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The Worlds of Splicing and Chromatin Collide

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1. Introduction

A. Integration of transcription and splicing

1. Chromatin, transcription, and splicing.

Both transcription and splicing take place in a nuclear environment which, at face value, may seem refractory to the efficiency afforded by the coupling of both processes. This environment, chromatin, was once viewed as only a passive packaging system for genetic material, with very little contribution to the variety of nuclear activities occurring within and around it. However, overwhelming evidence now points to the chromatin environment as being highly dynamic, and an active player in nuclear activities. Residues on all four histone N-termini (also known as tails) have been shown to be post-translationally modified in a variety of ways. Many of these modifications have been found to be recognized by factors involved in the regulation of gene expression and are associated with particular activating or repressive states, leading to the proposal of a "histone code" that directs (contributes to) the activity of gene regulatory factors ([1]). In addition to compositional changes, chromatin structure has also been proven to be dynamic. Specific enzymes have been characterized to utilize the energy from ATP hydrolysis to physically disrupt histone-DNA contacts, "remodeling" chromatin and altering the accessibility of DNA ([2]). These chromatin remodelers can slide nucleosomes along the DNA template, and even remove individual or subset of histones or entire nucleosomes at a particular genetic locus. In addition to changes in nucleosome (histone octamer plus 146 base-pairs of DNA) density at the primary level, chromatin structure can also be altered at a secondary level, exhibiting the ability to form compacted (and de-compacted) structures. Overall, the role of chromatin and factors acting upon it in the regulation of transcription (generally referred to as *epigenetics*) has become a well-studied topic, and a much better understanding of gene regulation has lead to many important breakthroughs in the fields of cellular differentiation, development, and disease. Because of the popularity of epigenetics and the knowledge that transcription and splicing are not mutually-exclusive, the role of chromatin in splicing is becoming an important area of research foci as well.

2. Co-transcriptionality.

Nuclear processes that involve the generation and manipulation of messenger RNA (mRNA) occur within remarkable spatial and temporal proximity. These processes include transcription, 5′ capping, splicing, and polyadenylation. Two of these mechanisms,

transcription and splicing, are utilized by the cell to create phenotypic variation from otherwise identical genotypes. Both complex and elegant, these two nuclear events provide an explanation as to how organisms with relatively identical genetic information can differ severely in appearance and behavior. All aspects of transcription (initiation, elongation, termination, activation, repression, etc.) have been the subject of intense research focus since the first articulation of the central dogma of molecular biology: *DNA>RNA>protein* ([3]). The molecular mechanisms of RNA splicing have been increasingly investigated throughout the past few decades, with its importance highlighted by the fact that more than 90% of all human genes undergo alternative splicing ([4,5]). Although each transcription and splicing processes were initially studied and characterized as being mutually exclusive, mounting evidence has proven them to occur simultaneously and in concert, maximizing the efficiency and fidelity of mature transcript synthesis. The first evidence for the coupling of transcription and splicing, or "co-transcriptionality", came almost 25 years ago, as electron microscopy on transcripts from *Drosophila* embryos revealed co-transcriptional splicing ([6]). Shortly after, on a more specific basis, the human gene *dystrophin* was described as being co-transcriptionally spliced ([7]). Underscoring the need for efficiency in transcript synthesis and processing, this 2400 kb gene takes up to 16 hours to be transcribed. As the distinct factors and mechanisms involved in both processes have been comprehensively elucidated, a rather clear picture as to how the cell integrates transcription and splicing has begun to emerge. In fact, two models have recently been proposed to explain this "coupling" process: kinetic and recruitment ([8]). The kinetic coupling model proposes that the rate of transcription elongation by RNA polymerase II (RNA pol II), the enzyme responsible for transcription catalysis, directly modulates splicing decisions. Recruitment coupling revolves around the association and interaction of transcription factors with the splicing machinery. These models are not mutually exclusive, and focus on two critical nuclear elements which will be fully explored in this chapter: the C-terminal domain of pol II and the chromatin environment in which both transcription and splicing take place.

3. Transcription and the RNA pol II CTD.

RNA polymerase II catalyzes the transcription of eukaryotic genes and is distinct among RNA polymerases because of the presence of a repetitive heptapeptide sequence within its Carboxyl Terminal Domain (CTD) ([9]; Figure 1). This sequence, Tyr-Ser-Pro-Thr-Ser-Pro-Ser ($Y_1S_2P_3T_4S_5P_6S_7$), is repeated 52 times in mammals, and has been found to be necessary for the transcription of endogenous genes ([10]). The potential for large amounts of post-translational modification (PTM), especially phosphorylation, exists on the pol II CTD, especially phosphorylation. In fact, the phosphorylation of two specific serine residues (Ser2 and Ser5) within the RNA pol II CTD is directly related to transcription initiation and elongation, as well as pre-mRNA capping and polyadenylation ([11]). In terms of initiation, phosphorylation of Ser5 on the promoter-bound RNA pol II CTD is accomplished by cyclin-dependent kinase 7 (cdk7) which is a component of the basal transcription factor TFIIH ([12]). At this point, additional components of the transcription machinery are able to assemble. However, another phosphorylation event, this time on Ser2, is necessary for promoter "clearance" and pol II elongation. The transcription elongation factor P-TEFb (Positive Transcription Elongation Factor b) is the kinase responsible for Ser2 phosphorylation, as it relieves the elongation-inhibitory effects of the factors DSIF (DRB-Sensitive Inducing Factor) and NELF (Basal Embryonic LHRH Factor) ([13]). The presence of 46 Ser2 residues and 51 Ser5 residues allows for a control mechanism of the rate of

elongation, a main principle of the kinetic model of transcription-splicing coupling, discussed in-depth later in this chapter.

