Pre-mRNA splicing: Role of epigenetics and implications in disease

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A B S T R A C T

Epigenetics refer to a variety of processes that have long-term effects on gene expression programs without changes in DNA sequence. Key players in epigenetic control are histone modifications and DNA methylation which, in concert with chromatin remodeling complexes, nuclear architecture and microRNAs, define the chromatin structure of a gene and its transcriptional activity. There is a growing awareness that histone modifications and chromatin organization influence pre-mRNA splicing. Further there is emerging evidence that pre-mRNA splicing itself influences chromatin organization. In the mammalian genome around 95% of multi-exon genes generate alternatively spliced transcripts, the products of which create proteins with different functions. It is now established that several human diseases are a direct consequence of aberrant splicing events. In this review we present the interplay between epigenetic mechanisms and splicing regulation, as well as discuss recent studies on the role of histone deacetylases in splicing activities.

Introduction

RNA splicing is a complex regulatory process that plays a major role in gene expression regime and proteome diversity. Splicing occurs co-transcriptionally, while the pre-mRNA remains bound to chromatin by RNA polymerase II (Brody and Shav-Tal, 2011). Over the last few years, much has been learned about the regulatory network of splicing regulation involving transcription mechanisms and...
chromatin modifications. Splicing reactions can be constitutive, where introns are removed and all the exons are retained in the pre-mRNA, or alternative, where exons and introns can be excised or may be present in variable ways in the mature pre-mRNA. Aberrant regulations of splicing mechanisms, whether constitutive or alternative, have been implicated in a number of diseases and cancers. Targeting splicing as a therapeutic intervention is a rapidly moving field of biomedical research. In this review, we will provide a brief overview of splicing and will discuss the involvement of splicing factors, serine/arginine-rich proteins, in diseases and cancers. We will highlight the interplay between epigenetic mechanisms and splicing regulation, as well as review of recent studies highlighting the role of histone deacetylases in splicing activities.

A snapshot: regulation of splicing

In human cells, approximately 95% of the multi-exon genes yield alternatively spliced transcripts (Hnilicova and Stanek, 2011; Ip et al., 2011). Pre-mRNA splicing is a highly regulated step-wise process catalyzed by a multicomponent machine (the spliceosome), consisting of RNA and protein (Kalsotra and Cooper, 2011; Sperling et al., 2008; Wahl et al., 2009). The spliceosome consists of uridine rich U1, U2, U4, U5, and U6 small nuclear ribonucleoproteins (snRNPs) and a large number of non-snRNP proteins (Rappsilber et al., 2002; Ritchie et al., 2009; Wahl et al., 2009). The spliceosome recognizes the consensus sequences (e.g. 5′ and 3′ splice sites) at exon–intron boundaries, assembled onto them, and catalyze the splicing reaction (Black, 2003; Wahl et al., 2009). In addition to these, branch point sequence, polypyrimidine tract, cis- (present within pre-mRNA) and trans-regulatory elements (cellular factors, proteins and RNA) are also necessary for proper splicing regulation (Black, 2003; Wahl et al., 2009). Cis-acting elements, also known as splicing enhancers and silencers, play a major role in spliceosome assembly and splice site selection. They are involved in constitutive as well as alternative splicing mechanisms. Trans-acting factors, such as serine/arginine-rich (SR) proteins or hnRNPs (heterogeneous nuclear ribonucleoproteins), can interact with the spliceosome complex, cis-regulatory elements or proteins bound to cis-elements, thereby controlling the splicing outcomes (Black, 2003; Shepard and Hertel, 2009). Apart from these, several studies have reported alternative splicing can be explained how transcription affects splicing: the kinetic model and recruitment model (Kornblihtt et al., 2004; Munoz et al., 2010). According to the kinetic model, variation in the RNA polymerase II elongation rate modulates the splice site recognition efficiency of spliceosome complex, thus determines the inclusion or exclusion of alternative exons (de la Mata et al., 2003). The recruitment model proposes that splicing and transcription are coupled by physical and functional interaction of RNA polymerase II carboxyl-terminal domain (CTD) with the pre-mRNA processing factors, where CTD functions to recruit the factors (Egloff and Murphy, 2008; Kornblihtt et al., 2004). Presumably, both of the models are involved in alteration of splicing outcomes; however the detailed molecular and mechanical basis of the models still needs further investigation.

