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Review

Review

Coupling of alternative splicing and alternative polyadenylation

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Abstract

RNA splicing and 3′-cleavage and polyadenylation (CPA) are essential processes for the maturation of RNA. There have been extensive independent studies of these regulated processing events, including alternative splicing (AS) and alternative polyadenylation (APA). However, growing evidence suggests potential crosstalk between splicing and 3′-end processing in regulating AS or APA. Here, we first provide a brief overview of the molecular machines involved in splicing and 3′-end processing events, and then review recent studies on the functions and mechanisms of the crosstalk between the two processes. On the one hand, 3′-end processing can affect splicing, as 3′-end processing factors and CPA-generated polyA tail promote the splicing of the last intron. Beyond that, 3′-end processing factors can also influence the splicing of internal and terminal exons. Those 3′-end processing factors can also interact with different RNA-binding proteins (RBPs) to exert their effects on AS. The length of 3′ untranslated region (3′ UTR) can affect the splicing of upstream exons. On the other hand, splicing and CPA may compete within introns in generating different products. Furthermore, splicing within the 3′ UTR is a significant factor contributing to 3′ UTR diversity. Splicing also influences 3′-end processing through the actions of certain splicing factors. Interestingly, some classical RBPs play dual roles in both splicing and 3′-end processing. Finally, we discuss how long-read sequencing technologies aid in understanding the coordination of AS-APA events and envision that these findings may potentially promote the development of new strategies for disease diagnosis and treatment.

Key words alternative polyadenylation, alternative splicing, crosstalk, RNA processing, RNA regulation

Introduction

In eukaryotes, mRNA synthesis occurs within the cell nucleus, where nascent RNA undergoes a series of processing events to generate mature transcripts, including 5′ capping, splicing, and 3′-end processing. Each of these steps has alternative regulation, such as alternative splicing (AS) in skipping or including an exon and alternative polyadenylation (APA) in selectively choosing proximal or distal polyadenylation site (pPAS or dPAS), or even intronic polyadenylation site (iPAS). These regulatory mechanisms play crucial roles in shaping the final repertoire of mature transcripts, thereby contributing to the complexity of protein isoforms. Hence, both AS and APA contribute significantly to the mRNA diversification and differences in the proteome in many organisms [1,2].

Alternative splicing leads to variations in transcripts through exon skipping, inclusion, intron retention, and *etc.*, determined by the selection of alternative splicing sites [1,3]. Meanwhile, the 3′-end formation in eukaryotes represents a pivotal step in mRNA maturation, involving the recognition of polyadenylation signals and subsequent cleavage and polyadenylation events [2,4]. These two processing events, the majority of which are co-transcriptional, have been studied independently. Contemporary research has revealed an intricate interplay between splicing and 3′-end processing mechanisms [5]. Notably, the coordination of these events is pertinent to APA and AS. This review starts with a brief introduction of the molecular machineries and processes of RNA splicing and 3′-end processing and aims to provide a comprehensive review of the progress on the coupling mechanisms between APA and AS.

Fundamentals of Splicing and 3′-End Processing

Splicing is a highly regulated molecular process characterized by intricate interactions involving spliceosomes, cis-acting elements, and trans-acting proteins. The spliceosome, a dynamic ribonucleoprotein (RNP) complex, plays a central role in identifying core regulatory sequences within pre-mRNAs, including 5′ splice sites (5′SS), 3′ splice sites (3′SS), branch point sites (BPS), and

polypyrimidine tracts [6]. Comprising five major small nuclear ribonucleoprotein particles (snRNPs)—U1, U2, U4/U6, and U5, the principal U2-type spliceosome—orchestrates the removal of approximately 99% of intron sequences [6]. Additionally, various protein factors interact with the human splicing machinery, with heterogeneous nuclear ribonucleoproteins (hnRNPs) and serine/arginine-rich (SR) proteins among the classical trans-acting proteins regulating splicing via splicing silencers and enhancers, respectively [7,8]. The formation of the spliceosome involves sequential interactions among multiple components. Notably, the U1 snRNP recognizes the 5′SS, while the U2 auxiliary factor (U2AF) complex identifies the 3′SS and polypyrimidine tract, marking an intron. Splicing factor 1 (SF1) binds to the branch point sequence to form complex E. Subsequently, SF1 is displaced by U2 snRNA, which binds to the BPS, leading to the formation of complex A. Subsequent recruitment of the U4/U6·U5 tri-snRNP complex forms complex B, leading to a series of conformational changes culminating in catalytically active complex C formation. Finally, two-step catalytic reactions complete the splicing process [9–13].

Cleavage-and-polyadenylation adds a poly(A) tail to mRNA, and this modification enhances mRNA translation, augmenting its functional capacity, and shields mRNA from degradation. The 3′-end processing machinery comprises four major subunits, including cleavage and polyadenylation specificity factor (CPSF), cleavage stimulation factor (CstF), cleavage factor I and II (CFIm and CFIIm), and other factors such as poly(A) polymerase (PAP), nuclear poly(A) binding protein (PABPN), and symplekin. The CFIm, CPSF, and CstF complexes bind to pre-mRNA via specific recognition motifs—upstream UGUA element, core AAUAAA motif, and downstream GU-rich sequences, respectively—acting synergistically to catalyze cleavage and polyadenylation reactions [14–18]. Interestingly, a recent study has elucidated a novel APA mode named sequential polyadenylation (SPA) for many genes, where the distal polyadenylation site is processed first, anchoring the transcript in the chromatin/nuclear matrix. Subsequently, this transcript serves as a substrate for further 3′-end processing at the proximal polyadenylation site [19]. Recently, SPA has been reported to regulate RNA post-transcriptional m^6A modification in coupled with RNA nuclear retention [20].

Alternative splicing significantly contributes to the complexity of the transcriptome in multicellular eukaryotes, including seven major types classified by splicing pattern: exon skipping or cassette exon, alternative 5′ splice site (A5SS), alternative 3′ splice site (A3SS), mutually exclusive exon (MXE), intron retention (IR), alternative promoter or alternative first exon, and alternative last exon [21]. The diversity of the 3′ UTR can arise from various mechanisms, including genetic variations, RNA modifications, alternative cleavage and polyadenylation, and alternative splicing [22]. APA, a prevalent phenomenon in metazoan transcripts encoding proteins [23], primarily occurs within the 3′ UTR (UTR-APA), resulting in transcripts encoding the same protein but with varying UTR lengths [24]. UTR-APA impacts mRNA stability, translation, nuclear export, and localization [1]. When alternative polyadenylation sites reside within internal introns or exons, this process is termed coding region APA (CR-APA) [25]. CR-APA leads to the expression of distinct protein isoforms, which regulate gene expression through various mechanisms. Thus, APA can be categorized into four types: tandem 3′ UTR APA, intronic APA, alternative terminal exon APA, and internal exon APA [26].

The interactions between splicing and polyadenylation are inherently interconnected, as potential splicing sites and poly(A) sites mutually intertwine along the pre-mRNAs (Figure 1). Next, we summarize recent progress on the coupling of the two processes in three sections: (1) Regulation and mechanisms of 3′-end processing on alternative splicing; (2) How splicing regulates 3′-end processing vice versa? (3) The dual roles of RNA binding proteins in regulating both splicing and 3′-end processing (Figure 1). Finally, we will discuss future directions, including new techniques to quantify the coupling of the two events, especially the long-read sequencing technique, and the potential avenues of detecting and targeting these processes in disease diagnostics and therapies.

Regulation and Mechanisms of 3′-End Processing on Alternative Splicing

Initial studies have revealed that the cleavage and polyadenylation process is not an isolated event but rather a tightly coordinated mechanism that occurs concurrently with splicing. Splicing of the terminal intron can promote CPA; mutations within the 3′ splice site of the final intron impede both splicing and polyadenylation [27]. Conversely, mutations weakening the “AAUAAA” motif have been shown to notably reduce the splicing efficacy of introns, particularly the terminal one [28].

CPA factors and poly(A) tail affect the splicing of the last intron

The cleavage and polyadenylation process, a crucial step in eukaryotic mRNA maturation, is intricately regulated by various factors that influence splicing dynamics. Among these factors, CFIm25, a CFIm complex, plays a pivotal role in modulating polyadenylation site selection and, consequently, alternative splicing outcomes. CFIm25 facilitates the utilization of distal polyadenylation sites by binding to upstream of the distal polyadenylation site [29,30], where its interaction with pre-mRNA promotes the assembly of other processing factors and impedes the usage of proximal polyadenylation signals [30]. Notably, in HeLa cells, loss of CFIm25’s activity induces the selection of the proximal polyadenylation site for the TIMP-2 gene, and interestingly, it also causes retention of the last intron [30] (Figure 2A), although the mechanism of which remains elusive. These findings implicate the intricate interplay between polyadenylation site utilization and alternative splicing, particularly in the context of the 3′ UTR, highlighting the regulatory role of CPA factors in post-transcriptional mRNA processing [31].

