The Biology of RNA-Protein Complexes

PROGRAM AND ABSTRACTS

October 11th-14th 2017, Thon-Dittmer Palais, Regensburg, Germany

GENERAL INFORMATION

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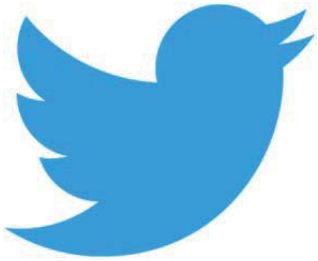

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About the SFB960

Ribonucleoproteins (RNPs) are cellular multicomponent assemblies whose biological functions depend on the interaction between RNA and proteins. The dynamic process of RNP assembly and regulation of RNP function represents the main interest of the Collaborative Research Centre SFB960. The SFB960 is supported by the Deutsche Forschungsgemeinschaft (DFG) since July 2011 establishing a strong local scientific network at the University of Regensburg. It currently unites 13 research and two core/service projects focusing on biogenesis and regulation of RNPs which are all directly or indirectly involved in the synthesis or regulation of ribosomes. Multisubunit complexes interacting with the rRNA synthesizing machineries, with argonaute-containing complexes or with nascent or mature ribosomes are especially interesting. This enables comparative analyses, which will reveal common principles and specific aspects of important RNP-related processes.

To further the knowledge of how ribosomes and other RNPs are synthesized and regulated, the SFB960 organizes international scientific meetings on a regular basis. The symposium 2017 "The biology of RNA-protein complexes" is already the third international conference organized by members of the SFB960, in which leading scientists of the RNP field exchange their thoughts and present their ongoing work in a hopefully stimulating environment.

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SCHEDULE

Wednesday, October 11th

13:00 **Registration starts:** Thon-Dittmer-Palais, 1st floor

Keynote Lectures

15:30 **Welcome Address**

15:45 **Roy Parker:** RNP Granules in Health and Disease

16:15 **Reinhard Lührmann:** Cryo-EM snapshots of the highly dynamic spliceosome

17:00 **Alan Hinnebusch:** A network of molecular interactions restricts stable preinitiation complex assembly to optimal translation start codons *in vivo*

17:45 **Dinner** at Thon-Dittmer-Palais

18:30 **Posters and Drinks –** at the 'großer Dollingersaal'
Even poster numbers

Thursday, October 12th

Session: Transcription

Session chair: Dina Grohmann

09:00	**Welcome Address:** Prof. Dr. Udo Hebel, President of the University of Regensburg
09:10	**Evgeny Nudler:** Structural Principles of Transcription-coupled DNA Repair
09:35	**Stefan Knauer:** Exploring RNA Polymerase Regulation by NMR spectroscopy
09:50	**Albert Weixlbaumer:** Regulation of Transcription Elongation by NusA – biochemical and structural studies
10:05	**David Dulin:** A network of pauses traps the bacterial RNA polymerase and its nascent transcript on the promoter
10:20	**Finn Werner:** Molecular Mechanisms of Transcription in Archaea
10:45	**coffee break**
11:15	**Dylan Taatjes:** Non-coding RNAs, Mediator, and Mediator kinases
11:40	**Steven Hahn:** Fuzzy Free-for-All: Retention of Fuzzy Binding in the Multi-Domain Interactions of Transcription Activator Gcn4 and Mediator Subunit Med15
12:05	**Zoltan Villanyi:** Ccr4-Not is at the core of the gene expression circuitry
12:20	**Christoph Müller:** Structural biology of RNA polymerase I and III transcription
12:45	**Lunch break**

Session: RNA processing

Session chair: Jan Medenbach

14:00 **Julian König:** iCLIP-based modeling uncovers 3' splice site definition: how U2AF65 specificity relies on regulation by co-factors

14:15 **Štěpánka Vaňáčová**: N6-methyladenosine demethylase FTO targets pre-mRNAs and regulates alternative splicing and 3' end processing

14:40 **Michaela Müller-McNicoll:** Cellular differentiation state modulates the mRNA export and retention activities of SR proteins

15:05 **Daniela Lazzaretti:** Insights into specific RNA recognition mediated by human Staufen1 from the crystal structure of its minimal RNA-binding region in complex with a physiological dsRNA target

15:20 **coffee break**

15:50 **Oliver Mühlemann:** Dissecting the functions of SMG5, SMG7 and PNRC2 in nonsense-mediated mRNA decay of human cells

16:15 **Remco Sprangers:** The Rrp4-exosome complex recruits and channels substrate RNA by a unique mechanism

16:30 **Michael Sattler:** Decoding regulatory protein-RNA interactions in gene regulation using integrated structural biology

17:00 **Sponsored Seminar:**
Nicolas Piganeau
Whole Transcriptome Analysis: Choose the right tool for the job

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17:30 **Guided tour of the old town**

starting right outside the Thon-Dittmer-Palais on Haidtplatz

19:00 **Conference Dinner at 'Haus Heuport'**

Friday, October 13th

Session: Ribosome biogenesis and function

Session chair: Sébastien Fereirra-Cerca and Jorge Perez-Fernandez

I – Fundamental aspects of ribosome biogenesis in Eukaryotes

09:00 **Ed Hurt:** Mechanism of ribosome biogenesis and its link to nucleocytoplasmic transport

09:25 **Brigitte Pertschy:** Coordination of distant 40S ribosomal subunit maturation events

09:40 **Susan J. Baserga:** High throughput discovery of novel regulators of human ribosome biogenesis

10:05 **Cameron Mackereth:** A novel dsRNA-binding module from a nuclear prolyl isomerase

II – Ribosome biogenesis and disease

10:20 **Nicholas Watkins:** The 5S RNP provides the link between ribosome biogenesis and the tumour suppressor p53

10:35 **Antje Ostareck-Lederer:** Interaction of hnRNP K and rpS19 and its function in erythroid maturation

10:50 **coffee break**

III – Ribosome function

11:20 **Daniel N Wilson:** Ribosome-targeting antibiotics and mechanisms of bacterial resistance

11:45 **Axel Innis:** Discovery and characterization of ribosome-arresting peptides by High-throughput Inverse Toeprinting

12:00 **Nenad Ban:** Extensions, extra factors and extreme complexity: Ribosomal structures provide insights into eukaryotic translation

12:25 **Kristyna Poncova:** The small ribosomal protein RPS3 and its functions

12:45 **Lunch break**

IV – Translation control and quality control

13:45 **Georg Auburger:** Efficient Prevention of Neurodegenerative Diseases by Depletion of Starvation Response Factor Ataxin-2

14:00 **Nagammal Neelagandan:** Mutant TDP-43 selectively affects translation of specific mRNAs in cultured cortical neurons and motor neuron-like cells

14:15 **Keynote Lecture - Nahum Sonenberg:** Ribosome quality control of premature polyadenylated mRNAs by a unique E3 ubiquitin ligase and RNA-binding protein

15:00 **coffee break**

Session: non-coding RNAs

Session chair: Markus Kretz

15:30 **Leemor Joshua-Tor:** Mad about U: regulating the let7 pre-miRNA

15:55 **Peter Sarnow:** From bench to clinical trial: microRNA 122 as an antiviral target for hepatitis C virus

16:20 **Claus-D. Kuhn:** The role of piRNAs in planarian regeneration

16:35 **Sara K. Eisenbart:** A nickel-regulated small RNA represses expression of multiple major virulence factors in *Helicobacter pylori*

16:50 **Lisa-Katharina Maier:** Self-targeting and gene repression with the CRISPR-Cas system of *Haloferax volcanii*

17:05 **Markus T. Bohnsack:** METTL16 is a N6-methyladenosine (m6A) methyltransferase that targets pre-mRNAs, lncRNAs and the U6 snRNA

17:20 **Sven Diederichs:** lncRNA & Ribonucleoproteins in Cancer

17:45 **Utz Fischer:** Mechanistic dissection of UsnRNP biogenesis and its role in disease

18:10 **Dinner** at Thon-Dittmer-Palais

18:45 **Posters and Drinks –** at the ‘großer Dollingersaal’
Odd poster numbers

Saturday, October 14th

Session: RNA systems biology

Session chair: Andrea Bleckmann

09:00 **Vicent Pelechano:** Genome-wide study of transcription complexity and ribosome dynamics

09:25 **Jörg Vogel:** Grad-seq discovers the third domain of small RNA-mediated gene regulation in bacteria

09:50 **Nicholas Jaé:** Functional characterization of hypoxia-regulated lncRNAs in endothelial cells

10:15 **Kathi Zarnack:** High-throughput screening for splicing regulatory elements

10:30 **coffee break**

11:00 **Award Ceremony: Poster prizes** Gunter Meister

Sponsored by the Federation of European Biochemical Societies (FEBS), the SFB960, and the Faculty of Biology and Pre-Clinical Medicine of the University of Regensburg

Session: RNA systems biology (continued)

11:15 **Jernej Ule:** Regulation of gene expression by proteins bound to LINE-derived RNA elements

11:40 **Dorothee Staiger:** iCLIP determines the target repertoire and binding landscape of the clock-regulated RNA-binding protein AtGRP7

11:55 **Cynthia Sharma:** Functional characterization of the CRISPR-Cas9 system of the foodborne-pathogen *Campylobacter jejuni*

12:20 **Closing remarks**

ORAL ABSTRACTS

O1

RNP Granules in Health and Disease

Anthony Khong[1], Briana Van Treeck[1], Tyler Matheny[1], Bhalchandra Rao[1], David S.W. Protter[1], Yuan Lin[2], Saumya Jain[1], Joshua R. Wheeler[1], Michael Rosen[2], and **Roy Parker**[1]

[1]University of Colorado and HHMI, Boulder, CO, USA, [2]Department of Biophysics, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA

Stress granules are non-membranous mRNP assemblies that form when translation is limiting and are related to pathological aggregates observed in neurodegenerative diseases. Analysis of stress granules from yeast and mammalian cells identifies a diverse proteome within stress granules including many ATP-dependent remodeling complexes. RNA-Seq also reveals a diverse transcriptome within stress granules. Genetic experiments demonstrate that numerous remodeling complexes affect both the assembly and disassembly of eukaryotic stress granules. Such remodeling complexes may also play a role in the assembly and disassembly of RNA-protein aggregates that form in some human diseases.

Using RNP granules as an example, we provide evidence for an assembly mechanism of large cellular structures wherein specific protein-protein, RNA-RNA, or protein-RNA interactions create an assembly, which is stabilized by promiscuous interactions of intrinsically disordered regions (IDRs) of assembled components. Such IDRs may remain in a dynamic weak interaction and/or transition to more stable amyloid-like assemblies in the context of long lived assemblies. This synergistic assembly mechanism illuminates RNP granule assembly, and explains why many components of RNP granules, and other large dynamic assemblies, contain IDRs linked to specific protein-protein or protein-RNA interaction modules. We suggest assemblies based on combinations of specific interactions and promiscuous IDRs are common features of eukaryotic cells.

O2

Cryo-EM snapshots of the highly dynamic spliceosome

Reinhard Lührmann

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The spliceosome catalyses the removal of the intron from nuclear pre-mRNAs and assembles initially into a pre-catalytic ensemble, termed complex B, which contains the snRNPs U1, U2 and the U4/U6.U5 tri-snRNP and numerous non-snRNP proteins. For catalytic activation the spliceosome undergoes a major structural rearrangement, mediated by the Brr2 RNA helicase, yielding the activated spliceosome (B^{act} complex). The final catalytic activation of the spliceosome requires an additional restructuring step by the RNA helicase Prp2, generating the B* complex which catalyses the first step of the splicing reaction, yielding the C complex. Subsequently the catalytic center of the spliceosome has to be remodeled by the RNA helicase Prp16 to generate the C* complex as a pre-requisite for second step catalysis.

Using cryo electron microscopy we have investigated the 3D structure of the human U4/U6.U5 tri-snRNP complex and several purified spliceosomal complexes (in collaboration with Holger Stark; MPIbpc, Göttingen). Our tri-snRNP model reveals how the spatial organization of Brr2 RNA helicase prevents premature U4/U6 RNA unwinding in isolated human tri-snRNPs and how the Sad1 protein likely tethers Brr2 to its pre-activation position, far away from its U4/U6 RNA substrate. The Cryo-EM structure of the human B complex reveals that most tri-snRNP proteins undergo significant structural rearrangements during integration of the tri-snRNP into the B complex. These include formation of a partially-closed Prp8 conformation that creates, together with Dim1, a 5’ splice site binding pocket, displacement of Sad1, and rearrangement of Brr2 such that it now contacts its U4/U6 substrate and is poised for the subsequent spliceosome activation step. The molecular architecture of several B-specific protein suggests they are involved in negatively regulating Brr2, positioning the U6/5’ss helix and/or stabilizing the B complex structure. Our data also indicate substantial differences in the early activation pathways between human and yeast spliceosomes. The Cryo-EM structure of the purified S. cerevisiae Bact complex reveals how the first step reactants (i.e. the 5'splice site and the branch site adenosine) are sequestered by protein prior to catalysis and provide insights into the molecular remodeling events that must be facilitated by Prp2 in order to generate a catalytically active B* spliceosome. Finally, I will also present the Cryo-EM 3D structure of a human C* complex, which has undergone the Prp16-mediated remodeling step. Our combined studies show that the spliceosome is an extremely dynamic molecular machine which undergoes dramatic large-scale structural changes during one round of pre-mRNA splicing.

O3

A network of molecular interactions restricts stable preinitiation complex assembly to optimal translation start codons in vivo

Anil Thakur[1], Jinsheng Dong[1], Colin Aitken[2], Pilar Martin Marcos[3], Fujun Zhou[2], Charm Karunasiri[2], Jagpreet Nanda[2], Laura Marler[1], Jyothsna Visweswaraiah[1], Tanweer Hussain[4], Jose L. Llácer[4], Michael Zeschnigk[5], Mercedes Tamame[3], Jon R. Lorsch[2], V. Ramakrishnan[4], and **Alan G. Hinnebusch**[1]

[1]Laboratory of Gene Regulation and Development, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892; [2]Laboratory on the Mechanism and Regulation of Protein Synthesis, Eunice K. Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892, USA; [3]Instituto de Biología Funcional y Genómica (IBFG) CSIC/Universidad de Salamanca, Salamanca, Spain; [4]MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK; [5]Institute of Human Genetics & Eye Cancer Research Group, Faculty of Medicine, University Duisburg-Essen, Essen, Germany

In the scanning mechanism of translation initiation, a preinitiation complex (PIC) comprised of the 40S ribosomal subunit, initiation factors 1, 1A and 3, and a ternary complex (TC) of eIF2-GTP and initiator $tRNA_i$ attaches to mRNA and scans the leader for an AUG codon in optimal context. eIF1 stimulates recruitment of TC with $tRNA_i$ bound in a metastable state (P_{OUT}) suitable for scanning, and opposes transition to a more stable P_{IN} state at the start codon, thus necessitating eIF1 release on AUG recognition. Structures of reconstituted PICs revealed an open conformation of the 40S subunit at a near-cognate AUC codon, and a more closed configuration at AUG. In the closed state, eIF1 shifts its position on the 40S and its β-hairpin loops are distorted to avoid a clash with $tRNA_i$. Substitutions in eIF1 loop-2 that remove ionic repulsion or create attraction with $tRNA_i$ increase initiation at both UUG codons (Sui^- phenotype) and AUGs in poor context in vivo, and stabilize TC binding at UUG start codons in reconstituted PICs in vitro. Thus, the loop-2/$tRNA_i$ clash destabilizes the P_{IN} state to disfavor suboptimal start codons. eIF2β contacts eIF1 and $tRNA_i$ exclusively in the open complex, and substitutions at the eIF2β/eIF1 interface likewise confer Sui^- phenotypes, indicating that eIF1:eIF2β interactions also impede rearrangement to P_{IN} at suboptimal start sites. In contrast, the unstructured N-terminal tail (NTT) of eIF1A contacts the codon:anticodon helix only in the closed/P_{IN} state, and substituting its conserved basic residues suppresses initiation at UUG codons (Ssu^- phenotype) and destabilizes TC binding at UUG codons in vitro. Ribosome profiling of the NTT substitution eIF1A-R13P reveals decreased initiation at AUGs in poor context genome-wide, implicating the NTT in selecting suboptimal start codons. Similar genetic/biochemical findings identify roles for conserved residues in ribosomal proteins at the mRNA exit (uS7) and entry (uS3) channels in stabilizing the closed/P_{IN} conformation via contacts with eIF2α (uS7) or mRNA (uS3), to enable recognition of suboptimal start sites. The eIF3 is a central player in PIC recruitment to mRNA, and we used model mRNAs lacking contacts with the 40S entry or exit channels in reconstituted PICs to identify a critical role for eIF3a in stabilizing PIC:mRNA interactions at the exit channel, and an ancillary role at the entry channel that is functionally redundant with uS3 residues that contact mRNA. We propose that eIF3 and 40S proteins collaborate to fix the mRNA in the exit and entry channels of the ribosome and thereby stabilize the closed/P_{IN} conformation at the start codon. Thus, multiple interactions within the PIC serve to stabilize the open or closed states and set the proper stringency level for initiation at non-optimum start codons in vivo.

O4

Structural Principles of Transcription-coupled DNA Repair

Venu Kamarthapu[1], Fangfang Zheng[2], Liqiang Shen[2], Britney Martinez[1], Vitaly Epshtein[1], Yu Zhang[2], and **Evgeny Nudler**[1]

[1] Department of Biochemistry and Molecular Pharmacology, Howard Hughes Medical Institute, New York University School of Medicine, New York, New York 10016, USA [2]Key Laboratory of Synthetic Biology, Chinese Academy of Sciences Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, 200032, China

Nucleotide excision repair (NER) is an evolutionarily conserved, multistep process that can detect a wide variety of DNA lesions. RNA polymerase (RNAP) stalled at DNA lesions mediates the recruitment of NER enzymes to the damage site thereby expediting the recognition of DNA damage by NER components – the process known as transcription-coupled DNA repair (TCR). Recently, we have described a new pathway for TCR in E. coli, where UvrD plays the central role by pulling stalled RNAP backwards, away from the site of DNA damage, thereby allowing repair enzymes to gain access to DNA lesions. Transcription elongation factor NusA and small molecular alarmone ppGpp play important role in this process. Here we present a structural model for the recruitment of UvrD by E. coli RNAP to enable TCR. We map and functionally validate RNAP-UvrD interactions by a combination of X-ray crystallography, crosslinking-coupled mass spectrometry (XL-MS), fast photo-oxidation of proteins (FPOP-MS), and genetic analysis. We identify the extensive interacting surface between UvrD and RNAP and propose the model of UvrD-mediated RNAP backtracking based on the positioning of UvrD with respect to the DNA scaffold in the elongation complex. We further identify the "β pincer" module of EcRNAP and the C-terminal domain of UvrD (EcUvrD-CTD) as one of the main contacting points between the two enzymes. Disrupting those interactions by mutations in RNAP or UvrD severely compromises TCR in vivo.

O5

Exploring RNA Polymerase Regulation by NMR spectroscopy

Stefan H. Knauer, Philipp Zuber, Benjamin Dudenhöffer, Birgitta M. Wöhrl, Kristian Schweimer, and Paul Rösch

Lehrstuhl Biopolymere und Forschungszentrum für Bio-Makromoleküle, Universität Bayreuth, Bayreuth, Germany

Multisubunit RNA polymerases (RNAPs) transcribe all cellular genomes and are highly regulated by numerous transcription factors. Only little information about dynamics, conformational rearrangements, and transient interactions of RNAPs is available, which, however, is crucial for a comprehensive understanding of transcription regulation in atomic detail.

Here we present approaches to study Escherichia coli RNAP (400 kDa) by NMR spectroscopy in solution to gain insights into its dynamics and interaction with transcription factors. First, we developed an efficient procedure to assemble active RNAP from separately produced subunits, allowing the specific labeling of individual constituents [1]. Using [1H,13C]-labeled Ile, Leu, and Val methyl groups in deuterated proteins as probes (methyl group labeling) we recorded [C,H] correlation spectra of complete RNAP and of methyl group labeled β' subunit within reconstituted, deuterated RNAP, setting the basis for the study of transcription factor/nucleic acid binding [1]. Further, we developed approaches to determine the RNAP binding surfaces of transcription factors and to follow conformational changes within those factors upon RNAP binding using [1H,13C]-labeled methyl groups of Val, Leu, and Ile in deuterated transcription factors as probes [2]. This allowed us, for example, to determine the RNAP binding surface of the N-terminal domain (NTD) of N-utilization substance (Nus) A and thus to create a model of the NusA-NTD:RNAP complex. Finally, we used these approaches to explore the recruitment mechanism of the transformer protein RfaH to the paused transcription elongation complex (TEC) by directly following RfaH binding to the TEC and by directly observing the structural transformation of its C-terminal domain.

References

[1] J. Drögemüller, M. Strauß, K. Schweimer, B.M. Wöhrl, S.H. Knauer, and P. Rösch, Sci Rep. 5, 10825 (2015)
[2] J. Drögemüller, M. Strauß, K. Schweimer, M. Jurk, P. Rösch, and S.H. Knauer, Sci Rep. 5, 16428 (2015)

O6

Regulation of Transcription Elongation by NusA – biochemical and structural studies

Xieyang Guo, Alexander Myasnikov, James Chen, Gabor Papai, Maria Takacs, **Albert Weixlbaumer**

IGBMC - Institute of Genetics and Molecular and Cellular Biology Department of Integrated Structural Biology Illkirch, France

During the first step of gene expression, a universally conserved enzyme called RNA Polymerase (RNAP) transcribes RNA from a DNA template in every living cell. Transcription is a fundamental, yet incompletely understood process. Crystal structures of RNAP core as well as RNAP elongation complexes (ECs) have revolutionized our understanding of transcription. This groundwork paved the way to address more complex questions regarding the regulation of transcription and the influence of transcription factors.

Transcriptional regulation impacts every aspect of biology including a growing list of human diseases. One way to regulate transcription is through a process called transcriptional pausing, which plays a major role in pro- and eukaryotes. Pausing is a temporary interruption of RNA extension during the elongation phase. Importantly, pausing can be further modulated by transcription factors.

We will report on our progress using biochemical and structural approaches to gain mechanistic insights on the regulation of transcription by a conserved bacterial transcription factor called NusA. NusA is involved in stabilizing paused states of RNAP and also has a stimulatory role in transcription termination. We determined single particle cryo-EM reconstructions of functional elongation complexes of E. coli RNAP and NusA and will describe the implications for pausing and transcription termination.

O7

A network of pauses traps the bacterial RNA polymerase and its nascent transcript on the promoter

David Dulin, Anssi M. Malinen, David L. V. Bauer, Jacob J. W. Bakermans, Martin Kaller, Zakia Morichaud, Ivan Petushkov, Konstantin Brodolin, Andrey Kulbachinskiy and Achillefs N. Kapanidis

FAU Erlangen-Nürnberg, Oxford University

Transcription in bacteria is controlled by multiple molecular mechanisms that ensure precise regulation of gene expression. Initial RNA synthesis by promoter-bound RNAP holoenzyme was recently identified to be interrupted by pauses, which determinants and relationship with productive RNA synthesis and abortive initiation remain poorly understood. Here, we employed single-molecule FRET to disentangle the pausing-related reaction pathways of initial transcription. We present further evidences that region σ3.2, constitutes a barrier at 6 nt RNA (ITC6), halting the RNAP. The ITC6 pause is a physiological sensor that directs the holoenzyme either towards productive transcription or towards a long-lived unscrunching–scrunching pathway that does not necessarily lead to transcript release (and instead is associated with blocking of transcription initiation). This demonstrates that abortive RNA release and the loss of scrunched DNA conformation are not as tightly coupled as previously thought. The above molecular mechanism renders the productivity of initial transcription sharply dependent and potentially regulated by NTP concentration.