Role of splicing proteins in human disease and cancer

Till the early 1980’s it was unclear how the limited number of human genes could give rise to a large variety of proteins. However, this question was resolved with the realization that alternative splicing could generate several different transcripts and different proteins from a single gene. Alternative splicing was first documented with the Adenovirus 2 major late messages (Leff et al., 1986). About two-thirds of the human genome contain one or more alternatively spliced exons as observed by microarray profiling and expressed sequence tags-cDNA sequencing (Johnson et al., 2003). Splice variants created through alternative splicing can have opposing functions and thereby can alter the biological outcome (Bae et al., 2000; Bingle et al., 2000). This is quite evident with human BCL-X, MCL1, CASP-9, CED-4, CASP-2/ICH-1 and HTID-1 genes, which encode both pro- and anti-apoptotic variants (Wu et al., 2003). Though alternative splicing is regulated in response to signal transduction pathways, disease state, cell cycle progression and developmental process, it is the splice site sequences and the proteins that bind to these sequences that direct alternative splicing (Barash et al., 2010; Grabowski and Black, 2001; Irimia and Blencowe, 2012; Kalsotra and Cooper, 2011; Martinez et al., 2012; Palusa et al., 2007; Pick...
et al., 2004; Wang and Cooper, 2007). The sequential process for splice site recognition and splice variant formation is modulated by several cis- and trans-acting factors (Cartegni et al., 2002; Wang and Cooper, 2007). In human disease, aberrant pre-mRNA splicing may be a consequence of mutations in cis-acting regulatory sequences and/or in the trans-acting factors. Further altered expression of the trans-acting factors can also impact pre-mRNA splicing.

Several human diseases such as spinal muscular atrophy, retinitis pigmentosa, Hutchinson–Gilford progeria syndrome, medium-chain acyl-CoA dehydrogenase deficiency and some cancers are a direct consequence of aberrant splicing events due to mutations in the critical cis-acting sequences guiding splicing events (Dredge et al., 2001; Faustino and Cooper, 2003; Tazi et al., 2009; Zaharieva et al., 2012). Genomic mutation and RNA editing are some of the cis-acting mutations leading to changes in splicing of pre-mRNA (Maas, 2012; Maas et al., 2001). Conversion of adenosine to inosine is the common type of RNA editing in mammalian cells. The RNA splicing machinery recognizes the inosine as a guanine, resulting in the formation or elimination of donor or acceptor splice sites (Dominissini et al., 2011).

The serine/arginine-rich (SR) family protein and heterogeneous nuclear ribonucleoprotein (hnRNP) family function as splicing enhancers or silencers by acting as trans-acting factors to influence RNA splicing (Maas et al., 2001; Tacke and Manley, 1999). About 12 SR proteins (SRSF3/SRp20, SRSF2/SC35, SRSF8/SRp46, SRF11/SRp54, SRSF9/SRp30c, SRSF1/ASF/SF2/SRp30a, SRSF5/SRp40, SRSF6/SRp55, SRSF4/SRp75, SRSF7/9G8, SRSF10/SRp38, SRSF12/SRp35) have been identified in human so far (Manley and Krainer, 2010). These trans-acting factors can bind to cis-acting elements and hence play role in modulating exon inclusion either as positive regulator or negative regulator. Among the splicing regulatory proteins, SR proteins bind to 5’ splice site or to exonic enhancer to induce splicing. These proteins also bind to sequences in the introns of pre-mRNA where they interfere with spliceosome assembly in order to repress mRNA splicing. This event was evident from SR proteins involvement in inhibition of adenosiral IIIa pre-mRNA splicing by interfering with U2 snRNP recruitment to the 3’ splice site (Kanopka et al., 1996). Recent studies report that SF3B1 is mutated in chronic lymphocytic leukemia (mutation occurring in 15% of patients), with the mutation being associated with the aggressive form of chronic lymphocytic leukemia (poorer prognosis) (Wang et al., 2011). The SF3B1 mutation altered splicing (Quesada et al., 2011; Wang et al., 2011). SF3B1 is involved in the splicing of MCL1 transcripts (Moore et al., 2010).