Furthermore, both PABPN1 and poly(A) tail exert significant influence on the splicing of the terminal intron. PABPN1, a prominent player in RNA metabolism, serves as a major poly(A) binding protein within the nucleus, facilitating poly(A) tail synthesis by stimulating the activity of poly(A) polymerase [32]. Additionally, PABPN1 modulates alternative polyadenylation by inhibiting the recognition of proximal polyadenylation sites, as evidenced by studies demonstrating increased utilization of proximal polyadenylation signals upon PABPN1 deletion [33]. Notably, Li *et al.* [34] discovered that PABPN1 acts as a splicing enhancer for weaker terminal introns, particularly at the 3′ splice site. Within the nucleus, many pre-mRNAs are often polyadenylated prior to complete splicing of their introns. As the poly(A) tail lengthens, it can recruit PABPN1, which subsequently recruits RNA-binding motif protein 26 (RBM26) and RBM27, facilitating the

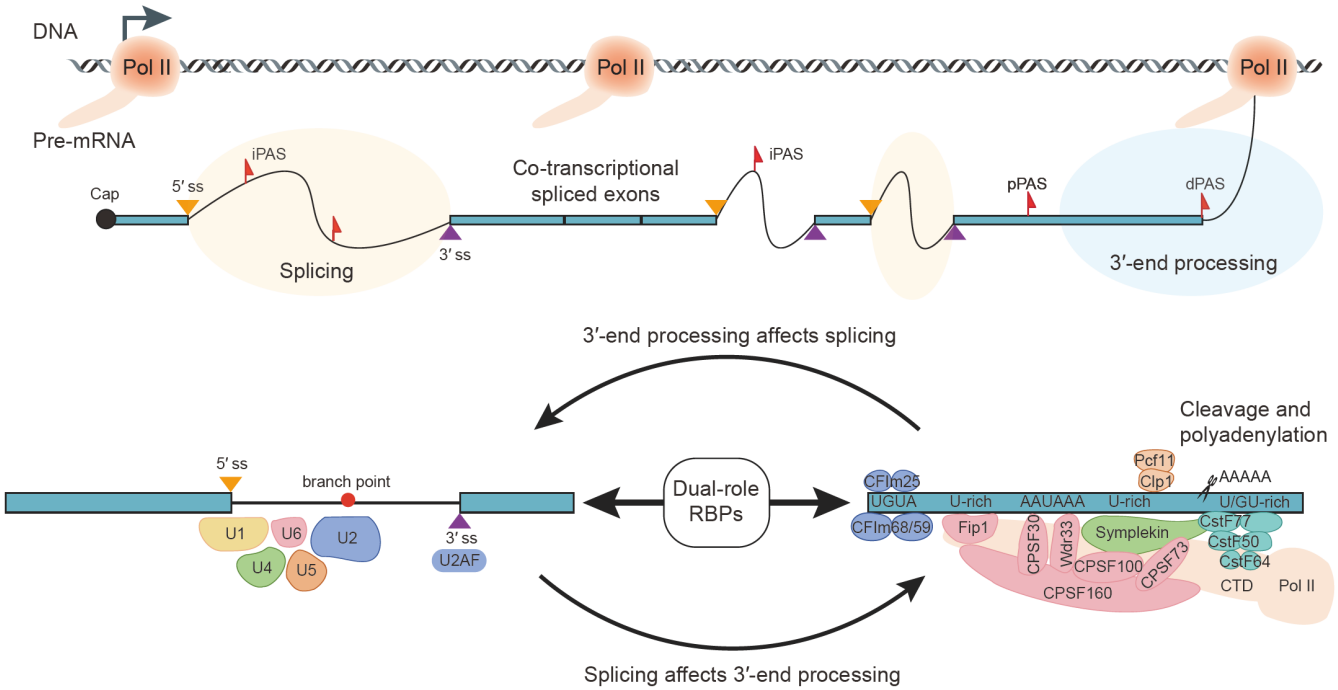

Figure 1. Schematic diagram illustrating the coupling between splicing and 3′-end processing The sequence signals of splicing (5′ or 3′ splicing site, ss) and 3′-end processing (PASs) are interleaving in many pre-mRNAs. The two processes are mutually regulated through multiple mechanisms, including dual-role RNA binding proteins (RBPs).

recognition of the 3′ splice site by the U2 snRNP, thereby enhancing post-transcriptional splicing of the weaker intron (Figure 2B). Moreover, the length of the poly(A) tail also influences terminal intron splicing. As reported [34], poly(A) tail length enhances the splicing of the terminal intron, with longer poly(A) tails exhibiting increased effectiveness within a certain range. This phenomenon may be attributed to the recruitment of more PABPN1 by longer poly(A) tails, consequently enhancing terminal intron splicing. However, the causal relationship between poly(A) tail length and splicing efficacy requires further investigation.

CPA factors affect the splicing of internal and terminal exon

Further investigations have revealed that the impact of tailing factors on splicing extends beyond the terminal intron, reaching to regulate the splicing of internal exons. One such example involves the critical splicing factor CPSF1, whose depletion results in enhanced inclusion of exon 6 in the interleukin 7 receptor (IL7R) gene [35]. Intriguingly, IL7R intron 6 has a weak 5′SS and harbors a consensus polyadenylation signal (AAUAAA) close to the 5′SS. The mutation of this signal similarly enhances exon 6 inclusion without concomitant cleavage and polyadenylation. These results suggested that CPSF1's interaction with intronic IL7R pre-mRNA impedes spliceosome binding to the splice site of exon 6; however, whether this is a widespread mechanism merits further studies. This interference may have implications for individual T cell ontogeny and the pathogenesis of multiple sclerosis (Figure 2C). Additionally, altered expression of CPSF1 facilitates the selection of the intronic cryptic exon 3 (CE) polyadenylation signal in the androgen receptor (AR) gene, leading to the recruitment of the CPSF complex. This event drives the synthesis of multiple AR variant mRNAs and impedes androgen-independent growth of castration-resistant prostate cancer (CRPC) cells [36]. In models of head and neck squamous cell carcinoma, aberrant CPSF1 expression disrupts alternative splicing events of cancer-related genes, including UBE2C and TGFBI, thereby increasing the likelihood of tumorigenesis [37]. Furthermore, in hepatocellular carcinoma (HCC), CPSF1 expression is markedly elevated and correlates with unfavorable survival outcomes. Knockdown of *CPSF1* leads to increased alternative polyadenylation and alternative splicing events, shedding light on the mechanisms of post-transcriptional regulation in HCC. This suggests CPSF1 as a potential prognostic biomarker and therapeutic target for HCC [38].

The coupling of these two processing events requires protein-protein interactions between splicing factors and 3′-end processing factors on the terminal exon. Experimental evidence suggests that point mutations in polyadenylation signals can impede splicing of adjacent upstream introns [28]. Moreover, inhibition or depletion of key CPA factors such as CPSF has been shown to hinder splicing of upstream introns, possibly by disrupting the function of the U2 snRNP cofactor large subunit U2AF65 [39–41]. The interplay between the CPA apparatus and splicing extends further, with CPA factors enhancing splicing before polyadenylation site cleavage occurs [39,42], which involves a series of steps. Initially, the splicing factor U2AF65 interacts with the CPA factor CFIm. Subsequently, in the remodeling step, CFIm is displaced from U2AF65 by poly(A) polymerase. Finally, U2 snRNP binds to CPSF, facilitating the cooperative interaction between splicing and CPA [43] (Figure 2D). *In vivo*, the Pol II CTD also stimulates coupling between splicing and 3′-end processing [44], probably by loading or recruiting both splicing and 3′-end processing factors.

