

## Mini-Symposium

# "Long non-coding RNAs at the heart of development and disease"

Centre Hospitalier Universitaire Vaudois (CHUV) Lausanne, 16 September 2016

Auditorium Mathias Mayor, CHUV main building, floor 8

Organizers: Ana-Claudia Marques & Samir Ounzain

### PROGRAM

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|----------------------|---|
| 8:00 – 8:30          | Welcome coffee  |
| <b>8:30-8:40</b>     | <b>Welcome: Ana-Claudia Marques</b><br>Department of Physiology, University of Lausanne   |
| <b>8:40 -9:25</b>    | <b>John Rinn</b><br>Broad Institute of MIT and Harvard, Cambridge, MA, USA<br><b><i>Regulatory Roles of RNA Repeats in Human Health and Disease</i></b>   |
| <b>9:25-10:10</b>    | <b>Alena Shkumatava</b><br>Unité de Génétique et Biologie du Développement, U934 / UMR3215, Institut Curie - Centre de Recherche, Paris, France<br><b><i>Dissecting functions and mechanisms of vertebrate lncRNAs</i></b>                |
| 10:10 – 10:30        | Coffee Break  |
| <b>10.30-11.15</b>   | <b>Phillip Grote</b><br>Institute of Cardiovascular Regeneration, Centre for Molecular Medicine, Goethe University, Frankfurt am Main, Germany<br><b><i>Studying Novel lncRNA Loci Involved in Cardiovascular Development In Vivo</i></b> |
| <b>11.15-12.00</b>   | <b>Samir Ounzain</b><br>Experimental Cardiology Unit, University of Lausanne Medical School, Lausanne, Switzerland<br><b><i>The long noncoding RNA WISPER controls cardiac fibrosis and remodelling</i></b>                               |
| 12.00 – 14.00        | Lunch   |
| <b>14:00 – 16:00</b> | <b>Afternoon workshops for PhD students with symposium speakers</b>   |

**This mini-symposium will be accredited by the Association of Cantonal Veterinarians (SCAV), section Lausanne, as a half day of continuing education.**

This meeting is free of charge but for organization purposes we would like participants (limited to a maximum number of 120) to register by filling the form [here](#) prior to September 1, 2016.

The UNIL doctoral school attributes 1.0 ECTS for PhD students who present a signed participation form for the mini-symposium (0.25 ECTS morning session, 0.75 ECTS afternoon session).

For additional information, please contact Dr. Ulrike Toepel ([ulrike.toepel@unil.ch](mailto:ulrike.toepel@unil.ch)).

## Talk abstracts

### John Rinn

Broad Institute of MIT and Harvard, Cambridge, MA, USA

#### ***Regulatory Roles of RNA Repeats in Human Health and Disease***

Our research program aims to understand long noncoding RNAs (lncRNAs) biology: from identifying and mapping thousands of lncRNA loci -- to developing numerous mutant lncRNA mouse models demonstrating the important impact of lncRNA loci in development and disease -- to dissecting the molecular modalities and mechanisms of lncRNAs in transcriptional and epigenetic regulation. This combinatorial and multi-faceted approach is critical to advance lncRNA biology to a more detailed understanding of their role in nuclear architecture. Here I will focus on a specific lncRNA termed FIRRE (Functional Intergenic Repeat RNA). FIRRE is mendelian inherited in human disease and has a fascinating mechanism of organizing multiple chromosomes in the nucleus. I will discuss our efforts to elucidate FIRREs role from mouse to molecules.

### Alena Shkumatava

Unité de Génétique et Biologie du Développement, U934 / UMR3215, Institut Curie - Centre de Recherche, Paris, France

