Abstract

This experimental work of thesis was performed at the International Centre for Genetic Engineering and Biotechnology (ICGEB, AREA Science Park, Padriciano, Trieste) in the Human Molecular Genetics Group, under the scientific direction of Prof. Franco Pagani.
The project was developed during the 2012-2015 academic years.

Spliceosome and Microprocessor complex (MPC) are processing machineries that act and cleave precursor (pre)-mRNA to generate spliced mature transcripts and mature microRNAs (miRNAs), respectively. The presence, on the nascent transcript, of several *cis*-acting regulatory elements allows the correct recognition of the intron-exon junctions by the spliceosome, while the hairpin sequences that form typical RNA secondary structures are recognized and cleaved by the MPC. I have explored the functional relationship between these two machineries focusing on a peculiar class of miRNAs, the Splice site Overlapping (SO)-miRNAs, whose hairpin secondary structure is juxtaposed to an intron-exon junction.
In the first part of my Ph.D. project I worked on the evolutionarily conserved SO-miR-34b, whose hairpin is juxtaposed to an acceptor splice site of a non-coding transcriptional unit.
Using a minigene approach, I identified a consensus branch point (BP) located in the hairpin and a purine-rich exonic splicing enhancer (ESE) that are strictly required for the correct selection of the 3' splice site (ss). Splicing inhibition owing to 3'ss mutation or ESE deletion increases miR-34b expression level. Moreover, depletion of MPC components Drosha and DGCR8 abolishes miR-34b production, improving splicing efficiency, while their overexpression has the opposite effect. Therefore, the processing of 3' SO-miR-34b is regulated in an antagonistic manner by the spliceosome and the MPC, due to the competition between these two processing machineries on the nascent transcript.

To better understand the relationship between splicing and SO-miRNAs on a global basis, I focused on the SF3b1 factor, an essential splicing component of the U2 snRNP complex, frequently mutated in different hematological malignancies like chronic lymphocytic leukemia. I carried out a global analysis of miRNA changes in SF3b1 depleted cells.

Small RNA-seq showed that, in comparison to other miRNAs, SO-miRNAs are significantly upregulated in the SF3b1 depleted cells, indicating that splicing has a direct influence on the biosynthesis of SO-miRNAs.

To evaluate in a more physiological context the crosstalk between these two machineries, I focused on three SO-miRNAs, miR-936, miR-4260 and miR-711, expressed in keratinocytes. Their host transcripts, the COL17A1, LAMB3 and COL7A1 genes, are expressed in the basal layer of epidermis and are down regulated during calcium-induced keratinocytes differentiation. Quantitative analysis of mRNAs and miRNAs expression levels showed that keratinocyte differentiation is associated to a decreased mRNAs expression and to an increased biosynthesis of the corresponding SO-miRNAs. Exogenous administration of these three miRNAs inhibits keratinocytes proliferation, confirming their contribution to keratinocytes differentiation.

Interestingly, both SF3b1 silencing and keratinocyte differentiation did not induce changes in the splicing pattern of corresponding SO-miRNA exons.

Analysis of published chromatin RNA sequencing data showed that Drosha depletion leads to extensive transcriptional readthrough downstream the SO-pri-miRNA hairpins, suggesting that the MPC might induce premature transcriptional termination of SO-miRNA transcripts.

The competition between the spliceosome and the MPC may represent a novel mechanism to regulate the production of mature mRNAs and miRNAs from a shared precursor RNA transcript. In some cases, SO-miRNAs may function as dead-end processing signals inside genes. The activation of these signals, either by an increase in MPC activity or by a reduction in splicing, might induce the biosynthesis of the SO-miRNA and a concomitant production of shorter transcript that is rapidly degraded.