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Review article

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The regulation and function of post-transcriptional RNA splicing

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Abstract

Eukaryotic RNA transcripts undergo extensive processing before becoming functional messenger RNAs, with splicing being a critical and highly regulated step that occurs both co-transcriptionally and post-transcriptionally. Recent analyses have revealed, with unprecedented spatial and temporal resolution, that up to 40% of mammalian introns are retained after transcription termination and are subsequently removed largely while transcripts remain chromatin-associated. Post-transcriptional splicing has emerged as a key layer of gene expression regulation during development, stress response and disease progression. The control of post-transcriptional splicing regulates protein production through delayed splicing and nuclear export, or nuclear retention and degradation of specific transcript isoforms. Here, we review current methodologies for detecting post-transcriptional splicing, discuss the mechanisms controlling the timing of splicing and examine how this temporal regulation affects gene expression programmes in healthy cells and in disease states.

Sections

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Introduction

The discovery of split genes in 1977 (refs. 1,2) radically changed our understanding of eukaryotic gene expression. Unlike their prokaryotic counterparts, eukaryotic genes exhibit an architecture in which protein-coding exons are interrupted by non-coding introns. This gene organization requires a sophisticated processing mechanism to remove introns and generate functional mRNA. This splicing process is mediated by the spliceosome, a dynamic ribonucleoprotein (RNP) complex, which consists of five distinct small nuclear RNPs (snRNPs U1, U2, U4, U5 and U6) composed of one small nuclear RNA (snRNA) and multiple proteins. Each snRNP binds the pre-mRNA in a sequential manner: first, U1 recognizes the 5′ splice site, then U2 binds the branch point sequence, followed by recruitment of the tri-snRNP (U4/U6.U5). After several conformational changes, the spliceosome catalyses two successive transesterification reactions to remove the intron and join the exons (Fig. 1, reviewed in ref. 3).

Soon after the discovery of splicing, gene-specific studies showed that splicing can occur either during transcription (co-transcriptionally) or after transcription is completed (post-transcriptionally)[4–11]. Moreover, the timing of splicing was shown to have a substantial impact on alternative splicing, a process in which different combinations of exons and/or introns are retained in the final mRNA[12]. Alternative splicing occurs in >95% of multi-intron human genes and enables extensive diversification of the proteome and regulation of mRNA and protein levels[13–16]. Nevertheless, the relative contribution to splicing regulation and the biological impact of co-transcriptional and post-transcriptional splicing have remained unclear.

In recent years, technological advances such as long-read sequencing have substantially improved our understanding of the timing of splicing relative to transcription[17–21]. Simultaneously, mounting evidence has highlighted post-transcriptional splicing as a contributor to critical regulatory mechanisms in development, stress response and disease[22–24].

This Review describes our current understanding of post-transcriptional splicing in metazoans, the methodological approaches available for its study, how it differs from co-transcriptional splicing as well as its regulation and biological functions. For a comprehensive discussion of co-transcriptional splicing function and regulation, we refer readers to other excellent recent reviews[16,25].

Detection of post-transcriptional splicing

The question of where and when splicing occurs has been key in the field since its inception. The development of high-throughput RNA sequencing (RNA-seq) techniques along with modern microscopy-based approaches have expanded our understanding of the temporal and spatial aspects of splicing. In this section, we discuss recent advances in molecular techniques to study splicing timing, their advantages and limitations.

Biochemical and sequencing approaches

In pioneering work, Baurén and Wieslander[8] isolated RNA from microdissected polytene chromosomes and from the nucleoplasm in the dipteran *Chironomus tentans*. This first physical isolation of chromatin-associated and nucleoplasmic RNAs showed that some introns are excised on chromatin, whereas others are removed in the nucleoplasm, that polyadenylation occurs before removal of the last intron in most pre-mRNAs and that excision efficiency varies across introns[8,10,11]. Soon after, Wuarin and Schibler[26] demonstrated for the first time that mammalian nascent RNA synthesized by RNA polymerase II (Pol II) can be isolated through fractionation of nuclei into a chromatin pellet containing DNA, histones, Pol II and nascent RNA. Comparison of this chromatin-associated fraction to nucleoplasmic RNA revealed that both co-transcriptional and post-transcriptional splicing occurs in selected mammalian genes[26,27]. This approach was then extended to the entire transcriptome by combining subcellular fractionation and short-read or long-read RNA-seq (subcellular RNA-seq) to separately analyse cytoplasmic, nucleoplasmic and chromatin-associated RNA populations in yeast, *Drosophila* S2 cells and fly heads, mouse liver and human cells[17,28–33] (Fig. 2a). These high-throughput sequencing studies provided the first transcriptome-wide measurements of co-transcriptional and post-transcriptional splicing, estimating the proportion of post-transcriptional splicing to range between 30% and 45% of introns in mammalian cells (Table 1). One of the main caveats of these studies is that they quantify the steady-state proportion of spliced and unspliced transcripts and assume that introns within chromatin-associated transcripts would have been subsequently excised. Although these transcripts can also be degraded[34], most human introns in protein-coding genes are successfully spliced independently from their splicing timing[35] (see the section 'Fate of partially spliced chromatin-associated transcripts'). Furthermore, many studies have assumed that all chromatin-associated RNAs are engaged with an elongating Pol II, which has been challenged by the separate analysis of co-transcriptional and post-transcriptional RNA populations through poly(A) selection of chromatin-associated RNA (Table 1). Numerous approaches have been developed to measure the absolute timing of splicing, but these methods typically do not distinguish between co-transcriptional or post-transcriptional splicing (reviewed elsewhere[36]). Overall, subcellular RNA-seq studies remain the gold standard to analyse splicing timing relative to transcription and have revealed key insights about the extent of post-transcriptional splicing and its regulatory mechanisms.

Microscopy techniques

In contrast to subcellular sequencing, microscopy approaches visualize an individual locus as its RNA is synthesized, spliced and released from the transcription site, providing both temporal and spatial information (Fig. 2b). Most analyses of splicing timing using microscopy have relied on reporter genes that are visible through their association with the bacteriophage MS2 and/or PP7 coat proteins fused with a fluorescent tag[37–39]. Other studies have used single-molecule fluorescent in situ hybridization (smFISH) of reporter or endogenous genes[40,41]. These studies confirmed that both co-transcriptional and post-transcriptional splicing occurs in mammalian cells. For instance, the excision of an intron within a β-globin reporter gene was shown to occur 10-fold faster and much more frequently (87%) post-release from the transcription site[37]. In another study, analysis of 10 endogenous human introns via bacteriophage hairpins showed that the probability of post-transcriptional excision is greater than co-transcriptional removal for 6 of them[42]. Consistently, for introns within four endogenous pre-mRNAs analysed by smFISH and expansion microscopy, at least one intron per gene can be excised post-transcriptionally[41]. A notable limitation of microscopy-based analyses lies in their focus on a subset of introns, predominantly within reporter gene constructs, which may not accurately represent the broader landscape of endogenous intronic behaviour. Furthermore, hairpin insertions potentially perturb native splicing kinetics, which could lead to systematic overestimation of post-transcriptional splicing events. By contrast, in some studies, splicing is considered post-transcriptional only when

