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Review

Transcriptional elongation and alternative splicing ☆

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ABSTRACT

Alternative splicing has emerged as a key contributor to proteome diversity, highlighting the importance of understanding its regulation. In recent years it became apparent that splicing is predominantly cotranscriptional, allowing for crosstalk between these two nuclear processes. We discuss some of the links between transcription and splicing, with special emphasis on the role played by transcription elongation in the regulation of alternative splicing events and in particular the kinetic model of alternative splicing regulation. This article is part of a Special Issue entitled: RNA polymerase II Transcript Elongation.

1. Introduction

The expression of protein coding genes is a multi-step process with complex regulation at multiple levels. Eukaryotic protein coding genes are transcribed by RNA polymerase II (Pol II) into pre-mRNA which is capped at its 5′ end and polyadenylated at its 3′ end, ensuring the stability of the newly synthesised molecule, and is further processed by the elimination of noncoding interspersed sequences called introns and the joining of the remaining sequences, the exons, in the process of RNA splicing. Different regulatory sequences along the introns, termed the 5′ splice site, branch point, polypyrimidine tract and 3′ splice site, are bound by five different snRNPs and a myriad of accessory proteins in a fixed sequence of steps that end in the assembly of the catalytically active form of a megacomplex called the spliceosome, which mediates intron removal and exon ligation [1]. Proteins of the serine/arginine-rich (SR) and hnRNP families are also implicated in exon recognition and splicing regulation. Besides the processing factors and the regulatory sequences in *cis*, a key actor for pre-mRNA maturation is RNA Pol II itself, in particular the C-terminal domain, or CTD, of the largest subunit Rpb1. The CTD consists of a series of repeats of the consensus sequence YSPTSPS, 26 in yeast and 52 in humans, which can be subjected to a variety of post-translational modifications including glycosylation [2], proline isomerization [3], and, crucially, phosphorylation. Five out of the seven residues can be phosphorylated, and serine 2 and serine 5 phosphorylations play an important part in Pol II transcription. Serine 5 phosphorylation by CDK7, a subunit of the preinitiation complex factor TFIIH, marks promoter clearance and the start of pre-mRNA synthesis; serine 2 phosphorylation by CDK9, the cyclin dependent kinase of the elongation factor P-TEFb, or by CDK12 or CDK13 [4], is more prevalent further downstream on the gene and is associated with elongation. Transcription of endogenous genes in the context of chromatin is severely impaired in the absence of the CTD [5]. The RNA exit channel of Rpb1 is in close proximity to the CTD, which is known to bind several processing factors, acting as a bridge between them and the nascent RNA. The enzymes responsible for 5′ cap addition and the 3′ processing factor complexes CPSF and CstF are recruited to phosphorylated CTD, and CTD truncation hampers capping and pA cleavage in vivo [6,7]. Splicing was also reported to be inhibited by the absence of the CTD [7], which is consistent with the fact that in the absence of the CTD there is less accumulation of snRNPs and SR splicing regulatory proteins at the sites of transcription in vivo [8].

Although most exons are included in the mRNA constitutively, there are particular exons that can be skipped, as a whole or partially through the use of alternative 3′ ss or 5′ ss, in some of the mRNA molecules; conversely, some introns can be retained in the final product. These alternative pathways of RNA splicing give rise to different mRNA species that will code for proteins that differ in their sequence, sometimes affecting their localization, stability or function; alternative splicing can also lead to a shift of the reading frame that creates premature stop codons and targets the mRNA for degradation through nonsense mediated decay (NMD) [9,10]. The outcome of alternative

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splicing events can change in response to stimuli or cell cycle progression, and in multicellular organisms many events follow tissue or developmental specific splicing patterns. Therefore, alternative splicing is subjected to tight regulation ensuring that the right proportion of the different isoforms is produced in any given situation. Based on the number of genes found in *C. elegans* and *D. melanogaster* it had been estimated that a mammal with a complex nervous system would harbor about 100,000 different genes; in contrast, only 23,000 protein coding genes have been identified in the human genome [11]. Post-transcriptional modifications allow single genes to code for different peptides, and in particular the fact that more than 90% of human genes are alternatively spliced [12,13] highlights the role of alternative splicing in expanding proteomic diversity in complex organisms.

