Regulation of alternative splicing: Functional interplay with epigenetic modifications and its implication to cancer

Article *in* WIREs RNA · September 2023

DOI: 10.1002/wrna.1815

CITATIONS
0

READS
23

3 authors, including:

Ning Wang
Chinese Academy of Sciences
8 PUBLICATIONS **54** CITATIONS
SEE PROFILE

Yue hu
Chinese Academy of Sciences
6 PUBLICATIONS **23** CITATIONS
SEE PROFILE

Received: 23 February 2023 | Revised: 26 June 2023 | Accepted: 30 June 2023

DOI: 10.1002/wrna.1815

ADVANCED REVIEW

WIREs RNA WILEY

Regulation of alternative splicing: Functional interplay with epigenetic modifications and its implication to cancer

Ning Wang[1,2] | **Yue Hu**[1,2] | **Zefeng Wang**[1,2]

[1]Bio-Med Big Data Center, CAS Key Laboratory of Computational Biology, CAS Center for Excellence in Molecular Cell Science, Shanghai Institute of Nutrition and Health, Shanghai, China

[2]University of Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing, China

Correspondence
Zefeng Wang, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing, China.
Email: wangzefeng@picb.ac.cn

Funding information
CAS Interdisciplinary Innovation Team; National Natural Science Foundation of China, Grant/Award Numbers: 31730110, 32030064, 91940303; the National Key Research and Development Program of China, Grant/Award Numbers: 2018YFA0107602, 2021YFA1300503; the NIH Basic Research in Cancer Health Disparities Award, Grant/Award Number: R01CA220314; the Starry Night Science Fund at Shanghai Institute for Advanced Study of Zhejiang University, Grant/Award Number: SN-ZJU-SIAS-009; the Strategic Priority Research Program of Chinese Academy of Sciences, Grant/Award Number: XDB38040100; the type A CAS Pioneer 100-Talent program

Edited by: Li Yang, Associate Editor and Jeff Wilusz, Editor-in-Chief

Abstract

Eukaryotic gene expression is intricately regulated at multiple levels. The protein-coding genes are first transcribed as pre-mRNAs in the nucleus and undergo a series of RNA processing steps before being transported into the cytoplasm for translation. During RNA processing, most human genes (>95%) undergo alternative splicing to generate multiple mRNA isoforms from a single gene, which effectively diversifies the genome complexity. Since the splicing of most genes occurs co-transcriptionally, the regulation layers of gene expression often show functional interactions with each other. In this review, we provide a brief overview of alternative splicing regulation in three different layers (controlled by the splicing machinery, transcription process, and chromatin structure), emphasizing the regulatory roles of epigenetic modifications and the crosstalk between these layers. Specifically, we categorize the major effects of the epigenetic modifications on alternative splicing into three different types: by affecting transcription rate, splicing factor recruitment, or the expression/activity of splicing factor. The dysregulation of epigenetics and splicing are extremely common in cancer, we also discuss the potential mechanisms of how epigenetic changes can lead to splicing dysregulation and their functional consequences. We aim to provide insights into the complicated regulation of different gene expression layers, which will shed light on the novel approaches to modulate disease-related splicing dysregulation.

This article is categorized under:

RNA Processing > 3′ End Processing
RNA Processing > Splicing Mechanisms
RNA Processing > Splicing Regulation/Alternative Splicing
RNA in Disease and Development > RNA in Disease

KEYWORDS

alternative splicing, cancer pathogenesis, co-transcriptional splicing, DNA methylation, epigenetic modifications, histone modification

Ning Wang and Yue Hu contributed equally to this study.

1 | INTRODUCTION

Transcription and precursor messenger RNA (pre-mRNA) splicing are the fundamental steps in gene expression. In 1977, Sharp and Roberts's laboratories independently made the landmark discovery that adenovirus genes are separated by intervening noncoding sequences (Berget et al., 1977; Chow et al., 1977), which are post-transcriptionally excised from the mature mRNA through a process known as RNA splicing. This discovery was later recognized as a general phenomenon for most eukaryotic genes, which are transcribed as pre-mRNAs containing both introns and exons. The introns were subsequently removed through RNA splicing to generate mature mRNAs that only contain exon sequences. During splicing, the selective inclusion or exclusion of certain exons can produce different mRNA isoforms, known as alternative splicing (AS). AS considerably increases the complexity of human transcriptome. Furthermore, the alternatively spliced exons tend to code important domains in protein-coding genes (Sahebi et al., 2016), resulting in proteins with distinct activities.

The mechanism and regulation of AS have been extensively studied in recent years. The splicing reactions are catalyzed by the spliceosome, a large protein/RNA complex that recognizes the splice sites and branch point in pre-mRNA to remove the introns in a multi-step process (Matera & Wang, 2014; Shi, 2017). In addition, many *cis*-regulatory elements recruit auxiliary splicing factors to pre-mRNAs, which either facilitate or inhibit splicing reactions (Wang & Burge, 2008). As a key step of gene expression, RNA splicing is closely associated with human health. The mutations in splice sites, regulatory *cis*-elements, spliceosome components, and regulatory splicing factors often lead to splicing dysregulation and various diseases (Scotti & Swanson, 2016).

In addition to the regulation at the RNA level, splicing of most human genes happens co-transcriptionally, expanding splicing regulation to multiple levels (Figure 1; Giono & Kornblihtt, 2020; Kornblihtt et al., 2004). Early researches using electron microscopy revealed that the formation of intronic loop and ribonucleoprotein particles happens while the nascent transcripts are still tethered to DNA, indicating that mRNA splicing occurs prior to transcript release (Beyer et al., 1981; Beyer & Osheim, 1988). Subsequent studies showed that splicing factors are localized at the sites of transcription of intron-containing genes (Huang & Spector, 1996; Misteli et al., 1997; Neugebauer & Roth, 1997), and the in situ hybridization with splice junction probes detected spliced mRNAs at their gene loci (Zhang et al., 1994), suggesting a spatial and functional coupling between transcription and splicing. With the recent development of new technologies, including various chromatin immunoprecipitation assays and high throughput sequencing, researchers have gained many new insights into the extent and kinetics of co-transcriptional splicing (Neugebauer, 2019). The functional linkage between splicing and transcription suggests an integrative regulation that allows the two processes to interact with each other (Herzel et al., 2017).

Furthermore, the transcription process incorporates additional regulatory modes to co-transcriptional splicing. Owing to the linkage between transcription and splicing, any factors that affect transcription may also have an effect, either directly or indirectly, on RNA splicing. Such regulation includes the RNA polymerase II elongation rate, transcription factor activity, chromatin structure, nucleosome assembly, etc., all of which may be influenced by epigenetic modifications. Therefore, a multidimensional understanding of splicing regulation is needed to dissect the complex functional relationships among these regulatory pathways (Braunschweig et al., 2013). In this review, we focused on

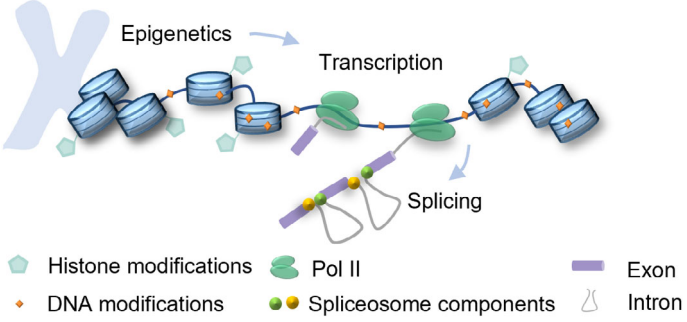

FIGURE 1 Summary of co-transcriptional regulation of splicing. Most splicing is spatially and temporally linked to transcription. Spliceosome assembly and binding to nascent mRNA is prior to transcript release from transcription machinery (Pol II). Transcription is influenced by epigenetic factors. Due to the close connection between splicing and transcription, epigenetic factors are playing a role in the splicing decision and regulation process.

the multidimensional regulation of AS, with emphasis on the regulatory roles of epigenetic modifications on AS and the crosstalk between different regulatory layers. The readers are referred to other great reviews on the general regulation of splicing. Since the dysregulations of both AS and epigenetic modification are important molecular hallmarks of human cancers, we also summarized the implications of this regulation crosstalk on cancer pathogenesis, which may shed light on the new therapeutic strategies for cancers.

2 | REGULATION OF AS IN THREE LEVELS

There are three different levels at which the regulation of AS might take place (Figure 2). At the RNA level, *cis*-acting elements specifically interact with *trans*-acting splicing factors, which can either inhibit or promote the usage of nearby splice sites (Ule & Blencowe, 2019). At the transcription level, the initiation and elongation of RNA polymerase II (Pol II) on specific genes can affect the dynamics of splice site recognition by spliceosome (Guo & Price, 2013; Shukla & Oberdoerffer, 2012). Finally, chromatin structure and some epigenetic modifications, such as histone modification or DNA methylation, alter the Pol II elongation rate or affect splicing factors to regulate splicing (Luco et al., 2011; Zhu et al., 2018). More details on splicing regulation and its link with transcription and epigenetic markers are explained below.

2.1 | Regulation of AS by *cis*-acting RNA elements and *trans*-acting protein factors

Since the discovery of RNA splicing, many studies have been focused on the splicing mechanism and regulation of AS. The major spliceosome is a large ribonucleoprotein (RNP) complex containing more than 100 proteins and 5 different small nuclear RNAs (snRNAs). During splicing, the 5′ splice sites are first recognized by U1 snRNP, and the 3′ splice sites/branchpoints are recognized by U2 snRNP, which is followed by the pairing of U1 and U2 snRNAs to form an exon definition complex (Bonnal et al., 2020; Matera & Wang, 2014). The pairing of U1 and U2 snRNPs is a critical step in defining splicing specificity. Subsequently, a series of rearrangements occur in the RNP complex, with additional snRNPs recruited to undergo stepwise assembly into the active spliceosome that catalyzes splicing reaction (Shi, 2017; Will & Luhrmann, 2011).

The recognition and pairing of 5′ and 3′ splice sites may be interfered by other competing sites, resulting in different AS products. Therefore, the strength and relative positions of the competing splice sites often affect the dynamic of spliceosome recognition, which may regulate AS (Yu et al., 2008). In addition to splice sites, pre-mRNAs contain multiple *cis*-acting splicing regulatory elements (SREs) that specifically regulate AS by recruiting *trans*-acting splicing factors (Figure 2a). According to the position and regulatory activity, the *cis*-acting SREs have been classified into exonic

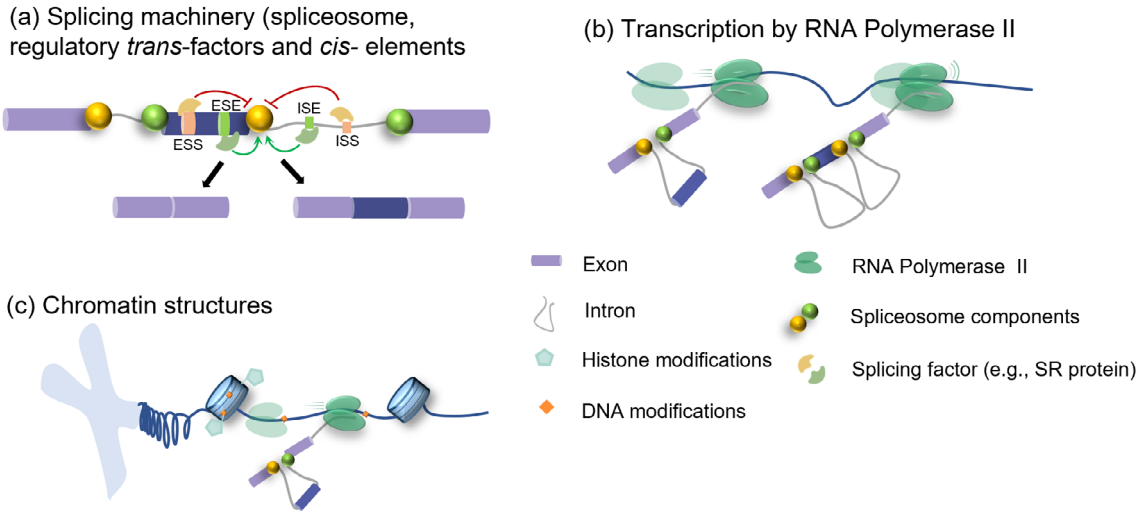

FIGURE 2 Regulation of alternative splicing in three layers: (a) *cis*-acting elements and *trans*-acting splicing factor at the splicing regulation level; (b) Pol II elongation rate at the co-transcription level; (c) chromatin structure at the epigenetics regulation level.

splicing enhancers or silencers (ESEs or ESSs) and intronic splicing enhancers or silencers (ISEs or ISSs). Splicing factors, such as SR proteins and hnRNPs, specifically bind to SREs and influence splice site recognition and/or spliceosome assembly. In addition, these *cis*-acting SREs and cognate *trans*-factors often exhibit context-dependent activities (Fu & Ares Jr., 2014; Goren et al., 2006; Wang et al., 2013), that is, the same SRE may function as either a splicing enhancer or silencer depending on the relative exon/intron context of its binding sites. For a specific splicing factor, a detailed RNA binding map at the transcriptome scale is critical to illustrate its activity in regulating AS (Ule et al., 2006; Xue et al., 2009). The *trans*-acting splicing factors may play a critical role in regulating certain biological processes. For example, the expression shift of PTB to nPTB is crucial for controlling neuronal cell fate (Hu et al., 2018), which may hold the key to treating many neurodegenerative diseases or repairing damaged brains (Dai et al., 2022).

