The reciprocal regulation between splicing and 3’-end processing

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Most eukaryotic precursor mRNAs are subjected to RNA processing events, including 5’-end capping, splicing and 3’-end processing. These processing events were historically studied independently; however, since the early 1990s tremendous efforts by many research groups have revealed that these processing factors interact with each other to control each other’s functions. U1 snRNP and its components negatively regulate polyadenylation of precursor mRNAs. Importantly, this function is necessary for protecting the integrity of the transcriptome and for regulating gene length and the direction of transcription. In addition, physical and functional interactions occur between splicing factors and 3’-end processing factors across the last exon. These interactions activate or inhibit splicing and 3’-end processing depending on the context. Therefore, splicing and 3’-end processing are reciprocally regulated in many ways through the complex protein–protein interaction network. Although interesting questions remain, future studies will illuminate the molecular mechanisms underlying the reciprocal regulation. © 2016 The Authors. WIREs RNA published by Wiley Periodicals, Inc.

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INTRODUCTION

In eukaryotic cells, most precursor mRNAs (pre-mRNAs) consist of protein-coding regions, exons, and intervening regions, introns. The pre-mRNAs are subjected to splicing to remove introns and to join flanking exons. In addition to splicing, the pre-mRNAs are also subjected to 5’-end capping and 3’-end processing, to become mature mRNAs. For a long time, the pre-mRNA processing events were studied independently; however, since the early 1990s, many laboratories have demonstrated that these processing events are reciprocally regulated. The following findings support reciprocal regulation. First, many RNA processing factors are recruited to the C-terminal domain of RNA polymerase II (Pol II); therefore, the factors have chances to regulate each other on the transcription apparatus. In addition, because these events are carried out co-transcriptionally in most cases, such factors can exist on pre-mRNA just after synthesis of the binding sites of processing factors, suggesting that the factors affect each other on the pre-mRNA. Second, a spliceosomal component, U1 small ribonucleoprotein particle (snRNP), is much more abundant than the other spliceosomal components: U2, U4, U5, and U6 snRNPs (e.g., 1,000,000 molecules/cell of U1 snRNP vs 200,000 molecules/cell of U5 in HeLa cells). However, these components form the spliceosome in equal stoichiometry, suggesting that U1 snRNP has extra-splicing function(s). Third, interaction between splicing factors and 3’-end processing factors is supposed to be required for exon definition of the last exon (see below). Therefore, it is reasonable that there is a functional coupling between splicing and 3’-end processing. This review focuses on such reciprocal regulation between splicing and 3’-end processing (e.g., cleavage and polyadenylation). Each mechanism affects the efficiency of the other mechanism positively and negatively through complicated interactions.
interacting networks among the protein and RNA factors involved in these two processing events.

SPLICING FACTORS AND 3'-END PROCESSING FACTORS

Splicing Factors
The splicing reaction is carried out by the spliceosome, a macromolecular ribonucleoprotein complex, and more than 100 nonspliceosomal proteins. The spliceosome consists of five subcomponents, U1, U2, U4, U5, and U6 snRNPs (Figure 1). The components recognize consensus sequences located near the 5’ and 3’ ends of introns: 5’ splice sites, 3’ splice sites, branch point sequences (BPSs), and polypyrimidine tracts. For spliceosome formation, U1 snRNP recognizes the 5’ splice site, and the U2 auxiliary factor (U2AF), a heterodimer consisting of U2AF65 and U2AF35, recognizes both the 3’ splice site and the polypyrimidine tract, which is located just upstream of the 3’ splice site. In addition, SF1 binds to the BPS to form complex E. SF1 is replaced by U2 snRNP to form complex A, then U4/U6.U5 tri-snRNPs join the complex to form complex B. Finally, several conformational changes occur to form the catalytic active form, complex C, followed by the two-step catalytic reaction to accomplish the splicing reaction.