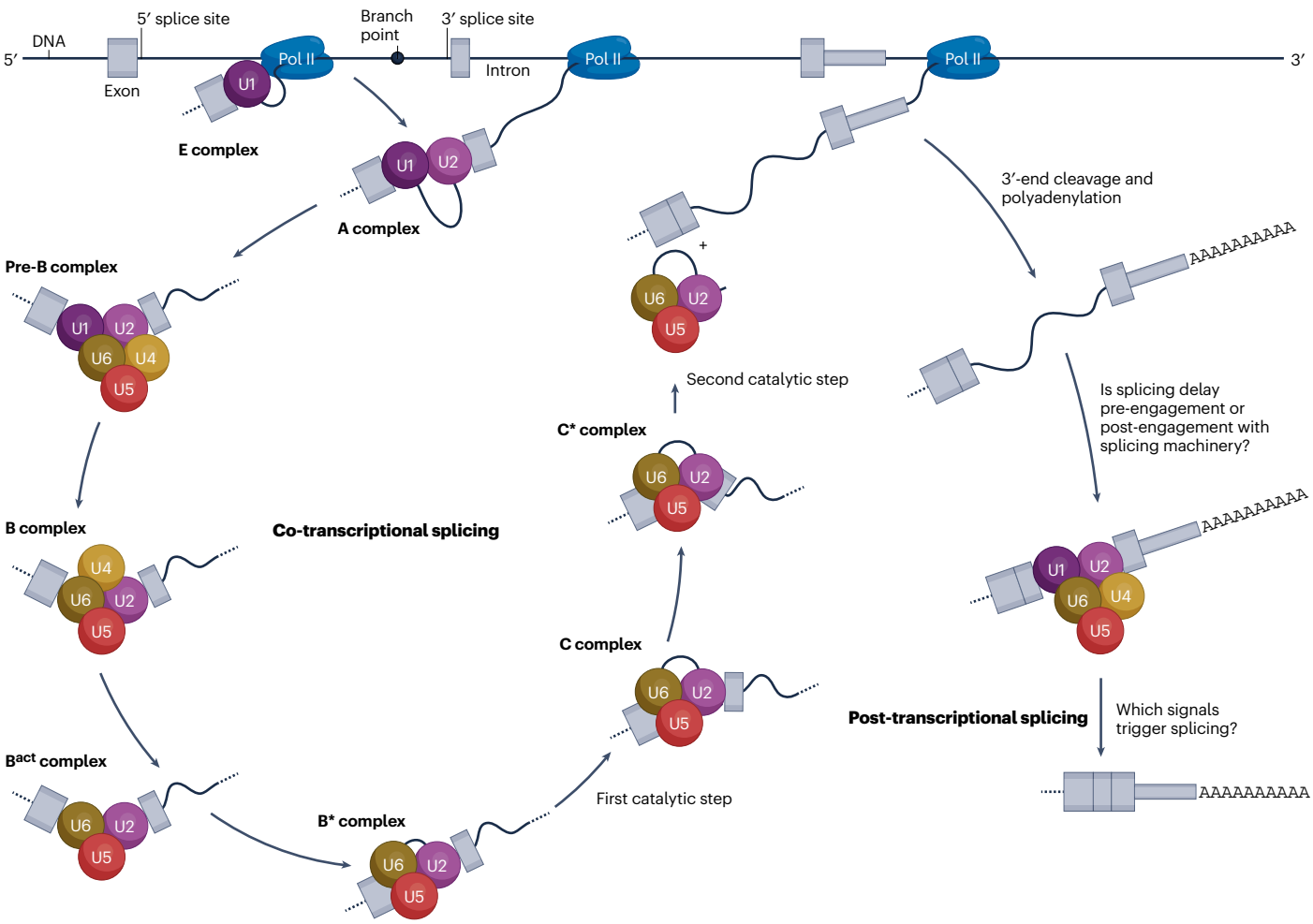


Fig. 1 | Overview of co-transcriptional and post-transcriptional splicing. Spliceosome assembly occurs through a multistep process (complexes E to B^{act}) characterized by protein exchanges and conformational changes, followed by two catalytic steps that are accomplished by catalytically activated complexes B* and C* (ref. 157). Coloured circles labelled U1 to U6 represent small nuclear ribonucleoproteins (snRNPs). Co-transcriptional splicing (left) begins with co-transcriptional deposition of U1 snRNP on the 5′ splice site. U1 snRNP remains in contact with RNA polymerase II (Pol II) as it continues to transcribe further downstream, forming a growing intron loop[158], followed by spliceosome assembly and splicing. Post-transcriptional splicing is preceded by 3′-end cleavage and polyadenylation (right). The individual steps of spliceosome assembly are presumed to be the same for both co-transcriptional and post-transcriptional splicing, but are only shown once for simplicity. It is unclear whether the delay associated with post-transcriptional splicing occurs before spliceosome assembly or during one of the earlier assembly steps. The signals that trigger the eventual splicing reaction are also unknown. Exons are shown as rectangles and introns are displayed as lines.

it occurs away from the transcription site[37,40,42], which thereby fails to capture chromatin-associated post-transcriptional splicing and potentially underestimates overall post-transcriptional splicing. Despite these technical constraints and the variability in measured rates of post-transcriptional splicing, studies have convincingly established the prevalence of post-transcriptional splicing in mammalian cells.

Extent and localization of post-transcriptional splicing

Traditionally, chromatin-associated RNAs have been viewed as molecules undergoing active synthesis. However, multiple lines of evidence now demonstrate that mature, polyadenylated transcripts can remain on chromatin long after transcription has finished. In this section, we examine the evidence for post-transcriptional chromatin retention of polyadenylated transcripts, delve into the molecular mechanisms and regulatory logic governing the fate of partially spliced retained transcripts and explore potential functional roles of this retention in gene regulation.

Post-transcriptional retention of pre-mRNA on chromatin

The traditional view that protein-coding transcripts are released into the nucleoplasm immediately after 3′-end cleavage and polyadenylation (CPA) has been challenged by recent evidence. Both microscopy-based and sequencing-based studies have revealed that transcripts can remain associated with chromatin for extended periods. For instance, in mouse embryonic stem cells (mESCs), the abundance

of polyadenylated RNAs from the average protein-coding gene on chromatin matches that found in the cytoplasm[31]. This retention pattern has been observed across different systems, from β-globin minigenes showing accumulation of polyadenylated RNAs near transcription sites[38] to activated mouse macrophages retaining partially spliced transcripts on chromatin[32].

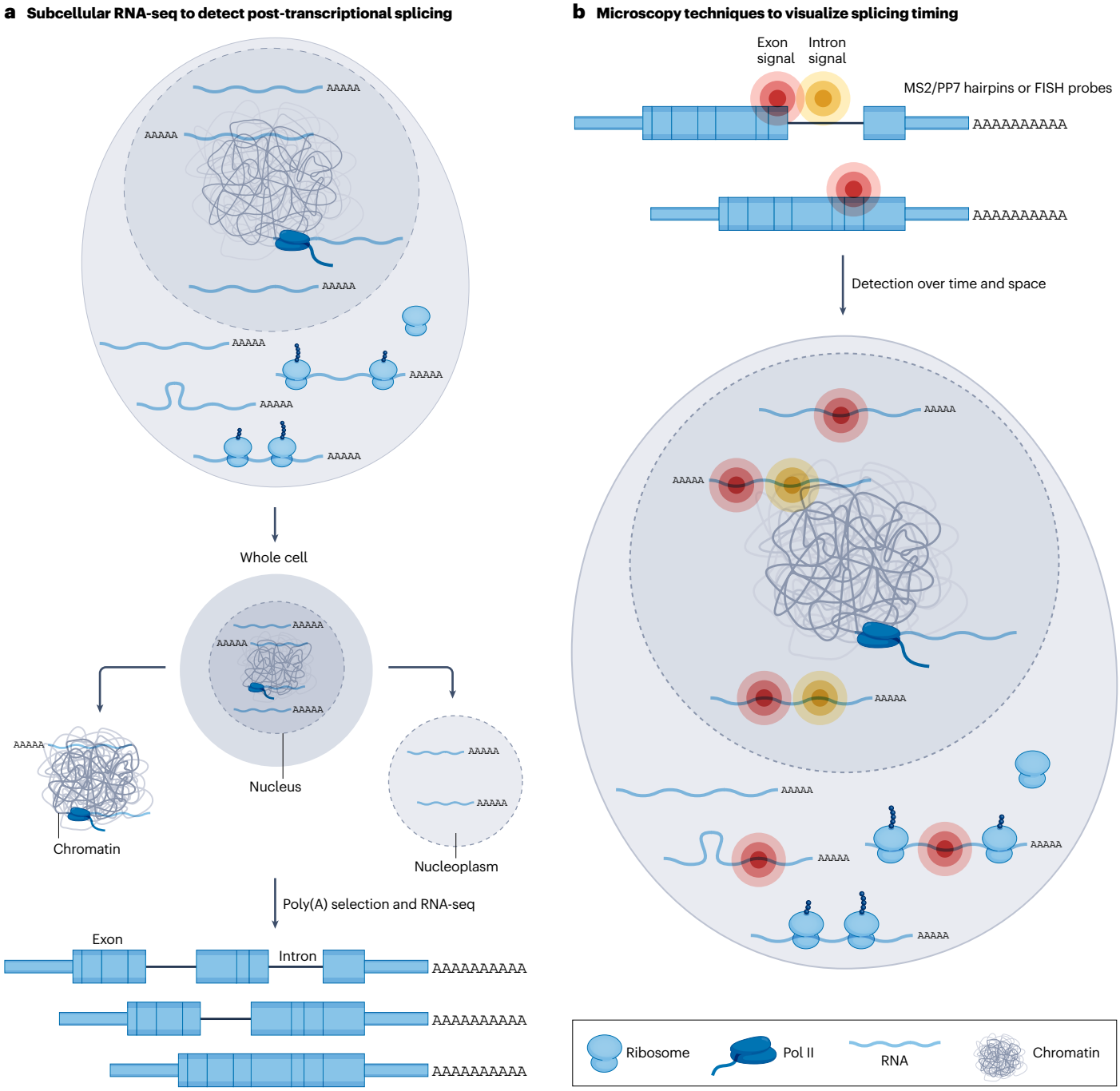


Fig. 2 | Experimental approaches to study post-transcriptional splicing. **a**, Subcellular RNA sequencing (RNA-seq) consists of biochemical cellular fractionation to separate cytoplasmic, nucleoplasmic and chromatin-associated RNAs. Individual fractions are then analysed by high-throughput RNA-seq. The addition of a poly(A) selection step enables specific analysis of post-transcriptional intron retention and splicing. **b**, Microscopy using reporter genes with hairpins recognized by the MS2/PP7 coat proteins or with single-molecule fluorescent in situ hybridization (smFISH) probes enables the detection of splicing in space and time. Probes or hairpin sequences in introns (yellow colour) appear when the intron is synthesized and disappear after splicing, whereas probes or hairpin sequences in exons (red colour) remain present throughout the RNA lifetime. The intronic signal is typically observed only in the nucleus, whereas the exonic signal is present in the nucleus and the cytoplasm.

Table 1 | Transcriptome-wide detection and extent of post-transcriptional intron retention and splicing

Species or cell type	Experimental approach	Calculation	Proportion of post-transcriptional splicing or intron retention	Ref.
Human K562 cells	Cellular fractionation + NGS of pA^+ and pA^- nuclear and cytoplasmic fractions, and total chromatin fraction	coSI = EE/(EE + EI/2) (0: fully unspliced, 1: fully spliced)	27% of introns are PTR (coSI ≤ 0.75 in pA^+ nuclear RNA and coSI ≥ 0.95 in pA^+ cytoplasmic RNA)	30
Human K562 cells	Cellular fractionation + direct nanopore RNA sequencing of pA^+ chromatin	Fraction PTR per intron = unspliced reads/(spliced + unspliced reads)	38% of introns have >10% retention on chromatin, >75% genes with at least 1 PTR intron	17
HeLa cells	Detection of spliceosome hyper-phosphorylation by western blot or immunofluorescence	NA	≤20% of spliceosomes involved in post-transcriptional splicing	155
Human cell lines (Hf1, HCT116, HeLa, K562, MiaPaCa, Panc1)	30 min BrU labelling + pulldown of labelled transcripts + NGS	SI = EE/(EE + EI/2) (0: fully unspliced, 1: fully spliced)	<50% of introns excised within 30 min of labelling	156
Mouse embryonic stem cells	Cellular fractionation + NGS of A^+ and total RNA in chromatin, nucleoplasmic and cytoplasmic fractions	Fraction of intron inclusion = EI/2/(EI/2 + EE) (0: fully spliced, 1: fully unspliced)	30% of introns (inclusion levels on chromatin ≥ 0.1), 70% genes with ≥1 PTR intron	31
Mouse liver	Cellular fractionation + NGS of pA^- RNA from chromatin fraction	Ratio of intronic to exonic reads adjacent to 3′ splice site (0: fully spliced, 1: fully unspliced)	Median = 0.55, no genes with all introns with ratio ≤ 0.1	29
Drosophila S2 cells and *Drosophila* heads	Cellular fractionation + NGS of pA^- RNA from chromatin fraction	Intron retention ratio = intron signal/exon signal for entire gene (with length normalization)	13% of introns with >50% retention on chromatin 42.6% of genes with ≥1 intron with >50% retention on chromatin	28
		Ratio of intronic to exonic reads adjacent to 3′ splice site (0: fully spliced, 1: fully unspliced)	Median = 0.25	29
Arabidopsis thaliana	Cellular fractionation + cDNA nanopore sequencing of chromatin RNA	Proportion of reads with introns all spliced, partially spliced or all unspliced, detection of pA tail	30% of chromatin-associated pA^+ transcripts are partially spliced	55

BrU, bromouracil; coSI, completed splicing index; EE, exon–exon junction reads; EI, exon–intron and intron–exon junction reads; NA, not available; NGS, next-generation sequencing; pA, poly(A); PTR, post-transcriptionally retained; SI, splicing index.

