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Review

Chromatin, DNA structure and alternative splicing

Nicolás Nieto Moreno, Luciana E. Giono, Adrián E. Cambindo Botto, Manuel J. Muñoz, Alberto R. Kornblihtt *

Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-UBA-CONICET) and Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, C1428EHA Buenos Aires, Argentina

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ABSTRACT

Coupling of transcription and alternative splicing via regulation of the transcriptional elongation rate is a well-studied phenomenon. Template features that act as roadblocks for the progression of RNA polymerase II comprise histone modifications and variants, DNA-interacting proteins and chromatin compaction. These may affect alternative splicing decisions by inducing pauses or decreasing elongation rate that change the time-window for splicing regulatory sequences to be recognized. Herein we discuss the evidence supporting the influence of template structural modifications on transcription and splicing, and provide insights about possible roles of non-B DNA conformations on the regulation of alternative splicing.

1. Introduction

The unpacked DNA content in a single nucleus of human diploid cells is around 2 m long, since the distance between consecutive base pairs is 0.34 nm and our genome is 6×10^9 bp-long. Thus, to fit inside a ≈10 μm-diameter nucleus, DNA is highly condensed in a nucleoprotein complex named chromatin, organized in nucleosomes, each of which wraps 147 bp of DNA in two turns around a histone octamer, which implies a 40-fold DNA length reduction. These octamers are composed by two H3–H4 and two H2A–H2B dimers, and an additional histone H1 that protects the internucleosomal DNA linker. Further condensation is obtained by supercoiling of DNA. This condensation is not arbitrary, and DNA inside the nucleus is organized in functional domains comprising regions that are transcribed and regions that remain silent [1].

Gene expression, as other processes involving the information encoded in DNA, implies local and transient changes in chromatin structure: nucleosomes have to disassemble and the DNA double helix has to melt for RNA polymerase II to generate a complementary RNA strand. Parameters like the underlying DNA sequence and its chemical modifications, chromatin composition and compaction, as well as post-translational modifications (PTMs) of histones, affect transcription by two means: either by the specific recruitment of factors that interact with RNAPII, the nascent RNA or chromatin itself, or simply by affecting RNAPII elongation rate along genes.

This review will focus on DNA and chromatin structures, transcriptional regulation and their influence on mRNA processing.

2. Transcription

RNA polymerase II (RNAPII) is the enzyme responsible of the synthesis of mRNA and many non-coding RNAs (ncRNAs). Similarly to RNA polymerases I and III, the other two mammalian DNA-dependent RNA polymerases, RNAPII has a large subunit in which resides the catalytic activity, and a set of other essential structural subunits. Distinctively, its large subunit, Rpb1, has a rather unique carboxy-terminal domain (CTD) composed of 52 repeats of the heptapeptide consensus YSPTSPS [2,3]. This domain acts as an interacting platform for factors involved in regulation of transcription and the four main processes coupled to it: capping, splicing, polyadenylation [4] and termination [5].

The 7 amino acids composing each CTD repeat can suffer post-translational modifications (PTMs). Tyrosines, threonines and serines can be phosphorylated. Threonines and serines can be glycosylated. Non-consensus residues, such as arginines and lysines can be ubiquitylated, acetylated, SUMOylated or methylated. Also, prolines can be isomerized between their cis- and trans-conformations [4,6]. These PTMs have been shown to be essential to modulate CTD's interactions with its partners, and therefore

* Corresponding author.
E-mail address: ark@fbmc.fcen.uba.ar (A.R. Kornblihtt).

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act on the regulation of transcription and pre-mRNA processing. For more detailed reviews on RNAPII CTD, transcription and processing, see [2,4,6].

As the first step of gene expression, transcription of DNA into mRNA by RNAPII is a tightly regulated process. It starts with the binding of TBP to the promoter and the subsequent binding of other General Transcription Factors (GTFs) in a stepwise fashion. RNAPII is recruited, constituting the pre-initiation complex (PIC). Following, TFIIE and TFIIH are added to the PIC and allow promoter melting to start transcription [7]. Within the first 30–60 bp downstream transcription start site (TSS) RNAPII tends to get paused and accumulate in a highly stable complex in what is known as the promoter proximal pause. Paused RNAPII is stabilized by NELF and DSIF. Release of this pause is triggered by the action of the transcription elongation factor P-TEFb that phosphorylates NELF, DSIF and RNAPII CTD in Ser2 [8]. Interestingly, it was recently published that this checkpoint that allows the switch to productive elongation is not limited to genes exhibiting a promoter proximal pause, but is a general feature of all genes [5].

For years, a simplistic model has been accepted referring to RNAPII phosphorylation, according to which Ser5 phosphorylation (Ser5P) by Cdk7, as part of TFIIH was a hallmark of transcription initiation, whereas Ser2P by Cdk9, as part of P-TEFb, was the hallmark of elongation. However, the actual picture is much more complex. The Carmo-Fonseca and Proudfoot groups recently reported a high-throughput analysis of RNAPII phosphorylation based on native elongating transcript sequencing (NET-seq) [9]. They found that the CTD is poorly phosphorylated at the transcription start site, but acquires both Ser2P and Ser5P at the beginning of the gene. Besides, at the end of the gene, Ser2P becomes predominant. Surprisingly, they detected high peaks of Ser5P in exons, suggesting that this modification may induce a pause in transcription to allow the nascent exon to be recognized by the splicing machinery. Though fundamental, this approach still does not provide information about the association of different combinatorial CTD modifications and the dynamics of transcription.

The average elongation rate of RNAPII along the genes is around 2–3 kb/min, but its actual speed is highly variable. In fact, GRO-seq analyses showed a 4-fold variation in elongation rates of different 17-β estradiol and TNF-α inducible genes. Moreover, RNAPII increases its elongation rate as it transcribes along the genes [10], presumably a consequence of a gradual maturation of the elongation complex. John Lis' group confirmed this in a genome-wide scale using GRO-seq in combination with RNAPII initiation or promoter-proximal pause escape inhibition with triptolide or flavopiridol, respectively. This allowed them to perform a multivariate analysis to determine the influence of different gene features in RNAPII elongation rates. Exon density, CpG content and methylation, as wells as H3K79me2 levels, showed up as the main factors that regulate elongation, while the contribution of other factors like promoter architecture, nucleosome occupancy and other chromatin marks appeared to be secondary. Moreover, when focusing on what happens at specific points in the gene body, they noticed a decrease in elongation rate in exons [11,12], consistent with a role of elongation rate in splicing. This result is in agreement with the splicing-dependent pause in transcription reported by Beggs and colleagues [13].

3. Transcription and alternative splicing

Splicing of pre-mRNA consists in the concerted removal of internal sequences of the pre-mRNA (i.e. introns) and ligation of sequences that are part of the mature mRNA (i.e. exons). This process is catalyzed by the spliceosome, a ribonucleoprotein complex [14] highly conserved in eukaryotes, and occurs in virtually all of our genes. Alternative splicing is the mean by which two or more different mRNAs can be produced from a single gene by the alternative inclusion/exclusion of particular sequences of the pre-mRNA. Thus, a single gene can produce protein isoforms with different and even opposite functions. As an example, the exclusion of an alternative sequence of Bcl-x gene results in a shorter protein that acts as a dominant negative, competing with the full-length protein that has a role in promoting apoptosis [15]. It is estimated that around 95% of our genes are affected by alternative splicing, the main mechanism by which a huge diversity of proteins is obtained from a limited number of genes. This property helps to explain the fact that animals of different complexity levels have a similar number of genes. For instance, while humans have 19.000 genes, *Drosophila melanogaster* has 14.000 genes and *Caenorhabditis elegans* has 20.000 genes, the proportion of genes affected by alternative splicing is much higher in humans (95%) [16], than in Drosophila (46%) [17] or *C. elegans* (25%) [18].