3’-End Processing Factors
3’-end processing is one of the most important processes for maintaining the integrity of the transcriptome. The poly(A) tail at the 3’ end that is added posttranscriptionally stimulates translation from the mRNA and transport of the mRNA to the cytoplasm, and also protects the mRNA from degradation. Cleavage and polyadenylation at the 3’ end is carried out by a large protein complex containing four major subcomplexes, including cleavage and polyadenylation specificity factor (CPSF), cleavage stimulation factor (CsfF), cleavage factor I (CF Im), and cleavage factor II (CF IIm) (Figure 2). In addition to them, poly(A) polymerase (PAP), nuclear poly(A) binding protein (PABPN), and symplekin contribute to the reaction. All the proteins except for PABPN are necessary for the cleavage reaction; however, only CPSF, PAP, and PABPN are sufficient for polyadenylation. CPSF, which binds to the polyadenylation signal (PAS), is required for both cleavage and polyadenylation reactions. The PAS is a hexamer located 10–30 nucleotides upstream of the cleavage site, and its consensus sequence is A[A/U] UAAA. CstF recognizes the downstream element (DSE), which is U or GU rich and is located approximately 30 nucleotides downstream of the cleavage site. CsfF interacts with CPSF to stabilize their binding to DSE and PAS, respectively. CF Im interacts with UGUA motifs located upstream of PAS. These factors recognize pre-mRNA through their binding motifs and carry out the cleavage and polyadenylation reaction coordinately.

U1 SNRNP INHIBITS 3’-END PROCESSING

U1A Autoregulates Its Expression
As described above, U1 snRNP is much more abundant than the other components, suggesting that U1 snRNP has extra-splicing function(s). A series of studies revealed that components of U1 snRNP regulate gene expression as an extra-splicing function. The first example of such regulation was the autoregulation of U1A, a component of U1 snRNP. U1A exists as a U1 snRNP-bound form, a free form, and a pre-mRNA-bound form and, when dissociated from U1 snRNA, U1A shuttles between the nucleus and the cytoplasm. These findings indicate that U1A has an extra-splicing function. U1A is well conserved in vertebrates at both mRNA and protein levels. Interestingly, not only the protein-coding region but also the 3’-untranslated region (UTR) is well conserved.
Viruses Take Advantage of U1 snRNP to Regulate Their Gene Expression

Almost at the same time as the reports of U1A autoregulation, U1 snRNP was found to regulate cleavage site selection in bovine papillomavirus type 1 (BPV-1). After infection, a group of viral genes called early genes are initially expressed and then late genes are expressed only after terminal transformation of the infected cells; however, the molecular mechanism underlying the restricted expression of the late genes was not known.

The BPV-1 genome has two PASs, the proximal PAS and the distal PAS. The proximal PAS is located between the early genes and the late genes, and the distal PAS is located at the 3’ end of the late genes (Figure 4(a)). Furth et al. found a short sequence located just upstream of the distal PAS that serves to downregulate the late gene expression during the early phase of infection. Deletion of this short sequence increases late gene mRNA levels; however, the sequence does not affect the stability of the polyadenylated mRNA. Interestingly, the short sequence contains a 9-nt sequence that is identical to the 5’ splice site. Expression of the late genes was upregulated by introducing mutations to the 5’ splice site-like sequence; in contrast, late gene expression was suppressed by the expression of a compensatory mutant of U1 snRNA, which base pairs with the mutated 5’ splice site-like sequence. Therefore, binding of U1 snRNP to the 5’ splice site-like sequence upstream of the PAS seems to be necessary for the inhibition of late gene expression, which is consistent with a study using reporter constructs. It is noteworthy that the 5’ splice site-like sequence is not involved in splicing. Further study revealed the molecular mechanism underlying the downregulation of the late genes. A gradual decrease in poly(A) length was observed by the addition of an increasing amount of purified U1 snRNP; therefore, U1 snRNP seems to downregulate late gene expression through inhibition of polyadenylation, similar to U1A autoregulation. Other characteristics that are common with U1A autoregulation are the interaction with PAP, and the requirement of the C-terminal domain of PAP for the inhibition of polyadenylation. Because of the similarity to U1A autoregulation, U1A was expected to be the responsible protein; however, U1-70K was found to be necessary and sufficient for the inhibition in this case. Considering the requirement of two U1A molecules for the autoregulation, it is likely that U1A is not the responsible factor in this case, because U1 snRNP contains only one molecule of U1A. This idea was supported by an experiment showing that an engineered U1 snRNA harboring two U1A-binding sites is able to inhibit polyadenylation. As U1-70K has four inhibitory motifs, one molecule of U1-70K may be sufficient for the inhibition of polyadenylation and the control of gene expression of BPV late...
Human papillomavirus type 16 (HPV-16) has an inhibitory element that contains four such tandem 5′ splice site-like sequences; therefore, some viruses, including BPV and HPV, may hijack U1 snRNP to control their own gene expression.36,39,40 Taken together, U1 snRNP binds to the 5′ splice site-like sequence located just upstream of the PAS and inhibits polyadenylation through the interaction between U1-70K and PAP, resulting in destabilization of the late genes (Figure 4(b)). More recently, it was revealed that mammalian U1A mRNA has a 5′ splice site-like sequence, which regulates the level of U1A expression, in addition to U1A-binding sites in its terminal exon.41 This was the first report to show that U1 snRNP suppresses not only viral genes but also endogenous mammalian genes. In addition to U1A and U1-70K, several nuclear proteins contain the inhibitory motif(s); therefore, other proteins might control the expression of a variety of pre-mRNAs through the inhibition of polyadenylation (see below).38

