JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

13th TRR81 PhD Minisymposium

"Molecular mechanism of gene regulation"

Monday, 30 March, 2020 11:00 am

Ernst-Leitz Hörsaal, 2nd floor

Institute of Biochemistry, University of Giessen, Friedrichstrasse 24, Giessen, Germany

Invited speakers:

1) Francesco Gualdrini,
Humanitas Research Hospital, Milan, Italy *(Gioacchino Natoli lab)*

2) Sofia Luciana Battaglia,
Department of Pathology and Center for Cancer Research, Massachusetts General Hospital and Harvard Medical School, Boston, USA *(Bradley E. Bernstein lab)*

3) Kseniia Lysakovskaia,
Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany *(Patrick Cramer lab)*

4) Jente van Staalduinen,
Department of Cell Biology, Erasmus MC, Rotterdam, Netherlands *(Frank Grosveld lab)*

5) Louisa Hill,
Research Institute of Molecular Pathology, IMP, Vienna, Austria *(Meinrad Busslinger lab)*

6) Simona Capponi,
Department of Urology, University of Freiburg, Germany *(Marc Timmers lab)*

Local organizer: Francesca Ferrante and Aleksandra Turkiewicz (Tilman Borggrefe lab)
Sponsoring: DFG – TRR81 (Chromatin Changes in Differentiation and Malignancies)

Program:

11:00h – 11:15h: **Welcome, Introduction**
Francesca Ferrante & Aleksandra Turkiewicz, Chair (JLU, University of Giessen, Germany)

11:15h – 12:00h: **Francesco Gualdrini**
"Transcriptional control of macrophage function"

12:00h – 12:45h: **Sofia Luciana Battaglia**
"Connecting genes and regulatory elements by single-molecule analysis of chromatin accessibility and DNA methylation"

12:45h – 14:15h: **Lunch break**

14:15h – 15:00h: **Kseniia Lysakovskaia**
"Mechanisms of transcriptional regulation during human cell type specification"

15:00h – 15:45h: **Jente van Staalduinen**
"Live visualization of genomic loci in mouse embryonic stem cells"

15:45h – 16:15h: ***Coffee Break***

16:15h – 17:00h: **Louisa Hill**
"Role of Pax5 in controlling loop extrusion and V_H gene recombination at the *Igh* locus"

17:00h – 17:45h: **Simona Capponi**
"Understanding the molecular mechanism of X-linked dystonia-parkinsonism: from alternative splicing to epigenetics"

19.00h: **Speakers dinner**

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Francesco Gualdrini
Humanitas Research Hospital, Milan, Italy *(Gioacchino Natoli lab)*

Transcriptional control of macrophage function

Macrophages are crucial mediators of the inflammatory response, regulating fundamental processes such as phagocytosis, antimicrobial defense and tissue repair. These diverse biological outcomes are brought about by the coordinated rewiring of signaling, transcriptional and metabolic networks in response to micro-environmental cues. Transcription factors (TFs) are major players capable of relay information delivered by signaling pathways to the transcriptional machinery via an array of co-regulators with disparate biochemical activities and functions.
In the lab we are investigating how signal-induced TFs are capable of exerting their activities within the chromatin context and how their activation over time relates to specific phenotypic outcomes.

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Sofia Luciana Battaglia
Department of Pathology and Center for Cancer Research, Massachusetts General Hospital and Harvard Medical School, Boston, USA *(Bradley E. Bernstein lab)*

Connecting genes and regulatory elements by single-molecule analysis of chromatin accessibility and DNA methylation

Our central goal is to understand the principles that underlie enhancer-promoter connectivity and link regulatory DNA elements to their target genes. We hypothesize that (1) the activity state of regulatory elements and genes is highly dynamic and correlated at the single-molecule level and (2) that certain genetic variants or haplotypes affect the activity of one or more cis-regulatory elements leading to gene expression variation in specific cell types. We aim to identify sets of regulatory elements and genes by simultaneously measuring their correlated chromatin accessibility and DNA methylation on single DNA molecules of immune loci in immune cell types. We developed a method that combines marking open chromatin (as in NOMe-seq) with nanopore sequencing to capture ~100 kb loci with >200x coverage in a single run using the MinION device from Oxford Nanopore Technologies. Sequencing of ultra-long reads allows us to separate alleles by haplotype to study allele-specific gene regulation. We demonstrate our method by capturing allele-specific differences in DNA methylation and chromatin accessibility of imprinted genes in embryonic stem cells. Lastly, we applied our method to activated and resting primary CD4+ T cells and aim to identify enhancer-promoter pairs that activate the expression of certain genes after T cell stimulation.

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Kseniia Lysakovskaia,
Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany *(Patrick Cramer lab)*

Mechanisms of transcriptional regulation during human cell type specification

Transcription in living cells is a precisely regulated and coordinated process. One of the key regulatory steps is promoter-proximal RNA polymerase (Pol II) pausing, which involves transient pausing of Pol II ~50 bp downstream of the transcription start site prior to the transition into productive elongation. Modulation of promoter-proximal pausing has been associated with various signaling pathways including development, differentiation, cell proliferation, and stress or damage responses ensuring rapid transcription activation upon certain stimuli. Among all of these processes, the change of cellular identity appears to be the most intriguing and poorly understood in the context of Pol II pausing. Therefore, in our current work we address this question by using human cell transdifferentiation as a model system. We employ a `multi-omics` approach that has been recently developed in our group and allows the estimation of transcriptional kinetics parameters in human cells. To model Pol II kinetics, we measured RNA synthesis by Transient Transcriptome sequencing (TT-seq) and genome-wide occupancy of Pol II associated with the nascent transcripts by mammalian Native Elongation Transcript sequencing (mNET-seq) over the time of transdifferentiation. From the high-resolution data, we could calculate and compare productive initiation frequencies and pause durations for the target genes involved in the transdifferentiation process. Our results provide novel insights into transcriptional regulation by Pol II promoter-proximal pausing during a human cell type specification switch.

