ADDENDUM to the PROGRAM for RNA 2019

June 11 to 16, 2019
ICE Congress Centre, Kraków

June 9, 2019

Corrections, withdrawals and new abstracts received after the program book was printed.

SESSION CHANGES

Friday, June 14 9:00 – 11:45
Plenary session 3: Mechanisms of Translation **Auditorium Hall**

Session chair has been changed to Michael Mathews, Rutgers New Jersey Medical School

Friday, June 14 16:45 – 19:00
Concurrent session 5: Non-coding RNAs: Long & Short **Auditorium Hall**

Session chair has been changed to Igor Ulitsky, Weizmann Institute of Science

ABSTRACT CHANGES and WITHDRAWALS

Corrected to author list

30 Structural basis of RNA transport: Tropomyosin 1 – Kinesin high-resolution structure and its interaction with RNA

Lyudmila Dimitrova-Paternoga, Pravin Kumar Ankush Jagtap, Anna Cyrklaff, Imre Gaspar, Janosch Hennig, Anne Ephrussi

Updated abstract

135 Structural determination of the yjdF riboswitch from *Staphylococcus aureus*

A. Ponce-Salvatierra[*I], *R. Pluta*[*I], *E. Skowronek*[I], *M. Orlowska*[I], *B. Baginski*[I], *M. Kurkowska*[I], *E. Purta*[I], *P. Ghosh*[I], *T. Wirecki*[I], *F. Stefaniak*[I], *J.M. Bujnicki*[I]
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[*] *The authors contributed equally to the work that is presented and should be considered as joint first authors.*

Riboswitches are regulatory 5' untranslated regions of mRNA molecules that specifically bind small molecules, resulting in conformational changes that regulate the production of proteins encoded by the mRNA. In recent years, riboswitches came under the spotlight as they are promising new targets in biomedicine.

The yjdF riboswitch, present in many bacteria, regulates the gene expression of the yjdF protein-coding gene. The function of the yjdF protein is unknown, although it is possible that its function will be related to detoxification for the ligand that regulates the gene expression. Although the yjdF riboswitch is known to bind a variety of compounds, its natural ligand remains unknown (Li et al, 2016).

To understand how the yjdF riboswitch regulates gene expression in bacteria, we decided to determine and validate its structure through an integrative approach involving X-ray crystallography, SAXS, chemical probing, and associated experimental and computational analyses.

I will present the structures of different yjdF riboswitch crystallization constructs. These structures, stabilized through a wealth of tertiary contacts, show that the yjdF riboswitch can adopt multiple conformations in the absence of its ligand, and hint towards important structural elements that may play a role in the ligand sensing mechanism.

Although ligand-bound structures will be required in the future, it is equally important to understand the structure of ligand free riboswitches. The comparison between ligand-bound and ligand-free riboswitch structures has the potential to reveal the atomic-scale rearrangements arising from ligand binding, and that ultimately translate into a genetic switch. Our work provides the first insight into the structural organization of the yjdF riboswitch in a ligand-free state and sets the basis for further structural and computational studies.

Replacement abstract

252 Cap-dependent linker ligation increases specificity for full-length products compared to template switch reaction

Pamela Moll, Musashi Tsujita, Florian Kabinger, Tomas Dozd, Michael Ante, Andreas Tuerk, Torsten Reda, and Alexander Seitz
Lexogen GmbH, Vienna, Austria

