MBG YOUNG INVESTIGATOR SYMPOSIUM 2020 - AARHUS UNIVERSITY

Wednesday 12 August 2020 - Zoom link https://aarhusuniversity.zoom.us/j/61143180543	
15.55	Welcome by Ditlev Brodersen, Chair of the MBG YI committee
16:00-16:30	**Davide Seruggia** Boston Children's Hospital, Dana-Farber Cancer Institute, Harvard Medical School, USA **Coding and non-coding elements in the control of gene expression**
16:30-17:00	Closed session
17:05-17:35	**Broder Schmidt** Stanford University, Department of Biochemistry, USA **'Phasing in' on the properties and functions of sticky disordered proteins**
17:35-18:05	Closed session

Thursday 13 August 2020 - Zoom link https://aarhusuniversity.zoom.us/j/61071576423	
09:30	Welcome by Ditlev Brodersen, Chair of the MBG YI committee
09:35–10:05	**Sandro Baldi** Ludwig-Maximilians-University, Group of Prof. Peter Becker, Munich, Germany **Revealing chromatin regularity – and what chromatin regularity reveals**
10:05-10:35	Closed session
10:40–11:10	**Sueli Marques Spencer** Karolinska Institutet, SciLife Lab, Stockholm, Sweden Title - pending
11:10 –11:40	Closed session
11:40–12:20	Break
12:20–12:50	**Stephanie Heinrich** Institute of Biochemistry, ETH Zurich, Switzerland **Quantitative single-molecule analysis of gene expression regulation in vivo**
12:50 –13:20	Closed session
13:25 –13:55	**Oleksiy Kovtun** Cambridge Biomedical Campus, MRC Laboratory of Molecular Biology, Cambridge, UK **Cryo-electron tomography reveals organisation of key molecular machines controlling membrane trafficking**
13:55 – 14:25	Closed session
14:30–15:00	**Matthias Schlichting** Brandeis University / HHMI, Waltham, USA **Neuromodulation is key to keeping track of time in Drosophila**
15:00–15:30	Closed session

Coding and non-coding elements in the control of gene expression

Davide Seruggia
Boston Children's Hospital, Dana-Farber Cancer Institute, Harvard Medical School, USA

How transcription factors and chromatin modifiers control gene expression, and how regulatory elements such as enhancers drive cell-type specific transcription are fascinating problems that in-situ targeted perturbations and next-generation sequencing (NGS) are helping to solve. In my presentation, I will describe my efforts in studying genes and protein complexes critical to control transcription, as well as in evaluating the functions of non-coding regulatory sequences. I will describe my work using CRISPR screens in mouse embryonic stem cells, and focus on two members of the SAGA complex, Taf5l and Taf6l, that are required for self-renewal1. Next, I will talk about red blood cells and describe strategies to de-repress fetal hemoglobin in human cells and in the mouse by targeting precise protein domains within the NuRD repressive complex2. Moving to the non-coding part of the genome, I will present data showing the impact of mutations at non-coding sequences, in the mouse3. Finally, I will introduce my future plans towards analysis of non-coding DNA in leukemia.

1. Seruggia D, Oti M, Tripathi P, et al. TAF5L and TAF6L Maintain Self-Renewal of Embryonic Stem Cells via the MYC Regulatory Network. Mol Cell. April 2019. doi:10.1016/j.molcel.2019.03.025
2. Sher F1, Hossain M1, Seruggia D1, et al. Rational targeting of a NuRD subcomplex guided by comprehensive in situ mutagenesis. Nat Genet. 2019;51(7):1149-1159. doi:10.1038/s41588-019-0453-4 (1 these authors contributed equally)
3. Seruggia D, Fernández A, Cantero M, Pelczar P, Montoliu L. Functional validation of mouse tyrosinase non-coding regulatory DNA elements by CRISPR-Cas9-mediated mutagenesis. Nucleic Acids Res. 2015;43(10). doi:10.1093/nar/gkv375

'Phasing in' on the properties and functions of sticky disordered proteins

Broder Schmidt
Stanford University, Department of Biochemistry, USA

Many key regulators of gene expression, including transcription factors (TFs), RNA-binding proteins (RBPs) or nucleoporins (Nups), contain intrinsically disordered regions (IDRs) that do not fold into defined tertiary structures. IDRs typically are fast evolving protein domains of low sequence complexity that often are important drivers of intracellular phase separation. However, the role of IDRs and phase separation in organizing gene expression remain poorly understood. To determine how IDRs work on both the molecular and cellular level, my work combines random, saturation ('deep') and targeted mutagenesis (guided by evolutionary sequence analysis) with in vitro reconstitution assays, quantitative in vivo reporter assays and genetic screens.

In this presentation, I will contrast the functions of IDRs and phase separation in nuclear transport and RNA processing. While I found that IDR-mediated phase separation allows the formation of nuclear pore-like permeability barriers at physiological conditions, I also discovered that phase separation-deficient mutants of the neurodegeneration-linked RNA-binding protein TDP43 remain functional in splicing. This finding challenges the emerging view that IDRs generally work through phase separation and underscores the need for novel approaches to disentangle the biophysical properties and biochemical functions of IDRs. Indeed, my research emphasizes that we are only beginning to understand how cells use IDRs and leaves us with lots of exciting work for the future.