2. Coupling between transcription and splicing

During the transcriptional cycle, elongating RNA Pol II travels along the gene in a 5′ to 3′ direction, synthesizing an alternation of exons and introns with their splicing regulatory sequences that are available to the processing machinery before transcription termination occurs, thus raising the possibility of cotranscriptional recognition and excision of introns. Beyer and Osheim [14] presented the earliest evidence of cotranscriptional splicing in their EM images of *Drosophila* chromatin spreads which show what can be interpreted as spliceosome assembly, intron loop formation and intron excision on structures identified as growing RNA chains tethered to their coding DNA. Isolation by microdissection of Balbiani ring-associated and free nucleoplasmic RNA in *Xenopus* embryos showed that intron excision can precede transcript release [15]. Tennyson et al. [16] provided proof of cotranscriptional splicing in their RT-PCR analysis of the human dystrophin gene. Following induction, they could detect accumulation of spliced transcripts at the 5′ end of the gene before transcription reached the 3′ end. Subsequently, splicing factor recruitment to transcription sites was detected by chromatin IP [17,18] or immunofluorescence [19–22], and was shown to be prior to transcript release in a reporter system [22]. In recent years, numerous studies have assessed the extent of cotranscriptional splicing, both on individual genes and using genome wide approaches. In all eukaryotes studied so far, the majority of introns are removed before cleavage at the poly A site and release of the transcript [21–27], although introns near the 3′ end of genes or introns surrounding alternative exons are less likely to be excised cotranscriptionally [23,25,27]. Pandya-Jones and Black [23] compared intron removal in two human genes, c-Src and fibronectin, in chromatin-associated and free nucleoplasmic RNA, and found that intron removal follows a predominant 5′ to 3′ order along the genes, and that most splicing reactions occur in the chromatin fraction, even for alternative splicing events. Using RNA FISH probes against exonic and intronic sequences, Brody et al. [21] were able to detect unspliced RNA only at the transcription site; they also presented evidence that polyadenylated unspliced RNA can accumulate at the transcription locus, with little or no diffusion to the nucleoplasm. In contrast, using single molecule imaging techniques, Vargas et al. [25] identified unspliced precursors in the nucleoplasm in conditions that favour post-transcriptional splicing such as inhibition of exon inclusion by specific regulatory proteins; however, only the introns surrounding the alternative exon are retained in the nucleoplasmic transcripts.

Notably, splicing is predominantly cotranscriptional in *Saccharomyces cerevisiae* [26], despite the fact that the average distance between introns and poly A signals would be too short for splicing to occur before termination [28], the necessary time for splicing completion being given by Pol II pausing on the terminal exons [26]. Interestingly, cotranscriptional splicing was found to be the most efficient pathway in a model of splicing kinetics in budding yeast [29].

Cotranscriptional splicing reduces the need for post-transcriptional processing of pre-mRNA, allowing for quicker transcriptional responses to stimuli. Besides, cotranscriptional recruitment of SR protein SRSF1 (previously known as SF2/ASF) prevents R-loop formation between the growing RNA chain and the template DNA strand that would otherwise lead to double strand break and genomic instability [30]. Crucially, spatial and temporal proximity of transcription and splicing opens the possibility of mutual influence, and indeed a growing body of evidence has established that both processes are functionally coupled. Splicing has been shown to affect transcription in a variety of fashions. For example, it has been shown that in yeast Pol II pauses at the 3′ end of introns, and splicing factor recruitment and CTD serine 2 phosphorylation are first detected during this pause [31]. Thus, splicing may act as a checkpoint in the transition from serine 5-phosphorylated polymerase to fully elongating serine 2-phosphorylated polymerase. In metazoans, splicing repressor snRNP 7SK sequesters P-TEFb, the CTD serine 2 cyclin-kinase, and hinders elongation [32], suggesting the existence of a similar mechanism. In another instance of crosstalk between splicing and transcription, the yeast splicing complex Prp19 was found to be necessary for efficient transcription by Pol II [33]. Prp19 recruits the THO subcomplex (a part of the nuclear export associated TREX complex) which is necessary for Pol II elongation and prevents R-loop formation [34]. Besides, human mutations that affect spliceosome recruitment enhance the accumulation of stalled Pol II downstream of the poly A site of human genes, pointing to a connection between splicing and transcription termination [35].