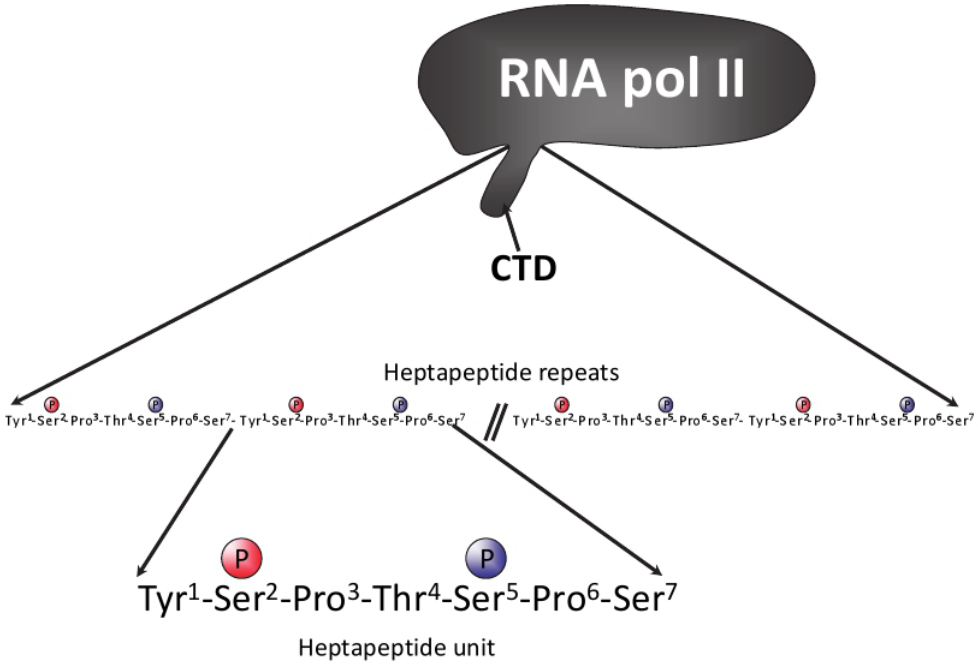

Fig. 1. Structure and composition of the RNA pol II large subunit.
The C-Terminal Domain (CTD) contains up to 52 repeats of the heptapeptide unit YSPTSPS. Note that the consensus sequence of the heptapeptide repeats 26-52 is often degenerated. Post-translational modifications of heptade residues affect the RNA pol II functions. Phosphorylation of Serine 5 (by TFIIH and cyclin-dependent kinase 7) is important for promoter clearance during initiation and elongation. Phosphorylation of Serine 2 (by P-TEFb and cyclin-dependent kinase 9) is associated with elongation and transcriptional termination.

4. Control of RNA splicing.

RNA splicing, catalyzed by the spliceosome, a large RNA-protein complex composed of five small nuclear ribonucleoproteins (snRNPs), provides the cell with an additional level of phenotypic complexity without the need for additional transcript generation ([14]). Control of splicing can occur in "cis" through regulatory sequences in pre-mRNA, as well as "trans" by factors that bind and act upon these sequences. An example of these factors is the SR proteins which act in the control of splice site recognition by affecting spliceosome assembly ([15]). It is the control of splice site recognition which provides the major mechanism by which RNA splicing is regulated. Splice sites within introns have been found to have differing "strengths" which affect their ability to be recognized and acted upon by components of the splicing machinery. This form of splicing regulation is directly related to the control of transcription elongation, both through the kinetic and recruitment models

mentioned earlier. Therefore, "co-transcriptional" splicing provides the cell with the advantages of increased efficiency of transcript generation and processing, preventing mRNA degradation and back-hybridization with DNA ([16]).

5. Kinetic model of co-transcriptional splicing.

The kinetic model of co-transcriptional splicing revolves around the concept that the rate of RNA pol II elongation directly affects splice site recognition and spliceosome assembly ([17]; Figure 2). The rate by which RNA pol II transcribes along the length of a gene can be affected by two factors: the phosphorylation level of Ser5 and Ser2 on the RNA pol II CTD, as well as the chromatin structure which encapsulates the gene being transcribed. In a nutshell, fast elongation, which occurs when the RNA pol II CTD is hyperphosphorylated and/or the chromatin of the gene being transcribed has a low nucleosome density, favors the inclusion of downstream exons with "strong" splice sites (Figure 3). In contrast, when the RNA pol II CTD is hypophosphorylated and/or the nucleosome density of the transcribed gene is increased, a slow elongation rate allows enough temporal flexibility for the splicing machinery to assemble on upstream, "weaker" splice sites. Initial experiments supporting this concept showed that using "slow" RNA pol II mutants or inserting pausing