Human immunodeficiency virus (HIV) also controls its gene expression by using U1 snRNP. HIV has duplicated long terminal repeats (LTRs) flanking the viral genome (Figure 4(c)).42 Each LTR contains a transcription start site and a PAS that is located just downstream of the transcription start site; therefore, the PAS within the 5′ LTR needs to be inhibited for the whole genome to be transcribed. Indeed, the PAS within the 5′ LTR is rarely used; however, the detailed molecular mechanism of this inhibition was unknown. Ashe et al. reported that the 5′ splice site located just downstream of the 5′ LTR is responsible for inhibiting cleavage at the cleavage site in the 5′ LTR.43,44 In fact, insertion of an intron downstream of the PAS inhibits cleavage at the cleavage site, whereas introducing mutations in the 5′ splice site stimulates cleavage at the cleavage site.43,44 Because a 5′-end-engineered U1 snRNA that base pairs just downstream of the mutated 5′ splice site restores the inhibitory activity, binding of U1 snRNP to the 5′ splice site or in the vicinity of the 5′ splice site is required for the inhibition of cleavage at the cleavage site in the 5′ LTR of HIV (Figure 4(c)). The same effect was observed in an experiment using reporter plasmids.37 Interestingly, although the inhibition is a splicing-independent function of U1 snRNP, insertion of a strong 3′ splice site at the downstream of the 5′ splice site stimulates the inhibition, suggesting that the 3′ splice site and binding proteins at the site strengthen the interaction of U1 snRNP to the 5′ splice site.44 As increased spacing between the poly(A) site and the 5′ splice site reduced the inhibitory activity,44 a physical interaction between U1 snRNP and cleavage and polyadenylation factors may be necessary for the inhibitory activity. Among the components of U1 snRNP, U1-70K is the protein responsible for the inhibition of cleavage and polyadenylation at the cleavage site in the 5′ LTR; however, U1-70K is not involved in the inhibition of cleavage in adenovirus L3.37 Therefore, the necessary factor for the inhibition might be different based on the context.

**Engineered U1 snRNP for the Regulation of Gene Expression**

As described above, the binding of U1 snRNP upstream of PAS can inhibit polyadenylation.30,37,38 This extra-splicing function can be applied for gene expression.
expression regulation. Expression of 5′-end-engineered U1 snRNAs that anneal to their target mRNAs through the 5′ end causes inhibition of polyadenylation and downregulation of their target genes.46–48 Because a one-nucleotide change affects the efficiency of the inhibition, the strength of U1 snRNA binding to pre-mRNA is critical for the inhibition.48,49 Although spacing between the U1 snRNP-binding site and the PAS has a small effect on the inhibitory efficiency, insertion of an intron between the U1-binding site and the poly(A) site decreases the inhibition level, suggesting that the U1-binding site must be located in the last exon.47 These findings should be helpful in the design of mutated U1 snRNAs for gene silencing. Goraczniak et al. developed an advanced method for gene silencing based on these findings.50 They used U1 Adapter oligonucleotides that anneal to both the 5′ end of U1 snRNA and the target pre-mRNAs to tether U1 snRNP on the targets. Transfection of a U1 Adaptor oligonucleotide reduced gene expression of the target gene; therefore, this strategy may be an additional method for gene silencing.50