As mentioned above, RNAPII elongates the nascent transcript at an average of 2–3 kb/min, but its speed suffers dramatical changes along the gene. Diverse trans- and cis-acting factors have been reported to regulate RNAPII elongation, either by steric interaction, or by acting as co-activators/co-inhibitors or introducing PTMs to the elongation complex. Multiple lines of evidence confirm that splicing is mostly co-transcriptional. These include deep-sequencing of nascent transcripts [9,19] and the detection of mRNAs already spliced, components of the spliceosome and splicing factors in the chromatin fraction [20–25]. Moreover, pre-mRNA splicing is coupled to transcription, meaning there is a crosstalk between these two processes by which they influence each other [26]. Although coincidence in time of transcription and splicing is probably a need for coupling, simultaneous occurrence of these processes is not sufficient to guarantee the mutual influence of the transcription and splicing machineries [27].

Coupling of transcription and splicing has been demonstrated by the fact that transcription by an RNAPII lacking its CTD results in inefficient splicing [28]. This was further confirmed by the finding that the same transcriptional unit, comprising an alternative exon, shows different alternative exon inclusion/exclusion ratios under the control of different RNAPII promoters [29,30]. Also, transcription factors [31,32], coactivators [33], transcription enhancers [34], and proteins with dual activity as transcription and splicing factors [35] as well as chromatin remodelers [36] and factors that alter chromatin structure [37–39] modulate alternative splicing. Two alternative models have been proposed to explain the coupling of transcription and alternative splicing: the kinetic coupling model and the recruitment model (Fig. 1). The proposal of two alternative models has a rather illustrative objective, for it is evident that not only these models are not mutually exclusive, but also what most probably happens is a combination of both.

One of the determining factors in coupling of transcription and splicing is the kinetics at which transcript elongation occurs. As splicing sites and regulatory sequences on the RNA (splicing enhancers and silencers) emerge, these are recognized by the spliceosome machinery and splicing factors. Sequences that induce pauses on transcription [40], as well as chromatin compaction and presence of factors that block RNAPII [39], increase the time window for weak splicing sites to be recognized before a strong site located downstream is synthesized (Fig. 1A). On the other hand, a high elongation rate gives more chances for strong sites to be recognized over weak sites.

Depending on the context, low elongation rates or transcriptional pauses within an alternative exon or its downstream intron can induce an increase or a decrease in exon inclusion. For instance, if the alternative exon is preceded by a weak 3′ splice site (3′-SS) and the downstream exon by a strong 3′-SS, a low elongation rate will promote exon inclusion, as happens with the exon

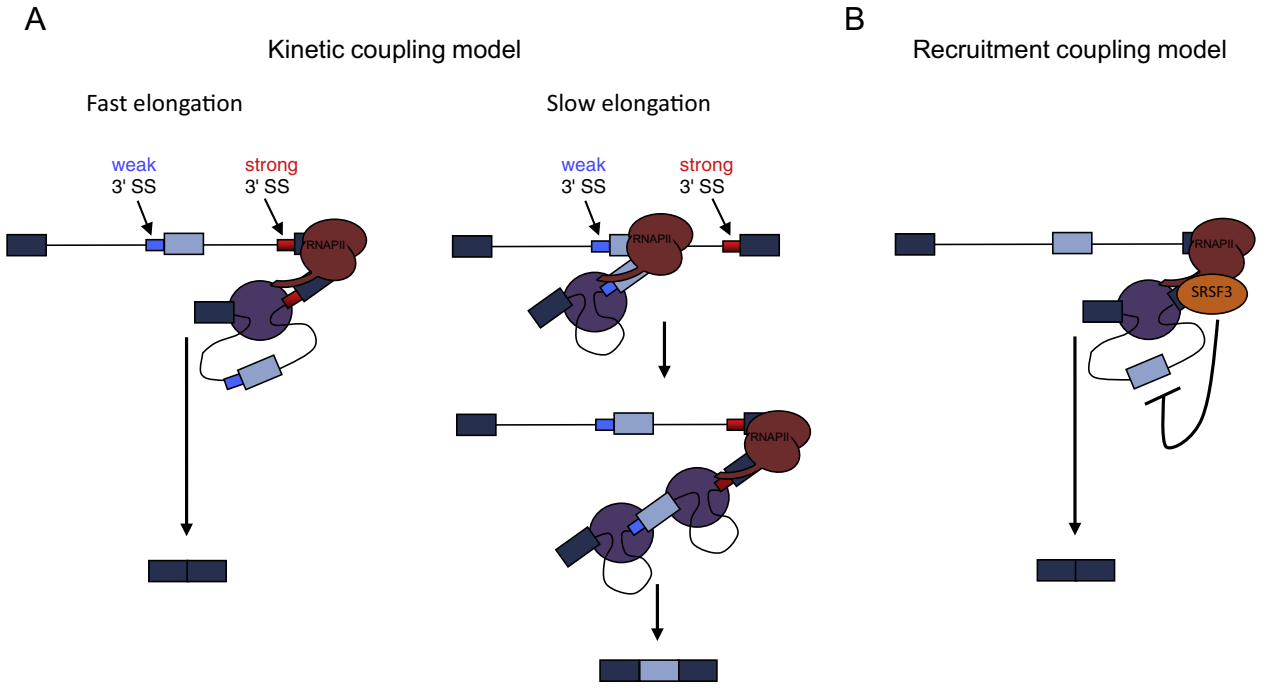

Fig. 1. Coupling of transcription and alternative splicing can be explained by two non mutually exclusive models. (A) The rate of transcriptional elongation can affect alternative splicing decisions in the context of the kinetic coupling model. In the example represented, an alternative exon is flanked by a weak 3′ splice site (3′-SS) followed by a constitutive exon flanked by a strong 3′-SS. At high transcriptional elongation rates, there is a short time window between the synthesis of both 3′-SS, and the strong 3′-SS competes with the weak 3′-SS for the commitment of the splicing machinery, promoting alternative exon skipping. On the other hand, when the transcription elongation rate is low, there is more time for the weak 3′-SS to be recognized before the strong 3′-SS is synthesized, promoting exon inclusion. (B) In the recruitment model, splicing factors that are recruited to the transcription machinery can affect alternative splicing decisions. In the example depicted, the splicing factor SRSF3 interacts with RNAPII CTD and inhibits the inclusion of the alternative exon into the mature mRNA.

33 (E33), of the human fibronectin gene. If both exons have strong splice sites, but inclusion of the alternative exon is negatively regulated by an splicing silencer placed in the upstream intron, a low elongation rate will increase the opportunity of this regulatory element to be recognized favoring exon skipping. This is the case of exon 9 of the CFTR gene, whose exclusion is induced by a low elongation rate [41].

The kinetic coupling of transcription and alternative splicing has been corroborated by multiple approaches. In 1998, Roberts et al. showed that the insertion of DNA sequences that induce pauses in transcription modulate alternative splicing. Their working model was a minigene containing the alternative exon 3 of α-tropomyosin, whose splicing is modulated by two regulatory elements, one upstream and one downstream of exon 3 (named URE and DRE, respectively). Exclusion of exon 3 is mediated by the binding of a protein complex to both regulatory elements. Thus, they reasoned that a delay in the synthesis of the DRE would result in higher inclusion of exon 3. To test this hypothesis, they cloned, in the downstream intron, but upstream the DRE, three different DNA sequences previously reported to induce pauses in transcription: the C2 and α2 elements of the human complement C2 and α-globin genes and a four tandem repeat of G_5AG_5 (MAZ_4), the consensus sequence for the binding of MAZ zinc finger protein. Consistently with their hypothesis, all three pause elements induced an upregulation of exon 3 inclusion [40], thus becoming the first evidence of pause elements affecting alternative splicing decisions. Alternative splicing regulation by a transcription pause site was also shown to happen in Bcl-x gene, in which the factor TCERG1 promotes elongation and reduces the effect of this pause [42].

