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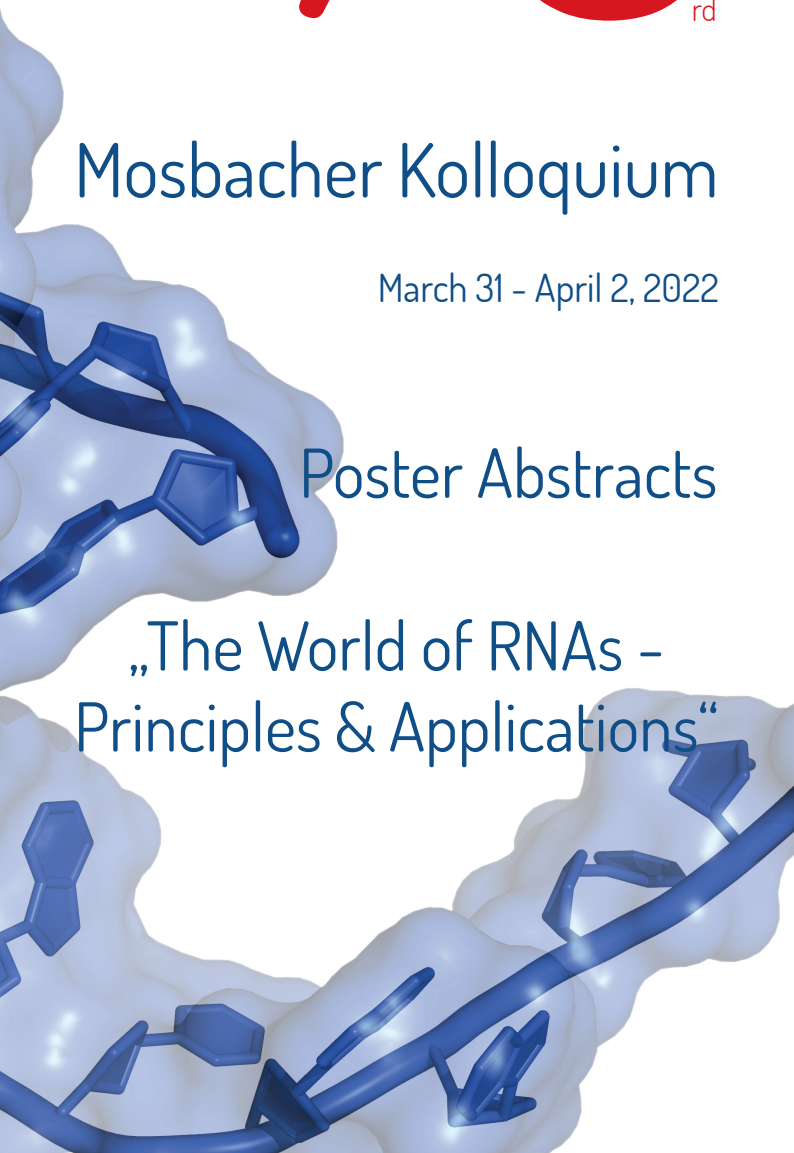
Poster Abstracts

„The World of RNAs – Principles & Applications“



P 01

A scalable method for identifying the protein interactomes of individual RNAs by quantitative mass spectrometry

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RNA-binding proteins determine the fate of RNAs in cells. Dissecting the interactions of individual RNAs with the cellular proteome can illuminate mechanisms of gene regulation and functions of non-coding RNAs. Most methods rely on antisense-capture to isolate RNAs via sequence features (e.g. poly(A)-tails) or target specific RNAs with oligonucleotides. While these methods illuminated the composition of ribonucleoprotein complexes (RNPs) and recently charted the interactions of viral RNAs in infected cells, they require large amounts of material, limiting their scope.

To address this, we are developing a new approach combining advances in phenol-chloroform-based extraction of UV crosslinked RNPs with a specific RNA release to obtain protein interactomes of individual RNAs in cells. Compared to RNA antisense purification (RAP-MS), this requires >10-fold less input for quantitative MS. This allows for higher scalability and could enable high-throughput identification of single RNA-bound proteomes.

We targeted well-known RNPs of and identified captured proteins by quantitative MS. Comparing to RAP-MS, we show near comprehensive capture of known factors with virtually no background. This led us to develop the approach into a versatile platform for RNA interactome

studies. We expect this work to accelerate the systematic discovery of RNA-protein interactions and to enable dissection of RNA species unamenable to common methods due to low abundance or lack of shared sequence features.

O 01
Sensitive localization of viral RNA in cells by direct RNA padlock probing and in-situ sequencing

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Influenza viruses are important human pathogens that are responsible for annual epidemics and occasional pandemics. A striking feature of influenza is its segmented genome composed of 8 negative stranded RNA segments (vRNAs). Although genome segmentation complicates virus assembly, it allows segment exchange between viruses by genetic reassortment. So far, this assembly and reassortment process is poorly understood because RNA-RNA associations in cells are difficult to study.

Spatial transcriptomics offers a way to follow the dynamics of viral infection at the single cell level, whilst retaining positional information that cannot be obtained in bulk assays. Single molecule fluorescent in situ hybridization is a common technique to visualize RNAs, but spectral overlap limits the analysis of only a few RNAs at a time and smFISH cannot distinguish between similar RNAs such as during co-infection. We have established a sensitive in-

situ sequencing technique based on direct RNA targeting padlock probes (PLP) in combination with sequencing by synthesis. PLPs offer great discriminatory power and we show that direct RNA targeting using an RNA specific ligase greatly improves the efficiency of the technique. We further increase sensitivity towards single molecule detection using multiple PLPs. Finally, we show that in situ sequencing of barcodes within the loop region enables highly multiplexed RNA detection and the visualisation all 8 influenza vRNAs and mRNAs in infected cells.

P 02
Large-scale identification of RBP-RNA interactions by RAPseq refines essentials of post-transcriptional gene regulation

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Post-transcriptional gene regulation is orchestrated by RNA binding proteins (RBPs) and their interactions with RNA molecules.

We developed a novel, in vitro, RBP-centric technology that discovers and quantifies RBP binding sites in any given transcriptome/species of interest. RAPseq is an in vitro binding assay between a recombinant purified RBP and native total RNA which if chemically fragmented prior to the binding assay to a median size of ~50 nucleotides.

The bound RNA molecules are recovered and NGS is used as a read-out.

We produced an RNA modification depleted total RNA substrate by T7 in vitro transcription of the original native RNA. We used the two substrates in parallel to profile the modification dependent and independent RNA interactome of YTHDF1 and classified which of its binding sites in the human transcriptome are affected by the presence/absence of RNA modifications discovering that mRNAs coding for chromatin regulators were the ones with the highest m^6A-dependency in terms of YTHDF1 binding. In addition, we further demonstrate the power of RAPseq by 1. providing binding cooperativity maps for two splicing factors; 2. describe how vertebrate evolution has shaped the binding properties of a highly conserved RBP by profiling all vertebrate orthologs with RAPseq; 3. assessing the impact on the RNA interactomes of RBPs mutated in cancer and 4. provide a resource of RNA interactomes for 26 novel non-canonical RBPs.

P 03
Homologous recombination by prokaryotic Argonautes

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Prokaryotic Argonautes (pAgos) are diverse nucleic acid guided nucleases involved in defense against mobile genetic elements. Recently, a pAgo from Natronobacterium gregoryi (NgAgo) has been shown to stimulate

homologous recombination between genomic and invading plasmid DNA. On this poster we describe our investigation of pAgo-stimulated homologous recombination.

R 01
Identification of RNA- based regulatory pathways affecting T cell – cancer cell communication

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The infiltration of T cells into tumors is a major step which is essential for an effective anti-cancer immune response. However, T cells are often prevented to efficiently invade tumors due to a repulsive tumor microenvironment. Infiltration of T cells is mediated by the interaction of endothelial adhesion molecules as ICAM-1 and integrins like LFA-1 on T cells. We hypothesize that soluble molecules which are expressed, post-transcriptionally regulated and then secreted by cancer cells may modulate the LFA-1 – ICAM-1 interaction and thus inhibit T cell infiltration into tumors.

To identify such molecules, we designed a RNAi screening approach to investigate regulatory events originating from cancer cells, which interfere with T cell adhesion and trafficking. We focus our screen on RNA-based regulatory pathways including e.g. RNA-binding proteins (RBPs) and the microRNA (miRNA) pathway. By using highly specific siPOOLs against different targets, we aim to identify

processes within cancer cells which lead to T cell exclusion from tumors. For the screening experiments, recombinant human ICAM-1-Fc protein is purified from mammalian cells and cell adhesion assays are performed using T cells treated with cancer cell tissue culture supernatants. Secreted molecules in the supernatants induce reduced or increased T cell binding to the immobilized ligand. These endogenous targets or target pathways identified by the screening will be further validated and functionally characterized.

T 01
Establishment of an assay to assess the immunogenicity of a therapeutic antisense oligonucleotide in clinical samples

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The immunogenicity of biotherapeutics is an important aspect of the safety program in clinical trials and is first of all assessed by analyzing the occurrence of anti-drug antibodies (ADAs) in response to the treatment of patients. Although RNA therapeutics, such as antisense oligonucleotides (ASOs) are much smaller than recombinant proteins, they still are able to elicit ADAs in patients. E.g. in about 65 % of patients treated with mipomersen (approved by FDA as Kynamro) the occurrence of ADA indicated an immunogenicity that can be even higher than that observed for most therapeutic antibodies[1]. Due to the small size of RNA therapeutics and their characteristics to bind serum proteins, it is often a challenge however to establish an assay to detect ADAs

in human blood samples that fulfills the acceptance criteria of regulatory authorities.

With this poster we will present the technical hurdles when establishing a highly sensitive assay for detection of ADAs against an ASO.

[1] European Medicines Agency (EMA) Assessment Report of Kynamro. 21. March 2013

T 02
Targeted Delivery of Antisense Oligonucleotides for the Treatment of Erythropoietic Protoporphyria by Splicing Modulation in a Mouse Model of Human EPP

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Erythropoietic Protoporphyria (EPP) is a genetic disorder that manifests primarily through severe photo-sensitivity. It is caused by an accumulation of protoporphyrin nine (PPIX), the penultimate product in the haem biosynthesis pathway; either due to a lack-of-function of the terminal enzyme (FECH) or an overregulation of the initial enzyme (ALAS). The most common underlying genotype is a loss-of-function FECH allele in trans to a single nucleotide polymorphism leading to aberrant splicing and subsequent degradation of FECH mRNA. Current treatment options are sparse and disease management mainly focuses on avoiding exposure to sunlight which comes with a tremendous decrease in quality of life. Despite great leaps in EPP treatment, the

therapeutic need is not met - especially with regards to quality of life and long-term health. We have previously shown that a splice-switching oligonucleotide (SSOs) comprising 2'-methoxyethyl chemistry is able to correct splicing of FECH mRNA; thereby increasing active FECH enzyme, leading to lower levels of toxic PPIX. Here we present how oligo-bioconjugates can further increase their selective uptake and delivery, while maintaining therapeutic activity. Ultimately, we introduce a new model of splicing-dependent EPP in the house mouse.

R 02
Dynamics of human tRNA repertoires as a function of cell identity

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Transfer RNA abundance impacts translation dynamics, and tRNA defects are linked to neurological diseases and cancer. Eukaryotic genomes encode hundreds of highly similar tRNA genes transcribed from conserved internal promoters by RNA Polymerase III (RNAPIII). How tRNA abundance is regulated in diverse cellular contexts is poorly understood, partly due to hurdles to tRNA quantitation. We recently overcame these hurdles with modification-induced misincorporation tRNA sequencing (mim-tRNAseq), which enables accurate quantitation of tRNA pools at single-transcript resolution. By combining mim-tRNAseq with measurements of chromatin status and RNAPIII occupancy during differentiation of human induced pluripotent stem

cells (hiPSC) into neural and cardiac lineages, we discovered lineage-specificity of human tRNA pools that is largely determined by RNAPIII gene sampling and occupancy strength. A third of the ~600 predicted human tRNA genes are not occupied in any cell type, have promoters divergent from the consensus sequence, and are found in closed chromatin. The remaining two-thirds are bound by RNAPIII in hiPSC while progressively smaller subsets are sampled during differentiation. Depletion of MAF1, a global repressor of RNAPIII, maintains hiPSC-like RNAPIII tRNA occupancy in neural progenitors and perturbs neural fate acquisition. Our data reveal that in human cells, tRNA gene expression is selective, context-specific, and established in a MAF1-dependent manner.