The temporal dynamics of this retention have been precisely measured using subcellular TimeLapse-seq, a technique that combines metabolic labelling with subcellular fractionation, RNA-seq and mathematical modelling to measure the kinetics of RNA life cycles[43]. In human K562 cells, mRNAs show a median chromatin half-life of 50 min, with the transcripts of some genes remaining chromatin-associated for 170 min or longer. Notably, these retention times often exceed the duration required for transcription itself, indicating that these transcripts remain chromatin-bound well after their synthesis is complete. Furthermore, comparison of chromatin and nuclear half-lives showed that transcripts spend most of their nuclear life on chromatin rather than freely diffusing in the nucleoplasm[43]. It is worth noting that metabolic labelling with high concentrations of nucleotide analogues for extended time periods can affect ribosomal RNA production[44], gene expression or splicing[45]. However, metabolic labelling at low concentrations for short time periods, such as those used in the above study (50–500 µM for up to 2 h in NIH-3T3 and K562 cells, respectively), minimally perturbs gene expression, subcellular mRNA localization and splicing[18,36,43,45] and thus should not negatively affect study results.

Full-length pre-mRNAs analysed by smFISH and expansion microscopy were found to move more slowly in a 'proximal zone' near the transcription site before diffusing into the nucleoplasm or near nuclear speckles[41]. High-resolution microscopy showed that RNAs in this proximal zone are no longer associated with the transcription site. Therefore, splicing in this zone happens post-transcriptionally, despite the biochemical association of the RNA with chromatin[41]. Together, these observations establish that chromatin retention of protein-coding transcripts post-synthesis is a common feature of gene expression regulation.

Recent research has revealed an important aspect of nuclear organization that influences how we interpret these findings. Nuclear speckles maintain stable physical associations with chromatin[43,46–48] (Box 1), which seems to be functionally important for efficient splicing[49,50]. However, it also means that biochemical fractionation techniques collect both directly chromatin-bound RNAs and nuclear speckle-associated RNAs. Although this makes precise quantification of chromatin-associated versus nuclear speckles-associated transcripts challenging, the evidence for functional coupling between nuclear speckles and chromatin suggests that these associations reflect genuine biological organization rather than technical limitations. In sum, multiple reports have established that post-transcriptionally retained introns maintain associations with either chromatin directly (Table 1) or chromatin-associated speckles[17,28–31,48,51,52] (Box 1).

Fate of partially spliced chromatin-associated transcripts

Polyadenylated chromatin-associated transcripts with retained introns face two potential fates: either they undergo successful splicing to produce mature mRNAs[17,53] or they are targeted for degradation[34].

Although the precise quantitative balance between splicing and nuclear decay requires further assessment, multiple lines of evidence indicate that productive splicing is a main outcome for post-transcriptionally retained introns. Degradation of intron-retaining transcripts occurs primarily through nuclear degradation mechanisms rather than cytoplasmic nonsense-mediated decay (NMD), as demonstrated by unchanged retention levels when NMD factors are depleted[31,54–56]. When nuclear degradation of intron-containing transcripts does occur, it happens through mechanisms involving the nuclear exosome and its cofactors (reviewed elsewhere[57,58]). The pathway involving the poly(A) tail exosome targeting connection, which requires the nuclear poly(A) binding protein (PABPN1) and RNA hyperadenylation[59–62], seems to be predominant, but distinct pathways involving the nuclear pore protein TPR or the TRAMP complex have also been described[56].

Subcellular TimeLapse-seq revealed that only about 4% of expressed protein-coding genes produce transcripts that are predicted to undergo nuclear degradation (PUND)[43]. Interestingly, PUND transcripts have higher intron retention levels and longer poly(A) tails on chromatin than non-PUND transcripts, indicating that they may be degraded through the poly(A) tail exosome targeting pathway. Consistently, PUND transcripts, particularly those containing retained introns, are stabilized upon knockdown of the nuclear exosome catalytic subunit EXOSC10. Nevertheless, PUNDs represent a small proportion of all expressed protein-coding genes[43].

Most retained introns are eventually excised to produce functional mRNAs, as demonstrated through multiple complementary approaches. Mathematical modelling combined with transient transcriptome sequencing showed that most introns, even long-lived ones, are successfully spliced[35]. Studies of inflammatory response genes provide further support — as levels of pre-mRNA containing long-lived introns decrease, there is a corresponding rise in mature mRNA levels, indicating successful processing rather than decay[53,63]. The emerging picture reveals post-transcriptional splicing as primarily a regulatory mechanism to control gene expression timing, rather than a quality control checkpoint leading to RNA decay. The ability to hold transcripts in a partially processed state, followed by regulated splicing, provides cells with an additional layer of control over when proteins are produced. A key outstanding question is what regulatory features distinguish partially spliced chromatin-associated transcripts that are destined for productive splicing from those that are fated for degradation.

Box 1 | Subnuclear organization and post-transcriptional splicing

Nuclear organization is not homogeneous or static. There are multiple subnuclear membrane-less structures with dedicated functions such as nucleoli, Cajal bodies, speckles and paraspeckles (reviewed elsewhere[159–161]). Moreover, the spatial genome organization and how loci organize within subnuclear structures affect gene expression and splicing[52,162–165].

Nuclear speckles are biomolecular condensates in the nucleus that are densely packed with post-transcriptional RNA and RNA-processing factors[166–171]. The functional role of nuclear speckles — whether they serve primarily as storage bodies for splicing factors or as sites where active splicing occurs — remains an active area of investigation in the field[166,172]. Nevertheless, active spliceosomes have been observed to colocalize with nuclear speckles and accumulate when splicing catalysis is inhibited through the knockdown of spliceosomal protein CDC5L[155]. Upon re-expression of CDC5L, these active spliceosomes complete splicing[155], suggesting that post-transcriptional splicing can occur in nuclear speckles. Interestingly, splicing factors required for splice site recognition and activation display enhanced recruitment to nuclear speckles in response to stress, which coincides with increased post-transcriptional splicing efficiency of immediate early genes dependent on p38 mitogen-activated protein kinase[173]. This finding indicates that nuclear speckles are actively remodelled in response to stress at the levels of protein and RNA composition.

Spatial genome organization and transcript localization within the nucleus influence RNA processing and are associated with specific intron features. Genes with short GC-rich introns tend to be in the nuclear centre, and the corresponding transcripts are associated with nuclear speckles. Genes with longer introns and lower GC content concentrate at the nuclear periphery and their transcripts are associated with the nuclear lamina[52,162], a dense fibrillar network of intermediate filaments and associated proteins that lines the inner nuclear membrane.

Although nuclear speckle-associated and lamina-associated transcripts exhibit distinctive features, they both have the highest levels of intron retention compared with other subnuclear domains[52]. Lamina-associated retained introns are enriched in genes involved in microtubule and chromosome organization and non-coding RNA processing, whereas nuclear speckles-associated retained introns are found in genes controlling RNA processing, translation and cell cycle progression[52]. Moreover, about 31% of nuclear speckles-associated retained introns overlap with previously annotated detained introns, whereas lamina-associated retained introns show only 12% overlap[52]. Given the enrichment of introns with detained intron characteristics in nuclear speckles, these structures likely serve as hubs for post-transcriptional splicing regulation, whereas lamina-associated introns may represent a distinct regulatory class.

In addition, multiple lines of evidence have shown a connection between nuclear speckles and both co-transcriptional and post-transcriptional splicing efficiency. On the one hand, proximity of gene loci to nuclear speckles is associated with increased co-transcriptional splicing efficiency of the corresponding pre-mRNAs[49,50]. On the other hand, the transcriptome of nuclear speckles is enriched for transcripts with post-transcriptionally excised introns[51,52,162] and the corresponding gene loci were found to be more proximal to nuclear speckles than loci encoding speckle-depleted transcripts[51]. Disrupting nuclear speckle formation leads to the retention of introns in the nuclear speckle transcriptome, but not in other transcripts, suggesting that nuclear speckles directly contribute to post-transcriptional splicing efficiency[51]. However, although these findings establish nuclear speckles as critical hubs for post-transcriptional splicing regulation, the molecular mechanisms that coordinate transcript localization with splicing timing remain to be elucidated. Understanding these mechanisms will be essential for deciphering how nuclear organization contributes to gene regulation through post-transcriptional splicing.