Further evidence of kinetic coupling was given by the use of drugs or agents that inhibit elongation, such as UV irradiation of the cells [43], DRB and campothecin [44], as well as by the use of drugs that loosen chromatin structure, and thereby allow a faster elongation speed [39] or factors that promote elongation [32]. The most direct demonstration of the kinetic coupling implied the use of slow RNAPII mutants. One of these polymerases was initially described in *D. melanogaster* [45], and bears a single amino acid substitution in the large catalytic subunit Rpb1. Greenleaf and coworkers showed that the slower elongation rate of this RNAPII mutant, named C4, is not due to lower catalysis, but to higher difficulties to overcome pauses, and its decreased elongation rate has been confirmed to occur in vivo [46]. Transcription by this polymerase increased fibronectin E33 inclusion, consistently with the kinetic coupling model. Also, it alters alternative splicing of the gene *ultrabithorax* (Ubx) in *D. melanogaster* [47]. Thus, this polymerase became a suitable and versatile tool to evaluate kinetic effects on alternative splicing, and reinforces the idea that transcriptional pauses play an important role in alternative splicing decisions.

On the other hand, the transcriptional machinery can interact with factors that affect splicing, which illustrates the recruitment model of coupling. The carboxy-terminal domain of RNAPII (CTD) plays a fundamental role [4], as it works as an interacting platform for factors that affect splicing [48]. Interestingly, these factors can be recruited even in the transcriptional promoter [49]. An illustrative example of the role of the CTD in the recruitment of splicing factors is given by the SR protein SRSF3 (formerly named SRp20): this splicing factor inhibits E33 inclusion and its knockdown prevents this effect promoting its inclusion (Fig. 1B). When the gene is transcribed by a mutant RNAPII lacking the CTD, knockdown of SRSF3 has no effect, suggesting that its interaction with the CTD is necessary to recruit SRSF3 [50]. Although constitutive splicing occurs co-transcriptionally, catalysis of alternative splicing is often

post-transcriptional [51]. Nevertheless, coupling is given because factors that alter splicing are recruited co-transcriptionally to the nascent pre-mRNA [52].

An example in the interface of both models is given by the DBIRD complex. This complex interacts with the nascent transcript and the transcribing RNAPII and promotes skipping of an alternative exon by enhancing elongation through AT-rich sequences. When DBIRD components are knocked-down, RNAPII elongates slower through these regions and alternative splicing patterns change [53].

4. How the template affects transcription and alternative splicing

As discussed above, RNAPII elongation rate and pauses imposed to transcription constitute important factors in the regulation of alternative splicing. Thus, in this section we will discuss the DNA and chromatin features, such as histone variants and PTMs, DNA methylation and DNA structure that affect transcription and therefore play a role in alternative splicing decisions.

4.1. Histone modifications and nucleosomes

Chromatin constitution has become, in the last years, one of the most studied factors that modulate transcription. Histones suffer PTMs such as methylation, acetylation, phosphorylation and ubiquitylation, among others. These PTMs can occur both in the amino acids forming part of the core of histones or in their protruding amino-terminal tails (also named histone tails), being the latter the most studied. Histone core PTMs affect chromatin structure by modifying the histone–DNA and histone–histone interactions and thereby altering the compaction of chromatin, what in turn leads to changes in regulation of processes involving DNA, including transcription. Also, these modifications may affect the binding of histone chaperones [54]. The histone tails are 40 amino acid domains that protrude from the nucleosomes. These domains, rich in basic amino acids, are subject of a variety of PTMs. Lysine modifications on histone tails, in particular acetylation and methylation, are the best characterized PTMs. Arginines can also be methylated, and histone tails suffer other modifications such as phosphorylation, glucosamination, ubiquitylation, SUMOylation and polyADPribosylation. These modifications act as adaptors for the binding of proteins. For example, the chromatin remodeler CHD1 binds to tri-methylated lysine 4 of H3 [55], while heterochromatin protein 1 (HP1) binds to H3K9me3 [56]. It is unclear whether modifications of histone tails have an effect *per se* aside from the recruitment of specific factors. Some authors claim that the change in histone tails net charge by acetylation or phosphorylation affects the electrostatic interaction of histones with DNA. In this sense, acetylation of lysine ε-amino residue turns a positive charge into a non-charged chemical group and this is supposed to open chromatin by weakening DNA-histone interactions [57]. Others argue that histone tail modifications may modulate internucleosomal interactions or act as adaptors for the binding of specific effectors, that in turn act as chromatin remodelers, transcription regulators, etcetera [58].

Nucleosomes act as physical roadblocks for progression of transcription, as DNA wrapped around histones has to be unwrapped for RNAPII to transcribe it. Indeed, the first nucleosome in the transcribed region (also named the +1 nucleosome) is highly positioned and has a role in promoter proximal pausing [59]. RNAPII dynamics through nucleosomes has been studied in detail by Carlos Bustamante's group, with an in vitro system of transcription [60]. In this study, they observed that nucleosomes impose a barrier for RNAPII progression, increasing pause density and duration and decreasing RNAPII apparent velocity. According to their model, when RNAPII finds a nucleosome on its way along DNA, it tends to pause and backtrack. RNAPII lacks the ability to unwrap nucleosomes, so it has to happen either spontaneously or via the action of chromatin remodelers. But what happens with the histones and how do nucleosomes re-assemble when RNAPII transcribes a region? It has been proposed that DNA forms a loop that allows DNA behind RNAPII to interact with the histones of partially unwrapped nucleosomes. This would permit an *in cis* histone transfer to DNA, upstream the original position of the nucleosome, and guarantee the correct re-establishment of nucleosome positioning after transcription. Other in vitro studies performed by Michelle D. Wang's group, suggest that the positive torque exerted by RNA polymerase on DNA can contribute to destabilize H2A-H2B dimers from nucleosomes, and thus favor their disassembly in the way of RNAPII [61]. In any case, nucleosome disassembly and reassembly in vivo are probably facilitated by the action of chromatin remodelers and histone chaperones [62]. In a more recent publication, Bustamante's group used their in vitro transcription system to study in detail the influence of different histone tail modifications and direct histone-DNA interactions on transcription [58]. Besides, DNA sequence *per se* can inhibit the progression of transcription [63], and this effect seems to be amplified by nucleosomes. Nascent RNA, on the other hand, reduces pause duration and prevents backtracking, as observed when RNase A is added to this in vitro transcription system [58], probably because it stabilizes the elongation complex. Not to be ignored, our genome encodes histone variants, such as H2A.Z and H3.3, that can replace canonical histones and change nucleosome stability, as well as give place to more compact or loose nucleosome associations, by changing their chemical properties or recruiting particular remodeling factors and histone chaperones [62,64]. Expectedly, there is a crosstalk between transcription and chromatin remodeling, as transcription itself can recruit chromatin remodeling factors and histone modifying proteins [65].

In summary, histone variants and histone tail PTMs can either silence or activate gene expression and influence alternative splicing depending on their effect on chromatin structure or the factors they recruit. In this sense, particular PTMs have been characterized as transcription repressing or activating marks [65]. It is easy to envision how changes in chromatin compaction can affect alternative splicing through kinetic coupling with transcription, as a more closed chromatin will result a more difficult template for RNAPII to transcribe. In fact, a role of chromatin constitution in alternative splicing regulation was first shown by our group in 2001. Using a replicative plasmid harboring an alternative splicing reporter minigene, it was observed that the replication of the plasmid, a process that results in a correctly structured chromatin within the plasmid, provokes higher exon inclusion levels in the mature mRNA. These results suggested that chromatin would impose pauses and delays in transcript elongation that, in turn, would affect alternative splicing in the paradigm of the kinetic model of coupling [31]. A role of chromatin structure in alternative splicing was later confirmed by our group and others. For instance, it was observed that upon neuronal cell depolarization, nucleosomes around the alternative exon 18 of NCAM gene get hyper-acetylated in H3K9 (H3K9ac), leading to an increase in exon skipping. H3K9ac is a hallmark of relaxed chromatin and this change in alternative splicing is consistent with a higher elongation rate of RNAPII modulating alternative splicing [39]. In turn, fibronectin E33 inclusion is favored by a tighter chromatin conformation, driven by siRNA-directed di-methylation of H3K9me2 and tri-methylation of H3K27 close to the alternative exon [66]. Another report shows that H3K9 methylation recruits HP1γ, leading to a more compact chromatin due to internucleosomal interactions that, in turn, results in a decrease in RNAPII elongation rate and affects alternative splicing decisions [38]. Finally,

histone PTMs can also act as adaptors for the binding of factors that regulate splicing: MRG15 binds to H3K36me3 and modulates alternative splicing events via the recruitment of the splicing factor PTB to the nascent mRNA [37].