**U1 snRNP Protects the Integrity of the Transcriptome**

As described above, U1 snRNP can regulate gene expression in some viruses and mammalian cells through interaction with the terminal exon.31,36,41,43,44 So, does U1 snRNP control gene expression only at the terminal exon in mammalian...
Most pre-mRNAs show intron accumulation after functional knockdown of U1 snRNP using the U1 antisense morpholino oligo (AMO), which anneals to the 5' end of U1 snRNP and prevents the interaction between U1 snRNP and 5' splice sites. In addition to the intron accumulation, numerous pre-mRNAs also show an abrupt decrease in the amount of pre-mRNA within 3–5 kb from the transcription start site, although spliceostatin A (SSA), a potent splicing inhibitor that binds to U2 snRNP, does not cause this phenomenon, suggesting that the decrease is a consequence of U1 inhibition but not splicing inhibition. Surprisingly, the short pre-mRNAs have a poly(A) tail at their 3' ends, and a PAS is located approximately 20 nucleotides upstream of the poly(A) tail, suggesting that the pre-mRNAs are subjected to premature cleavage and polyadenylation (PCPA) in the U1 snRNP-inhibited cells. U1 snRNP, which binds to the 5' splice site located upstream of the PAS, seems to protect pre-mRNA from PCPA, because introducing a mutation at the 5' splice site causes PCPA even without U1 AMO treatment. Interestingly, U1 AMO treatment causes further stimulation of PCPA induced by the mutation at the 5' splice site. This result suggests that U1 snRNPs bind to the pre-mRNA not only at the 5' splice site but also at other sites, and that such U1 snRNPs are also able to inhibit PCPA (Figure 5). Taken together, the inhibition of cleavage and polyadenylation is not a gene-specific regulatory mechanism, but is prevalent over the whole genome to protect the integrity of the transcriptome.

This is related to the finding that cleavage site selection of HIV mRNA is regulated by U1 snRNP. In both cases, U1 snRNP inhibits the cleavage reaction for the transcription of full-length pre-mRNA; however, there is a considerable difference between these two cases. U1 snRNA binding downstream of the PAS inhibits cleavage of HIV pre-mRNA; however, U1 snRNP inhibits PCPA when it binds upstream of the PAS. Although Vagner et al. reported that U1 snRNA binding upstream of PAS inhibits polyadenylation, and U1 snRNA binding downstream of PAS inhibits the cleavage reaction using a reporter construct, the U1 snRNP binding to the upstream region may be able to inhibit cleavage depending on the genes or neighboring regions. In addition, U1 snRNPs binding to sequences other than the 5' splice site also contribute to the inhibition; therefore, U1 snRNP binding to the sequence downstream of the poly(A) site may play an auxiliary role, or several U1 snRNPs may function cooperatively. To clarify the detailed molecular mechanism, further genome-wide studies together with biochemical experiments should be performed.

The majority of higher eukaryotic genes harbor multiple cleavage and polyadenylation sites, and some of which are reported to be cleaved and