On the other hand, the effects of RNA Pol II transcription on splicing are far reaching. *In vitro* and *in vivo* studies show that splicing of Pol II transcribed RNA is more efficient than splicing of pre-synthesized RNA or T7 polymerase-transcribed RNA [36,37]. Using an *in vitro* transcription and splicing assay, Hicks et al. [38] show that this effect may have to do with a more efficient recruitment of the spliceosome leading to enhanced transcript stability rather than with differences in the kinetics of intron removal.

Perhaps the most interesting instance of coupling between splicing and transcription came with the discovery that transcription can affect the outcome of alternative splicing events. As early as 1988, Eperon and colleagues [39] found that the use of an alternative 5′ ss embedded in a stem that hindered its usage in vitro was impaired in vivo only if the structure was short. They reasoned that longer stems needed more time to assemble, allowing recruitment of the splicing machinery, and speculated that the threshold length of the stem for the use of the alternative 5′ ss was determined by the rates of splicing factor recruitment and Pol II elongation.

More direct evidence of a link between splicing and transcription was presented a decade later in the work of Cramer et al. [40]. The authors showed that the level of inclusion of a widely studied alternative exon, human fibronectin exon 33 or EDI, changes with the identity of the Pol II promoter used to drive minigene transcription. They proposed that changes in promoter sequence can lead to differential recruitment of splicing factors to Pol II, which then travel along with the polymerase to their targets and change the balance of skipping and exclusion. Additionally to this model of splicing factor recruitment by Pol II, a kinetic model whereby the rate of Pol II elongation conditions the recruitment of splicing factors to competing sites was proposed [41]. Subsequently, numerous cases of alternative splicing events regulated by transcription have appeared in the literature, with examples both of specific recruitment of Pol II-associated factors and regulation by elongation rates. We will discuss some of the recent developments in the following sections.

3. Recruitment model

The C-terminal domain of RNA Pol II plays a key role in the functional coupling between transcription and processing, acting as a scaffold for capping and 3′ processing factors that are delivered to the

growing RNA chain. In contrast, few splicing factors have been shown to bind the CTD stably. David et al. [42] reported a direct interaction between Pol II CTD and U2AF65, the protein that recognizes the polypyrimidine tract. The SR protein SRSF3 inhibits FN EDI inclusion and it was shown that this effect depends on the CTD, as a mutant polymerase with a truncated CTD abolishes EDI upregulation upon SRSF3 knockdown [43]. In a classical example of the recruitment model, PGC-1 was shown to modulate FN EDII exon inclusion only when it was able to bind the promoter of the gene [44].

A recent paper by Huang et al. [45] has uncovered a role of Mediator subunit MED23 in alternative splicing regulation. Multisubunit Mediator complex physically links transcription activators or repressors bound to enhancers or silencers with the basal transcription factors on core promoters. A large number of RNA processing factors were found to bind MED23, including alternative splicing and polyadenylation regulator hnRNP L, which specifically interacts with MED23 in vitro and in vivo. hnRNP L binding to promoter sequences was downregulated upon MED23 knockdown, and most targets of hnRNP L alternative splicing regulation were also shown to be regulated by MED23.

4. Kinetic model

Smith and colleagues presented a strong case for a link between alternative splicing regulation and Pol II elongation kinetics in a study of α-tropomyosin (TM) exon 3 splicing [41]. They detected an increase of exon 3 inclusion in vivo using minigenes engineered to have pause sites or spacer elements upstream of a negative regulatory sequence in intron 3, indicating that a lag between transcription of exon 3 and synthesis of the downstream regulatory element allowed exon commitment to inclusion before repressor binding. In agreement with the cotranscriptional mechanism they postulated, no effect of the pause sites or spacer sequences was detected *in vitro*. Subsequently, the previously mentioned promoter effect on fibronectin EDI inclusion was found to be dependent on Pol II elongation, with a positive correlation between promoter ability to enhance continuous transcription elongation and EDI skipping [46,47]. As the 3′ ss of the intron upstream of EDI is weak compared to the competing downstream 3′ ss, which is less than 2 kb apart, one possible explanation is that fast Pol II transcription leads to competition between both splice sites within seconds, favoring the use of the strong site downstream, whereas slow elongation allows more time for spliceosome recruitment to the weak site before the synthesis of the strong site, enhancing inclusion (Fig. 1). In agreement with this model higher levels of EDI inclusion are seen if elongation rate is slowed through chromatin condensation [47–49], P-TEFb or topoisomerase I inhibitors [50,51], or transfection of mutant slow polymerases [52,53]. Transfection of a plasmid coding for an α-amanitin-resistant variant of Rpb1 with a point mutation that confers intrinsically low processivity followed by α-amanitin treatment enhanced EDI inclusion compared to α-amanitin resistant but otherwise wt polymerase [52], a very strong piece of evidence in favor of the kinetic model.

