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Long range chromatin organization

A new layer in splicing regulation?

Luciana I Gómez Acuña and Alberto R Kornblihtt*

Laboratorio de Fisiología y Biología Molecular, Departamento de Fisiología, Biología Molecular y Celular, IFIBYNE-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires; Buenos Aires, Argentina

Splicing is a predominantly co-transcriptional process that has been shown to be tightly coupled to transcription. Chromatin structure is a key factor that mediates this functional coupling. In light of recent evidence that shows the importance of higher order chromatin organization in the coordination and regulation of gene expression, we discuss here the possible roles of long-range chromatin organization in splicing and alternative splicing regulation.

Recent evidence indicates that pre-mRNA splicing occurs co-transcriptionally, i.e., before RNA polymerase II (RNAP II) has reached the end of the gene and while the transcript is associated to chromatin.^{1,2} Co-transcriptional splicing seems to be prevalent for most introns and in those introns whose excision was demonstrated to take place post-transcriptionally, splice site commitment and spliceosome assembly on the pre-mRNA were shown to occur mostly co-transcriptionally.^{1,3} Multiple levels of alternative splicing regulation have been described.⁴ On the one hand, the presence and relative position of regulatory elements on the pre-mRNA determine the recruitment of splicing factors and spliceosome components,⁵ whose abundance, activity and intracellular localization are subjected to regulation.⁶ On the other hand, as a consequence of the spatio-temporal coordination, both transcription and splicing were shown to be functionally coupled.⁴ In one of the mechanisms, known as “recruitment coupling,” splicing factors can be recruited to splice sites by the transcription machinery,⁷⁻⁹ which may

also affect alternative splicing outcomes through the differential recruitment of these factors to promoters and enhancers as well as by alternative promoter usage.^{10,11} Alternatively, in the mechanism known as “kinetic coupling,” RNAP II elongation rate was shown to regulate alternative splicing by modulating the relative timing by which splice sites and regulatory sequences are transcribed and thus exposed to splicing factor binding and spliceosome component assembly.^{4,12,13} RNAP II elongation rate can be regulated both by factors recruited at gene promoters or along gene bodies and by the chromatin configuration of the DNA template. This introduces chromatin structure as another regulatory layer of alternative splicing.^{4,14} Chromatin is a highly dynamic structure that serves as an active platform for protein recruitment through specific histone marks and DNA methylation. Similar to the behavior of several protein complexes involved in transcriptional activation or repression, splicing factors can also be recruited to chromatin by direct or indirect binding to specific histone marks.^{15,16} The elongation control of alternative splicing is also dependent on how tightly DNA is wrapped around nucleosomes together with the capacity of the transcribing machinery to overcome these nucleosomal physical barriers.^{17,18} DNA packed into chromatin is also specified by histone marks that elicit the recruitment of nonhistone proteins with enzymatic activity that further modify chromatin structure.¹⁹ All together, nucleosome positioning,^{20,21} histone marks that determine a more loosened or compact

Keywords: alternative splicing, coupling between transcription and splicing, chromatin, long-range chromatin interactions.

Abbreviations: RNAP II, RNA polymerase II; FISH, Fluorescence in Situ Hybridization; DHS, DNase hypersensitivity sites; 3C, Chromosome Conformation Capture; LCR, Locus control region; CTCF, CCCTC-binding factor