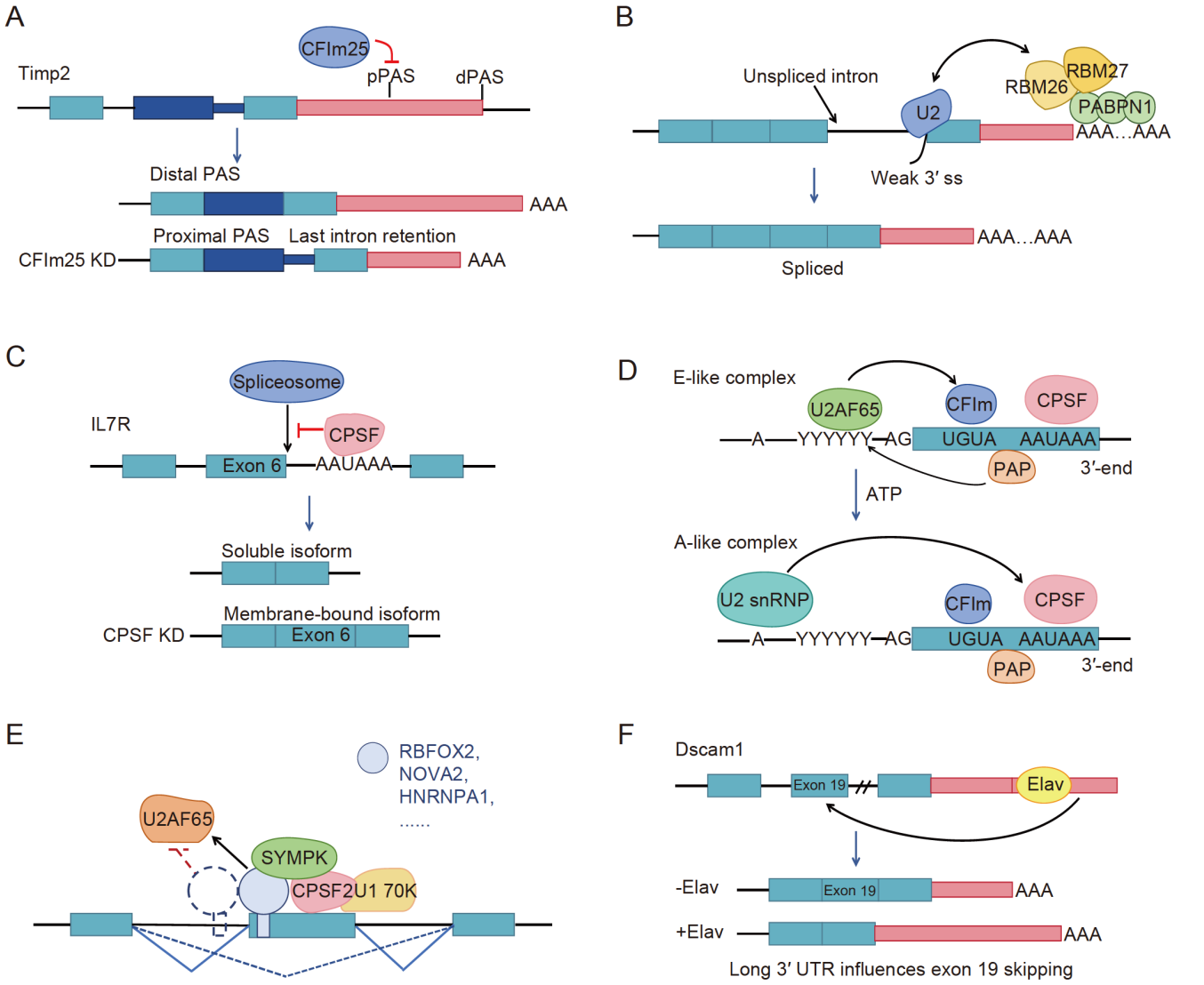

Figure 2. Regulation and mechanisms of 3′-end processing on alternative splicing (A) *CFIm* knockdown results in the formation of a short 3′ UTR transcript for Timp2, accompanied by retention of the last intron. (B) PABPN1 facilitates the splicing of a last intron with a weak 3′ splice site by recruiting RBM26/RBM27. (C) CPSF binding to the non-functional AAUAAA motif in the intron inhibits splicing of the cryptic exon. (D) Splicing factors and 3′-end processing factors interact on the terminal exon. (E) 3′-End processing factors, together with RBFOX2, NOVA2, HNRNPA1, and other RBPs, mediate exon inclusion and exclusion. (F) Elav promotes the usage of the long 3′ UTR of *Dscam1*, in coupled with the skipping of upstream exon 19.

3′-End factors affect splicing through RNA binding proteins

3′-End processing factors exert unexpected influences on alternative splicing via different RBPs. One such RBP is RBFOX2, known for its role in promoting exon inclusion or exclusion. Interestingly, two mRNA 3′-end processing factors, CPSF and SYMPK, act as cofactors of RBFOX2. RBFOX2-regulated exons can be categorized into two groups: those where CPSF/SYMPK is crucial for selective splicing, and those where CPSF/SYMPK is neither required nor present in the vicinity. For CPSF/SYMPK-regulated exons, RBFOX2 facilitates exon inclusion by interacting with and recruiting CPSF/SYMPK to pre-mRNAs. Subsequently, CPSF/SYMPK promotes the recruitment of U1 70K and U1 snRNP to the 5′ splice site, thereby facilitating exon inclusion. Conversely, on exons where RBFOX2 promotes exclusion, the binding of RBFOX2 and CPSF/SYMPK interferes with the binding of U2AF at the upstream 3′ SS, leading to exclusion of the exon. Moreover, CPSF/SYMPK serves as a cofactor for other splicing regulators such as NOVA2 and HNRNPA1, which also modulate exon inclusion and exclusion similar to RBFOX2 [45] (Figure 2E). This revelation sheds light on the intricate molecular mechanisms underlying the crosstalk between mRNA 3′-end formation factors and the spliceosome, offering valuable insights into AS regulation.

3′ UTR-mediated exon skipping

The preceding examples elucidate the impact of 3′-end processing factors on splicing, offering insights into potential interaction mechanisms and coupling between alternative polyadenylation

and alternative splicing. However, while these examples indicate that individual factors can influence AS processes, they provide only partial evidence of such interactions. Two hypothetical scenarios have been proposed to elucidate the relationship between APA events and upstream AS events. In the first scenario, APA and AS events are considered to be mechanistically uncoupled, operating relatively independently of each other. Conversely, the second scenario posits that APA and AS events are mechanistically coupled, suggesting that mRNA isoforms exhibiting a specific AS event may exhibit a selective preference for a particular APA type [31]. To test this hypothesis, the following examples may be utilized.

Zhang *et al.* [46] utilized Pull-a-Long-Seq (PL-seq), a long-read sequencing method based on cDNA capture, to explore the intricate relationship between alternative splicing and alternative polyadenylation. Their study focused on Drosophila late embryos and heads, revealing cryptic exon splicing events in 23 genes associated with the 3′ UTR. Notably, the Dscam1 gene was identified to undergo APA, generating a short 3′ UTR isoform (Dscam1-S) and a long 3′ UTR isoform (Dscam1-L). Elav protein was found to bind to the proximal PolyA site of Dscam1 pre-mRNA, promoting the biogenesis of the long 3′ UTR isoform (Dscam1-L). Long-read sequencing data showed that Dscam1-L exhibits a preference for skipping upstream exon 19 while containing the long 3′ UTR, whereas Dscam1-S retains exon 19 and has a short 3′ UTR [47] (Figure 2F).

Intriguingly, the skipping of exon 19 in Dscam1-L leads to alterations in the Dscam1 amino acid sequence, resulting in an abnormal protein. Deletion of the Dscam1-L protein was found to impair the growth of Drosophila axons, indicating the critical role of ELAV-mediated exon skipping and variable polyadenylation of the Dscam1 gene in axon growth. Further investigations using minigene reporter experiments revealed that exon 19 and its flanking introns alone are insufficient to promote exon skipping. Elav-induced exon 19 skipping was observed only in the presence of the long 3′ UTR. However, the precise mechanism underlying specific 3′ UTR selection and the coupling of exon skipping remains unclear. It is possible that these 3′ UTRs confer distinct RNA stability through variations in the length of polyA tails or via microRNA and RBP targets within the 3′ UTR [48]. Moreover, differences in the expression of these RNA isoforms across vairous cell types and subcellular localizations may also contribute to their functional impact.

Additionally, the long 3′ UTRs of the Dys and Khc-73 genes were identified to modulate cassette exon splicing in the short 3′ UTR [46]. These findings underscore the complex interplay between APA, AS, and mRNA processing factors in regulating gene expression and cellular functions. Elucidating these mechanisms further promises to enhance our comprehension of RNA biology and its impact on biological processes.

Regulation and Mechanisms of Splicing on 3′-End Processing

The regulation of splicing and 3′-end processing encompasses many intricate mechanisms, involving the competition of the two processes, splicing within 3′ UTR, and diverse splicing factors. This will be discussed in detail below.