Nanopore enables full-length sequencing of RNA or cDNA. A fast and easy way to obtain full-length cDNA is the commonly used template switch reaction. The reverse transcriptase (RT) adds non-templated nucleotides (preferentially C's) at the end of a transcript which hybridize to abundant template switch oligos. However, non-templated nucleotides can also be added to fragmented RNAs or premature termination sites of the RT. Artificial Spike-in transcripts are an essential part to monitoring the quality of an RNA-Seq experiment, to control NGS sample preparation, base callers and algorithms by adding a ground truth to the NGS experiment. We used Lexogen SIRV™ set 3 containing 69 Spike-In RNA Variant controls, simulating alternative splicing of 7 SIRV genes plus antisense transcription, plus 92 ERCC Spike in controls (External RNA Controls Consortium Spike-In controls, Thermo Fisher Scientific Inc.). ERCCs are monoexonic but cover a concentration range of 6 orders of magnitude. TeloPrime is a full-length cDNA preparation kit offered by Lexogen. Exceptional 5'-Cap specificity is achieved with the proprietary CAP dependent linker ligation. We capped SIRV set 3 using the Vaccinia Capping Enzyme and protocol from NEB (M2080). Universal Human Reference RNA (UHRR) was spiked in with the capped SIRV set 3 before the sample was subject to a controlled degradation. Nanopore sequencing libraries were made either by using the template switching protocol or a modified TeloPrime protocol (v3) from intact and degraded RNA aliquots. The new TeloPrime v3 Nanopore libraries contain a 12 nt unique molecular Index (UMI) that is introduced with the RT primer, enabling to account for sequencer and PCR errors in high coverage NGS data. Degraded RNA resulted in shorter libraries for the less Cap-sensitive template switching protocol, but little delay in the PCR cycles, while for TeloPrime v3 degraded RNA libraries resulted in significantly less amplifiable library. The analyses of apparent transcript start site distributions by Nanopore sequencing showed a higher cap specificity for TeloPrime v3 than for the template switching protocol. Hence, TeloPrime v3 enables an increased accuracy for transcript 5' end detection.

Corrected author list

384 Coregulation of primary microRNA processing by the SR proteins SRSF1 and SRSF3

Marija Dargyte[1], Christina Palka[2], Jon Howard[1], Sol Katzman[3], Michael Stone[2], Jeremy Sanford[1]
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Poster withdrawn

401 Grad-Seq in mycobacteria

Updated abstract

416 Predicting CRISPR-Cas9 sgRNA on-target activity while minimizing off-target potential

Giulia I. Corsi, Ferhat Alkan, Jan Gorodkin

The RNA-guided DNA endonuclease CRISPR-associated protein 9 (Cas9) is a precise and widely used genome-editing tool. Recent studies have shown that the ability of Cas9 to cleave a target DNA largely depends on structural characteristics and sequence composition properties of the guide RNA (gRNA). Based on this, several computational tools have been developed to support researchers in designing gRNAs that optimize Cas9 on-target activity. However, numerous aspects

remain poorly understood, including the role of gRNA-DNA binding properties and the impact that off-targets events have on cleavage efficiency.

In this study, we model the on-target cleavage efficiency of Cas9-gRNA complexes with a Gradient Boosting Regressor tree and use the resulting model to analyse gRNAs off-targets effects on a dataset of known Cas9-gRNA on-target cleavage efficiencies. On top of features employed by previous prediction algorithms, we introduce novel energy-related properties, recently proposed for off-target predictions[1], as features to the model. These properties are favoured by the learning model compared to the commonly used sequence-derived features and improve prediction accuracy. Our results indicate that the off-target potential of gRNAs has significant importance to predict on-target cleavage efficiency, which also suggests the presence of unexplored off-target events in the dataset. As a result, our model provides optimized on-target efficiency predictions that minimize off-target effects.

[1]Alkan, F., Wenzel, A., Anthon, C., Havgaard, J. H. & Gorodkin, J. CRISPR-Cas9 off-targeting assessment with nucleic acid duplex energy parameters. *Genome Biol.* **19**, 177 (2018)

Corrected author list

456 Exploring the role of 5 methyl cytosine modification in RNA metabolism of mitochondria

Dhiru Bansal[1], Jon Price[1], Yaara Or-Rosenfeld[4], Lina Vasiliauskaite[4], Alan Hendrick[4], Oliver Rausch[4], Eric Miska[1,2,3]

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Poster withdrawn

468 HBV hijacks TENT4 to stabilize its RNA via mixed tailing

Replacement abstract

524 Prediction of dynamic interactions of RNA with magnesium ions.

Masoud Amiri Farsani[1], Michał Boniecki[1], Pritha Ghosh[1], Filip Stefaniak[1] and Janusz M. Bujnicki[1]

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RNA plays crucial roles in the coding, decoding, regulation, and expression of genes. Riboswitches represent RNA molecules, whose structures can be modulated upon small molecule binding. They typically occur within the protein-non-coding parts of messenger RNA (mRNA) and regulate the translation of the protein-coding parts. As riboswitches are common in bacteria and rarely occur in eukaryotes, they are emerging as a potential target for new and selective antibacterial drugs. Thus, it is essential to know how ligands interact with RNA and how an RNA folds in the presence of ligands.