2.2 | Co-transcriptional regulation of AS

For most pre-mRNAs, the spliceosome components are recruited during transcription rather than post-transcriptionally. Therefore, AS can be naturally affected by the different transcriptional regulatory pathways. The early evidence for a functional link between the Pol II transcription and splicing came from replacing the promoter of a protein-coding gene with an RNA Pol I or III promoter, the resulting chimeric gene was transcribed, but the RNA was not spliced (Sisodia et al., 1987; Smale & Tjian, 1985). Later studies discovered that transcription elongation, concurrent with pre-mRNA splicing, significantly impacts AS regulation. The fluctuation of Pol II elongation process, in particular, may be affected by the local gene sequences, and thus variations in Pol II elongation rate can alter the splicing patterns by influencing the splice site recognition and pairing (Figure 2b; Chen et al., 2011). The elongation process may also determine the length of the "free to fold" region of pre-mRNA, which in turn can affect the dynamic structure of nascent RNAs and directly impact the accessibility of the splice sites by splicing factors (Eperon et al., 1988). Moreover, cells treated with SV40 T antigen and herpesvirus VP16, which have opposite effects on Pol II elongation, have different AS products (Kadener et al., 2001; Kadener et al., 2002). According to the "window of opportunity" model, altering the transcription rate may regulate splicing by modulating the accessibility of *cis*-regulatory elements on the nascent pre-mRNA, thereby influencing the recruitment of both positive and negative splicing factors (Fong et al., 2014; Saldi et al., 2016). Generally, a lower Pol II elongation rate or internal pause may promote the inclusion of the alternative exon, whereas a higher elongation rate does the opposite (de la Mata et al., 2003; Dujardin et al., 2013). This general regulation rule was further supported by a recent profile of cancer-associated AS events, which revealed that the short alternative exons are preferably excluded due to the elevated transcription rate in most cancers (Zhang et al., 2022).

To efficiently coordinate the spliceosome components at the transcription site, the CTD (C-terminal domain) repeat of Pol II large subunit plays a critical role in the interactions with splicing factors. The mammalian Pol II CTD contains 52 tandem heptapeptide repeats (YSPTSPS), which are dynamically phosphorylated during different phases of transcription. The CTD can interact with over 100 proteins actively involved in almost all RNA processing pathways, including RNA capping, cleavage/polyadenylation, and splicing (Giono & Kornblihtt, 2020). The CTD mutant lacking Ser2 phosphorylation can impair the recruitment of U2AF65 and U2 (Gu et al., 2013). The phosphorylation of the CTD can also impact the Pol II elongation rate by recruiting transcription factors to promoters and enhancer elements (Hsin & Manley, 2012).

The RNA splicing and transcription can occur with a certain degree of "kinetic competition" (Coulon et al., 2014). A study of isolated chromatins associated with nascent RNA showed that Pol II usually pauses within the terminal exons, allowing sufficient time for intron excision before the release of transcript (Carrillo Oesterreich et al., 2010; Carrillo Oesterreich et al., 2011). Similarly, another study demonstrated that yeast Pol II accumulates at 3′ ends of introns, which is associated with splicing reaction and splicing factor recruitment (Alexander et al., 2010). A splicing-dependent transcriptional checkpoint may exist at the 3′ ends of the intron to promote splicing (Carrillo Oesterreich et al., 2010). These findings suggest an interesting possibility that RNA splicing can reciprocally influence pol II elongation. In support of this notion, the binding of SR proteins to nascent transcript has been shown to induce transcription initiation and Pol II elongation via interaction with elongation factor P-TEFb (Eberhardy & Farnham, 2002; Ji et al., 2013). This reciprocal regulation between transcription and AS may imply that the same set of regulatory factors may control transcription and splicing in a bidirectional manner.

2.3 | AS regulation mediated through epigenetic modifications

The transcription of RNA by Pol II co-occurs with the exchange and displacement of core histones, which is tightly controlled by numerous protein complexes that modify chromatin structure (Kulaeva et al., 2007). Therefore the chromatin structure may play a role in AS regulation by affecting Pol II elongation and other co-transcriptional regulation steps, such as imposing pauses or delays in transcript elongation (Figure 2c).

The first evidence of AS regulation through chromatin structure was found in the exon inclusion of fibronectin E33, which is sensitive to the replication-mediated chromatinization status and the histone deacetylases inhibitor TSA (Kadener et al., 2001; Nogues et al., 2002). Another study showed that hormone-sensitive promoters could change the AS of a CD44 reporter gene by recruiting co-regulators to remodel chromatin (Auboeuf et al., 2002). In addition, the alternative exon of a splicing reporter minigene is included at a higher level during DNA replication of that minigene (Nieto Moreno et al., 2015), implying a link between DNA structure and AS outcome.

In eukaryotic cells, the chromatin is organized by repeated nucleosomes that comprise an octamer of histones (two molecules of each H2A, H2B, H3, and H4 histones) wrapped with approximately 147 bp DNA (Quina et al., 2006). Each nucleosome is linked to the next by a small segment of linker DNA. The DNA and nucleosomes are further compressed and packaged into a tight structure to stabilize genetic information. During the DNA replication cycle and the gene transcription process, the chromatin structure undergoes dynamic changes, which is affected by many covalent modifications on both DNA, histones, and other chromatin-associated proteins (Bannister & Kouzarides, 2011; Li et al., 2007). There are dozens of common chemical modifications on DNA and histones, collectively called epigenetic modifications, because they often affect inheritable phenotypes without changing DNA sequences.

In addition to the chromatin structures, epigenetic modifications can directly affect transcription initiation and elongation, thereby specifically regulating the AS of particular genes. For example, the hyperacetylation of H3K9ac (a hallmark of relaxed chromatin) around the NCAM gene promotes the skipping of NCAM exon 18 upon neuronal cell depolarization (Schor et al., 2009). In addition, the histone acetyltransferase complex physically interacts with U2 snRNP proteins in both yeast and humans (Gunderson & Johnson, 2009; Martinez et al., 2001), and the histone arginine methyltransferase CARM1 interacts with U1 snRNP proteins (Cheng et al., 2007), suggesting a role of chromatin modification complexes in facilitating the assembly of the spliceosome complex onto pre-mRNA. These effects are independent of transcription elongation, implying that chromatin structure plays a more direct role in splicing factor recruitment.

As mentioned above, Pol II elongation and pauses imposed on transcription, constitute important factors in splicing regulation. It is easy to envision how changes in chromatin compaction could affect AS through kinetic coupling with transcription, as more compacted chromatin will result in a more difficult template for Pol II to transcribe. Furthermore, epigenetic activation of transcription may increase the levels of splicing factors in general (Schor et al., 2013), which in turn affects AS. To reflect the intertwined regulation network of epigenetic modification and the co-transcriptional regulation of AS, we summarize three simplified regulatory models by considering the controls of chromatin compaction, Pol II elongation rate, splicing factor recruitment, and epigenetic modifications on specific genes encoding splicing regulators (Allo et al., 2009; Curado et al., 2015; Jordaan et al., 2013; Sun et al., 2020; Wang et al., 2019; Zheng et al., 2018)(see below for detailed discussion).

3 | EPIGENETIC MODIFICATIONS AND THEIR EFFECT ON THE REGULATION OF TRANSCRIPTION AND AS

Several studies have demonstrated that epigenetic modifications can control which parts of the genome are transcribed and how they are spliced. Chromatin organization is impacted by nucleosome density and positioning, as well as by specific histone modifications and DNA methylation. Recent research has identified DNA methylation and histone modifications as potential regulators of AS (Rahhal & Seto, 2019; Zhou et al., 2014). Certain histone modifications were demonstrated to regulate AS by either directly recruiting splice machinery or indirectly modulating transcription elongation (Hu et al., 2020). Moreover, DNA methylation regulates splicing primarily through coupling with Pol II elongation and thus affecting splice site recognition (Gelfman et al., 2013).

Over 50 histone modifications can modulate the chromatin states and affect the binding affinity of regulatory proteins to histones or DNA. Histone modifications in promoter regions are predictive of gene expression levels (Karlic et al., 2010), emphasizing their importance for various cellular functions. For example, active transcription initiation

sites are marked by the enrichment of H3K4me3 and H3K9ac (Chen et al., 2011) and depletion of H3K27me3 (Estaras et al., 2013), whereas the active transcription elongation is preferentially marked with H3K36me3 but depletion of H3K27me3 (Zhang et al., 2015). The involvement of histone modification in splicing regulation was first suggested by the surprising discovery that the exonic and intronic regions of genes are differentially marked with H3K36me3 (Kolasinska-Zwierz et al., 2009) Additional histone modifications, such as H3K9me2, H3K9me3, H3K79me1, H3K27me1, H3K27me2, H3K9ac, H3K18ac, and H3K27ac, are more prevalent in exons and are associated with the inclusion levels of exons (Schwartz et al., 2009; Spies et al., 2009; Wang et al., 2008). Such regulation is closely associated with splicing regulation in cancers. The inclusion of short exons is significantly associated with histone markers of transcription initiation and elongation according to a recent study (Zhang et al., 2022). Another study showed that the recruitment of histone deacetylases 1 (HDAC1) by ZNF827 can reduce Pol II progression and alter splicing in EMT regulatory genes, thus affecting brain development and cancer metastasis (Sahu et al., 2022). These examples demonstrated that variations in chromatin compaction might affect AS through kinetics coupled with transcription (Naftelberg et al., 2015; Nieto Moreno et al., 2015).

DNA methylation occurs predominantly on the cytosine in CpG dinucleotides, where a methyl group is added to the 5 positions of the cytosine ring by DNA methyltransferases (DNMTs) (Jones & Liang, 2009; Moore et al., 2013). DNA methylation plays important regulatory roles during embryogenesis, genomic imprinting, X chromosome inactivation, ontogenesis, and differentiation (Allo et al., 2009; Bestor, 2000; Bird, 2002; Flores et al., 2012; Li et al., 1992; Okano et al., 1999). Furthermore, the specific genomic regions with dynamic changes in DNA methylation overlap with transcription factor binding sites, which is linked to their targeted regulation (Lev Maor et al., 2015; Moore et al., 2013). However, it is yet unclear how methylation affects the binding of splicing factors, as well as splicing regulatory elements and genic sequences. Recently it has been found that DNA methylation may play a more confounding role in regulating gene expression: DNA methylation in promoter regions is associated with transcriptional repression, whereas methylation in gene bodies is generally associated with high expression levels (Deaton & Bird, 2011). More studies have revealed a connection between DNA methylation and AS, with exon sequences tending to have higher methylation levels than flanking intron sequences (Laurent et al., 2010).