Many transcripts undergo post-transcriptional splicing

To delineate the scope of post-transcriptional splicing, the levels of introns within the fraction of polyadenylated chromatin-associated RNA have been analysed in various biological systems by RNA-seq (Table 1). Most mammalian introns (~70%) show high levels of excision on chromatin-associated RNA[28–31]. However, 27–40% of mammalian introns are present at high levels in polyadenylated, chromatin-associated RNA[17,28–31]. Most expressed genes (>70%) contain at least one post-transcriptionally retained intron[17,31]. By contrast, nucleoplasmic RNA contains fewer introns[10,11,31,32,64], and transcripts transit rapidly through this compartment towards the cytoplasm[43], suggesting that most post-transcriptional splicing occurs on chromatin (Fig. 3).

Comparisons between species revealed a higher level of splicing completion on chromatin in *Drosophila* cells than in mouse liver (Table 1). Although some *Drosophila* genes have all introns ≥90% excised co-transcriptionally on chromatin, there were no mouse genes in which all introns reached this level of co-transcriptional-splicing, suggesting that post-transcriptional splicing is more frequent in mammalian cells[29]. Furthermore, comparison of *Drosophila* S2 cells and fly heads showed similar co-transcriptional and post-transcriptional splicing proportions, but distinct populations of introns were retained post-transcriptionally, demonstrating that post-transcriptional splicing is context-specific[28]. In *Arabidopsis thaliana*, >40% of chromatin-associated RNA is polyadenylated, of which one third is partially spliced[55], indicating a similar proportion of post-transcriptional splicing in plants and mammals. Taken together, these findings indicate that over one-third of introns in diverse eukaryotic species persist post-transcriptionally, suggesting that they serve fundamental roles in gene regulation.

Splicing as a rate-limiting step for transcript release from chromatin

Several studies indicate that post-transcriptional splicing may be a rate-limiting step for transcript release from chromatin (Fig. 3). Polyadenylated reporter RNAs are retained on chromatin longer if they contain more introns[38], and chromatin release of these reporter RNAs is modulated by the levels and phosphorylation state of specific serine–arginine (SR) proteins[39]. These SR splicing factors are concentrated in nuclear speckles (Box 1). Upon nuclear speckle disassembly, levels of hyperphosphorylated SR proteins increase, which correlates with enhanced release of spliced mRNAs from their sites of transcription[39]. These data suggest a model in which the finite pool of splicing factors acts as a rate-limiting determinant, resulting in transcription site retention of intron-rich pre-mRNAs until splicing completion[39].

Studies of endogenous genes have reinforced the connection between splicing and chromatin release. A delay of up to tens of minutes occurs between transcript synthesis and splicing completion on chromatin for induced genes during macrophage activation[53]. Accumulation of transcripts in the nucleoplasm coincides with the removal of the slowest introns on chromatin, further indicating that splicing completion is the limiting factor for transcript release[53].

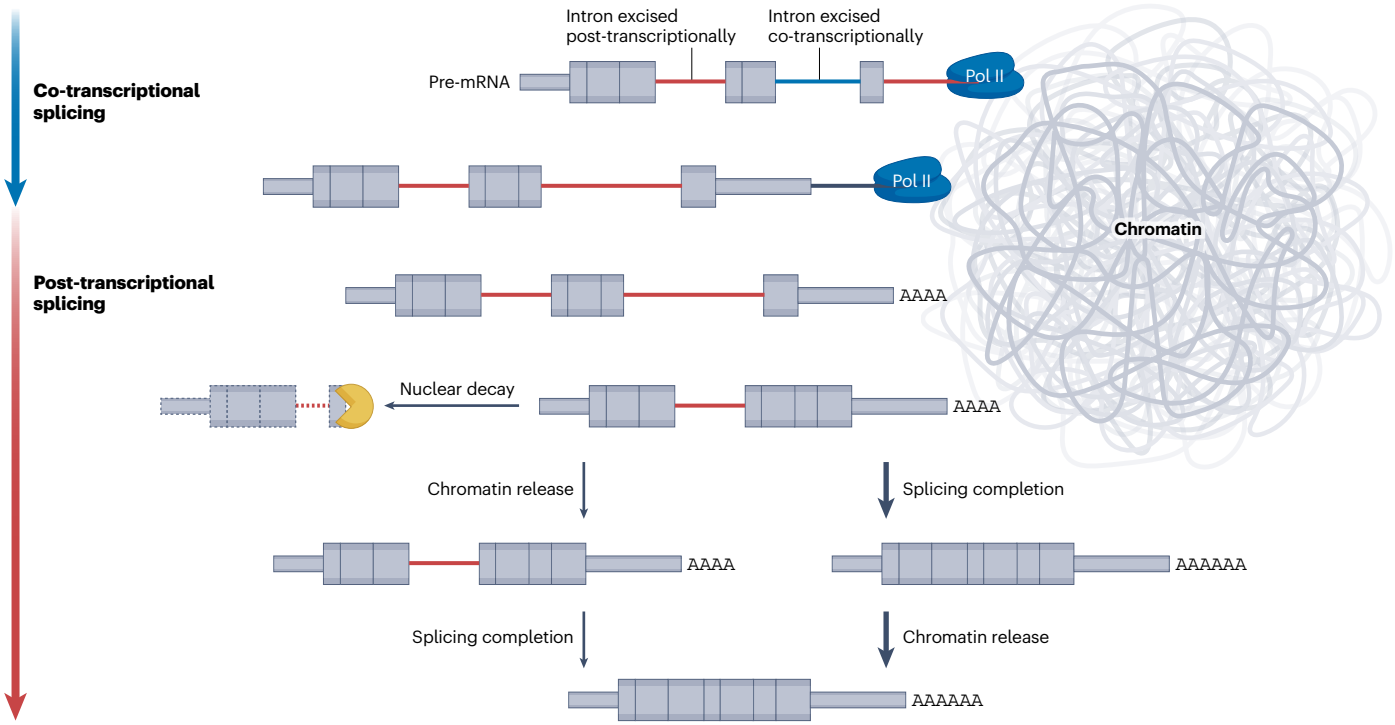


Fig. 3 | Splicing as a rate-limiting step for chromatin release. Co-transcriptionally excised introns (blue lines) are removed while the transcript is still being synthesized by RNA polymerase II (Pol II), whereas post-transcriptionally retained introns (red lines) are still present in polyadenylated chromatin-associated RNA. Pre-mRNAs remain tethered to chromatin, or nuclear speckles that are physically associated with chromatin, until they are completely spliced, at which point they are released (thick arrows, right). Chromatin release followed by splicing in the soluble nucleoplasm (thin arrows, middle) is considered less likely, given the low amount of introns and the short amount of time spent by transcripts in this fraction. Nuclear decay (thin arrows, left) can also occur for some partially spliced transcripts through the nuclear exosome (yellow shape).

Similar regulation occurs during development — during differentiation of mESCs to neurons, chromatin-associated transcripts contain introns that are highly retained during one differentiation stage but are efficiently excised at another[31]. For example, the *Gabbr1* mRNA is almost absent in the cytoplasm of mESCs but is present at high levels in neurons, whereas its pre-mRNA remains chromatin-associated with two non-excised introns across all stages of differentiation. These findings suggest that the retention of fully synthesized polyadenylated transcripts on chromatin could be controlled by splicing, even for long amounts of time. Furthermore, delayed splicing may have a critical role in the developmental expression of some genes[31], as discussed in more detail subsequently (see 'Retained and detained introns').

Although most transcripts eventually complete splicing and are released from chromatin, PUND transcripts are characterized by higher levels of retained introns compared with transcripts from other genes. Among isoforms encoded by PUND genes, only those containing retained introns are targeted for exosome-mediated nuclear degradation[43]. For these transcripts, nuclear degradation seems to represent a quality control mechanism if they take too long to be spliced (Fig. 3) or are not needed in a particular context. Notably, PUNDs are enriched for genes encoding splicing factors, suggesting a possible feedback mechanism regulating splicing factor levels through incomplete splicing and nuclear decay.

These studies collectively indicate that splicing completion serves as a rate-limiting step regulating the timing of transcript release from chromatin, thereby providing an additional layer of gene expression control. The coordinated regulation of splicing completion, chromatin retention and selective degradation allows precise control over when and whether transcripts become available for nuclear export and translation.

Special cases of post-transcriptional splicing

Some introns exhibit preferential post-transcriptional excision following 3′-end processing and polyadenylation. In this section, we focus on three well-characterized categories: terminal introns, detained introns and introns flanking alternative exons.