Genome-wide analyses show that nucleosomes are preferentially positioned in exons, which is consistent with the fact that the average size of an exon is around 150 bp, almost the same length of DNA that forms part of a nucleosome. Moreover, exonic nucleosomes are enriched in particular histone marks, as H3K36me3 [67–69]. Interestingly, there is a negative correlation between exon positioning, histone marks and the strength of the splice sites, suggesting these features may help recognize exons flanked by weak splice sites [69,70]. Despite nucleosomes have a preference for GC sequences, and human exons have a higher GC content, the nucleosome enrichment in exons is only partially explained by this sequence bias [67,69]. Interestingly, this enrichment of nucleosomes in exons is also seen in non-transcribed genes, suggesting nucleosome marking of exons is independent of transcription [69]. This feature is conserved across species, including *C. elegans*, *D. melanogaster* and mammals.

Regarding alternative exons, histone marks are reduced when compared to constitutive exons [71]. This is consistent with histone marks and nucleosomes contributing to exon recognition by a decrease of elongation rate [11]. For a detailed review on alternative splicing and chromatin modifications refer to [72].

4.2. DNA methylation

Eukaryotes can methylate DNA cytosines in C5 (5mC). In particular, 5mC is widespread in vertebrate genomes, contrasting with what happens in plants and invertebrates, where it is restricted to repetitive DNA sequences and actively transcribed loci. In mammals, methylation occurs primarily on CpG dinucleotides, although in neurons, embryonic stem cells and oocytes it also happens in other sites. CpG islands are genomic elements enriched in mammalian promoters and their hypermethylation leads to the repression of the downstream gene, presumably due to a more compacted chromatin. Indeed, heavily methylated DNA is observed in transcriptionally repressed loci, like imprinted genes and inactive X chromosome in mammals, and it is a hallmark of constitutive heterochromatin [73].

DNA methylation is established and maintained by DNA methyltransferases (DNMTs), a family of highly conserved proteins. DNA demethylation competes with DNA methylation and it can happen in a passive way, due to inefficient remethylation after replication or by an active pathway comprising the progressive oxidation of 5mC ending in the reestablishment of unmethylated cytosine [73]. Refer to [74] for an up-to-date review on DNA methylation, that includes the proposal of an stochastic DNA methylation model.

DNA methylation has been shown to influence alternative splicing decisions. In this sense, two articles show opposite effects of DNA methylation. Whereas DNA methylation of sequences coding for alternative exons can promote exon inclusion via recruitment of MeCP2 [75], DNA methylation can also promote alternative exon skipping by preventing the recruitment of the chromatin-insulating factor CTCF, that would act as a roadblock for RNAPII elongation [76].

4.3. DNA conformation

The canonical structure of DNA, published in 1953 by Watson and Crick, also called B-DNA structure, is a double helical right-handed molecule and the most common conformation adopted in the eukaryotic cell nucleus. Nevertheless, two other double helical DNA structures have been described: A-DNA, a right-handed, broader and more compressed along its axis conformation; and Z-DNA, a left-handed, thinner and less compressed conformation. These three DNA forms differ in many parameters, including angles formed by the base pairs, conformation of the pentose ring, geometry of the glycosyl bond and depth of minor and major grooves, among others [77]. It is thus expectable that DNA conformation will affect the quality and quantity of DNA interactors, as well as the transcriptional process [78]. Other non-B DNA structures will be discussed below.

A- and B-DNA actually constitute a family of conformations with intermediate structures [79]. Though in vitro A-DNA conformation is favored by low humidity and high salt concentration, it does also occur in vivo. For example, the TATA box consensus sequence is supposed to be prone to adopt an A-like conformation, and this conformation is an important intermediate step in the formation of the characteristic structure induced after the binding of TBP [80]. On the other hand, Z-DNA formation implies a radical change in DNA structure, passing from a right-handed to a left-handed double helix, and thus contributes to alleviate negative supercoiling. Its formation is specially biased by the sequence, occurring in $(CG)_n$ and $(TG)_n$ tracts [81]. Additionally, DNA methylation on C5 of cytosines lowers the free energy difference from B-DNA to Z-DNA, resulting in an increase on Z-DNA population [82]. There are proteins that specifically bind to Z-DNA and the first one to be described was ADAR1, a dsRNA adenosine deaminase. This protein is recruited to Z-DNA formed in the proximity of a transcribing RNAPII, and edits the nascent RNA turning adenosines into inosines [81]. It is important to note that DNA conformations coexist within the same chromosome, and even within the same gene. Thus, while transition from B to A-DNA can be explained by a tightening of the DNA helix, B to Z-DNA transition implies an inversion in the helix sense, that results in two bases extruded from the double helix [83].

As Z-DNA, there are other non-B DNA conformations that reduce DNA molecule free energy due to negative supercoiling, i.e. cruciform DNA, triplex (or H-DNA), G-quadruplex and slipped DNA [1]. The formation of these structures is dependent on the DNA sequence, being cruciforms formed in inverted repeats (i.e. palindromic sequences) of more than 6 nucleotides long. Triplex DNA is formed in mirror repeats, in which the double helix of one of the repeats melts and one strand of it forms a triple helix stabilized by Hoogsteen bonds (a type of hydrogen bonds between bases different from those described by Watson and Crick) with the other repeat, leaving a single strand of DNA. G-quadruplexes are non-helical structures formed by guanine tetrads interacting by Hoogsteen bonds, stacked and stabilized by monovalent cations. Slipped DNA forms in DNA repeats with no spacer and creates two strands of single-stranded DNA [84,85]. Non-B DNA conformations differ from the B-DNA substrate RNAPII uses as template, so they may play a role in alternative splicing by affecting elongation rates or inducing pauses in transcription. Additionally, these structures have effects on the chromatin state. Nucleosome formation is impaired in non-B DNA, both due to its physical characteristics and also because of the specific proteins that bind to these particular structures [86].

Many studies have been performed to assess a role for triplex formation on transcription. As it is deeply reviewed by Van Dyke [87], triplex DNA can either impede the binding of specific factors to their target DNA sequences, thus promoting or inhibiting transcription, or have a direct effect by arresting RNAPII elongation. Indeed, a wide variety of in vitro and in vivo studies suggest a transcriptional inhibitory role for triplex DNA. This was observed invading specific DNA sequences with triplex forming oligonucleotides (TFOs), or by assessing the roles of the naturally occurring inter- or intra-molecular triplexes. Indeed, a recent publication of the Hanawalt group shows that triplex DNA can block RNAP T7

elongation in vitro [88]. Additionally, triplex DNA can act as an anchor for the binding of chromatin modifying factors [89]. Regarding G-quadruplexes, when located in the template strand they may cause a steric blockage of transcription, impeding RNAPII elongation [85]. G-quadruplex forming sequences (G4 motifs) are located in around 50% of our gene promoters with a particular enrichment in oncogenes and regulatory genes versus housekeeping or tumor suppressor genes [90], and are predominant in the non-template strand of our genes. A crosstalk between non-B DNA and chromatin features seems likely, as nucleosomes and CpG methylation are depleted in G4 motifs [91], and cells deficient in Rev1, a protein involved in G-quadruplex denaturation during replication, loose certain histone marks around G4 motifs, such as H3K4me3 and H3K9/14ac [92]. Besides, as well as G-quadruplexes or triplex DNA, the single-stranded DNA that results from the formation of these structures can recruit particular factors that promote or inhibit transcription, as it happens with hnRNP K binding a C-rich tract of the VEGF promoter [93]. Refer to [86] for a detailed review on how non-B DNA structures might interfere with transcription.