**FIGURE 5** | U1 snRNP inhibits PCPA. Most mammalian genes have cryptic poly(A) sites and U1 snRNP protects pre-mRNAs from PCPA. The cryptic 5' splice sites have varying affinities for U1 snRNP, allowing for differential usage of the PAS and cryptic PAS elements. A slight decrease in the relative amount of U1 snRNP causes transcript shortening.
polyadenylated depending on circumstances.\textsuperscript{55,56} Many 3′ UTRs have regulatory sequences including miRNA-binding sites\textsuperscript{54,57,58}; therefore, selection of the cleavage site may affect the fate of mRNAs. Studies using a novel high-throughput sequencing strategy revealed that the relative amount of U1 snRNP against pre-mRNAs affects cleavage site selection.\textsuperscript{53} Berg et al. compared the effect of complete functional inhibition of U1 snRNP and that of moderate inhibition, which was insufficient for splicing inhibition, on the transcriptome. Complete U1 inhibition caused PCPA near the transcription start site, whereas moderate U1 inhibition caused PCPA at more distal sites compared with complete U1 inhibition,\textsuperscript{53} suggesting that the amount of functional U1 snRNP affects cleavage and polyadenylation site selection. Neuronal cells use this mechanism to produce shorter isoforms, which are specific to activated neuronal cells.\textsuperscript{53} Upon neuronal activation, many pre-mRNAs, including homer-1 and dab1, show a shift in the cleavage and polyadenylation site from the distal one to the proximal one.\textsuperscript{59,60} The shift was also observed in neuronal cell lines after neuronal activation, and by moderate U1 inhibition using U1 AMOs,\textsuperscript{53} suggesting that U1 snRNP controls gene length in activated neuronal cells. Contrary to expectations, the amount of U1 snRNA was not affected during neuronal activation; however, nascent transcripts that should be protected by U1 snRNP were upregulated,\textsuperscript{53} indicating that a decrease in the relative amount of U1 snRNP to pre-mRNA results in the use of proximal cleavage and polyadenylation sites (Figure 5). Indeed, overexpression of U1 snRNA suppressed this shift.\textsuperscript{53} This transcript shortening is not limited to activated neurons, but was also observed in cancer cells and proliferating cells.\textsuperscript{61,62} In addition, cleavage and polyadenylation site selection is tissue specific,\textsuperscript{63,64} suggesting that the selection may regulate tissue-specific gene expression. Therefore, U1 snRNP may regulate these cleavage and polyadenylation site selections to control transcript length and this function may be a physiological function to regulate gene expression in response to environmental cues.

More recently, U1 snRNP was found to control the directionality of transcription.\textsuperscript{65} Pol II is able to initiate transcription in both directions from promoters; however, Pol II produces long transcripts from the sense-coding regions, while transcripts from antisense upstream regions are short and unstable.\textsuperscript{66-68} In the sense-coding regions, 5′ splice site-like sequences are enriched, while the PAS is the least abundant sequence, relative to antisense upstream regions.\textsuperscript{65} Therefore, the production of long transcripts from the sense-coding regions and short transcripts from the upstream antisense regions may be explained by the biased distribution of 5′ splice site-like sequences and PASs. Namely, the mRNAs from sense-coding regions are protected by U1 snRNP, and the mRNAs from upstream antisense regions are downregulated by PCPA (Figure 6). Supporting this idea, functional inhibition of U1 snRNP causes an increase in short transcript levels from the sense-coding regions to the same level as that from the antisense upstream regions.\textsuperscript{65} Interestingly, the sense-coding regions seem to have gained 5′ splice site-like sequences and lost PASs during evolution. Therefore, the use by cells of the extra-splicing function of U1 snRNP to inhibit cleavage and polyadenylation may be very ancient.

**RECIPIROCAL REGULATION OF SPlicing AND CLEAVage AND POLYADENYLATION**

**Splicing factors Stimulate 3′-End Processing**

So far, I have introduced several examples of inhibition of 3′-end processing by U1 snRNP or its components, U1A and U1-70K. Next, this review focuses on the positive interaction between splicing factors and 3′-end processing factors. In the exon definition model, U1 snRNP and U2 snRNP interact with each other across an exon to enhance the binding of snRNPs to the pre-mRNA.\textsuperscript{15} However, U2 snRNP does not have a binding partner across the last exon for this definition, because there is no downstream 5′ splice site. Therefore, the 3′-end processing factors are good candidates for the interacting partner of U2 snRNP for last exon definition to stimulate splicing, and the interaction is thought to also stimulate 3′-end processing.

The intron located upstream of PAS stimulates cleavage and polyadenylation.\textsuperscript{8,9,69} Interestingly, 5′ splice site deletion does not affect the efficiency, whereas introducing a 3′ splice site mutation abolishes polyadenylation; therefore, 3′ splice sites and splicing factors binding to the sites are important for the stimulation. U2AF65 was found to be the splicing factor responsible for the stimulation.\textsuperscript{70,71} Within the U2AF65 protein, the N-terminal region is necessary and sufficient for the stimulation of cleavage and polyadenylation. The N-terminal region interacts with CF Im59, recruits it to PAS, and consequently stimulates 3′-end processing.\textsuperscript{71} However, the N-terminal region contains consensus sequences of the PAP inhibitory motif, as in U1A and U1-70K; therefore, the region may be capable of inhibiting
PAP. Indeed, tethering the region on pre-mRNA inhibits polyadenylation, presumably through inhibition of PAP activity; therefore, U2AF65 may stimulate and inhibit 3′-end processing depending on the substrates, interacting partner, and cellular and/or experimental conditions (see below). In addition to U2AF65, U2 snRNP is also involved in the coupling. Introducing mutations at the BPS and degradation of U2 snRNA suppresses polyadenylation. This finding also supports protein factors binding to or in the vicinity of the 3′ splice site are important for the coupling.