Terminal introns

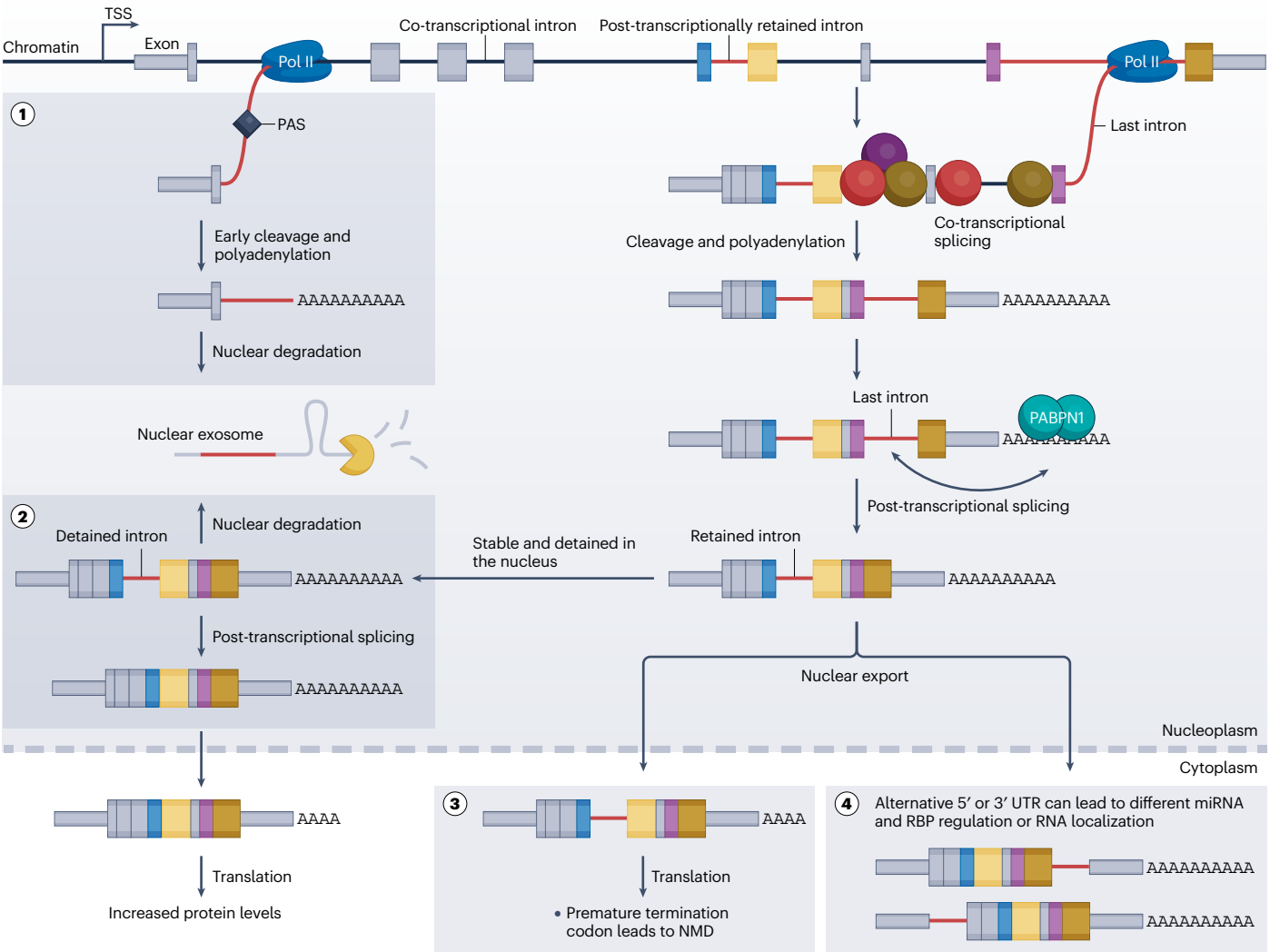
Terminal introns — defined as the last intron at the 3′-end of a transcript — are one of the best-characterized classes of post-transcriptionally excised introns[10,11,65] (Fig. 4a). Early in vitro experiments suggested that polyadenylation must occur before terminal intron excision[66]. Furthermore, 3′-end cleavage and a poly(A) tail stimulate terminal intron excision[11,65,67], an effect that is mediated through the poly(A) signal[68]. This phenomenon was not observed in all genes[69], which suggests transcript specificity. Reciprocally, polyadenylation was stimulated in the presence of an upstream intron[70–72], an effect that was found to rely on the presence of a splice acceptor and the recruitment of splicing factor U2AF65 but not on the splicing reaction itself[68,73,74]. Moreover, in vitro transcription-coupled processing assays showed that after 3′-end cleavage, RNAs remain anchored to Pol II through the CPA machinery and splicing factors, when introns are still present[75]. Either polyadenylation or terminal intron removal can happen first, but both steps are necessary to dismantle the CPA and splicing complexes, which triggers the release of the transcript from Pol II (ref. 75). Numerous splicing and 3′-end processing factors have been implicated in the coupling between these two processes[65,76–82]. These experiments led to a model in which binding of the splicing machinery at the terminal intron 3′-end and of the CPA machinery near the poly(A) site, and interaction between the two complexes, gives rise to an 'exon definition complex' that is essential for both splicing and 3′-end cleavage[75,83,84].

In mammalian cells, terminal introns are frequently removed post-transcriptionally, after polyadenylation but while transcripts are still on chromatin[53]. Terminal introns are generally still present in transcripts that are not cleaved at the 3′-end, whereas the majority are excised in cleaved transcripts that remain associated with chromatin[18–20]. Whereas uncleaved and unspliced transcripts are considered to be committed to eventual 3′-end processing and splicing in some studies[18,53], other studies have suggested that they represent 'dead-end' transcripts that will be degraded rather than processed[19]. In favour of the post-transcriptional splicing model, terminal introns retained in the absence of the nuclear poly(A) binding protein PABPN1 are not affected by the depletion of components of the nuclear decay machinery[81], providing evidence that transcripts with unspliced terminal introns are not widely destined for degradation. Beyond the fate decision between splicing and degradation, the molecular mechanisms controlling terminal intron excision are still being uncovered. Interestingly, a recent study suggested that the presence of a poly(A) tail is not sufficient to promote splicing, but that the tail must also achieve an optimal length[82]. Although CPA clearly influences terminal intron excision, future experiments will be necessary to elucidate the role of poly(A) tail length, and splicing and CPA factors in the post-transcriptional excision of terminal introns.

Retained and detained introns

Retained introns are introns that are stably retained in transcripts after cleavage and polyadenylation. Some of these intron-retention events are formed by the use of early cleavage and polyadenylation sites (PAS) within introns, resulting in retention of the intronic sequences upstream of the PAS (Fig. 4a, part 1). Approximately 20% of human genes contain at least one intronic PAS event[85,86], with these sites showing higher evolutionary conservation among humans, mice and rats when located towards the 3′-end of genes[85]. Interestingly, if the retained intron and PAS are close to the transcription start site, in particular within the first intron, the resulting short transcripts are often degraded by the nuclear exosome[87,88] (Fig. 4a, part 1). When retained introns are further away from the transcription start site, their transcripts can form a stable pool of nuclear transcripts (Fig. 4a, part 4) or be exported to the cytoplasm, where if they harbour a premature termination codon, they are degraded through NMD[89–91] (Fig. 4a, part 3). Alternatively, transcripts with retained introns have been shown to form stable cytoplasmic mRNAs with alternative untranslated regions (UTRs)[31], bind-specific RNA-binding proteins (RBPs) and microRNAs[92] and/or yield alternative peptides of physiological relevance[85,93–95] (Fig. 4a, part 4).

A retained intron that leads to a stable pool of nuclear mRNA is called a detained intron. Transcripts containing these introns are detained in the nucleus until they are fully spliced and exported or degraded within the nucleus[54,96,97]. Thus, detained intron-containing transcripts are not subjected to NMD, unlike other retained intron-containing transcripts[54,96,97] (Fig. 4, part 2). Detained introns possess several distinguishing characteristics that set them apart from non-retained introns. They tend to be shorter in length and exhibit elevated GC content, whereas their 5′ and 3′ splice sites are typically weaker than those found in non-retained introns[54,96,98,99]. Additionally, detained introns display greater nucleotide-level conservation compared with their non-detained intron counterparts[54]. These distinct molecular features, coupled with their unique nuclear retention properties, suggest that detained introns represent a specialized class


a Post-transcriptional splicing can lead to different RNA fates
TSS
Chromatin
Exon
Pol II
Co-transcriptional intron
Post-transcriptionally retained intron
Pol II
1
PAS
Early cleavage and polyadenylation
AAAAAAAAAA
Nuclear degradation
Nuclear exosome
Last intron
Co-transcriptional splicing
Cleavage and polyadenylation
AAAAAAAAAA
Last intron
PABPN1
AAAAAAAAAA
Post-transcriptional splicing
2
Detained intron
Nuclear degradation
AAAAAAAAAA
Post-transcriptional splicing
AAAAAAAAAA
Stable and detained in the nucleus
Retained intron
AAAAAAAAAA
Nuclear export
Nucleoplasm
Cytoplasm
AAAA
Translation
Increased protein levels
3
AAAA
Translation
• Premature termination codon leads to NMD
4
Alternative 5′ or 3′ UTR can lead to different miRNA and RBP regulation or RNA localization
AAAAAAAAAA
AAAAAAAAAA

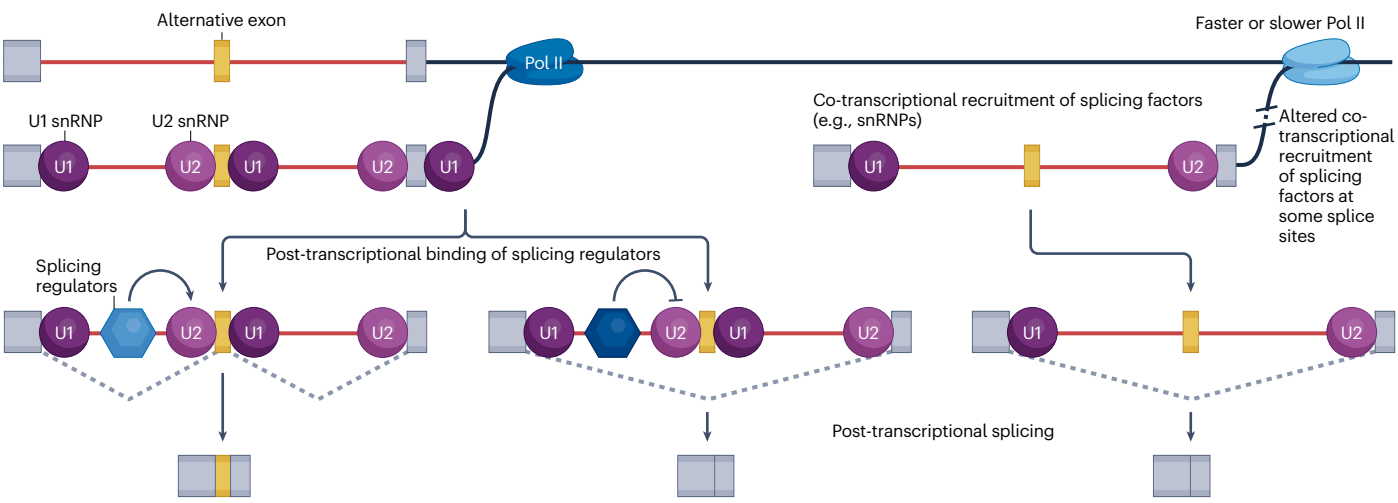

b Alternative splicing regulation: co-transcriptional recruitment versus post-transcriptional splicing
Alternative exon
Pol II
Faster or slower Pol II
U1 snRNP
U2 snRNP
U1
U2
U1
U2
U1
Co-transcriptional recruitment of splicing factors (e.g., snRNPs)
U1
U2
Altered co-transcriptional recruitment of splicing factors at some splice sites
Post-transcriptional binding of splicing regulators
Splicing regulators
U1
U2
U1
U2
U1
U2
U1
U2
U1
U2
Post-transcriptional splicing

