2023-12-19

International PhD Program IC-PhD

PhD thesis project

2024 Call for application

A DNA/RNA crosstalk: The role of splicing in transcriptional regulation

General information

Call	2024
Reference	2024-06-LUCO
Keyword(s)	Chromatin; histone modifications; alternative splicing; CRISPR/dCasRx; splicing editing; RNA Polymerase II; elongation rate; ChIP

Director(s) and team

Thesis director(s)	Reini Luco
Research team	Chromatin and RNA splicing
Research department	UMR3348 - Genome Integrity, RNA and Cancer

Description of the PhD thesis project

Abstract

To increase protein diversity and acquire new phenotypic traits despite a limited coding genome, the cell takes advantage of the alternative processing of the molecules of pre-mRNA into different mature mRNAs. This alternative splicing impacts more than 70% of the human genes and is involved in almost every cellular process, highlighting the importance of understanding how it is regulated. Interestingly, different teams amongst ours have uncovered a novel regulatory layer for alternative splicing, which are chromatin modifications and their impact in the 3D genome organization. Indeed, either by modulating the RNA polymerase II elongation rate, or by inducing recruitment of specific chromatin-adaptor complexes, histone and DNA modifications can change splicing factors binding capacity to the pre-mRNA. However, it is not so clear yet whether this cross-talk can go the other way around, which would mean that splicing regulators can also induce the recruitment of specific chromatin modifiers and/or transcriptional regulators to the DNA during co-transcriptional RNA splicing, which would impact RNA Pol II elongation and thus transcription.

In this proposal, we aim at studying the bidirectional cross-talk between histone modifications and alternative splicing regulation using innovative CRISPR tools to edit the epigenome (dCas9) or the pre-mRNA (dCasRx). We will target alternatively spliced genes in which we already have evidence of a role for histone marks in splicing regulation, such as FGFR2 and CTNND1, and try new ones of relevance for cancer, such as PKM and SCRIB. This will be the first time that pre-mRNA splicing is tested for its direct impact in chromatin organization and transcription, avoiding indirect effects from the use of splicing inhibitory drugs or repression of the splicing regulators involved. Results from this project will help better understand the cross-talk existing between these two regulatory layers essential for gene expression: chromatin and splicing.

Background

To have a full protein, a coding gene needs to be transcribed, spliced, exported to the cytoplasm and then translated. Each of these steps was thought to be independent, but we now know that there are intimately related in complex molecular cross-talks in which the absence of one can impair the progression of the other. In this project, we are interested in the transcription-splicing cross-talk. It has already been shown that RNA polymerase II elongation rate and chromatin modifications can impact alternative splicing. It has also been suggested that repression of splicing using inhibitory drugs or knocking-down key splicing regulators, such as SC35, can impact gene transcription. However it is not entirely clear yet to what extent splicing regulators can impact chromatin modifiers and therefore Pol II kinetics, 3D chromosome organization and ultimately transcription. In the team, we have recently set up a new CRISPR system in which an RNA targeting dCas13 protein, called dCasRx, can be targeted to a specific RNA motif along the exon pre-mRNA to compete with recruitment of key splicing regulators, which induces a change in the alternative splicing of this exon. This RNA splicing editing system is highly exon-specific, with little off-target effects, and can efficiently induce more than 50% change in exon inclusion levels. We thus propose to use this editing system to induce highly localized changes in splicing, one exon at a time, either by i) interfering with splicing by impairing recruitment of the spliceosome, or by ii) changing alternative splicing patterns to study the impact in chromatin, RNA Pol II dynamics and thus transcription. By inhibiting spliceosome recruitment we expect to impact splicing check-points, while by interfering with specific splicing regulators we expect to impact much more locally the chromatin and RNA Pol II dynamics. Different experimental approaches will be used for studying this transcriptional-chromatin-splicing cross-talk at exons known to be dependent on changes in chromatin and at exons with no evidence of a role for chromatin in their regulation. This will be the first time that such cross-talk is tested in such a locus specific way for highly specific and direct effects. If we want to modify gene expression for therapeutic purposes, we need to unravel all the players involved and their regulatory cross-talks for efficient editing.