A relatively minute change in SR family protein levels can lead to deregulation of alternative splicing event, resulting in changes in cellular behavior and expression levels of various protein isoforms (Ghigna et al., 1998; Mukherji et al., 2006; Pind and Watson, 2003; Stickeler et al., 1999). Induced expression of certain SR proteins is correlated with preneoplasia and intermediate stage of mouse mammary cancer. For instance, expression of specific SR proteins, such as SRSF5, increases during mammary carcinogenesis (Stickeler et al., 1999). This suggests a role for these splicing regulatory proteins in early tumorigenesis. Several studies have documented changes in SR protein levels during tumorigenesis (Ghigna et al., 1998). The mRNA levels of several SR proteins were lower in tumors than in the normal tissue (e.g. mRNA levels of SRSF1, SRSF5, SRSF6, and SRSF4 were lower in nonfamilial colon adenocarcinomas than in normal) (Ghigna et al., 1998).

Alterations in SR proteins expression during tumorigenesis may result in changes in splicing. For example, altered expression of SR proteins in mouse mammary adenocarcinomas results in change in the splicing pattern of CD44, a protein involved cell–cell interactions, producing metastatic CD44 variants (Naor et al., 2002; Stickeler et al., 1999). Effect of SR protein alteration in CD44 is of note as several human cancer cells exhibit CD44 splice variant v5, v6 and v7 by exon inclusion, providing a rationale for using it as biomarker for cancer (Jang et al., 2011; Olsson et al., 2011). In another study, overexpression of polyprimidin tract-binding protein (PTBP) and SRSF3 was correlated with increased splice variants of the multidrug resistance-associated protein 1 (MRP1/ABCC1) as well as CD44 in ovarian tissue as an early event in the ovarian tumorigenesis (He et al., 2004, 2007). Knockdown of either PTBP or SRSF3 inhibited anchorage independent growth and cell growth of ovarian tumor cells. Analyzing the cell cycle progression after reducing SRSF3 levels using siRNA in ovarian cancer cell lines A2780, IGROV1 and SK-OV-3, apoptosis mediated by down regulation of BCL2 was observed (He et al., 2011). Thereby this result indicates the role of SRSF3 in cell growth, cell survival and cell cycle progression, and is considered as a proto-oncogene (Jia et al., 2010). Interestingly where some SR proteins, such as PTBP, showed increased levels during ovarian tumorigenesis others (SRSF1 and
U2AF65) remain unchanged (He et al., 2007). In tissue samples of breast cancer patients increased expression of cell trafficking receptor CD44 variants, CD44v2, CD44v3, CD44v5, and CD44v6 was found in accordance with increased presence of SRSF5 (Huang et al., 2007). Furthermore, in an experimental approach to analyze the expression of different SR proteins in MCF7 and HBL100 breast cell lines, higher level of SR proteins (SRSF4, 5, 6, 9) and altered expression of CD44 variants were found (Pind and Watson, 2003). Though the above mentioned studies link altered levels of certain SR proteins in different cancer cells with altered splicing; however, some SR proteins remained unchanged throughout the tumorigenesis. This raises the question as to the possible mechanisms by which the levels of certain SR proteins change during different stages of tumorigenesis and metastasis. Therefore, it will be important to study the expression profile of all SR proteins and their interacting partners in cancerous and non-cancerous cell in order to decipher their role in disease condition.