To aid comprehension of the complex relationship between epigenetic regulation and AS, we have classified the regulatory mechanisms of how epigenetic modifications may affect AS into models: (i) by altering Pol II elongation rate, (ii) by acting as an adaptor to recruit splicing factors, and iii) by directly affecting the expression of splicing regulators (Figure 3).

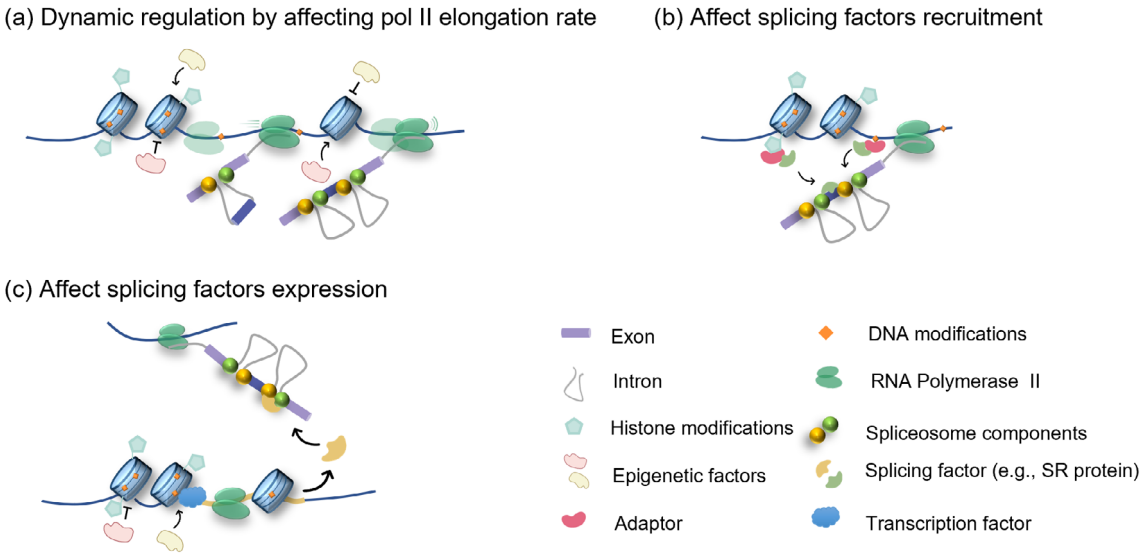

FIGURE 3 Three regulatory mechanisms of epigenetic modification affecting alternative splicing. (a) When altering the state of epigenetic modifications (DNA methylated/unmethylated, histone modification/absence of histone modification), related factors change in their binding ability to promote/suppress Pol II elongation rate. Slow Pol II elongation results in exon inclusion, while fast Pol II elongation results in exon skipping. (b) Some specific proteins can bind to epigenetic modifications and act as an adaptor, recruiting splicing factors to participate in alternative splicing. (c) Epigenetic modifications occur directly on splicing factors or their upstream regulatory genes, leading to the expression change of genes encoding splicing factors that cause extensive alteration in AS.

3.1 | Epigenetic modifications regulate AS by altering the transcription elongation rate

In the first model, DNA methylation/demethylation and histone modification may alter Pol II elongation rate or cause transcription pauses by placing obstacles in transcription elongation, thereby affecting the splicing time window and the AS patterns (Figure 3a). Extensive research has found that DNA methylation and AS are strongly correlated (Flores et al., 2012; Lev Maor et al., 2015). For example, CD45 is alternatively spliced in its exon 5 during lymphocyte differentiation, and unmethylated DNA in exon 5 can modulate its splicing via recruiting the transcription insulator CTCF (Shukla et al., 2011). CTCF, also known as CCCTC-binding factor, is a DNA-binding protein that is involved in numerous cellular processes (Alharbi et al., 2021). When DNA is unmethylated, the binding of CTCF can trigger local Pol II pause and facilitate the inclusion of upstream weak exons. Methylated DNA will prevent the binding of CTCF, thereby promoting fast Pol II elongation and exon skipping on mature mRNA (Ong & Corces, 2014). In addition, the general inhibition of DNA methyltransferase (DNMT) by 5′-aza-2′-deoxycytidine was found to decrease efficiencies of transcription elongation and impact splicing (Yang et al., 2014).

The methylated DNAs are bound by specific proteins, most notably by the methyl-CpG binding protein 2 (MeCP2), which can slow down the Pol II elongation rate. MeCP2 was the first identified member of the family of proteins with methyl-CpG-binding domains and transcriptional repressor domains (Fuks et al., 2003; Nan et al., 1993). Additionally, it participates in post-transcriptional gene regulation (Cheng et al., 2017; Nan et al., 1997). MeCP2 has been demonstrated to bind to YB-1, a component of messenger ribonucleoprotein particles that play a key role in the regulation of splicing (Young et al., 2005). MeCP2 binds to methylated DNA of many genes and regulates a large set of AS events across transcriptome (Cheng et al., 2017). Mechanistically, MeCP2 may recruit histone deacetylases (HDAC) that can promote local histone hypoacetylation, leading to Pol II pausing and exon inclusion (Jones et al., 1998; Maunakea et al., 2013). In certain alternatively spliced regions that lack DNA methylation, the absence of MeCP2 binding generally leads to fast Pol II elongation and skip of weak splice sites (Fuks et al., 2003; Maunakea et al., 2013; Wong et al., 2017).

Similar to DNA methylation, AS can also be regulated by nucleosome organizations that alter Pol II elongation rate. The chromatin markers H3K9me2 and H3K9me3 are associated with transcriptional repression (Miao & Natarajan, 2005) and contribute to splicing regulation when bound by heterochromatin protein 1 (HP1). The evolutionarily conserved HP1 exists in three distinct isoforms: HP1α, HP1β, and HP1γ. HP1α and HP1γ independently identify and bind H3K9me2 and H3K9me3. The H3K9me3 modifications are enriched in the chromosomal region encompassing the CD44 variable exons, and are recognized by HP1γ, which facilitates the inclusion of the alternative exons by reducing the local transcriptional elongation rate.(Saint-Andre et al., 2011). Similarly, H3K9me2 was discovered to be associated with AS of exon EDI in the Fibronectin (FN1) gene. When the siRNAs were used to target the region close to exon EDI, it was found to induce the formation of H3K9me2 marks on the corresponding chromatin region, which were recognized by HP1α to reduce Pol II elongation rate, resulting in increased inclusion of nearby alternative exon (Allo et al., 2009; White et al., 2008). In addition, H3K9ac is a characteristic of relaxed chromatin and leads to an increase in Pol II elongation rate, resulting in exon skipping in NCAM (Schor et al., 2009). These studies support the first model that AS can be regulated by epigenetic modifications through their effects on Pol II elongation rate.

3.2 | Epigenetic modifications function as adaptors to recruit splicing regulatory factors

In addition to altering the rate of Pol II elongation, epigenetic markers can directly regulate splicing by recruiting splicing factors, predominantly via mediator proteins that act as adaptors (Figure 3b). Generally, the alternative exons have lower DNA methylation levels than constitutively spliced exons (Oberdoerffer, 2012; Saint-Andre et al., 2011). Furthermore, the 5′ splice site regions have higher DNA methylation levels than 3′ splice site regions (Gelfman & Ast, 2013). DNA methylation can induce the H3K9me3 modification on the methylated alternative exons, which can be bound by HP1 that serves as a mediator protein between DNA methylation and splicing. Mechanistically, HP1 proteins accumulated on the DNAs of alternative exons can recruit splicing factors to exert their regulatory effect on these alternative exons (Bannister et al., 2001; Lev Maor et al., 2015). These splicing factors are presumably transferred onto the transcribed mRNA precursor and thus regulate AS (Allo et al., 2009; Yearim et al., 2015). For example, the retained introns were found to be associated with the reduction of DNA methylation and MeCP2 binding, which can in turn recruit splicing factors (such as Tra2b) to promote intron

splicing (Wong et al., 2017). MeCP2 was also reported to interact with many other splicing regulatory factors, including LEDGF and DHX9, to affect AS events in the brain cortex (Li et al., 2016). In addition, MeCP2 is a key subunit of the *large assembly* of splicing regulators(LASR), and thus can affect splice site selection by serving as a bridge between methylated DNA and the LASR (Jiang et al., 2021). These studies reveal a mechanism linking DNA methylation and alternative splicing by the recruitment of splicing factors.

Precious studies have provided additional evidences that histone markers can regulate AS, such as the ability of certain histone modifications to recruit splicing factors via chromatin-binding proteins (Luco et al., 2010). For instance, the H3K36me3 marker is recognized by MRG15 (MORF-related gene 15) that directly recruits the polypyrimidine-tract-binding protein (PTB) to the intronic splicing silencer element, thereby causing the skipping of an alternative exon (Iwamori et al., 2016). The complex of H3K36me3-MRG15-PTB forms a splicing-chromatin co-transcriptional architecture that maintains the proximity of the splicing machinery to chromatin (Hayakawa et al., 2007; Luco et al., 2010; Zhang et al., 2006). In addition, the H3K36me3 markers are also recognized by the Psip1(PC4 and SRSF1 interacting protein 1), which further regulates AS by recruiting the SR protein SRSF1 to specific gene regions encompassing alternative exons (such as exon 5 of Diap2 and exon 7 of Vcan) (Pradeepa et al., 2012). These findings suggest that histone markers also have a more direct impact on splicing, as certain histone modifications recruit splicing factors via adaptor proteins.

3.3 | Epigenetic regulations directly affecting splicing factors

In addition to the general effect, the epigenetic modifications may also occur directly in specific genes encoding splicing factors or their upstream regulators, leading to the expression change of splicing factors that causes extensive alteration in AS (Figure 3c). A variety of splicing regulators, such as hnRNP and SR proteins, bind to multiple regulatory *cis*-elements in pre-mRNAs to control alternative splice site usage. DNA methylation is known to silence gene expression, while different histone modifications can either activate or silence genes (Li et al., 2007). Therefore, epigenetic modifications may directly affect the expression of splicing regulators, resulting in various gene exon inclusion and exclusion. For example, scaffold/matrix-associated region-binding protein 1 (SMAR1) was found to negatively regulate AS via HDAC6-mediated histone deacetylation in the gene encoding RNA binding protein Sam68, which in turn regulates splicing of CD44 in breast cancer (Nakka et al., 2015). Furthermore, epithelial splicing regulatory protein 1 (ESRP1) is an RNA-binding protein that mediates the AS of several genes associated with actin dynamics, cell polarity, and cell–cell adhesion during EMT (Warzecha & Carstens, 2012). The elevated expression of transcription factor E2F1 and increased CpG hydroxy methylation in the E2F1 binding motif can induce ESRP1 expression, resulting in extensive splicing changes in breast carcinoma (Ashok et al., 2021). Additionally, epigenetic modifications can affect the binding of splicing regulator proteins/transcription factors to their binding motif. For example, the DNA methylation of the intronic regions of a gene can reduce the binding of splicing factors, resulting in aberrant splicing of the gene (Maunakea et al., 2013). In summary, increasing evidence suggests that the epigenetic modifications at specific regions may directly affect the expression or RNA binding activity of specific factors, thus affect AS of their corresponding targets.

4 | FUNCTIONAL INTERACTION BETWEEN AS AND EPIGENETIC REGULATION

The regulation of AS and its impact on epigenetic modifications and chromatin structure have garnered increasing attention. Recent evidence suggests a reciprocal relationship between the regulation of splicing and epigenetics (Figure 4). Notably, spliceosomal components and splicing factors have been reported to affect chromatin structure (Kornblihtt et al., 2004; Schwartz & Ast, 2010). For example, a core spliceosomal component, U1 snRNP, was reported to alter chromatin organization when binding to the 5′ ss of a pre-mRNA (Keren-Shaul et al., 2013). In addition, overexpression of the splicing factors SRSF2 and SRSF1 promotes nucleosome depletion, while overexpression of hnRNPA1 increases nucleosome occupancy (Maslon et al., 2014).