Due to the complexity of biological molecules, their interactions cannot be quickly inferred from simple mathematical equations derived from first principles. In order to overcome this problem, we have resorted to a knowledge-based approach. To this end, our group has developed SimRNA, a method for RNA 3D structure prediction and folding simulations [1]. It considers RNA in a coarse-grained representation, samples the RNA 3D conformational space with the Monte Carlo method and uses a statistical potential for identifying physically realistic RNA 3D structures. Currently, we are developing various extensions of this method that will enable the modeling of interactions of RNA with other molecules. Recently, we developed a prototypical method for modeling the interactions of RNA with magnesium ions, which are among the simplest and the most commonly observed ligand in RNA structures. Our new method successfully predicts preferred Mg^{2+}-binding sites in RNA structures. We are currently extending this approach to bigger organic ligands, which will help us in the development of a general purpose method for prediction of RNA-ligand 3D structures.

[1] Boniecki et al., *Nucleic Acids Res.*, 2016

Poster withdrawn

659 Plasma miR-320a as a liquid biopsy suppresses non-small cell lung cancer progression through AKT3 and its associated pathways

Poster withdrawn

714 Disruption of autoregulatory feedback mechanisms of the minor spliceosome core di-snRNP proteins 65K and 48K, leads to cell cycle defects.

Poster withdrawn

720 Changes of phosphorylation drive alternative splicing modulation by mild heat shock in human cells

Added Wednesday in topic area Targeting RNA for Therapy

900 ANTISENSE INDUCTION OF NATURAL SECRETED SOLUBLE DECOY RTK (sdRTK) VARIANTS INHIBITS SIGNALING AND TUMOR GROWTH IN TREATMENT-REFRACTORY LUNG CANCER

Trushar Rathod[1], Prasad Subramaniam[1], Jeong Park[1], Lee Spraggon[2], Elisa de Stanchina[2], Luca Cartegni[1]
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The differential inclusion of functional domains by alternative splicing or polyadenylation can lead to the expression of both pathogenic or non-pathogenic variants from many cancer-associated genes. We developed a novel therapeutic approach which employs antisense manipulation of RNA processing to induce negative dominant variants of oncogenes, to reverse pathological signaling in cancer. Here, we describe a number of novel secreted soluble decoy isoforms produced by an U1-snRNP-dependent alternative intronic polyadenylation (IPA) mechanism.

Most forms of cancers, and especially lung cancer, implicate the aberrant activity of receptor tyrosine kinases (RTKs), such as EGFR or MET. Moreover, functional crosstalk of EGFR with MET and other RTKs has been reported in tumors and both have emerged as a drivers or mechanisms of resistance to therapy, thus making them attractive target for cancer therapeutics. There are still very limited therapeutic options for MET-dependent tumors, while in the case of EGFR, current treatments are severely limited by the appearance of TKI resistance from secondary somatic mutations in the EGFR gene, such as the T790M mutation (>50% of biopsies) in the EGFR kinase domain following erlotinib or gefitinib treatment. Even next generation, T790M-targeted TKIs, such as Osimertinib, are overseeded by the appearance of EGFR mutations (e.g. C797S). As most of these tumors are still EGFR-dependent, EGFR remains a prime therapeutic target in lung cancer.

Multiple natural soluble decoy isoforms exist for most RTKs, including EGFR and MET, which depend on the differential inclusion of functional domains by alternative splicing. We employ antisense oligo nucleotide (ASO) to reprogram RTK pre-mRNA processing in order to increase expression of mRNA variants that encode for dominant-negative, soluble decoys RTKs (sdRTKs), at the expense of the oncogenic full-length receptor. These alternative splicing isoforms are generated via intronic polyadenylation (IPA) of pre-mRNA in a U1-snRNP (U1) -dependent manner. More specifically, we are able to induce the sdEGFR or sdMET variants by using specific ASOs, designed to block the upstream U1 binding site and thus activate the appropriate IPA sites. This approach effectively induces the expression of potent natural inhibitors of EGFR and MET signaling in treatment-refractory lung cancer models in vitro and in vivo, leading to suppression of downstream pathways and tumor growth inhibition. The ASO-induced natural sdRTK compounds function in a dominant-negative manner and induce dramatic cell death in NSCLC cells harboring multiple activating and resistance EGFR mutations, and thus provides a novel alternative strategy for treatment of refractory NSCLC, to overcome resistance mediated by the EGFR secondary mutations (including T790M, C797S and others), or to target MET-dependent cancers.