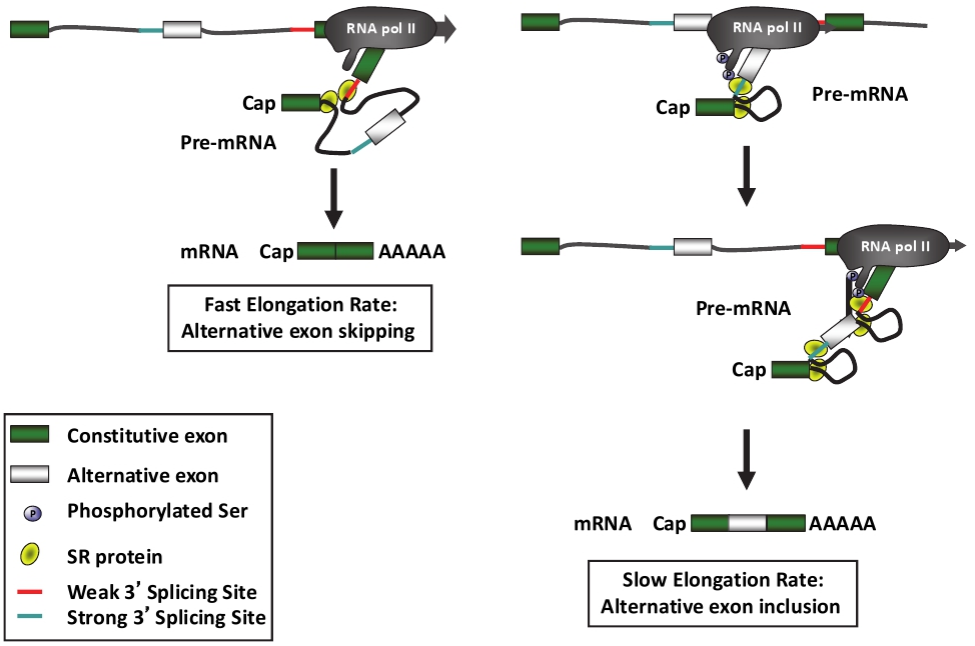

Fig. 2. Kinetic model for co-transcriptional splicing.
The regulation of alternative splicing is modulated by the rate of elongation of RNA pol II. Fast elongation rate (Left panel) results in more frequent exon skipping. The rate of elongation can be influenced by the level of CTD Ser 2 and Ser 5 phosphorylation. The weak 3′ splicing site is indicated in blue and the strong 3′ splicing site in red. Slower elongation (Right panel) results in inclusion of the alternative exon (Grey rectangle) between the two constitutive exons (Green rectangles). Cap: 7′methyl guanosine; AAAAA: poly A tail

elements in reporter minigenes favors "weak" exon inclusion in the fibronectin and fibroblast growth factor receptor 2 (FGFR2) genes ([18], [19]). The fact that there are 46 Ser2 and 51 Ser5 residues in mammalian CTDs provide a sort of "gas pedal" mechanism for the control of elongation rate, and therefore splicing decisions. In an intriguing example, the chromatin remodeling factor SWI/SNF which interacts with RNA pol II, splicing factors, and spliceosome-associated proteins, can cause inclusion of a block of exons in the middle of the CD44 gene by stalling RNA pol II through a phosphorylation status switch from phospho-Ser2 to phospho-Ser5 ([20]). Further evidence for this intragenic "brake" control mechanism comes from the transient accumulation of phospho-serine 5 on the RNA pol II CTD around the 3′ end of yeast introns ([21]). This pausing before an exon is suggestive of a splicing-dependent transcriptional checkpoint which holds any further transcription until spliceosome assembly is accomplished.