Furthermore, emerging evidences suggest that regulation of AS can directly affect histone modifications. The splicing regulator, HuR, can induce local histone acetylation in regions surrounding alternative exons (Zhou et al., 2011). Consistently, disruption of HuR binding sites leads to increased cassette exon inclusion and reduction of H3 and H4

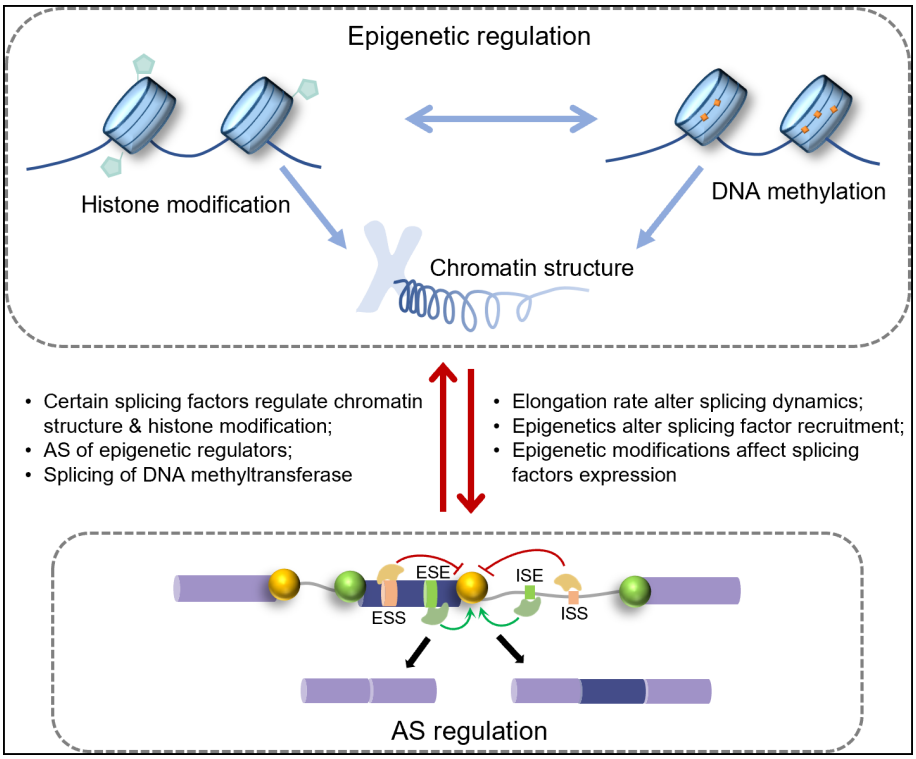

FIGURE 4 Crosstalk between AS and epigenetic regulation: The epigenetic markers, such as chromatin structure, histone modification, and DNA methylation, have been closely associated with the splicing regulation. All these regulation layers are inextricably intertwined and may have a reciprocal relationship. The crosstalk between epigenetic regulation and AS is emphasized by the red arrows, with the potential mechanisms listed on each side.

acetylation on the nucleosomes formed at the surrounding exon (Zhou et al., 2011). In addition, H3 and H4 hyperacetylation also promotes a more relaxed chromatin structure, a faster Pol II elongation rate, and increased exon skipping (Zhou et al., 2014). Mutations on splice sites were found to affect the methylation of histone H3K36, suggesting that the histone modifications were affected by the exon definition (Kim et al., 2011). Global inhibition of splicing by spliceostatin resulted in a rapid repositioning of H3K36me3 away from 5′ ends in favor of 3′ ends (Kim et al., 2011). These findings indicate that co-transcriptional splicing machinery influences the establishment of normal histone modification patterns.

In addition to the impacts on histone modifications, AS can also influence DNA methylation by regulating the splicing of epigenetic factors. A typical splicing factor, PTBP1, was found to directly affect DNA methylation via regulating AS of Dnmt3b (Iannone et al., 2023). Specifically, PTBP1 was found to activate the splicing of an alternatively retained intron 6 in DNMT3B, thereby increasing the Dnmt3b expression level and ensuring normal DNA methylation in pluripotent stem cells (Iannone et al., 2023).

Furthermore, various epigenetic modifications are inextricably interconnected. Many methyl-CpG binding proteins, including MeCP2, MBD1, and Kaiso, localize to DNA methylated promoters and recruit a protein complex that contains HDACs and histone methyltransferases (Jones et al., 1998; Martin Caballero et al., 2009), suggesting DNA methylation can alter the histone modifications to affect chromatin structure. Conversely, histone modifications have been shown to impact DNA methylation. For example, mutations in the histone H3K9 methyltransferase gene lead to decreased DNA methylation (Stancheva, 2005). Additionally, Dnmt3b-dependent DNA methylation at pericentric repeats requires Suv39h-mediated H3K9 methylation via a HP1α-Dnmt3b interaction (Lehnertz et al., 2003; Zhao et al., 2016).

These discoveries have profound implications in connecting splicing regulation with chromatin structure, histone modification, and DNA methylation, underscoring the multi-layered regulation of AS. Understanding the dynamic interplay between these different regulatory levels remain a challenge, but unraveling these intricate mechanisms holds great potential for advancing our comprehension of gene expression regulation.

TABLE 1 Common epigenetic modifications associated with AS regulation in cancer.

Epigenetic modifications	Related factors	AS events	Regulatory mechanisms	Disease	References
DNA methylation	MeCP2	Various targeted AS events	Adaptor	Colorectal carcinoma	(Maunakea et al., 2013)
	ESRP1 E2F1	Various targeted AS events	Splicing factor	Breast cancer	(Ashok et al., 2021)
	ESRP1	FGFR2 exon IIIc exclusion	Adaptor	Gastric Cancer	(Teles et al., 2019)
	CELF2	Intron retention in ULK1 CARD10	Pol II elongation	Breast cancer	(Pique et al., 2019)
H3K36me3	MRG15 PTB	FGFR-2 exon IIIb exclusion	Adaptor	Lung cancer	(Sanidas et al., 2014)
		Intron retention in DVL2	Pol II elongation	Colorectal cancer	(Yuan et al., 2017)
		ATG5 exon 2 exclusion	Pol II elongation	Renal cell carcinomas	(Gonzalez-Rodriguez et al., 2020)
		CDH1 exon 8 exclusion	Pol II elongation	Gastric cancer	(Curado et al., 2015; Li et al., 2015)
H3K9me3	JMJD1A	AR exon 3b inclusion	Adaptor	Prostate cancer	(Fan et al., 2018)
	HP1γ	CD44 variant exon inclusion	Pol II elongation	Cervical cancer	(Saint-Andre et al., 2011)
H3K9me2	HP1γSRSF1	VEGFA exon 6A inclusion	Adaptor	Osteosarcoma and breast cancer	(Salton et al., 2014)
Histone deacetylation	SAM68	CD44 variant exon inclusion	Splicing factor	Breast cancer	(Nakka et al., 2015)
Histone acetylation (H3, H4 acetylation)	SRSF1	MCL1 exon 2 inclusion	Pol II elongation	Colorectal carcinoma	(Khan et al., 2014; Khan et al., 2016)
		E-cadherin exon 11 inclusion	Pol II elongation	Chronic lymphocytic leukemia	(Jordaan et al., 2013)
Histon hyperacetylation	PHF5A	KDM3A intron retention	Pol II elongation	Colorectal cancer	(Wang et al., 2019)
H3K9me3 H3K27me3 H3K9 acetylation	Snail	CPEΔN variant	Adaptor	Lung cancer	(Sun et al., 2020)
H3K9ac H3K27ac H3K4me3		Various gene exon inclusion	Pol II elongation	Chronic myelogenous leukemia, hepatocellular carcinoma, adenocarcinoma	(Curado et al., 2015)
H3K4me3 H3K36me3	PHF5A	Intron retention in FASTK	Pol II elongation	Breast Cancer	(Zheng et al., 2018)
H3K9me2 H3K27me3	HP1α	FN1 gene inclusion of ED1	Adaptor	Hepatocellular carcinoma	(Allo et al., 2009)

5 | FUNCTIONAL IMPLICATION OF EPIGENETIC REGULATION OF AS IN CANCER

The dysregulation of epigenetics and AS are closely related to human diseases, especially cancers. Most oncogenes, such as TP53, KRAS, APC, and PIK3CA, have frequent mutations that affect their splicing in cancers, many of these mutations play a direct role in driving tumorigenesis (Wang et al., 2020). Furthermore, epigenetic changes caused by

mutation or aberrant gene expression are linked to cancer because they can cause the silencing of tumor suppressor genes or the activation of oncogenes (Baylin & Jones, 2016), which can affect multiple cancer-associated cellular process including cell growth, differentiation, cell cycle regulation, DNA repair, signal transduction, and metabolism.

The complex regulation of splicing is essential for the normal physiological functions of human cells. Splicing is tightly regulated in normal cells to ensure that proteins are produced properly. However, mutations in splicing machinery and splicing regulatory elements can also lead to aberrant splicing and cancer. Aberrant splicing can result in the production of abnormal proteins, which can disrupt the normal functioning of the cell and lead to cancer (Wang & Aifantis, 2020). Mutations in splicing machinery can lead to the skipping of important regulatory elements, resulting in the expression of oncogenes or the silencing of tumor suppressor genes, both of which can promote cancer (Kaida et al., 2012). Early studies indicated that several human diseases, including spinal muscular atrophy, retinitis pigmentosa, cystic fibrosis, and Hutchinson-Gilford progeria, are caused by a single aberrant splicing event (Narayanan et al., 2017). In addition to the genetic factors, epigenetic modifications also contribute to alternative pre-mRNA splicing patterns. For example, mutations in the histone acetyltransferase p300 can lead to aberrant splicing of the dystrophin gene, resulting in Duchenne Muscular Dystrophy (Colussi et al., 2011). Mutations in the functional enzymatic component of the Polycomb Repressive Complex 2 (PRC2) can lead to aberrant splicing of the BRCA1 gene, which is associated with an increased risk of breast cancer (Gonzalez et al., 2011; Puppe et al., 2009).

Dysregulation of epigenetic modifications and abnormal AS each both have crucial roles in tumor progression (Gimeno-Valiente et al., 2022; Yu et al., 2021). However, the combinatorial effects of these regulations on tumorigenesis remain to be discovered. Several studies have made the connection between the dysregulation of epigenetics and AS in various cancer. In Table 1, we summarized recent studies that link epigenetic modifications to AS in various cancers. We included information on a specific epigenetic modification type, related regulatory factors, cancer types, abnormal splicing events, and regulatory mechanisms. The connection between the regulations of transcription and splicing may provide critical new insight into cancer treatment (Imbriano & Belluti, 2022). For example, the key regulator of transcription elongation, MYC, is a frequent mutation in multiple cancers. The cancers with MYC mutations are especially vulnerable to small chemicals inhibiting spliceosomal functions (Hsu et al., 2015; Koh et al., 2015), probably due to the extra pressure imposed by elevated transcription on the splicing machinery. By understanding the mechanistic models of epigenetic modifications and AS, new strategies can be developed to target the epigenetic markers and other cancer-related AS, which could lead to more effective cancer treatment.

6 | CONCLUSION AND FUTURE PERSPECTIVES

In this review, we have presented an integrated view of the regulation of AS in different layers, with emphasis on the regulatory roles of epigenetic modifications and the crosstalk between these layers. Significant progress has been made recently to reveal the intricate connection between epigenetics and AS events in various model systems. In order to reflect this intertwined regulation network, we summarized three simplified regulatory models. Additionally, the involvement of epigenetic modifications in splicing regulation may provide novel mechanistic insights into the development and progression of cancer, and representative examples were also listed in this review. Epigenetic therapy has several advantages in cancer treatment, as it can increase the precision of targeting and enhance the efficacy of treatments for specific cancer subtypes. New therapies targeting both epigenetic modifications and AS regulation have great potential in cancer prevention and therapy. Future studies may concentrate on how epigenetic alterations impact widespread splicing dysregulation in cancer and other diseases, with the aims to explore new therapeutic opportunities.

