MBG YOUNG INVESTIGATOR SYMPOSIUM 2021 - AARHUS UNIVERSITY

Tuesday June 1 – zoom link: https://aarhusuniversity.zoom.us/j/64681940276	
Time	**Event**
13:55	Welcome by Ditlev Brodersen, Chair of the MBG YI committee.
14:00 – 14:30	**Francesc Xavier Bofill de Ros** *MicroRNA isoforms: A tale of two non-canonical ends*
14:30 – 15:00	Closed session
15:05 – 15:35	**Martin Machyna** *Hybridize and capture: A new insight into RNA localization and function at nucleotide resolution*
15:35 – 16:05	Closed session

Wednesday June 2 – zoom link: https://aarhusuniversity.zoom.us/j/69888241119	
Time	**Event**
12:00 – 12:05	Welcome by Ditlev Brodersen, Chair of the MBG YI committee.
12:05 – 12:35	**Ahmad Jomaa** *Mechanism of co-translational protein targeting to the endoplasmic reticulum by the mammalian signal recognition particle*
12:35 – 13:05	Closed session
13:10 – 13:40	**Robertas Ursache** *Plant cell wall remodelling during normal growth and in response to environmental changes"*
13:40 – 14:10	Closed session
14:10 – 14:20	Break
14:20 – 14:50	**Hanneke Vlaming** *Screening thousands of transcribed coding and non-coding regions reveals sequence determinants of RNA polymerase II elongation potential*
14:50 – 15:20	Closed session

MicroRNA isoforms: A tale of two non-canonical ends

Francesc Xavier Bofill-De Ros
RNA Biology Laboratory, National Institutes of Health (NIH) – National Cancer Institute

MicroRNAs (miRNAs) are a class of non-coding RNAs with important regulatory functions. In animals, miRNAs silence gene expression by binding to partially complementary sequences within target mRNAs. It is well-established that miRNAs recognize canonical target sites by base-pairings at their 5' region known as the seed region. However, the development of biochemical methods has identified many novel, non-canonical target sites, suggesting additional modes of miRNA-target association. My work in this field has revealed that internal loops disrupting the tertiary structure of primary miRNAs can trigger alternative cleavage by DROSHA. As a result of this alternative processing on their 5' end, miRNAs change their target specificity (Bofill-De Ros et al. Cell Rep. 2019). More recently, I found that primary miRNA with suboptimal structures facilitate the recruitment of RNA binding proteins modulating the accuracy of their processing. Interestingly, a global analysis of miRNA processing in tumors reveals a widespread increase in misprocessed miRNAs as a consequence of the dysregulation of several RNA-binding proteins (Bofill-De Ros et al. submitted). Although alternative cleavage sites by DROSHA and DICER1 during biogenesis contribute to the production of isoforms on both ends, most miRNA 3' end isoforms are a result of post-maturation modifications. In collaboration, we found that mono-uridylation of mature miRNAs by TUT4/7 on their 3' ends can expand target mRNA recognition (Yang*, Bofill-De Ros* et al. Mol. Cell 2019). We also uncovered that TUT4/7 are responsible for the oligo-uridylation of specific miRNAs, thus making them substrates for degradation by the exonuclease DIS3L2 (Yang*, Shao*, Bofill-De Ros* et al. Nature Comm. 2020). Overall, this work has provided evidence that gene regulation by miRNAs is a dynamic process not only determined by their overall abundance but also through the regulation of their target association by their 5' and 3' ends.

Hybridize and capture: A new insight into RNA localization and function at nucleotide resolution

Martin Machyna
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Chromatin modifying complexes regulate gene expression by changing the local chromatin context and thereby effecting RNA production. RNA also plays important roles in gene regulation by keeping chromatin in an open state, maintaining nuclear compartments such as Cajal bodies, and guiding chromatin modifying complexes to distal sites. An example of an RNA capable of targeting protein complexes are long non-coding RNAs (lncRNAs), which play a vital role in gene dosage compensation in mammals and flies. In male flies, roX2 RNA assembles with proteins into the Male-Specific Lethal (MSL) complex that binds to high-affinity sites (HAS) on the X chromosome. From these sites, the MSL complex spreads and upregulates the neighboring active genes on the whole chromosome. Despite extensive research in the area of chromatin-associated RNA complexes, it remains poorly understood what determines their precise targeting and spreading over the vast areas of chromatin or how stable their localization patterns are. This is partly due to limited specificity of the enrichment methods such as ChIP or CHART. I therefore developed a new method by combining enhanced nucleotide chemistry with DNA nanotechnology, which led to dramatic improvement of RNA enrichment specificity. For roX2, more than 90% of the signal could be attributed to the true MSL complex localization in comparison to ~30% of the previous approaches. This improvement became essential for detecting an unexpectedly rapid redistribution of MSL complex on X chromosome and discovering novel sites of autosomal accumulation in cells under acute stress conditions. Precise detection of roX2 localization also supported a model where chromatin insulators and local chromatin contacts guide chromatin-associated complexes to distal sites. Applying this approach further to all mature RNAs will enable to study general mechanisms and functional aspects of RNA retention on chromatin.