O 02
Function and mechanisms of long pAgos

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Eukaryotic Argonautes (eAgos) are key components in RNA interference (RNAi). In contrast, homologous proteins found in prokaryotes (pAgos) function as immune systems, and it was recently shown that they are involved in homologous recombination. On this poster we describe the progress on the characterization of long pAgos proteins.

R 03
The inner nuclear membrane protein Lem2

coordinates RNA degradation at the nuclear periphery

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Transcriptionally silent chromatin often localizes to the nuclear periphery. However, whether the nuclear envelope (NE) is a site for post-transcriptional gene repression is unknown. Here we demonstrate that, in S. pombe, the highly conserved NE protein Lem2 regulates nuclear exosome-mediated RNA degradation. Lem2 deletion causes accumulation of RNA precursors and meiotic transcripts, and de-localization of an engineered exosome substrate from the nuclear periphery. Lem2 does not directly bind RNA but instead physically interacts with the exosome-targeting MTREC complex, as well as its human homolog PAXT, to promote the recognition and recruitment of substrate RNAs. This pathway acts largely independently of nuclear bodies where exosome factors assemble. Nutrient availability modulates the repressive role of Lem2 in controlling meiotic transcripts, implying that this pathway is environmentally responsive and may be switched off during the mitosis-to-meiosis switch. Our work reveals that multiple, spatially distinct degradation pathways exist, among which Lem2 coordinates RNA surveillance of meiotic transcripts and non-coding RNAs by recruiting exosome co-factors to the nuclear periphery.

P 04
Introduction of a circular SRP RNA in the thermoacidophilic archaeon Sulfolobus acidocaldarius

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Signal recognition particles (SRP) are ubiquitous ribonucleoprotein complexes in all domains of life that mediate membrane targeting of proteins with signal peptides. Archaeal SRPs consist of an approx. 300 nt long SRP RNA and the proteins SRP54, SRP19 and L7Ae. Usually, the SRP RNA occurs as a linear molecule, however the hyperthermophilic organism Thermoproteus tenax was found to possess a permuted, intron-containing SRP RNA gene which is processed into a circular SRP RNA by its moonlighting tRNA splicing machinery. The closely related crenarchaeon Sulfolobus acidocaldarius harbors a linear SRP RNA with canonical termini. To elucidate the evolution and potential benefits of a circular SRP RNA in hyperthermophilic organisms, we designed an intron-containing, permuted SRP RNA gene for S. acidocaldarius based on the T. tenax structure. RNA-seq analysis detected the circular SRP RNA variant, as well as the linear version, in total RNA extracted from Sulfolobus. In vitro experiments with the purified subunits of the tRNA splicing endonuclease from S. acidocaldarius confirmed processing of the SRP RNA precursor, highlighting this complex' potential for a moonlighting activity. We aim to replace the original SRP RNA gene with its permuted variant in Sulfolobus using the pop-in/pop-out method to gain further insight into the evolution of this peculiar SRP RNA variant.

O 03
Investigating the role of RNA in H2AX-mediated DNA damage repair

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In the last decade, multiple studies used several distinct approaches to detect interaction between proteins and RNA proteome-wide. Our group established a proteome-wide quantitative screen to identify RNA-dependent proteins by measuring the RNA-dependence of their interactome using density gradient centrifugation and mass spectrometry (CaudronHerger et al., Mol Cell 2019 - http://R-DeeP.dkfz.de). The histone variant H2AX, whose phosphorylation coordinates the essential steps of DNA repair and is used as a marker for DNA damage, was identified as RNA-dependent in this screen. Our preliminary results confirm direct RNA binding to H2AX by photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP), which is specific to H2AX and not observed for other histones. Further, we found that isolated degraded RNA led to phosphorylation of H2AX in cell lysates. The project further aims to characterize the H2AX-binding RNAs and to unravel their role in the H2AX-mediated DNA damage response, and to investigate the impact of RNA damage upon cytostatic agents by correlating the effects on RNA integrity and phosphorylation of H2AX. Overall, we elucidate the control of the H2AX-mediated DNA damage response by the direct interaction of H2AX to RNAs.

O 04
Correlating the conformational dynamics of the DEAD-box helicase eIF4A with translation efficiencies

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The initiation of eukaryotic translation is a very tightly regulated process requiring the interplay of several translation initiation factors (eIFs). The role of eIF4A, an ATP-dependent RNA helicase, is the resolution of secondary structure elements within the 5′-untranslated regions (5′-UTR) of mRNA to enable ribosome recruitment and scanning. Previous studies from our lab have shown that the factors eIF4B and eIF4G jointly stimulate eIF4A ATPase and unwinding activity by accelerating switching of eIF4A between closed and open states (Andreou & Klostermeier, 2014; Harms et al., 2014). The activity of eIF4A is additionally modulated by single-stranded regions in RNA substrates (Andreou et al., 2019). Recent genome-wide studies have identified eIF4A-dependent mRNAs in yeast (Sen et al., 2015). In this study, we investigate the connection between eIF4A conformational dynamics and the efficiency of mRNA translation. To achieve this goal, we have generated yeast strains from which individual translation initiation factors can be depleted. In in vitro translation assays using these extracts, we monitored the translation efficiency of mRNAs in which the 5′-UTRs of eIF4A-dependent mRNAs are fused to a reporter gene (Firefly luciferase). eIF4A conformational dynamics is

monitored in single-molecule FRET experiments, ideally under the same conditions. As a proof of principle, luciferase reporter assays were performed in wildtype yeast cells to test the effects of 5′ UTRs of the eIF4A dependent genes on translational efficiencies. Our results indicate that with different 5′ UTRs, there are differences in both, the eIF4A conformational equilibrium as well as efficiency of mRNA translation. To understand the sequences of the conformational states and the kinetics of conformational changes of eIF4A interaction with the 5′ UTRs, smFRET experiments will be performed using TIRF microscopy under translating conditions.

References:

[1] A.Z. Andreou, U. Harms, D. Klostermeier, Single-stranded regions modulate conformational dynamics and ATPase activity of eIF4A to optimize 5′-UTR unwinding, Nucleic Acids Res. 47 (2019) 5260–5275. https://doi.org/10.1093/nar/gkz254.

[2] N.D. Sen, F. Zhou, N.T. Ingolia, A.G. Hinnebusch, Genome-wide analysis of translational efficiency reveals distinct but overlapping functions of yeast DEAD-box RNA helicases Ded1 and eIF4A, Genome Res. 25 (2015) 1196–1205. https://doi.org/10.1101/gr.191601.115.

[3] U. Harms, A.Z. Andreou, A. Gubaev, D. Klostermeier, eIF4B, eIF4G and RNA regulate eIF4A activity in translation initiation by modulating the eIF4A conformational cycle, Nucleic Acids Res. 42 (2014) 7911–7922. https://doi.org/10.1093/nar/gku440.

[4] A.Z. Andreou, D. Klostermeier, eIF4B and eIF4G Jointly Stimulate eIF4A ATPase and Unwinding Activities by Modulation of the eIF4A Conformational Cycle, Journal of Molecular Biology. 426 (2014) 51–61. https://doi.org/10.1016/j.jmb.2013.09.027.

T 03
Enzymatic cascade for photocaging of the mRNA 5′ cap

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Methyltransferases (MTases) have been used for the selective photocaging of DNA and mRNA. This reaction requires S-adenosyl-L-methionine (SAM, AdoMet) analogues bearing a photocaging group as cofactor. While chemical synthesis of AdoMet analogues is costly and laborious, engineered methionine adenosyltransferases (MATs) have been exploited for the in situ generation of AdoMet analogues in cascade reactions with MTases. We show that an engineered MAT from Methanocaldococcus jannaschii (MjMAT L147A/I351A) is 308-fold more efficient at converting ortho-nitrobenzyl-(ONB)-homocysteine than the wildtype enzyme. This variant, termed PC MjMAT, is active over a broad range of temperatures and compatible with MTases from mesophilic organisms. Additionally, we solved the crystal structures of wildtype and PC MjMAT in complex with AdoONB and a red-shifted derivative thereof to guide further engineering.

R 04
The influence of epidermal piRNAs on the immune defense in Schmidtea mediterranea

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PIWI proteins and their co-bound small RNAs, piwi interacting RNAs (piRNAs), are widely known for silencing transposable elements both transcriptionally and post-transcriptionally. The primary source of piRNAs are piRNA clusters that house remnants of transposable elements and coding genes as potential targets for silencing. However, recent studies revealed additional functions of piRNAs in a wide variety of animals, such as koalas or mosquitoes, by establishing their link to innate immunity. As the TRAF gene family (important transducers of innate immunity) is greatly expanded in the planarian flatworm S. mediterranea and represents prime targets of piRNAs, we hypothesized that there might also exist a connection between epidermal piRNAs and the innate immune response in S. mediterranea. To that end, I characterized the planarian epidermal piRNA and mRNA response in response to immune stimuli, and I studied the TRAF expansion in S. mediterranea in more detail. Taken together, my experiments uncover a potential role of piRNAs in planarian innate immune system, a connection that is likely present in the entire order Tricladida.

O 05
A novel binding platform consisting of three MademoiseLLE domains links the key RNA transporter to endosomes

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Transport of mRNAs coupled with local translation ensures the spatiotemporal regulation of gene expression. Active transport along the cytoskeleton is a common mechanism of mRNA localization in highly polarized cells.

In our model organism Ustilago maydis, a phytopathogenic filamentous fungus, Rrm4 is the key RNA binding protein that mediates the long-distance transport of mRNAs. Loss of rrm4 leads to disturbed hyphal growth and reduced pathogenicity. Together with the accessory Poly A binding protein Pab1, and an essential adaptor protein Upa1, Rrm4 forms the messenger ribonucleoprotein (mRNP) complex and, is transported along the microtubule cytoskeleton by hitchhiking on Rab5a-positive early endosomes. Both Rrm4 and Pab1 have MademoiseLLE (MLLE) domain(s) in their C terminal, loss of which results in severe growth defect comparable to the loss of full-length proteins. MLLE domains are conserved across eukaryotes. However, the MLLE domains of Pab1 and Rrm4 are very specific to their respective binding partners.

Using the structural biology approach, we found that Rrm4 has a sophisticated multi MademoiseLLE domain binding platform. Subsequently, we analyzed the role of the individual MLLE domains in vitro and in vivo and show that the third MLLE domain is essential for the endosomal attachment and polar growth whereas the first and second MLLE domains play an accessory role. Finally, we determined the structure of the MLLE-PAM2L complex by X-ray crystallography.