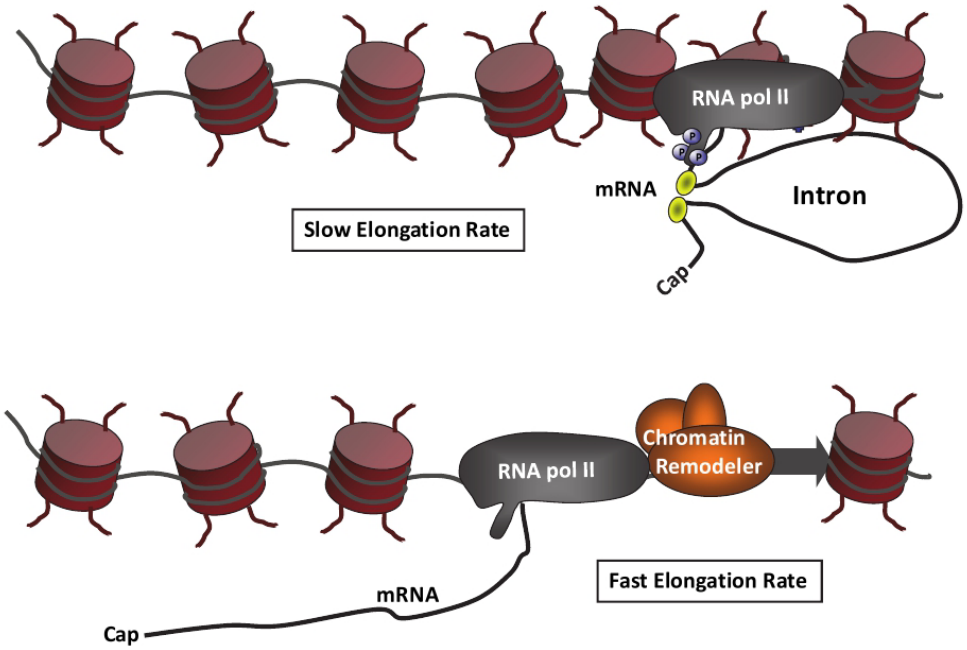

Fig. 3. Modulation of RNA pol II elongation rate is related to nucleosome density. Nucleosomes, through their position and/or density can affect the RNA pol II elongation rate. Low density would privilege a fast elongation rate and lead to exon skipping. This may be facilitated by the presence of chromatin remodeling complexes that can promote nucleosome displacement through sliding or partial loss of histones. The higher nucleosome density would reduce the RNA pol II elongation rate and favor exon inclusion.

In terms of chromatin structure altering elongation rate and splicing, on genes regulated by the chromatin-remodeler SWI/SNF, the ATPase subunit Brahma (Brm) has been shown to contribute to transcription-splicing crosstalk by decreasing the elongation rate (through alterations in nucleosome density patterns) and facilitating recruitment of the splicing machinery to variant exons with suboptimal splice sites ([20]). Conversely, treatment with

the histone deacetylase inhibitor, Trichostatin A (TSA) facilitates a more "open" chromatin conformation, stimulating elongation rates and causing inhibition of the fibronectin exon EDI inclusion ([22]). Much more evidence exists that relates chromatin structure and composition to the regulation of both transcription and splicing, independent of elongation rate and the kinetic model of "co-transcriptionality". These concepts, including chromatin as a recruiter of both transcription and splicing factors, nucleosome positioning in delineating critical transcription and splice sites, and the involvement of chromatin modifications and modifiers in both transcription and splicing, will be discussed later in detail in this chapter.

6. Recruitment model of co-transcriptional splicing.

The recruitment model of co-transcriptional splicing is similar to the kinetic model in the sense that it revolves around the RNA pol II CTD (Figure 4). Specifically, the recruitment model involves the allosteric regulation of splicing decisions through interactions with the elongation machinery mediated by the RNA pol II CTD ([23]). The most clear-cut example of the recruitment model involves the RNA pol II CTD, the SR protein SRp20, and the alternative exon EDI of the fibronectin gene ([24]). SRp20 has an inhibitory effect on the

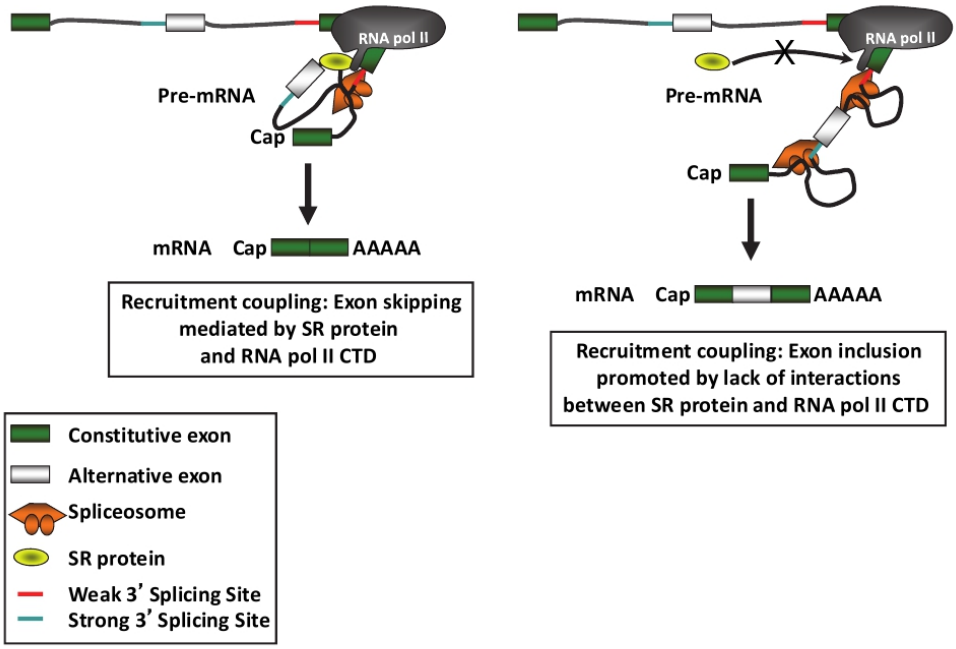

Fig. 4. Recruitment model for co-transcriptional splicing.
In this model, the recruitment of splicing factors SR protein(s) (yellow ovals) is mediated by interactions with the RNA pol II CTD. Binding of SR proteins, such as SRp20, to the CTD favors alternative exon skipping (Left panel). Loss of SR protein interactions with the CTD prevents formation of a proper ternary complex and leads to alternative exon inclusion (right panel). This model is based on work by de la Mata et al. ([24]).