Batsché et al. [54] showed that Brm, a subunit of the chromatin remodeling complex SWI/SNF, interacts *in vivo* with core spliceosomal components and with Sam68, a protein that promotes inclusion of the nine clustered variant exons of the CD44 gene and that is phosphorylated by the ERK MAP kinase. Upon activation of the MAP kinase signaling pathway, Brm causes Pol II accumulation and Sam68 peaking at the variant exon region of CD44 and enhances variant exon inclusion.

A role for the CTD in the kinetic regulation of alternative splicing was found in recent years [53]. CTD serine 5 and serine 2 hyperphosphorylation beyond normal physiological levels was observed after UV irradiation, and this hyperphosphorylation was coincidental with slow transcription elongation and with an increase of the proapoptotic isoforms of the genes Bcl-x and caspase 9 and of EDI inclusion. Transfection of Rpb1 phosphomimetic mutants with all serines at either position 5 or 2 replaced by glutamic acid, which also exhibit slow elongation rates, produced the same changes in alternative splicing.

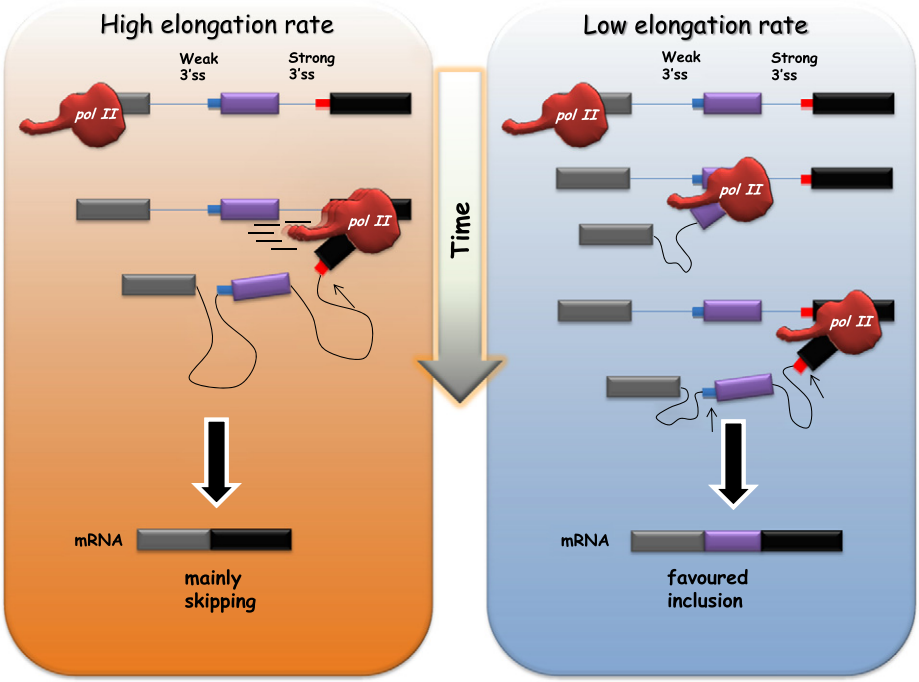

Fig. 1. Kinetic coupling between transcription and splicing. Left panel: in the case of a high RNA pol II elongation rate, both the weak (blue) and the strong (red) 3′ splice sites are presented together to the splicing machinery, resulting in a competition between them. In this scenario, only the strong splice site is recognized (arrow) as a splice site, leading to exon skipping. Right panel: slowing down RNA pol II elongation gives more time to the splicing machinery to recognize the weak 3′ splice site by preventing the competition between the splice sites. Then, the weak 3′ splice site is recognized as a real splice site (indicated by an additional arrow), leading to exon inclusion.

Two new reports show splicing regulator recruitment by the transcriptional machinery as described by the recruitment model but which affect splicing through changes in the elongation rate, in keeping with the kinetic model. Splicing-related transcription elongating factor TCERG1 regulates Bcl-x splicing by relieving a transcriptional pause; interestingly, its recruitment to minigenes is promoter specific [55]. A novel protein complex called DBIRD is shown to bind directly to Pol II and to regulate the splicing of a subset of exons embedded in A–T rich sequence, a substrate that is particularly difficult for Pol II transcription, presumably by facilitating elongation through the A–T rich tracts, as is shown by Pol II accumulation over the regulated exons following DBIRD knockdown [56].