Fig. 4 | Types of post-transcriptional splicing and connection to transcript fate. a, Different fates of retained introns and their regulatory mechanisms. Intron retention close to the transcription start site (TSS) can lead to the use of an early intronic cleavage and polyadenylation site (PAS), leading to termination and nuclear degradation of the short transcript by the nuclear exosome (part 1). Intron retention after cleavage and polyadenylation further along the gene body can form a pool of transcripts that are stably retained in the nucleus and contain detained introns (part 2). The fate of these transcripts is diverse: they can be targeted for degradation (part 2, upper arrow) or spliced and exported to the cytoplasm in response to regulatory signalling (part 2, lower arrow). Some transcripts with post-transcriptionally retained introns are exported to the cytoplasm where they can be targeted for nonsense-mediated decay (NMD) (part 3) or they can form stable transcripts with alternative 5′ and 3′ untranslated regions (UTRs) harbouring different microRNA (miRNA) and RNA-binding protein (RBP) binding sites (part 4). **b**, Representation of splicing regulation for introns flanking alternative exons, which are enriched for post-transcriptional splicing. U1 and U2 small nuclear ribonucleoproteins (snRNPs) are recruited co-transcriptionally. Inclusion (bottom left) or exclusion (bottom middle) of the alternative exon (yellow) is determined by splicing regulators (light and dark blue hexagons), some of which may require more time to bind post-transcriptionally (left). Polymerase II (Pol II) elongation speed could influence the co-transcriptional recruitment of splicing factors, depicted by the absence of snRNPs at the splice sites flanking the alternative exon, altering alternative splicing patterns even if splicing happens post-transcriptionally (right).

of retained introns that evolved to serve specific regulatory functions distinct from those of other retained introns.

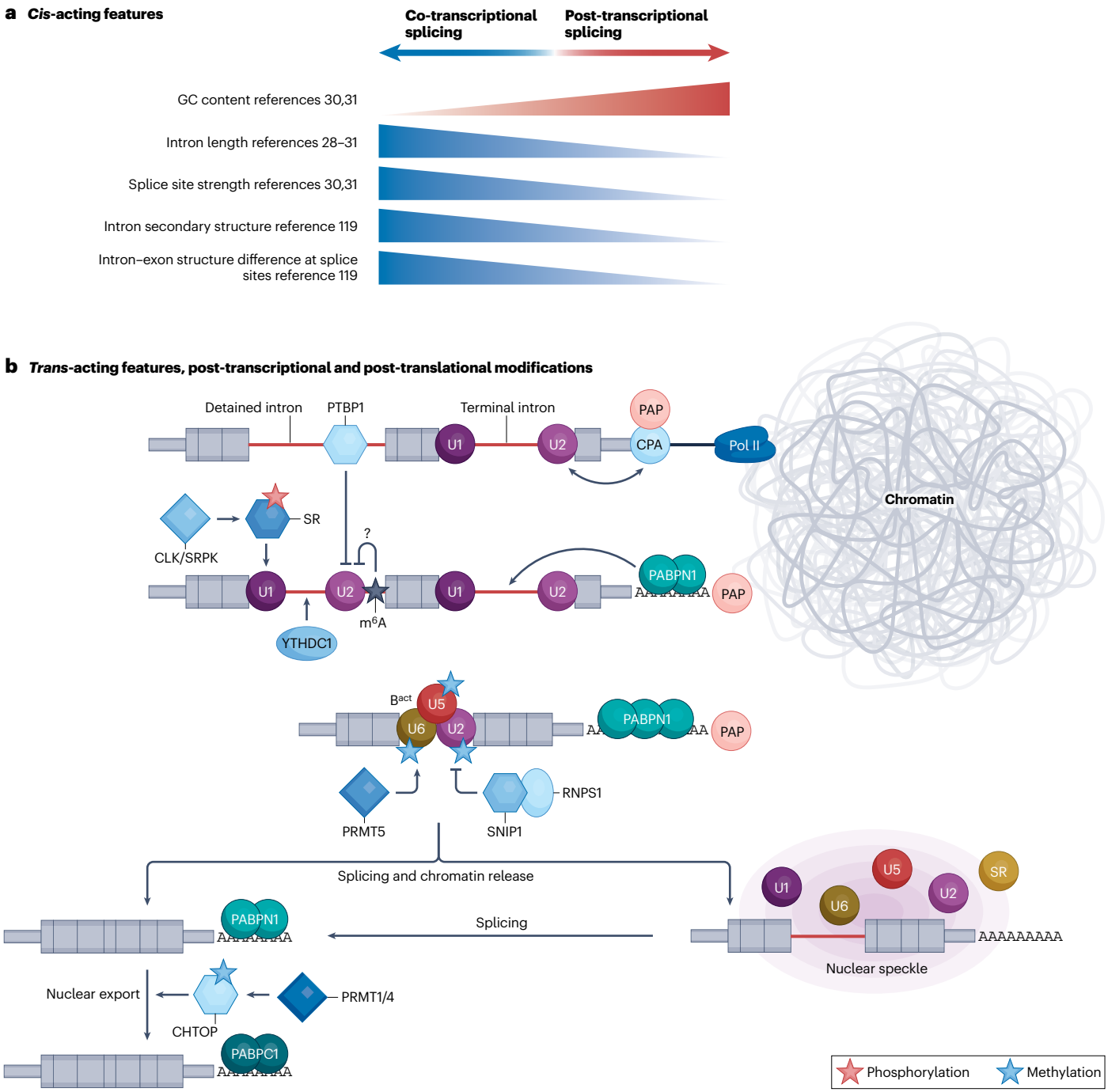
Detained introns can meet distinct fates that have an impact on gene regulation and serve important biological functions. Understanding these diverse outcomes and their regulation has revealed how cells leverage post-transcriptional splicing to achieve precise control over gene expression programmes. In neurons, activity-dependent detained intron excision enables rapid gene expression changes without requiring new transcription, allowing cells to quickly respond to different stimuli[100,101]. During development, detained intron regulation has critical roles in cellular differentiation programmes. For example, in spermatogenesis, coordinated retention and removal of detained introns help orchestrate the precise timing of gene expression during meiotic progression[99]. Similarly, during erythropoiesis, dynamic regulation of detained introns contributes to the complex gene expression changes required for red blood cell maturation[97]. The immune system also uses detained intron regulation, as seen during $CD4^+$ T cell activation where intron detention helps coordinate the rapid cellular response to immunological challenges[102]. The regulatory potential of detained introns extends to stem cell biology, where their excision controls muscle stem cell activation and the transition from quiescence[103]. In the nervous system, detained introns have multiple roles in the control of gene expression during neurogenesis[22,31,56] and memory[104]. These diverse examples highlight how cells have evolved to use detained intron regulation as a versatile mechanism to achieve temporal control over gene expression across different physiological contexts[103,104].

An extreme case of post-transcriptional splicing regulation is the increased intron detention and nuclear sequestration of hundreds of transcripts in response to heat shock, whereas transcripts required for the stress response escape widespread splicing inhibition[24,105,106]. Under normal physiological conditions, the transcripts affected by heat shock display higher levels of post-transcriptional splicing, whereas unaffected transcripts are spliced predominantly co-transcriptionally[24]. This pattern suggests that heat shock specifically targets and inhibits post-transcriptional splicing mechanisms. Detained intron excision has also been observed as part of a regulatory feedback loop that modulates the post-translational modification O-linked *N*-acetylglucosamine (O-GlcNAc), which increases in response to glucose levels[107]. Differential detained intron removal in pre-mRNAs encoding enzymes of the O-GlcNAc pathway regulates corresponding mRNA levels. For instance, inhibition of O-GlcNAc transferase (OGT), which catalyses O-GlcNAc addition, increases detained intron excision and productive splicing of the *OGT* pre-mRNA. Inhibition of O-GlcNAcase, which removes O-GlcNAc, has the opposite effect on *OGT* detained intron excision. Inhibition of these enzymes also results in excision changes for thousands of detained introns in other genes, indicating that O-GlcNAc is an important regulator of detained introns[108].

Two primary models have been proposed to explain detained intron regulation. The 'reservoir model' hypothesizes that transcripts containing detained introns constitute a pool of pre-mRNAs that can be rapidly spliced and exported according to cellular needs, allowing increased mRNA levels or production of new isoforms without re-initiating transcription[109]. The alternative 'dead-end model' postulates that based on stimuli received, detained introns are either excised co-transcriptionally to produce functional mRNAs, or retained post-transcriptionally, leading to nuclear decay[109].

The dead-end model was demonstrated for the last intron of *MAT2A*, which encodes an *S*-adenosyl methionine (SAM) synthetase. In response to SAM depletion, excision of the last intron increases through enhanced co-transcriptional splicing efficiency of newly synthesized pre-mRNAs, rather than post-transcriptional splicing of existing transcripts[110]. This regulation might depend on N^6-methyladenosine (m^6A) modifications at the 3′ splice site, which inhibit splicing[111]. Since SAM is the methyl donor for m^6A deposition[112], this creates a self-regulatory loop through detained intron regulation by m^6A[110]. Three other detained introns in *CLK1* and *OGT* also follow the dead-end model[109].