inclusion of the *fibronectin* EDI exon, and this effect is mediated by the RNA pol II CTD. When a RNA pol II mutant lacking the CTD is present, SRp20 is not recruited to the site, and EDI inclusion is greatly enhanced. Long before this evidence was presented, a trend connecting the recruitment of splicing factors with transcript elongation mediated by the RNA pol II CTD was emerging. When genes are placed under the control of RNA polymerase I, III, or bacteriophage T7 RNA polymerase promoters, transcription occurs, but splicing is greatly affected ([25]; [26]; [27]). Logically, the recruitment of splicing factors to proper slice sites is dependent on the RNA pol II CTD ([28]) and deletion of the CTD affects all aspects of RNA processing in the β-globin gene ([29]). The most obvious factor(s) playing a role in the recruitment model of co-transcriptional splicing are the SR proteins. Virtually all members of the SR family are known to interact with RNA pol II, as well as other splicing factors and components of the spliceosome ([30]). Additional factors which interact with both the pol II CTD (phorphorylated or un-phosphorylated), as well as integral components of the spliceosome include the elongation factors CA150 ([31]) and SPT6 ([32]), as well as the transcriptional regulators TRAP150/Med23 ([33]), TFII H ([34]), PSF/p54nrb ([35]), and EWS-Fli and NOR1 ([36]).

Many factors that affect chromatin structure and composition also interact with members of both the elongation and splicing machinery, consequently playing a role in the recruitment model of co-transcriptionality as well. These factors primarily include chromatin remodelers and histone modifiers. Evidence for these chromatin-associated factors acting as "adaptor" molecules, bridging both processes and playing roles in both proposed models for the coupling of transcription and splicing is overwhelming. Therefore, additional sections in this chapter have been included to explore their multi-faceted activity in full detail.

7. Incorporation of both kinetic and recruitment models of co-transcriptionality.

Just as the nuclear processes of transcription and splicing have proven to be non mutually-exclusive, the two models proposed to explain the coupling of both mechanisms have to be integrated to fully understand the concept. For example, the modulation of RNA pol II's elongation rate is directly linked to the recruitment of specific factors involved in altering CTD phosphorylation status and/or nucleosomal density at a particular locus. In the same vein, increasing or decreasing the rate of RNA pol II elongation has an unequivocal impact on the temporal requirement for spliceosome assembly at a particular splice site. An excellent example of this kinetic/recruitment "feedback loop" involves the CD44 gene and the chromatin-remodeling factor SWI/SNF ([20]). SWI/SNF interacts with the U1 and U5 snRNPs (two essential components of the spliceosome), as well as the splicing factor Sam68, at a block of alternative exons inside *CD44*. This binding promotes RNA pol II stalling through a CTD phosphorylation switch from Ser 2 to Ser 5, favoring the inclusion of the block of exons in mature *CD44*. In a similar mechanism, membrane depolarization of neural cells affects splicing of the NCAM gene by altering intragenic histone acetylation patterns, affecting the local chromatin structure, rate of RNA pol II elongation, and the distribution of RNA pol II and splicing machinery surrounding splice sites ([37]).

2. Chromatin as a dynamic active structure

A. Nucleosome density and position

1. Nucleosome position and exon location.

After more than 20 years of research, the role of chromatin as a dynamic structure necessary to regulate the initiation, elongation and termination phases of transcription has now been

clearly established ([38]; [39]; [40]). However, despite this vast effort to understand the intricate events leading to these regulatory events, the precise role of chromatin and the location and density of nucleosomes has remained fairly elusive during the process of splicing. Nucleosomes are composed of a stretch of 146 bp of DNA wrapped around an octamer of histone proteins (two H2A, two H2B, two H3, and two H4 histones) generating the basic unit of chromatin, and contribute to chromatin compaction and structure. Clear evidence have been presented to indicate that the two events, transcription and splicing, are coordinated. As early as 1988, electron microscopy of *Drosophila* embryo showed that nascent transcripts were undergoing active splicing ([6]). Direct evidence of co-transcriptional splicing of a specific gene (human *dystrophin*) was later confirmed ([7]). The link with chromatin was later established through the demonstration of preferential association of efficiently excised introns with the chromatin-bound nuclear fraction ([41]). But, in order to get a better understanding of the relationship between nucleosome location and density, we have had to wait until technological advances provided us with an efficient way of mapping nucleosomes, first on an individual gene basis, then over the entire genome. Next-generation sequencing, using various technical platforms (Illumina™, Roche 454 sequencing, pyrosequencing), following micrococcal nuclease (MNase) digestion of chromatin from various organisms and cell types, has allowed precise mapping of nucleosome distribution over the entire genome ([42]; [43]; [44]; [45]; [46]). The results demonstrated that there is indeed a higher nucleosome occupancy around exons, an occurrence that was observed across kingdoms from plants to mammals in both gametes and somatic cells ([44]). This strongly advocate for a role for both nucleosome positioning and density in defining exons and introns locations and boundaries.