Splicing competes with intronic cleavage and polyadenylation

For many genes, especially with long introns, where there are often intronic PASs, the two processes (splicing and polyadenylation) would compete with each other, and intronic PASs tend to be preferentially recognized when large introns have weak 5′ splice sites [49]. Maria Vlasenok *et al.* [50] defined a phenomenon termed spliced polyadenylated introns (SPIs). Through polyA-read analysis and 3′ end sequencing, SPI is observed to undergo both splicing and cleavage-and-polyadenylation. SPI represents an intermediate formed when splicing and CPA machinery operate simultaneously with transcription elongation. If CPA proceeds faster than splicing, IPA results in truncated transcripts. Conversely, if splicing outpaces CPA, introns containing PAS are excised, with PAS components degraded as part of the lariat. If PAS-mediated cleavage within the intron occurs after spliceosome assembly and is committed to splicing, the second catalytic step of splicing removes the lariat and all CPA products, forming SPI. The enrichment of PAS within introns and the presence of SPI collectively indicate competitive and cooperative interactions between splicing and CPA processes (Figure 3A). Co-transcriptional pre-mRNA splicing may function to salvage eukaryotic transcripts from premature transcription termination.

3′ UTR splicing

3′ UTR diversity also encompasses internal 3′ UTR splicing. While splicing research traditionally emphasizes changes in protein-coding regions, the presence of introns within the 3′ UTR can lead to alternative splicing that alters 3′ UTR length. For transcripts of the heterogeneous nuclear ribonucleoprotein D (hnRNPD/AUF1) gene, splicing of exon 9 and intron 9 can generate four alternatively spliced AUF1 3′ UTR variants. This intronic splicing within the 3′ UTR regulates AUF1 expression, suggesting a significant role in gene expression regulation [51].

Another example is the Arc gene, where splicing of its 3′ UTR introns induces its own nonsense-mediated mRNA decay (NMD) and also facilitates mRNA translation efficiency, revealing a novel mechanism in neuronal post-transcriptional gene regulation that confines Arc expression to be transient and bursting [52].

Another noteworthy factor, Cstf64, has the potential to be an AS regulator. Knockdown of *Cstf64* impacts splicing events within the 3′ UTR of hnRNP A2/B1, culminating in the reduced protein level of hnRNP A2/B1. Notably, the 3′ UTR of hnRNP A2/B1 harbors three potential alternative polyadenylation sites. Using the most distal APA site makes the splice sites in the last exon available and generates multiple unique spliced isoforms. Among these isoforms, hnRNP B1 isoform is susceptible to nonsense-mediated mRNA decay [53]. Consequently, knockdown of *Cstf64* leads to an increase in the abundance of the unstable hnRNP B1 isoform, thereby contributing to a reduction in protein levels [31].

Recent pan-cancer analyses indicate that widespread upregulation of isoforms with splicing within 3′ UTR drives tumorigenesis. Notably, CTNNB1 3′ UTR splicing emerges as one of the most consistently dysregulated events across cancers. Experimental results demonstrate that CTNNB1 3′ splicing transcripts (3′SPs) are major translational isoforms, predominantly localized in the cytoplasm, whereas 3′ full-length transcripts (3′FLs) are primarily located in the nucleus. These findings indicate that an increase in nuclear CTNNB1 3′ UTR splicing events generates more 3′SP

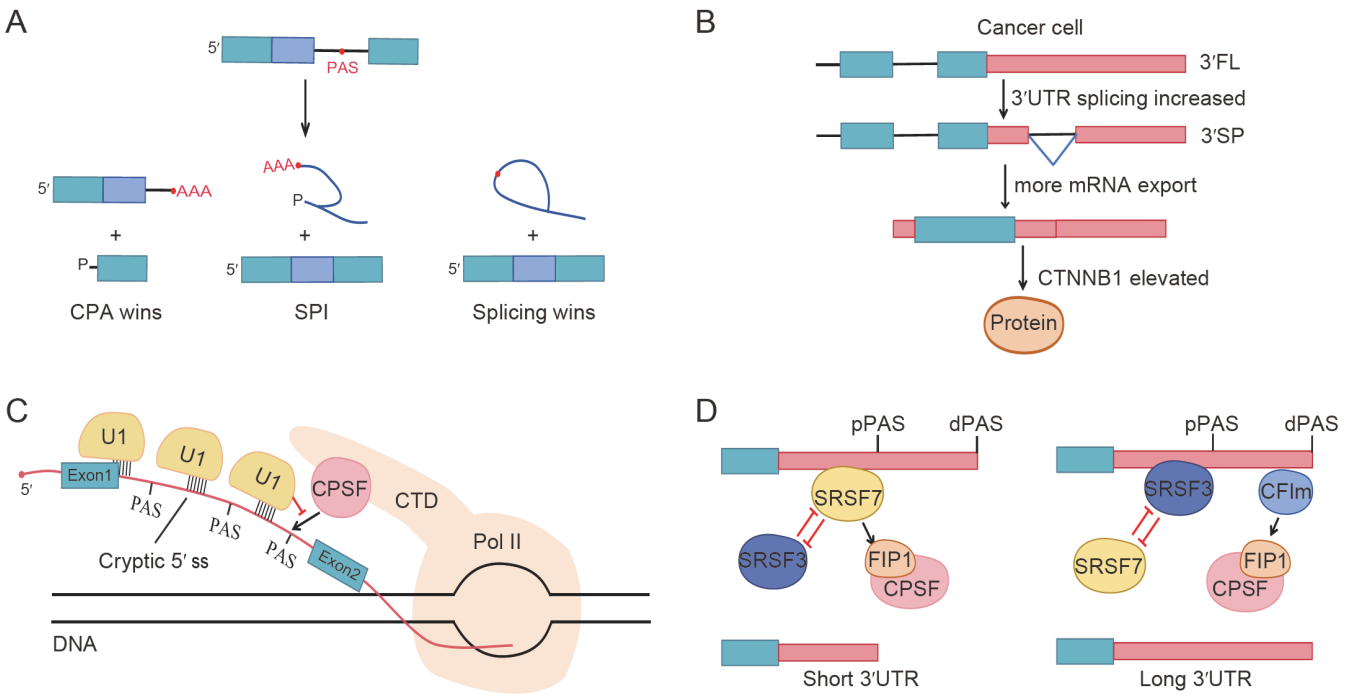

Figure 3. Regulation and mechanisms of splicing on 3′-end processing (A) Splicing competes with intronic cleavage and polyadenylation to form IPA, SPI or lariat. (B) Schematic depiction of how increased 3′ UTR splicing may promote carcinogenesis. (C) U1 snRNP serves as a crucial factor in safeguarding pre-mRNA from premature cleavage and polyadenylation, thereby ensuring the production of full-length mRNA transcripts. (D) SRSF7 and SRSF3 exert opposing effects on APA. SRSF7 promotes the utilization of the proximal polyadenylation site, leading to the formation of a short 3′ UTR. Conversely, SRSF3 facilitates the use of the distal polyadenylation site, promoting the generation of a long 3′ UTR.

transcripts. Subsequent cytoplasmic translation of these transcripts leads to the overexpression of CTNNB1, potentially conferring oncogenic advantages to cancer cells [54] (Figure 3B). Thus, 3′ UTR diversity not only enhances overall transcriptomic and proteomic diversity but also fuels cancer development and progression.

Splicing factors affect APA

The aforementioned findings underscore the bidirectional relationship between cleavage-and-polyadenylation and splicing processes, wherein CPA can influence splicing, and vice versa. Notably, certain splicing factors also exert regulatory effects on alternative polyadenylation. For instance, U1 snRNP, a pivotal component of the spliceosome machinery, plays a crucial role in safeguarding pre-mRNAs from premature cleavage and polyadenylation. Upon binding to nascent transcripts through complementary pairing with homologous sequences, U1 snRNP effectively inhibits cleavage and polyadenylation at cryptic polyadenylation sites, thereby facilitating the production of full-length mRNAs [55]. Conversely, a decline in the availability of splicing factors or disruption in splice site recognition can lead to enhanced intronic polyadenylation. Diminished levels of U1 snRNP result in the preferential utilization of proximal PASs over normal PASs, giving rise to truncated mRNA transcripts [56] (Figure 3C).

In addition to the mechanisms mentioned earlier, SNRNP70, a component of the spliceosomal U1 snRNP, can drive phase transitions of PABPN1 to reduce its inhibitory activity at proximal polyA sites. It can also recruit CPSF6 to promote the usage of proximal polyA sites [57]. The above phenomenon raises another question: why frequently used PASs are not inhibited by U1 snRNP? Shi *et al.* [58] found that actionable PASs are often located near regions that lack optimal U1 snRNP docking sites. In addition, the RNA-RNA base-pairings between short 5′-SS-like motifs (U(A/G)(A/G)G) and the 5′-end sequences of U1 snRNA are not strong enough to stably assemble U1 snRNP, which prevents its effect on these PASs. Deng *et al.* [59] discovered that U1 snRNP binding at the 5′ SS may promote the binding of U1A (U1 small nuclear ribonucleoprotein A) near the intronic PAS region, thereby interfering with the recruitment of the 3′-end processing factor CstF64 and inhibiting the cleavage of intronic PASs in pre-mRNA. In HeLa cells, inhibition of U4 snRNP leads to premature CPA events similar to those resulted from U1 snRNP inhibition [60].