However, the two models are not mutually exclusive, as *CLK1* splicing follows both the reservoir and dead-end pathways[54,109,113]. In normal cells, some unspliced transcripts could constitute a reservoir, whereas the remainder are targeted for decay. Upon CLK1 deficiency, cells both splice existing *CLK1* transcripts from the reservoir and increase co-transcriptional splicing of newly synthesized *CLK1* transcripts[109]. Both models of detained intron-mediated gene regulation occur in response to signalling pathway activation. In response to neuronal stimulation, for instance, rapid excision of nuclear detained introns produces a fast wave of increased cytoplasmic gene levels independently from new transcription[100,101]. The specificity of this response is remarkable — different stimuli trigger the processing of distinct subsets of detained intron-containing transcripts[101]. Conversely, some detained intron-containing transcripts are degraded upon neuronal activation[101], suggesting the existence of molecular mechanisms that can sort transcripts between these different fates. Although the complete regulatory mechanisms remain to be fully elucidated, recent work has revealed key players in this process, including the SR protein kinase (SRPK) family, and components of stimulus-specific signalling pathways such as MAPK and CAMK[101]. These findings suggest a model in which cellular signalling cascades can selectively modulate the activity of splicing regulators to control specific subsets of detained introns. However, several important questions remain unresolved, including



how cells determine which detained intron-containing transcripts to splice or degrade, how different stimuli achieve specificity in targeting distinct pools of transcripts and what additional factors contribute to the remarkable precision of this regulation.

Introns flanking alternative exons

Introns that flank alternative exons are more likely to be excised post-transcriptionally[30,31,40,54] and have slower excision kinetics than constitutive introns[27–29,40,53]. For example, 67% of introns flanking alternative exons are excised post-transcriptionally compared with 30% of constitutive introns in mESCs[31]. Similarly, during human neuronal differentiation, introns flanking alternative exons are removed even later than neighbouring post-transcriptionally excised introns, indicating a temporal hierarchy wherein regulated introns require extended processing time[17]. These delayed kinetics for splicing alternative exons perhaps allow more time for the correct choice to be made between alternative splice sites.

The rate of Pol II transcription influences alternative splicing outcomes by controlling the temporal window available for splice site recognition and regulatory element binding[114–118]. How can slowly

Fig. 5 | Regulation of post-transcriptional splicing. a, *Cis*-acting features have been associated with the tendency of an intron to be excised co-transcriptionally or post-transcriptionally[28–31,119]. The darker and higher sides of the triangles indicate that the features are more pronounced for the corresponding mode of splicing (co-transcriptional or post-transcriptional), whereas the lighter and more narrow sides of the triangles indicate that the features are less pronounced. **b**, Post-transcriptional splicing is regulated by several *trans*-acting features. Post-transcriptionally retained introns are depicted in red. Excision of the terminal intron is regulated by reciprocal interactions between U2 small nuclear RNP (snRNP) and the cleavage and polyadenylation machinery (CPA). As the poly(A) polymerase (PAP) synthesizes the poly(A) tail, PABPN1 monomers bind side by side and may promote removal of the terminal intron. Polypyrimidine tract binding protein 1 (PTBP1) inhibits splicing of detained introns by binding to the polypyrimidine tract and preventing recruitment of U2 snRNP. SR proteins modulate detained intron excision and are regulated through dynamic phosphorylation by the kinases: Cdc2-like kinase (CLK) and SR protein kinase (SRPK). SNIP1 was found to pause splicing at the B^{act} stage, whereas PRMT5 promotes splicing through methylation of snRNP components. PRMT1/4 promotes nuclear export of spliced mRNAs through methylation of the nuclear export factor CHTOP. Post-transcriptional modifications (for example, m^6A), which are detected by their reader YTHDC1, and localization of the pre-mRNA to nuclear speckles (Box 1), which are enriched in splicing factors such as snRNPs, are also associated with post-transcriptional splicing efficiency. Once in the cytoplasm, PABPC1 replaces PABPN1 on the poly(A) tail. CPA, cleavage and polyadenylation machinery; PAP, poly(A) polymerase; Pol II, polymerase II.

excised introns be impacted by Pol II elongation? A recent study showed that slower transcription modulates co-transcriptional RNA folding of introns flanking alternative exons, which are removed post-transcriptionally[119]. These structure changes are predictive of whether inclusion of the alternative exon is increased or decreased upon slower transcription, suggesting that co-transcriptional RNA folding influences post-transcriptional alternative splicing[119]. Furthermore, considering that many splicing factors physically interact with Pol II during transcription elongation[16], transcription elongation speed can affect the commitment to splicing by influencing the recruitment of splicing factors to splice sites and regulatory elements, rather than or in combination with splicing timing[114,120] (Fig. 4b). Additional analyses are required to determine whether exons regulated by transcription rate and by delayed removal of flanking introns overlap or represent two distinct groups of exons regulated by different mechanisms.

Regulation of post-transcriptional splicing

A key outstanding question in the field is how an intron is fated to be removed co-transcriptionally or post-transcriptionally. Studies have shown that the relative order of intron removal, both co-transcriptionally and post-transcriptionally, occurs consistently and is conserved between cell types[17,18,121,122], indicating that splicing order is tightly controlled. However, we currently lack a comprehensive understanding of the regulation of post-transcriptional splicing and how it differs from and/or crosstalks with co-transcriptional splicing regulation. In this section, we review factors and physiological responses that have been found to specifically regulate post-transcriptional splicing.

Pre-mRNA features

Several *cis*-acting features were found to be moderately associated with co-transcriptional versus post-transcriptional excision, including splice site strength, intron and exon length, exonic GC content and chromatin marks[28–30]. For instance, a deep neural network trained to predict subcellular intron retention[31] revealed that introns with high excision in all subcellular compartments tend to be longer and have stronger splice sites. By contrast, introns retained on chromatin post-transcriptionally but not present in the cytoplasm (indicative of post-transcriptional splicing) tend to have higher GC content and a greater probability of nucleosome positioning in the first or last 100 nt of the intron. Introns retained in all fractions, including the cytoplasm, have weaker splice sites, higher probability of containing stop codons, lower conservation of the first and last 100 nt compared with co-transcriptionally or post-transcriptionally excised introns and tend to be located in the 5′-UTR or 3′-UTR[31]. These findings indicate that post-transcriptionally excised introns are distinguishable from introns that are stably retained in the cytoplasm (Fig. 5a).

The structural properties of introns also correlate with their splicing timing. Introns that are excised co-transcriptionally tend to be more structured than introns that are removed post-transcriptionally[119]. Because introns are typically more structured than their adjacent exons, most splice sites are characterized by sharp structural transitions at 5′ and 3′ splice sites. Co-transcriptionally excised introns show steeper structural transitions at their splice sites, indicative of a greater structure difference with their adjacent exons, whereas post-transcriptionally excised introns have smaller structure transitions at their splice sites[119].

When examining the specific timing and order in which post-transcriptional splicing occurs, genetic variants provide additional evidence for sequence-based regulation. Analysis of sets of post-transcriptionally excised introns showed that the relative order of their removal correlates with specific genetic variants, both within splice sites and in more distal intronic regions[123]. These distal variants likely influence splicing timing by modulating RNA structure, splicing regulatory elements or RNA-binding protein sites.

Changes in post-transcriptional RNA modifications contribute to the regulation of post-transcriptional splicing. m^6A modification has emerged as a context-dependent regulator of post-transcriptional splicing. For instance, during neuronal differentiation, m^6A is linked to detained intron excision in *Scratch1*, which encodes a transcription factor that drives differentiation[22]. The *Scratch1* transcript accumulates in the nucleus of neuronal progenitors until differentiation signals trigger both increased m^6A modification and detained intron excision, enabling nuclear export. Blocking m^6A prevents *Scratch1* splicing and maintains cells in an undifferentiated state, but a direct link among *Scratch1* splicing, m^6A and differentiation is still missing[22]. The relationship between m^6A and splicing is complex and context-dependent. For instance, m^6A marks at 3′ splice sites inhibit splicing of transcripts encoding SAM synthetase in mammals and *Caenorhabditis elegans* as part of a feedback loop[111], although m^6A marks generally correlate with accelerated splicing in genome-wide studies[124]. During thermal stress, sequestration of the nuclear m^6A reader YTHDC1 in stress bodies promotes intron retention[105]. However, what determines transcript specificity in m^6A regulation of detained introns or how m^6A marks mechanistically affect splicing in response to differentiation or stress signals is unknown. Furthermore, the role of m^6A in regulating splicing is under debate, as most m^6A marks are not deposited close to splice sites, are mostly added post-transcriptionally to spliced mRNAs[125–127], and knockout of the methyltransferase *Mettl3* has minimal effects

on splicing[125]. These findings indicate that although m^{6}A can influence splicing in specific developmental and stress contexts, further investigations are required to determine to what degree m^{6}A acts as a universal splicing modulator.

Trans-acting factors

The regulation of post-transcriptional splicing involves multiple *trans*-acting factors, with spliceosomal components and splicing regulators playing a central role. Post-translational modification of these RBPs enables further fine-tuning of splicing regulation. Emerging evidence indicates that splicing factors create a regulated network that controls the timing and efficiency of splicing.

Core spliceosomal components, such as spliceosomal snRNAs, seem to have an important role in post-transcriptional splicing regulation. Moderate depletion of U1, U2, U4 and U6 snRNAs in human cell lines, which induces gene-specific alternative splicing changes without widespread splicing dysfunction, leads to increased retention of short GC-rich introns, which are common features of post-transcriptionally retained introns[128]. This finding was further supported by studies of the *NMF291*$^{-/-}$ mice, which carry a mutation in *Rnu2–8*, one of the genes encoding U2 snRNA. These mice exhibit widespread retention of short GC-rich introns within nuclear-retained polyadenylated transcripts, demonstrating the importance of proper U2 snRNA function in post-transcriptional splicing regulation[129,130]. Notably, *Snip1*, a genetic modifier of *NMF291*$^{-/-}$ mice, encodes a protein that interacts with detained intron-containing transcripts and components of the activated spliceosome B^{act}, but not with the catalytically activated B* spliceosome. This selective interaction pattern suggests that SNIP1 functions as a molecular brake, temporarily pausing splicing at specific regulated detained introns[130] (Fig. 5b).