P 05
Deciphering mRNA target specificity of the multi-domain RNA-binding protein IMP3

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IMP3 (Insulin-like growth factor-2 mRNA-binding protein) belongs to a conserved family of oncofetal multi-domain RNA-binding proteins. It comprises two N-terminal RRM domains and four C-terminal KH domains that are arranged in pairs, while all KHs and one RRM contribute to RNA binding. IMP3 plays a role in various post-transcriptional processes. Pathologically, IMP3 has been shown to protect oncogenic target mRNAs from degradation, thereby serving as a tumor marker. Structural data on single tandems of IMP3 are available except for KH3-4, but remain inadequate in answering the specificity achieved by IMP3 for its target mRNAs. To this end, atomic pictures of full-length IMP3 with and without target RNA motifs are essential. In this context, we here provide systematic structural studies with tandem combinations, starting from the KH1-4 di-tandem. As IMP3 shows an intrinsic tendency to oligomerize via KH3-4, we have optimized protein production, and have successfully grown initial crystals of IMP3 KH1-4. Using EMSA experiments with a 72mer RNA containing target motifs for all four KHs, we determined the RNA-binding contributions of each KH domain by combinations of impaired RNA-binding KH knockout mutants. Initial studies towards atom-resolved structures of IMP3-RNA complexes are ongoing. With our integrated approach, we intend to study the domain-interdependency within IMP3 for achieving target RNA specificity and

examine the role of pre-formed or induced target RNA structure.

E 01
Wobble Uridine tRNA Modifications Are Essential For Correct Translation Dynamics And Ribosome Movement Along mRNA

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Chemical tRNA modifications are key for tRNA processing and function. In particular, tRNA modifications of the anti-codon loop are essential to optimize codon-anticodon interaction. 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U) is located at wobble uridines (U_{34}) of the cognate tRNAs that decode AAA, CAA, GAA. Cells lacking this modification show codon-specific translational slowdown and contain aggregated protein.

Here we show that the slowdown can be reverted by Paromomycin, confirming that slowdowns are caused by a lack of fully functional tRNAs. To understand how codon-specific translation defects impact ribosome dynamics, we used reporters to monitor ribosome stalling. These reporters showed strong evidence for ribosome collisions. To confirm this result, we performed ribosome profiling of queued ribosomes in yeast cells deficient in U_{34} modification. Strikingly, we detected ribosome queueing upstream of AAA and CAA codons, establishing ribosome collisions upstream of local translation defects. Knocking out the ribosome quality control (RQC) pathway enhanced

ribosome queueing, providing further evidence of ribosome collisions. Importantly, inactivation of the RQC did not only result in an increase of ribosome collisions, but at the same time improved cell viability. This shows that the RQC contributes to the phenotypes of codon-specific translation defects. Finally, the RQC is not beneficial under all conditions and its induction needs to be precisely controlled.

E 11
The RNA modification m6A affects RNA binding and functions of SRSF7

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The N^6-methyladenosine (m^6A) modification affects all steps of the mRNA life cycle, including splicing, polyadenylation and mRNA export. This pleiotropy is caused by different RNA-binding proteins (RBPs) that recognize m^6A and mediate various downstream effects. Besides 'reading' m^6A marks, RBPs also affect m^6A deposition or are repelled by it.

Recently, the RBP SRSF7 was shown to promote m^6A deposition, regulating the stability of a small set of mRNAs important for cell proliferation. SRSF7 plays essential roles in the regulation of alternative splicing, but it has also diverse non-splicing functions – it forms nuclear condensates, regulates mRNA export and modulates alternative polyadenylation (APA). SRSF7 binds upstream of

proximal polyadenylation sites (pPASs) and enhances their usage generating mRNAs with short 3'UTRs. To test whether m^6A deposition affects APA we performed miCLIP2 followed by m^6Aboost and determined the methylome of P19 cells. Integration with SRSF7 iCLIP data revealed that m^6A sites are enriched at pPAS, overlap with SRSF7 binding sites and its binding motif (GAYGAY) is similar to the m^6A DRACH motif. To test whether SRSF7 affects m^6A deposition and APA, we generated P19 hGRAD cell lines for the rapid degradation of SRSF7 to minimize compensatory effects. We performed DART-Seq upon acute SRSF7 depletion and integrated changes in m^6A levels and 3'UTR length using DaPars. The data will illuminate the interplay of SRSF7, m^6A and the regulation of APA.

E 02
Substrates and functionality beyond pseudouridylation of tRNA PUS enzymes in pathogenic Epsilonproteobacteria

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Pseudouridine synthases (PUS) catalyze the post-transcriptional isomerization of uridine into pseudouridine (Ψ), a universally conserved modification of tRNAs and rRNAs in all domains of life. Little is known about global RNA substrate maps and functions of PUS enzymes in bacteria. Here, we explore potential roles of bacterial tRNA PUS by identifying their RNA substrates and function in

Campylobacter jejuni and Helicobacter pylori. Compared to E. coli, these Epsilonproteobacteria have a limited set of PUS that could facilitate their characterization: TruA, TruB, and TruD (CjTruD) in C. jejuni and TruA and TruD (HpTruD) in H. pylori. We show that deletion of truD in C. jejuni leads to a severe growth defect and deletion of truA leads to increased growth compared to the wildtype strain. In contrast, neither deletion of truD nor truA affects the growth of H. pylori. To map Ψ in RNA substrates, we performed Pseudo-seq in RNAs from wild-type and PUS mutant strains. This analysis revealed 33 Ψ sites in tRNAs and rRNAs. For example, CjTruD modifies tRNAGlu, which is present in one copy in C. jejuni genome, at position 13. We tested if HpTruD can complement the function of CjTruD. Surprisingly, while HpTruD is able to rescue the growth phenotype, it is unable to restore Ψ in the tRNAGlu. A similar result is observed when complementing with a catalytically inactive CjTruD. Together, this suggests that TruD might have an unexplored function beyond RNA modification.

T 04
Manipulating the activities of a gut microbiota model bacterium with antisense nucleic acids

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RNA is a specific, programmable molecule that lends itself for targeted interventions. Antisense technology exploits RNA basepairing to manipulate gene expression. Targeting

the translation initiation region of essential mRNAs has antimicrobial promise (Good & Nielsen, 1998). However, to what extent the technology can be used to manipulate the microbiota is little understood. The emerging model species Bacteroides thetaiotaomicron has versatile metabolic capacities that shape the gut ecosystem (Wexler & Goodman, 2017). Here, drawing from peptide nucleic acids (PNAs) coupled with cell-penetrating peptides (CPPs), we aim to establish antisense RNA technology in B. thetaiotaomicron to exploit its vast metabolic potential. First, we screened fluorescently tagged CPPs and observed overall efficient uptake into Bacteroides cells. To read out targeting efficiency, we designed PNAs against fabG, an essential gene involved in envelope fatty acid synthesis. Submicromolar concentrations of the CPP-PNA evoked a dose-dependent growth inhibition. Interestingly, this effect was carbon source-dependent, so we hypothesize that cell surface structures can influence uptake. We will test, combining single-cell approaches with defined mutant strains. We will also dissect the mechanisms of – and optimize – PNA-mediated target control. This knowledge may allow us to deliberately switch on or off metabolic traits in this abundant gut bacterium, with potential in biotechnology and microbiome editing.

R 05
Sibling Team Work – Interplay of a family of regulatory RNAs with an RNA sponge in Caulobacter crescentus

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Small RNAs (sRNAs) contribute significantly to the post-transcriptional control of gene expression in bacteria. By engaging in direct base-pairing interactions with target transcripts, sRNAs may influence mRNA translation and stability. In some cases, a given bacterium expresses multiple, seemingly homologous sRNAs, so-called sibling sRNAs. It is generally not well understood why the cell has several copies of nearly identical regulators, how it integrates the activity of multiple sRNA homologs into established regulatory circuits, and whether the siblings act redundantly.

The alpha-proteobacterium Caulobacter crescentus encodes a family of four αr8 sibling sRNAs, which share conserved sequence elements and a characteristic structure. In contrast to previously investigated sibling RNAs, the αr8 sRNAs are expressed from independent genomic loci under different environmental conditions (including carbon starvation and iron deficiency), indicating sibling-specific transcriptional input. We have performed comparative transcriptome analyses to determine the target spectra of each family member, and have identified both conserved as well as individual interactions. Intriguingly, our data also revealed the presence of a stress-responsive sponge RNA that binds to and facilitates αr8 turn-over. Using both in vivo as well as in vitro approaches, we have untangled a complex post-transcriptional network orchestrating carbon metabolism when Caulobacter switches between feast and famine.

R 06
Characterization of an sRNA involved in the σB–mediated stress response in C. difficile

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As part of its infection cycle, the obligate anaerobic human pathogen C. difficile encounters several environmental stressors. sRNAs are known regulators of stress associated pathways in pathogenic bacteria. Although >40 sRNAs have been identified in C. difficile, conditions relevant for sRNA expression and target interaction remain largely unknown.

In this study, we investigated the σB-dependent sRNA nc028 in C. difficile 630. σB primarily activates genes in response to reactive radicals and antimicrobial compounds. In accordance, northern blot analysis revealed an upregulation of nc028 during oxidative and iron limiting conditions. To identify nc028 targets, we pulse-expressed nc028 followed by RNA-seq and mass spectrometry, revealing an overlapping set of 12 targets to be differentially regulated. These included the operon forming CoA-disulfide reductase CDIF630_01996 and the putative nitrite/sulfite reductase subunit CDIF630_01995. Furthermore, a direct interaction of nc028 with the operon was supported by in silico and in vitro approaches. An E. faecalis homolog of CDIF630_01996 has been associated with the sulfide/reactive sulphur species (RSS) homeostasis system, suggesting a regulatory function for nc028 in sulfide stress response

In summary, we propose a role for nc028 in σB–mediated stress response in the nosocomial pathogen C. difficile. Ongoing work will further characterize the sRNA-mRNA interaction and its impact on stress related post-transcriptional regulation.

R 07
Mechanistic insights into the regulation of the guanidine-II riboswitch

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Riboswitches are cis regulatory elements that can regulate gene expression in bacteria. The guanidinium binding riboswitch guanidine-II consists of two GC rich hairpins with an ACGR loop motif where ligand binding takes place. Binding of guanidinium to the loops leads two a kissing interaction between the two hairpins via their CG base pairs and rotation of the loop purins at the fourth position out of the binding pocket. The aptamer region with the hairpins is followed by the Shine-Dalgarno sequence. In a proposed mechanism the kissing loop formation sequesters the anti-Shine-Dalgarno sequence positioned in the linker between the hairpins and thereby leads to activation translation of the downstream genes.

Using single molecule FRET spectroscopy we investigate the guanidine-II riboswitch of E. coli controlling gene expression of the small multidrug efflux pump SugE. Using strategic labeling inside the P1 hairpin, the linker or close to the P2 and SD sequence we are able to propose a mechanistic model for the regulation of this riboswitch. With multiple distance vectors, we characterize the ligand depending formation of the kissing hairpin and the role of the linker for its stability. Furthermore, we are first to not only analyze isolated hairpins or the aptamer domain, but also incorporating the effects of the expression platform including the SD sequence and the translational start codon in our model.

O 06
The structure of the RTCB-Archease complex reveals the activation principle of the human tRNA ligase

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The non-conventional splicing of intron-containing pre-tRNAs is an essential RNA maturation mechanism in all eukaryotic cells. During tRNA splicing, the human tRNA ligase complex (tRNA-LC) catalyses the sealing of the tRNA exon halves. In addition, RTCB plays an important role in mRNA processing during the unfolded protein response. The RNA ligase RTCB provides the catalytic core of the pentameric human tRNA-LC. Multiple-turnover ligation by RTCB is dependent on Archease, a small promoting protein factor. Here, we present the biochemical and structural analysis of the interaction between RTCB and Archease. Using in vitro reconstitution, we characterise the interaction between RTCB and Archease and its dependence on the nucleotide GTP and metal ions. The crystal structure of the dimeric RTCB-Archease complex reveals an important role of Archease in activation of RTCB. Conserved amino acids of Archease reach into the active site of RTCB promoting formation of a covalent RTCB-GMP intermediate, which is the initial step of the ligation reaction. Using structure-guided mutagenesis, we define the role of individual residues during the activation mechanism. Taken together, these results illuminate the structural determinants of the RTCB-Archease interaction and shed light onto the

molecular mechanism behind the promoting effect of Archease on RTCB-mediated RNA ligation.