#### ***Dissecting functions and mechanisms of vertebrate lncRNAs***

Thousands of long intervening noncoding RNAs (lincRNAs) have been identified in mammals. To better understand functions and evolution of these enigmatic RNAs, we identified more than 550 lincRNAs in zebrafish, an established vertebrate model for development. Although zebrafish lincRNAs share many characteristics with mammalian lincRNAs, only 5% have detectable sequence similarity with putative mammalian counterparts, typically restricted to short regions of high conservation. To understand if evolutionary pressure on conserved lincRNA sequences is associated with their important biological functions, we are generating multiple genetic zebrafish mutants of the ultra-conserved lincRNA motifs using Crispr/Cas9 genome editing. We are subsequently analyzing the impact of lincRNA loss-of-function on normal embryonic development and adult animals. For one of the conserved lincRNAs that we called linc5, we recognized that the conserved region of linc5 contains an unusually complementary, near-perfect microRNA miRNA site that is highly conserved in all examined vertebrates. To determine functions of linc5 in vivo, we used reverse genetics and generated linc5 mutants in zebrafish, mouse and mouse embryonic stem cells (mESC). Both, zebrafish and mouse mutants show a significantly disturbed reaction in behavioral tests. Using primarily our mouse model, we are investigating the exact molecular function of the linc5-miRNA pairing. Moreover, we aim to identify proteins binding conserved regions of linc5 using a novel high-throughput method we recently developed in the lab.

**Phillip Grote**

Institute of Cardiovascular Regeneration, Centre for Molecular Medicine, Goethe University, Frankfurt am Main, Germany

***Studying Novel lncRNA Loci Involved in Cardiovascular Development in Vivo***

The development of functional organs such as the heart requires precise spatio-temporal control of gene regulatory networks. Over several decades, protein-coding genes that are essential for heart development and function were identified, and most important insights came from genetic experiments performed on these genes in vivo. In recent years, unparalleled efforts in transcriptome sequencing uncovered that most of the mammalian genome gives rise to RNA that does not code for proteins. Although many of these RNAs might be non-functional as a molecule and rather indicative of transcriptional activity at the RNA originating locus, for some of these RNAs essential functions were already demonstrated.

We generated a differential transcriptome dataset of tissues from the early mouse embryo, including the heart tube. Bioinformatic profiling of this dataset identified several hundred long non-coding RNAs (lncRNAs), expressed specifically in the forming heart. Some of these RNAs originate from loci in the proximity to developmentally essential mRNA genes. We genetically ablate these lncRNA genes in mouse embryonic stem cells (ESCs), utilizing several different strategies for genome manipulation including CRISPR/Cas9. Direct generation of mouse embryos via tetraploid morula aggregation from such genetically altered ESCs, allows rapid profiling of in vivo consequences of the loss of lncRNA loci.

We created an allelic series of a lncRNA locus that gives rise to two different lncRNAs in the embryonic heart. Genetic ablation of the entire locus results in embryonic lethality, caused by a range of deformations in the early embryo, while removing only one of the two lncRNAs causes specifically an increased heart size in the mid-gestation embryo and no other visible defects. We are currently investigating the genetic interaction of these two RNAs in embryo development and the formation of the heart in particular.

**Samir Ounzain**

Experimental Cardiology Unit, University of Lausanne Medical School, Lausanne, Switzerland

***The long noncoding RNA WISPER controls cardiac fibrosis and remodelling***

Long noncoding RNAs (lncRNAs) are emerging as powerful regulators of cardiac development and disease. However, our understanding of these molecules in cardiac fibrosis is limited. Using an integrated genomic screen, we identified *WISPER* (Wisp2 SuPer-Enhancer associated RNA) as a cardiac fibroblast-enriched lncRNA that potentially regulates cardiac fibrosis. *WISPER* expression is significantly correlated with cardiac fibrosis both in a murine myocardial infarction (MI) model and in human patients suffering with aortic stenosis. *In vitro* loss-of-function approaches using modified antisense oligonucleotides (ASOs) demonstrated that *WISPER* is a heart-enriched cell-type specific regulator of cardiac fibroblast survival, proliferation and migration. Accordingly, ASO-mediated silencing of *WISPER* *in vivo* attenuated MI-induced fibrosis and remodelling in both preventative (injection pre-injury) and therapeutic (injection post-injury) experimental scenarios. Functionally, *WISPER* globally regulates cardiac fibroblast gene expression programs critical for extracellular matrix (ECM) deposition, cell identity, survival and proliferation. Together, our findings identify *WISPER* as a novel cardiac fibroblast-enriched super-enhancer associated lncRNA that represents an attractive therapeutic target to prevent cardiac fibrosis and pathological remodelling.