Several other splicing factors have been identified to influence polyadenylation processes [61]. Among these, serine/arginine-rich splicing factors (SRSFs) are crucial in regulating selective splicing. Notably, SRSF3 has been implicated in promoting cellular senescence through alternative polyadenylation mechanisms. In a study by Shen *et al.* [62], transcriptome-wide APA analysis was performed using polyadenylation sequencing (PA-seq) and strand-specific sequencing methods. The analysis revealed a global shortening of the 3′ UTR in human and mouse cells following *SRSF3* knockdown. Importantly, among the genes exhibiting 3′ UTR shortening, *SRSF3* displayed a higher binding density at the proximal polyadenylation site than at the distal site. Furthermore, the effects of 3′ UTR shortening were recapitulated by the overexpression of three candidate genes (*PTEN*, *PIAS1*, and *DNMT3A*), all of which have been linked to senescence-associated phenotypes. These findings

provide compelling evidence supporting the notion that deletion of SRSF3 triggers cellular senescence through APA mechanisms.

SRSF7 shares similarity with SRSF3 in its binding affinity upstream of the proximal polyadenylation signal. However, these two splicing factors exert opposing effects on 3′ UTR length regulation. The distinct protein structural domain of SRSF7 facilitates the recruitment of the cleavage factor FIP1, thereby promoting the utilization of the proximal polyA site and leading to the formation of a short 3′ UTR. Conversely, SRSF3 counteracts the action of SRSF7 by directly antagonizing its function. Specifically, SRSF3 favors the utilization of the distal polyA site, thereby facilitating the formation of a long 3′ UTR. Moreover, SRSF3 indirectly modulates the levels of active CFIm through selective splicing, orchestrating the assembly of non-productive cleavage complexes. This complex interplay ultimately enhances the utilization of the distal PAS while inhibiting the use of the proximal PAS, thereby promoting the generation of a long 3′ UTR [63] (Figure 3D).

RBPs Play Dual Roles in AS and APA

There are RBPs that exhibit dual functionalities in both alternative splicing and alternative polyadenylation. For instance, the ELAV protein is known for its multifaceted roles in mRNA processing. In Drosophila, ELAV, specifically expressed in neurons, plays a pivotal role in inducing the expression of long 3′ UTR and facilitating the skipping of upstream exons. Within neuronal contexts, ELAV/Hu RBP binds to the downstream UUGUUUU motif of the polyadenylation signal, thereby exerting local repression on the cleavage and polyadenylation process. Subsequently, CPA cleaves transcripts at the downstream PAS, leading to the generation of neural-extended 3′ UTR isoforms [64,65]. This intricate regulatory mechanism underscores the dynamic interplay between RNA-binding proteins and mRNA processing machinery, contributing to generating transcriptomic diversity essential for neuronal function and plasticity.

Another illustrative example involves the Drosophila Srrm234 gene, which exhibits a distinctive neural-specific alternative last exon isoform harboring the eMIC domain. Notably, the RNA-binding protein ELAV plays a pivotal role in orchestrating the selection of this last exon with ELAV binding sites (UUUNUUU motifs) at the 3′ end. By repressing the proximal polyadenylation signal site, ELAV facilitates the expression of the eMIC-containing isoform. Subsequently, this isoform plays a crucial role in directing the widespread inclusion of neural microexons [66] (Figure 4A). This intricate interplay between alternative polyadenylation and splicing underscores their collaborative roles in executing essential functions within the cellular transcriptomic landscape. Such coordination between APA and splicing mechanisms highlights the dynamic regulatory networks that govern mRNA processing, ultimately contributing to cellular diversity and functionality.

The loss of MBNL protein function is the underlying cause of myotonic dystrophy (DM), a genetic disorder characterized by muscle weakness and other symptoms. Throughout development, the MBNL protein family exerts significant influence over alternative splicing. Specifically, MBNL1 and MBNL2 modulate AS in muscle and brain development by binding to nascent transcripts. MBNL1 preferentially recognizes the YGCY motifs and promotes exon inclusion when binding to motifs downstream of the 5′SS, whereas it facilitates exon skipping when binding to motifs upstream of the 3′SS [67]. Additionally, an important binding site for the MBNL protein family resides within the 3′ UTR of target RNA. Research conducted by Batra *et al.* [68] shed light on the widespread dysregulation of alternative polyadenylation resulted from loss of MBNL function in cellular and animal models. This dysregulation manifests as a shift in APA patterns in adult tissues towards those typically observed in the fetal stage. Through techniques such as HITS-CLIP and minigene reporter analysis, it was demonstrated that MBNL proteins directly regulate APA by binding to specific sites. When the MBNL binding site overlaps with the polyadenylation site, it inhibits the recruitment of 3′-end processing factors. Conversely, when the MBNL protein predominantly binds with the upstream site, it activates 3′-end processing at the downstream site. Furthermore, aberrant activation of normally

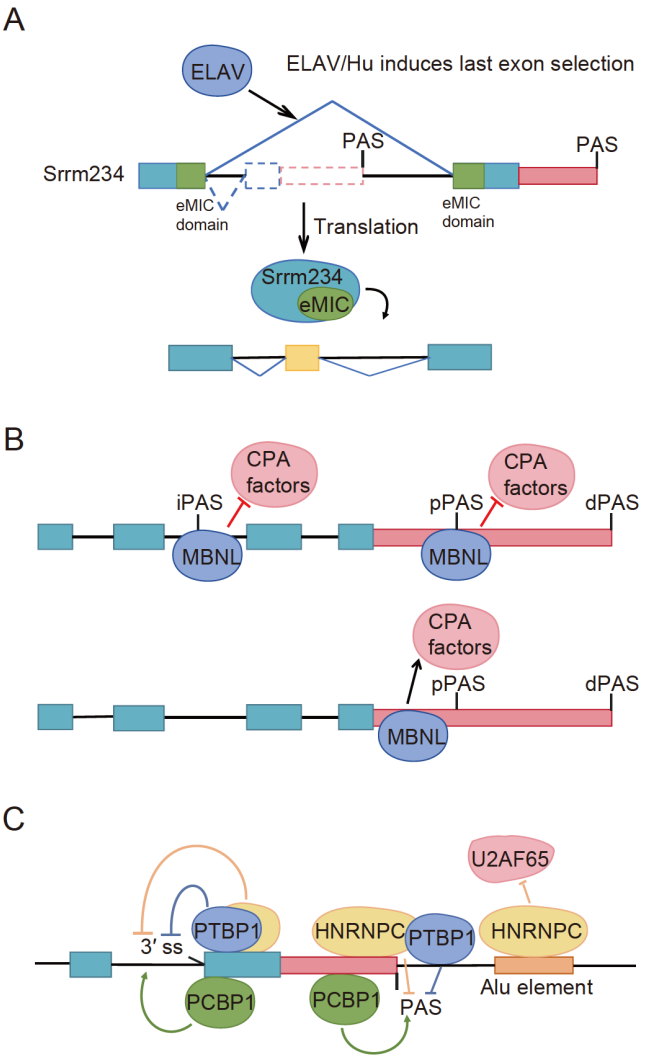

Figure 4. Dual-role RBPs in AS and APA (A) ELAV facilitates the expression of the eMIC-containing Drosophila Srrm234 isoform, which mediates microexon inclusion. (B) The MBNL protein exhibits a dual regulatory role in AS and APA. When the binding site of MBNL protein overlaps with the polyadenylation site, it impedes the recruitment of 3′-end processing factors, thereby influencing APA. Conversely, when MBNL protein binds to upstream of the polyadenylation site, it activates 3′-end processing at the downstream site, thereby modulating APA. (C) The splicing factors HNRNPC, PTBP1, and PCBP1 also modulate cleavage and polyadenylation.

expressed polyadenylation sites during embryonic development and silencing of adult polyadenylation sites are observed upon inhibition of MBNL protein activity by CUG^{exp} RNA. Hence, it is evident that MBNL proteins exert dual regulatory control over both AS and 3′-end processing of target gene transcripts (Figure 4B). This intricate interplay underscores the multifaceted roles of MBNL proteins in post-transcriptional regulation, highlighting their significance in cellular homeostasis and disease pathology.