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R 08
Giant phages hijack post-transcriptional regulation and translation in the host

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The Gram-negative bacterium Pseudomonas aeruginosa is a major cause of nosocomial infections due to an intrinsic antibiotic resistance, a large genome, and wealth of regulatory mechanisms. Stress drives regulation and adaptation, hence we asked how the cellular complexome changes upon application of a biological stress in form of a phage infection for which the molecular mechanisms are still poorly understood. To illuminate how the phage transcriptome and proteome merge with cellular complexes and affect RNA-based regulation, we analysed RNA and protein complexes in glycerol gradients coupled to sequencing and mass spectrometry (Gerovac et al. 2021, Grad-seq: Smirnov et al. 2016). We selected the giant bacteriophage ΦKZ that encodes for hundreds of uncharacterized factors but no non-coding RNAs (Wicke et al., 2021). Notably, we observed phage transcripts that sedimented like non-coding RNAs. In the host, non-coding RNAs shifted in sedimentation profiles towards ribosomal fractions together with a high load of phage transcripts. Translation of host transcripts was inhibited. Strikingly, we observed phage proteins in ribosomal fractions that do not match with known translation factors and could mediate new modes of post-transcriptional control. In conclusion,

the translation machinery is not just overwhelmed by phage transcripts but also post-transcriptionally regulated, which makes new opportunities for biotechnological applications.

E 03
Insights into the processivity of NAD-RNAs in Sulfolobus acidocaldarius

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The discovery of NAD^+ as a 5´ cap in bacteria challenged the knowledge that only eukaryotes present RNA caps. Further studies showing NAD-RNAs in eukaryotes suggested that this RNA modification is ubiquitous to the tree of life. Still, the presence and function of NAD-RNAs in Archaea remained an open question. Here, by combining LC-MS and NAD captureSeq, we quantified the total levels of NAD-RNAs and determined which RNAs are capped in S. acidocaldarius and H. volcanii. A complementary dRNA-Seq analysis evidenced that NAD transcription start sites (TSS) present a well-defined promoter region and mostly correlate to primary TSS. Curiously, the population of NAD-RNAs in the two archaeal subjects differs, with S. acidocaldarius possessing more capped small ncRNAs and leader sequences. Multiple Nudix family proteins are involved in the processing of mRNA caps. A similarity search provided four Nudix protein candidates in S. acidocaldarius. In vitro assays and knockout strains

evidenced that these proteins do not have deNADing activity and are not essential. Finally, we show that the exonuclease Sso-aCPSF2 preferentially degrades NAD-RNAs, suggesting that this enzyme might have evolved to take over the role of NAD-RNA processivity in S. acidocaldarius in a similar fashion to DXO, Rai1, and Xrn1. In summary, this study characterizes the NAD-RNA landscape in two archaeal organisms and unravel insights into its metabolism in S. acidocaldarius.

E 04
Identification of FAD-capped RNAs: the FAD CaptureSeq

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RNA presents a vast functional diversity, ranging from regulation to even cellular defense. This fact evidences that some of these roles rely only on the molecule's sequence and structure, but also on RNA modifications, which are now considered to be important regulators of RNA function and metabolism. However, of the many RNA modifications known to date, few are found at the 5′ terminus of RNA, with the canonical eukaryotic m7G as the paradigmatic example. In the search for new RNA modifications, the coenzyme NAD was first discovered as a new RNA conjugate in bacteria. Although this was originally regarded as prokaryotic-specific, noncanonical NAD capping of RNA was also found in several eukaryotes, such as yeast, plant, and human cell lines. Apart from NAD, other nucleotide

analogs, such as the riboflavin-derived coenzyme FAD, were also found to cap RNA. Even though FAD-capped RNAs have already been detected and quantified in vivo, the identification of the specific RNAs carrying a FAD-cap still needs new methodologies that can selectively isolate these molecules. Here, several affinity-based enrichment strategies are proposed to develop a new FAD CaptureSeq protocol which will enable the study of RNAs bearing the FAD-cap. The study of new noncanonical caps has helped to unveil a new paradigm in epitranscriptomics, stimulating future efforts to investigate new regulatory processes, which may finally link cellular metabolism to gene expression.

R 09
Enhancer RNAs stimulate Pol II pause release by harnessing multivalent interactions to NELF

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Enhancer RNAs (eRNAs) are long non-coding RNAs arising from the transcription of activated enhancers. eRNA transcripts likely employ various mechanisms to induce target gene transcription, e.g. in neurons, they were suggested to facilitate the release of promoter-proximally paused RNA polymerase II (Pol II) into productive transcription elongation. However, a direct proof for this mechanism and the molecular determinants of eRNA function were undisclosed. Thus, using biochemical approaches we could demonstrate that eRNAs indeed

trigger the specific dissociation of NELF from the paused elongation complex and thereby facilitate Pol II pause release. We found eRNAs not to exert their function through common structural or sequence motifs. Instead, eRNAs that exhibit a length >200 nucleotides and that contain unpaired guanosines make multiple, allosteric contacts with NELF subunits -A and -E to trigger efficient NELF release. We confirmed our in vitro findings by NELF-E directed eCLIP-seq experiments in mouse primary neurons, which demonstrated that NELF-crosslinks to eRNAs are evenly distributed along the entire eRNA sequence. Taken together, our study reveals the molecular determinants of eRNA function for the first time, and mechanistically links eRNAs to Pol II pause release. Furthermore, our study suggests that eRNAs could bypass the canonical P-TEFb dependent pause release pathway and hence provides new insight into the complex network of metazoan transcription regulation.

R 10
Synthetic Riboswitches for the Analysis of tRNA Processing by eukaryotic RNase P Enzymes

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Removal of the 5' leader region is an essential step in the maturation of tRNA molecules in all domains of life. This reaction is catalyzed by various RNase P activities, ranging from ribonucleoproteins with ribozyme activity to protein-only forms. In Escherichia coli, the efficiency of RNase P

mediated cleavage can be controlled by computationally designed riboswitch elements in a ligand-dependent way, where the 5' leader sequence of a tRNA precursor is either sequestered in a hairpin structure or presented as a single-stranded region accessible for maturation. In the presented work, the regulatory potential of such artificial constructs is tested on different forms of eukaryotic RNase P enzymes – two protein-only RNase P enzymes (PRORP1 and PRORP2) from Arabidopsis thaliana and the ribonucleoprotein of Homo sapiens. The PRORP enzymes were analyzed in vitro as well as in vivo in a bacterial RNase P complementation system. We also tested in HEK293T cells whether the riboswitches remain functional with human nuclear RNase P. While the regulatory principle of the synthetic riboswitches applies for all tested RNase P enzymes, the results also show differences in the substrate requirements of the individual enzyme versions. Hence, such designed RNase P riboswitches represent a novel tool to investigate the impact of the structural composition of the 5'-leader on substrate recognition by different types of RNase P enzymes

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E 05
Investigating NAD-capped RNAs in mammalian cells

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The methylguanosine cap protecting the 5'end of messenger RNA in eukaryotes has an important effect on the fate of the RNA and is associated to crucial processes such as splicing, nucleus export, translation initiation, and

turnover. Interestingly, metabolites containing a nucleotide (e.g., NAD+, FAD) have been identified to be covalently linked to RNA in a cap-like structure. This recent discovery raises new questions about the roles, as well as the capping and decapping mechanisms of these non-canonical caps. In order to investigate the effects of NAD caps on RNA metabolism, we aim to establish a method to pull down NAD-capped RNAs, followed by sequencing. We plan to select adequate conditions affecting the tightly regulated homeostasis of NAD+ in human cells, and therefore gain an insight into the production, the stability and the physiological relevance of NAD-capped RNAs.

O 07
Expanding the transcriptomic toolbox in prokaryotes by Nanopore sequencing of RNA and cDNA molecules

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RNA sequencing dramatically changed our view of transcriptome architectures and allowed for ground-breaking discoveries in RNA biology. Recently, sequencing of full-length transcripts based on the single-molecule sequencing platform from Oxford Nanopore Technologies (ONT) was introduced and is widely employed to sequence eukaryotic and viral RNAs. However, experimental approaches implementing this technique for prokaryotic transcriptomes remain scarce. Here, we present an experimental and bioinformatic workflow for ONT RNA-seq in the bacterial model organism Escherichia coli. Our study highlights critical steps of library preparation and

computational analysis and compares the results to gold standards in the field. Furthermore, we evaluate the applicability and advantages of different ONT-based RNA sequencing protocols, including direct RNA, direct cDNA, and PCR-cDNA. We find that (PCR)-cDNA-seq offers improved yield and accuracy compared to direct RNA sequencing. Notably, (PCR)-cDNA-seq is suitable for quantitative measurements and can be readily used for accurate detection of transcript 5´ and 3´ boundaries, analysis of transcriptional units and transcriptional heterogeneity. In summary, based on our study, we show that Nanopore RNA-seq to be a ready-to-use tool allowing rapid, cost-effective, and accurate annotation of multiple transcriptomic features. Thereby Nanopore RNA-seq holds the potential to become a valuable alternative method for RNA analysis in prokaryotes.

R 11
Thermo-modular small RNA in Yersinia pseudotuberculosis

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As food-borne pathogen, Yersinia pseudotuberculosis requires the ability to respond rapidly to sudden changes. The thermal shift from environment to host rewires the bacterial metabolism towards pathogenesis. Many virulence-related genes are controlled by RNA thermometers (RNATs) that regulate translation initiation by temperature-induced structural changes.

This study aims to extend the concept of 5'UTR-encoded RNATs to small RNAs (sRNAs), as they are integral to bacterial gene regulation in response to environmental changes. RNA structuromics revealed compelling conformational differences at 25°C and 37°C in the sRNA OmrA (1,2) suggesting that target gene regulation might be regulated by temperature. We found a drastically reduced half-life of OmrA at 37°C compared to 25°C, whereas the half-life of a point-mutated stabilized OmrA variant was significantly extended at 37°C. Transcriptome analyses showed an increased spectrum of OmrA targets at 37°C compared to 25°C.

Our structural analysis suggests the formation of a stem-loop structure, which masks an U-rich motif in OmrA at lower temperatures. Melting of this structure at host body temperature of 37°C seems to reduce the half-life of OmrA as well as increase its target spectrum, which in-turn suggests an increased turn-over rate as sRNA-mRNA complexes are rapidly degraded.

(1) Righetti et al., PNAS (2016)
(2) Twittenhoff et al., Nucleic Acids Res (2020)

O 08
Genetic interactions and re-wired signaling pathways in Dicer-deficient cancer cells

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Deregulated expression of individual microRNAs (miRNAs) can cause developmental defects as well as cancer. The endoribonuclease Dicer is responsible for the maturation of almost all miRNAs in mammalian cells. Consequently, germline depletion of Dicer in murine knockout models results in organismal lethality. In contrast, deletion of Dicer in a tissue- or cell-type specific manner in mice can be tolerated, and even a few human DICER1 knockout cell lines have been established. Intriguingly, these cell systems display a strongly reduced proliferation rate. However, the underlying molecular mechanisms responsible for this aberrant phenotype are largely unknown. We addressed this question using transcriptional profiling as well as a genome-wide CRISPR/Cas9 functional genomics approach which allowed us to map, for the first time, the genetic interaction landscape of DICER1 within a human cancer cell line. These experiments unraveled a novel and surprising function of Dicer and the endogenous miRNA system in reprogramming the outcome of a dichotomous signaling pathway whose oncogenic or tumor suppressive function depends on the expression of Dicer and certain miRNAs. Utilizing a reconstitution assay allowed us to identify a miRNA family that was able to rescue the loss-of-function phenotype of Dicer knockout cells. Last but not least, our cellular analyses discovered an unexpected pathway of apoptosis induction in Dicer-deficient cells which might have clinical implications.