AUTHOR CONTRIBUTIONS

Zefeng Wang: Supervision (lead); writing – review and editing (equal). **Ning Wang:** Visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Yue Hu:** Visualization (equal); writing – original draft (equal); writing – review and editing (equal).

ACKNOWLEDGMENTS

The authors want to thank Dr. Sirui Zhang from Harvard Brigham and Women's Hospital for useful discussion and suggestions on this manuscript.

FUNDING INFORMATION

This work is supported by the Strategic Priority Research Program of Chinese Academy of Sciences (XDB38040100 to Zefeng Wang), the National Key Research and Development Program of China (2021YFA1300503 and 2018YFA0107602 to Zefeng Wang), the National Natural Science Foundation of China (32030064, 91940303, and 31730110 to Zefeng Wang), the Starry Night Science Fund at Shanghai Institute for Advanced Study of Zhejiang University (SN-ZJU-SIAS-009) and the NIH Basic Research in Cancer Health Disparities Award to Steven R. Patierno and Jennifer A. Freedman (R01CA220314). Zefeng Wang is also supported by CAS Interdisciplinary Innovation Team and the type A CAS Pioneer 100-Talent program.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Zefeng Wang https://orcid.org/0000-0002-6605-3637

RELATED WIREs ARTICLE

Alternative-splicing defects in cancer: Splicing regulators and their downstream targets, guiding the way to novel cancer therapeutics

REFERENCES

Alexander, R. D., Innocente, S. A., Barrass, J. D., & Beggs, J. D. (2010). Splicing-dependent RNA polymerase pausing in yeast. *Molecular Cell*, *40*(4), 582–593. https://doi.org/10.1016/j.molcel.2010.11.005

Alharbi, A. B., Schmitz, U., Bailey, C. G., & Rasko, J. E. J. (2021). CTCF as a regulator of alternative splicing: New tricks for an old player. *Nucleic Acids Research*, *49*(14), 7825–7838. https://doi.org/10.1093/nar/gkab520

Allo, 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., & Kornblihtt, A. R. (2009). Control of alternative splicing through siRNA-mediated transcriptional gene silencing. *Nature Structural & Molecular Biology*, *16*(7), 717–724. https://doi.org/10.1038/nsmb.1620

Ashok, C., Ahuja, N., Natua, S., Mishra, J., Samaiya, A., & Shukla, S. (2021). E2F1 and epigenetic modifiers orchestrate breast cancer progression by regulating oxygen-dependent ESRP1 expression. *Oncogene*, *10*(8), 58. https://doi.org/10.1038/s41389-021-00347-6

Auboeuf, D., Honig, A., Berget, S. M., & O'Malley, B. W. (2002). Coordinate regulation of transcription and splicing by steroid receptor coregulators. *Science*, *298*(5592), 416–419. https://doi.org/10.1126/science.1073734

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research*, *21*(3), 381–395. https://doi.org/10.1038/cr.2011.22

Bannister, A. J., Zegerman, P., Partridge, J. F., Miska, E. A., Thomas, J. O., Allshire, R. C., & Kouzarides, T. (2001). Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature*, *410*(6824), 120–124. https://doi.org/10.1038/35065138

Baylin, S. B., & Jones, P. A. (2016). Epigenetic determinants of cancer. *Cold Spring Harbor Perspectives in Biology*, *8*(9), a019505. https://doi.org/10.1101/cshperspect.a019505

Berget, S. M., Moore, C., & Sharp, P. A. (1977). Spliced segments at the 5′ terminus of adenovirus 2 late mRNA. *Proceedings of the National Academy of Sciences of the United States of America*, *74*(8), 3171–3175. https://doi.org/10.1073/pnas.74.8.3171

Bestor, T. H. (2000). The DNA methyltransferases of mammals. *Human Molecular Genetics*, *9*(16), 2395–2402. https://doi.org/10.1093/hmg/9.16.2395

Beyer, A. L., Bouton, A. H., & Miller, O. L., Jr. (1981). Correlation of hnRNP structure and nascent transcript cleavage. *Cell*, *26*(2 Pt 2), 155–165. https://doi.org/10.1016/0092-8674(81)90299-3

Beyer, A. L., & Osheim, Y. N. (1988). Splice site selection, rate of splicing, and alternative splicing on nascent transcripts. *Genes & Development*, *2*(6), 754–765. https://doi.org/10.1101/gad.2.6.754

Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes & Development*, *16*(1), 6–21. https://doi.org/10.1101/gad.947102

Bonnal, S. C., Lopez-Oreja, I., & Valcarcel, J. (2020). Roles and mechanisms of alternative splicing in cancer - implications for care. *Nature Reviews Clinical Oncology*, *17*(8), 457–474. https://doi.org/10.1038/s41571-020-0350-x

Braunschweig, U., Gueroussov, S., Plocik, A. M., Graveley, B. R., & Blencowe, B. J. (2013). Dynamic integration of splicing within gene regulatory pathways. *Cell*, *152*(6), 1252–1269. https://doi.org/10.1016/j.cell.2013.02.034

Carrillo Oesterreich, F., Bieberstein, N., & Neugebauer, K. M. (2011). Pause locally, splice globally. *Trends in Cell Biology*, *21*(6), 328–335. https://doi.org/10.1016/j.tcb.2011.03.002

Carrillo Oesterreich, F., Preibisch, S., & Neugebauer, K. M. (2010). Global analysis of nascent RNA reveals transcriptional pausing in terminal exons. *Molecular Cell*, *40*(4), 571–581. https://doi.org/10.1016/j.molcel.2010.11.004

Chen, Y., Jorgensen, M., Kolde, R., Zhao, X., Parker, B., Valen, E., Wen, J., & Sandelin, A. (2011). Prediction of RNA polymerase II recruitment, elongation and stalling from histone modification data. *BMC Genomics*, *12*, 544. https://doi.org/10.1186/1471-2164-12-544

Cheng, D., Cote, J., Shaaban, S., & Bedford, M. T. (2007). The arginine methyltransferase CARM1 regulates the coupling of transcription and mRNA processing. *Molecular Cell*, *25*(1), 71–83. https://doi.org/10.1016/j.molcel.2006.11.019

Cheng, T. L., Chen, J., Wan, H., Tang, B., Tian, W., Liao, L., & Qiu, Z. (2017). Regulation of mRNA splicing by MeCP2 via epigenetic modifications in the brain. *Scientific Reports*, *7*, 42790. https://doi.org/10.1038/srep42790

Chow, L. T., Gelinas, R. E., Broker, T. R., & Roberts, R. J. (1977). An amazing sequence arrangement at the 5′ ends of adenovirus 2 messenger RNA. *Cell*, *12*(1), 1–8. https://doi.org/10.1016/0092-8674(77)90180-5

Colussi, C., Rosati, J., Straino, S., Spallotta, F., Berni, R., Stilli, D., Rossi, S., Musso, E., Macchi, E., Mai, A., Sbardella, G., Castellano, S., Chimenti, C., Frustaci, A., Nebbioso, A., Altucci, L., Capogrossi, M. C., & Gaetano, C. (2011). Nepsilon-lysine acetylation determines dissociation from GAP junctions and lateralization of connexin 43 in normal and dystrophic heart. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(7), 2795–2800. https://doi.org/10.1073/pnas.1013124108

Coulon, A., Ferguson, M. L., de Turris, V., Palangat, M., Chow, C. C., & Larson, D. R. (2014). Kinetic competition during the transcription cycle results in stochastic RNA processing. *eLife*, *3*, e03939. https://doi.org/10.7554/eLife.03939

Curado, J., Iannone, C., Tilgner, H., Valcarcel, J., & Guigo, R. (2015). Promoter-like epigenetic signatures in exons displaying cell type-specific splicing. *Genome Biology*, *16*, 236. https://doi.org/10.1186/s13059-015-0797-8

Dai, S., Wang, C., Zhang, C., Feng, L., Zhang, W., Zhou, X., He, Y., Xia, X., Chen, B., & Song, W. (2022). PTB: Not just a polypyrimidine tract-binding protein. *Journal of Cellular Physiology*, *237*(5), 2357–2373. https://doi.org/10.1002/jcp.30716

de la Mata, M., Alonso, C. R., Kadener, S., Fededa, J. P., Blaustein, M., Pelisch, F., Cramer, P., Bentley, D., & Kornblihtt, A. R. (2003). A slow RNA polymerase II affects alternative splicing in vivo. *Molecular Cell*, *12*(2), 525–532. https://doi.org/10.1016/j.molcel.2003.08.001

Deaton, A. M., & Bird, A. (2011). CpG islands and the regulation of transcription. *Genes & Development*, *25*(10), 1010–1022. https://doi.org/10.1101/gad.2037511

Dujardin, G., Lafaille, C., Petrillo, E., Buggiano, V., Gomez Acuna, L. I., Fiszbein, A., Godoy Herz, M. A., Nieto Moreno, N., Munoz, M. J., Allo, M., Schor, I. E., & Kornblihtt, A. R. (2013). Transcriptional elongation and alternative splicing. *Biochimica et Biophysica Acta*, *1829*(1), 134–140. https://doi.org/10.1016/j.bbagrm.2012.08.005

Eberhardy, S. R., & Farnham, P. J. (2002). Myc recruits P-TEFb to mediate the final step in the transcriptional activation of the cad promoter. *The Journal of Biological Chemistry*, *277*(42), 40156–40162. https://doi.org/10.1074/jbc.M207441200

Eperon, L. P., Graham, I. R., Griffiths, A. D., & Eperon, I. C. (1988). Effects of RNA secondary structure on alternative splicing of pre-mRNA: Is folding limited to a region behind the transcribing RNA polymerase? *Cell*, *54*(3), 393–401. https://doi.org/10.1016/0092-8674(88)90202-4

Estaras, C., Fueyo, R., Akizu, N., Beltran, S., & Martinez-Balbas, M. A. (2013). RNA polymerase II progression through H3K27me3-enriched gene bodies requires JMJD3 histone demethylase. *Molecular Biology of the Cell*, *24*(3), 351–360. https://doi.org/10.1091/mbc.E12-07-0561

Fan, L., Zhang, F., Xu, S., Cui, X., Hussain, A., Fazli, L., Gleave, M., Dong, X., & Qi, J. (2018). Histone demethylase JMJD1A promotes alternative splicing of AR variant 7 (AR-V7) in prostate cancer cells. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(20), E4584–E4593. https://doi.org/10.1073/pnas.1802415115

Flores, K., Wolschin, F., Corneveaux, J. J., Allen, A. N., Huentelman, M. J., & Amdam, G. V. (2012). Genome-wide association between DNA methylation and alternative splicing in an invertebrate. *BMC Genomics*, *13*, 480. https://doi.org/10.1186/1471-2164-13-480

Fong, N., Kim, H., Zhou, Y., Ji, X., Qiu, J., Saldi, T., Diener, K., Jones, K., Fu, X. D., & Bentley, D. L. (2014). Pre-mRNA splicing is facilitated by an optimal RNA polymerase II elongation rate. *Genes & Development*, *28*(23), 2663–2676. https://doi.org/10.1101/gad.252106.114

Fu, X. D., & Ares, M., Jr. (2014). Context-dependent control of alternative splicing by RNA-binding proteins. *Nature Reviews Genetics*, *15*(10), 689–701. https://doi.org/10.1038/nrg3778

Fuks, F., Hurd, P. J., Wolf, D., Nan, X., Bird, A. P., & Kouzarides, T. (2003). The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *The Journal of Biological Chemistry*, *278*(6), 4035–4040. https://doi.org/10.1074/jbc.M210256200

Gelfman, S., & Ast, G. (2013). When epigenetics meets alternative splicing: The roles of DNA methylation and GC architecture. *Epigenomics*, *5*(4), 351–353. https://doi.org/10.2217/epi.13.32

Gelfman, S., Cohen, N., Yearim, A., & Ast, G. (2013). DNA-methylation effect on cotranscriptional splicing is dependent on GC architecture of the exon-intron structure. *Genome Research*, *23*(5), 789–799. https://doi.org/10.1101/gr.143503.112