Objectives

1. To change the alternative splicing of chromatin-associated spliced exons one at a time in a highly localized way using the CRISPR/dCasRx system in our MCF10a-Snail-ER epithelial model system to study EMT.
2. To test the impact in histone modifications, 3D chromatin organization, RNA polymerase II kinetics and RNA transcription upon splicing editing of a specific splicing event/gene.
3. To identify how this chromatin modifier-splicing regulator cross-talk is possible.
4. To study the importance of this cross-talk in EMT progression

Experimental approaches

Key alternatively spliced exons for EMT progression previously shown to be regulated by chromatin modifications will be targeted with the CRISPR/dCasRx system to induce a change in splicing. Control exons not impacted by chromatin modifications will be also targeted and studied in parallel for specific effects. Two types of splicing editing will be performed for comparison: interference with the spliceosome core complex and specific interference with key splicing regulators. These gene loci will be then studied by ChIP to analyze the changes in chromatin modification before and after the localized change in splicing. RNA polymerase II elongation rate and RNA polymerase II enrichment levels will be also assessed by ChIP and DRB-RTqPCR to study nascent RNA. If changes in splicing impact Pol II elongation rate, drugs impacting Pol II will be used to rescue the splicing phenotype. Finally, we will look for the chromatin modifiers recruited at the chromatin level and test whether their binding is dependent on the presence of the splicing regulators competed away by targeting of the dCasRx to the pre-mRNA. Finally, if changes in splicing impact the chromatin environment of the whole locus, which impacts its transcription, we will also study the impact in EMT progression using well-established migration and invasion assays.

International, interdisciplinary & intersectoral aspects of the project

We are collaborating with Tom Misteli (NIH) and Gary Stein (University of Vermont College of Medicine) in the USA to understand the role of 3D chromatin organization in splicing regulation using high-resolution microC data in our cellular model system, MCF10a. Out of these collaborations, we will identify interesting alternatively spliced exons to be targeted to test the role of splicing in chromatin conformation.

We are in collaboration with Thierry Dubois (Institut Curie-Paris, Translational Research Dpt), an expert in arginine methylation and gene expression, to understand the role of CARM1 in alternative splicing. Based on our preliminary data, we will certainly look into the role of splicing in recruiting these arginine methyltransferases and therefore in gene expression.

In collaboration with Daniela Verga at Curie Orsay (Chemistry and Modelling for Biology of Cancer Unit), we are studying the role of DNA and RNA G4 quadruplexes in alternative splicing regulation. She is synthesizing chemical ligands to stabilize these tertiary structures and test the effect in splicing. If successful, we will also test the impact in the chromatin.

Recent publications

1. Sahu S, Agirre E, Inayatullah M, **Luco RF**, Belmonte J, Tiwari V. A complex epigenome-splicing crosstalk governs epithelial to mesenchymal transition in metastasis and brain development. Nature Cell Biology 2022, 24(8) PMID: 35941369.

2. Segelle A., Núñez-Álvarez Y., Oldfield, A., Webb K.M., Voigt P., **Luco RF***. Histone marks regulate the epithelial-to-mesenchymal transition via alternative splicing. Cell Reports 2022, 38(07) PMID: 35172149

3. Villemin JP, Lorenzi C, Oldfield A, Cabrillac MS, Ritchie W and **Luco RF***. A cell-to-patient machine learning transfer approach uncovers novel basal-like breast cancer prognostic markers amongst alternative splice variants. BMC Biology 2021, 19(1):70

4. Agirre E., Oldfield A., Segelle A., Bellora N. and **Luco RF***. Splicing-associated chromatin signatures: a combinatorial and position-dependent role for histone marks in splicing definition. Nature Communications 2021, 12(1)

5. Gonzalez I, Munita R, Agirre E, Dittmer TA, Gysling K, Misteli T and **Luco RF***. A long non-coding RNA regulates alternative splicing via establishment of a splicing-specific chromatin signature. Nature Structural Molecular Biology 2015, 22(5):370-6.

Expected profile of the candidate

- Master degree or equivalent
- Highly motivated
- With background in molecular biology, particularly in RNA biology or Chromatin/Epigenetics
- Curious
- With initiative (resourceful)