SR proteins are modified by phosphorylation and acetylation, and these modifications can change the activity of these proteins. Dephosphorylation of SR proteins can modulate the alternative splicing of BCL2L1 (BCL-X) and CASP-9 (Chalfant et al., 2002). Moreover, hyperphosphorylated SRSF10 resulted in the increased G2/M population of SK-OV-3 and BT-474 cell lines (Mukherji et al., 2006). Proteins involved in pre-mRNA splicing are also modified by methylation. The coactivator-associated arginine methyltransferase 1 (CARM1), which methylates H3 at R17 and is a transcriptional coactivator, methylates RNA binding proteins ELAV1/HuR, SNRPB/SmB, SNRPC/U1C and SF3B4/SAP49 (Kuhn et al., 2011). CARM1 has a role in alternative splicing. In the splicing of CD44 pre-mRNA, CARM1 promoted variant exon skipping (Cheng et al., 2007). Understanding the impact of the variety of reversible modifications that the splicing factors undergo on alternative splicing is an area of intense investigation.

SRSF1 in disease and cancer

SRSF1 is a sequence-specific RNA binding factor that promotes spliceosome formation by binding to exonic splicing enhancers during pre-mRNA splicing (Cho et al., 2011). SRSF1 plays a role in regulating splicing and induction of translation initiation (Michlewski et al., 2008), nonsense mediated mRNA decay (Zhang and Krainer, 2004), mRNA stability and export (Huang et al., 2003; Michlewski et al., 2008; Zhang and Krainer, 2004). Further, SRSF1 has become a crucial factor to be considered as a cancer biomarker as it is found to be associated with tumorigenesis (Ezponda et al., 2010; Karni et al., 2007). SRSF1 is found to be over-expressed in many tumor types, and binds to other oncogenic proteins during tumorigenesis (Pedraza-Farina, 2006). For example, MYC interacts with SRSF1 through its RRM domain (Anzukow et al., 2012). SRSF1 can promote breast cancer and is considered a proto-oncogene (Anzukow et al., 2012; Karni et al., 2007, 2008; Sinclair et al., 2003). Slight changes in the expression of SRSF1 can transform immortal mouse fibroblasts (Karni et al., 2007). Through its function in alternative splicing, SRSF1 plays a role in promoting expression of pro-tumorigenic isoforms of tumor suppressor BIN1 and the kinases Mnk2 and S6K1 in murine immortal fibroblasts (Michlewski et al., 2008). Similarly, increased SRSF1 expression shifts alternative splicing of BCL2L1 (BCL-X), MCL1, and CASP-2 and CASP-9 toward anti-apoptotic forms (Moore et al., 2010). Induction of SRSF1 shifted alternative splicing toward isoforms lacking pro-apoptotic activity, providing evidence that a change in SRSF1 levels can affect several downstream splicing factors and the expression of genes by changing alternative splicing (Anzukow et al., 2012). Thus, overexpression of SRSF1, as observed in many tumor types, results in the altered splicing of several pre-mRNAs, giving rise to proteins that support tumorigenesis and metastasis (Ge et al., 1999; Karni et al., 2007).

Epigenetic control of alternative splicing: a two-way communication

In recent years, substantial evidences have been accumulated suggesting a major role of epigenetic mechanisms in the alternative splicing regulation. Some of the key players of this regulatory network involve: (1) nucleosome occupancy or positioning and exon–intron architecture, (2) DNA methylation, (3) post-translational modifications (PTM) of histones, (4) chromatin–splicing adaptor complexes, and (5) chromatin remodeling complexes. Nucleosomes and five methyl cytosine (5meC) are non-uniformly distributed along the body of transcribed genes. Nucleosomes and 5meC are enriched on exons relative to introns, and nucleosomes exhibit preferential positioning at exon–intron and intron–
exon boundaries (Chodavarapu et al., 2010; Dhami et al., 2010; Hodges et al., 2009; Schwartz et al., 2009; Shukla and Oberdoerffer, 2012; Spies et al., 2009; Tilgner et al., 2009). The phenomenon of nucleosomal positioning is evolutionary conserved and independent of transcription, GC content or DNA sequences (Andersson et al., 2009; Nahkuri et al., 2009; Schwartz et al., 2009). However, alternatively spliced exons are relatively less enriched in nucleosomes in comparison to the constitutive exons, correlating nucleosome positioning with alternative splicing outcomes (Schwartz et al., 2009). Nucleosomes are suggested to function as ‘speed bumps’ to impose barrier to RNA polymerase II elongation, and thereby affects splicing, which supports the kinetic model of splicing regulation (Carrillo et al., 2011; Schwartz and Ast, 2010).