R 12
Distinct mechanisms keep Skipper-1 under control

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Skipper-1 is a chromovirus-type retrotransposon that accumulated in (peri-)centromeric regions of the Dictyostelium discoideum genome. We determined that Skipper-1 expression and mobility are controlled by two independent RNA interference pathways. In the cytoplasm, the Argonaute protein AgnA and the RNA-dependent RNA Polymerase (RdRP) RrpC inhibit Skipper-1 translation by small RNAs. Dysregulation of either protein disrupts this posttranscriptional gene silencing (PTGS) and leads to Skipper-1 mobilization. Transcriptional silencing of Skipper-1 in the nucleus is mediated by the RdRP RrpA, the Dicer-like protein DrnB and the Argonaute protein AgnB. Skipper-1 transcripts accumulated in mutants lacking any of these proteins, but retrotransposition was still blocked by cytoplasmatic PTGS. DrnB and RrpA co-localized in the nucleus with Skipper-1 genomic DNA and the heterochromatin marker H3K9me3. We suggest that Skipper-1 transcription is reduced via small RNA-induced heterochromatization of its genomic copies, whereas Skipper-1 amplification is suppressed in the cytoplasm by a small RNA-induced silencing complex and we discuss evolutionary implication of its complex control.

O09
β-propeller domains as novel RNA binding modules

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In 2015, the RNP crystal structure of the β-propeller NHL domain of the Drosophila protein Brain tumor (BRAT) was solved by our group in complex with its specific target RNA motif, presenting a novel type of RNA binding domain. We hence speculated that β-propeller protein domains generally present highly variable structures, enabling specific protein-RNA contacts. The WD40 domain defines one of the most ubiquitous and best-studied sub-classes of the β-propeller protein family, so far however mainly known for its function in protein-protein interaction. Based on previous results, we hypothesized that some WD40 domains may furthermore specifically interact with mRNAs.

To closer investigate the nature of potential RNA binding WD40 protein candidates, we established an improved in vivo Bind-n-Seq (BNS) protocol in our lab. By re-transcription of initially selected target RNA motifs, our protocol allows us to perform two target selection rounds with highly specific RNA binding in tandem. Consequently, both recognition as well as the enrichment of specific RNA motifs from a random pool of short RNAs are significantly increased compared to common BNS protocols out in the field. During our studies, our BNS together with further experiments emphasized amongst others the pbody component EDC4 as promising RNA binding candidate. With our further work we hope to contribute to previously published studies, focusing on the WD40 β-propeller domain as novel exciting and important RBD in cells.

O 10
Description of a canavanine utilization operon in

the novel bacterium Pseudomonas canavaninivorans

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Canavanine, the δ-oxa-analogue of arginine, is produced as one of the main nitrogen storage compounds in legume seeds and has repellent properties. Its toxicity originates from incorporation into proteins as well as arginase-mediated hydrolysis to canaline that forms stable oxims with carbonyls. So far no pathway or enzyme has been identified acting specifically on canavanine. Here we report the isolation of Pseudomonas canavaninivorans 1 from bean rhizosphere that utilizes canavanine as sole carbon and nitrogen source. We characterize a novel PLP-dependent enzyme, canavanine-γ-lyase, catalyzing the elimination of hydroxyguanidine from canavanine to subsequently yield homoserine. The findings broaden the diverse reactions that the versatile class of PLP-dependent enzymes is able to catalyze. We describe additional homoserine dehydrogenase, aspartate semialdehyde dehydrogenase and ammonium-aspartate-lyase activities facilitating canavanine utilization. We demonstrate that this novel pathway is also found in the Rhizobiales symbionts of legumes. Additionally, we identified a standalone B3/4 protein which can edit mischarged canavanyl-tRNAArg to prevent the incorporation into proteins. Our results could have implications for the establishment and maintenance of the legume rhizosphere and may contribute to its better understanding which is of upmost importance as legumes

are valuable crops for food security in face of challenges such as climate change.

P 06
RNA-protein interactions shape the architecture of eukaryotic H/ACA RNPs

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H/ACA Box ribonucleoprotein complexes are responsible for the sequence specific pseudouridylation of ribosomal and spliceosomal RNA in Eukaryotes and Archaea. The complex consists of a snoRNA with two hairpin structures with an internal loop where the enzymatic reaction takes place on a substrate RNA which is recruited via base pairing. Eukaryotic H/ACA complexes contain two sets of four core proteins Cbf5, Nop10, Gar1 and Nhp2, with Cbf5 being the catalytic subunit, which bind to each of the hairpins. Using yeast snR81 as a model RNA, we investigated structural dynamics of this RNP and characterized its overall architecture.

Using single-molecule FRET spectroscopy on complexes with different RNA and protein labeling sites, we could demonstrate how successive binding of the core proteins shapes the overall RNP on individual hairpins. Surprisingly, these effects are diverging for the two hairpins of snR81, suggesting specific requirements for folding and RNP formation.

Since the spatial arrangement of the two hairpin RNPs is poorly characterized, we placed strategic labels in proteins as well as the RNA, and investigated the position of the hairpins relative to each other. We find that within the fully assembled complex, interactions between protein domains and the RNA govern the overall architecture of this complex, and that eukaryote-specific protein domains play an important role in facilitating the RNP assembly process.

O 11
Profiling Intracellular RNA-Protein Interactions by Proximity Biotinylation

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RNA-protein interactions modulate various processes inside the living cell, including the biogenesis, localization, stability and processing of RNAs and proteins [1]. To better understand the role of RNA-protein interactions, we now present an approach to identify intracellular RNA-protein interactions by combining proximity biotinylation [2] with in-situ hybridization.

Our approach allows the identification of RNA binders directly inside cells without prior knowledge and does not require any genetic manipulation of the cells. We show the functioning of tool by identifying protein binders to bulk mRNA, to the ACTB transcript, and to the long non-coding RNA MALAT1. In contrast to state-of-the-art pulldown assays, our method can also be applied to compare RNA-

protein binding profiles between two distinct cellular states, e.g. w/wo oxidative stress or knockdown.

References
[1] S. Gerstberger, M. Hafner, T. Tuschl Nature Reviews Genetics 2014, Vol. 15, 829-845.
[2] M. Ramanathan, K. Majzoub, [...] P. A. Khavari Nature Methods 2018, Vol. 15, No. 3, 207-212.

O 12
The interferon-inducible antiviral MxB GTPase promotes capsid disassembly and genome release of herpesviruses

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Host proteins sense viral products and induce defense mechanisms, particularly in immune cells. Using cytosolic extracts from macrophages, we have reconstituted capsid-cytosol complexes, and identified the large dynamin-like GTPase MxB as one of several interferon-inducible proteins interacting with capsids of herpes simplex virus type 1 (HSV-1). Electron microscopy analyses showed that cytosols with MxB, but not with mutated MxB, had the remarkable capability to disassemble the icosahedral capsids of HSV-1.

Cell lines stably expressing MxB (Schilling et al. 2018, JVI) or upon tetracycline induction both restricted HSV-1 protein expression and virus production, while MxB mutants did not impair infection. We currently characterize the subcellular localization of different FLAG-tagged MxB constructs, in order to determine whether MxB binds and targets incoming or progeny nuclear or cytosolic capsids. Moreover, we investigate the subcellular localization of incoming HSV-1 CLICKable genomes or incoming capsids tagged with mCherry to test whether MxB also induces capsid disassemly in the cytosol or the nucleus of infected cells.

Our data suggest that MxB senses herpesviral capsids, mediates their disassembly, and thereby restricts the efficiency of nuclear targeting of incoming capsids and/or the assembly of progeny capsids. The resulting premature release of viral genomes from capsids may enhance the activation of DNA sensors, and thereby amplify the innate immune responses.

O 13
Molecular insights into the remodelling of roX lncRNA during Drosophila dosage compensation

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Dosage compensation provides an ideal model system to study the contribution of RNA–protein interactions and role of lncRNAs in epigenetic regulation. Maleless (MLE), an evolutionarily conserved RNA helicase and RNA chaperone Upstream of N-ras (UNR) are best known for their role in remodelling roX lncRNA during dosage compensation in Drosophila. This remodelling is required for the assembly of the functional dosage compensation complex (DCC) on the male X chromosome. However, how these multi-domain RNA binding proteins recognize and remodel roX RNA is unknown. Here, we address this using a combination of structural (Cryo-EM, NMR and crystallography), biophysical, biochemical and cell biology approaches. Our data provides molecular insights into the recognition and unwinding of roX2 RNA by MLE and shows that MLE undergoes drastic structural changes to bind the roX2 RNA. Mutations which compromise conformational changes of MLE also reduce RNA binding and the ability to integrate roX2 RNA into the dosage compensation complex. In addition, UNR and MLE remodel and stabilize the roX2 RNA to an alternative conformation competent for integration into the dosage compensation complex. These data provide new insights into how lncRNAs are remodelled to modulate gene regulation and how DExH-type helicases process their RNA targets.

O 14
ReLo: a simple colocalization assay to identify and characterize physical protein-protein interactions

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Characterizing protein-protein interactions (PPI) is fundamental for understanding biochemical processes. Many methods have been established to identify and study direct PPIs. However, screening and investigating physical PPIs involving large and poorly soluble proteins, such as many RNA-binding proteins, remains challenging.

Here, we present ReLo, a simple cell culture-based method that allows detection and investigation of PPIs in a cellular context. ReLo is suitable to define the protein domains that mediate a PPI and to screen for interfering point mutations. ReLo also allows the investigation of PPIs that depend on a specific conformation of one interaction partner, as examined using a DEAD-box RNA helicase, and those that depend on posttranscriptional protein modification, specifically symmetric arginine dimethylation. Further, ReLo is sensitive to drugs that mediate or interfere with a specific PPI. Importantly, through studies on the CCR4-NOT deadenylase multi-subunit complex, we demonstrate that ReLo detects specifically direct, but not indirect PPIs. Finally, ReLo can be applied to describe the binding topology of subunits within multi-protein complexes. Together, ReLo represents a simple, quick, and versatile tool to identify and study binary PPIs, as well as to characterize multi-subunit complexes, which together enables the comprehensive, initial description of direct PPI networks.

O 15
Revealing how tRNA splicing defects cause pontocerebellar hypoplasia using brain organoids

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Pontocerebellar hypoplasia type 2 A (PCH2A) is a rare, severe neurodevelopmental disorder. And has as hallmarks a cerebellum and pons of reduced size. PCH2A is characterized by a single point mutation in TSEN54, which encodes a protein required for tRNA splicing. Despite its unique genetic cause and stereotypical clinical manifestation, the pathological mechanism at the cellular and molecular level is currently unknown. We aim to address this gap in knowledge by investigating PCH2A pathology using human brain organoids of patient-derived iPSCs. We characterize the cellular developmental trajectories in cerebellar organoids, since it is the brain region where hypoplasia is observed. Subsequently, we aim to determine cell type-specific molecular changes in PCH2A through single-cell RNA-sequencing of organoids. We expect that our study will elucidate key disease mechanisms of PCH2A and have implications for other disorders induced by defects in tRNA splicing. Importantly, we will determine at which time point during cerebellar development differences between PCH2A and control organoids emerge. Hence, we will answer the question if PCH2A pathology emerges through the degeneration of existing structures, or whether the formation of the cerebellum is already altered. We will thus reveal a possible interplay between disrupted neurodevelopment and neurodegeneration. Our long-term goal is to define a therapeutic approach for PCH2A.