The presence of single nucleosome units at specific locations overlapping with single exons appears to correlate with the evolutionary conserved average size of ~150 bp observed in mammalian exons ([42]; [43]). This may indicate a role for conserved exon-specific nucleosome positioning sequences aimed at maintaining and defining the identity of exonic regions. The strength of the splice site (likelihood to have efficient splicing) appears to be proportional to the nucleosome density, arguing that nucleosome positioning and density not only affect exon definition and identity, but also contribute to splicing efficiency.

2. Role for DNA sequence in nucleosome positioning.

The possibility of a loosely defined set of "exon-specific DNA sequences" is suggested by computational modeling experiments that were capable of predicting "exon-associated" nucleosome locations matching the ones determined using genome-wide sequencing ([42]; [43]). These exon-specific sequences, with increased nucleosome density over exons, displayed a higher GC content when compared to their counterpart intron sequences ([42]; [43]). As CpG dinucleotides can undergo methylation, a modification that can affect both nucleosome positioning ([47]; [48]) and transcription elongation rate, it appears reasonable to envision a role for CpG methylation in splicing. Supporting this observation, several recent studies have indicated a correlation between DNA methylation and the levels of exon-specific histone post-translational modifications (positive correlation with H3K36me3 and negative correlation with H3K4me2; ([48]; [49]; see next section on "Histone PTMs").

3. Nucleosome density and RNA pol II elongation rate.

The ability to control splicing by modulating RNA pol II elongation rate, referred to as the kinetic model, where slower transcription equates to more efficient splicing of alternative exons, is likely to be influenced by all above-mentioned epigenetic regulatory events. An

obvious connection between the "chromatin effect", mediated by nucleosome position and/or density, and the rate of elongation of transcription on alternative splicing (Figure 3) is supported by the comparison of experimental results obtained from *in vitro* splicing assay performed using cell-free extracts and *in vivo*-based experiments ([50]). The rate of transcription was demonstrated to affect the size of RNA loops generated and to also influence the use of alternative splicing sites. The effect of elongation rate on alternative splicing has since been confirmed using RNA Pol II mutants displaying a slow rate of elongation ([24]; [51]) or cells harboring a mutated form of the elongation factor TFIIS ([52]). The presence of nucleosomes has long been known to affect efficiency of transcription. Based on the nucleosome position mapping with regard to transcribed regions, the increased nucleosome density observed at exon locations is expected to significantly decrease the RNA pol II elongation rate, and therefore favor efficient splicing. However, to this day, the ultimate experiment to fully determine the precise nature of the relationship between nucleosome position and splicing involving the simultaneous relocation of nucleosomes and the precise mapping of splicing sites has not been performed.

B. Histone Post-Translational Modifications (PTM).

1. Identification of exon-specific histone PTMs.

The complex nature of chromatin can be significantly modulated by altering the position of nucleosomes through sliding, loss of histone subsets, or re-location of complete histone octamers ([53]) (See "Chromatin Remodelers and Splicing"). In addition to these histone composition changes, the dynamic nature of chromatin and the recruitment of specific factors are strongly dependent on histone post-translational modifications ([1]). These modifications include lysine acetylation, lysine methylation, serine phosphorylation, ADP ribosylation, and ubiquitination. Specific lysine and serine modifications have been associated with genes actively transcribed or repressed. For example, histones H3 and H4 lysine acetylation, histone H3 lysine 4 di- or tri-methylation (H3K4me2; H3K4me3) and histone H3 Serine 10 and Serine 28 phosphorylation (H3S10P, H3S28P) are strongly associated with actively transcribed genes, and histone H3 lysine 9 and lysine 27 di- and tri-methylation (H3K9 me2, H3K9me3, H3K27me2, H3K27me3) are considered to be markers of repressed genes ([54]). A connection between RNA splicing and changes in histone PTMs was demonstrated in the early 2000's. Using Trichostatin A, a histone deacetylase inhibitor, researchers demonstrated that changes in the status of histone lysine acetylation was influencing the regulation of alternative splicing of *fibronectin* exon 33 ([55]; [22]) and of a CD44 reporter gene ([56]). As histone acetylation is considered a marker of actively transcribed genes, this result was by no mean surprising. It can provide support to, both the kinetic model, where histone acetylation can influence the rate of RNA pol II elongation, as well as the model where timing of splicing is described as regulated by the timing of splicing factors recruitment, an event likely to be affected by histone PTMs. But, as was the case for nucleosome positioning, the use of genome-wide analysis of the distribution of histone PTMs has provided a much clearer image of the role of these events in splicing. To date genome-wide maps for 42 different histone PTMs have been generated and matched to exon-intron locations ([57]). After normalization to account for increased nucleosome density over exonic regions, the results show enrichment at exon of tri-methylated histone H3 Lysine 36 (H3K36me3), tri-methylated histone H3 Lysine 4 (H3K4me3), and di-methylated histone H3 Lysine 27 (H3K27me2), and depletion of tri-methylated histone H3 Lysine 9 (K3K9me3) ([58]; [46]) over exonic sequences. The role of H3K36me3 in splicing

was further confirmed by studies indicating its association with actively transcribed regions enriched in constitutive exons ([59]; [58]) with a concomitant increased nucleosome occupancy ([58]; [42]). Additional evidence for the role of H3K36me3 and H3K4me3 were provided by experiments monitoring splicing efficiency as a function of methylation levels of H3K36 and H3K4 (over-expression or down-regulation of H3K36- or H3K4-specific methyl-transferases; [60]).