In addition to nucleosomes, genome wide mapping of histone PTMs reveal a non-random distribution pattern with relative enrichment of some specific modifications in exons, attributing their role in splicing (Andersson et al., 2009; Kolasinska-Zwierz et al., 2009; Schwartz et al., 2009; Spies et al., 2009). Trimethylation of H3K36, monomethylation of H3K79, H4K20 and H2BK5 and mono, di, and trimethylation of H3K27 are enriched on exons (Andersson et al., 2009; Dhami et al., 2010; Schwartz et al., 2009; Spies et al., 2009). Some of these modifications have been linked to the splicing regulation via change in chromatin configuration and RNA polymerase II elongation behavior. Histone PTMs can also interact with the splicing factors such as SRSF1 (also called ASF/SF2) and splicing machinery with the help of chromatin-splicing adaptor complexes which act as a scaffold, providing another mechanism of splicing regulation [reviewed in (Luco et al., 2011; Luco and Misteli, 2011)]. Furthermore, chromatin remodeling complexes, such as SWI/SNF (SWItch/Sucrose Non Fermentable) and CHD1 (chromodomain helicase DNA binding protein 1), have been reported to modulate splicing with consequences in splicing efficacy and spliceosome assembly as well as by regulating the rate of RNA polymerase II elongation (Batsche et al., 2006; Sims et al., 2007; Tyagi et al., 2009). These complexes interact with several members of spliceosome complex and splicing proteins and are recruited by specific histone PTMs. For example, trimethylation of histone H3 on Lys4 (H3K4me3), can create a binding site for CHD1 which associates with snRNPs, and facilitates their recruitment (Sims et al., 2007). These studies illustrate the interplay between epigenetic mechanisms and splicing regulation.

The MED23 subunit of Mediator interacts with hnRNP L. Mediator is a multiprotein complex that acts as an integrator of various signaling pathways and is a critical component of the RNA polymerase II transcription initiation apparatus (Conaway and Conaway, 2011; Spaeth et al., 2011). MED23 interacts with the splicing machinery and regulates alternative splicing of several hnRNP L targets (Huang et al., 2012).

There is emerging evidence that pre-mRNA splicing itself influences chromatin organization (Kim et al., 2011), providing a means of two-way communication between chromatin organization and the splicing regulation. Studies show that splicing mechanisms are required to establish and to maintain epigenetic marks such as trimethylation of H3K36 (de Almeida et al., 2011; Kim et al., 2011). H3K36me3 is considered as a major mark of exons and transcriptional activation (Edmunds et al., 2008; Luco et al., 2010). Genome wide mapping with high throughput sequencing reveals the increased accumulation of H3K36me3 mark toward the 3′ end of genes and in intron-containing genes, the enrichment of H3K36me3 mark is higher than intronless genes (Barski et al., 2007; de Almeida et al., 2011; Kolasinska-Zwierz et al., 2009; Schwartz et al., 2009). This observed phenomenon is irrespective of gene sizes, transcription activities and nucleosomal occupancies. Pharmacological inhibition of splicing or siRNA-mediated knockdown of splicing factor (e.g. SAP130) leads to the reduced level of H3K36me3 with consequences of reduced recruitment of histone methyltransferase, HYPB/Setd2 (KMT3A) (de Almeida et al., 2011). However, activation of splicing or change in the splicing patterns (e.g. shift from exon skipping to inclusion), exhibits opposing effects. Furthermore, blockage of splicing by mutating the splice site (e.g. deletion of 3′ splice site) or by use of splicing inhibitor such as spliceostatin A cause the relative shift of H3K36me3 mark, which is a generalized observation on thousands of genes containing introns, but not on intronless genes (Kim et al., 2011). Tom Misteli and colleagues reported a connection between H3K36me3 and pre-mRNA splicing (Luco et al., 2010). MRG15, which binds to the polypyrimidine tract-binding protein, binds to H3K36me3. SetD2 (KMT3A), the enzyme catalyzing H3K36me3, binds to the elongating, phosphorylated RNA polymerase II (Sun et al., 2005). Knocking down SetD2 did not impact elongation but
altered pre-mRNA splicing (Edmunds et al., 2008; Luco et al., 2010). MRG15 can form complexes with HDAC2 and KAT5/TIP60, but whether either of these enzymes are co-loaded onto the body of transcribed genes remains to be shown (Doyon et al., 2004). The splicing inhibitor, meamycin, reduced H3K36me3 levels without altering elongation rates or chromatin-associated RNA (de Almeida et al., 2011).