O 16
Characterisation and investigation of SMIM24 in cancer

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Small integral membrane proteins are involved in various physiological processes such as immune cell activation, cell migration, cellular differentiation, transport and cell-cell fusion. Small integral membrane protein 24 (SMIM24) is a type I single-pass membrane protein, belonging to a family of integral membrane proteins . In cancer, studies have reported a link between SMIM24 expression levels and prognosis. According to our preliminary data, the SMIM24 expression level is upregulated in liver metastasis as compared to primary tumour and human cancer cell line suggesting its role in metastasis and/or tumour progression. Recent studies have shown that SMIM24 is upregulated in colorectal cancer and leukaemia whereas it is downregulated in renal and pancreatic cancer. However, the functional role of SMIM24 in cancer remains obscure. This study aims to characterise and delineate the role of SMIM24 in cancer. Immunoprecipitation followed by Mass spectrometry analysis of SMIM24 has revealed putative interacting partners (FANCI, ATR, RFC, BRCA1, PARP1) and their possible role in the DNA replication/repair pathway. Currently, we are validating the mass spec data to understand the mechanistic function of SMIM24. To further assess the effect of SMIM24 on growth, cell cycle, migration and invasion, SMIM24 knockout cell lines were generated using CRISPR/Cas9 gene-editing system and are currently analysing the phenotypes associated with SMIM24 knockout in cancer progression.

R29
Identification and molecular basis of host antiviral protein ZAP-S as an inhibitor of SARS-COV-2 viral gene switch

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Programmed ribosomal frameshifting (PRF) is a fundamental gene expression event in many viruses, including SARS-CoV-2. It allows production of essential viral, structural and replicative enzymes that are encoded in an alternative reading frame, thus acting like a gene switch. Despite the importance of PRF for the viral life cycle, it is still largely unknown how and to what extent cellular factors alter mechanical properties of frameshift RNA elements and thereby impact virulence. This prompted us to comprehensively dissect the interplay between the SARS-CoV-2 frameshift RNA element and the host proteome. Using antisense oligo purification coupled with mass spectrometry we identified that the short isoform of the zinc-finger antiviral protein (ZAP-S) is a direct regulator of PRF in SARS-CoV-2 infected cells. ZAP-S overexpression strongly impairs frameshifting and inhibits viral replication. Employing in vitro ensemble microscope thermophoresis, DMS-MaPseq and single-molecule optical tweezers techniques, we further demonstrate that ZAP-S directly interacts with the SARS-CoV-2 RNA and interferes with the folding of the frameshift RNA element. Together, these data identify ZAP-S as a host-encoded inhibitor of SARS-CoV-2 frameshifting and expand our understanding of RNA-based gene regulation1.

1 Zimmer, M.M., Kibe, A., Rand, U. et al. The short isoform of the host antiviral protein ZAP acts as an inhibitor of SARS-CoV-2 programmed ribosomal frameshifting. Nat Commun12, 7193 (2021)

O 17
The essential DEAD-box ATPase Dbp2 functions in nuclear RNA surveillance

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Across kingdoms, DEAD-box ATPases are involved in multiple aspects of RNA metabolism, including RNA maturation and decay, regulation of translation, RNA granule homeostasis, and the modulation of non-coding RNA activity. At the molecular level, they act as RNA remodelling helicases and their various biological functions are thought to depend on their ability to rearrange structural RNA elements and remodel entire mRNPs through RNA unwinding and annealing activities. Here, we focus on the essential DEAD-box protein Dbp2 (homologue of DDX5), which is recruited to transcribing RNA polymerase II in Schizosaccharomyces pombe and has been proposed to mediate early mRNP remodelling. Using comparative proteomics, we link Dbp2 to specific co-transcriptional RNA processing events and to nuclear RNA surveillance. In the absence of Dbp2, RNA accumulates in nuclear granules, suggesting a role for the DEAD-box ATPase in licensing RNAs for export from the nucleus.

O 18
Synthetic riboswitches designed for tetracycline-inducible gene expression in human cell culture

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Synthetic ribowitches represent ideal tools for controlling transgene expression. The potential use ranges from applications in synthetic biology, functional genomics, and pharmaceutical target validation. Riboswitches sensing ligands with suitable pharmacological properties and their high dynamic ranges might be exploited for therapeutic purposes. Based on alternative splicing, we were able to develop a set of synthetic riboswitches in human cells with high fold changes. By placing the 5'-splice site into the stem of a tetracycline-sensing aptamer, exon skipping can be regulated, allowing switchable gene expression. Aside from the splicing context, another strategy is the combination of a tetracycline-sensing aptamer with nucleolytic ribozymes. With this, we aim for a robust and universal tool to achieve high switching effects with tetracyline addition. Since first attempts resulted in moderate fold changes, we screened and compared several small ribozymes on their self-cleaving activity in order to find promising candidates as a starting point to develop efficient synthetic riboswitches and further test them in vivo.

E 06
The role of tRNA position 37 modifications in translation and translation-associated diseases

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Chemical modifications of transfer RNAs (tRNAs) are crucial to optimize translational fidelity and efficiency, tRNA aminoacylation and tRNA structure. Hypomodified tRNAs are associated with neurological diseases and cancer. However, how hypomodified tRNAs contribute to the etiology of these diseases is largely unknown.

To better understand the role of tRNA modifications in human diseases, I inactivated tRNA modifying enzymes targeting position 37 in human cells. This position is located 3' adjacent to the anticodon and contains 9 distinct modifications in human tRNAs. Importantly, hypomodified tRNAs led to decreased cell growth and global translation. I am currently performing in-depth analyses of translational dynamics in these mutants using ribosome profiling. Reading-frame information and ribosomal RNA (rRNA) contamination levels are crucial parameters that determine library quality. I have therefore optimized these parameters for human samples. I found that combining RNaseI and MNase results in a strikingly improved mapping resolution in contrast to using both enzymes separately. Furthermore, I am optimizing rRNA depletion by targeting specific contaminants through biotinylated oligonucleotides. This optimized protocol will allow me to perform the first systematic high-resolution analysis of tRNA modification mutants in human cells to determine how tRNA modifications tune translation and how they are linked to translation-associated diseases.

P 07
Functional characterization of Pseudomonas oleovorans Type IV-A CRISPR-Cas activity in vitro

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CRISPR-Cas systems provide adaptive immunity against invading nucleic acids in prokaryotes. Class 1 CRISPR-Cas systems are most common in nature and are characterized by multi-subunit ribonucleoprotein complexes for target interference. Type IV CRISPR-Cas systems belong to Class 1, but lack nucleases for target degradation. Here, we studied a Type IV-A CRISPR-Cas system of Pseudomonas oleovorans, which contains an operon coding for four Cas proteins in addition to the helicase DinG and a CRISPR array. We identified the first crRNA of the system to perfectly match the host gene pilN and aimed to evaluate the biological relevance of this self-targeting mechanism. Analyses of transcripts and expression levels showed a downregulation of the pil operon in the native P. oleovorans strain compared to a CRISPR array deletion strain. This activity resembles CRISPRi methodology which uses dCas9 or effectors without nucleases to block the transcription machinery. Our data indicates that Type IV-A CRISPR-Cas systems can function in host gene regulation and represent a natural CRISPRi system. Furthermore, recombinant protein production enabled first structural analyses of Type IV-A CRISPR-ribonucleoprotein complexes (crRNPs), revealing effector complexes that resemble Type I crRNPs.

O 19
Structure-guided functional analysis of yeast tRNA ligase

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Non-conventional RNA splicing is an essential RNA maturation step during tRNA processing as well as during the unfolded protein response. This splicing reaction differs vastly from spliceosome-mediated processing of mRNAs in the nucleus. Non-conventional splicing is a cytosolic, fully enzyme-catalysed reaction. The intron is first cut out by a splicing endonuclease complex leaving two RNA exons, which are further ligated by an RNA ligase, named Trl1 in fungi. Trl1 is a tripartite enzyme that catalyses the ligation reaction in three reaction steps. The final activity of an adenylyltransferase domain (LIG) connects both exon-ends to yield the mature RNA molecule. Recent studies revealed the crystal structure of the Trl1-LIG domain with its two subdomains (SDs). The N-terminal SD resembles a typical adenylyltransferase structure, whereas the C-terminal SD adopts an unique all helical fold. The precise coordination of both exon-ends and the function of the individual SDs of Trl1 have remained unknown. Here, we tested the impact of the C-terminal SD on the adenylylation activity and the ligation reaction of the Trl1-LIG domain. Structure-guided mutagenesis revealed an essential role of the C-terminal SD during ligation and regulation of non-conventional splicing. Further, these results will help us to understand the enzymes mode of action to get an idea how both RNA ends are selected and coordinated for their ligation.

R 14
A 3´UTR-derived, processed small RNA modulates

flagellar biogenesis by repression of the anti-sigma factor FlgM in Campylobacter jejuni

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In Campylobacter jejuni, as for many pathogens, flagella are a crucial virulence factor, enabling traversal through the viscous mucus layer of the human intestine. Flagellar biogenesis is known to involve hierarchical transcriptional control of each component, with class I genes transcribed from sigma-70-dependent promoters, and class II and class III genes transcribed with the help of alternative sigma factors, RpoN and FliA, respectively. In contrast to transcriptional control, less is known about post-transcriptional regulation of flagellar biosynthesis cascades by small regulatory RNAs (sRNAs). Here, we have characterized a conserved Campylobacter sRNA, CJnc230, encoded downstream of the RpoN-dependent flagellar hook structural protein FlgE, and found that three ribonucleases are involved in sRNA maturation. RNase III cleaves off the sRNA from the flgE mRNA, while RNase Y and PNPase process CJnc230 at its 3´ end. We identified a regulator of flagella-flagella interactions as well as the anti-sigma-28 factor FlgM as targets of CJnc230 repression. CJnc230 overexpression led to de-repression of FliA and subsequent upregulation of class III flagellar genes such as the major flagellin flaA, culminating in an increase in motility and flagellar length. Overexpression of the FliA-dependent sRNA CJnc170 instead led to reduced motility suggesting sRNA-mediated regulation of C. jejuni flagellar biosynthesis through balancing of the hierarchically expressed components.

P 08
Short pAgo systems interfere with invading nucleic acids

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While long prokaryotic Argonaute proteins have been shown to mediate DNA-guided DNA interference, the mechanisms and function of short prokaryotic Argonaute proteins are poorly understood. We discovered that short prokaryotic Argonaute and associated TIR-APAZ (SPARTA) form complexes that mediate RNA-guided detection of invading DNA and trigger NAD depletion in cells. Here, we describe how SPARTA systems are activated in vitro and in vivo. This provides mechanistic and functional insights into the activity of SPARTA systems.

O 20
Integrative target prediction for bacterial sRNAs

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Bacterial small RNAs (sRNAs) are involved in maintaining cellular physiology and adapting bacteria to changing environments and stress conditions. Generally, sRNAs base pair with partially complementary regions within mRNAs, impacting on the translation or stability of these targets. However, sRNA target identification remains challenging as computational and experimental approaches suffer from inherent limitations. In this project, we borrow statistical techniques from the field of meta-analysis to exploit the synergy between orthogonal approaches to sRNA target prediction. Specifically, we integrate MS2 affinity purification and sequencing to determine cellular RNAs that physically interact with a given sRNA of interest (Lalaouna, 2015), in silico prediction of sRNA-mRNA interactions (Wright, 2014), and sRNA pulse-expression followed by measurement of genome-wide expression changes (Papenfort, 2006). Our method calculates a combined significance value for each gene over all datasets. We benchmarked the pipeline on the well-studied PinT sRNA from Salmonella enterica for which many direct targets and regulatory mechanisms are known (Westermann, 2016; Kim, 2019; Santos, 2021). Our approach "re-discovered" established PinT targets, while also identifying new candidates that are currently being validated. Together, this study provides a blueprint for high-confidence sRNA target identification through the principled integration of multiple datasets.

P 09
Binding preferences of the SARS-CoV-2 nucleocapsid for genomic RNA elements

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The virus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) possesses a large RNA genome of almost 30kb, flanked by highly-conserved regulatory untranslated regions (UTRs). Genome packaging into virions is crucial for viral progression. Key player of this process is the versatile nucleocapsid protein (N) - one of four coronaviral structural proteins - that is further involved in RNA transcription. N harbors five functionally distinct parts, including two folded domains, the RNA-binding NTD and the dimerization-mediating CTD, flanked by intrinsically disordered regions.