Gimeno-Valiente, F., Lopez-Rodas, G., Castillo, J., & Franco, L. (2022). Alternative splicing, epigenetic modifications and cancer: A dangerous triangle, or a hopeful one? *Cancers (Basel)*, *14*(3), 560. https://doi.org/10.3390/cancers14030560

Giono, L. E., & Kornblihtt, A. R. (2020). Linking transcription, RNA polymerase II elongation and alternative splicing. *The Biochemical Journal*, *477*(16), 3091–3104. https://doi.org/10.1042/BCJ20200475

Gonzalez, M. E., DuPrie, M. L., Krueger, H., Merajver, S. D., Ventura, A. C., Toy, K. A., & Kleer, C. G. (2011). Histone methyltransferase EZH2 induces Akt-dependent genomic instability and BRCA1 inhibition in breast cancer. *Cancer Research*, *71*(6), 2360–2370. https://doi.org/10.1158/0008-5472.CAN-10-1933

Gonzalez-Rodriguez, P., Engskog-Vlachos, P., Zhang, H., Murgoci, A. N., Zerdes, I., & Joseph, B. (2020). SETD2 mutation in renal clear cell carcinoma suppress autophagy via regulation of ATG12. *Cell Death & Disease*, *11*(1), 69. https://doi.org/10.1038/s41419-020-2266-x

Goren, A., Ram, O., Amit, M., Keren, H., Lev-Maor, G., Vig, I., Pupko, T., & Ast, G. (2006). Comparative analysis identifies exonic splicing regulatory sequences—The complex definition of enhancers and silencers. *Molecular Cell*, *22*(6), 769–781. https://doi.org/10.1016/j.molcel.2006.05.008

Gu, B., Eick, D., & Bensaude, O. (2013). CTD serine-2 plays a critical role in splicing and termination factor recruitment to RNA polymerase II in vivo. *Nucleic Acids Research*, *41*(3), 1591–1603. https://doi.org/10.1093/nar/gks1327

Gunderson, F. Q., & Johnson, T. L. (2009). Acetylation by the transcriptional coactivator Gcn5 plays a novel role in co-transcriptional spliceosome assembly. *PLoS Genetics*, *5*(10), e1000682. https://doi.org/10.1371/journal.pgen.1000682

Guo, J., & Price, D. H. (2013). RNA polymerase II transcription elongation control. *Chemical Reviews*, *113*(11), 8583–8603. https://doi.org/10.1021/cr400105n

Hayakawa, T., Ohtani, Y., Hayakawa, N., Shinmyozu, K., Saito, M., Ishikawa, F., & Nakayama, J. (2007). RBP2 is an MRG15 complex component and down-regulates intragenic histone H3 lysine 4 methylation. *Genes to Cells*, *12*(6), 811–826. https://doi.org/10.1111/j.1365-2443.2007.01089.x

Herzel, L., Ottoz, D. S. M., Alpert, T., & Neugebauer, K. M. (2017). Splicing and transcription touch base: Co-transcriptional spliceosome assembly and function. *Nature Reviews Molecular Cell Biology*, *18*(10), 637–650. https://doi.org/10.1038/nrm.2017.63

Hsin, J. P., & Manley, J. L. (2012). The RNA polymerase II CTD coordinates transcription and RNA processing. *Genes & Development*, *26*(19), 2119–2137. https://doi.org/10.1101/gad.200303.112

Hsu, T. Y., Simon, L. M., Neill, N. J., Marcotte, R., Sayad, A., Bland, C. S., Echeverria, G. V., Sun, T., Kurley, S. J., Tyagi, S., Karlin, K. L., Dominguez-Vidana, R., Hartman, J. D., Renwick, A., Scorsone, K., Bernardi, R. J., Skinner, S. O., Jain, A., Orellana, M., ... Westbrook, T. F. (2015). The spliceosome is a therapeutic vulnerability in MYC-driven cancer. *Nature*, *525*(7569), 384–388. https://doi.org/10.1038/nature14985

Hu, J., Qian, H., Xue, Y., & Fu, X. D. (2018). PTB/nPTB: Master regulators of neuronal fate in mammals. *Biophysics Reports*, *4*(4), 204–214. https://doi.org/10.1007/s41048-018-0066-y

Hu, Q., Greene, C. S., & Heller, E. A. (2020). Specific histone modifications associate with alternative exon selection during mammalian development. *Nucleic Acids Research*, *48*(9), 4709–4724. https://doi.org/10.1093/nar/gkaa248

Huang, S., & Spector, D. L. (1996). Intron-dependent recruitment of pre-mRNA splicing factors to sites of transcription. *The Journal of Cell Biology*, *133*(4), 719–732. https://doi.org/10.1083/jcb.133.4.719

Iannone, C., Kainov, Y., Zhuravskaya, A., Hamid, F., Nojima, T., & Makeyev, E. V. (2023). PTBP1-activated co-transcriptional splicing controls epigenetic status of pluripotent stem cells. *Molecular Cell*, *83*(2), 203–218.e209. https://doi.org/10.1016/j.molcel.2022.12.014

Imbriano, C., & Belluti, S. (2022). Histone marks-dependent effect on alternative splicing: New perspectives for targeted splicing modulation in cancer? *International Journal of Molecular Sciences*, *23*(15), 8304. https://doi.org/10.3390/ijms23158304

Iwamori, N., Tominaga, K., Sato, T., Riehle, K., Iwamori, T., Ohkawa, Y., Coarfa, C., Ono, E., & Matzuk, M. M. (2016). MRG15 is required for pre-mRNA splicing and spermatogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(37), E5408–E5415. https://doi.org/10.1073/pnas.1611995113

Ji, X., Zhou, Y., Pandit, S., Huang, J., Li, H., Lin, C. Y., Xiao, R., Burge, C. B., & Fu, X. D. (2013). SR proteins collaborate with 7SK and promoter-associated nascent RNA to release paused polymerase. *Cell*, *153*(4), 855–868. https://doi.org/10.1016/j.cell.2013.04.028

Jiang, Y., Fu, X., Zhang, Y., Wang, S. F., Zhu, H., Wang, W. K., Zhang, L., Wu, P., Wong, C. C. L., Li, J., Ma, J., Guan, J. S., Huang, Y., & Hui, J. (2021). Rett syndrome linked to defects in forming the MeCP2/Rbfox/LASR complex in mouse models. *Nature Communications*, *12*(1), 5767. https://doi.org/10.1038/s41467-021-26084-3

Jones, P. A., & Liang, G. (2009). Rethinking how DNA methylation patterns are maintained. *Nature Reviews Genetics*, *10*(11), 805–811. https://doi.org/10.1038/nrg2651

Jones, P. L., Veenstra, G. J., Wade, P. A., Vermaak, D., Kass, S. U., Landsberger, N., Strouboulis, J., & Wolffe, A. P. (1998). Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nature Genetics*, *19*(2), 187–191. https://doi.org/10.1038/561

Jordaan, G., Liao, W., & Sharma, S. (2013). E-cadherin gene re-expression in chronic lymphocytic leukemia cells by HDAC inhibitors. *BMC Cancer*, *13*, 88. https://doi.org/10.1186/1471-2407-13-88

Kadener, S., Cramer, P., Nogues, G., Cazalla, D., de la Mata, M., Fededa, J. P., Werbajh, S. E., Srebrow, A., & Kornblihtt, A. R. (2001). Antagonistic effects of T-Ag and VP16 reveal a role for RNA pol II elongation on alternative splicing. *The EMBO Journal*, *20*(20), 5759–5768. https://doi.org/10.1093/emboj/20.20.5759

Kadener, S., Fededa, J. P., Rosbash, M., & Kornblihtt, A. R. (2002). Regulation of alternative splicing by a transcriptional enhancer through RNA pol II elongation. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(12), 8185–8190. https://doi.org/10.1073/pnas.122246099

Kaida, D., Schneider-Poetsch, T., & Yoshida, M. (2012). Splicing in oncogenesis and tumor suppression. *Cancer Science*, *103*(9), 1611–1616. https://doi.org/10.1111/j.1349-7006.2012.02356.x

Karlic, R., Chung, H. R., Lasserre, J., Vlahovicek, K., & Vingron, M. (2010). Histone modification levels are predictive for gene expression. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(7), 2926–2931. https://doi.org/10.1073/pnas.0909344107

Keren-Shaul, H., Lev-Maor, G., & Ast, G. (2013). Pre-mRNA splicing is a determinant of nucleosome organization. *PLoS One*, *8*(1), e53506. https://doi.org/10.1371/journal.pone.0053506

Khan, D. H., Gonzalez, C., Cooper, C., Sun, J. M., Chen, H. Y., Healy, S., Xu, W., Smith, K. T., Workman, J. L., Leygue, E., & Davie, J. R. (2014). RNA-dependent dynamic histone acetylation regulates MCL1 alternative splicing. *Nucleic Acids Research*, *42*(3), 1656–1670. https://doi.org/10.1093/nar/gkt1134

Khan, D. H., Gonzalez, C., Tailor, N., Hamedani, M. K., Leygue, E., & Davie, J. R. (2016). Dynamic histone acetylation of H3K4me3 nucleosome regulates MCL1 pre-mRNA splicing. *Journal of Cellular Physiology*, *231*(10), 2196–2204. https://doi.org/10.1002/jcp.25337

Kim, S., Kim, H., Fong, N., Erickson, B., & Bentley, D. L. (2011). Pre-mRNA splicing is a determinant of histone H3K36 methylation. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(33), 13564–13569. https://doi.org/10.1073/pnas.1109475108

Koh, C. M., Bezzi, M., Low, D. H., Ang, W. X., Teo, S. X., Gay, F. P., Al-Haddawi, M., Tan, S. Y., Osato, M., Sabo, A., Amati, B., Wee, K. B., & Guccione, E. (2015). MYC regulates the core pre-mRNA splicing machinery as an essential step in lymphomagenesis. *Nature*, *523*(7558), 96–100. https://doi.org/10.1038/nature14351

Kolasinska-Zwierz, P., Down, T., Latorre, I., Liu, T., Liu, X. S., & Ahringer, J. (2009). Differential chromatin marking of introns and expressed exons by H3K36me3. *Nature Genetics*, *41*(3), 376–381. https://doi.org/10.1038/ng.322

Kornblihtt, A. R., de la Mata, M., Fededa, J. P., Munoz, M. J., & Nogues, G. (2004). Multiple links between transcription and splicing. *RNA*, *10*(10), 1489–1498. https://doi.org/10.1261/rna.7100104

Kulaeva, O. I., Gaykalova, D. A., & Studitsky, V. M. (2007). Transcription through chromatin by RNA polymerase II: Histone displacement and exchange. *Mutation Research*, *618*(1–2), 116–129. https://doi.org/10.1016/j.mrfmmm.2006.05.040

Laurent, L., Wong, E., Li, G., Huynh, T., Tsirigos, A., Ong, C. T., Low, H. M., Kin Sung, K. W., Rigoutsos, I., Loring, J., & Wei, C. L. (2010). Dynamic changes in the human methylome during differentiation. *Genome Research*, *20*(3), 320–331. https://doi.org/10.1101/gr.101907.109

Lehnertz, B., Ueda, Y., Derijck, A. A., Braunschweig, U., Perez-Burgos, L., Kubicek, S., Chen, T., Li, E., Jenuwein, T., & Peters, A. H. (2003). Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Current Biology*, *13*(14), 1192–1200. https://doi.org/10.1016/s0960-9822(03)00432-9

Lev Maor, G., Yearim, A., & Ast, G. (2015). The alternative role of DNA methylation in splicing regulation. *Trends in Genetics*, *31*(5), 274–280. https://doi.org/10.1016/j.tig.2015.03.002

Li, B., Carey, M., & Workman, J. L. (2007). The role of chromatin during transcription. *Cell*, *128*(4), 707–719. https://doi.org/10.1016/j.cell.2007.01.015

Li, E., Bestor, T. H., & Jaenisch, R. (1992). Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell*, *69*(6), 915–926. https://doi.org/10.1016/0092-8674(92)90611-f