O8

Molecular Mechanisms of Transcription in Archaea

Finn Werner

UCL ISMB

We are following a multidisciplinary approach to study the molecular mechanisms of transcription using both eury- and crenarchaeal model systems, ranging from the molecular- to the systems level. I will present recent insights into the mechanisms underlying transcription processivity, and the way by which virus infection-induced transcription factors appropriate the host transcription apparatus.

O9

Non-coding RNAs, Mediator, and Mediator kinases

Jonathan D. Rubin, Charli B. Fant, Meagan Esbin, Zachary Poss, William Old, Robin D. Dowell, **Dylan J. Taatjes**

University of Colorado, Boulder

The human Mediator complex regulates RNA polymerase II (pol II) activity genome-wide, and has been implicated in binding of functionally relevant non-coding RNAs. We have begun to explore how non-coding RNAs might interact with and/or regulate Mediator function. Our results suggest Mediator is a promiscuous binder of RNAs, analogous to the PRC2 complex. Mediator controls pol II function in part by relaying regulatory signals from DNA-binding transcription factors (TFs) to the pol II enzyme. Conversely, Mediator can regulate TF function through the Mediator-associated kinases CDK8 and CDK19, as DNA-binding TFs are common substrates for Mediator kinases. Using a combination of approaches, including SILAC-based phosphoproteomics, GRO-Seq, and *in vitro* transcription assays, we have begun to uncover basic mechanisms by which the human CDK8 module, and CDK8 kinase activity *per se*, regulates pol II transcription at various stages. The CDK8 module appears to function cooperatively with pol II elongation factors and its kinase activity affects enhancer RNA (eRNA) transcription directed by stimulus-specific TFs. Taken together, these results establish new mechanistic roles for Mediator in transcription regulation and provide a more comprehensive understanding of Mediator kinase function.

O10

Fuzzy Free-for-All: Retention of Fuzzy Binding in the Multi-Domain Interactions of Transcription Activator Gcn4 and Mediator Subunit Med15

Lisa M. Tuttle[1,2], Derek Pacheco[1], Linda Warfield[1], Jie Luo[3], Jeff Ranish[3], Rachel E. Klevit[2] and **Steven Hahn**[1]

[1]Fred Hutchinson Cancer Research Center, Seattle, WA, USA [2]Department of Biochemistry, University of Washington, Seattle, WA, USA, [3]Institute for Systems Biology, Seattle, WA, USA

Transcriptional regulation is often mediated by interactions between intrinsically disordered activation domains (ADs) and transcription coactivators. These interactions often involve multiple activation regions and multiple binding sites as is the case for the tandem ADs of yeast transcription factor Gcn4 and the four activator binding domains (ABDs) of Med15, a subunit of the Mediator transcription coactivator complex. While interaction of multiple ADs and ABDs appears to be a common theme in transcription activation, the mechanism of how these multi domain interactions contribute to the affinity and specificity of AD function was not known.

To address these questions, we used a combination of protein crosslinking, transcription activity assays, biochemistry, and NMR structural analysis to characterize the interactions between the tandem ADs of Gcn4 and the four activator binding domains of Med15, individually and as part of the larger Gcn4-Med15 complex. Our data support a model where, instead of a specific higher order structure being formed, there is a fuzzy free-for-all involving the hydrophobic patches of the tandem ADs with the four structured domains in the activator-binding region of Med15. Our results provide a general model for the mechanism of many activators and demonstrate how multiple weak fuzzy interactions can combine to generate a biologically significant and specific interaction.

O11

Ccr4-Not is at the core of the gene expression circuitry

Olesya Panasenko[1], **Zoltan Villanyi**[1], Ishaan Gupta[2], Christopher Hughes[3], Lars M. Steinmetz[2,4], Peña Chou Cohue[5], Syam Somasekharan[5], Vikram Panse[6], Martine A. Collart[1]

[1] Department of Microbiology and Molecular Medicine, University of Geneva, Faculty of Medicine, [2] European Molecular Biology Laboratory [3] British Columbia Cancer Research Agency [4] Stanford University [5] ETH Zurich [6] Vancouver Prostate Centre

The Ccr4-Not complex is a conserved multi-protein complex that regulates gene expression at all stages, from production of the mRNA in the nucleus to its degradation in the cytoplasm. Challenging the general understanding of gene expression that considers transcription and translation to be independent processes, we recently demonstrated that translation efficiency is determined during transcription elongation through imprinting of ribosomal protein mRNAs with Not1, the central scaffold of Ccr4-Not. We also determined that Not5-dependent Not1 association with specific mRNAs was important during translation for assembly of protein complexes such as RNA polymerase II and the SAGA histone acetyltransferase. This regulation of transcription during translation and translation during transcription places the Ccr4-Not complex at the core of the gene expression circuitry. More recently we have focused on understanding the mechanisms by which co-translational assembly of protein complexes is regulated and involves Not1. Our results indicate the existence of Not1-containing assemblysomes.

O12

Structural biology of RNA polymerase I and III transcription

Christoph W. Müller

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RNA polymerase I (Pol I) and RNA polymerase III (Pol III) mainly synthesize non-coding RNA components required for ribosome assembly and protein synthesis in eukaryotes. Pol I synthesizes precursor ribosomal RNA, whereas Pol III produces small RNAs including tRNAs, 5S RNA and U6 snRNA. Pol I and Pol III transcription is carefully regulated in healthy cells, while misregulation of Pol I and Pol III transcription has been observed in a variety of cancers.

During the last years, we determined molecular structures of 14-subunit Pol I and 17-subunit Pol III unbound and in complex with different transcription scaffolds by X-ray crystallography and cryo-EM that revealed the dynamics of both transcription machineries as well as their specific adaptions allowing them to fulfill their distinct transcription tasks. The Pol I and Pol III structures also served as starting points for assembling the Pol I and Pol III pre-initiation complexes (PIC) followed by their cryo-EM analysis. In yeast, the minimal Pol I PIC includes Pol I, the transcription factor Rrn3 and Core Factor (CF) composed of subunits Rrn6, Rrn7 and Rrn11, while Pol III minimally requires three-subunit transcription factor TFIIIB with subunits TPB, Brf1 (B-related factor 1) and Bdp1.

The Pol I PIC structure reveals an unusual architecture where TFIIB-related factor Rrn7 binds promoter DNA considerably more upstream compared to TFIIB and Brf1 in the Pol II and Pol III PICs, respectively. In the Pol III PIC, TFIIIB participates in an intricate interaction network with the Pol III-specific heterotrimer that traps promoter DNA above the Pol III cleft and leads to DNA melting. These and other differences and similarities between the Pol I, Pol II and Pol III PICs will be discussed.

O13

iCLIP-based modeling uncovers 3’ splice site definition: how U2AF65 specificity relies on regulation by co-factors

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Alternative splicing generates distinct mRNA isoforms and is crucial for proteome diversity in eukaryotes. The RNA-binding protein (RBP) U2AF65 is central to splicing decisions, as it recognizes 3' splice sites and recruits the spliceosome. We establish '*in vitro* iCLIP' experiments, in which recombinant RBPs are incubated with long transcripts, to study how U2AF65 recognizes RNA sequences and how this is modulated by *trans*-acting RBPs. We measure U2AF65 affinities at hundreds of binding sites, and compare *in vitro* and *in vivo* binding landscapes by mathematical modeling. We find that *trans*-acting RBPs extensively regulate U2AF65 binding *in vivo,* including enhanced recruitment to 3' splice sites and clearance of introns. Using machine learning, we identify and experimentally validate novel *trans*-acting RBPs (including FUBP1, BRUNOL6 and PCBP1) that modulate U2AF65 binding and affect splicing outcomes. Our study offers a blueprint for the high-throughput characterization of *in vitro* mRNP assembly and *in vivo* splicing regulation.

O14

N6-methyladenosine demethylase FTO targets pre-mRNAs and regulates alternative splicing and 3' end processing

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N6-methyladenosine (m^6A) is the most abundant base modification found in messenger RNAs (mRNAs). The reversibility and dynamics of m^6A in mRNAs has been linked to a number of regulatory steps in the mRNA metabolism. The major focus of many works was on the role of the methylase complexes and the mechanisms and function of demethylation remaines rather unexplored. There are at least two mRNA m6A demethylases in mammals, ALKBH5 and FTO. We have performed comprehensive transcriptome-wide analyses of FTO targets in human cells. Our results reveal FTO as a potent regulator of nuclear mRNA processing events such as alternative splicing and 3'end mRNA processing. Using cross-linking and immunoprecipitation coupled to high-throughput sequencing (CLIP-seq) we reveal that FTO binds preferentially to pre-mRNAs in intronic regions, in the proximity of alternatively spliced (AS) exons and poly(A) sites. Depletion of FTO by gene knockout results in substantial changes in pre-mRNA splicing with prevalence of exon skipping events. FTO binding was highly enriched in the skipped exons, indicating its direct role in AS regulation. The AS phenotype could be reproduced from a heterologous reporter system and rescued with episomal expression of WT, but not mutant FTO. Deletions of intronic region containing m^6A-linked DRACH motif resulted in partial diminution of FTO KO AS phenotype. In summary, these experiments demonstrated, that the splicing effects are dependent on the FTO catalytic activity *in vivo* and are mediated by m^6A. Our results reveal for the first time the dynamic connection between FTO RNA binding and demethylation activity that influences several mRNA processing events.

O15

Cellular differentiation state modulates the mRNA export and retention activities of SR proteins

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SR proteins function in nuclear pre-mRNA processing, mRNA export and translation. To investigate their cellular dynamics, we developed a quantitative assay, which detects differences in nucleo-cytoplasmic shuttling among seven canonical SR protein family members. As expected, SRSF2 and SRSF5 shuttle poorly in HeLa cells but surprisingly display considerable shuttling in pluripotent murine P19 cells. Combining iCLIP and mass spectrometry, we show that elevated arginine methylation and lower phosphorylation of co-bound SRSF2 enhance shuttling of SRSF5 in P19 cells by modulating protein-protein and protein-RNA interactions. Moreover, SRSF5 is bound to pluripotency-specific transcripts such as Lin28a and Pou5f1/Oct4 in the cytoplasm. SRSF5 depletion reduces and overexpression increases their cytoplasmic mRNA levels, suggesting that enhanced mRNA export by SRSF5 is required for the expression of pluripotency factors. Remarkably, neural differentiation of P19 cells leads to dramatically reduced SRSF5 shuttling. Our findings indicate that post-translational modification of SR proteins underlies the regulation of their mRNA export activities and distinguishes pluripotent from differentiated cells.

O16

Insights into specific RNA recognition mediated by human Staufen1 from the crystal structure of its minimal RNA-binding region in complex with a physiological dsRNA target

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Members of the Staufen (Stau) protein family have conserved functions at several steps of post-transcriptional gene regulation. In *Drosophila*, Stau is involved in the intracellular localization of mRNAs required for anterior-posterior axis determination during oogenesis, neuroblast asymmetric cell division in the developing brain, and long term memory (LTM) formation in the adult brain. Mammals express two Stau paralogs, which mediate mRNA transport in neurons, and function in neuronal morphogenesis and plasticity. In addition, mammalian Stau proteins are involved in a form of translation-dependent mRNA degradation known as Stau-mediated decay (SMD). All these functions require Stau to target specific mRNAs.

Stau family members contain several copies of the dsRNA-binding domain (dsRBD), although only two of these (dsRBDs 3 and 4) are required for RNA binding. dsRBDs primarily recognize RNA secondary structures in a sequence-independent manner, raising the question of how Stau proteins are able to recognize their targets.

Here we present the crystal structure of human Stau1 (hStau1) minimal RNA-binding region in complex with a double stranded RNA (dsRNA) from a physiological target. The structure shows how hStau1 dsRBDs sense dsRNA structure through contacts with the sugar-phosphate backbone, and reveals positions at which base-specific contacts are formed to achieve target specificity. RNA-dependent dimerization of hStau1, as well as dsRBD flexibility, could further modulate substrate recognition.

O17

Dissecting the functions of SMG5, SMG7 and PNRC2 in nonsense-mediated mRNA decay of human cells

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The term “nonsense-mediated mRNA decay” (NMD) originally described the degradation of mRNAs with premature translation-termination codons (PTCs), but its meaning has recently been extended to be a translation-dependent post-transcriptional regulator of gene expression affecting 3-10 % of all mRNAs. The degradation of NMD target mRNAs involves both exonucleolytic and endonucleolytic pathways in mammalian cells. While the latter is mediated by the endonuclease SMG6, the former pathway has been reported to require a complex of SMG5-SMG7 or SMG5-PNRC2 binding to UPF1. However, the existence, dominance and mechanistic details of these exonucleolytic pathways are divisive. Therefore, we have investigated the possible exonucleolytic modes of mRNA decay in NMD by examining the roles of UPF1, SMG5, SMG7 and PNRC2 using a combination of functional assays and interaction mapping. Confirming previous work, we detected an interaction between SMG5 and SMG7 and also a functional need for this complex in NMD. In contrast, we found no evidence for the existence of a physical or functional interaction between SMG5 and PNRC2. Instead, we show that UPF1 interacts with PNRC2 and that it triggers 5’-3’ exonucleolytic decay of reporter transcripts in tethering assays. PNRC2 interacts mainly with decapping factors and its knockdown does not affect the RNA levels of NMD reporters, indicating that PNRC2 is an important mRNA decapping factor but it is not required for NMD.

O18

The Rrp4-exosome complex recruits and channels substrate RNA by a unique mechanism

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The exosome is a large molecular machine involved in RNA degradation and processing. Here we address how the trimeric Rrp4 cap enhances the activity of the archaeal enzyme complex. Using methyl-TROSY NMR methods we identified a 50-Å long RNA binding path on each Rrp4 protomer. We show that the Rrp4 cap can thus simultaneously recruit three substrates, one of which is degraded in the core while the others are positioned for subsequent degradation rounds. The local interaction energy between the substrate and the Rrp4-exosome increases from the periphery of the complex toward the active sites. Notably, the intrinsic interaction strength between the cap and the substrate is weakened as soon as substrates enter the catalytic barrel, which provides a means to reduce friction during substrate movements toward the active sites. Our data thus reveal a sophisticated exosome-substrate interaction mechanism that enables efficient RNA degradation.

O19

Decoding regulatory protein-RNA interactions in gene regulation using integrated structural biology

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RNA plays essential roles in virtually all aspects of gene regulation, which involve the recognition of cis regulatory RNA sequences by RNA binding proteins (RBPs). Most eukaryotic RBPs are multi-domain proteins that comprise multiple structural domains to mediate protein-RNA or protein-protein interactions. We employ integrated structural biology to unravel the molecular mechanisms involving these regulatory RNP (ribonucleoprotein) complexes. For these studies, solution NMR-spectroscopy and SAXS/SANS provide unique information on functionally important dynamics and are combined with X-ray crystallography and electron microscopy to elucidate the structural mechanisms and dynamics of regulatory RNPs. Examples will be presented that highlight the role of conformational dynamics in RNA recognition and protein-protein interactions in the regulation of eukaryotic pre-mRNA splicing, translation and RNA stability.

O20

Mechanism of ribosome biogenesis and its link to nucleocytoplasmic transport

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Ribosomes consisting of ribosomal RNA and ribosomal proteins are the machines that synthesize the proteins of the cell. In eukaryotes, the two ribosomal subunits (60S and 40S subunit) are first assembled in the nucleolus before export to the cytoplasm. Ribosome biogenesis is not only complicated but the most energy consuming process in growing cells, and thus requires extensive regulation and coordination. Eukaryotic ribosome synthesis is initiated by transcription of a large rRNA precursor, which is subsequently modified and processed to 25S, 5.8S and 18S rRNA with a concomitant assembly of the ribosomal proteins. At the beginning of ribosome synthesis, a huge (90S) precursor particle is formed that is subsequently split to induce the formation of the pre-60S and pre-40S particles, which each follow separate biogenesis and export routes. During ribosome synthesis about 200 non-ribosomal factors and 100 small non-coding RNAs (snoRNAs) transiently work on the evolving ribosomal subunits to facilitate their assembly, maturation and transport. We study ribosome formation *in vivo* in the yeast *Saccharomyces cerevisiae* and exploit a eukaryotic thermophile, *Chaetomium thermophilum*, for structural studies. In my talk I will summarize our recent findings on the mechanism of ribosome biogenesis and its link to nucleocytoplasmic transport, which were obtained from *in vitro* assays combined with genetic investigations and structural studies including electron microscopy and x-ray crystallography.

O21

Coordination of distant 40S ribosomal subunit maturation events

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The synthesis of eukaryotic ribosomes is an intricate process involving a complex maturation cascade with a multitude of intermediates which travel from the site of initial assembly of ribosomal proteins with ribosomal RNA in the nucleolus through the nucleoplasm and are then exported into the cytoplasm. On that path, they are transiently bound by more than 200 ribosome assembly factors which participate in various aspects of their maturation, including rRNA processing, assembly of ribosomal proteins, restructuring events and release of assembly factors.

We are studying the maturation of freshly exported precursor particles of the small 40S ribosomal subunit, which still contain ~10 ribosome assembly factors bound at different important functional sites of the 40S. Among them is Rio2, an ATPase positioned on the intersubunit side of the 40S subunit in the so called "head region". Rio2 hydrolyses ATP and is believed to thereby trigger structural re-arrangements. Additionally, ATP hydrolysis results in Rio2 dissociation from pre-40S particles. Another assembly factor, Ltv1 is also positioned in the 40S head, albeit distantly from Rio2 on the solvent exposed side. Its release involves phosphorylation by a protein kinase Hrr25 and allows for the stable incorporation of ribosomal protein Rps3 into 40S subunits. Intriguingly, despite the distant positioning of Rio2 and Ltv1 on opposing sides of the 40S head, we show that their release is mutually linked. ATP hydrolysis by Rio2 is not only a pre-requisite for Rio2 dissociation, but is also required for Ltv1 release. Vice versa, Ltv1 phosphorylation triggers its liberation from pre-40S particles, but is also necessary for Rio2 release. These results raise the question how such tight communication between physically distant maturation events is possible. Notably, a ribosomal protein, Rps20, is positioned on the solvent side of the 40S subunit, where it interacts with Ltv1 and Rps3. Additionally Rps20 has an extension diving deep into the 40S subunit and reaching almost through the 40S head, thereby making contacts with 18S rRNA helix 31, which was previously shown to be also bound by Rio2. In this study, we provide evidence that Rps20 is involved in Rio2 and Ltv1 release events, and might thereby coordinate these spatially distant steps in pre-40S maturation.

O22

High throughput discovery of novel regulators of human ribosome biogenesis

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It is well known that in cancer an increased size and number of nucleoli correlates with increased malignant potential. Nucleoli make ribosomes, the essential protein synthesizers of the cell. Previously we have shown (PLoS Genetics 2012) that disruption of nucleolar function in mammalian cells by depletion of the ribosome biogenesis factors UTP4 or NOL11 causes a change in the number of nucleoli per cell from 2-3 to only 1. Taking advantage of this relationship between number and function, we exploited this assay to discover new regulators of nucleolar function in human cells via a high-content, high-throughput, genome-wide siRNA screen. This screen successfully identified approximately 200 proteins whose depletion caused a change in the number of nucleoli per cell from 2-3 to only 1. The function of a subset of these hits in ribosome biogenesis has been validated by oligonucleotide deconvolution. Of these high-confidence, validated hits, 20 were chosen for further study, including both nucleolar and non-nucleolar proteins. While the nucleolar proteins are directly involved in ribosome biogenesis, the non-nucleolar proteins likely reflect new pathways of nucleolar regulation in mammalian cells. Out of the 20 tested hits, we have identified new roles for 17 hits in regulating either tRNA polymerase I transcription of the ribosomal DNA (7/20), the processing of the pre-ribosomal RNA (16/22) and/or global protein synthesis (14/20). This genome-wide analysis exploits the relationship between nucleolar number and function to discover unexpected and diverse cellular pathways that regulate the making of ribosomes and paves the way for further exploration of the links between ribosome biogenesis and human disease.

Supported by NIH R01GM0115710 and by the Yale Cancer Center.

O23

A novel dsRNA-binding module from a nuclear prolyl isomerase

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Prolyl isomerases are defined by a catalytic domain that facilitates the cis-trans interconversion of proline residues. In most cases, additional domains are also present in these enzymes that add important biological functions. While several isomerases have been located in the nucleus, very little is known about the range of native substrates and the cellular function of nuclear FK506-binding proteins (FKBPs). In order to gain insight into the biological role of the nuclear prolyl isomerase FKBP25, we have performed proteomic assays to identify partners of FKBP25 and have identified that these interactions occur via an RNA-dependent mechanism based on a previously unidentified dsRNA-binding domain. The proximity dependent biotin identification (BioID) approach provides an historical snapshot of neighboring protein interactions to FKBP25 fused to the E. coli biotin ligase BirA. Biotinylated proteins in the FKBP25-BirA sample belong to RNA-binding proteins and ribosome biogenesis factors, including 22 ribosomal proteins and several proteins involved in the early maturation events of ribosome production. These FKBP25-associated proteins were further validated by using a complementary co-immunopurification assay. Strikingly, the addition of RNase A in a follow-up assay strongly reduced the interacting partners of FKBP25, implicating a role for RNA in mediating the contacts. Furthermore, we find that RNA is necessary for the localization and recruitment of FKBP25 to nucleolar sites of ribosome biogenesis, with FKBP25 co-localizing with the rRNA transcription factor UBF in an RNA-dependent manner. We therefore predicted a direct interaction with RNA to explain these findings, and a series of in vitro experiments were used to elucidate the nature of the RNA association by FKBP25. We find that FKBP25 in fact harbours a module that binds specifically to double-stranded RNA (dsRNA) and is selective for dsRNA over dsDNA, ssRNA and ssDNA. A minimal dsRNA length of eight base-pairs is required for the interaction, and in the context of ribosome biogenesis this interaction also occurs with nucleolin-bound rRNA stemloop structures. The atomic details were used to design a minimal mutation to abrogate the interaction with RNA in vitro, and this mutant also prevents interaction with RNA in vivo. We hypothesize that the repertoire of substrates for FKBP25 is defined by cellular localization, granted in this case by selective binding to dsRNA.

O24

The 5S RNP provides the link between ribosome biogenesis and the tumour suppressor p53

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The 5S RNP (RPL5, RPL11 and the 5S rRNA) is an essential assembly intermediate of the large ribosomal subunit that is also a key signalling factor important for the cellular reaction to stalled or reduced levels of ribosome production. Perturbations in ribosome production lead to the accumulation of the 5S RNP, which binds to, and inhibits MDM2, the main regulator of the tumour suppressor p53, resulting in p53 activation. The activation of p53, a transcription regulator, leads to anti-proliferative responses such as cell cycle arrest, apoptosis or senescence. The 5S RNP-MDM2 pathway has been shown to be induced in several genetic diseases caused by defects in ribosome biogenesis (ribosomopathies) and is central to the cell cancer defence systems. Indeed, the ribosomal protein RPL5, which is mutated in the more severe forms of Diamond Blackfan anaemia, has been described as a “cancer gene” in several tumour types.

We have shown that defects in either large or small ribosome subunit production, even cytoplasmic steps, lead to p53 activation through the 5S RNP-MDM2 pathway. Indeed, this pathway monitors ribosome biogenesis and p53 activation occurs prior to changes in ribosomal subunit levels. Our data also shown that a block in ribosome biogenesis results in the rapid accumulation of millions of copies of the 5S RNP that sequester the cellular MDM2 pool. Interestingly, the 5S RNP-MDM2 complex remains bound to p53 suggesting that the 5S RNP can directly modulate p53 activity. We have shown that RPL5, in free (non-ribosomal) 5S RNP, is phosphorylated and that this modification controls both ribosome biogenesis and cellular signalling. Finally, we have found that the splicing factor SRSF1 is required for efficient 5S RNP recruitment into the ribosome as well as the repression of MDM2 activity. Taken together, our data indicate that p53 signalling and ribosome production are intimately linked via the 5S RNP and that this link is critical to regular cellular growth and function.