U1 snRNP, as well as U2 snRNP, functions in the coupling. Binding of U1 snRNP near the PAS activates polyadenylation. U1A positively regulates 3′-end processing through interaction with the upstream sequence element (USE), which stimulates polyadenylation efficiency of the SV40 late PAS. The binding of U1A to the USE is required for efficient cleavage and polyadenylation because addition of an excess amount of USE inhibits cleavage and polyadenylation. In contrast, addition of stem-loop II of U1 snRNA, which is the U1-binding site, has no effect, suggesting that U1A, which is free from U1 snRNA, is able to stimulate cleavage and polyadenylation. As U1A affects polyadenylation efficiency, a physical interaction between U1A and 3′ processing factors was expected. Among these factors, CPSF160 interacts with U1A. The N-terminus of U1A binds to CPSF160 and the C-terminal RRM of U1A is important for the binding with pre-mRNA; therefore, U1A recruits and stabilizes CPSF160 on the target pre-mRNA and enhances the polyadenylation reaction. Interestingly, however, an excess amount of U1A inhibits polyadenylation, suggesting that such an amount of U1A inhibits PAP (see below). Therefore, U1A has a dual function, both to activate and inactivate polyadenylation depending on the local concentration of U1A around PASs and its binding partner. Supporting this idea, the N-terminal and C-terminal of U1A are required for the activation and inactivation of polyadenylation, respectively. Furthermore, U1 snRNP binds to the CF Im complex, presumably through direct interaction between U1-70K and CF Im25. This binding may contribute to the coupling of splicing and 3′-end processing.

3′-End Processing Factors Stimulate Splicing

As described above, many splicing factors and 3′-end processing factors interact with each other and the interaction stimulates 3′-end processing. Next, I introduce several studies that uncovered the novel function of 3′-end processing factors that enhances...
splicing efficiency. It was reported that introducing a mutation at PAS greatly reduces splicing efficiency.6,79 Interestingly, if the pre-mRNA has multiple introns, only the terminal intron is affected by the mutation, suggesting that protein–protein interaction of splicing factors and 3′-end processing factors on the last exon is required for the coupling. However, the molecular mechanism of the stimulation had not been revealed. Kyburz et al. revealed that CPSF contributes to the stimulation of splicing.73 Furthermore, Vagner et al. reported that PAP stimulates splicing at the adjacent upstream intron through stabilization of U2AF65 to the intron.79 PAP enhances the binding of U2AF65 to the adjacent upstream intron and consequently recruits the spliceosome to the intron, stimulating splicing activity. U2AF65 has two short stretches of the PAP-binding sequence, similar to U1A and U1-70K,38,79 which interact with the C-terminal region of PAP, suggesting that the direct interaction between PAP and U2AF65 is the key to the coupling. As described above, U2AF65 recruits a 3′-end processing factor, CF Im59, to the 3′ end, and stimulates 3′-end processing;70,71 therefore, splicing factors and 3′-end processing factors may recruit each other and form a stabilized complex on the target pre-mRNA, resulting in reciprocal stimulation of efficiency.

REMAINING QUESTIONS

This review introduced examples of reciprocal regulation between splicing factors and 3′-end processing factors and the molecular mechanisms underlying the regulation. Interestingly, these factors positively and negatively regulate each other depending on the context. However, some questions remain.