Beyond core spliceosomal components, various splicing factors have crucial roles in regulating post-transcriptional splicing through distinct mechanisms. For instance, the polypyrimidine tract binding protein 1 (PTBP1) regulates the splicing levels of several introns defined as post-transcriptionally excised on chromatin[31]. PTBP1 depletion leads to some of these chromatin-retained introns showing increased excision in both the nucleoplasm and cytoplasm, suggesting that PTBP1 normally acts to prevent splicing and release of these transcripts. This regulatory role was shown to be particularly important in neuronal development, where the natural downregulation of PTBP1 promotes splicing of neuronal-specific transcripts containing detained introns, facilitating their nuclear export and augmented expression[56]. Many splicing factors also exhibit autoregulation through post-transcriptional splicing[131,132]. For instance, the Fused in Sarcoma (FUS) splicing factor inhibits its own splicing when present at high levels, resulting in intron retention and nuclear detention, thereby reducing the amount of cytoplasmic FUS available for translation[131]. This autoregulatory mechanism is disrupted in amyotrophic lateral sclerosis, in which pathogenic variants in the FUS nuclear localization signal impair its nuclear import. The reduced nuclear FUS levels result in decreased intron detention and increased splicing of *FUS* transcripts, ultimately leading to elevated cytoplasmic FUS levels that may contribute to disease pathogenesis. Although these examples highlight specific regulatory mechanisms, splicing timing likely depends on the combined action of multiple RBPs[18,35,121], and additional work is required to define their specific roles in post-transcriptional splicing (Fig. 5b).

Post-translational modifications of RBPs, particularly phosphorylation and methylation, have a central role in regulating post-transcriptional splicing. Reversible phosphorylation of splicing factors is a key mechanism to regulate their function[133–142]. Notably, SR proteins — a group of splicing factors that are functionally and structurally related[143–146] — are dynamically phosphorylated in their serine–arginine-rich domain (RS domain) by two families of protein kinases that interact with each other: SRPKs and Cdc2-like kinases (CLKs)[147,148].

Both CLKs and SRPKs have been implicated in the regulation of post-transcriptional splicing[54,101]. Small-molecule inhibition of SRPKs showed stimulus-specific regulation of some detained introns in response to neuronal activity[101]. CLK1 was identified as a regulator of detained introns upon their initial description. Using chemical inhibitors of CLK1 (refs. 149,150), multiple studies have shown that this kinase can upregulate and downregulate specific detained introns post-transcriptionally[54,113,150]. CLK1 itself is regulated through excision of detained introns: upon reduction of *Clk1* mRNA levels, its detained introns are excised post-transcriptionally, leading to increased nuclear export of *Clk1* mRNA, thus restoring CLK1 protein levels[54,113]. In mESCs, phosphorylation of SRSF4 by CLK1 regulates a subset of detained introns[54]. Phosphorylation of SR proteins by CLK1 is also responsible for the recovery of post-transcriptional splicing after thermal stress in human cells[106,151].

Beyond phosphorylation, protein arginine methylation represents another critical PTM in post-transcriptional splicing regulation. Protein arginine methyltransferases (PRMTs) were found to modulate post-transcriptional but not co-transcriptional splicing through methylation of spliceosomal Sm proteins and the nuclear export factor CHTOP[152]. Symmetric dimethylarginine formation on Sm proteins by PRMT5 is necessary for the post-transcriptional splicing of detained intron-containing transcripts and their escape from chromatin, providing further evidence that splicing is a prerequisite for transcript release[152,153]. Conversely, asymmetric dimethylarginine formation on CHTOP by PRMT1/4 is required for detained intron-containing transcripts to be exported[152]. In the absence of CHTOP, splicing levels of these transcripts increase, suggesting that longer nuclear residence time promotes splicing or degradation[152].

The dysregulation of post-transcriptional splicing in disease contexts reveals its critical importance in maintaining normal cellular function. In mice, PRMT5 was identified as a factor needed for glioblastoma cell proliferative fitness[23]. In these aggressive brain tumours, PRMT5 becomes aberrantly activated and promotes the excision of specific detained introns in transcripts encoding cell cycle regulators. This enhanced detained intron excision leads to increased expression of proliferation-associated genes, creating a feedforward loop that drives tumour growth[23]. Notably, this mechanism seems to represent a pathological hijacking of a normal developmental process — during neurogenesis, PRMT5 levels are naturally reduced, resulting in increased intron detention within the same transcripts encoding cell cycle regulators. This observation suggests that detained intron excision by PRMT5 controls cell proliferation in both physiological and pathological contexts[23]. The fundamental role of this pathway in glioblastoma has important therapeutic implications. Both genetic and pharmacological approaches to inhibit PRMT5 have shown promise in animal models by preventing excision of these regulated detained introns, thereby suppressing tumour growth[23,154]. These findings not only highlight how cancer cells can co-opt post-transcriptional splicing mechanisms but also demonstrate how understanding the basic biology of detained intron regulation can reveal new therapeutic vulnerabilities.

Glossary

Alternative splicing
A regulated process by which different combinations of exons and/or introns from a single gene can be included or excluded in the final mRNA, allowing multiple protein isoforms to be produced from a single gene.

Alternative untranslated regions
(UTRs). Different versions of untranslated regions that can be included in the final mRNA through alternative splicing, alternative polyadenylation or alternative transcription start sites.

Branch point
An intronic adenosine, typically located 18–40 nt upstream of the 3′ splice site, whose 2′-OH group attacks the 5′ splice site in the first step of splicing, creating a characteristic branched intermediate (lariat).

Cleavage and polyadenylation
(CPA). The process of cutting the primary transcript at a specific site and adding a poly(A) tail to create the 3′-end of mature mRNA.

Cleavage and polyadenylation sites
(PAS). Specific sequences in the pre-mRNA that signal where the transcript should be cleaved and polyadenylated.

Detained intron
A class of introns that are retained in nucleus-localized transcripts until specific signals trigger their removal and export of the transcript from the nucleus, serving as a regulatory mechanism for gene expression.

m^{6}A
N^6-methyladenosine, an RNA chemical modification consisting of a methyl group added to the nitrogen at position 6 of adenosine.

Metabolic labelling
A technique in which cells are grown with nucleotide analogues that are incorporated into newly synthesized RNA, which can be specifically isolated or detected using biochemical methods, allowing to track new RNA synthesis and processing.

MicroRNAs
Small non-coding RNAs (21–23 nt) that regulate gene expression post-transcriptionally, generally through binding sites in 3′ untranslated regions.

mRNA
A class of RNA molecules that unlike non-coding RNAs carry coding information and are translated into proteins by ribosomes.

Nonsense-mediated decay
(NMD). A quality control mechanism that degrades mRNAs containing premature stop codons in the cytoplasm.

Nuclear degradation
The breakdown of RNA molecules within the nucleus, serving as a quality control mechanism and regulatory process. This occurs through multiple pathways including the nuclear exosome.

Nuclear speckles
Membrane-less nuclear compartments enriched in pre-mRNA splicing factors, RNA-processing factors and partially processed mRNAs.

PUND
Genes encoding transcripts that are predicted to undergo nuclear degradation rather than be processed into mRNA based on subcellular fractionation, metabolic labelling, mathematical modelling and/or knockdown of nuclear exosome subunits.

RNA polymerase II
(Pol II). The enzyme responsible for transcribing all eukaryotic protein-coding genes and many non-coding RNA genes. Its largest subunit contains a C-terminal domain that undergoes dynamic phosphorylation during transcription, coordinating various RNA processing events.

RNA-binding proteins
(RBPs). Proteins that bind to RNA, typically by recognizing specific sequences or structures, and regulate processes including splicing, polyadenylation, export, localization, stability and translation.

Serine–arginine (SR) proteins
A family of proteins that have crucial roles in constitutive and alternative splicing. They are characterized by one or more RNA recognition motif(s) and a domain rich in serine–arginine dipeptides (RS domain). Dynamic phosphorylation of the RS domain regulates their activity, localization and function.

Splice sites
Specific sequences at the start (5′-end) and end (3′-end) of introns that are recognized by the spliceosome.

Subcellular fractionation
Separation of subcellular compartments through differential centrifugation and/or biochemical extraction, yielding cytoplasmic, nucleoplasmic and chromatin-associated fractions.

Conclusions

Analyses of splicing in time and space have revealed that chromatin retention of polyadenylated pre-mRNA and post-transcriptional splicing are frequent features of mammalian gene expression that are intricately connected. Post-transcriptional splicing seems to be a rate-limiting step for transcript release from chromatin, thereby controlling when the mRNA becomes available for nuclear export and eventual translation. This delayed splicing offers additional opportunities for regulation, from modulation of alternative splicing to context-specific removal of detained introns, enabling the maturation of distinct mRNAs to meet cellular needs during development, in response to various stimuli and in disease states.

Although *cis*-regulatory elements and *trans*-acting factors that modulate post-transcriptional splicing have begun to be uncovered, several key questions remain. The complex molecular mechanisms that determine splicing timing require further investigation. The precise mechanisms by which post-transcriptionally spliced transcripts escape nuclear quality control pathways, and how cells determine whether to splice or degrade these transcripts, are not fully elucidated. Additionally, the therapeutic potential of targeting post-transcriptional splicing in diseases in which this process is dysregulated, such as in certain cancers, warrants further exploration. Recent technological advances in spatial transcriptomics, long-read sequencing and live-cell RNA imaging will provide unprecedented temporal and spatial resolution to address these questions. Combined with emerging tools for manipulating splicing timing and improved computational approaches for analysing complex splicing patterns, we are entering an exciting era that promises to reveal how cells leverage post-transcriptional splicing to achieve precise control over gene expression programmes in both health and disease.

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