O25

Interaction of hnRNP K and rpS19 and its function in erythroid maturation

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In erythropoiesis posttranscriptional control is essential to safeguard structural and metabolic transformation during enucleated reticulocyte to erythrocytes transition. Regulation of reticulocyte 15-lipoxygenase (r15-LOX) mRNA translation by hnRNP K, which constitutes a silencing complex at the 3'UTR DICE[1-3] secures initiation of timely mitochondria degradation by newly synthesized r15-LOX.

To elucidate how hnRNP K interferes with 80S ribosome formation to suppress r15-LOX mRNA translation initiation, we applied three independent interaction screens: 1] DICE RNA affinity chromatography from cytoplasmic extracts of K562 cells, which represent a premature erythroid state was combined with hnRNP K-immunoprecipitation[3]. 2] HnRNP K interacting proteins were enriched from extracts of K562 cells induced for erythroid maturation and non-induced cells[2]. 3] Considering alterations in hnRNP K arginine dimethylation during erythroid maturation[2,4], methylated and non-methylated His-hnRNP K[5] were employed to purify interactors from RNase A-treated K562 extracts.

All strategies identified the 40S ribosomal subunit protein rpS19. Mutations in rpS19 have been functionally linked to impaired erythropoiesis in Diamond Blackfan Anemia (DBA)[6]. Interestingly, hnRNP K - rpS19 interaction declines during erythroid maturation of K562 cells *in vitro* and *in vivo*. We studied the impact of differentially dimethylated arginine residues in hnRNP K on rpS19 binding in thermal shift assays. Furthermore we analyzed the disturbed hnRNP K interaction with DBA related rpS19 variants. Their function in r15-LOX mRNA silencing and erythropoiesis was examined in *in vitro* translation assays with recombinant rpS19 variants and by depletion from K562 cells.

[1]Ostareck D *et al.* (1997) *Cell*
[2]Naarmann IS *et al.* (2008) *JBC*
[3]Naarmann IS *et al.* (2010) *RNA*
[4]Ostareck-Lederer A *et al.* (2006) *JBC*
[5]Moritz B *et al.* (2014) *Biol. Chem.*
[6]Draptchinskaia N *et al.* (1999) *Nat. Gen.*

O26

Ribosome-targeting antibiotics and mechanisms of bacterial resistance

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The ribosome is one of the main antibiotic targets in the bacterial cell. Structures of naturally produced antibiotics and their semi-synthetic derivatives bound to ribosomal particles have provided unparalleled insight into their mechanisms of action, and they are also facilitating the design of more effective antibiotics for targeting multidrug-resistant bacteria. In this presentation, I will discuss the recent structural insights into the mechanism of action of ribosome-targeting antibiotics and the molecular mechanisms of bacterial resistance, in addition to the approaches that are being pursued for the production of improved drugs that inhibit bacterial protein synthesis.

REFERENCES

Arenz S, Wilson DN. (2016) Blast from the Past: Reassessing Forgotten Translation Inhibitors, Antibiotic Selectivity, and Resistance Mechanisms to Aid Drug Development. Mol Cell. 61(1):3-14.

Wilson DN. (2014) Ribosome-targeting antibiotics and mechanisms of bacterial resistance. Nat Rev Microbiol. 2014, 12(1):35-48.

Sohmen D, Harms JM, Schlunzen F, Wilson DN (2009) SnapShot: Antibiotic inhibition of protein synthesis I. Cell 138: 1248 e1241.

O27

Discovery and characterization of ribosome-arresting peptides by High-throughput Inverse Toeprinting

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During translation by the ribosome, nascent peptides sometimes block their own synthesis by interacting with the exit tunnel of the large ribosomal subunit. Although this process mainly depends upon the amino acid sequence of the ribosome-arresting peptide, it can also rely upon the sensing of a small molecule by the ribosome nascent chain complex, explaining its use for drug or metabolite-dependent gene regulation in bacteria and in eukaryotes. While numerous biochemical and structural studies have sought to dissect the molecular details of the arrest process, the ability of ribosome-arresting peptides to sense different types of small molecules and their impact as regulators of gene expression in nature are still largely unexplored.

Here, I will present our ongoing efforts to decipher the *arrest code* governing nascent chain-mediated translational arrest in bacteria. Specifically, I will describe High-throughput iNverse Toeprinting (HiNT), a method for the systematic analysis of nascent chain-associated ribosomal stalling events, and I will discuss its application to the study of drug-dependent translational arrest. By performing HiNT on an mRNA library encoding $>10^{11}$ random peptide sequences translated *in vitro* in the presence or absence of the macrolide antibiotic erythromycin, we could establish the complete sequence-specific arrest profile of this drug and compare the strength of drug-dependent pauses with those induced by short intrinsic arrest motifs. Importantly, successive cycles of HiNT should make it possible to evolve and enrich longer, less abundant arrest sequences present within the input library. We will therefore use HiNT to discover sequences that cause translational arrest in response to different small molecules. Given that the sites of action of arrest peptides coincide with those of known antibiotics and natural antimicrobial peptides, addressing the functional diversity and molecular basis of the arrest process will not only be key to understanding a central aspect of ribosome biology, but could also provide a handle for designing next-generation antibiotics.

O28

Extensions, extra factors and extreme complexity: Ribosomal structures provide insights into eukaryotic translation

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We are investigating bacterial and eukaryotic ribosomes and their functional complexes to obtain insights into the process of protein synthesis. Eukaryotic ribosomes are much more complex than their bacterial counterparts, require a large number of assembly and maturation factors during their biogenesis, use numerous initiation factors, and are subjected to extensive regulation. We have investigated the structures of eukaryotic ribosomes and their complexes involved in initiation and maturation and complexes involved in regulation of protein synthesis (1,2,3,4). These results provide insights into the architecture of the eukaryotic ribosome and into various eukaryotic-specific aspects of protein synthesis. Recently, using electron microscopy, we determined the complete molecular structure of the 55S mammalian mitoribosome. The maps that we calculated between 3.4 and 3.6 A resolution allowed de-novo tracing of a large number of mitochondrial specific ribosomal proteins and visualization of interactions between tRNA and mRNA in the decoding centre, the peptidyl transferase center, and the path of the nascent polypeptide through the idiosyncratic tunnel of the mammalian mitoribosome. Furthermore, the structure suggested a mechanism of how mitochondrial ribosomes, specialized for the synthesis of membrane proteins, are attached to membranes (5,6).

1. Rabl J, Leibundgut M, Sandro F. Ataide SF, Haag A and Ban N. (2010) Nature 331(6018):730-6.
2. Klinge S, Voigts-Hoffmann F, Leibundgut M, Arpagaus S, Ban N. (2011) Science, 334, 941-8.
3. Erzberger JP et al. (2014) Cell 158(5):1123-35.
4. Aylett CH et al. (2016) Science 351(6268):48-52
5. Greber BJ, Boehringer D et al. (2014) Nature. 505(7484):515-9.
6. Greber BJ, Bieri P, et al. (2015) Science. 348(6232):303-8.

O29

The small ribosomal protein RPS3 and its functions

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Eukaryotic translational initiation requires interaction of many protein factors with the 40S ribosomal subunit. These factors are called eukaryotic initiation factors (eIFs) and numerous studies have been published over the years describing their specific roles not only in translational initiation, but also in termination and ribosomal recycling. Small ribosomal proteins are key players in anchoring the eIFs to the ribosomal surface and perhaps even in modulating their function, but their exact roles remain largely unexplored. In this study, we focused on small ribosomal protein RPS3, which lies near the mRNA entry channel and is known to be a part of the latch mechanism modulating mRNA recruitment and movement of the ribosome along mRNA. In addition, RPS3 interacts with the TIF32 and TIF35 subunits of eIF3, recently implicated in controlling translation termination and promoting programmed stop codon readthrough. Indeed, our experiments identified two RPS3 variants with altered readthrough levels. Interestingly, both mutants seem to have an opposite effect on the efficiency of readthrough (increase vs. decrease), which most likely reflects their specific orientation towards other constituents of the latch mechanism.

O30

Efficient Prevention of Neurodegenerative Diseases by Depletion of Starvation Response Factor Ataxin-2

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Ataxin-2 (ATXN2) homologs exist in all eukaryotic organisms and may have contributed to their origin. Apart from a role in endocytosis, they are known for global effects on mRNA repair and ribosomal translation. Cell size, protein synthesis, and fat and glycogen storage are repressed by ATXN2 via mTORC1 signaling. However, specific liver mitochondrial matrix enzymes and the mitochondrial repair factor PINK1 require ATXN2 abundance. During periods of starvation, ATXN2 is transcriptionally induced and localized to cytosolic stress granules, where nuclear factors dock to compensate RNA pathology. These physiological actions were now revealed to be crucial for human neurodegenerative diseases, given that ATXN2 depletion is surprisingly efficient in preventing motor neuron and cerebellar atrophy, as demonstrated in mouse models, flies, and yeast.

O31

Mutant TDP-43 selectively affects translation of specific mRNAs in cultured cortical neurons and motor neuron-like cells

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TDP-43 is an RNA-binding protein implicated in several neurodegenerative diseases, particularly ALS and FTLD. In patients, numerous mutations have been identified in TARDBP, the gene that codes for TDP-43 suggesting a causal link between altered TDP-43 function and disease. TDP-43 is primarily a nuclear protein, but levels in the cytoplasm increase in disease, where it is the major component of aggregates in most forms of ALS. Cell and animal models indicate that TDP-43's RNA-binding activity is essential for toxicity and expression of mutant TDP-43 in motor neurons is sufficient for disease phenotypes. Thus, altered regulation of cellular RNAs by TDP-43 in motor neurons presumably drives disease. Previous studies have revealed TDP-43's direct RNA targets and function in many aspects of mRNA metabolism. However, it remains unresolved which effects on gene expression are the key drivers of disease. We report here that mutant TDP-43 can selectively modulate translation of specific mRNAs in neuronal cell lines and cultured primary neurons.

To examine possible effects of TDP-43 on translation of specific mRNAs, we applied the sensitive genome-wide ribosome footprint profiling method to mouse motor neuron-like cells and primary cortical neurons expressing either wild type TDP-43 or patient mutant protein. In each case we performed deep sequencing of ribosome "footprints" and corresponding mRNA samples from at least two independent replicates. Bioinformatic analysis provided a short list of candidate mRNAs that appear to be differentially regulated at the translational level in cells expressing the mutated protein. We analyzed a subset of these mRNAs via an independent approach, classical polyribosome profiling, and were able to validate selective effects of mutant TDP-43 on their translation. Strikingly, some of these mRNAs encode proteins that have already been implicated in neurodegenerative diseases. We also detected a subset of overlapping mRNAs in data sets from both models, highlighting a potential "common signature" for altered translation in the two different cell populations affected by disease (cortical and spinal motor neurons). Taken together, our data indicate that mutant alleles of TDP-43 from ALS patients can selectively modulate translation of specific mRNAs in mammalian cells.

O32

Ribosome quality control of premature polyadenylated mRNAs by a unique E3 ubiquitin ligase and RNA-binding protein

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Cryptic polyadenylation within coding sequences or incompletely removed introns produces aberrant transcripts that lack in-frame stop codons and are subject to ribosome-associated quality control (RQC). Premature polyadenylated mRNAs trigger ribosome stalling and activation of the RQC pathway, leading to degradation of the aberrant mRNA and nascent polypeptide, ribosome disassembly and recycling of its subunits. While ribosomal subunit dissociation and nascent peptide degradation are well-understood, the molecular sensors of aberrant mRNAs and their mechanism of action, especially in mammalian cells, remain largely unknown. We show that the unique RNA-binding protein and E3 ubiquitin ligase, Zinc Finger Protein 598 (ZNF598) may function as a sensor for detection of the premature polyadenylated mRNAs. PAR-CLIP assay revealed that ZNF598 crosslinks to tRNAs, mRNAs, and rRNAs, thereby placing the protein on translating ribosomes. Crosslinked reads originating from AAA-decoding $tRNA^{Lys}$(UUU) were 10-fold enriched over its cellular abundance, and poly-lysine encoded by poly(AAA), but not poly(AAG), induced RQC in a ZNF598-dependent manner. Sensing of premature polyA tails by ZNF598 triggered ubiquitination of the ribosomal proteins RPS3A, RPS10, and RPS20 requiring the E2 ubiquitin ligase UBE2D3 and thereby initiating RQC. Considering that human coding sequences are devoid of >4 repeated AAA codons, sensing of premature polyA by a specialized RNA-binding protein is a novel nucleic-acid-based surveillance mechanism for contributing to RQC.

O33

Mad about U: regulating the let7 pre-miRNA

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The pluripotency factor Lin28 inhibits the biogenesis of the let-7 family of mammalian microRNAs. Lin28 is highly expressed in embryonic stem cells and has a fundamental role in regulation of development, glucose metabolism and tissue regeneration. Alternatively, Lin28 overexpression is correlated with the onset of numerous cancers, while let-7, a tumor suppressor, silences several human oncogenes. Lin28 binds to precursor let-7 (pre-let-7) hairpins, triggering the 3' oligouridylation activity of TUT4/7. The oligoU tail added to pre-let-7 serves as a decay signal, as it is rapidly degraded by the exonuclease Dis3L2. Disruption of this pathway has been associated with pediatric kidney cancer and other cancers as well. In somatic cells, in the absence of Lin28, TUT4/7 promotes let-7 biogenesis by catalyzing single uridine addition to a subset of pre-let-7 miRNAs. I will discuss our studies toward the molecular basis and mechanism of Lin28 mediated recruitment of TUT4/7 to pre-let-7, and its effect on the uridylation activity of TUT4/7, switching it from a monouridylation activity to an oligouridylase, and the subsequent degradation of pre-let-7 by Dis3L2.

O34

From bench to clinical trial: microRNA 122 as an antiviral target for hepatitis C virus

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Liver-specific microRNA miR-122 is known to bind at two adjacent sites that are close to the 5' end of the hepatitis C virus (HCV) RNA genome, resulting in upregulation of viral RNA abundance. Extensive analyses have shown that miR-122 regulates the turnover of HCV RNA. Specifically, cellular 5'-3' exonucleases XRN1 and XRN2 have been shown to degrade the viral, 5' triphosphate-containing RNA in the absence of miR-122. The main function of miR-122 in the liver involves the upregulation of cholesterol biosynthesis by a mechanism that involves the downregulation of an Insig1 mRNA isoform that encodes an inhibitor of cholesterol biosynthesis. This finding prompted studies in which miR-122 was sequestered in the liver after injection of modified antisense oligonucleotides (miR-122 antagomir). Phase I and phase II clinical trials showed that single subcutaneous injections resulted in loss of HCV in infected patients (Regulus Therapeutics (Carlsbad, CA). Curiously, HCV rebound in some of the patients. Analysis of this virus, isolated from serum of patients, revealed that the viral RNA genome harbored a single C3U change that is predicted to change the interaction between miR-122 and HCV RNA from a G-C to a G-U base pair in their 3' end pairing. The C3U revertant virus does grow in liver Drosha-/- cells, albeit very poorly, where miR-122 is absent. This data suggests that the C3U HCV RNA can persist in patient serum, which lacks miR-122, by folding into an alternate RNA structure or by formation of specific RNA-protein complexes that stabilize the viral RNA. Indeed, in vivo proximity ligation assays have identified cellular factors that bind specific to the 5' end of the viral C3U genome. These findings argue that a specific HCV genotype can persist in an extrahepatic reservoir.

O35

The role of piRNAs in planarian regeneration

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Planarian flatworms possess fantastic regenerative abilities that can be attributed to the presence of stem cells all across their bodies. Intriguingly, PIWI proteins and PIWI-interacting RNAs (piRNAs) were found essential for proper stem cell function and hence regeneration in planarians. However, how the three planarian PIWI homologs (SMEDWI-1/2/3) contribute to regeneration is not understood.

Thus, employing biochemical techniques paired with next-generation sequencing, we set out to unravel the role of planarian piRNAs during regeneration. We show that SMEDWI-1 and SMEDWI-3 are cytoplasmic proteins and that they direct the post-transcriptional degradation of active transposons. In contrast, SMEDWI-2 is mostly nuclear and regulates transposons on the transcriptional level. Further analysis revealed that only piRNAs bound by SMEDWI-1 and -3 are derived from mRNAs in planarians. This raises the intriguing possibility that piRNAs in planarians directly regulate protein-coding mRNAs that are important for regeneration.

Using HITS-CLIP on planarian PIWI proteins we are currently exploring the role of the piRNA pathway in the post-transcriptional regulation of planarian mRNAs. In addition, we are employing ChIP-seq on heterochromatin marks to uncover the mechanism of transcriptional regulation by SMEDWI-2. Our results will help to establish how small RNAs contribute to the pluripotency of stem cells. In addition, understanding the role of piRNAs during planarian regeneration will facilitate attempts to grow human organs from pluripotent stem cells.

O36

A nickel-regulated small RNA represses expression of multiple major virulence factors in *Helicobacter pylori*

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The Gram-negative Epsilonproteobacterium *Helicobacter pylori* colonizes the stomachs of about 50% of the world's population and thereby leads to gastritis, ulcers, and gastric cancer. Several virulence factors including secreted effectors, exotoxins and outer membrane proteins have been described in *H. pylori* that allow this spiral-shaped bacterium to survive in the acidic environment of the human stomach and to interact with host cells. For many of these factors their contribution to pathogenicity has been well studied. However, how their expression is regulated is less understood. Whereas many genes important for pathogenicity are regulated at the transcriptional level, e.g. in response to low pH or the availability of metal ions, almost nothing is known about their regulation at the post-transcriptional level.

Based on a differential RNA-seq approach we had identified an unexpected number of more than 60 small RNA (sRNA) candidates in *H. pylori* strain 26695 (1). While their functions and targets remained largely unknown, we now report the characterization of an abundant and conserved sRNA, NikS (NikR-dependent sRNA), and show that it directly represses expression of several major virulence factors at the post-transcriptional level. Using *in vitro* and *in vivo* experiments, we demonstrate that NikS sRNA folds into a stem-loop structure with an extended loop region and directly binds to the mRNAs of multiple virulence genes with different single-stranded regions within its extended loop. Moreover, we demonstrate that expression of NikS itself is transcriptionally repressed in response to nickel stress through the transcriptional regulator NikR. In turn, the major virulence factors are post-transcriptionally repressed through NikS sRNA dependent on nickel availability. *In vitro* cell culture infection assays revealed that deletion and overexpression of *nikS* impacts host cell interaction, indicating that fine-tuning and coordinated regulation of virulence gene expression by NikS is important for pathogenesis. Overall, NikS represents the first potential virulence regulating sRNA from *H. pylori*.

Reference:

1. Sharma, C.M., Hoffmann, S., Darfeuille, F., Reignier, J., Findeiss, S., Sittka, A., Chabas, S., Reiche, K., Hackermuller, J., Reinhardt, R. *et al.* The primary transcriptome of the major human pathogen Helicobacter pylori. *Nature*, 2010, **464**, 250-255.

O37

Self-targeting and gene repression with the CRISPR-Cas system of *Haloferax volcanii*

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CRISPR-Cas systems are prevalent amongst archaea and well known for providing heritable immunity by incorporation of foreign DNA. The sequence information acquired is transcribed to crRNAs and incorporated into protein complexes, which can thus re-recognize the foreign DNA and trigger degradation. On the down-side, like the eukaryotic immune system, CRISPR-Cas systems are prone to auto-immunity if self-targeting spacers are acquired that lead to cleavage of host DNA.

Here, we investigated whether archaea can tolerate auto-immunity and elucidated the effects of self-targeting on cellular fitness in the halophilic archaeon *Haloferax volcanii*. To investigate the damage caused by CRISPR-Cas auto-immunity in *Haloferax*, we re-programmed the endogenous CRISPR-Cas system to target a non-essential gene involved in carotenoid biosynthesis. Upon loss or mutation of the ORF for phytoene dehydrogenase (*crtL*) the phenotype of *H. volcanii* colonies changes to white rather than red (wild type). Thus damages to this gene can be easily observed. We designed three crRNAs to target the chromosomal *crtL* gene and investigated the effect of targeting in detail.

We could show that in contrast to the observations in bacteria, self-targeting is neither lethal nor even strongly deleterious in *Haloferax*. Furthermore, our results imply that either efficient repair mechanisms, the highly polyploid genome, or the combination of both prevent lethality by CRISPR-Cas self-targeting.

O38

METTL16 is a N6-methyladenosine (m6A) methyltransferase that targets pre-mRNAs, lncRNAs and the U6 snRNA

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N^6-methyladenosine (m^6A) is a highly dynamic RNA modification that has recently emerged as a key regulator of gene expression. While many m^6A modifications are installed by the METTL3-METTL14 complex, others appear to be introduced independently, implying that additional human m^6A methyltransferases remain to be identified. Using crosslinking and analysis of cDNA (CRAC), we reveal that the putative human m^6A "writer" protein METTL16 binds to the U6 snRNA, as well as numerous lncRNAs and pre-mRNAs. We demonstrate that METTL16 is responsible for N^6-methylation of A43 of the U6 snRNA and identify the early U6 biogenesis factors La, LARP7 and the methylphosphate capping enzyme MEPCE as METTL16-interaction partners. Interestingly, A43 lies within an essential ACAGAGA sequence of U6 that base pairs with 5' splice sites of pre-mRNAs during the catalytic step of the splicing reaction. This suggests that METTL16-mediated modification of this site fine-tunes U6 snRNA-pre-mRNA interactions, thereby regulating 5' splice site recognition or spliceosome assembly on its substrate pre-mRNAs. The identification of METTL16 as an active m^6A methyltransferase in human cells expands our understanding of the mechanisms by which the m^6A landscape is installed on cellular RNAs.

O39

lncRNA & Ribonucleoproteins in Cancer

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Long non-coding RNAs (lncRNAs) and their protein interaction partners (RBPs) can play important roles in malignant diseases. The lncRNA MALAT1 is a marker and essential factor in the metastasis of lung cancer. It controls cell migration epigenetically and could serve as a therapeutic target. Numerous additional lncRNAs execute important functions in cancer initiation and progression. Many lncRNAs as well as all other classes often interact with proteins altering their confirmation, localization, function or activity. Hence, the elucidation of the composition and functional consequences of ribonucleoprotein complexes in health and disease is of great importance.

O40

Mechanistic dissection of UsnRNP biogenesis and its role in disease

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The formation of macromolecular complexes within the crowded environment of cells often requires aid from assembly chaperones. An elaborate system of assembly factors united in PRMT5- and SMN-complexes mediates formation of the common core structure of the pre-mRNA processing U snRNPs composed of seven Sm/Lsm proteins bound to snRNA. Core formation is initiated by the PRMT5-complex subunit pICln, which pre-arranges Sm/Lsm proteins into spatial positions occupied in the assembled snRNP. The SMN-complex then catalyzes snRNP formation by accepting these Sm/Lsm proteins and uniting them with snRNA. We have investigated how newly synthesized proteins are channeled into the assembly machinery to evade aggregation and/or mis-assembly. We show that Sm/Lsm proteins initially remain bound to the ribosome near the polypeptide exit tunnel. Release from the ribosome is dependent on pICln, which works in remarkable analogy to folding chaperones. The PRMT5-complex then ensures the formation of cognate Sm/Lsm heterooligomers and their guidance into the late assembly stage mediated by the SMN-complex. SMN, one key component of the SMN-complex is reduced in the neuromuscular disorder Spinal Muscular Atrophy (SMA). I will discuss how reduced activity of this protein may lead to this devastating disorder.

O41

Genome-wide study of transcription complexity and ribosome dynamics

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One of the biggest challenges in biology is to understand how apparently identical cells respond differently to the same stimulus. During the last decade, thanks to the development of genomics tools, research has uncovered extensive variability in the RNA molecules present within the cells.

In the past we have developed a diversity of novel genome-wide approaches to study eukaryotic gene expression using both budding yeast and mammalian cells. By simultaneously sequencing both the 5' and 3' ends of each RNA molecule (TIF-Seq), we showed that the complexity of overlapping transcript isoforms had been greatly underestimated even in a genetically homogeneous population of cells. More recently, we have shown how the existence of widespread co-translational mRNA degradation allows studying ribosome dynamics by sequencing mRNA degradation intermediates (5P-Seq).

