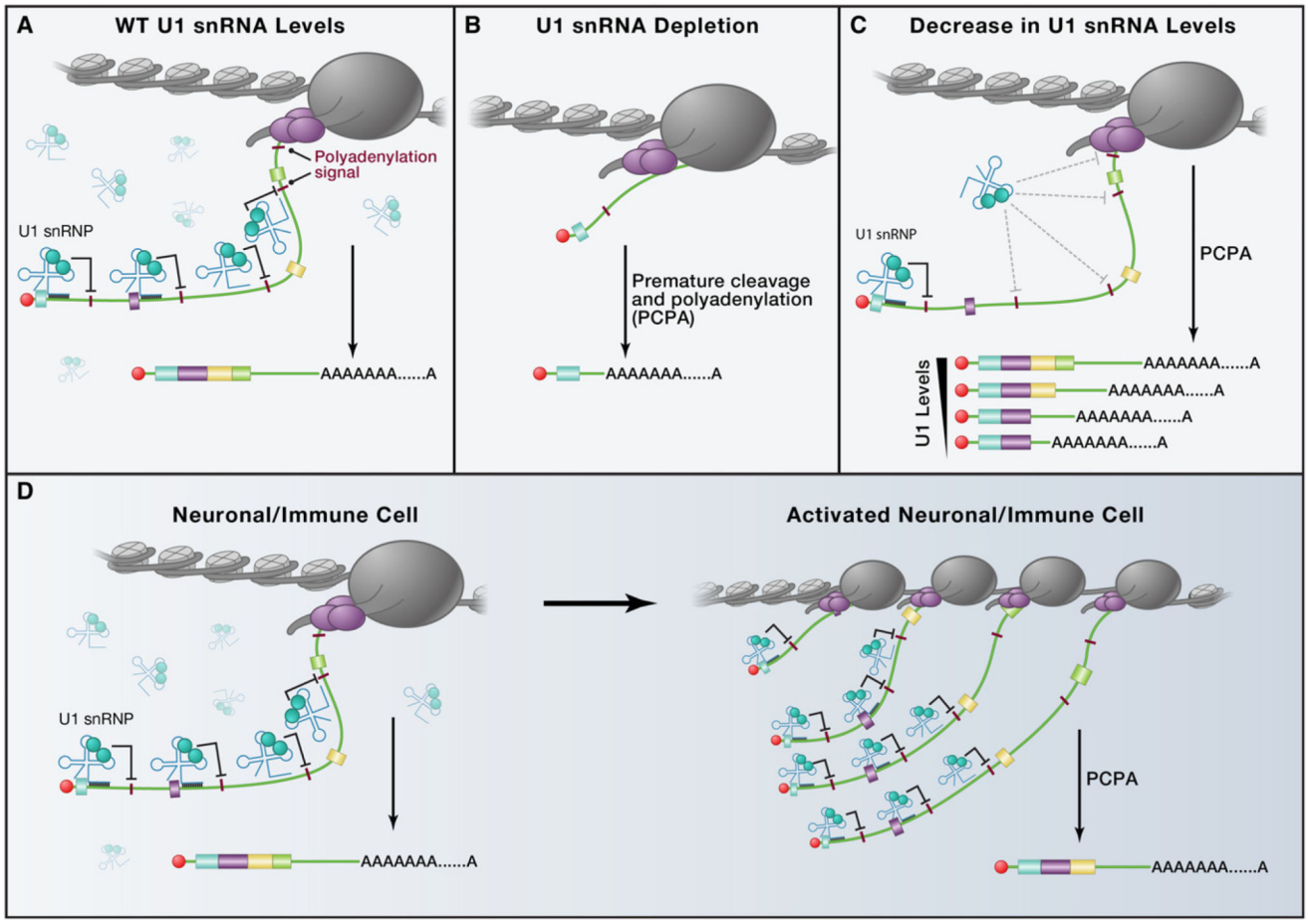

Figure 1. U1 Levels Determine the Use of Premature Cleavage and Polyadenylation Sites

(A) At wild-type levels of U1, U1 snRNA base pairing at the 5′ss inhibits the use of proximal PASs, leading to generation of a full-length transcript polyadenylated at the 3′ end. Potential cleavage sites far downstream of the 5′ss are also inhibited by the activity of U1, presumably via 5′ss-independent interactions (or perhaps by recognition of cryptic 5′ss signals).

(B) When U1 snRNA is depleted experimentally, as with AMO, it is not available to splice pre-mRNAs or to inhibit the use of PASs in the first intron, and as a consequence, short cleaved and polyadenylated RNAs are generated through PCPA.

(C) When U1 snRNA is decreased experimentally (by titrating AMO levels), the 3′ end of the RNA becomes shorter due to a decrease in U1-mediated protection from PCPA. A greater decrease in U1 snRNA levels leads to usage of cryptic PASs closer to the 5′ end of the RNA.

(D) 3′ UTR shortening and 3′ exon switching have been observed in activated neuronal and immune cells to produce shortened transcripts similar to the observed effects of decreasing U1 levels. Rapid and transient transcriptional upregulation of pre-mRNAs creates a shortage of U1 snRNA relative to the amount of nascent pre-mRNA, leading to the production of short isoforms under activated conditions (For example, *homer-1 long* is produced in unactivated neuronal cells, whereas *homer-1 short* predominates in activated cells). It is unclear whether other, perhaps unstable, isoforms are also produced.

For each panel, RNA polymerase II is shown synthesizing PAS-containing pre-mRNA from a chromatin template. The pre-mRNA is shown with exons (colored boxes), introns, the 5′ cap (red ball), and multiple PAS sequences. The purple ovals represent components of the polyadenylation machinery that associate with the C-terminal domain (CTD) of RNA polymerase II. The U1 snRNA is depicted in association with spliceosomal proteins (light blue ovals).