The polypyrimidine (Py) tract-binding proteins (PTBPs) combines with Py-rich motif at the 3′ splice site to inhibit the splicing of target exons [69,70]. In glioblastoma tumors, PTBP1 has been observed to bind to approximately 75-nucleotides downstream of the poly(A) site, where it antagonizes CSTF2 and inhibits the usage of the PAS [71]. hnRNPC can compete with U2AF65 to suppress the exonization of Alu elements to protect transcriptome integrity [72,73]. When HnRNPC binds to U-rich elements near the poly(A) site, it inhibits the usage of the poly(A) site [74]. The poly(C)-binding proteins (PCBPs) facilitate both splicing [75] and polyadenylation [76] (Figure 4C). It is anticipated that more dual-role RBPs will be revealed in future studies.

Perspectives

It is known that traditional RNA-seq is not well-suited for the precise identification of poly(A) sites. For that purpose, many library preparation methods that enrich fragments carrying poly(A) tails have been developed, which can be categorized into two types based on their strategies [77]. The first category is oligo(dT) priming-based, where mRNA molecules are reverse transcribed into cDNA using oligo(dT) primers, and encompasses techniques such as PA-seq [78], 3′-Seq [79], PAS-seq [80], 3PC [81], EXPRSS [82], MAPS [83], SAPAS [84], and WTTS-seq [85]. The second category is RNA manipulation-based, where adapters are added to the 3′-ends of RNA fragments, followed by reverse transcription using primers annealed to the adapters, including 3P-seq [86], 3′ READS [87], and PAT-seq [88]. It is a general strategy to integrate those high-resolution 3′-end sequencing technologies with RNA-seq to investigate the regulation of RNA processing.

Additionally, third-generation sequencing technologies offer more powerful approaches for studying various RNA processing steps, especially the coupled regulation of different events. The advancement of third-generation sequencing technologies has propelled long-read RNA sequencing (LRS) into a potent tool for investigating RNA biology. The leading long-read sequencing platforms include Pacific Biosciences (PacBio) REVIO system, Oxford Nanopore devices, and BGI CycloneSEQ released recently. Unlike short-read high-throughput sequencing methods, which typically generate reads shorter than 150 or 300 nucleotides, long-read sequencing can capture transcripts spanning tens of kilobytes without fragmenting RNA molecules, thus preserving transcript integrity [89]. Consequently, long-read RNA sequencing facilitates a comprehensive exploration of different mRNA processing events simultaneously at the single-transcript level. It enables the investigation of the interplay between various RNA processing phenomena, thereby enhancing our understanding of underlying biological processes [90].

Zhang *et al.* [46] introduced a targeted Nanopore long-read sequencing strategy named Pull-a-Long-Seq. Through PL-Seq, they identified 23 genes exhibiting AS linked to 3′ UTR variations in neuron-enriched tissues and quantified the regulatory role of ELAV in coordinating AS and APA. PL-seq (Pull-a-Long Seq) is a probe-based cDNA pulldown strategy, in which SMARTer cDNA synthesis is first performed using oligo(dT) primers, followed by cDNA PCR. Synthesized and biotinylated probes are then used to capture the target genes, followed by PCR amplification and Nanopore long-read sequencing. The targeted long-read single-molecule sequencing enables studying the coordination between alternative splicing and alternative polyadenylation in single transcripts with sufficient depth, different from the traditional NGS-based RNA-seq good at detecting local AS or APA events. PL-Seq offers a cost-effective approach and can be broadly applied to quantify linked AS-APA events across diverse organisms, tissues, and cell types. Furthermore, PL-seq holds promise for single-cell analysis, complementing single-cell RNA-seq methodologies in deciphering the interplay of co-transcriptional events within complex tissues. In addition, the removal of introns occurs predominantly in a defined sequence order during splicing. However, current global analyses of intron removal order are limited to adjacent pairs of introns. Multi-intron splicing order exceeding two introns is rare and has been achieved only in a few individual genes through RT-PCR or targeted next-generation sequencing. Therefore, long-read transcriptome sequencing represents a promising approach to studying the order of RNA processing events within single RNA molecules [91].

Dysregulation of splicing and 3′-end processing is implicated in the mechanisms of numerous diseases. For instance, alternative splicing of the 3′ UTR may be a widely exploited mechanism by cancer cells to produce transcripts resistant to nonsense-mediated decay and splicing-mediated decay, thereby promoting oncogene expression and tumorigenesis [54]. Therefore, new mechanisms uncovered through long-read transcriptome sequencing may elucidate the etiology of various diseases and play significant roles in both physiological and pathological contexts. Furthermore, discovering mechanisms coupling splicing and 3′-end processing could provide novel insights for developing new drug targets. It is possible that diseases caused by splicing dysregulation might be addressed through targeting 3′-end processing, or conversely. Thus, detecting and targeting these processes might represent new avenues for more precise diagnostics and therapies.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Tian B, Manley JL. Alternative polyadenylation of mRNA precursors. *Nat Rev Mol Cell Biol* 2017, 18: 18–30
2. Ule J, Blencowe BJ. Alternative splicing regulatory networks: functions, mechanisms, and evolution. *Mol Cell* 2019, 76: 329–345
3. Bonnal SC, López-Oreja I, Valcárcel J. Roles and mechanisms of