Despite numerous publications on RNA-binding by SARS-CoV-2 N, high-resolution structural data of complexes with RNA is missing, and no systematic examination of its specificity for genomic RNA is available. However, a structural basis for specific interactions of N with viral RNAs would pave a way to new medication. We here give a detailed insight into the interaction of N's NTD with 5'-cis regulatory elements SL1-SL8, using an NMR-centered approach. We specify preferences of the NTD for sequences within the 5'UTR occurring in distinctive structural contexts. In fingerprint spectra, we further define signature residues located in the RNA-interacting regions of the NTD that are likely sensors for the distinction between specific vs. non-specific target sequences. Our results will serve as a basis for further studies that aim at understanding precise determinants of nucleocapsid RNA specificity.

O 21
Spatial single-bacteria gene expression profiling using fluorescence in situ hybridization and expansion microscopy

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Single-cell (sc)RNA-seq powerfully reports the physiological states of cells by measuring a census of all RNA molecules. In the context of bacterial infections, RNA-seq can reveal the interplay between the host and the pathogen with unprecedented resolution. However, bacteria appear to be highly intermingled in infected tissues, forming cellular communities that are impossible to isolate one by one and sequence. Furthermore, bacterial RNA molecules are labile and rare (

T 05
siRNA delivery to immune cells ex vivo - a potential for cell therapy reprogramming

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Effectivity of cell therapies (i.e. CAR T cells or donor lymphocyte infusions) is currently limited by initial phenotypic heterogeneity, which may be optimized using siRNAs. To achieve this an efficient delivery platform and a

basic understanding of the expected silencing duration are needed.

We found lipid–conjugate–mediated delivery to be more efficient than lipid–nanoparticle–mediated delivery in Jurkat cells and activated T cells (ATCs). siRNA uptake did not correlate with silencing nor with hydrophobicity of the lipid conjugate. The best silencing was achieved with the following lipid conjugates: myristic acid in dendritic cells and ATCs, divalent myristic acid in Jurkats, cholesterol in peripheral blood mononuclear cells. Live cell imaging showed different uptake kinetics, with cholesterol siRNA being quickest, followed by single, divalent and trivalent myristic acid conjugated siRNAs. Best siRNA candidates resulted in up to 99% silencing and IC50 of 4 nM.

Silencing duration (at least 50% efficiency) depended on the initial siRNA dose and varied between 15 and 30 cell divisions in Jurkat cells, up to 25 divisions in HeLa and at least 4 divisions in ATCs. Silencing duration also depended on the expression level of the target mRNA.

Conditions characterized here may enable siRNA use to potentiate cell therapies.

O 22
MED12 binds and activates Cyclin-dependent kinase 3

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Due to their oncogenic role in multiple malignancies, members of the cyclin-dependent kinase (CDK) family are important drug targets. This is also the case for CDK8, a colon cancer oncogene that, in addition to Cyclin C association, requires MED12 binding for its full activation. As atomic resolution structures, a prerequisite of drug development, are to date unavailable for the CDK8/Cyclin C/ MED12 complex, we aimed at identifying alternative CDKs that might also be activated by MED12. Intriguingly, the cell cycle kinase CDK3, that promotes G_0 exit by phosphorylating retinoblastoma protein (Rb), was shown to bind Cyclin C, making it a prime candidate for the formation of a ternary complex involving MED12. Indeed, I was able to endogenously express and purify human ternary CDK3/Cyclin C/MED12 complexes in vitro from insect cells. Moreover, using in vitro kinase assays I could confirm that the CDK3 complexes phosphorlyated Rb. Despite the fact that the T-loop of CDK3 harbors a potential phosphorlyation site, my experiments revealed that the MED12 activation helix, an element essential for MED12-driven CDK8 activation, is also needed for full CDK3 activity. Taken together, my experiments therefore reveal that MED12 enhances CDK3 activity by formation of a ternary CDK3/Cyclin C/MED12 complex, thus representing a potential functional analog to ternary CDK8/Cyclin C/MED12 complexes.

E 07
MePMe-seq: Enrichment and Identification of Methyltransferase Target Sites in RNA

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The most abundant internal modification in eukaryotic mRNA is N^6-methyladenosine (m^6A) which is introduced by the methyltransferases (MTases) METTL3-METTL14 as well METTL16 (Boccaletto et al, 2018; Dominissini et al, 2012; Meyer et al, 2012). The mRNA methylation state influences the mRNA metabolism, which then affects cell differentiation and development (Batista et al, 2014; Haussmann et al, 2016). Precise transcriptome-wide assignment of m^6A sites is of utmost importance. However, polymerase-based detection is challenging, as m^6A does not interfere with Watson-Crick base pairing. We developed a chemical biology approach called MePMe-seq that is independent of anti-m^6A antibodies and sequence preferences by filtering (Hartstock et al, 2018). Our approach relies on enzymatic propargylation of RNA, which can be performed with wild-type MTases either in vitro or metabolically in cellulo. After bioconjugation, enrichment, reverse transcription (RT) under optimized conditions to obtain 80 % of termination and purification, MTase target sites can be detected in mRNA and identified by next generation sequencing (NGS). Importantly, we were able to validate known m^6A sites and identify unreported sites. Direct comparison of metabolically labeled RNA with RNA modified by METTL16 in vitro revealed false positives in the in vitro studies. We conclude that metabolic labeling is superior and required for reliable identification of modification sites.

O 23
Creating a cellular toolbox to investigate the role of miRNAs in cancer

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MicroRNAs (miRNAs) are short, hairpin-derived RNAs, which post-transcriptionally repress the expression of target genes. The vast majority of miRNAs are generated by enzymes of the canonical pathway with key players being the ribonuclease III enzymes Dicer and Drosha. Moreover, the AGO2 protein is the main effector of miRNAs inside human cells. Our aim was the creation of a toolbox comprised of genetically engineered, isogenic cell lines lacking these key players to gain novel insights into their function in human cells. To this end we took advantage of the CRISPR/Cas9 system from S. pyogenes to generate individual knockout clones using one or two single guide RNA. Fluorescence-based single cell sorting after transfection of a lung adenocarcinoma cell line gave rise to single cell clones lacking mature miRNA expression or activity which led to a reduced proliferation. Hence, by depleting three key players of the miRNA biogenesis pathway we created valuable tools to thoroughly examine miRNA biogenesis including its influencing factors. For example, the miRNA deficient cells allow the investigation of interactions between RNA-binding proteins and their cellular targets with further focus on miRNA competition of certain interactions. Moreover, the toolbox facilitates the study of the complex interplay between (single) miRNAs and signal transduction pathways and thereby opens up a plethora of possibilities to explore crucial pathways and their interactions in lung cancer cells.

O 24
Messengers outer space: mRNA effectors in extracellular vesicles of the corn smut pathogen Ustilago maydis

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Extracellular vesicles (EVs) are emerging as important mediators of plant-pathogen interactions. Since EVs carry diverse molecules including RNA, examining EVs of phytopathogens could facilitate discovery of a broader spectrum of effectors secreted to manipulate host plants. The corn smut pathogen Ustilago maydis is a fungal model for membrane-associated mRNA transport, we have characterized the repertoire of mRNAs associated with its EVs (Kwon et al., 2021). For this purpose, EV isolation methods were developed for both filamentous cultures of U. maydis, as well as infected plant materials. Intact mRNAs were found in U. maydis EVs and a subset of mRNAs were enriched in EVs compared to the cells, suggesting a selective loading mechanism. Interestingly, the most highly enriched mRNAs in EVs encode metabolic enzymes that are upregulated during infection, inspiring the idea that EV-associated mRNAs may participate in reprogramming of host cell metabolism. Furthermore, mRNAs of known effectors and virulence-related proteins were also found in EVs. We hypothesize that U. maydis secretes mRNAs encoding effectors via EVs, which can be delivered and translated in the maize cell during infection. This would transfer the cost of pathogen effector protein production to the host, making for an economically effective strategy for the pathogen.

KWON, S., et al. (2021) mRNA Inventory of Extracellular Vesicles from Ustilago maydis. Journal of Fungi, 7, 562.

O 25
Grad-seq in Clostridioides difficile identifies KhpB as global RNA-binding protein and regulator of toxin production

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Most of our knowledge about regulatory networks comprising small noncoding RNAs (sRNAs) and RNA-binding proteins (RBPs) is derived from research in gram-negative model organisms, leaving the research in gram-positive species such as C. difficile behind.

We applied gradient profiling by sequencing (Grad-seq), a recently introduced approach for the reconstruction of global RNA/protein complexes, to native cell lysates of the gram-positive pathogen C. difficile. Genome-wide comparative analysis of the obtained sedimentation profiles of cellular proteins and transcripts, led to the identification of similar behaving sRNAs. To identify binding partners we applied RNA-bait dependent pulldowns. This led to the discovery of the widely conserved protein KhpB.

With RIP-seq we could show that KhpB is able to bind a large suite of RNAs including sRNAs and mRNAs, identifying it as a novel globally acting RBP in C. difficile. Altered steady-state levels or reduced half-lives of bound sRNAs could be observed with rifampicin stability assays. Characterization of a khpB knockout strain provided

evidence that KhpB impacts cell division and further revealed a role of this RBP in the regulation of toxin A production.

Our analysis of the KhpB protein in C. difficile indicates that homologues of this protein family are global acting RBPs that regulate conserved physiological functions as well as species-specific functions such as the regulation of toxin production.

E 08
An unbiased analysis of YTHDF2 protein functions

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N6-methyladenosine (m^6A) is one of the most abundant RNA modifications within coding and non-coding RNAs in eukaryotes. YT521-B homology domain family (YTHDF) are proteins able to recognize this modification and are thought to influence the translation efficiency and stability of m^6A containing mRNAs. Previous studies and data from Bio-ID experiments (Youn et al., 2018, Du et al., 2016) reported that several factors involved in RNA degradation such as CCR4-NOT complex proteins, AGO2, TNRC6, were detected as YTHDF protein interactors. By performing an immunoprecipitation of endogenous YTHDF2 followed by mass spectrometry analysis, we discovered that most of these interactions could not be reproduced in our lab conditions. Moreover, using the tethering system in several

RNA degradation machinery depleted cells we found out that YTHDF2 does not seem to be involved in any specific degradation pathway. In addition, we identified two new individual YTHDF2 phosphorylations in the YTH domain at position Y429 and S487. Those two sites have a little impact on YTHDF2 localization in vivo but significantly increase YTHDF2 binding affinity of a m^6A-modified RNA 12mer in vitro, hinting that they could play a pivotal role in the regulation of m^6A-marked mRNAs binding. In conclusion, our work provides a new perspective about the role of YTHDF2 in mRNA metabolism and its localization in the cell, highlighting that this is a field in constant development and more controls need to be done.

P 10
Targeted A-to-I and C-to-U RNA Editing using SNAP-fused effectors

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Of the numerous and manifold RNA modifications two major modifications attained great prominence in recent years. The adenosine deaminase acting on RNA (ADAR)-family performs adenosine to inosine (A-to-I) conversions on mRNA level. As inosine is interpreted as guanosine by the expression machinery, this way not only amino acid sequence is affected but also other processes (e.g. splicing). Besides adenosine, cytidines can also be deaminated

resulting in uracil (C-to-U) which is carried out by among others the APOBEC family.

We have shown in the past that ADARs can be exploited for site-directed A-to-I RNA editing by fusing the deaminase domain to a self-labeling protein tag. This SNAP-tag covalently binds to guideRNAs bearing O^6-benzylguanine derivatives. This way SNAP-ADARs can be directed to the target site (Vogel, Moschref et al. 2018). Moreover, we have not only shown that this technique can be transferred to APOBEC1 for targeted C-to-U editing but it can be combined with different protein tags for simultaneous A-to-I of endogenous transcript in cell culture (Stroppel, Latifi et al. 2021).