I will discuss our current efforts to study transcriptome complexity and its implications for gene expression regulation.

O42

Grad-seq discovers the third domain of small RNA-mediated gene regulation in bacteria

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RNA-seq can rapidly profile the expression of theoretically all RNA molecules in any given organism but the primary sequence of these transcripts is a poor predictor of cellular function. This has been particularly evident for the regulatory small RNAs of bacteria which dramatically vary in length and sequence within and between organisms.

We have established a new method (gradient profiling by sequencing; Grad-seq) to partition the full ensemble of cellular RNAs based on their biochemical behavior. Our approach enabled us to draw an RNA landscape of the model pathogen *Salmonella* Typhimurium, identifying clusters functionally related noncoding RNAs irrespective of their primary sequence. The map revealed a previously unnoticed class of transcripts that commonly interact with the osmoregulatory protein ProQ in *Salmonella enterica*. We show that ProQ is a conserved abundant global RNA-binding protein with a wide range of targets, including a new class of ProQ-associated small RNAs that are highly structured, and mRNAs from many cellular pathways. Our functional characterization of these small RNAs that suggests that they constitute a previously unrecognized third domain of RNA-mediated control in bacteria which rivals the scope of the well-established regulons of the small RNA-binding proteins, Hfq and CsrA.

By its ability to describe a functional RNA landscape based on expressed cellular transcripts irrespective of their primary sequence, our generic gradient profiling approach promises to aid the discovery of major functional RNA classes and RNA-binding proteins in many organisms.

Smirnov A, Förstner KU, Holmqvist E, Otto A, Günster R, Becher D, Reinhardt R, Vogel J (2016);*Grad-seq guides the discovery of ProQ as a major small RNA binding protein ;* PNAS 113(41):11591-6

Smirnov A, Wang C, Drewry LL, Vogel J (2017); *Molecular mechanism of mRNA repression in trans by a ProQ-dependent small RNA;* EMBO Journal 36(8):1029-1045

Gonzalez GM, Hardwick SW, Maslen SL, Skehel JM, Holmqvist E, Vogel J, Bateman A, Luisi BF, Broadhurst RW (2017); *Structure of the Escherichia coli ProQ RNA binding protein;* RNA 23(5):696-711

Chao Y, Li L, Girodat D, Förstner KU, Said N, Corcoran C, Śmiga M, Papenfort K, Reinhardt R, Wieden HJ, Luisi BF, Vogel J (2017); *In vivo cleavage map illuminates the central role of RNase E in coding and noncoding RNA pathways;* Molecular Cell 65(1):39-51

Westermann AJ, Förstner KU, Amman F, Barquist L, Chao Y, Schulte LN, Müller L, Reinhardt R, Stadler PF, Vogel J (2016) *Dual RNA-seq unveils noncoding RNA functions in host-pathogen interactions.* Nature 529:496-501

O43

Functional characterization of hypoxia-regulated lncRNAs in endothelial cells

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With the rise of next generation sequencing technologies it became evident that the human genome is almost pervasively transcribed, however, only a minor part of the genome actually accounts for protein coding exons. Consequently, the majority of all transcripts is non-coding. According to their size, non-coding RNAs can be divided into small (<200nt) and long non-coding RNAs (lncRNAs; >200nt). Whereas the role of distinct non-coding RNAs, e.g. microRNAs, is well understood, little is known about the detailed molecular mechanisms of lncRNAs. Recent studies suggest a broad functional spectrum including sponging of miRNAs, modulation of mRNA splicing and stability, or recruitment of chromatin modifying enzymes. Regarding endothelial cells (ECs), the response to hypoxia and the regulation of angiogenic activity are key events in the context of several diseases, however, the involvement of regulatory lncRNAs and their molecular function is not well understood.

To analyze the influence of hypoxia on lncRNA expression, we performed RNA deep sequencing in hypoxic (24h; 0.2% O_2) and normoxic ECs and identified 71 and 78 lncRNAs to be significantly up- or down-regulated, respectively. From these transcripts, we chose two robustly up-regulated and uncharacterized lncRNAs -named GATA6-AS and NTRAS- for a detailed investigation. First functional assays revealed that silencing of both candidate lncRNAs using LNA GapmeRs significantly alters the angiogenic activity of ECs, underlining their involvement in endothelial biology. Moreover, cellular fractionation showed a predominant nuclear localization of GATA6-AS and NTRAS and analysis of the respective gene loci ruled out *cis*-regulatory mechanisms. Thus, in order to get detailed insights into the molecular functions of these transcripts, we deployed antisense affinity purification followed by mass spectrometry to enrich for and analyze the respective endogenous RNA-protein-complexes. **1)** For GATA6-AS, this uncovered the chromatin modifying enzyme LOXL2, which catalyzes the oxidative deamination of trimethylated H3K4 residues, as a bound protein factor. Strikingly, gene expression profiling identified ~71% of GATA6-AS-regulated genes to be inversely regulated by LOXL2 silencing, including numerous angiogenesis-related genes, like PTGS2 and POSTN. Moreover, silencing of GATA6-AS significantly decreased H4K3me3 ChIP efficiencies on PTGS2 and POSTN promoters and in parallel reduced global H3K4me3 levels. Taken together, these results strongly argue for GATA6-AS acting as negative regulator of LOXL2 function. **2)** For NTRAS, affinity purification and mass spectrometry identified the splicing regulator hnRNPL to be strongly associated with the lncRNA, which is in line with a *bona fide* hnRNPL binding motif within the NTRAS sequence. Using RNA immunoprecipitation and RT-qPCR, we were able to confirm the observed interaction. Based on these findings, we assayed for a functional involvement of NTRAS in alternative splicing regulation by microarrays. Strikingly, silencing of NTRAS changed the splicing pattern of more than 400 genes, among those the well-known hnRNPL target TJP1. In detail, NTRAS was found to activate TJP1 exon 20 inclusion, which is repressed by hnRNPL. On a functional level, TJP1 is required for tight junction assembly, and we currently investigate the role of the "α-motif" encoded by exon 20 in the context of angiogenic integrity and permeability. In conclusion, our data demonstrates that the hypoxia-regulated lncRNA NTRAS controls endothelial cell functions putatively via modulating hnRNPL-dependent alternative splicing.

O44

High-throughput screening for splicing regulatory elements

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Alternative splicing constitutes a major step in eukaryotic gene expression and requires tight control of *trans*-acting factors that recognise *cis*-regulatory elements in the RNA sequence. However, the majority of *cis*-regulatory elements are poorly defined, and the impact of intronic and exonic sequence variants on the splicing outcome remains elusive.

Here, we establish a high-throughput screen to comprehensively identify all *cis*-regulatory elements that determine a particular splicing decision. As a prototype example, we screen a minigene harbouring the cancer-relevant alternative exon of the RON receptor kinase gene. A library of thousands of randomly mutagenised minigene variants is transfected as a pool into human HEK293T cells, and the splicing products are subsequently analysed via RNA sequencing. In parallel, DNA sequencing enables reliable point mutation discovery which are assigned to the corresponding splicing products via a unique barcode sequence. In addition to known *cis*-regulatory elements, we identify numerous previously unknown regulatory sites. This approach proves particularly powerful when combined with knockdown experiments which allows to connect *trans*-acting factors with their corresponding *cis*-regulatory elements. In summary, this novel screening approach introduces a tool to study the relationship of *cis*-regulatory elements, sequence variants and their impact on the splicing outcome, offering new insights into alternative splicing regulation and the implication of mutations in human disease.

O45

Regulation of gene expression by proteins bound to LINE-derived RNA elements

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The human genome contains over 1.5 million degenerate LINE-derived sequences, many of which are transcribed as part of longer genes. We analysed iCLIP, eCLIP and RNAseq data to find that many RNA-binding proteins (RBPs) bind LINE-derived RNA sequences, and thereby regulate gene expression. MATR3 and PTBP1 are particularly enriched, and both proteins repress splicing as well as 3' end processing within or nearby the bound LINEs, which affects both annotated and cryptic exons.

MATR3 is an RNA- and DNA-binding protein recently implicated in motor neuron disease, which has been reported to play multiple presumably distinct functions in enhancer activation and regulation of alternatively splicing. We show that LINEs are associated with both functions. MATR3 can repress splice sites in as far as 2kb distance to LINEs, which we demonstrate by mutagenesis experiments. Moreover, MATR3 and PTBP1 bind non-coding RNAs containing LINE sequences and are required for their expression.

We also analysed data from PTBP2-/- mouse brain, which confirms that PTBP proteins regulate tissue-specific splicing of LINE-derived exons, which are frequently species-specific. We conclude that RBPs are employed to proofread RNA processing at thousands of LINEs. We propose that the invasion of vertebrate genome by LINE and other retrotransposons has led to their co-evolution with RNA-binding proteins, which is crucial for the evolutionary processes that shape the species-specific gene expression patterns.

O46

iCLIP determines the target repertoire and binding landscape of the clock-regulated RNA-binding protein AtGRP7

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A key function for RNA-binding proteins in orchestrating plant development and environmental responses is well established. However, the lack of a genome-wide view on their *in vivo* binding targets and binding landscapes represents a gap in understanding the mode of action of plant RNA-binding proteins. Here, we adapt individual nucleotide resolution crosslinking immunoprecipitation (iCLIP) for genome-wide determining the binding repertoire of the circadian clock-regulated *Arabidopsis thaliana* glycine-rich RNA-binding protein *At*GRP7. iCLIP identified 858 transcripts with significantly enriched crosslink sites in plants expressing *At*GRP7-GFP and absent in plants expressing an RNA-binding-dead *At*GRP7 variant or GFP alone. To independently validate the targets, we performed RNA immunoprecipitation (RIP)-sequencing of *At*GRP7-GFP plants subjected to formaldehyde fixation. 452 of the iCLIP targets were also identified by RIP-seq, thus representing a set of high-confidence binders. *At*GRP7 can bind to all transcript regions with a preference for 3´untranslated regions. In the vicinity of crosslink sites, UC-rich motifs were overrepresented. Cross-referencing the targets against transcriptome changes in *At*GRP7 loss-of-function mutants or *At*GRP7-overexpressing plants revealed a predominantly negative effect of *At*GRP7 on its targets. In particular, elevated *At*GRP7 levels lead to damping of circadian oscillations of transcripts including *DORMANCY/AUXIN ASSOCIATED FAMILY PROTEIN2* and *CCR-LIKE*. Furthermore, several targets show changes in alternative splicing or polyadenylation in response to altered *At*GRP7 levels. Establishing iCLIP for plants to identify target transcripts of the RNA-binding protein *At*GRP7 paves the way to investigate the dynamics of posttranscriptional networks in response to exogenous and endogenous cues.

O47

Functional characterization of the CRISPR-Cas9 system of the foodborne-pathogen *Campylobacter jejuni*

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Based on a comparative RNA-seq analysis of multiple strains of the food-borne bacterial pathogen Campylobacter jejuni, we have detected strain-specific transcriptional output and small RNA (sRNA) repertoires (1). Our C. jejuni global transcriptome maps also revealed a minimal variant of the RNA-based CRISPR-Cas immune system of the type-II subtype, which relies on the host factor RNase III and a trans-encoded sRNA (tracrRNA) for maturation of crRNAs. This minimal type-II C system of C. jejuni employs a unique maturation pathway, where the CRISPR RNAs (crRNAs) are transcribed from individual promoters in the CRISPR repeats and thereby minimize the requirements for the maturation machinery. Type II CRISPR-Cas systems and their Cas9 nucleases naturally utilize crRNAs and tracrRNA to bind and cleave complementary invading DNA sequences. Besides a potential role in phage defense, Cas9 in the bacterial pathogen Francisella novicida, was reported to use an alternative scaRNA to repress an endogenous transcript and thereby affects virulence (2).

To study RNA-protein complexes in Campylobacter, we have set-up several approaches, such as co-immunoprecipitation combined with RNA-seq (RIP-seq) to identify the direct RNA substrates of an RNA binding protein (3). Our RIP-seq analysis of C. jejuni Cas9 revealed that not only the crRNAs and tracrRNA are abundantly bound by Cas9, but also uncovered several endogenous mRNAs that co-purify with Cas9. Using in vivo and in vitro approaches, we show that C. jejuni Cas9 mediates crRNA- and tracrRNA-dependent mRNA cleavage in the absence of a scaRNA. Moreover, we show that RNA cleavage by Cas9 can be reprogrammed in vitro by a single guide RNA. These findings suggest that the C. jejuni Cas9 is a promiscuous nucleic acid nuclease and could serve in applications of programmable RNA binding or cleavage.

1. Dugar, G., Herbig, A., Förstner, K.U., Heidrich, N., Reinhardt, R., Nieselt, K. and Sharma, C.M. (2013) High-Resolution Transcriptome Maps Reveal Strain-Specific Regulatory Features of Multiple Campylobacter jejuni Isolates. PLoS Genet, 9, e1003495.

2. Sampson, T.R., Saroj, S.D., Llewellyn, A.C., Tzeng, Y.L. and Weiss, D.S. (2013) A CRISPR/Cas system mediates bacterial innate immune evasion and virulence. Nature, 497, 254-257.

3. Dugar, G., Svensson, S.L., Bischler, T., Waldchen, S., Reinhardt, R., Sauer, M. and Sharma, C.M. (2016) The CsrA-FliW network controls polar localization of the dual-function flagellin mRNA in Campylobacter jejuni. Nature communications, 7, 11667.

POSTER ABSTRACTS (A-Z)

P1

Functional characterization of HMGN5, a specific RNA or nucleosome binding protein opening higher order structures of chromatin

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Packaging of DNA into higher order structure of chromatin has profound implications in the regulation of nuclear processes, like transcription, replication, recombination or DNA repair. The reversible opening and closing of chromatin domains is mediated by architectural proteins of the "high mobility group nucleosomal binding domain" (HMGN) family, which are known to reorganize chromatin structure and to regulate gene expression. However, the mechanisms underlying this process are still unclear. Here we use the human HMGN5 as a model to study the opening of higher order structure of chromatin using genome-wide analysis methods. We reveal that HMGN5 mainly associates with active promoters and regulatory regions like CpG islands, maintaining their accessible state. HMGN5 binding overlaps with PolII and CTCF binding sites and localizes to DNase I hypersensitive sites (DHSs). Actively regulated target genes belong to the group of genes involved in RNA metabolic processes. Surprisingly, we identified a specific RNA binding domain overlapping with the nucleosomal binding domain of HMGN5. Our data suggest a bimodal state or a switch that enables HMGN5 binding either chromatin or RNA since the HMGN5-bound RNAs have no functional relationship with the chromatin function of HMGN5. We suggest that HMGN5 has a dual function either regulating chromatin structure or RNA metabolism.

P2

Assembly, localization and translational control of WUS-/WOX-containing mRNPs in *A. thaliana*

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Plant reproduction, embryogenesis and development are regulated by a complex signaling network. *WUSCHEL*-related homeobox (*WOX*) superfamily members are key regulators of such developmental processes. This gene family is founded by *WUSCHEL* (*WUS*) and consists of 15 members, which are essential for stem cell maintenance in shoot apical meristem. Other members such as *WOX2*, *WOX8* and *WOX9* are essential for pro-embryo development. And *WOX5* is essential for root meristem formation.

Temporal and spatial control of mRNA translation is a precise mechanism to control the presence of gene products. This mechanism is found in all kingdoms of life and appears to be essential for asymmetric cell division, cell fate determination and many other developmental processes. In contrast to animals and fungi, to date little is known about mRNPs localization and translational control in plants. Using fluorescent whole-mount *in situ* hybridization (F-WISH) we could show that WOX2/5/8 and WUS mRNAs accumulate in globular structures. These globular structures indicate specific mRNPs formation, which might be essential for localization and translational control. I currently try to identify response elements for mRNP association using deletion and synthetic derivatives in F-WISH experiments. To characterize mRNP composition I perform RNA pull down experiments using specific RNA-tag or labeled antisense probes.

P3

Auxiliary Factors of the RNA helicase Prp43 in ribosome biogenesis

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Ribosome biogenesis is a complex, tightly regulated and highly energy demanding process. It needs participation of RNA-polymerases, 82 ribosomal proteins, 66 (small nucleolar RNA) snoRNAs and more than 200 non ribosomal proteins which carry out molecular rearrangements, modifications and rRNA processing steps within ribosomal precursors for maturation and functional assembly of ribosome. These include assembly factors, endo –and exonucleases, ATPases, transport factors, RNA helicases.

Among the RNA helicases, Prp43 DEAH/RHA helicase plays multiple roles at different stages in both 40S and 60S ribosomal subunit biogenesis and also participates in mRNA splicing. As Prp43 is responsible for essential processes, it necessitates the engagement of many coordinating auxiliary factors for the accomplishment of its functions. These co-factors of Prp43 include a group of proteins sharing a glycine-rich domain, termed G-patch domain. G-patch proteins Ntr1, Pxr1, Pfa1 and Cmg1 are known to stimulate ATPase and RNA helicase activity of Prp43. In mRNA splicing G-patch protein Ntr1 is responsible for Prp43 helicase activation, while the non G-patch auxiliary factor Ntr2 functions as adaptor for the recruitment of Prp43 to the spliceosome. Pxr1 and Pfa1 are involved in ribosome biogenesis.

Here we show that Prp43 is a component of ribosome precursor particles from 90S to both 60S and 40S subunit biogenesis. Pxr1 and Pfa1 activate Prp43 in ribosome biogenesis, with Pxr1 being mainly involved at the level of 90S particles, and Pfa1 presumably functioning at later stages of both 40S and 60S maturation. This finding indicates distinct roles of Pxr1 and Pfa1 during ribosome biogenesis. Along with Prp43 co-factors we also identified a G-patch protein Associated Factor named Gaf1. Gaf1 is a novel ribosome biogenesis factor involved at the level of 90S particles, as well as at early stages of 60S maturation. It is interesting to investigate the functional role of Gaf1 in ribosome biogenesis in association with Prp43.

P4

mRNPs assembly and localization during early embryogenesis in *Arabidopsis thaliana*

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The spatial-temporal control of mRNA translation is a precise mechanism to regulate the presence of gene products, which involves the formation of specific mRNA/protein complexes so-called mRNPs. Such mRNPs are well described in other organisms and appear to be essential for asymmetric cell division, cell fate determination and cell differentiation. Using fluorescent whole mount *in situ* hybridization (F-WISH), we could show that the mRNA of severla homeobox transcription factors accumulate in globular structures and localize at specific sites of the cell during embryogenesis. These globular structures indicate the formation of specific mRNPs, which might be essential for mRNA localization and translational control. We currently focusing on the mechanism of mRNP formation and its impact on plant developmental processes.

P5

High-throughput random mutagenesis screening unveils extensive regulation of RON alternative splicing by hnRNP H

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Alternative splicing increases the coding capacity of most human genes, and numerous diseases can arise from defects in this process. Control of alternative splicing is realized by cis-regulatory elements, such as RNA sequence and structure, which recruit trans-acting RNA-binding proteins. Although several of those interactions are already described in detail, we lack a comprehensive understanding of the regulatory code that underlies a specific splicing decision. Here, we established a high-throughput random mutagenesis screen to comprehensively study all cis-regulatory elements that control a selected splicing decision in the proto-oncogene RON. To this end, we generated a library of thousands of randomly mutagenized minigene variants and characterized the mutations present in the library using next-generation sequencing. Next, we transfected this library as a pool into human cells, and subsequently quantified the spliced isoforms by RNA sequencing. Importantly, we used a barcode sequence to tag the minigene variants and thereby linked mutations to their corresponding spliced products. We identify numerous sites involved in the regulation of the alternative exon that spread across the entire sequence space, including the surrounding introns and neighbouring constitutive exons. Furthermore, we find that the splicing factor hnRNP H acts as an extensive regulator of RON splicing via multiple cis-regulatory elements. Overall, our results unveil an unprecedented view on the complexity of splicing regulation at a single exon.

P6

Structural studies on novel MLLE-PAM2L interaction in dynamic endosome mediated mRNA transport

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Long-distance transport of macromolecules is essential for highly polarized cells such as fungal hyphae. In the plant pathogenic fungus *Ustilago maydis* active mRNA transport is carried out by early endosomes along microtubules.

During the transport, mRNAs are bound to the key RNA-binding protein Rrm4 on the cytoplasmic surface of the endosome. Poly-A binding protein (Pab1) is other predominantly found mRNA binding protein on endosomes. Rrm4 has three RNA recognition motifs (RRM) in its N-terminal and two Mademoiselle (MLLE) domains in its C-terminal. In contrast, Poly-A binding protein (Pab1) contains only one MLLE domain. However, both Rrm4 and Pab1 do not have a lipid binding domain hence cannot be directly linked to endosomes. MLLE domains are known to interact with proteins which contain polyA binding protein interacting motif (PAM2). In the past, we identified an adapter protein, with a PAM2 domain and a FYVE domain for lipid binding, it was named Upa1. Interestingly, in addition to PAM2 motif Upa1 also has a novel PAM2-like (PAM2L) motif which specifically interacts with MLLE domain from Rrm4 but not Pab1.

The objective of our study is to decipher the structural basis for the specificity of $MLLE^{Pab1}$- $PAM2^{Upa1}$ and $MLLE^{Rrm4}$ – $PAM2L^{Upa1}$ interactions by using biochemical, biophysical characterization and X ray crystallography.

P7

Identification of Proteins that Interact with Endogenously Produced tRNA Fragments

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Transfer RNAs (tRNAs) have been widely appreciated as fundamental components of the translational machinery, acting as adaptor molecules that are essential for mRNA decoding into proteins. In addition, tRNA molecules can be endonucleolytically cleaved under a wide variety of stress conditions through the activity of endonucleases, resulting in the production of distinct tRNA fragments. Although various tRNA fragments have been shown to modulate a range of biological processes, namely proliferation, stress responses and apoptosis, the full scope and mechanistic details of their biological impact are still poorly understood.

To better define the biological function of tRNA fragments, we have set out to identify human proteins, which interact with specific and endogenously produced tRNA fragments during the stress response. To that end, we employ a combination of biochemical approaches. Specific tRNA fragments are purified from human cells by chromatographic methods after exposure to stress or after transient expression of a tRNA endonuclease. RNA affinity capture is being performed using protein extracts obtained from stress-exposed cells allowing the identification of tRNA fragment-interacting proteins using mass spectrometry. This poster discusses the progress, bottlenecks and future directions of the project.

P8

Structural insights into ribosomal antitermination of *Escherichia coli*

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Transcription is the first step in gene expression and consists of three steps (initiation, elongation, termination) with RNA polymerase (RNAP) being the core machinery responsible for RNA synthesis. Each step of the transcription cycle is highly regulated by a variety of transcription factors. During antitermination RNAP is modified to suppress termination signals, allowing the expressing of downstream genes. In *Escherichia coli* (*E. coli*) the expression of the ribosomal (*rrn*) operons relies on antitermination. Processive *rrn* antitermination requires the assembly of a multicomponent complex and N-utilization substance (Nus) factors A, B, E, and G as well as ribosomal protein S4 are suggested to be part of it. Recently, SuhB, an inositol monophosphatase, was identified to be also essential for robust expression of the *rrn* operon[1]. The structural basis of *rrn* antitermination has so far remained elusive and in particular the roles of S4 and SuhB are not understood. Here we present first molecular insights into *rrn* antitermination based on a combinatorial approach of analytical size exclusion chromatography and solution state NMR spectroscopy. First experiments indicated that NusA, NusB, NusE and NusG together with S4 and SuhB can form a stable complex on *rrnG* RNA and in the presence of elongating RNAP. We then showed that the six domain protein NusA might play a central role in *rrn* antitermination as it directly interacts with S4 and SuhB. Whereas the NusA domain that binds to S4 remains to be determined, we identified SuhB to bind specifically to the acidic repeat (AR) 2 domain of NusA. We determined the SuhB interaction surface on NusA-AR2, which comprises the C-terminal helix of NusA-AR2 and overlaps with the binding site for the C-terminal domain of the α-subunit of RNAP as well as with the binding site for the N-terminal domain of NusG[2,3]. During elongation, and most probably during antitermination, NusA is tethered to RNAP *via* its NTD. Thus we propose that NusA-AR2 serves as general interaction platform for various transcription factors and may thus be responsible for the recruitment of SuhB during the assembly of the *rrn* antitermination complex.