alternative splicing in cancer — implications for care. *Nat Rev Clin Oncol* 2020, 17: 457–474
4. Mitschka S, Mayr C. Context-specific regulation and function of mRNA alternative polyadenylation. *Nat Rev Mol Cell Biol* 2022, 23: 779–796
5. Komili S, Silver PA. Coupling and coordination in gene expression processes: a systems biology view. *Nat Rev Genet* 2008, 9: 38–48
6. Wilkinson ME, Charenton C, Nagai K. RNA splicing by the spliceosome. *Annu Rev Biochem* 2020, 89: 359–388
7. Geuens T, Bouhy D, Timmerman V. The hnRNP family: insights into their role in health and disease. *Hum Genet* 2016, 135: 851–867
8. Howard JM, Sanford JR. The RNAissance family: SR proteins as multifaceted regulators of gene expression. *Wiley Interdiscip Rev RNA* 2015, 6: 93–110
9. Plaschka C, Newman AJ, Nagai K. Structural basis of nuclear pre-mRNA splicing: lessons from yeast. *Cold Spring Harb Perspect Biol* 2019, 11: a032391
10. Yan C, Wan R, Shi Y. Molecular mechanisms of pre-mRNA splicing through structural biology of the spliceosome. *Cold Spring Harb Perspect Biol* 2019, 11: a032409
11. Staley JP, Guthrie C. An RNA switch at the 5′ splice site requires ATP and the DEAD box protein prp28p. *Mol Cell* 1999, 3: 55–64
12. Laggerbauer B, Achsel T, Lührmann R. The human U5-200kD DEXH-box protein unwinds U4/U6 RNA duplices *in vitro*. *Proc Natl Acad Sci USA* 1998, 95: 4188–4192
13. Raghunathan PL, Guthrie C. RNA unwinding in U4/U6 snRNPs requires ATP hydrolysis and the DEIH-box splicing factor Brr2. *Curr Biol* 1998, 8: 847–855
14. Gruber AJ, Zavolan M. Alternative cleavage and polyadenylation in health and disease. *Nat Rev Genet* 2019, 20: 599–614
15. Elkon R, Ugalde AP, Agami R. Alternative cleavage and polyadenylation: extent, regulation and function. *Nat Rev Genet* 2013, 14: 496–506
16. Ogami K, Richard P, Chen Y, Hoque M, Li W, Moresco JJ, Yates JR, ***et al.*** An Mtr4/ZFC3H1 complex facilitates turnover of unstable nuclear RNAs to prevent their cytoplasmic transport and global translational repression. *Genes Dev* 2017, 31: 1257–1271
17. Casañal A, Kumar A, Hill CH, Easter AD, Emsley P, Degliesposti G, Gordiyenko Y, ***et al.*** Architecture of eukaryotic mRNA 3′-end processing machinery. *Science* 2017, 358: 1056–1059
18. Neve J, Patel R, Wang Z, Louey A, Furger AM. Cleavage and polyadenylation: ending the message expands gene regulation. *RNA Biol* 2017, 14: 865–890
19. Tang P, Yang Y, Li G, Huang L, Wen M, Ruan W, Guo X, ***et al.*** Alternative polyadenylation by sequential activation of distal and proximal PolyA sites. *Nat Struct Mol Biol* 2022, 29: 21–31
20. Tang P, Yang J, Chen Z, Du C, Yang Y, Zhao H, Huang L, ***et al.*** Nuclear retention coupled with sequential polyadenylation dictates post-transcriptional m6A modification in the nucleus. *Mol Cell* 2024, 84: 3758–3774.e10
21. Bhadra M, Howell P, Dutta S, Heintz C, Mair WB. Alternative splicing in aging and longevity. *Hum Genet* 2020, 139: 357–369
22. Hong D, Jeong S. 3′UTR diversity: expanding repertoire of RNA alterations in human mRNAs. *Molecules Cells* 2023, 46: 48–56
23. Tian B, Manley JL. Alternative cleavage and polyadenylation: the long and short of it. *Trends Biochem Sci* 2013, 38: 312–320
24. Mayr C. Evolution and biological roles of alternative 3′UTRs. *Trends Cell Biol* 2016, 26: 227–237
25. Di Giammartino DC, Nishida K, Manley JL. Mechanisms and consequences of alternative polyadenylation. *Mol Cell* 2011, 43: 853–866
26. Zhang Y, Liu L, Qiu Q, Zhou Q, Ding J, Lu Y, Liu P. Alternative polyadenylation: methods, mechanism, function, and role in cancer. *J Exp Clin Cancer Res* 2021, 40: 51
27. Dye MJ, Proudfoot NJ. Multiple transcript cleavage precedes polymerase release in termination by RNA polymerase II. *Cell* 2001, 105: 669–681
28. Niwa M, Berget SM. Mutation of the AAUAAA polyadenylation signal depresses *in vitro* splicing of proximal but not distal introns. *Genes Dev* 1991, 5: 2086–2095
29. Kubo T, Wada T, Yamaguchi Y, Shimizu A, Handa H. Knock-down of 25 kDa subunit of cleavage factor im in Hela cells alters alternative polyadenylation within 3′-UTRs. *Nucleic Acids Res* 2006, 34: 6264–6271
30. Zhu Y, Wang X, Forouzmand E, Jeong J, Qiao F, Sowd GA, Engelman AN, ***et al.*** Molecular mechanisms for CFIm-Mediated regulation of mRNA alternative polyadenylation. *Mol Cell* 2018, 69: 62–74.e4
31. Movassat M, Crabb TL, Busch A, Yao C, Reynolds DJ, Shi Y, Hertel KJ. Coupling between alternative polyadenylation and alternative splicing is limited to terminal introns. *RNA Biol* 2016, 13: 646–655
32. Kühn U, Gündel M, Knoth A, Kerwitz Y, Rüdel S, Wahle E. Poly(A) tail length is controlled by the nuclear poly(A)-binding protein regulating the interaction between poly(A) polymerase and the cleavage and polyadenylation specificity factor. *J Biol Chem* 2009, 284: 22803–22814
33. De Klerk E, Venema A, Anvar SY, Goeman JJ, Hu OH, Trollet C, Dickson G, ***et al.*** Poly(A) binding protein nuclear 1 levels affect alternative polyadenylation. *Nucleic Acids Res* 2012, 40: 9089–9101
34. Huang L, Li G, Du C, Jia Y, Yang J, Fan W, Xu Y-, ***et al.*** The polyA tail facilitates splicing of last introns with weak 3′ splice sites via PABPN1. *EMBO Rep* 2023, 24: e57128
35. Evsyukova I, Bradrick SS, Gregory SG, Garcia-Blanco MA. Cleavage and polyadenylation specificity factor 1 (CPSF1) regulates alternative splicing of interleukin 7 receptor (IL7R) exon 6. *RNA* 2013, 19: 103–115
36. Van Etten JL, Nyquist M, Li Y, Yang R, Ho Y, Johnson R, Ondigi O, ***et al.*** Targeting a single alternative polyadenylation site coordinately blocks expression of androgen receptor mRNA splice variants in prostate cancer. *Cancer Res* 2017, 77: 5228–5235
37. Sakai A, Ando M, Fukusumi T, Ren S, Liu C, Qualliotine J, Haft S, ***et al.*** Aberrant expression of CPSF1 promotes head and neck squamous cell carcinoma via regulating alternative splicing. *PLoS ONE* 2020, 15: e0233380
38. Chen S, Zhu Z, Yang X, Liu L, He Y, Yang M, Guan X, ***et al.*** Cleavage and polyadenylation specific factor 1 promotes tumor progression via alternative polyadenylation and splicing in hepatocellular carcinoma. *Front Cell Dev Biol* 2021, 9: 616835
39. Kyburz A, Friedlein A, Langen H, Keller W. Direct Interactions between Subunits of CPSF and the U2 snRNP contribute to the coupling of pre-mRNA 3′ end processing and splicing. *Mol Cell* 2006, 23: 195–205
40. Li Y, Chen ZY, Wang W, Baker CC, Krug RM. The 3′-end-processing factor CPSF is required for the splicing of single-intron pre-mRNAs *in vivo*. *RNA* 2001, 7: 920–931
41. Vagner S, Vagner C, Mattaj IW. The carboxyl terminus of vertebrate poly (A) polymerase interacts with U2AF 65 to couple 3′-end processing and splicing. *Genes Dev* 2000, 14: 403–413
42. Rigo F, Martinson HG. Functional coupling of last-intron splicing and 3′-end processing to transcription *in vitro*: the poly(A) signal couples to splicing before committing to cleavage. *Mol Cell Biol* 2008, 28: 849–862
43. Martinson HG. An active role for splicing in 3′-end formation. *Wiley Interdiscip Rev RNA* 2011, 2: 459–470
44. Bird G, Zorio DAR, Bentley DL. RNA polymerase II carboxy-terminal domain phosphorylation is required for cotranscriptional pre-mRNA splicing and 3′-end formation. *Mol Cell Biol* 2004, 24: 8963–8969
45. Misra A, Ou J, Zhu LJ, Green MR. Global promotion of alternative internal exon usage by mRNA 3′ end formation factors. *Mol Cell* 2015, 58: 819–831