However, in regulation of alternative splicing, the effect of H3K36me3 is not consistent. For neural cell adhesion molecule (NCAM) and fibroblast growth factor receptor 2 (FGFR2) genes, increased accumulation of H3K36me3 has been correlated with exon skipping (Luco et al., 2010; Schor et al., 2009). However, in CD45 and YPEL5 genes, it is reported that H3K36me3 has no role in regulating the splicing outcomes, rather this histone PTM is involved in exon definition (Huff et al., 2010). Reduced levels of H3K36me3 are also observed in alternative exons of these genes than in constitutive ones. This is in contrast with a recent report that alternative exons have higher levels of H3K36me3 relative to constitutive exons (for example, CD44 gene) (de Almeida et al., 2011). However, this may not be a prerequisite or a generalized mechanism for all genes. Depending on cellular contexts or particular splicing events, H3K36me3 may exhibits increased or decreased levels in alternative exons. All of these studies point toward the fact that deposition and maintenance of H3K36me3 is dependent on splicing and localized changes in this epigenetic mark can modulate the splicing activity. However, the detailed mechanisms of regulation still require further validation.

HP1γ is recruited to the coding region of transcribed genes by the elongating form of RNA polymerase II (Kwon and Workman, 2011a, 2011b). HP1γ recruits the histone chaperone complex FACT and has a similar distribution as H3K36me3 (Kwon et al., 2010; Kwon and Workman, 2011a). A recent study demonstrated that H3K9me3 and HP1γ, which binds to H3K9me3, was at greater levels on the variant exons of the CD44 gene (Saint-Andre et al., 2011). HP1γ played a role in the alternative splicing of CD44 pre-mRNA by slowing down the elongating RNA polymerase II, allowing the inclusion of the variant

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**Fig. 1.** Histone PTMs alter nucleosome structure facilitating or hindering elongation. A. Nucleosomes with H3K9me3 recruit HP1γ. HP1γ retards the movement of RNA polymerase II, allowing splicing events for the inclusion of variant exon (E2) to take place. B. HuR inhibits HDAC2 resulting in increased acetylation of nucleosomes. The highly acetylated (Ac) nucleosome will stay in the atypical form established by elongation. The atypical nucleosome will facilitate passage of RNA polymerase II, preventing the formation of spliceosome and the inclusion of alternate exons. In this situation exon 1 (E1) and exon 3 (E3) are spliced together, while alternate exon 2 (E2) is excluded.
exons (Fig. 1). Interestingly HP1γ was also bound to the CD44 pre-mRNA in the variant region, thus linking the pre-mRNA to the CD44 chromatin (Saint-Andre et al., 2011).

There is a growing list of readers that bridge a gap between pre-mRNA splicing components and histone PTMs along the body of the transcribed gene (Luco et al., 2011). SRSF1 potentially links U1-70K snRNP, involved in early spliceosome assembly, to histone H3 (Hnilicova and Stanek, 2011; Luco et al., 2011).