Although scarce evidence exists on whether non-B DNA conformations affect alternative splicing, the fact that non-B DNA has been shown to play roles in intragenic transcriptional dynamics makes investigation of alternative splicing regulatory mechanisms involving non-B DNA timely and interesting. A bioinformatics analysis showed a strong genome-wide correlation between non-B DNA forming sequences and alternative exons, suggesting that alternative splicing could be regulated by non-B DNA structures, either acting as a roadblock for RNAPII or as a platform for the binding of regulatory factors [84]. In this sense, it was shown that the splicing factor U2AF65 has affinity to triplex DNA [94]. However, not only proteins binding to these non-canonical nucleic acid structures may play a role in transcription and splicing: activity of helicases that resolve these structures, such as XPD/XPB [95], ChIR1 [96], BLM [97] or FANCJ [98], may modulate alternative splicing decisions. Further research has to be made in this area to get a clearer picture of the role of non-canonical nucleic acid structures in alternative splicing.

Transcription of G-rich sequences tends to form a highly stable DNA:RNA hybrid named R-loop. The co-transcriptional recruitment of splicing factors to the nascent pre-mRNA restricts its interactions with the template DNA and prevents R-loop formation, which can in turn be resolved by RNase H activity or by specific helicases [99]. These DNA:RNA hybrids can have an effect on transcription. In fact, Hanawalt and colleagues demonstrated that a G-rich homopurine stretch at the non-template strand blocks transcription by T7 RNA polymerase in vitro, presumably via the formation of R-loops. However, the mechanism is not clear, and it may be a consequence of RNA polymerase destabilization by a stable R-loop formation, or a collision of RNA polymerase with RNA:DNA hybrids formed previously in the template DNA [63]. Besides, R-loop formation can lead to the deposition of repressive chromatin marks as observed in mammalian gene terminators, thereby affecting transcription in an indirect manner [100]. Therefore, one could speculate that R-loop formation might lead to changes in alternative splicing decisions by affecting RNAPII elongation rate, either directly acting as roadblocks, or by inducing changes in chromatin constitution.

Importantly, these non-canonical structures are not exclusive of DNA and also occur in RNA. Regarding non-canonical RNA structures, splicing factors such as SRSF1, SRSF9 and proteins of the hnRNP family have been reported to interact with RNA G-quadruplexes [101]. It has been reported that the formation of an RNA G-quadruplex in intron 3 of the nascent mRNA of the gene *TP53* promotes intron 2 excision [102]. As well, it was shown that an RNA G-quadruplex increases splicing efficiency in *PAX9* intron 1

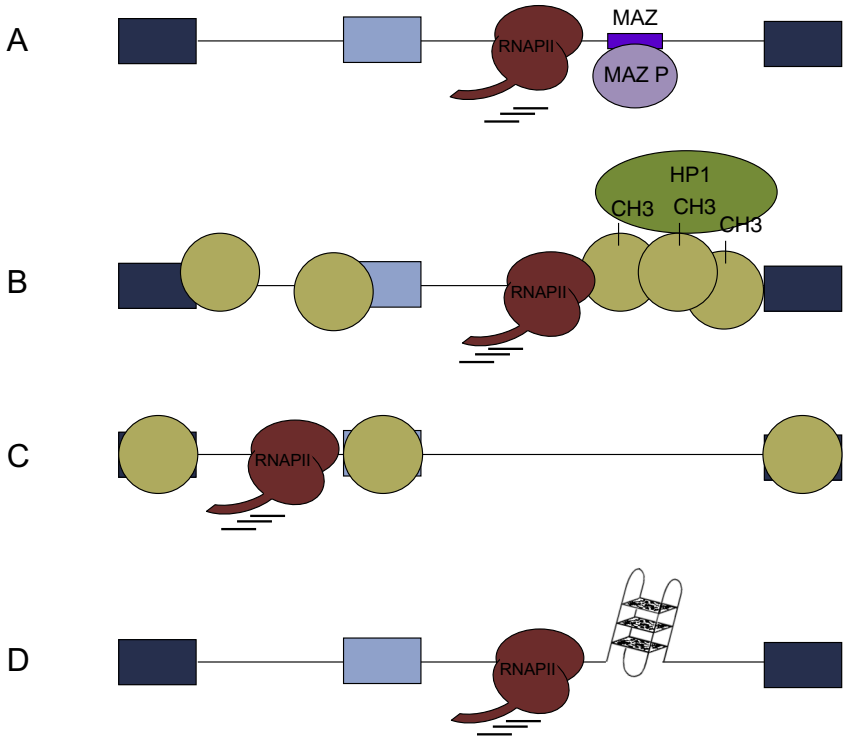

Fig. 2. Template features that act as roadblocks for RNAPII transcription progression can affect alternative splicing decisions by changing the time-window for splicing regulatory sequences to be recognized. (A) DNA sequences that recruit specific proteins, such as the MAZ sequences that bind the MAZ zinc finger protein [40]. (B) Chromatin compaction and histone PTMs [39,66]. (C) Nucleosomes positioned in exons [60,69]. (D) Non-B DNA structures [86].

[103]. RNA-binding proteins of the AFF family can modulate alternative splicing of a reporter minigene harboring an RNA G-quadruplex [104].

5. Concluding remarks

Coupling of transcription and mRNA splicing has been vastly confirmed by multiple approaches. This coupling is due to the recruitment of factors that modulate transcription and splicing, as well as a consequence of the kinetics of transcription [26]. The rate at which RNAPII transcribes can be modulated either by trans- or cis-acting factors including the template DNA or proteins bound to it. On the other hand, template features can participate in the recruitment of factors that modulate splicing directly [94]. Up to this point it becomes evident that the recruitment and the kinetic coupling models are not intertwined, but probably represent two faces of the same process.

In this review we went through the existing evidence of how these cis-acting factors modulate transcription. These factors can be classified in 4 different categories, i.e. (A) proteins that bind to DNA, (B) chromatin compaction and histone PTMs, (C) positioned nucleosomes and (D) template DNA conformation (Fig. 2). All of them have been shown to modulate transcription acting as roadblocks for RNAPII progression. Still, we are aware that the actual scenario might be more complicated, and a combination of these factors may be playing a role in alternative splicing decisions.