*Correspondence to: ark@fbmc.fcen.uba.ar

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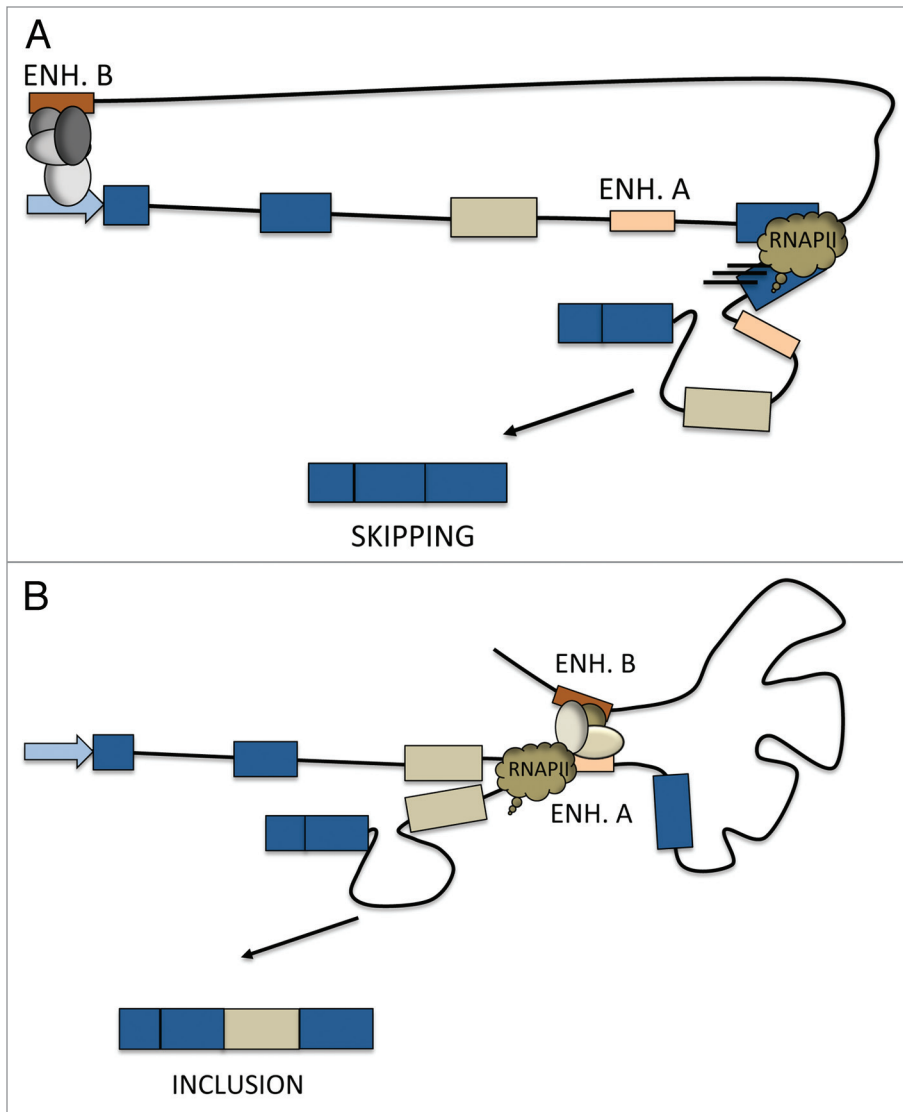


Figure 1. Speculative models by which enhancer usage might influence alternative splicing choices through changes in RNAP II elongation rates. **(A)** Enhancer B interacts with the gene promoter and determines the recruitment of elongation factors that enhance RNAP II elongation rate through the body of the gene, thus promoting alternative exon skipping. **(B)** The intragenic enhancer A, located in the intron downstream of the alternative exon, interacts enhancer B. The resulting loop formation and protein complex recruitment to the region induces RNAP II stalling upregulating alternative exon inclusion.

chromatin,²²⁻²⁶ and DNA methylation²⁷ regulate RNAP II elongation through the body of genes and modulate alternative splicing choices.

DNA packed into chromatin is not linearly nor randomly organized in the nucleus. Instead, a network exists where distant regions of the genome interact in a functional manner to elicit complex coordination and regulation of gene expression.^{28,29} Based on chromosome conformation capture (3C) technologies, genome-wide analyses of long range

genome interactions performed in various species, developmental stages and cell types upon various stimuli, are increasingly showing the importance of topology in transcriptional regulation.^{28,30-36} Enhancers interact with promoters of distant genes to activate transcription.³⁷ Pioneer work on the β globin gene cluster revealed for the first time a loop formation between an enhancer element of the locus control region (LCR) and the active β -globin gene, located 50 kbp downstream.³⁸ Most importantly, the

loop is necessary for the transcriptional activation of the β globin gene.³⁹⁻⁴¹ Genome-wide studies on the estradiol-mediated transcriptional activation in mammary human cells revealed that genes that have the estrogen receptor bound to their promoters without being involved in interactions with other regions of the genome display less activation upon estradiol treatment than those in which the estrogen receptor is engaged in long range interactions.³⁰ The human genome contains more enhancers than genes and more than 50% of these enhancers are located intragenically.^{42,43} Many of these intragenic enhancers were reported to act as alternative promoters of their host genes.⁴² Additionally, it was demonstrated that the distribution of enhancer-specific histone mark H3K4me1 is more specific of the cell type than that of the promoter-specific mark H4K4me3.⁴³ Chromatin interactions are not only restricted to promoters and enhancers. Promoters and the tails of active genes interact and this interaction seems to enhance transcriptional directionality.⁴⁴ Long range interactions were also described in the establishment of transcriptional repression domains by the Polycomb group proteins.⁴⁵

In view of the reported functional association between transcription and splicing, we wish to hypothesize that genome three-dimensional architecture can also have a role in alternative splicing regulation. Favoring this hypothesis, Mercer et al.⁴⁶ reported that a considerable fraction of exons significantly overlaps with DNase hypersensitivity sites (DHS-exons).⁴⁷ ENCODE data analysis revealed that several transcription factors and histone marks are enriched in DHS-exons, in comparison to non-DHS-exons.⁴⁶ Based on the specific transcription regulatory factors and histone marks that overlap with DHS-exons, these were grouped into promoter-like, enhancer-like and cohesin-like.⁴⁶ The latter refers to sites enriched in the CCCTC-binding factor (CTCF) and cohesins, proteins known to be involved in chromatin distant interactions, in particular of promoters with enhancers.⁴⁷ Promoter-like DHS exons show no evidence of acting as real promoters