[1] Singh, N.; Bubunenko, M.; Smith, C.; Abbott, D.M.; Stringer, A.M.; Shi, R.; Court, D.L.; Wade J.T. *mBio* **2016**, 7(2), e00114-16.

[2] Schweimer, K.; Prasch, S.; Sujatha, P. S.; Bubunenko, M.; Gottesman, M.E.; Rösch, P. *Structure* **2011**, 19(7), 945–954.

[3] Strauss, M.; Vitiello, C.; Schweimer, K.; Gottesman, M.E.; Rösch, P.; Knauer, S. H. *NAR* **2016**, 44(12), S. 5971–5982

P9

UPF1 in the cell nucleus

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UPF1 binds RNA and is an ATP-dependent RNA helicase belonging to the SF1 superfamily. Functionally, UPF1 is best known for its essential role in nonsense-mediated mRNA decay (NMD), a post-transcriptional quality control mechanism that recognizes transcripts with premature stop codons and efficiently degrades them. In addition to NMD, UPF1 is implicated in several nuclear processes including telomere homeostasis, S phase progression, DNA repair, and DNA replication. However, its exact roles in these processes are poorly understood and despite its shuttling capacity, the majority of UPF1 is found in the cytoplasm of unperturbed cells. We aim at a better understanding of UPF1's function(s) in the nucleus and its recruitment to RNA. RNA immunoprecipitation (RIP) showed that UPF1 associates with a diverse set of RNAs including both, coding and non-coding transcripts. Interestingly, nuclear long non-coding RNAs like ANRIL, GAS5 and FIRRE appear to be extensively bound with UPF1, pointing towards a recruitment of UPF1 to transcripts already in the nucleus and independent of their coding potential. We currently try to understand why some transcripts are highly enriched in UPF1 IPs while others lack UPF1 almost completely. So far, the molecular basis for this differential association of UPF1 with RNA is not understood. Further investigation of the proposed nuclear functions of UPF1 would greatly benefit from having a UPF1 mutant with a defective nuclear localization signal (NLS) that localizes exclusively to the cytoplasm. To this end, we try to functionally map the NLS of UPF1 by testing different UPF1 mutants in cells treated with leptomycin B, which blocks the nuclear export pathway.

P10

ALY RNA-binding proteins are required for nucleo-cytosolic mRNA transport and modulate plant growth and development in *Arabidopsis*

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The regulated transport of mRNAs from the cell nucleus to the cytosol is a critical step in the expression of protein-coding genes, linking transcript synthesis and processing with translation. Still only few plant factors of the mRNA export pathway have been functionally characterised. Flowering plant genomes encode several members of the ALY protein family that in other organisms act as mRNA export factors. Here we show that the four nuclear Arabidopsis ALY proteins (ALY1-4) are commonly detected in root and leaf cells, but they are differentially expressed in reproductive tissue. The subnuclear distribution of ALY1/2 differs from that of ALY3/4. ALY1 binds with higher affinity to ssRNA relative to dsRNA and ssDNA and it interacts preferentially with 5-methylcytosine modified ssRNA. Compared to the full-length ALY1 its individual RNA recognition motif binds RNA only weakly. The ALY proteins interact with the RNA helicase UAP56, suggesting a link to the mRNA export machinery. While individual aly mutants have wild type appearance, simultaneous inactivation of all four ALY genes in the 4xaly plants results in vegetative and reproductive defects including strongly reduced growth, altered flower morphology and partially abnormal ovules and female gametophytes that cause reduced seed production. Compared to control cells, in 4xaly cells polyadenylated mRNAs accumulate in the nuclei, supporting the role of Arabidopsis ALY proteins as plant mRNA export factors. The four ALY proteins might act partly redundantly in mRNA export, but differences in expression and subnuclear localisation suggest also distinct functions.

P11

Small RNA pathway components in the female gametophyte of *Arabidopsis thaliana*

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The reproductive lineage initiates when the meiocyte precursor cells specify and undergo a transition from a mitotic to a meiotic cell fate. Unlike animals, where the germline is established during early embryogenesis, the plant male and female reproductive lineages arise late during development, when the flowers are formed. Notably, the haploid products of meiosis (the spores) do not immediately differentiate to form the gametes but undergo further mitotic divisions in a process termed gametogenesis. As a result the haploid "gametophytic" generation is formed, comprising the gametes and accessory cells which are necessary for successful reproduction.

Specific Argonaute proteins and their small RNA targets are important for both animal and flowering plant germline development. The role of Argonautes and sRNAs during male and female sporogenesis has been intensively studied in rice and maize, demonstrating that epigenetic factors participate in intercellular interactions leading to the establishment of reproductive cell number and fate in plants.

However, it is not known whether small RNA pathway components are also important for female gametogenesis. The composition of small RNAs in the cells of the female gametophyte remains elusive and their gametogenesis or transgenerational role(s) are unexplored. Reasons for this lack of knowledge are their small size and inaccessibility, as they are deeply embedded in the sporophytic tissues of the ovule, and the ovary. Here, we will present our methodological approaches to identify small RNAs expressed in the cells of the female gametophyte as well as AGO effector ribonucleoprotein complexes present in the *Arabidopsis* egg cell.

P12

The *Arabidopsis* THO/TREX component TEX1 functionally interacts with MOS11 and modulates mRNA export and alternative splicing events

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TREX (transcription-export) is a multiprotein complex that plays a central role in the coordination of synthesis, processing and nuclear export of mRNAs. Using targeted proteomics, we identified proteins that associate with the THO core complex of Arabidopsis TREX. In addition to the RNA helicase UAP56 and the mRNA export factors ALY2-4 and MOS11 we detected interactions with the mRNA export complex TREX-2 and multiple spliceosomal components. Plants defective in the THO component TEX1 or in the mRNA export factor MOS11 (orthologue of human CIP29) are mildly affected. However, tex1 mos11 double-mutant plants show marked defects in vegetative and reproductive development. In tex1 plants, the levels of tasiRNAs are reduced, while miR173 levels are decreased in mos11 mutants. In nuclei of mos11 cells increased mRNA accumulation was observed, while no mRNA export defect was detected with tex1 cells. Nevertheless, in tex1 mos11 double-mutants, the mRNA export defect was clearly enhanced relative to mos11. The subnuclear distribution of TEX1 substantially overlaps with that of splicing-related SR proteins and in tex1 plants the ratio of certain alternative splicing events is altered. Our results demonstrate that Arabidopsis TEX1 and MOS11 are involved in distinct steps of the biogenesis of mRNAs and small RNAs, and that they interact regarding some aspects, but act independently in others.

P13

New insights into prevalence and functions of sigma-dependent pausing in bacteria

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The σ subunit associates with bacterial RNA-polymerase (RNAP) to assist it in promoter recognition and transcription initiation. Most bacteria contain one principal and several alternative σ subunits that have different promoter specificities and are responsible for gene expression under varying conditions. Transcription elongation is performed by the core enzyme of RNAP following promoter escape and σ subunit dissociation. However, the principal s^{70} subunit in *E. coli* can stay bound to the transcription elongation complex and recognize promoter-like sequences in initially transcribed regions, resulting in transcriptional pausing and increased s retention at later steps of elongation. This phenomenon was proposed to be limited to the principal σ subunit only. We showed that σ-dependent pausing can be observed with RNAPs from various bacteria. Furthermore, we found that alternative s subunits can also induce pausing. In particular, σ^{38} and σ^{24} subunits of *E. coli* induced efficient pausing at promoter-like sites in the transcribed DNA. In both cases, we detected formation of backtracked complexes that could be reactivated by Gre-factors, similarly to σ^{70}-dependent pausing. To understand the role of s-dependent pausing in gene expression, we designed several model systems based on the use of luciferase and *lac*Z reporters containing natural and artificially designed σ-pause sequences. We found that the presence of promoter-like sequences in the early transcribed regions significantly modulates the level of gene expression. In conclusion, our data suggest that σ-dependent pausing is a widespread phenomenon that can be induced by various types of σ subunits and modulate gene expression.

P14

A general method for rapid and cost-efficient large-scale production of 5' capped RNA

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The eukaryotic mRNA 5’ cap structure is indispensible for pre-mRNA processing, mRNA export, translation initiation, and mRNA stability. Despite this importance, structural and biophysical studies that involve capped RNA are challenging and rare due to the lack of a general method to prepare mRNA in sufficient quantities. Here, we show that the vaccinia capping enzyme can be used to produce capped RNA in the amounts that are required for large-scale structural studies. We have therefore designed an efficient expression and purification protocol for the vaccinia capping enzyme. Using this approach, the reaction scale can be increased in a cost-efficient manner, where the yields of the capped RNA solely depend on the amount of available uncapped RNA target. Using a large number of RNA substrates, we show that the efficiency of the capping reaction is largely independent of the sequence, length, and secondary structure of the RNA, which makes our approach generally applicable. We demonstrate that the capped RNA can be directly used for quantitative biophysical studies, including fluorescence anisotropy and high-resolution NMR spectroscopy. In combination with ^{13}C-methyl-labeled *S*-adenosyl methionine, the methyl groups in the RNA can be labeled for methyl TROSY NMR spectroscopy. Finally, we show that our approach can produce both cap-0 and cap-1 RNA in high amounts. In summary, we here introduce a general and straightforward method that opens new means for structural and functional studies of proteins and enzymes in complex with capped RNA.

P15

Depletion of the long non-coding RNA SINCR1 inhibits cell proliferation and induces senescence in liver cancer cells

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Hepatocellular carcinoma (HCC) is the sixth most common cancer in the population worldwide with a poor 5-year overall survival of less than 15% illustrating the need for deeper investigation into hepatocarcinogenesis. Long non-coding RNAs (lncRNAs) have emerged as critical players for regulating various pathological and physiological processes including HCC. In-spite of the clinical relevance, their functional role and molecular mechanisms remain poorly characterized. Here, we used a genome-wide screening approach to identify SINCR1 (Senescence Inducing Non Coding RNA 1) - an lncRNA induced by 5.57 fold in HCC. The depletion of SINCR1 in multiple HCC cell lines evokes a strong proliferation defect. SINCR1 depleted cells are arrested in G1/S resulting in a senescence-like phenotype. This strong phenotype is supported by proteomics profiles of SINCR1-depleted cells using triple-label SILAC experiments. Depletion of SINCR1 led to downregulation of genes such as CCNB1, TOP2A, CDK2, KIF11, MKi-67 and TYMS confirming the link to cell cycle processes. An *in vivo* RNA Affinity Purification approach identified direct interaction partners of SINCR1 with known roles in regulating G1/S transition and promoting Senescence. Further validation of these interaction partners will unravel its molecular mechanism in further detail. Our findings highlight SINCR1 as a crucial regulator of cancer cell proliferation and senescence in HCC

P16

Molecular mechanisms of Bdp1 in TFIIIB assembly and RNA polymerase III transcription initiation

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Eukaryotes encode at least three distinct RNA polymerases that transcribe the genome into functional and regulatory RNAs. Among them, RNA polymerase III (Pol III) synthesizes a diverse pool of abundant RNAs including tRNAs and the U6 spliceosomal RNA. Initiation of transcription by Pol III requires the activity of the initiation factor TFIIIB, a complex formed by Brf1 (or Brf2, B-related factor), TBP (TATA-binding protein), and Bdp1. TFIIIB recruits Pol III to the promoter and supports the transition from a closed to an open pre-initiation complex, a process dependent on the activity of the Bdp1 subunit. While some transcription factors are shared between Pol I, II and III, Bdp1 is unique to the Pol III system. Structural data showed that Bdp1 utilizes similar binding surfaces as the Pol II transcription factors TFIIA and TFIIF that promote the stability of the complex and suggest an auxiliary role in promoter opening. While Bdp1 does show DNA interactions similar to bacterial sigma factors it does not induce DNA melting alone, supporting the idea of a concerted interaction with Pol III subunits. Single-molecule FRET measurements on immobilized and freely diffusing molecules can provide information beyond the static image of the crystal structure. Here, we used time resolved single-molecule FRET measurements to gain insights into the architecture and dynamic assembly of TFIIIB on a U6 promoter [1]. Our measurements revealed that TBP only transiently binds and bends the U6 promoter (complex lifetime of 0.3 s). This is reminiscent of the dynamic archaeal TBP-promoter DNA interaction [2]. In contrast, the interaction of TBP with Pol II promoters lasts for minutes to hours. The transient TBP-DNA interaction is stabilized by Brf2 (complex lifetime of 6.5 min), locking the promoter in a single bent conformation. Subsequent binding of Bdp1 further stabilizes the complex to a lifetime of 9 min but does not alter the DNA conformation. These data suggest that additional initiation factors like Brf1/2 and Bdp1 evolved to stabilize the pre-initiation complex and to ensure efficient transcription from a subset of promoters.

[1] J. Gouge, N. Guthertz, K. Kramm, O. Dergai, G. Abascal-Palacios, K. Satia, P. Cousin, N. Hernandez, D. Grohmann, and A. Vannini, 'Molecular mechanisms of Bdp1 in TFIIIB assembly and RNA polymerase III transcription initiation', *Nat. Commun.*, vol. 8, no. 1, p. 130, 2017.

[2] A. Gietl, P. Holzmeister, F. Blombach, S. Schulz, L. V. Von Voithenberg, D. C. Lamb, F. Werner, P. Tinnefeld, and D. Grohmann, 'Eukaryotic and archaeal TBP and TFB/TF(II)B follow different promoter DNA bending pathways', *Nucleic Acids Res.*, vol. 42, no. 10, pp. 6219–6231, 2014

P17

Global transcriptional start site mapping in *Pyrococcus furiosus*: Transcriptional noise or part of archaeal gene regulation?

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Occupying a key position in the tree of life, archaeal organisms and their complex transcriptomes share molecular characteristics with Eukarya and Bacteria. We used differential RNA-seq (dRNA-seq)[1] to map transcriptional start sites (TSS) in a newly annotated genome of the hyperthermophilic archaeon *Pyrococcus furiosus (Pfu)*. To reach maximum transcriptome coverage, sequencing cDNA libraries were generated from RNA isolated from cells under 9 different growth conditions. Mapping results were complemented with a single-nucleotide resolution map of the whole transcriptome with a 6000 fold sequence coverage. In addition, TSS of genes with varying 5`-untranslated region (5`-UTR) lengths were confirmed using primer extension analysis. Using TSSpredator[2], more than 5800 TSS for about 2000 annotated genes have been identified and categorized as primary (pTSS), secondary, internal or antisense transcripts according to their relative gene position. Only 968 pTSS (~50% of all genes) have been detected with a median 5`-UTR length of 27 nt. Archaeal promoter motifs, consisting of a BRE-element and a TATA-box, were present for all 4 categories of TSS. Genes with strong promoters tend to be leaderless. Overall, extensive RNA-based regulation is consistent with results of other archaeal model organisms. Differential RNA-seq further revealed the presence of a bi-directional promoter motif upstream of almost 30% of all pTSS. So far, this has not been reported in archaea, while it is widespread in eukaryotic species[3,4]. A question that remains is, to what extent *Pfu´*s densely packed genome and low GC content (41%) result in these promoters, leading to transcriptional noise or if it is an energy efficient way for transcriptional regulation[5].

[1] Sharma, C. M., & Vogel, J. (2014). Differential RNA-seq: The approach behind and the biological insight gained. *Current Opinion in Microbiology*

[2] Dugar, G., Herbig, A., Förstner, K. U., Heidrich, N., Reinhardt, R., Nieselt, K., & Sharma, C. M. (2013). High-Resolution Transcriptome Maps Reveal Strain-Specific Regulatory Features of Multiple Campylobacter jejuni Isolates. *PLoS Genetics*

[3] Xu, Z., Wei, W., Gagneur, J., Perocchi, F., Clauder-Münster, S., Camblong, J., … Steinmetz, L. M. (2009). Bidirectional promoters generate pervasive transcription in yeast. *Nature*

[4] Jin, Y., Eser, U., Struhl, K., & Churchman, L. S. (2017). The Ground State and Evolution of Promoter Region Directionality. *Cell*

[5] Lloréns-Rico, V., Cano, J., Kamminga, T., Gil, R., Latorre, A., Chen, W.-H., … Lluch-Senar, M. (2016). Bacterial antisense RNAs are mainly the product of transcriptional noise. *Science Advances*

P18

NMR analysis conformational dynamics during UsnRNP assembly

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pICln is a highly conserved chaperone, which regulates the assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs). These are the building blocks of the spliceosome and thereby plays a crucial role in splicing of cellular pre-mRNAs. A hallmark of the snRNPs is a seven-membered ring of Sm proteins that binds to a uridine-rich RNA sequence in the U snRNAs. The biogenesis of snRNPs is tightly controlled and involves additional factors that ensure fidelity of correct snRNP assembly. One of these proteins, pICln assembles sub complexes of Sm proteins in the cytosol and mediates correct assembly of the complete Sm ring by the SMN (survival motor neuron) complex. We are studying the role of conformational dynamics and flexibility within intermediate complexes involving pICln, a subset of Sm proteins and various components of the SMN complex using NMR-spectroscopy. Furthermore, we would like to understand the role of the SMN Tudor domain and its interaction with the dimethylated arginines in the 8S complex and U snRNA binding. Preliminary results show that isotope-labelled pICln can be expressed, purified and incorporated into Sm protein complexes. To better understand the structural dynamics of pICln in these processes, we are using solution NMR relaxation experiments and small angle scattering experiments to study the role of pICln in the complex assembly

P19

SLAM-FRET: surveying site-specifically labelled eukaryotic biomolecular complexes on the single molecule level

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In the cell, biomolecules constantly form new interactions or dissociate from bindings partners to fulfill the myriad of tasks required for the survival of a cell. Misregulation of these processes often causes diseases. Oftentimes, conformational changes in a biomolecule initiate complex formation, dissociation or functional transitions. In order to exploit these processes for biotechnical applications or to target these reactions with suitable inhibitors, it is necessary to understand the detailed molecular mechanisms. In this context, single-molecule measurements, like single molecule FRET (smFRET), allow the precise and time-resolved interrogation of biological interactions and conformational changes on the single molecule level. In order to carry out fluorescence-based single-molecules studies, it is necessary to equip them with an organic fluorophore. Site-specific labelling of biological molecules is a challenging task, especially if human proteins are to be studied. Often it is not possible to produce eukaryotic proteins in a recombinant manner due to their complex posttranslational modifications essential for protein function preventing traditional fluorophore engineering approaches. To overcome these problems, we have established the site-specific labelling of endogenous mammalian proteins for single-molecule FRET measurements, a method we termed SLAM-FRET (site-specific labeling of mammalian proteins for single-molecule FRET measurements). Here we show that the SLAM-FRET method can be applied to human proteins and protein complex to carry out smFRET measurements and that the FRET-derived distances are in very good agreement with structural studies. Using this technique, we succeeded to carry out single-molecule FRET measurements using fluorescently labeled human Argonaute 2 (hAgo2). hAgo2 could not be produced in a fluorescently labelled form yet and consequently, single-molecule studies that follow the conformational changes of this protein throughout its activity cycle are lacking. Labelling two amino acids in hAgo2, it was possible to measure intramolecular FRET, which reports on an interdomain distance in hAgo2. Furthermore, we measured intermolecular FRET between hAgo2 and an interaction partner, in this case a fluorescent labeled guide RNA. Notably, the modified hAgo2 variants were fully posttranslationally modified and active. We also extended our method to survey a human protein complex via FRET, namely the RNA polymerase II subunits RPB4/7

P20

The C-terminal domain of *S. cerevisiae* Net1 is a potent activator of RNA polymerase I transcription in vivo and in vitro

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RNA polymerase I (Pol I) transcribes ribosomal DNA (rDNA) producing a precursor of the large ribosomal RNAs (rRNAs) in all eukaryotes. rRNA transcription is an essential process and accounts for more than half of total cellular transcription in proliferating cells. To achieve this high transcriptional output, a specific set of protein factors has evolved to support Pol I transcription initiation. Whereas some of these factors are highly conserved from yeast to human, others are conserved rather at the functional and structural level. In *S. cerevisiae* (hereafter called yeast) a minimal set of transcription factors composed of Rrn3, which associates with Pol I to form the initiation competent polymerase, and a three subunit complex called core factor support promoter dependent transcription *in vitro*. Another six subunit complex called upstream activating factor, which forms a complex with TATA binding protein, is required for Pol I transcription *in vivo*. Previously, it has been reported that a large protein, Net1, stimulates cell growth correlating with its ability to activate Pol I transcription. Net1 is a multifunctional protein, which fulfills additional important roles in regulating the cell cycle and silencing of transcription by RNA polymerase II at the rDNA locus. The mechanism by which Net1 stimulates Pol I transcription is unknown.

We show, that the Pol I stimulating function of Net1 can be attributed to an individual domain within the protein. Thus, the very C-terminal domain (CTD) of Net1, comprised of only 139 amino acids is required for normal cell growth and sufficient to partially rescue the growth defect observed in the absence of full-length Net1. In good correlation, the CTD enhances Pol I loading on rRNA genes *in vivo* and promoter-dependent transcription in a minimal transcription system *in vitro*. Interestingly, phosphorylation of the CTD appears to modulate its potential to stimulate Pol I transcription. The data suggests, that the Net1-CTD might be an important regulator of Pol I transcription and cell proliferation. Possible mechanisms how the CTD may influence Pol I transcription are discussed.

P21

A novel function of the La-related protein LARP7 in the modification of U6 snRNA

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The Lupus autoantigen La and the La-related proteins (LARPs) are RNA-binding proteins (RBPs) which exert their functions within a multitude of pathways in RNA biology. Of particular relevance is the interaction of the La protein with the 3'-end of all nascent RNA polymerase III (Pol III) transcripts, which has not merely a protective role but also supports the correct folding of these RNAs.

In terms of protein domain organization, as well as substrate binding characteristics, genuine La is most closely related to the LARP7 protein. So far, LARP7 has been mainly described as a component of the 7SK small nuclear ribonucleoprotein (snRNP) complex which negatively regulates RNA polymerase II (Pol II) by sequestering the positive transcription elongation factor b (P-TEFb). Within the 7SK snRNP, LARP7 stably associates with the 3'-end of the 7SK RNA, an approximately 330 nucleotides long non coding RNA transcribed by Pol III. In our studies we discovered a novel, 7SK snRNP-independent function of LARP7. We show that LARP7 specifically interacts with the spliceosomal U6 snRNA as well as with the small nucleolar RNAs (snoRNAs) which direct the 2'O-methylation of U6. Importantly, our investigations indicate that in the absence of LARP7 significantly less 2'O-methylations are deposited on the U6 snRNA. We finally analyze the impact of LARP7 depletion on the spliceosome.

P22

Circular RNA ZNF609 promotes the progression of colorectal cancer

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Non-coding RNAs can function as potent gene regulators and consequently, such RNA molecules have been implicated in many diseases including different types of cancer. Using an intrasplenic tumor model for colorectal cancer, we have identified several long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs) differentially expressed in primary tumors and liver metastases suggesting a role of these RNAs in tumor progression. Of the circRNA candidates, we have identified circular RNA ZNF609 (circZNF609), which is up-regulated in primary tumors as well as liver metastases in our mouse model. We developed a method for knocking down and overexpression of circZNF609 and established inducible stable cell lines for in vivo analyses. In a xenograft mouse model, overexpression of circZNF609 promotes colorectal cancer progression. Interestingly, we found that circZNF609 encodes for small peptides, which could potentially have function in tumor development. Using Flag reporter constructs and mutagenesis, we found that several ATGs (even exogenous ATGs) can be used to initiate translation from circZNF609. Moreover, circZNF609 protein localizes to the cytoplasm in several tested cell lines while the parental ZNF609 protein exclusively localizes to the nucleus. Mechanistically, mass spec analysis identified several interaction partners which bind to both circZNF609 and ZNF609 proteins suggesting an interesting mode of circZNF609 functions. Currently, we are developing methods for rescuing the phenotype of circZNF609 depletion and investigating further circZNF609 mechanisms.