References

[1] Lavelle, C. (2014) Pack, unpack, bend, twist, pull, push: the physical side of gene expression. Curr. Opin. Genet. Dev. 25, 74–84.
[2] Srivastava, R. and Ahn, S.H. (2015) Modifications of RNA polymerase II CTD: Connections to the histone code and cellular function. Biotechnol. Adv. 33, 856–872.
[3] Heidemann, M., Hintermair, C., Voß, K. and Eick, D. (2013) Dynamic phosphorylation patterns of RNA polymerase II CTD during transcription. Biochim. Biophys. Acta 1829, 55–62.
[4] Muñoz, M., de la Mata, M. and Kornblihtt, A. (2010) The carboxy terminal domain of RNA polymerase II and alternative splicing. Trends Biochem. Sci. 35, 497–504.
[5] Laitem, C., Zaborowska, J., Isa, N.F., Kufs, J., Dienstbier, M. and Murphy, S. (2015) CDK9 inhibitors define elongation checkpoints at both ends of RNA polymerase II-transcribed genes. Nat. Struct. Mol. Biol. 22, 396–403.
[6] Hsin, J.P. and Manley, J.L. (2012) The RNA polymerase II CTD coordinates transcription and RNA processing. Genes Dev..
[7] Sainsbury, S.B.C. and Cramer, P. (2015) Structural basis of transcription initiation by RNA polymerase II. Nat. Rev. Mol. Cell Biol. 16, 129–143.
[8] Zhou, Q., Li, T. and Price, D.H. (2012) RNA polymerase II elongation control. Annu. Rev. Biochem. 81, 119–143.
[9] Nojima, T., Gomes, T., Grosso, A.R., Kimura, H., Dye, M.J., Dhir, S., Carmo-Fonseca, M. and Proudfoot, N.J. (2015) Mammalian NET-Seq reveals genome-wide nascent transcription coupled to RNA processing. Cell 161, 526–540.
[10] Charles, G.D., Nasun, H., Xin, L., André, L.M., Leighton, C., John, T.L., Adam, S. and Kraus, W.L. (2013) Signaling pathways differentially affect RNA polymerase II initiation, pausing, and elongation rate in cells. Mol. Cell 50.
[11] Jonkers, I., Kwak, H. and Lis, J.T. (2014) Genome-wide dynamics of Pol II elongation and its interplay with promoter proximal pausing, chromatin, and exons. eLife 3, e02407.
[12] Jonkers, I. and Lis, J.T. (2015) Getting up to speed with transcription elongation by RNA polymerase II. Nat. Rev. Mol. Cell Biol. 16, 167–177.
[13] Alexander, R.D., Innocente, S.A., Barrass, J.D. and Beggs, J.D. (2010) Splicing-dependent RNA polymerase pausing in yeast. Mol. Cell 40, 582–593.
[14] Will, C.L. and Lührmann, R. (2011) Spliceosome structure and function. Cold Spring Harb. Perspect. Biol. 3.
[15] Boise, L.H., González-García, M., Postema, C.E., Ding, L., Lindsten, T., Turka, L. A., Mao, X., Nuñez, G. and Thompson, C.B. (1993) Bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 74, 597–608.
[16] Barash, Y., Calarco, J., Gao, W., Pan, Q., Wang, X., Shai, O., Blencowe, B. and Frey, B. (2010) Deciphering the splicing code. Nature 465, 53–59.
[17] Hansen, K.D., Lareau, L.F., Blanchette, M., Green, R.E., Meng, Q., Rehwinkel, J., Gallusser, F.L., Izaurralde, E., Rio, D.C., Dudoit, S. and Brenner, S.E. (2009) Genome-wide identification of alternative splice forms down-regulated by nonsense-mediated mRNA decay in Drosophila. PLoS Genet. 5.
[18] Ramani, A., Calarco, J., Pan, Q., Mavandadi, S., Wang, Y., Nelson, A., Lee, L., Morris, Q., Blencowe, B., Zhen, M. and Fraser, A. (2011) Genome-wide analysis of alternative splicing in *Caenorhabditis elegans*. Genome Res. 21, 342–348.
[19] Khodor, Y., Rodriguez, J., Abruzzi, K., Tang, C.-H.A., Marr, M. and Rosbash, M. (2011) Nascent-seq indicates widespread cotranscriptional pre-mRNA splicing in Drosophila. Genes Dev. 25, 2502–2512.
[20] Ameur, A., Zaghlool, A., Halvardson, J., Wetterbom, A., Gyllensten, U., Cavelier, L. and Feuk, L. (2011) Total RNA sequencing reveals nascent transcription and widespread co-transcriptional splicing in the human brain. Nat. Struct. Mol. Biol. 18, 1435–1440.
[21] Kotovic, K., Lockshon, D., Boric, L. and Neugebauer, K. (2003) Cotranscriptional recruitment of the U1 snRNP to intron-containing genes in yeast. Mol. Cell. Biol. 23, 5768–5779.
[22] Lacadie, S. and Rosbash, M. (2005) Cotranscriptional spliceosome assembly dynamics and the role of U1 snRNA:5′ss base pairing in yeast. Mol. Cell 19, 65–75.
[23] Listerman, I., Sapra, A. and Neugebauer, K. (2006) Cotranscriptional coupling of splicing factor recruitment and precursor messenger RNA splicing in mammalian cells. Nat. Struct. Mol. Biol. 13, 815–822.
[24] Pandya-Jones, A. and Black, D.L. (2009) Co-transcriptional splicing of constitutive and alternative exons. RNA (New York, NY) 15, 1896–1908.
[25] Tilgner, H., Knowles, D., Johnson, R., Davis, C., Chakrabortty, S., Djebali, S., Curado, J., Snyder, M., Gingeras, T. and Guigó, R. (2012) Deep sequencing of subcellular RNA fractions shows splicing to be predominantly co-transcriptional in the human genome but inefficient for lncRNAs. Genome Res. 22, 1616–1625.
[26] Kornblihtt, A.R., Schor, I.E., Alló, M., Dujardin, G., Petrillo, E. and Muñoz, M.J. (2013) Alternative splicing: a pivotal step between eukaryotic transcription and translation. Nat. Rev. Mol. Cell Biol. 14, 153–165.
[27] Lazarev, D. and Manley, J.L. (2007) Concurrent splicing and transcription are not sufficient to enhance splicing efficiency. RNA (New York, NY) 13, 1546–1557.
[28] McCracken, S., Fong, N., Yankulov, K., Ballantyne, S., Pan, G., Greenblatt, J., Patterson, S.D., Wickens, M. and Bentley, D.L. (1997) The C-terminal domain of RNA polymerase II couples mRNA processing to transcription. Nature 385, 357–361.
[29] Cramer, P., Cáceres, J., Cazalla, D., Kadener, S., Muro, A., Baralle, F. and Kornblihtt, A. (1999) Coupling of transcription with alternative splicing: RNA pol II promoters modulate SF2/ASF and 9G8 effects on an exonic splicing enhancer. Mol. Cell 4, 251–258.
[30] Cramer, P., Pesce, C., Baralle, F. and Kornblihtt, A. (1997) Functional association between promoter structure and transcript alternative splicing. Proc. Natl. Acad. Sci. U.S.A. 94, 11456–11460.
[31] Kadener, S., Cramer, P., Nogués, G., Cazalla, D., de la Mata, M., Fededa, J., Werbajh, S., Srebrow, A. and Kornblihtt, A. (2001) Antagonistic effects of T-Ag and VP16 reveal a role for RNA pol II elongation on alternative splicing. EMBO J. 20, 5759–5768.
[32] Nogués, G., Kadener, S., Cramer, P., Bentley, D. and Kornblihtt, A.R. (2002) Transcriptional activators differ in their abilities to control alternative splicing. J. Biol. Chem. 277, 43110–43114.
[33] Auboeuf, D., Dowhan, D., Kang, Y., Larkin, K., Lee, J., Berget, S. and O'Malley, B. (2004) Differential recruitment of nuclear receptor coactivators may determine alternative RNA splice site choice in target genes. Proc. Natl. Acad. Sci. U.S.A. 101, 2270–2274.
[34] Kadener, S., Fededa, J., Rosbash, M. and Kornblihtt, A. (2002) Regulation of alternative splicing by a transcriptional enhancer through RNA pol II elongation. Proc. Natl. Acad. Sci. U.S.A. 99, 8185–8190.
[35] Kornblihtt, A., de la Mata, M., Fededa, J., Munoz, M. and Nogues, G. (2004) Multiple links between transcription and splicing. RNA (New York, NY) 10, 1489–1498.
[36] Batsché, E., Yaniv, M. and Muchardt, C. (2006) The human SWI/SNF subunit Brm is a regulator of alternative splicing. Nat. Struct. Mol. Biol. 13, 22–29.
[37] Luco, R.F., Pan, Q., Tominaga, K., Blencowe, B.J., Pereira-Smith, O.M. and Misteli, T. (2010) Regulation of alternative splicing by histone modifications. Science (New York, NY) 327, 996–1000.
[38] Saint-André, V., Batsché, E., Rachez, C. and Muchardt, C. (2011) Histone H3 lysine 9 trimethylation and HP1γ favor inclusion of alternative exons. Nat. Struct. Mol. Biol. 18, 337–344.
[39] Schor, I.E., Rascovan, N., Pelisch, F., Alló, M. and Kornblihtt, A.R. (2009) Neuronal cell depolarization induces intragenic chromatin modifications affecting NCAM alternative splicing. Proc. Natl. Acad. Sci. U.S.A. 106, 4325–4330.
[40] Roberts, G., Gooding, C., Mak, H., Proudfoot, N. and Smith, C. (1998) Co-transcriptional commitment to alternative splice site selection. Nucleic Acids Res. 26, 5568–5572.
[41] Dujardin, G., Lafaille, C., de la Mata, M., Marasco, L.E., Munoz, M.J., Le Jossic-Corcos, C., Corcos, L. and Kornblihtt, A.R. (2014) How slow RNA polymerase II elongation favors alternative exon skipping. Mol. Cell 54, 683–690.
[42] Montes, M., Cloutier, A., Sanchez-Hernandez, N., Michelle, L., Lemieux, B., Blanchette, M., Hernandez-Munain, C., Chabot, B. and Sune, C. (2012) TCERG1 regulates alternative splicing of the Bcl-x gene by modulating the rate of RNA polymerase II transcription. Mol. Cell. Biol. 32, 751–762.
[43] Muñoz, M.J., Pérez Santangelo, M.S., Paronetto, M.P., de la Mata, M., Pelisch, F., Boireau, S., Glover-Cutter, K., Ben-Dov, C., Blaustein, M., Lozano, J.J., Bird, G., Bentley, D., Bertrand, E. and Kornblihtt, A.R. (2009) DNA damage regulates alternative splicing through inhibition of RNA polymerase II elongation. Cell 137, 708–720.