5. Transcription through chromatin structure

In vivo, Pol II elongation is hindered by the chromatin structure. The basic unit of DNA compaction in chromatin is the nucleosome, an octamer of the histone proteins H2A, H2B, H3 and H4 around which less than two turns of DNA are wrapped. Nucleosomes form a barrier to elongating Pol II and they partially disassemble and reassemble in order to allow for transcription. This process depends on the elongation rate [57], again connecting chromatin structure with transcription elongation rate and thus potentially splicing.

A hint of alternative splicing regulation through chromatin structure was the fact that two identical adenoviral genomes inserted in the same nucleus but at different stages of infection showed different stage-specific alternative splicing patterns [58]. Since the change in alternative splicing could not be justified either by differences in the template DNA sequences or in the abundance of cellular trans-acting factors, it was predicted that the cause was a stage-specific change in chromatin condensation of the templates. A transiently transfected reporter minigene for EDI alternative splicing that was allowed to replicate and therefore achieved a more compact chromatin structure showed enhanced EDI inclusion compared to a similar reporter lacking the eukaryotic origin of replication [47], directly linking nucleosome coverage, transcription elongation and splicing outcome in agreement with the kinetic model. Treatment with the histone deacetylase inhibitor trichostatin A (TSA) reverted this effect by promoting a more relaxed chromatin structure [51].

Thanks to the development of high throughput-based methods, these initial findings were extended by a series of outstanding reports that established the role of chromatin in basal and alternative splicing regulation but also the regulation of transcription elongation by splicing-controlled chromatin structure. In the next sections, we will discuss some of the most important recent publications in the field.

5.1. Nucleosome positioning

Genome wide analyses in a number of metazoans [59,60] have shown that nucleosomes are preferentially positioned in the central part of exons; notably, nucleosome positioning is stronger for exons with weak splice sites, whereas intronic sequences flanked by strong splice sites but that are not included in mRNAs show nucleosome depletion; alternative exon nucleosome positioning is proportional to exon inclusion [60]. Consistently, sequences found to disfavor nucleosome positioning were shown to peak at intronic regions that are adjacent to exons. Exonic nucleosome positioning was found to be independent of transcriptional activity, but histone modifications H3K36me3, and to a lesser extent H3K79me1, H4K20me1 and H2BK5me1 peak at exons and are proportional to transcription levels [59]. As nucleosomes are an obstacle for Pol II elongation, nucleosome positioning may enhance exon recognition by causing Pol II pausing and maybe CTD-associated basal splicing factor recruitment to splice sites. Besides, nucleosome positioning allows the addition of specific histone marks by Pol II-associated factors that may indicate the position of exons in subsequent rounds of transcription; accordingly, yeast H3K36 methyltransferase Set2 and its human homolog HYPB/Setd2 catalyze H3K36 trimethylation and are recruited to Ser2P Pol II [61].

5.2. Chromatin and histone marks in splicing regulation

A work that correlated changes in Pol II elongation and alternative splicing with an extracellular signal that triggers chromatin structure modification focused on Neural Cell Adhesion Molecule (NCAM) exon 18, an alternative exon cassette with increasing inclusion levels during neuronal differentiation [48]. Membrane depolarization of murine neuroblastoma cells with KCl caused general histone acetylation and induced NCAM exon 18 skipping, an effect that correlated with an increase of H3K9 acetylation, H3K36 trimethylation, and H4 acetylation, a more relaxed chromatin configuration around exon 18, and enhanced Pol II elongation through the region (Fig. 2).

The potential role of small RNAs in alternative splicing regulation by the establishment of closed chromatin states was highlighted in a recent work [49]. The use of siRNAs to trigger transcriptional gene silencing (TGS) against intronic or exonic sequences around EDI was shown to cause heterochromatin formation around the target sites and an enhancement in EDI inclusion.

More recently, the first evidence for alternative splicing regulation by a heritable epigenetic mark has been presented [62]. DNA methylation at CD45 exon 5 prevents recruitment of CTCF. In the absence of methylation, CTCF binds to exon 5 DNA and creates a transient roadblock to pol II elongation that favors exon 5 recognition at the pre-mRNA level and its inclusion into mature mRNA, constituting a new example of kinetic regulation of alternative splicing by endogenous factors (Fig. 2).