P23

CHD3 and CHD4 form distinct NuRD complexes with different yet overlapping functionality

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CHD3 and CHD4 (Chromodomain Helicase DNA binding protein), two highly similar representatives of the Mi-2 subfamily of SF2 helicases, are coexpressed in many cell lines and tissues and have been reported to act as the motor subunit of the NuRD complex (nucleosome remodeling and deacetylase activities). Besides CHD proteins, NuRD contains several repressors like HDAC1/2, MTA2/3 and MBD2/3, arguing for a role as a transcriptional repressor. However, the subunit composition varies among cell- and tissue types and physiological conditions. In particular, it is unclear if CHD3 and CHD4 coexist in the same NuRD complex or whether they form distinct NuRD complexes with specific functions. We mapped the CHD composition of NuRD complexes in mammalian cells and discovered that they are isoform-specific, containing either the monomeric CHD3 or CHD4 ATPase. Both types of complexes exhibit similar intranuclear mobility, interact with HP1 and rapidly accumulate at UV-induced DNA repair sites. But, CHD3 and CHD4 exhibit distinct nuclear localization patterns in unperturbed cells, revealing a subset of specific target genes. Furthermore, CHD3 and CHD4 differ in their nucleosome remodeling and positioning behaviour in vitro. The proteins form distinct CHD3- and CHD4-NuRD complexes that do not only repress, but can just as well activate gene transcription of overlapping and specific target genes.

P24

Composition of the *Arabidopsis* RNA polymerase II transcript elongation complex reveals interplay of elongation and mRNA processing factors

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Transcript elongation factors (TEFs) are a heterogeneous group of proteins that control the efficiency of transcript elongation by RNA polymerase II (RNAPII) of subsets of genes in the chromatin context. Using reciprocal tagging in combination with affinity-purification and mass spectrometry, we demonstrate that the TEFs SPT4/SPT5, SPT6, FACT, PAF1-C and TFIIS co-purified with each other and elongating RNAPII, while P-TEFb was not among the interactors. Additionally, NAP1 histone chaperones, ATP-dependent chromatin remodelling factors and some histone modifying enzymes including Elongator were repeatedly found associated with TEFs. Analysis of double-mutant plants defective in different combinations of TEFs revealed genetic interactions between genes encoding subunits of PAF1-C, FACT and TFIIS, resulting in synergistic/epistatic effects on plant growth/development. The analysis of sub-nuclear localisation, gene expression and chromatin-association did not provide evidence for an involvement of the TEFs in the transcription by RNAPI (and RNAPIII). The proteomics analyses showed also multiple interactions between the transcript elongation complex and factors involved in mRNA splicing and polyadenylation including an association of PAF1-C with the polyadenylation factor CstF. Our data establish that the *Arabidopsis* RNAPII transcript elongation complex represents a platform for interactions among different TEFs as well as for coordinating ongoing transcription with mRNA processing.

P25

Translational regulation of pollen development and pollen tube growth; implications for male-female communication

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The male gametophyte, highly organized haploid flower organ, offers an unique opportunity to analyze development and differentiation of single haploid cells, cell-cell interactions and recognition, cellular polarity and pollen tube tip growth. Transcriptional and posttranscriptional control of gene expression play a vital role during tobacco pollen maturation and tube growth. The need for a high rate of translation during pollen tube growth suggests a demand for a robust storage system that could withstand a long-term storage and transport, ongoing cellular morphogenesis, and yet deliver the message efficiently accompanied with instant translation. A number of pollen genes showed apparent expression discrepancy at mRNA and protein levels and their respective transcripts were shown to be associated with long-term stored ribonucleoprotein particles. Similarly to the role played in growing mammalian neurons, these particles represent pre-loaded complex machinery devoted to mRNA processing, transport, subcellular localization and protein synthesis. Here, we present functional, transcriptomic and proteomic characterisation of pollen storage ribonucleoprotein particles. In particular, we aimed to integrate our knowledge on the categorization of translationally regulated transcripts in developing pollen and to identify the mode of action of the translational repression and derepression of mRNAs stored in developing pollen and which have gradually activated during the progamic phase. We also discuss the selective activation of particular transcripts following male-female interaction in semi-in vivo conditions.

Acknowledgement: The authors gratefully acknowledge the financial support from Czech Science Foundation (projects 13-06943S, 15-16050S, 17-23183S).

P26

Discovery of RNA-protein complexes by Grad-seq

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RNA-binding proteins (RBPs) are important factors in the regulation of gene expression. In bacteria, three major families of regulatory RBPs are known: Hfq, ProQ and CsrA, all of which act by regulating gene expression on mRNA level. While Hfq and ProQ facilitate binding of small RNAs (sRNAs) to their target mRNAs and thereby lead to regulation, CsrA binds directly to mRNAs, altering their translation and stability. sRNAs are also a key factor in CsrA regulation, as they are able to sequester CsrA away from its mRNA targets. Furthermore, RBPs can be essential factors for bacterial virulence as it was shown for Hfq in the major human pathogen *Salmonella* Typhimurium. However, many bacterial species lack one or more of these three well-studied RBPs even though they express regulatory sRNAs, suggesting the existence of currently unknown RBPs. Using gradient profiling by sequencing (Grad-seq), we are investigating the existence of overlooked RBPs. For this, whole bacterial cell lysates are run on a linear glycerol gradient, leading to partitioning of all soluble content. Since Grad-seq is performed under native conditions, RNA-protein complexes stay intact and sediment as a whole according to their biochemical properties. These interactions can then be investigated by fractionation of the gradient followed by RNA-seq and mass spectrometry of each of those fractions. The combined analysis of the resulting data sets allows us to draw conclusions which RNAs might interact with which proteins.

P27

Translational repression of *nanos* mRNA in early fly oogenesis

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Tissue homeostasis requires a fine balance between germ cell maintenance and differentiation. In the female germline of *D. melanogaster* two to three germline stem cells (GSCs) reside at the tip of the germarium. Signaling from the surrounding tissue defines a stem cell niche that prevents differentiation. After mitotic division of a GSC, the two daughter cells adapt different cell fates: one remains a GSC to replenish the pool of stem cells, the other undergoes four synchronous cell divisions with incomplete cytokinesis to generate a cyst of sixteen cells, one of which develops into the oocyte. To initiate this differentiation process, production of the stem cell factor Nanos (nos) has to be transiently repressed. This requires the female-specific master regulator protein Sex-lethal (Sxl), which binds to *nos* mRNA to repress its translation. Failure to do so results in the formation of germ line tumors and sterility. Furthermore, the proteins Bag of marbles (Bam), Benign gonial cell neoplasm (Bgcn) and Mei-P26 have been implicated in Sxl-dependent regulation of *nos*, however, their molecular function remains unexplored. Here we report that both Bam and Bgcn can repress translation upon tethering to an RNA reporter, suggesting that they play an active role in *nos* repression and tissue homeostasis in the female *Drosophila* germline.

P28

Force-dependency of Cas9 target recognition investigated with a DNA origami-based nanoscopic force clamp

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The discovery of prokaryotic adaptive immune systems consisting of the so called CRISPR–Cas (clustered regularly interspaced short palindromic repeats (CRISPR)-associated) proteins and their applicability for genome editing in eukaryotes is one of the most exciting biochemical advances within the past years. Among the many CRISPR system variants, the type II CRISPR system is most suitable for gene editing applications. It contains a single effector protein, the endonuclease Cas9, which associates with an RNA duplex consisting of a CRISPR RNA (crRNA) and a trans-acting CRISPR RNA (tracrRNA) to specifically cleave double-stranded DNA (dsDNA) targets complementary to the crRNA. Additional to sequence complementarity, a protospacer adjacent motif (PAM) is required for efficient target recognition and cleavage. For genome editing applications, single guide RNAs (sgRNA), which are chimeras of crRNA and tracrRNA are commonly used to reduce the complexity of heterologous expression in eukaryotic cells. For editing purposes, sgRNA-associated Cas9 needs to be efficiently imported into the nucleus to encounter its target sequence. However, within the nucleus, genomic DNA is seldom easily accessible but often tightly packed within chromatin regions. The wrapping and bending of DNA induces tensions on the dsDNA and this might constitute a decisive factor for Cas9 efficiency and accuracy.

We employed our recently developed DNA origami-based nanoscopic force clamp (Nickels et al., 2016) to analyze whether the extent of strain on DNA influences Cas9 binding. This approach allows high throughput force measurements in the biological relevant piconewton range on the single-molecule level. Using the nanoscopic force clamp, we exerted a defined force on the target DNA and monitored Cas9 binding on the single-molecule level using a fluorescence resonance energy transfer (FRET) signal between the donor-labeled target DNA and the acceptor-labeled Cas9-bound crRNA as readout. We can show that the binding behavior of the Cas9-sgRNA to its target DNA changes in a force-dependent manner and that high forces abolish Cas9-sgRNA binding to its target DNA. These results suggest that Cas9 is a force-sensitive enzyme and that strained DNA regions in the genomic DNA will less efficiently recognized by Cas9.

P29

Visualising sequential pre-60S maturation by chemical inhibition

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Ribosomal subunit assembly is a highly dynamic process that is initiated in the nucleus and involves the action of more than 200 trans-acting factors, some of which accompany the pre-ribosomes into the cytoplasm and must be recycled back into the nucleus. Diazaborine arrests 60S subunit maturation at an early maturation step by inhibiting the AAA-ATPase Drg1 and preventing the cytoplasmic release and recycling of shuttling pre-60S maturation factors. We have taken advantage of the rapid onset of diazaborine inhibition to investigate the structural changes occurring during export of the 60S pre-ribosomal particle using single particle cryo-electron microscopy. We will present the structures of sequential late nuclear and early cytoplasmic pre-60S intermediates, supported by a comprehensive series of immunopurification and laser scanning microscopy experiments. Our results highlight the power of combining chemical inhibition with cryo-electron microscopy to interrogate the mechanisms of 60S subunit maturation with temporal resolution

P30

Insights into the evolutionary conserved regulation of Rio ATPase activity

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Eukaryotic ribosome biogenesis is a complex and dynamic process which requires the action of numerous ribosome assembly factors. Among them, the eukaryotic Rio protein family members (Rio1, Rio2 and Rio3) belong to an ancient conserved atypical protein kinase/ATPase family required for the maturation of the small ribosomal subunit (SSU). Recent structure-function analysis suggested an ATPase-dependent role of the Rio proteins to regulate their dynamic association with the nascent pre-SSU. However, the detailed molecular mechanism that allows the timely activation of Rio catalytic activity and consequent release of Rio proteins from the nascent ribosomal subunit remained to be determined. In addition, the ancient functional origin of the Rio proteins in ribosome biogenesis has not been characterized to date. In this work we provide functional evidence supporting an ancient role of the Rio proteins in the synthesis of the archaeal SSU. Moreover, we unravel a conserved ribosomal RNA-mediated cross-talk regulatory mechanism enabling timely stimulation of Rio2 catalytic activity and subsequent release of Rio2 from the nascent pre-40S particle. Based on our findings we suggest a unified release mechanism of the Rio proteins from the pre-SSU.

P31

Toolkits for the analysis of RNA metabolism in Archaea

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Archaea are widespread organisms colonizing almost every habitat on Earth. However, the molecular biology of archaea still remains relatively uncharacterized. RNA metabolism is a central cellular process, which has been extensively analyzed in both bacteria and eukarya. In archaea, however RNA metabolism in general and ribosome biogenesis in particular are still poorly characterized. In order to shed light on these fundamental processes, we are currently establishing and applying several methodologies allowing to analyze archaeal gene expression network dynamic in unprecedented detail. A summary of non-radioactive pulse labeling of RNA, using the nucleotide analog 4-thiouracil (4TU), as well as the applications of a rDNA-reporter system will be presented.

P32

SynthesisTakesOnePause! Screening for novel inhibitors of eukaryotic ribosome biogenesis.

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Ribosomes are outstanding molecular nanomachines that synthesize all cellular proteins and hence form the basis for cellular growth. Eukaryotic ribosome biogenesis, an enormously intricate and highly dynamic process, requires not only 80 ribosomal proteins and four ribosomal RNAs (rRNAs) but more than 200 ribosome assembly factors. In the course of this process these assembly factors perform molecular rearrangements, rRNA processing and modifications or are involved in the nuclear export of maturing ribosomal particles. Due to the high complexity and immense speed of ribosome synthesis, the specific functions of most ribosome assembly factors remain still elusive.
A valuable tool to gain deeper insights into these mechanisms is to block the rapidly ongoing ribosome formation at a certain step. To this end, we developed a screen to identify novel inhibitors that target either the 40S or 60S ribosomal subunit maturation. We discovered several compounds blocking ribosome biogenesis leading to various rRNA processing defects. Treatment with these individual drugs might provide a well-equipped toolbox to study the process with high temporal resolution.

P33

Identification of Circular RNA degradation pathways

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Circular RNAs (circRNAs) are highly abundant and evolutionary conserved non-coding RNAs. Several evidences indicate that circRNAs can regulate gene expression by titrating microRNAs or RNA binding proteins, controlling transcription and interfering with splicing. Although the biogenesis of circRNAs is considerably well understood, an intriguing question remains how circRNAs are ultimately degraded, as they are stable and resistant to exonucleolytic degradation. We aim to identify the cellular degradation pathways of circRNAs and the responsible endonucleases by a biochemical approach. Therefore, we first selected circRNA candidates and validated their expression in different cell lines. Then, using an enzymatic ligation method, we performed *in vitro* synthesis of selected circRNAs and confirmed their circularity. Finally, we measured the half-lives of the synthetic circRNAs in different cell lysates. Our data confirm the high stability of circRNAs and suggest that circRNAs are more sensitivity to degradation in cytoplasmic extracts. Altogether, our studies elucidate a new and fascinating aspect of circRNA biology, which has not been analysed so far.

P34

Triplexes on Nucleosomes: bases for chromatin regulation of triplex function

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The epigenetic landscape of chromatin and its associated gene expression programs is modulated through the interaction with non-coding RNAs (ncRNAs). However, the molecular mechanisms that allow ncRNA targeting to chromatin, favouring its stable and sequence specific binding, are still unclear to date. The ncRNAs can be targeted to chromatin via sequence specific binding proteins, or they do directly interact with DNA through sequence specific contacts, in form of RNA-dsDNA triple helix structures. The RNA binds to the major groove, stabilised by Hoogsteen bonds between the bases. It has been described that triplex sequences are involved in the regulation of gene expression, but experimental evidence for the formation of triplexes *in vivo* is still lacking. RNA-dsDNA triplex structures, in comparison with dsDNA, are unstable due to the charge repulsion forces and it is believed that the nucleosomes form a major obstacle for triplex formation. In the present study we analysed the effect of chromatin on the stability and sequence specific formation of a RNA-dsDNA triplex. Interestingly, we discovered that the triplex formation is stabilised by the nucleosomes. Our results show that the histone tails, especially histone H3 tail, do specifically contribute to the stable formation of triplexes in chromatin. Triplexes are formed in the linker regions, and even in the realm of the nucleosome. Genome-wide analyses revealed functional arrangements of nucleosome positioning and putative triplex targeting sites, correlating with specific states of gene activity and epigenetic modifications.

P35

Analysis of RNA Polymerase I transcribing nucleosomal templates

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RNA polymerase I (Pol I) is a specialized RNA polymerase which transcribes rRNA genes in eukaryotes synthesizing up to 60 % of the total cellular RNA content. The detailed molecular mechanism driving Pol I transcription is still unknown. Pol I-driven transcription occurs on DNA templates with a particular chromatin structure. We analyzed the ability of Pol I to transcribe through nucleosomes in direct comparison with RNA polymerases II and III (Pol II and Pol III). Purified Pol I and Pol III from *S. cerevisiae* transcribed *in vitro* assembled nucleosomal templates more efficiently than Pol II. Pol I processivity and speed were dependent on both, nucleotide concentration, and reaction temperature. Under suboptimal assay conditions the efficiency of Pol I to elongate through a single nucleosome was significantly reduced. It was previously published that Pol I subunits A49 and A12 are involved in Pol I processivity and/or RNA cleavage, thereby affecting transcription elongation. We present data how depletion of either one of these subunits or some of their domains influence subunit composition, Pol I elongation speed, Pol I processivity and Pol I passage through a nucleosome. The results of these *in vitro* experiments are correlated with *in vivo* analyses of Pol I and Pol I mutants lacking A49- or A12-domains with regard to their ability to support cellular growth or rDNA transcription.

P36

Drosophila Sister of Sex-lethal antagonizes Sxl-lethal-dependent splicing to maintain a male-specific gene expression pattern

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In *Drosophila* female development is governed by a single RNA-binding protein, Sex lethal (Sxl), that controls the expression of key factors involved in dosage compensation, germline homeostasis and the establishment of female morphology and behaviour. Sxl expression in female flies is maintained by an auto-regulatory, positive feedback loop with Sxl controlling splicing of its own mRNA. Until now, it remained unclear how males completely shut down the Sxl expression cascade and protect themselves against runaway protein production.

Here we identify the protein Sister of sex lethal (Ssx) as an antagonist of Sxl auto-regulatory splicing. Sxl and Ssx have a comparable RNA-binding specificity and compete for RNA regulatory elements present in the Sxl transcript. Ablation of the *ssx* gene results in a low level of productive Sxl mRNA splicing in male flies; and in cultured *Drosophila* cells, Sxl-induced changes to alternative splicing can be reverted by the expression of Ssx. In sum, this demonstrates that Ssx safeguards male animals against Sxl protein production to establish a stable, male-specific gene expression pattern.

P37

RNA-binding proteins in the male gametophyte

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Pollen, an extremely reduced bi-cellular or tri-cellular male reproductive structure of flowering plants, serves as a model for numerous studies covering a wide range of developmental and physiological processes. The pollen development and subsequent progamic phase represent two fragile and vital phases of plant ontogenesis, and pollen was among the first singular plant tissues thoroughly characterised at the transcriptomic level. Posttranscriptional control of gene expression including RNA accumulation and storage plays a vital role during tobacco pollen maturation and tube growth. Storage ribonucleoprotein particles were described in pollen and pollen tubes of tobacco and were shown to comprise ribosomal subunits, translationally silent mRNAs and RNA processing proteins. Following our previous transcriptomic studies, we identified several members of RNA-binding protein family involved in RNA processing and metabolism. Analysed proteins were shown to localise to male gametophyte vegetative or sperm cells and roots. Here we present functional characterisation and tissue-specific localisation of four from six pollen-expressed homologs (RNP1-6) in generative tissues.

The authors thankfully acknowledgement the financial support from Czech Science Foundation (grants No. 13-06943S and 15-16050S).

P38

eIF2 assembly by Cdc123: mechanism and link to cell proliferation control

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eIF2 is a central player in the initiation of mRNA translation. In its active, GTP-bound form eIF2 recruits the initiator methionyl-tRNA to the ribosome, participates in mRNA scanning and start codon recognition. eIF2 also serves an important regulatory function as part of the integrated stress response, where phosphorylation of eIF2 reduces global protein synthesis, but up-regulates the mRNA translation of a stress-responsive transcription factor gene. eIF2 is a complex of 3 protein subunits. Surprisingly, however, eIF2 heterotrimer formation does not occur autonomously in vivo, but requires a dedicated assembly factor called Cdc123, which binds the un-assembled eIF2-gamma subunit. Cdc123 is conserved among eukaryotic organisms and essential for cell viability in yeast and human cells. As indicated by recent structural studies, Cdc123 resembles ATP-Grasp enzymes and binds ATP. To further define the mechanism by which Cdc123 assembles the eIF2 complex, we studied various mutant versions of eIF2-gamma. The alpha- and beta-subunits bind to separate sites on eIF2-gamma. Mutation of either site disrupted binding of the respective subunit, but left binding of the other subunit intact. In contrast, a mutation in the Cdc123 binding site located in the C-terminal domain III of eIF2-gamma disrupted the binding of both, the alpha- and beta-subunits, to eIF2-gamma. These and further data suggest that Cdc123 may reshape eIF2-gamma by an allosteric mechanism to expose the alpha and beta binding sites. Interestingly, a mutation in eIF2-gamma close to the putative Cdc123 binding site causes a complex neurological disease in humans. Experiments are under way to check potential assembly defects of this mutated human eIF2-gamma version. Cdc123 was first identified in mammals due to its role in supporting cell proliferation. We confirmed that Cdc123 is needed for cell cycle entry in yeast. Moreover, depletion of eIF2 subunits caused a G1-specific cell cycle arrest in yeast. Thus, normal rates of protein synthesis and eIF2 function appear to be of particular importance for cell cycle entry. To better understand the eIF2-cell cycle linkage, we aim to determine how reduced eIF2 function affects the translation of various mRNAs in yeast by RNA sequencing of polysome-associated mRNAs.

P39

The *S. pombe* mRNA decapping complex recruits cofactors and an Edc1-like activator through a single dynamic surface

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The removal of the 5' 7-methylguanosine mRNA cap structure (decapping) is a central step in the 5'-3' mRNA degradation pathway and is performed by the Dcp1:Dcp2 decapping complex. The activity of this complex is tightly regulated to prevent premature degradation of the transcript. Here, we establish that the aromatic groove of the EVH1 domain of Schizosaccharomyces pombe Dcp1 can interact with proline-rich sequences in the exonuclease Xrn1, the scaffolding protein Pat1, the helicase Dhh1, and the C-terminal disordered region of Dcp2. We show that this region of Dcp1 can also recruit a previously unidentified enhancer of decapping protein (Edc1) and solved the crystal structure of the complex. NMR relaxation dispersion experiments reveal that the Dcp1 binding site can adopt multiple conformations, thus providing the plasticity that is required to accommodate different ligands. We show that the activator Edc1 makes additional contacts with the regulatory domain of Dcp2 and that an activation motif in Edc1 increases the RNA affinity of Dcp1:Dcp2. Our data support a model where Edc1 stabilizes the RNA in the active site, which results in enhanced decapping rates. In summary, we show that multiple decapping factors, including the Dcp2 C-terminal region, compete with Edc1 for Dcp1 binding. Our data thus reveal a network of interactions that can fine-tune the catalytic activity of the decapping complex.

P40

Evolution of RNA-binding proteins across different yeast species

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Unlike prokaryotic cells, where translation is directly coupled to transcription; eukaryotic cells are compartmentalized. The genetic material is preserved inside the nucleus, and mRNAs are subject to several post-transcriptional processes before being translated into proteins in the cytoplasm. RNA-binding proteins (RBPs) are key regulators of these tightly controlled gene regulatory network. RBPs are able to recognize RNA-recognition elements (RREs) on their target RNA, which may range from sequence motifs to structured three-dimensional folds. Thereby RBPs influence gene expression post-transcriptionally by regulating the "fate" of the RNA at different levels: transcription, capping, splicing, polyadenylation, cellular localization, translation, and RNA turnover. In the last years new methods were established to identify RBPs *in vivo*. We here apply the previously developed interactome capture approach to identify RBPs across a monophyletic group of 10 different yeast species covering 500 million years of evolution. Because of their relatively low complex genomes, yeast have been a widespread model system in biology. However, while all species originate from a common ancestor, they have taken different directions for their post-transcriptional gene regulation. For example, *S. pombe* preserved a more complex splicing machinery and alternative splicing patterns not found in *S. cerevisiae*. A comparative study based on interactome capture data allows to characterize RNA-binding proteins by their ability to bind mRNA rather than just based on overall sequence homology. We have previously shown by phylointeractomics that sequence homology does not necessarily equate to functional homology and a few amino acid exchanges can change a proteins' capability to bind to nucleic acids. Besides identification of divergent molecular evolution, we also aim to uncover new RBPs based on evolutionary conservation, and to trace the evolution of RBPs and their RBDs across several species.