Added Friday in topic area Biology and Mechanism of Small RNAs

901 The role of ABA unresponsive SnRK2 kinases in regulation of miRNA biogenesis in plant response to salinity

Adrian Kasztelan[1], Olga Sztatelman[1], Patrycja Plewka[2], Przemysław Nuc[2], Zofia Szweykowska-Kulińska[2], Artur Jarmołowski[2], Grażyna Dobrowolska[1]

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SNF1-related protein kinases subfamily 2 (SnRK2s) are plant specific enzymes that are the key players of responses to environmental stresses, especially osmotic stress caused by drought or salinity and also for abscisic acid (ABA) dependent development. Based on phylogenetic analysis, 10 members of this family have been classified into three groups. The classification correlates with their response to ABA. Kinases from group 1 are not responsive to ABA, kinases form group 2 are slightly or non-responsive whereas, SnRK2s comprising group 3 are strongly activated in response to ABA. To date numerous SnRK2s' targets have been identified and among them DNA- and RNA-binding proteins.

Several data published have shown that the group 3 SnRK2s regulate ABA-dependent gene expression via phosphorylation of transcription factors (mainly AREB-type transcription factors) and additionally are involved in miRNA biogenesis (Yan el al. 2017). Much less in known on the role of the SnRK2s nonregulated by ABA. Soma et al. (2017) have shown that kinases from this group are involved in regulation of gene expression in response to osmotic stress at the level of mRNA decay by phosphorylation of VARICOSE (VCS), an mRNA decapping activator. Comparative phosphoproteomic studies between an Arabidopsis snrk2.10 mutant, wild type, and plants overexpressing SnRK2.10 identified several proteins involved in miRNA biogenesis as potential SnRK2.10 targets (Maszkowska et al. 2019). This indicates that SnRK2.10 and possibly also other ABA-non-responsive SnRK2s might regulate miRNA biogenesis upon salinity stress. To test this hypothesis we performed sequencing of small RNA isolated from roots of the *snrk2* group 1 mutants and wild type Arabidopsis plants exposed to salt stress. The analysis of the NGS results indicate that indeed ABA-non-responsive SnRK2s have a great impact on a number of miRNA expressed both in control conditions and upon salinity stress.

This work was supported by National Science Centre (grant 2016/23/B/NZ3/03182)

Maszkowska et al., *Plant Cell Environ.* **2019**; 42:931–946
Soma et al., *Nature Plants* **2017**; 3:16204–16212
Yan et al., *PLoS Genet* **2017**; 13; 4:e1006753

Added Wednesday in topic area Targeting RNA for Therapy

902 Structure-activity Relationship of Raloxifene Analogs as Potential Hepatitis B Virus Therapeutics, through Targeting of Encapsidation Signal, Epsilon, of Viral Pregenomic RNA

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Despite the advent of an effective vaccine against HBV, 257 million individuals are living infected with chronic Hepatitis B virus (HBV) which results in cirrhosis and hepatocellular carcinoma. Current nucleos(t)ide analog treatments target viral reverse transcriptase, are successful at reducing viral load but do not clear the virus. HBV encapsidation signal, epsilon (HBVε), a cis-acting regulatory RNA element of HBV pregenomic RNA (pgRNA), is involved in pgRNA packaging, as well as signaling for viral polymerase presents a novel method for treatment. Potential disruption of these events by small molecule interaction with HBVε presents an attractive drug target. To start, a library of 26,000 immobilized small molecules were screened against a fluorescently labeled 61nt HBVε, identifying an FDA-approved drug, raloxifene, a selective estrogen receptor modulator (SERM), as warranting further investigation. Biophysical, biochemical, and in silico testing revealed raloxifene interacts with the flexible 6-nt bulge region of HBVε with an IC_{50} of 69 uM. Herein we present the chemical synthesis of a focused library of analogs, their biochemical evaluation using a dye displacement assay and NMR study to provide insight into structure-activity relationship of this class of HBVε small molecule binders. Substitutions at positions 2 and 3 of the benzothiophene core of raloxifene were explored. Analogs with various linker composition at the 3-position were tolerated and improved binding to HBVε was achieved through further manipulation of this side. Changes to the 4-hydroxy group of benzene ring at the 2-position however were less tolerated and is being further investigated. Furthermore, chemical shift perturbation studies along with molecular docking of this class of compounds provided molecular detail of interaction with HBVε, In summary, a class of raloxifene-based small molecules have been developed and fully characterized to target a novel motif of HBV