[44] Ip, J., Schmidt, D., Pan, Q., Ramani, A., Fraser, A., Odom, D. and Blencowe, B. (2011) Global impact of RNA polymerase II elongation inhibition on alternative splicing regulation. Genome Res. 21, 390–401.
[45] Coulter, D. and Greenleaf, A. (1985) A mutation in the largest subunit of RNA polymerase II alters RNA chain elongation in vitro. J. Biol. Chem. 260, 13190–13198.
[46] Boireau, S., Maiuri, P., Basyuk, E., de la Mata, M., Knezevich, A., Pradet-Balade, B., Bäcker, V., Kornblihtt, A., Marcello, A. and Bertrand, E. (2007) The transcriptional cycle of HIV-1 in real-time and live cells. J. Cell Biol. 179, 291–304.
[47] de la Mata, M., Alonso, C., Kadener, S., Fededa, J., Blaustein, M., Pelisch, F., Cramer, P., Bentley, D. and Kornblihtt, A. (2003) A slow RNA polymerase II affects alternative splicing in vivo. Mol. Cell 12, 525–532.
[48] Das, R., Yu, J., Zhang, Z., Gygi, M., Krainer, A., Gygi, S. and Reed, R. (2007) SR proteins function in coupling RNAP II transcription to pre-mRNA splicing. Mol. Cell 26, 867–881.
[49] Monsalve, M., Wu, Z., Adelmant, G., Puigserver, P., Fan, M. and Spiegelman, B. M. (2000) Direct coupling of transcription and mRNA processing through the thermogenic coactivator PGC-1. Mol. Cell 6, 307–316.
[50] de la Mata, M. and Kornblihtt, A.R. (2006) RNA polymerase II C-terminal domain mediates regulation of alternative splicing by SRp20. Nat. Struct. Mol. Biol. 13, 973–980.
[51] Vargas, D., Shah, K., Batish, M., Levandoski, M., Sinha, S., Marras, S., Schedl, P. and Tyagi, S. (2011) Single-molecule imaging of transcriptionally coupled and uncoupled splicing. Cell 147, 1054–1065.
[52] de la Mata, M., Lafaille, C. and Kornblihtt, A.R. (2010) First come, first served revisited: factors affecting the same alternative splicing event have different effects on the relative rates of intron removal. RNA (New York, NY) 16, 904–912.
[53] Close, P., East, P., Dirac-Svejstrup, A.B., Hartmann, H., Heron, M., Maslen, S., Chariot, A., Söding, J., Skehel, M. and Svejstrup, J.Q. (2012) DBIRD complex integrates alternative mRNA splicing with RNA polymerase II transcript elongation. Nature 484, 386–389.
[54] Tessarz, P. and Kouzarides, T. (2014) Histone core modifications regulating nucleosome structure and dynamics. Nat. Rev. Mol. Cell Biol. 15, 703–708.
[55] Flanagan, J.F., Mi, L.Z., Chruszcz, M., Cymborowski, M., Clines, K.L., Kim, Y., Minor, W., Rastinejad, F. and Khorasanizadeh, S. (2005) Double chromodomains cooperate to recognize the methylated histone H3 tail. Nature 438, 1181–1185.
[56] Bannister, A.J., Zegerman, P., Partridge, J.F., Miska, E.A., Thomas, J.O., Allshire, R.C. and Kouzarides, T. (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 410, 120–124.
[57] Bannister, A.J. and Kouzarides, T. (2011) Regulation of chromatin by histone modifications. Cell Res. 21, 381–395.
[58] Bintu, L., Ishibashi, T., Dangkulwanich, M., Wu, Y.Y., Lubkowska, L., Kashlev, M. and Bustamante, C. (2012) Nucleosomal elements that control the topography of the barrier to transcription. Cell 151, 738–749.
[59] Jimeno-Gonzalez, S., Ceballos-Chavez, M. and Reyes, J.C. (2015) A positioned +1 nucleosome enhances promoter-proximal pausing. Nucleic Acids Res. 43, 3068–3078.
[60] Hodges, C., Bintu, L., Lubkowska, L., Kashlev, M. and Bustamante, C. (2009) Nucleosomal fluctuations govern the transcription dynamics of RNA polymerase II. Science 325, 626–628.
[61] Sheinin, M.Y., Li, M., Soltani, M., Luger, K. and Wang, M.D. (2013) Torque modulates nucleosome stability and facilitates H2A/H2B dimer loss. Nat. Commun. 4, 2579.
[62] Petesch, S.J. and Lis, J.T. (2012) Overcoming the nucleosome barrier during transcript elongation. Trends Genet. 28, 285–294.
[63] Belotserkovskii, B.P., Neil, A.J., Saleh, S.S., Shin, J.H., Mirkin, S.M. and Hanawalt, P.C. (2013) Transcription blockage by homopurine DNA sequences: role of sequence composition and single-strand breaks. Nucleic Acids Res. 41, 1817–1828.
[64] Subramanian, V., Fields, P.A. and Boyer, L.A. (2015) H2A.Z: a molecular rheostat for transcriptional control. F1000Prime Rep. 7. 01.
[65] Campos, E.I. and Reinberg, D. (2009) Histones: annotating chromatin. Annu. Rev. Genet. 43, 559–599.
[66] Alló, M., Buggiano, V., Fededa, J.P., Petrillo, E., Schor, I., de la Mata, M., Agirre, E., Plass, M., Eyras, E., Elela, S.A., Klinck, R., Chabot, B. and Kornblihtt, A.R. (2009) Control of alternative splicing through siRNA-mediated transcriptional gene silencing. Nat. Struct. Mol. Biol. 16, 717–724.
[67] Schwartz, S., Meshorer, E. and Ast, G. (2009) Chromatin organization marks exon-intron structure. Nat. Struct. Mol. Biol. 16, 990–995.
[68] Andersson, R., Enroth, S., Rada-Iglesias, A., Wadelius, C. and Komorowski, J. (2009) Nucleosomes are well positioned in exons and carry characteristic histone modifications. Genome Res. 19, 1732–1741.
[69] Tilgner, H., Nikolaou, C., Althammer, S., Sammeth, M., Beato, M., Valcarcel, J. and Guigo, R. (2009) Nucleosome positioning as a determinant of exon recognition. Nat. Struct. Mol. Biol. 16, 996–1001.
[70] Spies, N., Nielsen, C.B., Padgett, R.A. and Burge, C.B. (2009) Biased chromatin signatures around polyadenylation sites and exons. Mol. Cell 36, 245–254.
[71] Kolasinska-Zwierz, P., Down, T., Latorre, I., Liu, T., Liu, X.S. and Ahringer, J. (2009) Differential chromatin marking of introns and expressed exons by H3K36me3. Nat. Genet. 41, 376–381.
[72] Luco, R.F., Allo, M., Schor, I.E., Kornblihtt, A.R. and Misteli, T. (2011) Epigenetics in alternative pre-mRNA splicing. Cell 144, 16–26.
[73] Schübeler, D. (2015) Function and information content of DNA methylation. Nature 517, 321–326.
[74] Jeltsch, A. and Jurkowska, R.Z. (2014) New concepts in DNA methylation. Trends Biochem. Sci. 39, 310–318.
[75] Maunakea, A.K., Chepelev, I., Cui, K. and Zhao, K. (2013) Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. Cell Res. 23, 1256–1269.
[76] Shukla, S., Kavak, E., Gregory, M., Imashimizu, M., Shutinoski, B., Kashlev, M., Oberdoerffer, P., Sandberg, R. and Oberdoerffer, S. (2011) CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing. Nature 479, 74–79.
[77] Dickerson, R.E. (1992) DNA structure from A to Z. Methods Enzymol. 211, 67–111.
[78] Bansal, M., Kumar, A. and Yella, V.R. (2014) Role of DNA sequence based structural features of promoters in transcription initiation and gene expression. Curr. Opin. Struct. Biol. 25, 77–85.
[79] Vargason, J.M., Henderson, K. and Ho, S. (2001) A crystallographic map of the transition from B-DNA to A-DNA. Proc. Natl. Acad. Sci..
[80] Flatters, D., Young, M., Beveridge, D.L. and Lavery, R. (1997) Conformational properties of the TATA-box binding sequence of DNA. J. Biomol. Struct. Dyn. 14, 757–765.
[81] A. Herbert, A. Rich, Left-handed Z-DNA: structure and function, in: Structural Biology and Functional Genomics, Springer: USA, 1999, pp. 53–77.
[82] Temiz, N.A., Donohue, D.E., Bacolla, A., Luke, B.T. and Collins, J.R. (2011) The role of methylation in the intrinsic dynamics of B- and Z-DNA. PLoS ONE 7.
[83] Ha, S.C., Lowenhaupt, K., Rich, A., Kim, Y.G. and Kim, K.K. (2005) Crystal structure of a junction between B-DNA and Z-DNA reveals two extruded bases. Nature 437, 1183–1186.
[84] Tsai, Z.T., Chu, W.Y., Cheng, J.H. and Tsai, H.K. (2014) Associations between intronic non-B DNA structures and exon skipping. Nucleic Acids Res. 42, 739–747.
[85] Bochman, M.L., Paeschke, K. and Zakian, V.A. (2012) DNA secondary structures: stability and function of G-quadruplex structures. Nat. Rev. Genet. 13, 770–780.
[86] Belotserkovskii, B.P., Mirkin, S.M. and Hanawalt, P.C. (2013) DNA sequences that interfere with transcription: implications for genome function and stability. Chem. Rev. 113, 8620–8637.
[87] M. Van Dyke, Do DNA triple helices and quadruplexes have a role in transcription? in DNA conformation and transcription, Springer, 2005, pp. 105–26.
[88] Pandey, S., Ogloblina, A.M., Belotserkovskii, B.P., Dolinnaya, N.G., Yakubovskaya, M.G., Mirkin, S.M. and Hanawalt, P.C. (2015) Transcription blockage by stable H-DNA analogs in vitro. Nucleic Acids Res..
[89] Buske, F.A., Mattick, J.S. and Bailey, T.L. (2011) Potential in vivo roles of nucleic acid triple-helices. RNA Biol. 8, 427–439.
[90] Brooks, T.A., Kendrick, S. and Hurley, L. (2010) Making sense of G-quadruplex and i-motif functions in oncogene promoters. FEBS J. 277, 3459–3469.
[91] De, S. and Michor, F. (2011) DNA secondary structures and epigenetic determinants of cancer genome evolution. Nat. Struct. Mol. Biol. 18, 950–955.
[92] Schiavone, D., Guilbaud, G., Murat, P., Papadopoulou, C., Sarkies, P., Prioleau, M.N., Balasubramanian, S. and Sale, J.E. (2014) Determinants of G quadruplex-induced epigenetic instability in REV1-deficient cells. EMBO J. 33, 2507–2520.
[93] Uribe, D.J., Guo, K., Shin, Y.J. and Sun, D. (2011) Heterogeneous nuclear ribonucleoprotein K and nucleolin as transcriptional activators of the vascular endothelial growth factor promoter through interaction with secondary DNA structures. Biochemistry 50, 3796–3806.
[94] Nelson, L.D., Bender, C., Mannsperger, H., Buergy, D., Kambakamba, P., Mudduluru, G., Korf, U., Hughes, D., Van Dyke, M.W. and Allgayer, H. (2012) Triplex DNA-binding proteins are associated with clinical outcomes revealed by proteomic measurements in patients with colorectal cancer. Mol. Cancer 11, 38.
[95] Gray, L.T., Vallur, A.C., Eddy, J. and Maizels, N. (2014) G quadruplexes are genomewide targets of transcriptional helicases XPB and XPD. Nat. Chem. Biol. 10, 313–318.
[96] Guo, M., Hundseth, K., Ding, H., Vidhyasagar, V., Inoue, A., Nguyen, C.H., Zain, R., Lee, J.S. and Wu, Y. (2015) A distinct triplex DNA unwinding activity of ChlR1 helicase. J. Biol. Chem. 290, 5174–5189.
[97] Chatterjee, S. and Pal, J.K. (2009) Role of 5′- and 3′-untranslated regions of mRNAs in human diseases. Biol. Cell 101, 251–262.
[98] Castillo Bosch, P., Segura-Bayona, S., Koole, W., van Heteren, J.T., Dewar, J.M., Tijsterman, M. and Knipscheer, P. (2014) FANCJ promotes DNA synthesis through G-quadruplex structures. EMBO J. 33, 2521–2533.
[99] Hamperl, S. and Cimprich, K.A. (2014) The contribution of co-transcriptional RNA:DNA hybrid structures to DNA damage and genome instability. DNA Repair (Amst) 19, 84–94.
[100] Skourti-Stathaki, K., Kamieniarz-Gdula, K. and Proudfoot, N.J. (2014) R-loops induce repressive chromatin marks over mammalian gene terminators. Nature 516, 436–439.