Histone deacetylases: modulator of alternative splicing

A role for histone acetylation regulated by lysine acetyltransferases (KATs) and histone deacetylases (HDACs), in RNA splicing and processing is currently gaining momentum, which adds an additional regulatory layer to splicing mechanisms. As such, KATs and HDACs are identified in spliceosome complexes (Rappsilber et al., 2002; Zhou et al., 2002). DEAD-box RNA helicases, for example p68 and p72, that have been shown to be involved in the splicing of pre-mRNAs, are also associated with class I HDACs, HDAC1, 2 and 3 (Mooney et al., 2010; Wilson et al., 2004). Also, an in vitro study revealed that KATs and HDACs are required for spliceosomal rearrangements and assembly (Kuhn et al., 2009). These indicate that HDACs must have a key role in splicing.

Multiple studies have demonstrated the association of histone acetylation with alternative splicing regulation (Gunderson et al., 2011; Hnilicova et al., 2011; Kuhn et al., 2009; Schor et al., 2009; Zhou et al., 2011). A recent study by Zhou et al. has suggested that a family of RNA binding protein, Hu proteins, can enhance the localized histone acetylation in regions at the alternative exons of NF1 and FAS genes, involving the functional interaction with the activity of histone deacetylases, in particular, HDAC2 (Zhou et al., 2011). From a mechanistic point of view, Hu proteins are co-transcriptionally recruited to the target genes, directly interact with HDAC2 and inhibit its activity. This localized change in chromatin structure leads to the increase in elongation rate and decreases inclusion of alternative exons in mature mRNA (Fig. 1). During transcription the nucleosome structure is perturbed, forming atypical structures (Czarnota et al., 1997; Locklear et al., 1990). Typically the cysteine residue at position 110 of histone H3 is buried in the interior of the nucleosome. However, the transcribed unfolded nucleosome has its H3 cysteine exposed, offering a tag to isolate and study transcribed nucleosomes (Chen-Cleland et al., 1993; Sun et al., 2002). The atypical nucleosome requires elongation to expose the nucleosome’s cysteinyl-thiol, and histone acetylation will maintain this unfolded structure (Walia et al., 1998). It is conceivable that through histone acetylation maintaining the unfolded nucleosome structure, subsequent rounds of transcription elongation are facilitated (Fig. 1).