P41

Transcriptome analysis of molecular subtypes of Glioma stem cells reveal differential expression of circRNAs and lncRNAs

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World Health Organization classifies Glioblastoma multiforme (GBM) as Grade IV astrocytoma, with a median survival rate of just 14 to 16 months. The solid tumours in GBM constitutes of a highly heterogeneous population of cells. Among these are a small group of cells termed brain tumour initiating cells (BTICs) or cancer stem cells (CSCs). These are often thought to be the contributors of resistance towards radiation and chemotherapy and hence relapse. The BTICs often contain a higher population of CD133 expressing cells; this can be used to enrich the stem cell-like cells. Recent studies also revealed that GBM from various patients could be classified into four molecular subtypes based on molecular signatures and mutations. These molecular subtypes have different degrees of response to therapy and hence varying degrees of survival rates among patients. Contribution of non-coding genome towards such a complex disease still requires investigation. In the present study, patient samples were classified into the two major subtypes, Mesenchymal and Proneural, based on marker gene expression. With special, focus on long non-coding RNAs (lncRNAs) and the circular RNAs (circRNAs) us performed RNA sequencing on cells raised from glioma patients. Primary cells raised in culture were sorted into CD133+ and CD133- cells or differentiated in-vitro. A whole transcriptome analysis of these fractions revealed the presence of lncRNAs with differential expression among subtypes and fractions. Unlike lncRNAs only a very few circRNAs exhibited differential expression. We are currently performing additional validation in additional cells from patient samples and functional characterisation on select lncRNAs and circRNAs

P42

The dedicated chaperone Acl4 escorts Rpl4 to its nuclear pre-60S assembly site

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In eukaryotic cells, most ribosomal proteins need to be transported from the cytoplasm where they are synthesised to their ribosome assembly site in the nucleus. Due to the high abundance and difficult physicochemical properties of r-proteins, their correct folding and fail-safe targeting to the assembly site depends largely on general, as well as highly specialized, chaperone and transport systems. Many r-proteins contain universally conserved or eukaryote-specific internal loops and/or terminal extensions, which were shown to mediate their nuclear targeting and association with dedicated chaperones in a growing number of cases. The 60S r-protein Rpl4 is particularly interesting since it harbours a conserved long internal loop and a prominent C-terminal eukaryote-specific extension. Here we show that both the long internal loop and the C-terminal eukaryote-specific extension are strictly required for the functionality of Rpl4. While Rpl4 contains at least five distinct nuclear localization signals (NLS), the C-terminal part of the long internal loop associates with a specific binding partner, termed Acl4. Absence of Acl4 confers a severe slow-growth phenotype and a deficiency in the production of 60S subunits. Nevertheless, Acl4 is not essential to produce ribosomes and strains lacking Acl4 quickly accumulate mutations that completely suppress their slow growth phenotype. By studying these mutations, we hope to uncover regulation pathways, protection mechanisms or assembly mechanisms of Rpl4

P43

Reconstitution of the Pol I preinitiation complex in *S. cerevisiae*

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In fast growing cells, up to 60% of total transcription is devoted to ribosomal RNA (rRNA) synthesis. In eukaryotes a specialized enzyme, RNA polymerase I (Pol I), synthesizes a polycistronic precursor rRNA which is the 35S rRNA in the yeast *S. cerevisiae.* This primary transcript, is processed to the mature 18S, 5.8S and 25S rRNAs. The transcription initiation factor CF (core factor) together with Pol I and the Pol I-bound initiation factor Rrn3 support Pol I-promoter dependent *in vitro* transcription at a basal level. Binding of Upstream-Activating-Factor (UAF) and TATA-Binding-Protein (TBP) to the upstream promoter element (UE) enhance Pol I transcription initiation *in vitro* and are required for Pol I-dependent rRNA synthesis *in vivo*. Recent studies presented high-resolution structures of a minimal Pol I-Rrn3-CF complex, leading to hypotheses about the mechanism of Pol I transcription initiation. Nevertheless, structural information about the complete pre-initiation complex is lacking and the detailed mechanism how Pol I recognizes its promoter and how it achieves its high transcriptional output remain to be further investigated. It was suggested that UAF, TBP and CF bind the promoter and form a platform to which Rrn3 bound Pol I is recruited. Earlier studies assigned positions -38 to +8 relative to the transcription start site as core promoter element (CE) responsible for CF binding and start site selection, and the activating upstream (cis)-element UE from -155 to -39. We developed a reconstituted *in vitro* transcription system using highly purified factors supporting promoter-dependent transcription and use gel shift assays to better define DNA interaction sites and functional cis-elements of the Pol I promoter. We want to understand the concerted assembly of UAF and TBP and their interaction with CF, Rrn3 and Pol I to form the full Pol I preinitiation complex as well as their role in stimulating Pol I activity. We discuss functional requirements of Pol I to initiate transcription considering the specific role of Pol I transcription factor UAF and TBP.

P44

When a pre-ribosome hits a roadblock: Using inhibitors to study the maturation of pre-ribosomal particles

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Eukaryotic ribosome biogenesis has emerged as one of the most energy consuming and elaborate cellular pathways. More than 200 trans-acting factors are needed to guarantee the correct processing and assembly of the individual RNA and protein components of a mature ribosome. All of these factors have to act in a timely and spatially strictly concerted manner to be able to produce a functioning ribosome at the end of the assembly line. One of the major challenges that slows down the elucidation of this pathway is its fast pace since the complete maturation of the rRNA, the major component of the ribosome, can be finished in minutes. Most available genetic and biochemical techniques only allow rather static pictures of individual steps representing only a glimpse of the dynamics and temporal order of events. Therefore we make use of low molecular weight inhibitors to induce roadblocks at specific points in the maturation of the ribosomal subunits. This allows a focused view on the final processing steps of the particles downstream of the block. Among others this enables us to gain new insights in the formation of export competent pre-ribosomes.

P45

Guardians of the ribosomal proteins

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The ribosome, an outstanding molecular machine, synthesizes all cellular proteins and is assembled as well as matured in a complex process involving more than 200 ribosome biogenesis factors. In the course of this process, most ribosomal proteins have to be transported into the nucleus and incorporated into the pre-ribosomal particles. Thereby, chaperones and importins protect the ribosomal proteins from aggregation and guard them from the cytoplasm through the Nuclear Pore Complex into the nucleus.

Up to the present only a few chaperones are identified that show an interaction with ribosomal proteins, but it is still unknown how chaperones escort these proteins efficiently to their location of assembly. In this study, we are looking for unknown specific chaperones, importins and other non-ribosomal interaction partners of ribosomal proteins to gain further insights into the mechanism of their assembly path. All ribosomal proteins of the 40S subunit were purified via Tandem Affinity Purification and potential interaction partners were identified by mass spectrometry. Subsequently, the most promising candidates, including importins and chaperones, are characterized using biochemical, genetic and cell biological methods to verify the interplay between the interaction partner and its ribosomal protein. Here, we want to exemplarily present potential new guardians of ribosomal proteins.

P46

Translational reprogramming under ER stress

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Endoplasmic reticulum (ER) stress triggers a signaling cascade to cope with the accumulation of misfolded proteins: the unfolded protein response (UPR). It aims to reinstate cellular homeostasis by adjusting transcription and translation. If cellular homeostasis cannot be reinstated, the UPR triggers apoptosis.

In recent years, the UPR has gained widespread attention due to its involvement in many different diseases such as diabetes, Alzheimer's, or various types of cancer. In most cancers, due to rapid growth and elevated protein synthesis in nutrient limited and hypoxic environment, tumor cells often exhibit high levels of ER stress which activates the UPR. Tumor cells exploit the pro-survival signals of the UPR while avoiding apoptosis even under continuous ER stress conditions. This has also been linked to resistance of chemotherapeutic treatment (e.g. in gliomas).

Despite its broad clinical importance, qualitative and quantitative models that describe the UPR in cancer cells are still missing. The aim of our consortium (Systems Biology of the Unfolded Protein Response in Glioma, SUPR-G) is to provide these models by system-wide analyses of the UPR. In our project we focus on UPR-dependent translational reprogramming in various different cell lines and under different conditions. To this end we globally analyze translation by employing ribosomal profiling, aiming to establish a quantitative model of the UPR in glioma to reveal potential therapeutic candidates.

P47

RNA matchmakers: exploring the RNA interactome of euryarchaeal Lsm proteins

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Sm and Sm-like (Lsm) proteins are key players in RNA metabolism and are found in all domains of life. Whereas eukaryotic and bacterial Lsm homologues have been extensively studied, the function of Lsm proteins from the archaeal domain of life is not well understood. In general, Sm/Lsm proteins are known as RNA binding proteins involved in RNA biogenesis and small RNA mediated regulation of gene expression. To shed light on the mechanisms that enables Sm/Lsm proteins to associate wtih with multiple RNA interaction partners, we are studying members of the Lsm family from two hyperthermophilic archaeal organisms: *Methanocaldococcus jannaschii* (Mja) and *Pyrococcus furiosus* (Pfu). While the PfuLsm protein resembles a heptamer and is a typical representative of archaeal Sm-like proteins, the hexameric MjaLsm protein is more closely related to bacterial Hfq, a protein known as RNA chaperone that facilitates interactions between small RNAs (sRNAs) and messenger RNAs (mRNAs). Therefore, besides analysing the mechanism of interaction, we aim to identify RNA interaction partners that associate with the archaeal PfuLsm and MjaLsm variants to gain insights into differences and similarities as well as into the biological function of Lsm proteins of these closely related euryarchaeal organisms. To this end, we recombinantly expressed PfuLsm and MjaHfq in *E.coli* and purified the multimeric proteins via a strep-tag. This allowed us to i) carry out a biochemical analysis of the RNA binding and RNA chaperon functions and ii) to co-purify RNA interaction partners by incubating the proteins with total cellular RNA isolated from the respective organisms followed by high-throughput sequencing. Additionally, we follow a experimental strategy that exploits the fact that *P. furiosus (*unlike *M.jannaschii)* is one of the few archaeal organims that can be genetically manipulated. We created a mutant *P. furiosus* strain that constitutively expresses strep-tagged PfuLsm. This allows us to co-purify and sequence RNAs bound to PfuLsm in the cellular context and to compare this dataset to the RNA interactome data of the recombinant PfuLsm variant.

P48

Differential expression of promoter and enhancer-associated long non-coding RNAs and their neighboring protein-coding genes in diabetic nephropathy

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The discovery of prokaryotic adaptive immune systems consisting of the so called CRISPR–Cas (clustered regularly interspaced short palindromic repeats (CRISPR)-associated) proteins and their applicability for genome editing in eukaryotes is one of the most exciting biochemical advances within the past years. Among the many CRISPR system variants, the type II CRISPR system is most suitable for gene editing applications. It contains a single effector protein, the endonuclease Cas9, which associates with an RNA duplex consisting of a CRISPR RNA (crRNA) and a trans-acting CRISPR RNA (tracrRNA) to specifically cleave double-stranded DNA (dsDNA) targets complementary to the crRNA. Additional to sequence complementarity, a protospacer adjacent motif (PAM) is required for efficient target recognition and cleavage. For genome editing applications, single guide RNAs (sgRNA), which are chimeras of crRNA and tracrRNA are commonly used to reduce the complexity of heterologous expression in eukaryotic cells. For editing purposes, sgRNA-associated Cas9 needs to be efficiently imported into the nucleus to encounter its target sequence. However, within the nucleus, genomic DNA is seldom easily accessible but often tightly packed within chromatin regions. The wrapping and bending of DNA induces tensions on the dsDNA and this might constitute a decisive factor for Cas9 efficiency and accuracy.

We employed our recently developed DNA origami-based nanoscopic force clamp (Nickels et al., 2016) to analyze whether the extent of strain on DNA influences Cas9 binding. This approach allows high throughput force measurements in the biological relevant piconewton range on the single-molecule level. Using the nanoscopic force clamp, we exerted a defined force on the target DNA and monitored Cas9 binding on the single-molecule level using a fluorescence resonance energy transfer (FRET) signal between the donor-labeled target DNA and the acceptor-labeled Cas9-bound crRNA as readout. We can show that the binding behavior of the Cas9-sgRNA to its target DNA changes in a force-dependent manner and that high forces abolish Cas9-sgRNA binding to its target DNA. These results suggest that Cas9 is a force-sensitive enzyme and that strained DNA regions in the genomic DNA will less efficiently recognized by Cas9.

P49

Reversible protein aggregation is a protective mechanism to ensure cell cycle restart after stress

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Protein aggregation is usually considered as an irreversible process responsible for several pathologies. However, recent evidence suggests that the aggregation of an increasing number of proteins is a reversible, highly-regulated physiological mechanism used by cells to adapt to stress conditions. Despite these findings, the molecular mechanisms underlying reversible protein aggregation and its relation to irreversible aggregation have remained elusive. Here, we characterize the mechanisms controlling reversible aggregation of yeast pyruvate kinase Cdc19 upon stress. We show that aggregation of Cdc19 is regulated by oligomerization and binding to allosteric regulators. Moreover, we identify a region of low compositional complexity (LCR) within Cdc19 that is necessary and sufficient for reversible aggregation. During exponential growth, shielding the LCR within tetrameric Cdc19 or phosphorylation of the LCR prevents unscheduled aggregation, while de-phosphorylation of the LCR is necessary for reversible aggregation upon stress. Importantly, we also demonstrate that Cdc19 aggregation triggers its localization to stress granules, and modulates their formation and dissolution. Moreover, recruitment to stress granules protects Cdc19 from stress-induced degradation, suggesting that stress granules act as storage compartments for proteins necessary to restart the cell cycle after stress relief. As LCRs are also responsible for irreversible aggregation of proteins causing several diseases, we predict that understanding the molecular mechanisms regulating reversible protein aggregation through LCRs will provide important functional insights into the regulation of protein aggregation in normal physiology and in diseases.

P50

Functional and structural analyses of natively purified rDNA chromatin domains from *S. cerevisiae*

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RNA polymerase I (Pol I) transcription of the ribosomal DNA (rDNA) is the main contributor to transcription in proliferating eukaryotic cells. Pol I produces rRNA precursor molecules which are finally processed into the 3 largest ribosomal RNAs (rRNAs) forming the structural scaffold of the ribosome. To reach the high transcriptional output Pol I is a specialized enzyme with an efficient transcription machinery. Additionally, rRNA genes are present in several hundred copies in each cell. Interestingly, not all of these copies are transcribed. In dividing yeast cells about 50% of the rRNA genes are kept in a nucleosomal, transcriptionally inactive state ("closed"). The other half of the copies are usually heavily transcribed and largely devoid of nucleosomes, which is referred to as the "open" chromatin state. The different chromatin states can undergo dynamic changes. Replication, for example, converts rDNA copies in the "closed" chromatin state, whereas transcription of Pol I is required to convert "closed" into "open" copies. With our research, we aim to elucidate the interplay between Pol I transcription and rDNA chromatin structure. We chose *S. cerevisiae* as model organism, since there is a rich knowledge about the interrelationship between Pol I transcription and chromatin structure *in vivo*. To analyse these processes in molecular detail, we established a purification approach to isolate native rDNA chromatin and analyse it in its composition and structure *in vitro* (Hamperl et al. 2014). Previous analyses were limited by the fact that the isolated rDNA chromatin domains were a mixture of both chromatin states, and that the native chromatin had not been rigorously used in functional assays. Here we present strategies to separate both chromatin states biochemically. We show preliminary results of single molecule analyses yielding insights into heterogeneity and individual properties of the rDNA chromatin states. Besides, we present first results of *in vitro* transcription experiments from native chromatin templates using a minimal purified Pol I transcription system. We believe that the use of a defined *in vitro* system will add to our knowledge about an important biological process

P51

Assembly and disassembly of the SSU procesome

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The synthesis of ribosomes is an essential process in all growing cells. During ribosome biogenesis, more than 200 assembly factors (AFs) facilitate the processing and folding of the ribosomal (r) RNA as well as the incorporation of the ribosomal proteins. In yeast cells, the rRNA constitutes more than 60% of the transcribed RNA, and around 80% of total cellular transcription is dedicated to the synthesis of ribosomes. Therefore, ribosome biogenesis consumes most of the cellular resources and requires a tight regulation.

The correct production of the **s**mall **su**bunit involves the formation of the **SSU** processome. The assembly of the SSU processome requires the transient association of more than 50 AFs with the nascent RNA. The AFs establish hierarchical interdependencies for their association in the SSU processome. However, the molecular mechanisms behind the hierarchical recruitment of AFs are not clear. Some of these AFs have been found in large protein complexes not engaged in ribosome assembly. Among them, the UTP-A/t-UTP complex has been characterized as the primary binder of the nascent rRNA.

Interestingly, the protein composition of the UTP-A/t-UTP complex is not clear. Among the UTP-A components, Pol5 has not been identified in the proteomes of SSU processome purified particles. Although Pol5 has been related with the synthesis of ribosomes its role during ribosome biogenesis has not been characterized. We present a detailed functional analysis of Pol5 on pre-rRNA processing and subunit assembly. Our results suggest a role of Pol5 in the synthesis of both ribosomal subunits. We discuss possible roles of Pol5 in recycling the UTP-A complex and adjusting the production of small and large subunits.

P52

Characterization of the cooperative function of the METTL3/METTL14 methyltransferase complex and investigation of the putative RNA methyltransferase METTL8

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More than hundred different post-transcriptional RNA modifications can be found in all classes of RNA. Processing and modification steps in mRNAs include 5` capping, addition of the poly (A) tail, splicing as well as the incorporation of base modifications such as N^6-adenosine methylation (m^6A) whose investigation was reignited in the last few years. Dynamic m^6A modifications are involved in fundamental cellular processes including gene expression, meiosis and stemness by regulating translation as well as inducing mRNA instability. The m^6A landscape in mRNA is catalyzed by a heterodimeric core complex composed of METTL3 - METTL14 together with the splicing factor WTAP. Here we confirm that METTL3 is the catalytically active subunit, while the RGG domain of METTL14 is essential for substrate recognition. Furthermore, we show that the phosphorylation site S399 of METTL14 does not influence the interaction between METTL3 and METTL14 neither methylation activity of this complex. Interestingly, phylogenetic analysis indicates a cluster of the METTL proteins 2, 6 and 8, which might represent a functionally related class of methyltransferases acting on RNA. So far, the functions and the biological relevance of these proteins are unknown and thus we plan to characterize the methyltransferase like protein 8 in molecular detail by using different biochemical assays. To better understand the biological role of the METTL8, we establish monoclonal antibodies against this METTL protein, Flp - In™ T-Rex™ cell lines and gene knockout by the CRISPR/Cas9 system. This toolbox will be ideal starting points to obtain indications of binding partners, promising target RNAs and potential consensus binding sequences.

P53

A synergistic network of interactions promotes the formation of in vitro processing bodies and protects mRNA against decapping

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Cellular liquid–liquid phase separation (LLPS) results in the formation of dynamic granules that play an important role in many biological processes. On a molecular level, the clustering of proteins into a confined space results from an indefinite network of intermolecular interactions. Here, we introduce and exploit a novel high-throughput bottom-up approach to study how the interactions between RNA, the Dcp1:Dcp2 mRNA decapping complex and the scaffolding proteins Edc3 and Pdc1 result in the formation of processing bodies. We find that the LLPS boundaries are close to physiological concentrations upon inclusion of multiple proteins and RNA. Within in vitro processing bodies the RNA is protected against endonucleolytic cleavage and the mRNA decapping activity is reduced, which argues for a role of processing bodies in temporary mRNA storage. Interestingly, the intrinsically disordered region (IDR) in the Edc3 protein emerges as a central hub for interactions with both RNA and mRNA decapping factors. In addition, the Edc3 IDR plays a role in the formation of irreversible protein aggregates that are potentially detrimental for cellular homeostasis. In summary, our data reveal insights into the mechanisms that lead to cellular LLPS and into the way this influences enzymatic activity.

P54

Plasmodium falciparum Nucleosomes Exhibit Reduced Stability and Lost Sequence Dependent Nucleosome Positioning

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The packaging and organization of genomic DNA into chromatin represents an additional regulatory layer of gene expression, with specific nucleosome positions that restrict the accessibility of regulatory DNA elements. The mechanisms that position nucleosomes in vivo are thought to depend on the biophysical properties of the histones, sequence patterns, like phased di-nucleotide repeats and the architecture of the histone octamer that folds DNA in 1.65 tight turns. Comparative studies of human and P. falciparum histones reveal that the latter have a strongly reduced ability to recognize internal sequence dependent nucleosome positioning signals. In contrast, the nucleosomes are positioned by AT-repeat sequences flanking nucleosomes in vivo and in vitro. Further, the strong sequence variations in the plasmodium histones, compared to other mammalian histones, do not present adaptations to its AT-rich genome. Human and parasite histones bind with higher affinity to GC-rich DNA and with lower affinity to AT-rich DNA. However, the plasmodium nucleosomes are overall less stable, with increased temperature induced mobility, decreased salt stability of the histones H2A and H2B and considerable reduced binding affinity to GC-rich DNA, as compared with the human nucleosomes. In addition, we show that plasmodium histone octamers form the shortest known nucleosome repeat length (155bp) in vitro and in vivo. Our data suggest that the biochemical properties of the parasite histones are distinct from the typical characteristics of other eukaryotic histones and these properties reflect the increased accessibility of the P. falciparum genome

P55

New players involved in plant fertilization

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The launch of seed development in flowering plants is initiated by the process of double fertilization. Two immobile sperm cells are delivered by the vegetative pollen tube towards the female gametophyte by guidance and attraction mechanisms. After pollen tube burst the two sperm cells are release and fuse with the egg and the central cell to form the precursor cells of the two major seed components, the embryo and endosperm, respectively. The underlying mechanisms involved in pollen tube guidance and sperm cell release are not fully understood and we are looking for new regulatory elements. So far, we could identify two peptides, expressed by the synergids, the major producer of pollen tube attractants, which might be involved in pollen tube guidance or sperm cell release. Both peptides localize to the filiform apparatus and are likely to be secreted. To unravel their functions during the process of pollen tube guidance and double fertilization, we will generate and characterize mutants and use synthesized peptides in fertilization experiments.

P56

Functional characterization of an RNase III-processed small RNA with an antisense partner in the food-borne pathogen *Campylobacter jejuni*

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Transcriptome mapping using deep sequencing has revealed many conserved candidate small RNAs (sRNAs) in *Campylobacter jejuni* (1), a major cause of foodborne illness, as well as in the related gastric pathogen *Helicobacter pylori* (2). These sRNAs represent an uncharacterized layer of post-transcriptional regulation that might control pathogenesis. Here, we are characterizing a pair of overlapping antisense sRNAs of *C. jejuni*, CJ-RepG and antisense-CJ-RepG (as-CJ-RepG). We show that CJ-RepG represents a functional homologue of *H. pylori* RepG (HP-RepG), an sRNA which directly targets a length-variable G-repeat in a target mRNA 5'UTR and thereby links phase variation to post-transcriptional regulation (3). Deletion of the locus encoding CJ-RepG and as-CJ-RepG upregulates PtmG, an enzyme in the legionaminic acid flagellin-glycosylation pathway. Regulation in the deletion strain can be complemented by either CJ-RepG alone (without its partner as-CJ-RepG) or by HP-RepG. CJ-RepG and HP-RepG directly interact with a G-rich sequence near the ribosomal binding site of *ptmG* mRNA, suggesting an analogous regulatory mechanism. Although CJ-RepG and HP-RepG appear to be functionally related sRNAs that target G-rich sequences, they have very different biogenesis pathways. While HP-RepG is transcribed as a mature primary transcript from a stand-alone gene, CJ-RepG is generated by processing and also has a processed antisense partner. Both CJ-RepG and as-CJ-RepG are processed by RNase III to smaller sRNAs derived from the 3'- and 5'-ends of their primary transcripts, respectively. Processing of as-CJ-RepG, but surprisingly not of CJ-RepG, requires its antisense partner. CJ-RepG is also expressed from two promoters to produce primary transcripts with different 5'-end lengths that are processed to the same 3'-derived ~70-nt sRNA product. Understanding the biogenesis of CJ-RepG and its antisense partner will provide insight into RNase E-independent sRNA processing, as well as how sRNAs themselves might be transcriptionally and post-transcriptionally regulated by multiple signals. Varying conservation of the asRNA promoter in *Campylobacter* raises questions about the function of the asRNA and whether it can regulate additional target genes independent of CJ-RepG. Finally, CJ-RepG and its target *ptmG* target affect *C. jejuni*-colonic cell interactions in a new tissue-engineered model for studying intestinal pathogens, indicating a role for the sRNA in virulence.