and native ChIP-seq of H3K4me3 revealed a decrease of the enrichment of this histone mark at DHS exons, supporting the notion that the observed transcription factor and promoter specific histone mark enrichment is most probably due to the interaction of DHS exons with promoters.⁴⁶ In fact, an enrichment of DHS-exons was observed in genomic regions involved in long-range chromatin interactions where the RNAP II pre-initiation complex is present.⁴⁶ Interactions between DHS-exons and promoters were also found to be cell-specific.⁴⁶ Similarly to promoter-like DHS-exons, the presence of enhancer specific histone marks and factors on enhancer like DHS exons can also be explained, at least partially, by interactions between these exons and enhancers.⁴⁶ In the same way, cohesin and CTCF associated DHS exons were found to be enriched in regions involved in interactions centered in CTCF.⁴⁶ It cannot be completely ruled out, however, that some enhancer- and promoter-like DHS exons actually function as alternative promoters or regulatory elements. Favoring the hypothesis that interactions of exons with promoters and enhancers might favor co-transcriptional exon recognition, it was found that Ser2-phosphorylated RNAP II accumulates more in DHS exons than in total exons.⁴⁶ Most interestingly, DHS exons were found to be enriched in alternative splicing events.⁴⁶

As hypothesized by Mercer et al.,⁴⁶ chromatin loops could bring in close proximity splicing and transcription machineries so that splicing factors recruited to gene promoters are efficiently delivered to splice sites. More than ten years ago, our lab showed, using splicing reporter minigenes, that the usage of an SV40 enhancer element located upstream of the minigene promoter induces exon skipping.⁴⁸ It was hypothesized that the enhancer element increased RNAP II elongation at the body of the minigene, with this increase being responsible for the observed exon skipping (Fig. 1A).⁴⁸ In view of the more recent evidence discussed, we can now also speculate that the enhancer might directly interact with the alternative exon region and favor

the delivery of factors that inhibit exon inclusion.⁴⁸ Consistently, in recent report a Mediator subunit was shown to be involved in alternative splicing regulation by interacting and recruiting the splicing inhibitor hnRNPL to alternative exons.¹⁰ Mediator is a protein complex known to interact with several transcription factors involved in the preinitiation complex and with cohesin that facilitates Mediator interaction with enhancers and promoters of active genes.⁴⁹ Suggestively, in the proximity of the alternative exon 4 of the *SLC2A2* gene, used to describe the Mediator effect on hnRNPL-regulated alternative splicing, an enhancer can be mapped according to the analysis of several histone marks (H3K4me3, H3K4me2, H3K4me2 and H3K27ac) and transcription factor binding in several cell lines.⁵⁰ This brings forward another intriguing possibility by which intragenic enhancer activity and loop formation might influence alternative splicing of neighboring exons (Alló, Gómez Acuña, Kornbliht et al., in preparation). Considering the mentioned prevalence in the intragenic location of enhancers,^{42,43} we can speculate that tissue specific enhancer usage might determine tissue specific alternative splicing outcomes.

Another matter that emerges when considering the possibility of constitutive and alternative splicing regulation by three dimensional chromatin organization is how RNAP II elongation is affected when transcribing through regions looped to distant regulatory elements by long range interactions. It was reported that DNA methylation antagonizes with CTCF recruitment to exon 5 of the *CD45* gene, which regulates splicing of the alternative exons 4–5 by modulating RNAP II elongation rate.²⁷ It was shown that CTCF binding induces a slowing down of transcription and thus favors exon inclusion. Further analysis revealed that the CTCF binding site is engaged in a chromatin loop with another distant genomic region.⁴⁶ Figure 1B depicts how chromatin interactions may favor exon recognition by slowing transcription immediately downstream of an alternative exon. As discussed above, many intragenic enhancers are involved in interactions with the promoter of the host gene or

other regions in a tissue-dependent manner, so their formation may also lead to alternative splicing regulation of neighboring exons. The dynamics of chromatin organization is currently being studied both by genome-wide and imaging technologies.²⁹ It is known that chromatin architecture undergoes changes during cell differentiation and proliferation.^{40,51} Hi-C experiments performed in human embryonic stem (ES) cells and fibroblasts show that chromatin is organized in discrete modules, named topological domains, separated by boundary regions enriched in CTCF. The position of the boundary regions and lower resolution domains are largely conserved between the cell lines, suggesting that the gross domain structure remains unchanged along differentiation.²⁸ However, interactions within each topological domain are very variable and depend on cell type-specific gene expression.^{28,32} It has been shown that looping does occur before gene activation but that effective transcriptional activation is associated with additional loop formation.³¹ Deeper genome-wide analysis confirmed that promoter-enhancer interactions pre-exist to gene activation in various cell types and upon different stimuli, being loop formation a strong predictor of gene activation.³² In view of these findings, the hypothesis that high order chromatin organization might determine cell-specific alternative splicing becomes of particular relevance and deserves further investigation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

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