First, why does U1 snRNP inhibit PCPA but not canonical polyadenylation at the 3′ end? It was suggested that the required protein factors for PCPA are the same as for canonical polyadenylation;31; therefore, U1 snRNP does not seem to inhibit PCPA-specific factors. Another possibility is that fewer U1-binding sites exist on the last exon. Because sense-coding regions have lost PCPA sites during evolution,65 it is possible that analogous selection pressure has caused last exons to have fewer U1-binding sites for efficient gene expression. It is also possible that formation of a stable complex containing 3′-end processing factors and U2 snRNP and/or U2AF on the last exon may prevent the inhibitory activity of U1 snRNP. For the inhibition of PCPA, direct interaction between U1 snRNP and 3′-end processing factors seems to be required.37,38,45,51 For the canonical PAS, U2 snRNP and/or U2AF65 bind to the adjacent upstream intron and have more chance to interact with PAP than U1 snRNP, because the spatial distance is less compared with that for U1 snRNP, which binds to the upstream 5′ splice site. Once the factors form a stable complex with U2 snRNP and/or U2AF, U1 snRNP may not bind to the 3′-end processing factors. If a PAS is located in an intron (i.e., PCPA site), U1 snRNP binding to the 5′ splice site or intron sequence could easily interact with PAP at the PCPA site because usually there is no 3′ splice site and polyypyrimidine tract upstream of the PCPA site. This possibility is supported by a report that insertion of an intron between the U1 snRNP-binding site and PAS diminishes the inhibitory activity of U1 snRNP.47 However, as binding of U1 snRNP and U1A to the last exon inhibits polyadenylation,38 U1 snRNP and its components can inhibit polyadenylation only when these factors strongly interact with the last exon and bind to PAP ahead of U2 snRNP and/or U2AF65. Therefore, inhibition of 3′-end processing by U1 snRNP might occur under limited circumstances, such as in U1A pre-mRNA and some viruses, or when U1 snRNP is tethered at the last exon.25,31,36,38 The major extra-splicing function of U1 snRNP is protecting pre-mRNA from PCPA and regulating gene expression.51,53,65

Second, does the PAP-binding motif of U2AF65 inhibit polyadenylation or not? The PAP-binding motifs of U1A and U1-70K inhibit PAP activity.38 U2AF65 contains a homologous sequence to the PAP-binding motifs and interacts with PAP through the motifs.72,79 Indeed, U1A, U1-70K and U2AF65 bind to the C-terminal region of PAP and the binding inhibits PAP activity.38,72 However, because PAP recruits U2AF65 to the upstream intron through the interaction to stimulate splicing,79 it is not plausible that PAP recruits U2AF65 to inhibit its own activity. The following may be able to explain this contradiction. Transient interaction between splicing factors and 3′-end processing factors contributes to the recruitment of their interacting proteins and to the stimulation of cooperative mRNA processing. However, tight and long-term interaction may inhibit enzymatic activity (Figure 7). U2AF65 inhibits polyadenylation through interaction with PAP; however, PAP recruits U2AF65 to the upstream intron. For the recruitment, once U2AF65 is recruited to the binding site, interaction between PAP and U2AF65 may not be necessary; therefore, short-term interaction is enough for the purpose. In contrast, for inhibition, U2AF65 has to continue to interact with PAP. In an analogous way, U1A recruits CPSF160 to
the 3'-end region, but the excess amount of U1A inhibits polyadenylation. Therefore, the strength and duration of the interaction may differentiate the outcome of the interaction between splicing factors and 3'-end processing factors, although other possibilities cannot be ruled out.

CONCLUSION

Pre-mRNA splicing and 3'-end processing are fundamental events that maintain the integrity of gene expression. These two events are reciprocally regulated in both positive and negative ways. U1 snRNP and its components regulate cleavage and polyadenylation in mammalian and viral mRNAs. U1 snRNP also inhibits PCPA to protect the integrity of the transcriptome, and regulates gene length and the direction of transcription. The reciprocal regulation on the last exon affects the efficiency of the events positively and negatively. For this regulation, protein–protein interaction is required and a very complicated interacting network can give rise to different outcomes of the interaction depending on the context. However, many unsolved questions still remain. For instance: why is the canonical PAS not suppressed by U1 snRNA? Additionally, how does the interaction between splicing and 3'-end processing factors result in different consequences? Further studies will illustrate the global picture of the detailed molecular mechanism of reciprocal regulation between splicing and 3'-end processing.

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