46. Zhang Z, Bae B, Cuddleston WH, Miura P. Coordination of alternative splicing and alternative polyadenylation revealed by targeted long read sequencing. *Nat Commun* 2023, 14: 5506
47. Zhang Z, So K, Peterson R, Bauer M, Ng H, Zhang Y, Kim JH, ***et al.*** Elav-mediated exon skipping and alternative polyadenylation of the dscam1 gene are required for axon outgrowth. *Cell Rep* 2019, 27: 3808–3817.e7
48. Kiltschewskij DJ, Harrison PF, Fitzsimmons C, Beilharz TH, Cairns MJ. Extension of mRNA poly(A) tails and 3′UTRs during neuronal differentiation exhibits variable association with post-transcriptional dynamics. *Nucleic Acids Res* 2023, 51: 8181–8198
49. Tian B, Pan Z, Lee JY. Widespread mRNA polyadenylation events in introns indicate dynamic interplay between polyadenylation and splicing. *Genome Res* 2007, 17: 156–165
50. Vlasenok M, Margasyuk S, Pervouchine DD. Transcriptome sequencing suggests that pre-mRNA splicing counteracts widespread intronic cleavage and polyadenylation. *NAR Genomics Bioinf* 2023, 5: lqad051
51. Wilson GM, Sun Y, Sellers J, Lu H, Penkar N, Dillard G, Brewer G. Regulation of AUF1 expression via conserved alternatively spliced elements in the 3′ untranslated region. *Mol Cell Biol* 1999, 19: 4056–4064
52. Paolantoni C, Ricciardi S, De Paolis V, Okenwa C, Catalanotto C, Ciotti MT, Cattaneo A, ***et al.*** Arc 3′ UTR splicing leads to dual and antagonistic effects in fine-tuning arc expression upon BDNF signaling. *Front Mol Neurosci* 2018, 11: 145
53. McGlincy NJ, Tan LY, Paul N, Zavolan M, Lilley KS, Smith CW. Expression proteomics of UPF1 knockdown in HeLa cells reveals autoregulation of hnRNP A2/B1 mediated by alternative splicing resulting in nonsense-mediated mRNA decay. *BMC Genomics* 2010, 11: 565
54. Chan JJ, Zhang B, Chew XH, Salhi A, Kwok ZH, Lim CY, Desi N, ***et al.*** Pan-cancer pervasive upregulation of 3′ UTR splicing drives tumourigenesis. *Nat Cell Biol* 2022, 24: 928–939
55. Kaida D, Berg MG, Younis I, Kasim M, Singh LN, Wan L, Dreyfuss G. U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation. *Nature* 2010, 468: 664–668
56. Berg MG, Singh LN, Younis I, Liu Q, Pinto AM, Kaida D, Zhang Z, ***et al.*** U1 snRNP determines mRNA length and regulates isoform expression. *Cell* 2012, 150: 53–64
57. Hu Z, Li M, Huo Z, Chen L, Liu S, Deng K, Lu X, ***et al.*** U1 snRNP proteins promote proximal alternative polyadenylation sites by directly interacting with 3′ end processing core factors. *J Mol Cell Biol* 2022, 14: mjac054
58. Shi J, Deng Y, Huang S, Huang C, Wang J, Xiang AP, Yao C. Suboptimal RNA–RNA interaction limits U1 snRNP inhibition of canonical mRNA 3′ processing. *RNA Biol* 2019, 16: 1448–1460
59. Deng Y, Shi J, Ran Y, Xiang AP, Yao C. A potential mechanism underlying U1 snRNP inhibition of the cleavage step of mRNA 3′ processing. *Biochem Biophys Res Commun* 2020, 530: 196–202
60. Feng Q, Zhao D, Lin Z, Li M, Xiang AP, Ye C, Yao C. U4 snRNP inhibits premature cleavage and polyadenylation of pre-mRNAs. *Proc Natl Acad Sci USA* 2024, 121: e2406710121
61. Millevoi S, Loulergue C, Dettwiler S, Karaa SZ, Keller W, Antoniou M, Vagner S. An interaction between U2AF 65 and CF Im links the splicing and 3′ end processing machineries. *EMBO J* 2006, 25: 4854–4864
62. Shen T, Li H, Song Y, Li L, Lin J, Wei G, Ni T. Alternative polyadenylation dependent function of splicing factor SRSF3 contributes to cellular senescence. *Aging* 2019, 11: 1356–1388
63. Schwich OD, Blümel N, Keller M, Wegener M, Setty ST, Brunstein ME, Poser I, ***et al.*** SRSF3 and SRSF7 modulate 3′UTR length through suppression or activation of proximal polyadenylation sites and regulation of CFIm levels. *Genome Biol* 2021, 22: 82
64. Carrasco J, Rauer M, Hummel B, Grzejda D, Alfonso-Gonzalez C, Lee Y, Wang Q, ***et al.*** ELAV and FNE determine neuronal transcript signatures through exon-activated rescue. *Mol Cell* 2020, 80: 156–163.e6
65. Wei L, Lee S, Majumdar S, Zhang B, Sanfilippo P, Joseph B, Miura P, ***et al.*** Overlapping activities of ELAV/Hu family RNA binding proteins specify the extended neuronal 3′ UTR landscape in drosophila. *Mol Cell* 2020, 80: 140–155.e6
66. Torres-Méndez A, Pop S, Bonnal S, Almudi I, Avola A, Roberts RJV, Paolantoni C, ***et al.*** Parallel evolution of a splicing program controlling neuronal excitability in flies and mammals. *Sci Adv* 2022, 8: eabk0445
67. Du H, Cline MS, Osborne RJ, Tuttle DL, Clark TA, Donohue JP, Hall MP, ***et al.*** Aberrant alternative splicing and extracellular matrix gene expression in mouse models of myotonic dystrophy. *Nat Struct Mol Biol* 2010, 17: 187–193
68. Batra R, Charizanis K, Manchanda M, Mohan A, Li M, Finn DJ, Goodwin M, ***et al.*** Loss of MBNL leads to disruption of developmentally regulated alternative polyadenylation in RNA-mediated disease. *Mol Cell* 2014, 56: 311–322
69. Singh R, Valcárcel J, Green MR. Distinct binding specificities and functions of higher eukaryotic polypyrimidine tract-binding proteins. *Science* 1995, 268: 1173–1176
70. Polydorides AD, Okano HJ, Yang YYL, Stefani G, Darnell RB. A brain-enriched polypyrimidine tract-binding protein antagonizes the ability of nova to regulate neuron-specific alternative splicing. *Proc Natl Acad Sci USA* 2000, 97: 6350–6355
71. Gruber AJ, Schmidt R, Ghosh S, Martin G, Gruber AR, van Nimwegen E, Zavolan M. Discovery of physiological and cancer-related regulators of 3′ UTR processing with KAPAC. *Genome Biol* 2018, 19: 44
72. Zarnack K, König J, Tajnik M, Martincorena I, Eustermann S, Stévant I, Reyes A, ***et al.*** Direct competition between hnRNP C and U2AF65 protects the transcriptome from the exonization of alu elements. *Cell* 2013, 152: 453–466
73. Tajnik M, Vigilante A, Braun S, Hänel H, Luscombe NM, Ule J, Zarnack K, ***et al.*** Intergenic *Alu* exonisation facilitates the evolution of tissue-specific transcript ends. *Nucleic Acids Res* 2015: gkv956
74. Gruber AJ, Schmidt R, Gruber AR, Martin G, Ghosh S, Belmadani M, Keller W, ***et al.*** A comprehensive analysis of 3′ end sequencing data sets reveals novel polyadenylation signals and the repressive role of heterogeneous ribonucleoprotein C on cleavage and polyadenylation. *Genome Res* 2016, 26: 1145–1159
75. Ji X, Park JW, Bahrami-Samani E, Lin L, Duncan-Lewis C, Pherribo G, Xing Y, ***et al.*** αCP binding to a cytosine-rich subset of polypyrimidine tracts drives a novel pathway of cassette exon splicing in the mammalian transcriptome. *Nucleic Acids Res* 2016, 44: 2283–2297
76. Ji X, Wan J, Vishnu M, Xing Y, Liebhaber SA. αCP Poly(C) binding proteins act as global regulators of alternative polyadenylation. *Mol Cell Biol* 2013, 33: 2560–2573
77. Cao J, Kuyumcu-Martinez MN. Alternative polyadenylation regulation in cardiac development and cardiovascular disease. *Cardiovasc Res* 2023, 119: 1324–1335
78. Derti A, Garrett-Engele P, MacIsaac KD, Stevens RC, Sriram S, Chen R, Rohl CA, ***et al.*** A quantitative atlas of polyadenylation in five mammals. *Genome Res* 2012, 22: 1173–1183
79. Elkon R, Drost J, van Haaften G, Jenal M, Schrier M, Oude Vrielink JA, Agami R. E2F mediates enhanced alternative polyadenylation in proliferation. *Genome Biol* 2012, 13: R59
80. Shepard PJ, Choi EA, Lu J, Flanagan LA, Hertel KJ, Shi Y. Complex and dynamic landscape of RNA polyadenylation revealed by PAS-Seq. *RNA* 2011, 17: 761–772
81. Mata J. Genome-wide mapping of polyadenylation sites in fission yeast reveals

widespread alternative polyadenylation. *RNA Biol* 2013, 10: 1407–1414
82. Rallapalli G, Kemen EM, Robert-Seilaniantz A, Segonzac C, Etherington GJ, Sohn KH, MacLean D, *et al.* EXPRSS: an illumina based high-throughput expression-profiling method to reveal transcriptional dynamics. *BMC Genomics* 2014, 15: 341
83. Fox-Walsh K, Davis-Turak J, Zhou Y, Li H, Fu XD. A multiplex RNA-seq strategy to profile poly(A+) RNA: application to analysis of transcription response and 3′ end formation. *Genomics* 2011, 98: 266–271
84. Fu Y, Sun Y, Li Y, Li J, Rao X, Chen C, Xu A. Differential genome-wide profiling of tandem 3′ UTRs among human breast cancer and normal cells by high-throughput sequencing. *Genome Res* 2011, 21: 741–747
85. Zhou X, Li R, Michal JJ, Wu XL, Liu Z, Zhao H, Xia Y, *et al.* Accurate profiling of gene expression and alternative polyadenylation with whole transcriptome termini site sequencing (WTTS-Seq). *Genetics* 2016, 203: 683–697
86. Jan CH, Friedman RC, Ruby JG, Bartel DP. Formation, regulation and evolution of caenorhabditis elegans 3′UTRs. *Nature* 2011, 469: 97–101
87. Hoque M, Ji Z, Zheng D, Luo W, Li W, You B, Park JY, *et al.* Analysis of alternative cleavage and polyadenylation by 3′ region extraction and deep sequencing. *Nat Methods* 2013, 10: 133–139
88. Harrison PF, Powell DR, Clancy JL, Preiss T, Boag PR, Traven A, Seemann T, *et al.* PAT-seq: a method to study the integration of 3′-UTR dynamics with gene expression in the eukaryotic transcriptome. *RNA* 2015, 21: 1502–1510
89. Oikonomopoulos S, Bayega A, Fahiminiya S, Djambazian H, Berube P, Ragoussis J. Methodologies for transcript profiling using long-read technologies. *Front Genet* 2020, 11: 606
90. Herzel L, Straube K, Neugebauer KM. Long-read sequencing of nascent RNA reveals coupling among RNA processing events. *Genome Res* 2018, 28: 1008–1019
91. Choquet K, Baxter-Koenigs AR, Dülk SL, Smalec BM, Rouskin S, Churchman LS. Pre-mRNA splicing order is predetermined and maintains splicing fidelity across multi-intronic transcripts. *Nat Struct Mol Biol* 2023, 30: 1064–1076