Li, R., Dong, Q., Yuan, X., Zeng, X., Gao, Y., Chiao, C., Li, H., Zhao, X., Keles, S., Wang, Z., & Chang, Q. (2016). Misregulation of alternative splicing in a mouse model of Rett syndrome. *PLoS Genetics*, *12*(6), e1006129. https://doi.org/10.1371/journal.pgen.1006129

Li, X. W., Shi, B. Y., Yang, Q. L., Wu, J., Wu, H. M., Wang, Y. F., Wu, Z. J., Fan, Y. M., & Wang, Y. P. (2015). Epigenetic regulation of CDH1 exon 8 alternative splicing in gastric cancer. *BMC Cancer*, *15*, 954. https://doi.org/10.1186/s12885-015-1983-5

Luco, R. F., Allo, M., Schor, I. E., Kornblihtt, A. R., & Misteli, T. (2011). Epigenetics in alternative pre-mRNA splicing. *Cell*, *144*(1), 16–26. https://doi.org/10.1016/j.cell.2010.11.056

Luco, R. F., Pan, Q., Tominaga, K., Blencowe, B. J., Pereira-Smith, O. M., & Misteli, T. (2010). Regulation of alternative splicing by histone modifications. *Science*, *327*(5968), 996–1000. https://doi.org/10.1126/science.1184208

Martin Caballero, I., Hansen, J., Leaford, D., Pollard, S., & Hendrich, B. D. (2009). The methyl-CpG binding proteins Mecp2, Mbd2 and Kaiso are dispensable for mouse embryogenesis, but play a redundant function in neural differentiation. *PLoS One*, *4*(1), e4315. https://doi.org/10.1371/journal.pone.0004315

Martinez, E., Palhan, V. B., Tjernberg, A., Lymar, E. S., Gamper, A. M., Kundu, T. K., Chait, B. T., & Roeder, R. G. (2001). Human STAGA complex is a chromatin-acetylating transcription coactivator that interacts with pre-mRNA splicing and DNA damage-binding factors in vivo. *Molecular and Cellular Biology*, *21*(20), 6782–6795. https://doi.org/10.1128/MCB.21.20.6782-6795.2001

Maslon, M. M., Heras, S. R., Bellora, N., Eyras, E., & Caceres, J. F. (2014). The translational landscape of the splicing factor SRSF1 and its role in mitosis. *eLife*, *3*, e02028. https://doi.org/10.7554/eLife.02028

Matera, A. G., & Wang, Z. (2014). A day in the life of the spliceosome. *Nature Reviews Molecular Cell Biology*, *15*(2), 108–121. https://doi.org/10.1038/nrm3742

Maunakea, A. K., Chepelev, I., Cui, K., & Zhao, K. (2013). Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. *Cell Research*, *23*(11), 1256–1269. https://doi.org/10.1038/cr.2013.110

Miao, F., & Natarajan, R. (2005). Mapping global histone methylation patterns in the coding regions of human genes. *Molecular and Cellular Biology*, *25*(11), 4650–4661. https://doi.org/10.1128/MCB.25.11.4650-4661.2005

Misteli, T., Caceres, J. F., & Spector, D. L. (1997). The dynamics of a pre-mRNA splicing factor in living cells. *Nature*, *387*(6632), 523–527. https://doi.org/10.1038/387523a0

Moore, L. D., Le, T., & Fan, G. (2013). DNA methylation and its basic function. *Neuropsychopharmacology*, *38*(1), 23–38. https://doi.org/10.1038/npp.2012.112

Naftelberg, S., Schor, I. E., Ast, G., & Kornblihtt, A. R. (2015). Regulation of alternative splicing through coupling with transcription and chromatin structure. *Annual Review of Biochemistry*, *84*, 165–198. https://doi.org/10.1146/annurev-biochem-060614-034242

Nakka, K. K., Chaudhary, N., Joshi, S., Bhat, J., Singh, K., Chatterjee, S., Malhotra, R., De, A., Santra, M. K., Dilworth, F. J., & Chattopadhyay, S. (2015). Nuclear matrix-associated protein SMAR1 regulates alternative splicing via HDAC6-mediated deacetylation of

Sam68. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(26), E3374–E3383. https://doi.org/10.1073/pnas.1418603112

Nan, X., Campoy, F. J., & Bird, A. (1997). MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. *Cell*, *88*(4), 471–481. https://doi.org/10.1016/s0092-8674(00)81887-5

Nan, X., Meehan, R. R., & Bird, A. (1993). Dissection of the methyl-CpG binding domain from the chromosomal protein MeCP2. *Nucleic Acids Research*, *21*(21), 4886–4892. https://doi.org/10.1093/nar/21.21.4886

Narayanan, S. P., Singh, S., & Shukla, S. (2017). A saga of cancer epigenetics: Linking epigenetics to alternative splicing. *The Biochemical Journal*, *474*(6), 885–896. https://doi.org/10.1042/BCJ20161047

Neugebauer, K. M. (2019). Nascent RNA and the coordination of splicing with transcription. *Cold Spring Harbor Perspectives in Biology*, *11*(8), a032227. https://doi.org/10.1101/cshperspect.a032227

Neugebauer, K. M., & Roth, M. B. (1997). Distribution of pre-mRNA splicing factors at sites of RNA polymerase II transcription. *Genes & Development*, *11*(9), 1148–1159. https://doi.org/10.1101/gad.11.9.1148

Nieto Moreno, N., Giono, L. E., Cambindo Botto, A. E., Munoz, M. J., & Kornblihtt, A. R. (2015). Chromatin, DNA structure and alternative splicing. *FEBS Letters*, *589*(22), 3370–3378. https://doi.org/10.1016/j.febslet.2015.08.002

Nogues, G., Kadener, S., Cramer, P., Bentley, D., & Kornblihtt, A. R. (2002). Transcriptional activators differ in their abilities to control alternative splicing. *The Journal of Biological Chemistry*, *277*(45), 43110–43114. https://doi.org/10.1074/jbc.M208418200

Oberdoerffer, S. (2012). A conserved role for intragenic DNA methylation in alternative pre-mRNA splicing. *Transcription*, *3*(3), 106–109. https://doi.org/10.4161/trns.19816

Okano, M., Bell, D. W., Haber, D. A., & Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*, *99*(3), 247–257. https://doi.org/10.1016/s0092-8674(00)81656-6

Ong, C. T., & Corces, V. G. (2014). CTCF: An architectural protein bridging genome topology and function. *Nature Reviews Genetics*, *15*(4), 234–246. https://doi.org/10.1038/nrg3663

Pique, L., Martinez de Paz, A., Pineyro, D., Martinez-Cardus, A., Castro de Moura, M., Llinas-Arias, P., Setien, F., Gomez-Miragaya, J., Gonzalez-Suarez, E., Sigurdsson, S., Jonasson, J. G., Villanueva, A., Vidal, A., Davalos, V., & Esteller, M. (2019). Epigenetic inactivation of the splicing RNA-binding protein CELF2 in human breast cancer. *Oncogene*, *38*(45), 7106–7112. https://doi.org/10.1038/s41388-019-0936-x

Pradeepa, M. M., Sutherland, H. G., Ule, J., Grimes, G. R., & Bickmore, W. A. (2012). Psip1/Ledgf p52 binds methylated histone H3K36 and splicing factors and contributes to the regulation of alternative splicing. *PLoS Genetics*, *8*(5), e1002717. https://doi.org/10.1371/journal.pgen.1002717

Puppe, J., Drost, R., Liu, X., Joosse, S. A., Evers, B., Cornelissen-Steijger, P., Nederlof, P., Yu, Q., Jonkers, J., van Lohuizen, M., & Pietersen, A. M. (2009). BRCA1-deficient mammary tumor cells are dependent on EZH2 expression and sensitive to Polycomb repressive complex 2-inhibitor 3-deazaneplanocin A. *Breast Cancer Research*, *11*(4), R63. https://doi.org/10.1186/bcr2354

Quina, A. S., Buschbeck, M., & Di Croce, L. (2006). Chromatin structure and epigenetics. *Biochemical Pharmacology*, *72*(11), 1563–1569. https://doi.org/10.1016/j.bcp.2006.06.016

Rahhal, R., & Seto, E. (2019). Emerging roles of histone modifications and HDACs in RNA splicing. *Nucleic Acids Research*, *47*(10), 4911–4926. https://doi.org/10.1093/nar/gkz292

Sahebi, M., Hanafi, M. M., van Wijnen, A. J., Azizi, P., Abiri, R., Ashkani, S., & Taheri, S. (2016). Towards understanding pre-mRNA splicing mechanisms and the role of SR proteins. *Gene*, *587*(2), 107–119. https://doi.org/10.1016/j.gene.2016.04.057

Sahu, S. K., Agirre, E., Inayatullah, M., Mahesh, A., Tiwari, N., Lavin, D. P., Singh, A., Strand, S., Diken, M., Luco, R. F., Belmonte, J. C. I., & Tiwari, V. K. (2022). A complex epigenome-splicing crosstalk governs epithelial-to-mesenchymal transition in metastasis and brain development. *Nature Cell Biology*, *24*(8), 1265–1277. https://doi.org/10.1038/s41556-022-00971-3

Saint-Andre, V., Batsche, E., Rachez, C., & Muchardt, C. (2011). Histone H3 lysine 9 trimethylation and HP1gamma favor inclusion of alternative exons. *Nature Structural & Molecular Biology*, *18*(3), 337–344. https://doi.org/10.1038/nsmb.1995

Saldi, T., Cortazar, M. A., Sheridan, R. M., & Bentley, D. L. (2016). Coupling of RNA polymerase II transcription elongation with pre-mRNA splicing. *Journal of Molecular Biology*, *428*(12), 2623–2635. https://doi.org/10.1016/j.jmb.2016.04.017

Salton, M., Voss, T. C., & Misteli, T. (2014). Identification by high-throughput imaging of the histone methyltransferase EHMT2 as an epigenetic regulator of VEGFA alternative splicing. *Nucleic Acids Research*, *42*(22), 13662–13673. https://doi.org/10.1093/nar/gku1226

Sanidas, I., Polytarchou, C., Hatziapostolou, M., Ezell, S. A., Kottakis, F., Hu, L., Guo, A., Xie, J., Comb, M. J., Iliopoulos, D., & Tsichlis, P. N. (2014). Phosphoproteomics screen reveals akt isoform-specific signals linking RNA processing to lung cancer. *Molecular Cell*, *53*(4), 577–590. https://doi.org/10.1016/j.molcel.2013.12.018

Schor, I. E., Gomez Acuna, L. I., & Kornblihtt, A. R. (2013). Coupling between transcription and alternative splicing. *Cancer Treatment and Research*, *158*, 1–24. https://doi.org/10.1007/978-3-642-31659-3_1

Schor, I. E., Rascovan, N., Pelisch, F., Allo, M., & Kornblihtt, A. R. (2009). Neuronal cell depolarization induces intragenic chromatin modifications affecting NCAM alternative splicing. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(11), 4325–4330. https://doi.org/10.1073/pnas.0810666106

Schwartz, S., & Ast, G. (2010). Chromatin density and splicing destiny: On the cross-talk between chromatin structure and splicing. *The EMBO Journal*, *29*(10), 1629–1636. https://doi.org/10.1038/emboj.2010.71

Schwartz, S., Meshorer, E., & Ast, G. (2009). Chromatin organization marks exon-intron structure. *Nature Structural & Molecular Biology*, *16*(9), 990–995. https://doi.org/10.1038/nsmb.1659

Scotti, M. M., & Swanson, M. S. (2016). RNA mis-splicing in disease. *Nature Reviews Genetics*, *17*(1), 19–32. https://doi.org/10.1038/nrg.2015.3

Shi, Y. (2017). Mechanistic insights into precursor messenger RNA splicing by the spliceosome. *Nature Reviews Molecular Cell Biology*, *18*(11), 655–670. https://doi.org/10.1038/nrm.2017.86