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Jente van Staalduinen
Department of Cell Biology, Erasmus MC, Rotterdam, Netherlands *(Frank Grosveld lab)*

Live visualization of genomic loci in mouse embryonic stem cells

Chromatin conformation capture (3C)-based techniques have shown that transcription is tightly linked to 3D genome organization. The folding of chromatin fiber inevitably determines the proximity of regulatory regions (e.g. enhancers) to their cognate genes [Furlong and Levine, 2018]. These experiments, however, are performed in fixed cell populations and the results therefore do not inform about the dynamics of locus configurations. In contrast, tracking of individual genomic loci during live cell imaging has the potential of elucidating conformation dynamics on a single locus level.

Recently, a non-intrusive DNA visualization approach was developed called the ANCHOR DNA labelling system. After directed genomic insertion of a non-repetitive short ANCH sequence (± 1kb), bacterial dimer OR proteins weakly bind to the 6-9 DNA binding sites per ANCH sequence. After the initial binding, ORs oligomerize and spread along the adjacent DNA, locally recruiting hundreds of molecules which fluorescently mark the sequence of interest. The weak DNA binding of the OR proteins do not induce heterochromatin formation and the ANCH sequences can be inserted very close to regulatory elements, such as enhancers and promoters [Germier et al. 2017].

In this project, we have adopted the ANCHOR DNA labelling system for mouse embryonic stem cells. We have inserted ANCH sequences from different bacterial species next to regulatory elements of a developmental gene which allows visualization and live tracking of this genomic locus by expression of ORs fused to fluorescent proteins. In this talk, I will discuss technical considerations of the ANCHOR DNA labelling system (genome editing, microscopy set-up e.g.) and how we plan to use this system to interrogate the dynamics of the chromatin landscape of a developmental gene locus during embryonic development.

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Louisa Hill
Research Institute of Molecular Pathology, IMP, Vienna, Austria *(Meinrad Busslinger lab)*

Role of Pax5 in controlling loop extrusion and V_H gene recombination at the *Igh* locus.

B cell immunity provides acute and long-term protection of the host against infection through the generation and secretion of high-affinity antibodies that recognize an almost unlimited number of pathogens. The enormous adaptive potential of B cells is generated through the random assembly of immunoglobulin gene segments by V(D)J recombination, which leads to a highly diverse B cell antigen receptor repertoire. This large diversity is only achieved if each of the 195 Variable (V_H) gene segments, which are arrayed over a 2.4-Mb region in the murine immunoglobulin heavy-chain (*Igh*) locus, have an equal opportunity to recombine with the proximal DJ_H-rearranged gene segment. This is mediated by Pax5-dependent contraction of the *Igh* locus during early B cell development. How Pax5 controls *Igh* contraction in pro-B cells is, however, not known.

The long-range genomic interactions that give rise to topologically associating domains (TADs) and chromatin loops are generated in a cohesin-dependent manner that likely involves the process of chromatin loop extrusion, during which cohesin binds the chromatin fiber and extrudes a continuously growing loop. Loop extrusion by cohesin can be blocked by extrusion barriers, such as the DNA-bound protein CTCF. As the *Igh* locus is three times larger than most TADs, it is currently unknown whether loop extrusion extends over the entire *Igh* locus to facilitate the participation of all V_H genes in V(D)J recombination.

Here, we demonstrate that locus contraction and recombination of distal V_H genes depend on cohesin-mediated loop extrusion along the entire *Igh* locus. Notably, expression of the cohesin-release factor Wapl is repressed by Pax5 specifically in pro-B and pre-B cells, which facilitates extended loop extrusion by increasing the residence time of cohesin on chromatin. Reduced Wapl expression causes global alterations of the three-dimensional chromatin architecture, indicating that the potential to recombine all V genes entails structural changes of the entire genome in pro-B cells.

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Simona Capponi,
Department of Urology, University of Freiburg, Germany *(Marc Timmers lab)*

Understanding the molecular mechanism of X-linked dystonia-parkinsonism: from alternative splicing to epigenetics

X-linked dystonia-parkinsonism (XDP) is an adult onset, neurodegenerative condition of the Philippines. The disease onsets with progressive loss of voluntary motor control replaced by severe motor contractions (dystonia) combined with or replaced by parkinsonism features. All XDP patients harbor the insertion of an SVA (SINE-VNTR-Alu) retrotransposon of the F-subclass into intron 32 of the *TAF1* gene, which resides on the X-chromosome. It has been proposed that insertion of this SVA affects expression and alternative splicing of *TAF1* mRNAs.

In order to investigate the effect of the XDP SVA on *TAF1* alternative splicing, we first characterized the splicing mechanism underlying its neuronal isoform named *TAF1-34'*. We identified the neuron-specific splicing factor SRRM4/nSR100 as necessary and sufficient to regulate temporal and spatial expression of *TAF1-34'* mRNA in the brain, to generate a neuronal-specific TFIID complex. We then investigated whether the presence of the XDP SVA could interfere with SRRM4 splicing program and affect the expression of *TAF1 34'* mRNA. The analysis of different brain regions of XDP patients, together with *in vitro* validations, suggested that the XDP SVA does not impair *TAF1-34'* splicing. Using *TAF1* mini-genes we demonstrated that the presence of this retrotransposon seems to act as transcriptional repressor of *TAF1* expression in different cell systems. These findings would suggest that the XDP SVA might scaffold heterochromatin nucleation to reduce *TAF1* expression.

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