Revealing chromatin regularity – and what chromatin regularity reveals

Sandro Baldi
Ludwig-Maximilians-University, Group of Prof. Peter Becker, Munich, Germany

The positioning of nucleosomes relative to DNA and their neighboring nucleosomes represents a fundamental layer of chromatin organization. Changes in nucleosome positioning and spacing affect the accessibility of DNA to regulatory factors and the formation of higher order chromatin structures. During my postdoc, I developed new tools to measure nucleosome spacing and chromatin regularity in vivo and to reconstitute physiological chromatin in vitro.
In a first project, we elucidated the formation and distribution of locally confined, regular nucleosome arrays. Such so-called phased arrays are commonly found in the vicinity of critical genomic elements such as gene promoters and often indicate the presence of regulatory sites. Devising new tools for analyzing genome-wide nucleosome maps, we generated a comprehensive inventory of phased arrays in the Drosophila genome. This revealed thousands of new potential regulatory sites. As a key result I identified Phaser, a previously uncharacterized but essential protein, which is one of the major determinants of phased arrays in flies. Importantly, this work introduced chromatin assembly with Drosophila embryo extracts as a powerful tool to reconstitute chromatin features on a global scale in vitro and to purify whole genomic features with associated proteins.

In a second project, I mapped nucleosome spacing and regularity on a genome-wide level. Previously, spacing and regularity could only be determined with high throughput at select sites with well-positioned nucleosomes. Therefore, I developed Array-seq, a new method that determines nucleosome spacing throughout the genome and even in repetitive regions such as heterochromatin. This revealed that nucleosome array regularity differs widely for chromatin states. Array-seq is a new tool in the repertoire of methods to assay chromatin structure and can now be used to assess changes in chromatin structure during development or in mutants.

Sueli Marques Spencer
Karolinska Institutet, SciLife Lab, Stockholm, Sweden
Title - pending

Quantitative single-molecule analysis of gene expression regulation in vivo

Stephanie Heinrich
Institute of Biochemistry, ETH Zurich, Switzerland

Compartmentalization is a fundamental principle of eukaryotic cellular organization that provides spatial and temporal control of gene expression. Importantly, the genetic information in the nucleus is separated from translation to protein in the cytosol, and the central process that enables information transfer is messenger RNA (mRNA) transport through nuclear pore complexes. Surprisingly, despite its fundamental nature, the molecular mechanism of uni-directional mRNA transport is poorly understood, which is mainly due to the lack of sensitive tools and functional assays to study this highly dynamic process. Recently, I overcame this major hurdle by (a) building and successfully employing a high-speed single-molecule imaging and data analysis platform (SimPI) which allows real-time single RNA imaging in vivo, and by (b) developing genetic and biochemical assays to acutely manipulate mRNA export components. These tools allowed me to quantitatively analyze individual steps of mRNA transport, which is a major step towards mechanistically dissecting the mRNA export machinery. Furthermore, I found that cells rewire mRNA export during stress by forming membraneless condensates of a nuclear mRNA export factor, thereby selectively exporting stress-induced mRNAs, while globally retaining bulk mRNAs in the nucleus. These findings establish a novel layer of gene expression regulation and provide key mechanistic insights into mRNA transport.

Cryo-electron tomography reveals organisation of key molecular machines controlling membrane trafficking

Oleksiy Kovtun
Cambridge Biomedical Campus, MRC Laboratory of Molecular Biology, Cambridge, UK

Over 30% of eukaryotic genes encode transmembrane proteins that rely on membrane transport via vesicles/tubules for their functional localisation to organelles. The precise turnover of protein cargo by membrane transport is essential in many processes including signalling, immunity, and cell polarity. Dedicated protein complexes known as coats initiate the assembly of transport carriers thereby orchestrating membrane trafficking. These molecular machines assemble ad hoc on the target membrane, oligomerise into a lattice-like coat, drive cargo selection and remodel the membrane, resulting in the formation of cargo-loaded compartments ready for transport.

I study coat complexes assembly and organisation in order to understand the molecular mechanisms of their function in trafficking. In particular, I use cryo-electron tomography and sub tomogram averaging as prime methods to enable structural studies of functional complexes on the membrane both in vitro and in situ. Here I present a pioneering experimental model of membrane-assembled Retromer and AP2/Clathrin coat complexes and discuss their implications for our understanding of membrane trafficking.

Neuromodulation is key to keeping track of time in Drosophila

Matthias Schlichting
Brandeis University / HHMI, Waltham, USA

Most organisms have developed circadian clocks which allow them to predict day/night transitions. Work over the last 40 years has identified several transcriptional feedback loops within clock neurons in the brain which provide animals with a sense of time. However, this molecular machinery is not sufficient to keep time as neuronal communication is essential for molecular and behavioral rhythms. G-Protein Coupled Receptors (GPCRs) are an essential part of this communication. To learn more about how they function in the clock network, we performed single cell RNA sequencing experiments on most of the clock neurons in Drosophila. Surprisingly, the expression of individual GPCRs is highly cell-type specific and many of their transcripts cycle in the course of a day, which may contribute to neuronal physiology. To investigate the function of these GPCRs, we generated a guide library for all 124 GPCRs encoded by the fly genome. This library allows for cell-specific and highly efficient mutations of GPCRs. As a proof-of-principle experiment, we performed pan-neuronal knockout experiments, which reproduced known behavioral phenotypes. We then focused on the dorsal neurons which are involved in sleep regulation. We identified several GPCRs that function in these neurons to regulate sleep structure, including neuropeptide (e.g. sNPF) and biogenic amine (e.g. dopamine) receptors. These data are further supported by anatomical studies tracing the downstream partners of dopamine or sNPF expressing neurons. Most importantly, GPCRs are not only involved in regulating sleep structure, they appear to be involved in seasonal adaptation. We showed recently that the clock neuron network changes its dynamics during long summer days using neuromodulation. Therefore, the tools we developed should be widely applicable and allow for new insights in Drosophila neurophysiology.