Changes in the splicing pattern of approximately 700 genes, mostly involved in cell signaling, differentiation and cell cycle regulation, have been identified with HDAC inhibitor treatment by splicing sensitive exon-arrays (Hnilicova et al., 2011). Toward the understanding of the mechanism as to how changes in histone PTMs alter the splicing of several genes, the current literature supports the kinetic model of transcriptional elongation or processivity, where a change in RNA polymerase II elongation rate acts as a sensor to decide the alternative splicing outcomes. For genes with alternative exons that have weak splice sites, a slowly moving RNA polymerase II results in the inclusion of the exons in the RNA. For example, depolarization of neuronal cell membrane induced enhanced acetylation of H3K9 surrounding the exon 18 (E18) of NCAM gene (Schor et al., 2009). However, the increased acetylation status was not detectable in the promoter region of the gene and was localized only in intragenic gene body region. This relaxed chromatin structure leads to an increased rate of RNA polymerase II elongation, preventing the inclusion of this exon in mature mRNA (Fig. 1). Furthermore, removal of depolarization signal can reverse the effect of acetylation and splicing outcomes. Also, the physiological response of membrane depolarization can be functionally mimicked by HDAC inhibitor, TSA treatment, with E18 exon skipping as well as open chromatin structure (Schor et al., 2009). This phenomenon is also observed for other genes such as fibronectin (Hnilicova et al., 2011). HDAC inhibitor mediated alternative splicing of this gene (exon 25 or EDB) has been attributed with increased RNA polymerase II processivity, which is correlated with increased histone H4 acetylation as well as with reduced association of one of the major splicing regulatory protein, SRSF5 (also called SRp40), along the alternative exon of the gene. In addition, siRNA-mediated deletion of HDAC1, but not HDAC2, had similar effect as HDAC inhibitor in the splicing
pattern change of this gene, suggesting HDAC1 is primarily involved in the splicing regulation of this gene (Hnilicova et al., 2011). However, it is still not clearly understood how RNA polymerase II slows down at some exons and whether variation in RNA polymerase II kinetics at the alternatively spliced exons occurs at all genes or only in a subset of genes. One possibility is the change in the dynamics of phosphorylation status of RNA polymerase II CTD from elongating Ser-2 phosphorylation to Ser-5 phosphorylation form, facilitates RNA polymerase II to slow down by recruitment of specific phosphatases or kinases (Munoz et al., 2010). However, it is also possible that a change in phosphorylation cycle of RNA polymerase II may not be the major player. Combinatorial effect of chromatin structure, histone PTMs and properties of template DNA sequences, which can act as recruiters for other proteins, may play key roles in determining the elongation rate, which needs to be studied in further detail. Furthermore, it is yet to be known whether change in RNA polymerase kinetics along a gene is a generalized mechanism to determine the splicing outcome in response to HDAC inhibitor treatment. In support of this, recently it has been shown in yeast (Saccharomyces cerevisiae, intron-containing genes, DBP2 and ECM33), the genetic deletion of multiple histone deacetylases Hos3 and Hos2, but not the single deletion of HDAC (either Hos2 or Hos3), caused an enhanced acetylation of H3K9 and H3K14 throughout the body of the genes, with a very slight change in the distribution pattern of RNA polymerase II (Gunderson et al., 2011). However, an aberrant regulation in the co-transcriptional spliceosome complex assembly is detected. Presumably, histone acetylation dynamics influences the dynamics of co-transcriptional spliceosome assembly and can affect splicing without altering the transcriptional elongation rate in yeast, indicating a direct role of chromatin structure in spliceosome complex recruitment.

Treatments to manipulate and correct splicing defects

Splicing-directed and epigenetic therapies are being actively pursued in animal models and clinical trials to treat diseases that are a consequence of aberrant splicing (Dredge et al., 2001; Osorio et al., 2011; Porensky et al., 2011; Riessland et al., 2010; Sanford et al., 2009; Tazi et al., 2009; Wang et al., 2012; Zaharieva et al., 2012). Morpholino oligonucleotides have been used successfully to alter the splicing of MCL1 (Shieh et al., 2009) and BCL2L1 (Taylor et al., 1999). Morpholino oligonucleotides blocking the 3′ acceptor and 5′ donor sites of exon 2 of the MCL1 pre-mRNA result increased levels of the MCL-1S transcript, inducing apoptosis in cancer cells (Shieh et al., 2009). An oligonucleotide positioned near the BCL-xL splice donor site, results in a marked increase in the BCL-xS transcript (Taylor et al., 1999). Spinal muscular atrophy is a disease caused by a defect in splicing (Tazi et al., 2009; Ververis and Karagiannis, 2011). Morpholino oligonucleotides have shown promise in treating spinal muscular atrophy (Porensky et al., 2011). There are many HDAC inhibitors in clinical trials (Li and Manley, 2006; Witt et al., 2009). The FDA-approved HDAC inhibitor SAHA (also called Vorinostat and Zolinza) has had success in ameliorating the spinal muscular atrophy phenotype in a mouse model (Riessland et al., 2010).

Conclusion

Major progress is being made in understanding the dynamic relationship between chromatin organization, transcription elongation and alternative pre-mRNA splicing. In near future, we can look forward to a frontier in the current research field as ‘consanguinity’ of chromatin and pre-mRNA splicing. A comprehensive understanding of the splicing code and interplay between epigenetics and pre-mRNA splicing will provide new insights into the complex network of gene regulations. Further, this research will open doors to develop better therapeutic strategies involving modulation of chromatin and/or splicing to treat human diseases that are a consequence of aberrant splicing.

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