Shukla, S., Kavak, E., Gregory, M., Imashimizu, M., Shutinoski, B., Kashlev, M., Oberdoerffer, P., Sandberg, R., & Oberdoerffer, S. (2011). CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing. *Nature*, *479*(7371), 74–79. https://doi.org/10.1038/nature10442

Shukla, S., & Oberdoerffer, S. (2012). Co-transcriptional regulation of alternative pre-mRNA splicing. *Biochimica et Biophysica Acta*, *1819*(7), 673–683. https://doi.org/10.1016/j.bbagrm.2012.01.014

Sisodia, S. S., Sollner-Webb, B., & Cleveland, D. W. (1987). Specificity of RNA maturation pathways: RNAs transcribed by RNA polymerase III are not substrates for splicing or polyadenylation. *Molecular and Cellular Biology*, *7*(10), 3602–3612. https://doi.org/10.1128/mcb.7.10.3602-3612.1987

Smale, S. T., & Tjian, R. (1985). Transcription of herpes simplex virus tk sequences under the control of wild-type and mutant human RNA polymerase I promoters. *Molecular and Cellular Biology*, *5*(2), 352–362. https://doi.org/10.1128/mcb.5.2.352-362.1985

Spies, N., Nielsen, C. B., Padgett, R. A., & Burge, C. B. (2009). Biased chromatin signatures around polyadenylation sites and exons. *Molecular Cell*, *36*(2), 245–254. https://doi.org/10.1016/j.molcel.2009.10.008

Stancheva, I. (2005). Caught in conspiracy: Cooperation between DNA methylation and histone H3K9 methylation in the establishment and maintenance of heterochromatin. *Biochemistry and Cell Biology*, *83*(3), 385–395. https://doi.org/10.1139/o05-043

Sun, J., Meng, D., Yu, T., Li, F., Zhang, G., Tian, X., Zhao, N., Li, G., Li, L., Wang, H., Liu, Y., Meng, Y., Ma, Y., Wan, Z., Bao, J., & Piao, H. (2020). N-terminal truncated carboxypeptidase E represses E-cadherin expression in lung cancer by stabilizing the snail-HDAC complex. *American Journal of Cancer Research*, *10*(3), 925–938. https://www.ncbi.nlm.nih.gov/pubmed/32266100

Teles, S. P., Oliveira, P., Ferreira, M., Carvalho, J., Ferreira, P., & Oliveira, C. (2019). Integrated analysis of structural variation and RNA expression of FGFR2 and its splicing modulator ESRP1 highlight the ESRP1(amp)-FGFR2(norm)-FGFR2-IIIc(high) Axis in diffuse gastric cancer. *Cancers (Basel)*, *12*(1), 70. https://doi.org/10.3390/cancers12010070

Ule, J., & Blencowe, B. J. (2019). Alternative splicing regulatory networks: Functions, mechanisms, and evolution. *Molecular Cell*, *76*(2), 329–345. https://doi.org/10.1016/j.molcel.2019.09.017

Ule, J., Stefani, G., Mele, A., Ruggiu, M., Wang, X., Taneri, B., Gaasterland, T., Blencowe, B. J., & Darnell, R. B. (2006). An RNA map predicting Nova-dependent splicing regulation. *Nature*, *444*(7119), 580–586. https://doi.org/10.1038/nature05304

Wang, E., & Aifantis, I. (2020). RNA splicing and cancer. *Trends Cancer*, *6*(8), 631–644. https://doi.org/10.1016/j.trecan.2020.04.011

Wang, Y., Bao, Y., Zhang, S., & Wang, Z. (2020). Splicing dysregulation in cancer: From mechanistic understanding to a new class of therapeutic targets. *Science China. Life Sciences*, *63*(4), 469–484. https://doi.org/10.1007/s11427-019-1605-0

Wang, Y., Xiao, X., Zhang, J., Choudhury, R., Robertson, A., Li, K., Ma, M., Burge, C. B., & Wang, Z. (2013). A complex network of factors with overlapping affinities represses splicing through intronic elements. *Nature Structural & Molecular Biology*, *20*(1), 36–45. https://doi.org/10.1038/nsmb.2459

Wang, Z., & Burge, C. B. (2008). Splicing regulation: From a parts list of regulatory elements to an integrated splicing code. *RNA*, *14*(5), 802–813. https://doi.org/10.1261/rna.876308

Wang, Z., Yang, X., Liu, C., Li, X., Zhang, B., Wang, B., Zhang, Y., Song, C., Zhang, T., Liu, M., Liu, B., Ren, M., Jiang, H., Zou, J., Liu, X., Zhang, H., Zhu, W. G., Yin, Y., Zhang, Z., … Luo, J. (2019). Acetylation of PHF5A modulates stress responses and colorectal carcinogenesis through alternative splicing-mediated upregulation of KDM3A. *Molecular Cell*, *74*(6), 1250–1263.e1256. https://doi.org/10.1016/j.molcel.2019.04.009

Wang, Z., Zang, C., Rosenfeld, J. A., Schones, D. E., Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Peng, W., Zhang, M. Q., & Zhao, K. (2008). Combinatorial patterns of histone acetylations and methylations in the human genome. *Nature Genetics*, *40*(7), 897–903. https://doi.org/10.1038/ng.154

Warzecha, C. C., & Carstens, R. P. (2012). Complex changes in alternative pre-mRNA splicing play a central role in the epithelial-to-mesenchymal transition (EMT). *Seminars in Cancer Biology*, *22*(5–6), 417–427. https://doi.org/10.1016/j.semcancer.2012.04.003

White, E. S., Baralle, F. E., & Muro, A. F. (2008). New insights into form and function of fibronectin splice variants. *The Journal of Pathology*, *216*(1), 1–14. https://doi.org/10.1002/path.2388

Will, C. L., & Luhrmann, R. (2011). Spliceosome structure and function. *Cold Spring Harbor Perspectives in Biology*, *3*(7), a003707. https://doi.org/10.1101/cshperspect.a003707

Wong, J. J., Gao, D., Nguyen, T. V., Kwok, C. T., van Geldermalsen, M., Middleton, R., Pinello, N., Thoeng, A., Nagarajah, R., Holst, J., Ritchie, W., & Rasko, J. E. J. (2017). Intron retention is regulated by altered MeCP2-mediated splicing factor recruitment. *Nature Communications*, *8*, 15134. https://doi.org/10.1038/ncomms15134

Xue, Y., Zhou, Y., Wu, T., Zhu, T., Ji, X., Kwon, Y. S., Zhang, C., Yeo, G., Black, D. L., Sun, H., Fu, X. D., & Zhang, Y. (2009). Genome-wide analysis of PTB-RNA interactions reveals a strategy used by the general splicing repressor to modulate exon inclusion or skipping. *Molecular Cell*, *36*(6), 996–1006. https://doi.org/10.1016/j.molcel.2009.12.003

Yang, X., Han, H., De Carvalho, D. D., Lay, F. D., Jones, P. A., & Liang, G. (2014). Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell*, *26*(4), 577–590. https://doi.org/10.1016/j.ccr.2014.07.028

Yearim, A., Gelfman, S., Shayevitch, R., Melcer, S., Glaich, O., Mallm, J. P., Nissim-Rafinia, M., Cohen, A. H., Rippe, K., Meshorer, E., & Ast, G. (2015). HP1 is involved in regulating the global impact of DNA methylation on alternative splicing. *Cell Reports*, *10*(7), 1122–1134. https://doi.org/10.1016/j.celrep.2015.01.038

Young, J. I., Hong, E. P., Castle, J. C., Crespo-Barreto, J., Bowman, A. B., Rose, M. F., Kang, D., Richman, R., Johnson, J. M., Berget, S., & Zoghbi, H. Y. (2005). Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(49), 17551–17558. https://doi.org/10.1073/pnas.0507856102

Yu, B., Yu, X., Xiong, J., & Ma, M. (2021). Methylation modification, alternative splicing, and noncoding RNA play a role in cancer metastasis through epigenetic regulation. *BioMed Research International*, *2021*, 4061525. https://doi.org/10.1155/2021/4061525

Yu, Y., Maroney, P. A., Denker, J. A., Zhang, X. H., Dybkov, O., Luhrmann, R., Jankowsky, E., Chasin, L. A., & Nilsen, T. W. (2008). Dynamic regulation of alternative splicing by silencers that modulate 5′ splice site competition. *Cell*, *135*(7), 1224–1236. https://doi.org/10.1016/j.cell.2008.10.046

Yuan, H., Li, N., Fu, D., Ren, J., Hui, J., Peng, J., Liu, Y., Qiu, T., Jiang, M., Pan, Q., Han, Y., Wang, X., Li, Q., & Qin, J. (2017). Histone methyltransferase SETD2 modulates alternative splicing to inhibit intestinal tumorigenesis. *The Journal of Clinical Investigation*, *127*(9), 3375–3391. https://doi.org/10.1172/JCI94292

Zhang, G., Taneja, K. L., Singer, R. H., & Green, M. R. (1994). Localization of pre-mRNA splicing in mammalian nuclei. *Nature*, *372*(6508), 809–812. https://doi.org/10.1038/372809a0

Zhang, P., Du, J., Sun, B., Dong, X., Xu, G., Zhou, J., Huang, Q., Liu, Q., Hao, Q., & Ding, J. (2006). Structure of human MRG15 chromo domain and its binding to Lys36-methylated histone H3. *Nucleic Acids Research*, *34*(22), 6621–6628. https://doi.org/10.1093/nar/gkl989

Zhang, S., Mao, M., Lv, Y., Yang, Y., He, W., Song, Y., Wang, Y., Yang, Y., Al Abo, M., Freedman, J. A., Patierno, S. R., Wang, Y., & Wang, Z. (2022). A widespread length-dependent splicing dysregulation in cancer. *Science Advances*, *8*(33), eabn9232. https://doi.org/10.1126/sciadv.abn9232

Zhang, T., Cooper, S., & Brockdorff, N. (2015). The interplay of histone modifications - writers that read. *EMBO Reports*, *16*(11), 1467–1481. https://doi.org/10.15252/embr.201540945

Zhao, Q., Zhang, J., Chen, R., Wang, L., Li, B., Cheng, H., Duan, X., Zhu, H., Wei, W., Li, J., Wu, Q., Han, J. D., Yu, W., Gao, S., Li, G., & Wong, J. (2016). Dissecting the precise role of H3K9 methylation in crosstalk with DNA maintenance methylation in mammals. *Nature Communications*, *7*, 12464. https://doi.org/10.1038/ncomms12464

Zheng, Y. Z., Xue, M. Z., Shen, H. J., Li, X. G., Ma, D., Gong, Y., Liu, Y. R., Qiao, F., Xie, H. Y., Lian, B., Sun, W. L., Zhao, H. Y., Yao, L., Zuo, W. J., Li, D. Q., Wang, P., Hu, X., & Shao, Z. M. (2018). PHF5A epigenetically inhibits apoptosis to promote breast cancer progression. *Cancer Research*, *78*(12), 3190–3206. https://doi.org/10.1158/0008-5472.CAN-17-3514

Zhou, H. L., Hinman, M. N., Barron, V. A., Geng, C., Zhou, G., Luo, G., Siegel, R. E., & Lou, H. (2011). Hu proteins regulate alternative splicing by inducing localized histone hyperacetylation in an RNA-dependent manner. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(36), E627–E635. https://doi.org/10.1073/pnas.1103344108

Zhou, H. L., Luo, G., Wise, J. A., & Lou, H. (2014). Regulation of alternative splicing by local histone modifications: Potential roles for RNA-guided mechanisms. *Nucleic Acids Research*, *42*(2), 701–713. https://doi.org/10.1093/nar/gkt875

Zhu, L. Y., Zhu, Y. R., Dai, D. J., Wang, X., & Jin, H. C. (2018). Epigenetic regulation of alternative splicing. *American Journal of Cancer Research*, *8*(12), 2346–2358. https://www.ncbi.nlm.nih.gov/pubmed/30662796

How to cite this article: Wang, N., Hu, Y., & Wang, Z. (2023). Regulation of alternative splicing: Functional interplay with epigenetic modifications and its implication to cancer. *WIREs RNA*, e1815. https://doi.org/10.1002/wrna.1815