In addition to the well-characterized H3K36me3, H3K4me3, H3K27me2, and H3K9me3, several other histone PTMs (H3K79me1, H4K20me1, H2BK5me1, H3K27 me1, 2, 3) have been occasionally reported to be differentially enriched or depleted in exons ([43]; [61]). However, these PTMs have not been consistently identified as consensus exon markers. These differences in histone PTM patterns observed may reflect different cell types, tissues, or variations in the technical analysis and normalization.

2. Synergy between DNA methylation and histone PTMs.

Based on genome-wide sequencing results, a correlation between the pattern of histone methylation and that of DNA methylation over exonic region suggests that these two epigenetic markers may act synergically to mark exons ([62]; [48]). More specifically, enrichment of H3K36me3 and depletion of H3K4me2 appear to correlate with increased CpG methylation over exonic regions ([48]; [49]).

3. Histone PTMs can mediate interactions between chromatin, splicing factors, and RNA.

Histone PTMs have been demonstrated to act as targets promoting the recruitment of specific regulatory factors for transcription, DNA repair, and other DNA-related events (for review, see [54]). Not surprisingly, a similar role for histone PTMs has been described in the context of RNA splicing, linking chromatin, RNA, and splicing factors (See Figure 5).

The intricacies of the interactions between RNA and chromatin remain poorly defined. Physical interactions between chromatin components and RNA, mediated by the Xist RNA, have been demonstrated to occur in the context of inactive X-chromosome (review by [63]). However, early work by chromatin research pioneers such as Drs. van Holde, Bradbury, and Kornberg, exploring the possibility of RNA-histone interactions in the context of individual or arrays of nucleosomes, failed to clearly identify defined mechanisms leading to specific interactions between core histones and RNA transcripts. Based on the physical properties and inherent charge of mRNA, electrostatic interactions would be predicted, even if they are transient, as suggested by studies showing an impeded mobility of pre-mRNA associated with the presence of nucleosomes affecting pre-mRNA diffusion away from the transcription site ([64]; [65]). The presence of histone tails that can protrude from the nucleosomes and the fact that these tails are highly positively charged provides additional support for conditions conducive to electrostatic interactions between mRNA and chromatin. As early work on investigating the mechanism of splicing had been performed using artificial transcription systems and *in vitro*-assembled chromatin, the importance and the role of histone PTMs in the process was not directly addressed in the interpretation of the results. The presence of multiple types of PTMs, that have now been clearly identified as contributing to various aspects of transcription, is extremely likely to affect RNA-histone interactions. In addition to the potential for direct physical contact between histone and mRNA, researchers suspected and started to investigate the involvement of bridging proteins or complexes, referred to as chromatin-adaptors acting, in regulating the process of RNA splicing. These chromatin-adaptors would provide a scaffold linking DNA, histones,

and the RNA splicing machinery. To date, four such chromatin-adaptors have been identified to interact with specific histone PTMs and splicing factors (see Table 1). Two of these chromatin-adaptors are associated with histone PTMs enriched in exons. MORF-related Gene 15 (MRG15), a transcription factor involved in embryonic development and cell proliferation, bridges pre-mRNA through interactions between its chromo-domain, H3K36me3, and the polypyridine tract-binding protein (PTB) splicing factor ([60]). The Chromo-Domain-Helicase-DNA-binding 1 protein (CHD1) mediates pre-mRNA interactions through contacts between H3K4me3 and U2 snRNP ([66]). The other two identified chromatin-adaptors mediate interactions through binding acetylated H3 (Gcn5 contacts U2snRNP, [67]), or H3K9me3 (Heterochromatin Protein 1 -HP1- recognizes hnRNPs, [68]). As genome-wide maps of histone PTMs, chromatin-associated proteins, and location of specific alternative splicing sites continue to be generated, we expect the number of identified chromatin-adaptors involved in splicing to increase.

Chromatin-Adaptor	Matching histone PTM	Splicing Factor
MRG15	H3K36me3	PTB
Gcn5	Acetylated H3	U2snRNP
CHD1	H3K4me3	U2snRNP
HP1α	H3K9me3	hnRNPs

Table 1. Chromatin-Adaptors, target PTMs, and Splicing Factors

C. Chromatin remodelers and splicing.

1. SWI/SNF and splicing.

Two separate chromatin remodeling factors have proven to play an integral role in not only transcription, but also splicing. First, the SWI/SNF chromatin remodeling complex was initially characterized as an ATP-driven motor that disrupts protein-DNA interactions, more specifically histone-DNA contacts within nucleosomes ([69] [70]). Because of this activity, SWI/SNF has proven to be inherently involved in the process of transcription, altering the accessibility of DNA to transcription factors. However, recent evidence has shown SWI/SNF to have an important role in splicing activity, mainly independent of its remodeling capability. Brahma (Brm), the catalytic subunit of SWI/SNF, was found to interact with several components of the spliceosome, as well as Sam68, a splicing enhancer ([20]). It also increased the accumulation of RNA pol II (Ser2 phosphorylated) on regions encoding variant exons of several genes including E-cadherin, BIM, cyclin D1, and CD44. It is postulated that Brm exerts its regulatory activity on splicing by slowing the RNA pol II elongation rate which facilitates recruitment of the splicing machinery to exons with "weaker" splice sites. In a perhaps more surprising role, Brm was shown to affect splicing at the RNA level ([71]). It was found to be incorporated into nascent pre-mRNPs, and human Brm and Brg1 (another SWI/SNF component) associate with RNPs. In addition, depleting SWI/SNF affects the abundance of alternative transcripts from a subset of genes. Overall, SWI/SNF has a role in both transcription and splicing, regulating not only the amount, but also the type of transcript generated.