[101] von Hacht, A., Seifert, O., Menger, M., Schutze, T., Arora, A., Konthur, Z., Neubauer, P., Wagner, A., Weise, C. and Kurreck, J. (2014) Identification and characterization of RNA guanine-quadruplex binding proteins. Nucleic Acids Res. 42, 6630–6644.

[102] Marcel, V., Tran, P.L., Sagne, C., Martel-Planche, G., Vaslin, L., Teulade-Fichou, M.P., Hall, J., Mergny, J.L., Hainaut, P. and Van Dyck, E. (2011) G-quadruplex structures in TP53 intron 3: role in alternative splicing and in production of p53 mRNA isoforms. Carcinogenesis 32, 271–278.

[103] Ribeiro, M.M., Teixeira, G.S., Martins, L., Marques, M.R., de Souza, A.P. and Line, S.R. (2015) G-quadruplex formation enhances splicing efficiency of PAX9 intron 1. Hum. Genet. 134, 37–44.

[104] Melko, M., Douguet, D., Bensaid, M., Zongaro, S., Verheggen, C., Gecz, J. and Bardoni, B. (2011) Functional characterization of the AFF (AF4/FMR2) family of RNA-binding proteins: insights into the molecular pathology of FRAXE intellectual disability. Hum. Mol. Genet. 20, 1873–1885.