

Abstract

In my thesis, I focused on several underexplored areas of RNA splicing regulation. In the first part, I analyzed how chromatin and transcription regulatory elements change pre-mRNA splicing. In the second part, I studied why long non-coding RNAs (lncRNAs) are spliced less efficiently than protein-coding mRNAs. Finally, I was testing the importance of intron for the activating function of lncRNAs.

It has been shown that chromatin and promoter identity modulate alternative splicing decisions. Here, I tested whether local chromatin and distant genomic elements that influence transcription can also modulate splicing. Using the chromatin modifying enzymes directly targeted to *FOSL1* gene by TALE technology, I showed that changes in histone H3K9 methylation affect constitutive splicing. Furthermore, I provide evidence that deletion of transcription enhancer located several kilobases upstream of an alternative exons changes splicing pattern of the alternative exon.

Many nascent lncRNAs undergo the same maturation steps as pre-mRNAs of protein-coding genes (PCGs), but they are often poorly spliced. To identify the underlying mechanisms for this phenomenon, we searched for putative splicing inhibitory sequences. Genome-wide analysis of intergenic lncRNAs (lincRNAs) revealed that, in general, they do not contain more splicing inhibitory sequences compared to PCGs. Using *ncRNA-a2* as a model, we provide evidence that its inefficient splicing is independent of chromatin or promoter sequence. On the contrary, we show that the intron sequence of *ncRNA-a2* is a major determinant of its inefficient splicing. Additionally, we provide experimental evidence that the strengthening of the 5' splice site and increasing the thymidine content in polypyrimidine tract significantly enhance lincRNA splicing. We further show that lincRNA exons contain less putative binding sites for SR proteins and are bound to a much lower extent by SR proteins than expression-matched PCGs. We propose that lincRNAs lack the cooperative interaction network that enhances splicing, which renders their splicing outcome more dependent on the optimality of splice sites.

Finally, we removed intron from *ncRNA-a2* and tested whether the splicing process is important for the function of an enhancer-like lncRNA. My results suggest the functionality of DNA element of *ncRNA-a2* locus rather the RNA product itself in the promoting transcription of

neighboring genes. However, we could not distinguish between these two possibilities thus future experiments have to be done to provide a definite answer.