FINAL REPORT 334000

THE ROLE OF EPIGENETICS IN THE REGULATION OF CELL-SPECIFIC ALTERNATIVE SPLICING PROGRAMS

Alternative splicing is a very general process essential to increase the protein diversity and complexity of our genome. It is an extremely well regulated process, with highly cell-specific splicing isoforms that have been shown to play important roles in a variety of biological processes from sex determination in Drosophila, to neuronal and muscular cell differentiation and pluripotency in mammals. Moreover, an increasing number of splicing events have been shown to be involved in several diseases, such as cancer, highlighting the importance of understanding how this process is so well regulated. Interestingly, in the past few years, we have realized that splicing is mainly a co-transcriptional process, and chromatin also play an important role in alternative splicing regulation. We have shown that alternatively spliced genes can be enriched in specific histone modifications important for the inclusion of the correct splice variant. These chromatin marks impact splicing by changing the RNA Polymerase II elongation rate and/or by favouring the recruitment of the splicing regulators to the pre-mRNA via protein-protein interaction with specific chromatin binding proteins that act as adaptors (see Figure below).

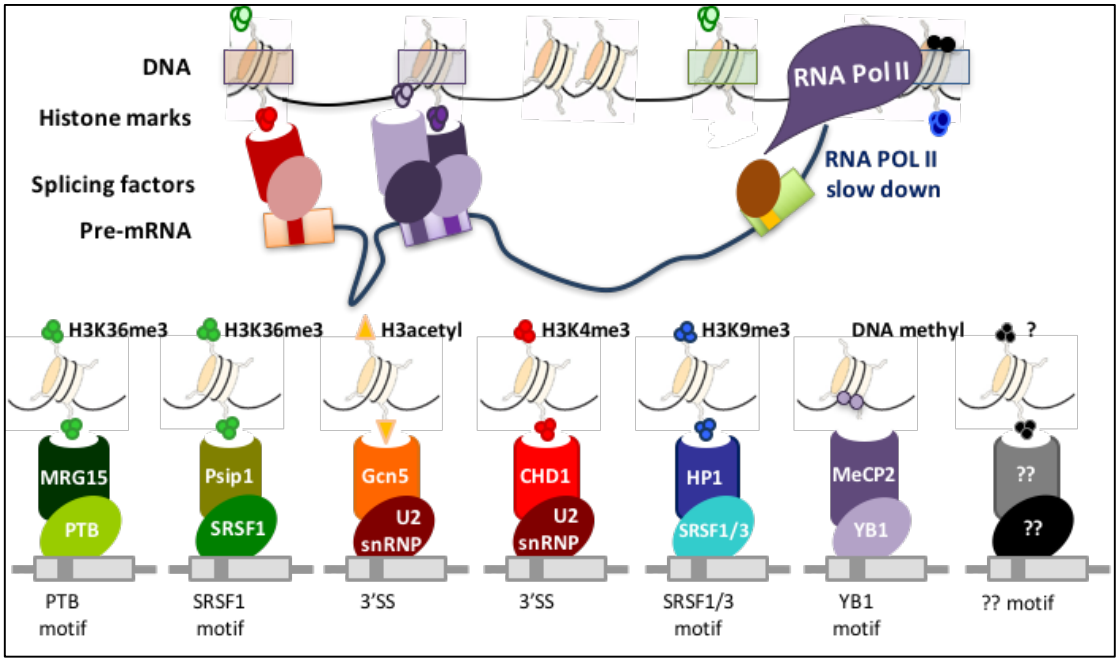

FIGURE. *Combinatorial effect of histone marks on alternative splicing by modulating recruitment of the splicing factors to the pre-mRNA via chromatin-adaptor complexes and/or RNA Pol II elongation rate. Examples of chromatin-adaptor complexes are shown below.*

Moreover, when studying how these splicing-related chromatin marks are regulated, we discovered a novel regulatory layer in alternative splicing that involves long non-coding RNAs. We showed that cell-specific lncRNAs, expressed within the alternatively spliced gene, have the capacity to recruit chromatin regulators to the gene locus, such as the Polycomb Repressive Complex 2, that create the chromatin signature necessary for inclusion of the correct splicing variant in a cell-specific manner. Moreover, we have also shown that histone modifications play a combinatorial role in the regulation of alternative splicing, increasing the complexity of and specificity of alternative splicing (manuscript published in Nat. Str. Mol. Biol. in 2015).

Taking into consideration the well-known role of epigenetics in transcriptional regulation and cell memory, we now hypothesize that chromatin modifications and lncRNAs might also play an important role in the establishment and maintenance of the highly cell-specific splicing programs.

To understand the role of histone modifications and lncRNAs in the regulation of cell-specific alternative splicing patterns, we have chosen as an inducible, highly dynamic and physiologically relevant model system the well-established epithelial-to-mesenchymal transition (EMT), which has been involved in early development and tumour progression and metastasis. **Our aim is to assess the real biological impact of histone modifications and lncRNAs in regulating cell-specific splicing, both at a global and gene-specific level, and to understand the molecular mechanisms behind these novel regulatory layers.**

To do so, we have first set up and fully characterized in the lab the EMT model system that best fit our needs and have started generating extensive transcriptomics and epigenomics data to identify novel chromatin and lncRNA regulators of splicing. To identify, in an unbiased way, novel chromatin marks to be tested in our model system, we have taken advantage of extensive available RNA-seq and ChIP-seq data from the ENCODE and ROADMAP Epigenomics project for computational analysis. Using machine learning approaches, we have identified novel histone modifications, that in a combinatorial way, differentially mark included from excluded alternatively spliced exons, and not constitutive ones. We have even shown, that a cell-specific switch in the levels of exon inclusion correlates well with a change in the chromatin signatures enriched along the regulated exons, supporting a role in splicing. Finally, we suggest that these distinctive combinations of chromatin marks along precisely the regulated exons might play a role in the final splicing outcome by modulating RNA Pol II elongation rate and the recruitment of specific splicing regulators, such as hnRNPs, to the pre-mRNA (Manuscript to be submitted soon to Nature Comm.).

Finally, following a more gene candidate approach, we have shown that there is a perfect correlation in time between early changes in enrichment of specific histone modifications along precisely the regulated exon and the first changes in alternative splicing very early during the onset of the EMT. There are other histone marks that also change their enrichment levels, but later during the EMT, suggesting different regulatory functions. We are now developing exon-specific strategies, based on the CRISPR/dCas9 system, to modulate those histone marks levels and test their effect on splicing and EMT progression. We are also looking for the mechanisms of splicing regulation dependent on these chromatin modifications.

Results from this project will prove for the first time the dynamic and physiological role of histone marks in the regulation and maintenance of alternative splicing patterns. It will also identify unsuspected new regulators of EMT splicing amongst lncRNAs, which could be used to impair EMT progression and tumour metastasis, as a more efficient and targeted therapeutic strategy to increase cancer prognosis.

Reini F. LUCO
Chromatin & Splicing team.
Institut de Genetique Humaine – CNRS-UM
141 rue de la Cardonille, Montpellier
reini.luco@igh.cnrs.fr
www.igh.cnrs.fr/en/research/departments/genome- dynamics/22-chromatin-and-splicing