P57

Structural and Functional Studies on the Role of Noc3p for Large Ribosomal Subunit Maturation in *Saccharomyces cerevisiae*

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Ribosomes are complex and highly conserved ribonucleoprotein particles that translate in cells of all domains of life messenger RNA into proteins. During ribosome production ribosomal RNA (rRNA) precursors undergo extensive processing and folding events and assemble with a large number of ribosomal proteins (r-protein) in a highly dynamic and defined manner. A multitude of small nucleolar RNAs (snoRNA) and biogenesis factors transiently interact with the maturing eukaryotic pre-ribosomal particles. Most of these factors are conserved from yeast to human and play important roles in ribosome maturation. Examples are the “Noc-proteins” Noc1p and Noc3p which contain a common homology domain and which are essential in yeast for early to intermediate steps of large ribosomal subunit rRNA maturation. Here, we analyzed the effect of Noc3p in vivo depletion on the assembly of yeast large ribosomal subunit precursors. Furthermore, we investigated how the r-protein assembly state influences pre-ribosomal recruitment and release of Noc3p. Our findings indicate that Noc3p coordinates rRNA processing with subunit assembly events in intermediate large ribosomal subunit precursors. We are currently studying by tertiary structure probing approaches possible structural transitions accompanying these Noc3p mediated maturation events and will report on the results.

P58

An improved, versatile RNA bind-and-seq method for direct assessment of RBP specificity

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RNA binding proteins (RBPs) play crucial roles in the regulation of gene expression, RNA metabolism and the response to viruses. Most RBPs contain RNA binding domains (RBDs) that recognize a coherent short sequence motif on the bound RNA and thus define the specificity of the interaction. Frequently, RBPs consist of a modular array of more than one RBD, which leads to greater flexibility of binding modes. Among the most common RBDs are the RNA recognition motif (RRM), zinc fingers, KH-domains and cold shock domains. Although the binding specificity of an increasing number of RBPs has been elucidated in recent years, the binding mode and specificity of the majority of canonical and even more so of non-canonical RBPs remains unknown.

Several techniques for the unbiased experimental assessment of the binding preferences of RBPs have been described including SELEX, RNA compete and RNA bind and seq (RBNS), in which a complex pool of RNA sequences is selected by an immobilized RBP and subsequently analyzed by microarray analysis or sequencing. We have established an improved RBNS protocol using very short (8-14mer) random sequences with only 4 invariant nucleotides for selection. After selection by the RBP of interest, the pool is read out by adapter ligation and deep sequencing. Specially designed adaptors allow for transcription of the insert and additional rounds of selection and sequencing.

We have tested our protocol with a number of purified, recombinant RBPs and find that the short insert length allows for an easy and robust detection of enriched motifs without the requirement for extensive sequencing or bioinformatic analysis. In addition we could show that it works with a whole range of protein formats and selection protocols using differently tagged or even untagged RBP preparations.

Importantly, we have been able to extend our analysis to full length RBPs overexpressed in mammalian cell cultures and even endogenous RBPs from cultured cells or tissue lysates. To this end, we immunopurify the RBP of interest using a specific antibody against the tag or protein and perform the RNA pool selection on the beads. This offers the possibility to directly analyze the specificity of RBPs purified from their native environment. A clear difference in the selected motif by the well-studied RBP hnRNPA1 between recombinant and endogenous proteins emphasizes the importance of this approach.

P59

Preliminary insights into bicoid–Exuperantia interaction

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Targeted mRNA localization in cells enables a spatially and temporally tight regulation of protein expression. This regulatory mechanism is crucial in *Drosophila melanogaster* oogenesis, first in determining the future oocyte within the stem cell niche, and subsequently in defining the anatomical axes of the egg leading to the body axes of the future adult fly. One of the canonical mRNAs in body axes formation is *bicoid*, which is produced in the nurse cells and transported to the oocyte anterior. *bicoid* is responsible for head-to-tail development of the embryo and its transport is accomplished in a RNA-protein complex dependent on the microtubule cytoskeleton and associated molecular motors. In this project, we aim at determining the role of one protein - Exuperantia (Exu) - which is part of the *bicoid*-protein complex and is involved in the localization mechanism of *bicoid* mRNA in the *Drosophila melanogaster* oocyte. Our strategy is to obtain both structural insights from purified protein as well as kinetic information of mRNA-protein interaction within the physiological setting of the oocyte. To fulfil the first aim, we cloned four different constructs of the target gene in plasmids of pET expression systems and expressed the protein in *E. coli*. We were able to express and purify one of Exu's constructs, after the optimization of the expression protocol by gene synthesis, using nickel affinity chromatography (IMAC) and ionic-exchange chromatography (IEC). The obtained protein was then concentrated to take the biomolecule into a supersaturation state and several crystallization conditions, from homemade and commercially available kits were screened using the vapor diffusion method. Obtaining good morphological diffracting crystals is considered the bottleneck of X-ray crystallography. Only after a two months incubation period and over 3500 drops, few promising hits were obtained. The putative protein crystals obtained were cryo-protected and sent for diffraction experiments at Diamond Light Source, United Kingdom. For the majority of the crystals its size was a limiting step, for some others the internal order was poor and the diffraction data did not yield a detailed structure. Presently, the biochemical information obtained allows us to uplift the study into a physiological situation and determine the interaction kinetics of Exu and *bicoid* in the oocyte, determine the dependence on the microtubule cytoskeleton more thoroughly and testing loading and transport using a *ex vivo* reconstitution approach.

P60

Establishment of monoclonal antibodies against modified RNA-bases

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More than one hundred different posttranscriptional modifications are found in all classes of RNA. Some are rather abundant and relatively well investigated, but others are infrequent and only poorly characterised. To better understand the biological roles of such modifications, we started to establish specific monoclonal antibodies against a variety of RNA base modifications. As antigens, we coupled the oxidized modified nucleosides of interest to the lysines of ovalbumin and injected them into rats. After immunization and cell fusion, the quality of the resulting hybridomas produced by the obtained fusion clones was determined in ELISA-screens, dot blot and RNA-IP experiments of total RNA. For further validation, we determined the enrichment factors of the different candidate antibodies. We performed RNA-IPs using *in vitro* transcribed RNA oligos containing either ^{32}P-UTP and the respective modified nucleotides or ^{32}P-ATP and unmodified bases. After complete digestion of the enriched RNA, the nucleotides were analysed via thin layer chromatography. The signals for the differently labelled nucleotides could then be compared and an enrichment factor could be defined. With this data, we could further characterize the candidate antibodies. Stable hybridoma cell lines were generated from the most specific and strongest binders. Using this very useful toolbox we aim at detecting specifically modified RNAs. Using RNAseq we plan to identify potential consensus sequences within modified RNA candidates, potentially necessary for the binding of writer or reader proteins. Putative consensus sequences might be ideal starting points for the identifications of catalytic enzymes that establish these modifications (writers) or proteins that recognize and bind them (readers).

P61

The ribosome biogenesis factor yUtp23/hUTP23 coordinates key interactions in the yeast and human pre-40S particle and hUTP23 contains an essential PIN domain

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One of the biggest challenges and energy-demanding processes in the cell is the production of the four mature ribosomal (r)RNA components of the small (40S) and large (60S) ribosomal subunits. During pre-rRNA processing, a number of timely and spatially regulated endo- and exonucleolytic cleavages release the mature 18S, 5.8S and 25S/28S rRNAs from a single precursor molecule. Three early pre-rRNA cleavages in yeast (y) and human (h) 18S rRNA maturation (at sites A0, A1 and A2 in yeast and sites A0, 1 and 2a in humans) require the correct assembly of a large ribonucleoprotein complex known as the SSU processome, which contains two proteins with evolutionarily conserved PIN endonuclease domains, yUtp24(Fcf1)/hUTP24 and yUtp23/hUTP23.

The yUtp24/hUTP24 PIN endonuclease is proposed to cleave at sites A1/1 and A2/2a, but the enzyme cleaving at site A0 is not known. In budding yeast, yUtp23 contains a degenerate PIN domain (only 2 out of 3 required catalytic residues are present) and likely plays a non-enzymatic role. In contrast, hUTP23 harbours three PIN domain active site residues, which could be sufficient for enzymatic activity. The yUtp23 protein functions together with a H/ACA snoRNP, snR30, while hUTP23 is associated with U17, the human snR30 counterpart.

Here, using *in vivo* RNA-protein crosslinking and gel shift experiments, we show that yUtp23/hUTP23 makes direct contacts with expansion sequence 6 (ES6) in the 18S rRNA sequence and that yUtp23 interacts with the 3' half of the snR30 snoRNA. Protein-protein interaction studies further demonstrate that yUtp23/hUTP23 directly interacts with the H/ACA snoRNP protein yNhp2/hNHP2, the RNA helicase yRok1/hROK1(DDX52) and the ribosome biogenesis factors yRrp7/hRRP7 and yUtp24/hUTP24. yUtp23/hUTP23 could therefore be central to the coordinated integration and release of ES6 binding factors and likely plays a pivotal role in remodelling this pre-rRNA region in both yeast and humans. Studies using RNAi-rescue systems in human cells further reveal that intact PIN domain and Zinc finger motifs in hUTP23 are essential for 18S rRNA maturation. This raises the exciting possibility that hUTP23 may be an active nuclease.

P62

Small but tough: structural and functional importance of the Argonaute linker helix

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Argonaute (Ago) proteins are key players in nucleic acid silencing processes in all domains of life. Whereas eukaryotic Agos are mostly restricted to RNA-guided silencing of complementary mRNAs, the substrates used by prokaryotic Agos are more diverse. The euryarchaeal Ago variant from *Methanocaldococcus jannaschii* (MjAgo) for example is able to slice target DNAs in a DNA guide-dependent and guide-independent manner. Although the substrates and biological functions of Ago proteins from different domains of life are disparate, they are structurally highly similar. Ago proteins are organized in a bilobal fashion with the N-terminal and the PAZ domain positioned in the N-lobe and the C-lobe consisting of the Mid and the catalytically active PIWI domain. Comparison of the human Ago2-guide RNA structure with the crystal structure of guide-bound MjAgo revealed the presence of a conserved helix positioned in the linker region between the C- and the N-lobe of Ago, called helix-7 in human Ago2 (hAgo2) and helix-8 in MjAgo[1,2]. This element was shown to be decisive for efficient target search of hAgo2 in the enormous amount of possible RNA targets in living organisms[2,3]. Furthermore, we were able to solve the crystal structure of the MjAgo apo enzyme[1]. Here, helix-8 is present in a bipartite state. We therefore conclude that guide binding induces the formation of a continuous helix. Mutation of a leucine to a proline in the bipartite motif disturbs formation of the full-length helix-8 and disruption of the helix negatively affects target cleavage[1,4]. Furthermore, if helix-8 is disrupted, substrate sequence and the identity of the 5'-nucleotide of the guide become important parameters that determine target cleavage efficiency[1,4]. This suggests that helix-8 is not only important for an efficient target search, but also for a correct positioning of a variety of guide-target duplexes within Ago. Since our biochemical data revealed that hAgo2 and MjAgo share many mechanistic features[5], we hypothesize that helix-7 might play an equally crucial role in hAgo2.

[1]Willkomm et al., Nat. Microbiol. (2017)
[2]Schirle et al., Science (2014)
[3]Chandradoss et al., Cell (2015)
[4]Zander et al., Nat. Microbiol. (2017)
[5]Willkomm et al., PloS One (2016)

P63

The conformational landscape and active conformation of the mRNA decapping complex

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Crystal structures of enzymes are indispensable to understanding their mechanisms on a molecular level. It, however, remains challenging to determine which structures are adopted in solution, especially for dynamic complexes. Here, we study the bilobed decapping enzyme Dcp2 that removes the 5' cap structure from eukaryotic mRNA and thereby efficiently terminates gene expression. The numerous Dcp2 structures can be grouped into six states where the domain orientation between the catalytic and regulatory domains significantly differs. Despite this wealth of structural information it is not possible to correlate these states with the catalytic cycle or the activity of the enzyme. Using methyl transverse relaxation-optimized NMR spectroscopy, we demonstrate that only three of the six domain orientations are present in solution, where Dcp2 adopts an open, a closed, or a catalytically active state. We show how mRNA substrate and the activator proteins Dcp1 and Edc1 influence the dynamic equilibria between these states and how this modulates catalytic activity. Importantly, the active state of the complex is only stably formed in the presence of both activators and the mRNA substrate or the m7GDP decapping product, which we rationalize based on a crystal structure of the Dcp1:Dcp2:Edc1:m7GDP complex. Interestingly, we find that the activating mechanisms in Dcp2 also result in a shift of the substrate specificity from bacterial to eukaryotic mRNA.

P64

Phosphorylation of Argonaute Proteins Affects mRNA Binding and is Essential for microRNA-guided Gene Silencing

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Argonaute proteins associate with microRNAs and are key components of gene silencing pathways. With such a pivotal role in gene silencing, these proteins represent ideal targets for regulatory post-translational modifications. Using quantitative mass spectrometry, we find that a C-terminal Serine/Threonine cluster is phosphorylated at five different residues in human. This conserved hyper-phosphorylation does not affect microRNA binding, localization or cleavage activity of human Ago2. However, mRNA binding is strongly affected. Strikingly, on Ago2 mutants that cannot bind microRNAs or mRNAs, the cluster remains unphosphorylated indicating a role at late stages of gene silencing. Interestingly, this mutant retains its capacity to produce and bind microRNAs and represses expression when artificially tethered to an mRNA. Altogether, our data suggest that the phosphorylation state of the Serine/Threonine cluster is important for Argonaute-mRNA interactions

P65

The novel long non-coding RNA EDClnc1 is indispensable for proper terminal keratinocyte differentiation

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Several thousand long non-protein coding transcripts >200 nt are encoded within the human genome. Although this large, newly discovered portion of the human transcriptome is still poorly characterized to date, a number of functional studies strongly indicate that long non-coding RNAs (lncRNAs) are highly functional relevant and not mere transcriptional waste. Full transcriptome sequencing of differentiated versus human progenitor keratinocytes revealed the strong upregulation of the previously undescribed lncRNA EDClnc1 (Epidermal Differentiation Complex lncRNA1) in differentiated keratinocytes. During further studies, we were able to pinpoint the transcript structure of EDClnc1 and showed that recent annotations within this gene locus are not valid for keratinocytes. The strong induction of EDClnc1 during epidermal differentiation already suggests a functional importance of EDClnc1 for keratinocyte differentiation and indeed, knockdown of EDClnc1 in human keratinocytes results in a severe downregulation of several key epidermal differentiation markers. Furthermore, an RNA-sequencing approach with EDClnc1 depleted organotypic tissues confirmed the necessity of EDClnc1 for proper differentiation and pointed to a possible epigenetic regulation mechanism of EDClnc1. In summary, we report the novel long non-coding RNA EDClnc1 which is indispensable for keratinocyte differentiation, including a comprehensive locus analysis and preliminary results regarding the mechanism of EDClnc1. These studies will help us to understand the exact molecular role of EDClnc1 during keratinocyte differentiation as well as the generation of skin diseases.

P66

Viewing pre-60S maturation at a minute's timescale

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The formation of ribosomal subunits is a highly dynamic process that is initiated in the nucleus and involves more than 200 trans-acting factors, some of which accompany the pre-ribosomes into the cytoplasm and have to be recycled into the nucleus. The inhibitor diazaborine prevents cytoplasmic release and recycling of shuttling pre-60S maturation factors by inhibiting the AAA-ATPase Drg1. The failure to recycle these proteins results in their depletion in the nucleolus and halts the pathway at an early maturation step. Here we made use of the fast onset of inhibition by diazaborine to chase the maturation path in real-time from 27SA2 containing pre-ribosomes localized in the nucleolus up to nearly mature 60S subunits shortly after their export into the cytoplasm. This allows for the first time to put protein assembly and disassembly reactions as well as pre-rRNA processing into a chronological context unraveling temporal and functional linkages during ribosome maturation.

P67

Structure and function of the KOW 4 and KOW 6 domains of the human transcription factor DSIF

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Eukaryotic transcription by the enzyme RNA polymerase II (RNAP II) is tightly regulated by different mechanisms. One important regulatory step is promoter proximal transcriptional pausing, which introduces an early block to RNAP II elongation after ca. 20 to 70 transcribed bases. The general transcription factor DRB (5,6,-dichloro-1-β-D-ribofuranosylbenzimidazole) sensitivity inducing factor (DSIF) is involved in this rate limiting step of RNAP II transcription of various cellular promoters. Human DSIF is a heterodimer, composed of a 14 kDa (hSpt4) and a 120 kDa (hSpt5) subunit which are the human homologues of Spt4 and Spt5 from *Saccharomyces cerevisiae*. The hSpt5 subunit contains a region that is homologous to the N-terminal domain of the bacterial transcription factor NusG (NGN). NusG/Spt5 proteins are conserved in all three kingdoms of life. hSpt4, which is not present in bacteria, interacts with hSpt5 via the NGN-domain. Furthermore, the NGN domain interacts with the Rpb1 and 2 subunits of RNAP II. The position of DSIF bound to RNAP II could be localized over the active center cleft in the clamp domain of RNA Pol II. Bacterial NusG and the archaeal Spt5 proteins consist of the NGN-domain and a C-terminal Kyprides-Ouzounis-Woese (KOW) domain. Eukaryotic Spt5 harbors several copies of KOW domains whose functions have not been studied in full detail. Upon phosphorylation DSIF appears to have an activating effect on RNAP II elongation. hSpt5 is known to interact with RNAP II through the region harbouring the KOW motifs. The KOW 1 domain is flexible and is located in a region between the clamp and the RNA exit tunnel of RNAP II. Although the structure of mammalian RNAP II has been solved, the function of the different KOW domains of human DSIF remain elusive. Structures of KOW 1, 2, 3 and 5 are available from yeast or *homo sapiens*, however the structures of KOW 4 and KOW 6 are still missing. To learn more about the function of human KOW 4 and 6, we determined their structures by solution nuclear magnetic resonance (NMR) analyses and performed interaction studies with nucleic acids and Rpb4/7. Both KOW domains exhibit a central β-barrel motif, however, additional structural features are present. Fluorescence and NMR-based titrations showed that only KOW 4 binds to nucleic acids, whereas no binding to nucleic acids could be detected for KOW 6.

P68

Regulation of bacterial transcription by the transformer protein RfaH

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Transcription is the first step in gene expression and RNA polymerase (RNAP) is highly regulated by a variety of transcription factors of which NusG proteins represent the only universally conserved class. RfaH is an operon-specific paralog of NusG in *Escherichia coli*. Its N-terminal domain (NTD) exhibits the typical NusG topology, but in contrast to the beta-barrel conformation of the C-terminal domain (CTD) of NusG proteins the RfaH-CTD is an alpha-helical hairpin that tightly binds to the RfaH-NTD, masking the RNAP binding site on RfaH-NTD and rendering RfaH autoinhibited. Upon recruitment of RfaH to elongating RNAP (transcription elongation complex, TEC) the domains dissociate and the RfaH-CTD transforms from its alpha-helical state into the beta-barrel typical for NusG proteins (Fig. 1)[1]. RfaH activation depends on an *operon polarity suppressor* (*ops*) element in the leader region of the RfaH-controlled operon. The *ops* sequence pauses the TEC so that the *ops* site in the non-template strand is exposed and accessible[2]. So far, the structural basis of RfaH regulation and its recruitment in particular are only poorly understood. Here we provide molecular details of the life cycle of RfaH obtained by applying solution state NMR spectroscopy on a supramolecular, multicomponent system. Due to the size of the system (RNAP: 390 kDa) [^{1}H, ^{13}C]-labeled Ile, Leu, and Val methyl groups in perdeuterated protein were used as NMR active probes. First, we aimed to identify the signal for RfaH recruitment. We showed that both *ops*DNA and RNAP bind to RfaH individually, but neither of the isolated interaction partners was able to trigger domain dissociation. We then assembled a TEC paused at the *ops* site (*ops*TEC) and demonstrated the recruitment of RfaH to this *ops*TEC by directly following RfaH binding and the transformation of its CTD. Quantitative analysis of the signal intensity allowed us to determine the *ops*TEC interaction site on RfaH. In combination with the crystal structure of the RfaH:*ops* complex that we solved we created a model of RfaH bound the *ops*TEC. Moreover, we showed that TEC-bound RfaH can bind to ribosomal protein S10, an interaction that is essential for the activation of translation as RfaH-controlled operons lack a Shine-Dalgarno sequence. Finally, displacement experiments confirmed that RfaH can be recycled into its autoinhibited state upon dissociation from the TEC.

[1] Burmann, B.M.; Knauer, S.H.; Sevostyanova, A., Schweimer, K., Mooney, R.A.; Landick, R.; Artsimovitch, I.; Rösch, P. *Cell* **2012**, *150*, 291-303

[2] Artsimovitch, I.; Landick, R. *Cell* **2002,** *109*, 193-203

P69

RNP-ID generates a picture of the molecular interactions during shuttling and mRNP export

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SR proteins belong to a family of RNA-binding proteins (RBPs) with essential functions as regulators of splicing. Additionally, some family members shuttle continuously between nucleus and cytoplasm and act as export adapters for the main mRNA export receptor NXF1 (Müller-McNicoll, 2016; Botti, 2017). We have shown that shuttling of SR proteins is influenced by the extent of NXF1 interaction, methylation of the NXF1 interaction region, binding to different RNA classes and the phosphorylation state of their RS domain (Botti, 2017). Recruited NXF1 subsequently dimerizes with its co-factor NXT1 to form a binding platform for nuclear export of cellular mRNAs via the nuclear pore complex.

To understand the regulation of mRNA export by SR proteins and NXF1 we developed an MS approach, based on the endogenous expression of GFP-tagged bait proteins from genomic loci, UV-crosslinking, differential RNase digest and stringent pulldown. This method, termed RNP-ID, allows the quantitative comparison of RNPs between cell types or in different cellular conditions and the extraction of diverse parameters describing the composition, modification and dynamics of RNPs. We applied this approach to SRSF5- and NXF1-GFP endogenously expressed in murine P19 cells. Comparing +UV to -UV samples in RNase-sensitive and RNase-resistant fractions we were able to devise protein:protein interactors and protein:RNA:protein interaction partners that bind in close proximity to the bait on the same RNA piece. Moreover we identified and quantified PTMs and RNA crosslinks as well as UV-induced disulfide bridges that provide information on transient protein interactions and the responsible protein domains. Using NXF1 as bait, we purified an active shuttling complex containing many nuclear pore proteins (NUPs) and export factors. Of interest, our sample preparation strategy allowed us for the first to monitor the complex path of NXF1 during its way through the nuclear pore including NUP, RBP and RNA interactions using UV induced RNA crosslinks and transient disulfide bridges. Modeling and integrating these transient interactions and RNA contacts including data for SRSF5 will help to understand the different shuttling capacities of SR proteins and their impact o the regulation of mRNP export by NXF1.

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VENUE INFORMATION & MAPS

Network access in the Thon-Dittmer-Palais

Information to access the local network ‘Stadt Regensburg’ will be provided on site.

The audio-visual team

To upload your talks on the provided computers, or to connect your own computer to the system, please contact Felix Grünberger (pictured) or any other member of the AV team.

A map of the old town with the conference sites marked can also be found here:

Downtown Regensburg