2. Chd1 and Splicing.

The chromatin-remodeler CHD1, which display significant similarities to the SWI/SNF complex, is a multi-faceted factor with roles in transcription activation, repression, elongation, termination, as well as the deposition of variant histones ([72]). Not surprisingly, novel research has emerged which links CHD1 activity to splicing as well. Yeast two-hybrid assays proved an interaction between CHD1 and the splicing proteins mKIAA0164, Srp20, and SAF-B ([73]). Also, splicing assays showed that Chd1 over-expression can affect alternative splicing. More convincingly, CHD1 was found to interact with the U2snRNP component of the spliceosome ([66]). This interaction was found to be facilitated by CHD1 binding to the tri-methylated histone H3 lysine 4 (H3K4me3) mark generally associated with transcriptional activation. Knockdown of both CHD1 and decreased H3K4me3 reduced the association with U2snRNP and affected splicing efficiency. These results led to the proposal of the existence of "chromatin-adaptor complexes" described earlier in this chapter.

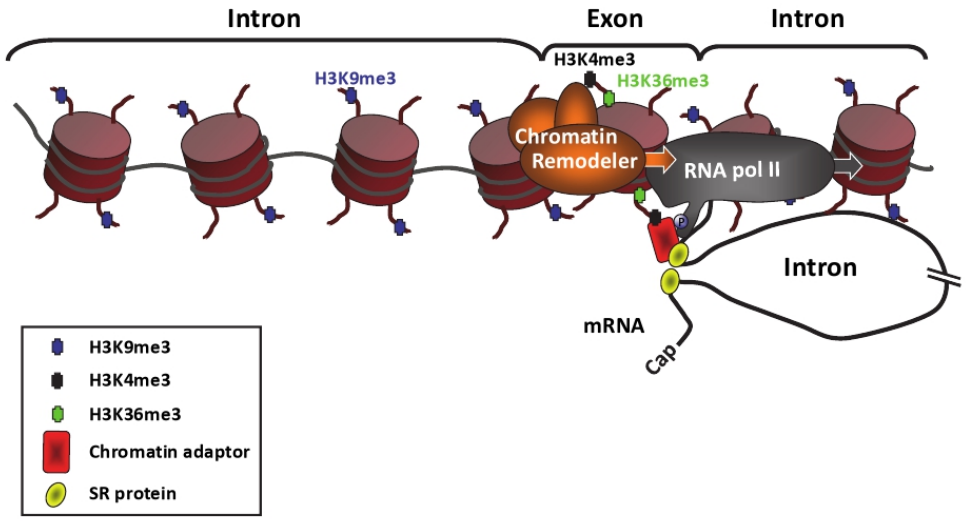

Fig. 5. Chromatin dynamics alter elongation and splicing activity mediated by RNA Pol II CTD.

Nucleosome's position and histone post-translational modifications affect the recruitment of chromatin-associated proteins (chromatin adaptors), which in turn can modulate the recruitment and/or binding of splicing factors to the pre-mRNA. Histone PTMs act as markers of introns (K3K9me3, blue oval) and exons (H3K4me3, black oval and H3K36me3, green oval). The exon-enriched modifications can contribute to the recruitment of splicing factors (yellow oval) and chromatin adaptors (red rectangle), resulting in a significant alteration in splicing efficacy. Chromatin remodelers (orange complex) can regulate the overall or local nucleosome density and position. Such variations can lead to changes in chromatin higher-order structure and modulation of the RNA pol II elongation rate ", affecting splicing decisions (see Figure 3).

3. Conclusion

By now, a distinct niche for chromatin and its interactors in the process of splicing has been established. The cellular process most commonly associated with chromatin, transcription, has been found to act in concert with splicing, increasing the efficiency and accuracy of mature transcript genesis. Two models have been proposed for the coupling of transcription and splicing, kinetic and recruitment, both orchestrated through the modulation and interactions of the RNA pol II C-terminal domain (see Figure 5 for an integrated model). In terms of chromatin structure, nucleosome density and positioning have both been found to have a direct impact on the location, recognition, and selection of splice sites. Post-translational modifications of histone tail residues also have a role in the delineation of splice sites. They are also known to be beacons and docking sites for factors that can simultaneously regulate both transcription and splicing. Chromatin remodelers have shown to interact with the transcriptional and splicing machinery, and are necessary for proper splicing efficiency. In sum, the role of chromatin in splicing is an ever-expanding subject of research and will prove to be for many years to come, as technology allows for a more precise determination of all involved players.

4. References

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