5.3. Regulation of transcription by splicing

It has long been known [63] that the presence of introns has a stimulatory effect on transcription, although the reasons of this phenomenon are controversial, as early work by Fong and Zhou [64] indicating a role for TAT-SF1-interacting snRNPs in enhancing elongation rate was challenged by a recent report that shows the same elongation rate for intron-containing and intronless genes as measured by FRAP [21]. Lin and colleagues [65] have shown that the SR proteins SRSF1 and SRSF2 are necessary for normal transcriptional activity and that SRSF2 depletion causes Pol II accumulation along a number of genes. They have also showed that SRSF2 binds CDK9, the kinase subunit of P-TEFb, and the elongation factor TAT-SF1, and that serine 2 phosphorylation is dependent of SRSF2.

Recently, de Almeida and colleagues [66] showed that H3K36 trimethylation levels are higher in intron-containing genes, a difference that is not due to nucleosome occupancy, and that HYPB/Setd2 recruitment is impaired by splicing inhibition, in agreement with genome wide analyses of nucleosome positioning that link this histone modification to exons. In parallel, Kim and colleagues [67] showed that intact splice sites are necessary for the maintenance of H3K36 trimethylation patterns. Mutant splice sites or the use of spliceostatin A produce a shift towards the 3′ end of genes in the peaks of H3K36 methylation.

Zhou et al. [68] show an interesting case of alternative splicing regulation of transcriptional activity through local chromatin modification. The four members of the Hu protein family are splicing regulators that bind to U- or AU-rich sequences and change the level of inclusion of target exons. Hu proteins interact with both Pol II and CDK9, and, interestingly, they interact with histone deacetylases HDAC1 and HDAC2 and are shown to inhibit HDAC2 in vitro, and upon binding to their target sites promote local hyperacetylation of histones and exon skipping by locally enhancing Pol II elongation.