Mechanism of co-translational protein targeting to the endoplasmic reticulum by the mammalian signal recognition particle

Ahmad Jomaa
Institute for Molecular Biology and Biophysics, Swiss Federal Institute of Technology, ETH Zurich

Almost 30% of the cell proteome consists of membrane and secretory proteins that are delivered co-translationally to the endoplasmic reticulum membrane (ER) in eukaryotes and to the plasma membrane in bacteria. The process involves the function of the universally conserved signal recognition particle (SRP) and its receptor (SR), in a multi-step pathway that recruits the translating ribosome to the Sec translocon on the membrane. Perturbation of this pathway has been linked to the onset of disease particularly to severe congenital neutropenia. Mammalian SRP and SR are compositionally and structurally more complex than their bacterial counterparts with a number of eukaryotic-specific features with unknown regulatory roles. How eukaryotic SRP and SR transition from cargo recognition in the cytosol to the late stages of the targeting pathway on the membrane is a long-standing question. Using a cell-free translation system from rabbit reticulocyte lysate, we trapped molecular snapshots in the targeting pathway and then determined their structures by single particle cryo-EM. We also resolved intermediates in the SRP pathway assembled with a mutant SRP54G226E, which is identified in patients with severe congenital neutropenia. Combined with biochemical experiments, our structures reveal the molecular mechanism for co-translational protein targeting to the ER by the mammalian SRP. The cryo-EM structures of complexes assembled with SRP mutants uncover intermediates along the targeting pathway that provide structural and functional basis for this disease in humans.

Plant cell wall remodelling during normal growth and in response to environmental changes

Robertas Ursache
Department of Plant Molecular Biology (DBMV), University of Lausanne

Plants are incredibly complex organisms capable of living in two opposite environments at the same time. While the aboveground organs provide energy by means of photosynthesis, their roots anchor the plants and allow assimilation of essential nutrients. As part of plant growth and adaptation, changes in cell walls take place in different parts of the plant body. In the root, to ensure an efficient growth and exploration of soil, the root tip cells continuously divide, transition into a differentiated state where they elongate and become specialized. The vascular cell walls undergo thickening, some enucleate, like phloem sieve elements, or die and lignify, like xylem, to become efficient in transporting. The endodermal tissue layer surrounding the vascular cylinder provides an extracellular diffusion barrier through a network of lignified cell walls, Casparian strips, supported by subsequent formation of suberin lamellae. Whereas lignification is thought to be irreversible, suberin display plasticity, which is crucial for plant adaptative responses. Although suberin is a major plant polymer, fundamental aspects of its biosynthesis and turnover have remained obscure. Recently, we showed that root endodermis has a specific, auxin-mediated transcriptional response dominated by cell wall remodeling genes. We identified two sets of GDSL lipases (GELPs). Out of more than a hundred proteins of the same family, five appeared to be essential for establishing a functional suberin barrier and another set can drive suberin degradation. The quintuple biosynthetic *gelp* mutant shows a complete absence of root suberin leading to increased sensitivity to osmotic stress. Such example served as an inspiration for my proposed future research and, using tissue-specific RNA-seq, optimized gene editing and advanced imaging, it is now possible to look deeper into multigene families, break their functional redundancy and asses their role in essential processes, such as cell wall remodeling.

Screening thousands of transcribed coding and non-coding regions reveals sequence determinants of RNA polymerase II elongation potential

Hanneke Vlaming
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Proper gene expression relies on RNA Polymerase II synthesizing the appropriate repertoire of messenger RNAs (mRNAs) from protein-coding genes. Productive elongation of full-length transcripts is essential for mRNA function, however what determines whether an engaged RNAPII molecule will terminate prematurely or transcribe processively remains poorly understood. Notably, despite a common process for transcription initiation across RNAPII-synthesized RNAs, RNAPII is particularly susceptible to termination when transcribing non-coding RNAs such as promoter upstream transcripts (PROMPTs) and enhancers RNAs (eRNAs). The variability in RNAPII behavior is striking at divergent mammalian promoters, where an mRNA and PROMPT are transcribed in close proximity to one another, initiating from indistinguishable sequence elements, yet have vastly different fates. This dichotomy suggests that differences arise during RNA elongation and lie within the transcribed sequences themselves.

To investigate the role of transcribed sequence on elongation potential, we developed a powerful system to screen the effects of thousands of INtegrated Sequences on Expression of RNA and Translation using high-throughput sequencing (INSERT-seq). We found that the lower GC content in PROMPTs and eRNAs, rather than specific sequence motifs, underlies the propensity for RNAPII termination on these transcripts. Further, we demonstrate that 5' splice sites exert both splicing-dependent and autonomous, splicing-independent stimulation of transcription, even in the absence of cleavage and polyadenylation signals. Together, our results reveal a potent role for transcribed sequence in dictating gene output at mRNA and non-coding RNA loci and demonstrate the power of INSERT-seq to identify sequence elements regulating transcription elongation.