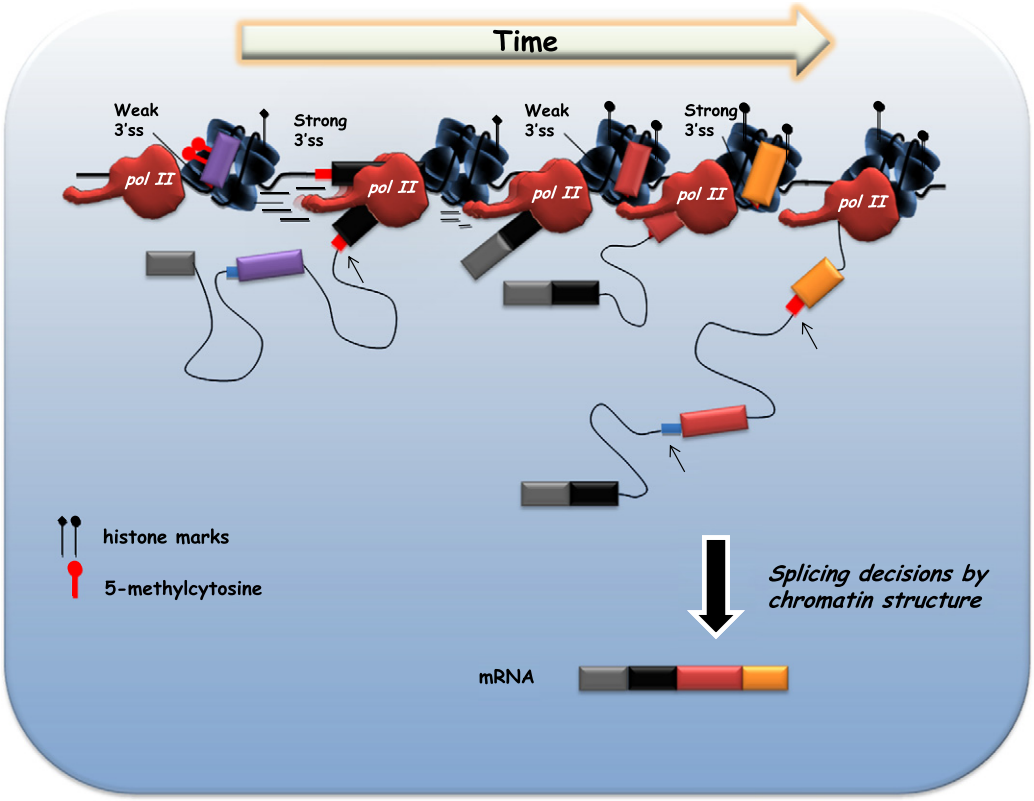

Fig. 2. Chromatin affects transcription-dependent splicing. A five-exon gene containing two alternative exons due to weak 3′ splice sites (blue) is present. At the beginning of the gene, a loose chromatin structure or, as in the case of CD45 exon 5 [62], the presence of 5-methylcytosine allows for a fast elongation rate leading to the skipping of the first alternative exon, only the strong splice site of the following exon being recognized by the splicing machinery (arrow). Further downstream, the more compact chromatin structure, due to histone modifications, induces slow down of RNA pol II elongation allowing for the recognition of the weak 3′ splice site (depicted by the yellow exclamation marks), leading to exon inclusion in agreement with the kinetic model of cotranscriptional splicing presented in Fig. 1.

6. Concluding remarks

RNA splicing is found to be predominantly cotranscriptional, as an ever growing corpus of evidence shows for a variety of eukaryotes. Cotranscriptional recruitment of splicing factors makes cross-regulation between splicing and transcription possible, and in particular it allows for transcriptional control of alternative splicing choices, expanding the possible mechanisms of regulation of this important step in gene expression. In contrast, the timing of intron cleavage once a particular stable spliceosomal complex is assembled does not seem to be so crucial. Though the order of intron removal follows loosely a 5′ to 3′ order, each gene displays a preferred pathway of intron removal that is seldom exactly linear [69]. Alternative exons were reported to be spliced post-transcriptionally more often than constitutive exons, a fact that has been accounted for by the need for simultaneous presentation of the competing sites. The very quick splice of exons to the next available 3′ ss would lead to the constitutive inclusion of all exons, abolishing the possibility of alternative splicing. On the other hand, simultaneous presentation of all the regulatory sequences is incompatible with kinetic regulation of alternative splicing, and in their study of intron removal Pandya-Jones and Black see quick elimination of the introns surrounding the kinetically regulated FN EDI [23].

In a similar study [70], the excision of introns around EDI was monitored under a number of conditions that affect its inclusion, such as mutagenesis of *cis* regulatory sequences, SR protein overexpression or reduced Pol II elongation. Notably, slowing down transcription affected EDI splicing without altering the order of removal of the introns around EDI, indicating that slow elongation allows the weak splice site upstream of EDI to recruit splicing factors and hence to create a commitment for EDI inclusion, without affecting the subsequent kinetics of intron removal.

The prevalence of cotranscriptional splicing does not necessarily reflect a prevalence of transcriptional regulation of alternative splicing. Until not long ago, AS events were explained in terms of tissue- or stage-specific expression of regulatory factors that bound target sequences independently of transcription, and coupling was seen only in highly artificial reporter systems. While AS uncoupled from transcription may still be the prevailing pathway in living organisms, examples of a different sort of regulation are starting to emerge. Three different stimuli, namely membrane depolarization [48], MAP kinase pathway activation [54], and UV irradiation [53], were shown to elicit changes in alternative splicing of endogenous genes of otherwise untreated cells. While the doses used may not be on scale with physiological stimuli, it is not at all unlikely that the pathways of kinetic splicing regulation found to operate in these three cases represent genuine pathways of AS regulation. TCERG1, previously known as CA150, was first described as a transcriptional elongation factor. It directly binds Pol II phospho CTD [71], and in a recent work it was shown to regulate Bcl-x splicing by changing Pol II elongation [55]. Interestingly, TCERG1 is subject to sumoylation [72] and there is evidence of reduced TCERG1 transcriptional activity upon SUMO binding, suggesting the possibility that TCERG1 may act as a link between a particular post-translational modification and a specific change in alternative splicing through elongation. The newly characterized DBIRD complex [56] and the splicing regulators of the Hu family [68] provide more examples of kinetic regulation of alternative splicing of cellular genes by endogenous factors. Notably, both DBIRD and Hu proteins seem to target defined sequence motifs and affect discrete sets of exons. Hu proteins are particularly interesting in the sense that they seem to affect alternative splicing by inducing small scale changes in chromatin, something already seen for NCAM exon 18 after depolarization.

Taken together, all these evidences suggest that kinetic coupling of transcription and alternative splicing is indeed a relevant regulatory pathway in living organisms.

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