Unfortunately, APOBEC1 fusion exhibited certain limitations and handling difficulties rendering it suboptimal. However, we were able to create a versatile and very powerful tool, exhibiting maximum programmability with extended codon scope and unmatched potency. This, in combination with our SNAP-ADAR approach constitutes an excellent platform for targeted A-to-I and C-to-U RNA editing.

T 06
Targeted elimination of a human pathogen Clostridioides difficile using antisense oligonucleotide-based therapeutics

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Antibiotic resistant bacteria are an increasing threat to human health worldwide and pose a demand for the development of novel antibacterial agents. Nucleic-acid-based therapeutics hold a great promise and can be rationally designed to site-specifically target genes of interest [1,2]. Clostridioides difficile is a gram positive, opportunistic human pathogen that exploits microbial dysbiosis due to prior antibiotic treatment and causes severe inflammation of the gut. We aim to design antisense oligonucleotide (ASO)-based drugs against C. difficile that will target transcripts involved in its viability and virulence. To identify the optimal ASO delivery molecules, we utilised a library of fluorescently labelled cell penetrating peptides (CPPs). Yet in contrast to many other bacteria, selected CPPs coupled to ASO analogues - peptide nucleic acids (PNA) - did not show efficient inhibitory effect in C. difficile. We therefore designed a "switch-on" reporter system that will enable an unbiased monitoring of ASO uptake and efficiency. Finally, to overcome challenges of ASO delivery we are exploiting alternative carrier approaches using amphiphilic bolasomes. Notably, such formulations allow the complexation of multiple ASOs within a single amphiphilic vesicle. We anticipate that combinatorial targeting of multiple transcripts may enhance bacterial elimination and diminish off-target responses.

[1] Roberts et al. 2020 Nat. Rev. Drug Discov.
[2] Vogel 2020 Mol. Microbiol

E 09
Deoxyribozymes as tool for analysis of RNA modifications

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The majority of cellular RNAs undergo posttranscriptional RNA modifications, which are key regulators of gene expression. Methylation is among the most abundant, evolutionary conserved, and dynamic RNA modifications, and reliable tools for detecting, quantifying, and validating methylated nucleotides are in high demand. We contribute deoxyribozymes as tools to interrogate the methylation state of RNA.

Using in vitro selection, we identified RNA-cleaving DNA enzymes that specifically recognize modified adenosines, N^6-methyladenosine (m^6A) and N^6-isopentenyladenosine (i^6A), differentiate the three natural monomethylated cytidine isomers 3-methylcytidine (m^3C), N^4-methylcytidine (m^4C), and 5-methylcytidine (m^5C), and distinguish them from unmodified cytidine. These DNAzymes exhibit broad RNA sequence scope as exemplified by the examination of m^3C- and m^5C- modified tRNAs. For example, m^3C-specific DNAzymes assisted in demonstrating the role of METTL8 for installing m^3C32 on human mt-tRNAThr/Ser(UCN). Our findings provide insights into DNA's catalytic abilities and offer synthetic tools to detect RNA modification. In order to accelerate this process, we developed deoxyribozyme sequencing (DZ-seq), a NGS-based strategy that allows massively parallel quantification of RNA cleavage activity directly from the sequencing data, and could enable the discovery of new deoxyribozymes for the plethora of RNA modifications.

R 15
A new Argonaute 2 splice variant influences microRNA function and melanoma cell viability

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Melanoma is a highly proliferative and invasive tumor with a steadily increasing incidence and a high mortality rate. Post-transcriptional gene regulation by microRNAs (miRNAs) plays an important role for melanoma development and progression. Using RNA-seq, we found a melanoma-specific upregulation of many miRNAs compared to normal human epidermal melanocytes (NHEM). In addition, we identified a strong downregulation of Argonaut 2 (AGO2), the main effector protein of miRNA function, in melanoma cells compared to NHEM or other cancer cell lines. We further detected a yet unknown AGO2 variant, AGO2-ex1/3, in melanoma cell lines and patient samples. This variant is translated into an N-terminally truncated AGO2 protein. Molecular dynamics simulations showed an increased interdomain flexibility due to the truncation, but the main protein structure remains largely intact. A knockdown of AGO2-ex1/3 in melanoma cells leads to reduced proliferation and increased apoptosis which was analyzed using XTT-assay, "real time cell analysis" and flow cytometry. RNA-seq after the knockdown revealed a strong regulation of miRNA target genes. This implicates an important functional significance of this AGO2 variant for the miRNA pathway. Our data about this previously unknown AGO2 variant provide insight into a fascinating, yet undiscovered cellular mechanism and could potentially lead to the development of a new therapeutic strategy by modifying the viability of melanoma cells.

O 26
Discovery of the first human 5'-3' RNA ligase

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Recently, we found that a previously uncharacterized human gene (only 4 entries in PubMed in January 2022) encodes a "5'-3' RNA ligase", an enzyme for which there is as yet no example in human cells. This enzyme is highly conserved in chordates but absent in lower eukaryotes. We found that the enzyme closes "broken" RNA strands. Functional characterization shows that this ATP-dependent RNA ligase forms a phosphodiester bond between 3'-OH and 5'-phosphate in single-stranded RNA termini via a three-step mechanism, including protein and RNA AMPylation.

We present results on the functional characterization of the enzyme and its role in stress-adaptive RNA repair at the cellular and organismal level. The data obtained indicate the importance of RNA strand repair for cellular and organismal fitness.

O 27
Comprehensive analysis of translation by ultra-rapid ribosome profiling from minute amounts of biological material

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Ribosome profiling provides genome-wide and comprehensive snapshots of translation based on the sequencing of ribosome-protected fragments (RPFs). Revealing the precise positions of ribosomes in a quantitative manner, it has e.g. provided evidence for pervasive translation and yielded novel mechanistic insights thus greatly advancing our understanding of cellular translation.

Here we present a simplified and extremely rapid workflow for ribosome profiling. An improved library preparation protocol enables the robust production of high-complexity libraries from as little as 0.1 pmol of RPFs at little effort and within 24 hours. This is achieved by combining several recently introduced innovations to sequencing library preparation into a single, streamlined protocol optimized for ribosome profiling: (1) Bead-based solid phase extraction offers convenient and rapid purification of reaction intermediates while minimizing loss of material, (2) an optimized strategy for PCR amplification prevents over-amplification to ensure high quality of the final libraries, (3) highly efficient ligation of adapters and newly designed primers enhance the sensitivity and facilitate experimental multiplexing, (4) degenerate nucleotides in the ligation adapters reduce ligation bias and allow the identification of PCR duplicates. In summary, our presented workflow can be applied to minute amounts of sample, is simple to perform, and faster and cheaper than current existing protocols.

P 11
The splicing factor PHF5A influences melanoma cell growth and viability

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Melanoma, a malignant tumor derived from melanocytes, is a highly aggressive form of cancer, because of its high rate of metastasis. The dysregulation of RNA-binding proteins (RBPs) is a crucial step in the development and progression of cancer. RBPs are involved in all RNA-associated cellular processes, such as transcription, splicing or translation. Therefore, RBPs are also important regulators of processes that participate in tumorigenesis, e.g. cell proliferation, migration or apoptosis. One important RBP is PHF5A, a subunit of the splicing factor 3B. We could show a strong upregulation of PHF5A in several melanoma cell lines compared to normal human epidermal melanocytes. After siRNA-mediated knockdown of PHF5A, we observed a complete inhibition of proliferation in many melanoma cell lines. Furthermore, the melanoma cells lost their specific ability to form colonies derived from a single cell. Flow cytometry analysis revealed a huge arrest in the G2 phase of the cell cycle, as well as an increase of apoptotic cells. To investigate if this observed phenotype is due to a defect in splicing, quantitative real time PCR was performed. It was possible to confirm dysregulated splicing in different genes. Our results indicate an important role of PHF5A for melanoma cell proliferation and viability. This makes PHF5A a potential promising target for future melanoma therapy approaches.

P 12
Single-molecule FRET measurements of the RISC loading complex reveal a hAgo2 conformation favourable for miRNA duplex loading

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RNA interference (RNAi) regulates posttranscriptional gene silencing in human cells. Central to RNAi is the RNA-induced silencing complex (RISC) that contains Argonaute 2 (hAgo2) protein loaded with a miRNA, which helps to recognize and bind complementary mRNA targets. Target binding leads to translational repression or direct cleavage by catalytically active hAgo2. Mature miRNAs are generated from precursor miRNA by Dicer. In the RISC loading complex (RLC) composed of Dicer, hAgo2 and a double-stranded RNA binding protein, a miRNA duplex is loaded into hAgo2. The molecular details of this process and the conformational state that allows interaction between Dicer and hAgo2 as well as miRNA loading are not well understood.

To answer these questions, we employed our recently developed method for site-specific labelling of endogenous mammalian proteins for single molecule (sm) FRET (SLAM-FRET) measurements. hAgo2 and Dicer were expressed in HEK cells and incorporation of an unnatural amino acid at two positions in hAgo2 was achieved allowing site-specific labelling of hAgo2 with a donor and acceptor fluorophore. We performed smFRET experiment with hAgo2 as part of the RLC by using a Dicer-directed antibody for immobilisation. Compared to directly immobilised hAgo2, we observed that hAgo2 in the RLC

adopts a more open conformation. Our data indicate that complex formation with Dicer induces an opening between the two lobes of hAgo2, which might facilitate miRNA duplex loading.

E 10
Direct Biotinylation in NAD captureSeq 2.0 – One Step into the Future

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Since its discovery as a non-canonical RNA modification, nicotinamide adenine dinucleotide (NAD) has been identified as an RNA 5′-cap in organisms amongst all kingdoms of life. A breakthrough in determining the sequences of NAD-capped RNAs was achieved by NAD captureSeq, presented by the Jäschke Lab in 2015. The central part of this sophisticated protocol involves a two-step modification of the NAD 5′-cap: First, the enzymatic substitution of the nicotinamide moiety by 4-pentyn-1-ol, followed by chemical modification via azide-alkyne-cycloaddition to introduce a biotin label. To date, all protocols for NAD-RNA sequencing are based on variations of this two-step strategy.

Here we developed biotin-coupled pyridine derivatives, e.g. 3-picolylamine biotin (3PAB), as novel substrates for ADPRC that allow for the specific, direct biotinylation of

NAD-RNA providing fast kinetics and high yield (>80% modification in

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R 16
Functional characterization of the novel long non-coding RNA P4 in epidermal tissue homeostasis

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In epidermal homeostasis, keratinocytes continually pass through strictly regulated stages of differentiation. Only a few long non-coding RNAs (lncRNAs) are functionally characterized in mature human epidermal tissue environment.

High-throughput sequencing revealed downregulation of the novel lncRNA P4 during differentiation. P4-Knockdown (KD) resulted in heavy upregulation of early and late epidermal differentiation marker genes. Gene ontology term analysis in P4-depleted tissue predicted a functional role in regulation of skin barrier formation and progenitor state.

KD-studies in organotypic tissue revealed massively thickened suprabasal layers and shared histopathological patterns with keratinizing skin diseases and keratoacanthomas, rapidly growing and spontaneously

regressing skin tumors. Increased proliferation rates were also detected in Bromdesoxyuridin (BrdU) labelling assays. In 2D cell culture, enhanced migration rates in scratch assays and restricted colony-forming capacity in P4-deficient keratinocytes were perceived.

Elevated expression of differentiation markers in P4-KD tissue might therefore not be directly associated to a function of P4 regulating differentiation. This might rather be a secondary effect upon a prevailing barrier impairment due to loss of P4, which increases proliferation rates of basal layer cells as compensative mechanism. This might also cause fluorescent dye retention in skin barrier assays and the observed hyperproliferative phenotype.

R 17
Functional characterization of lncRNA LINC00941 as regulator of human epidermal homeostasis

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Long non-coding RNAs (lncRNAs) have been shown to act as regulators of many cellular processes including epidermal homeostasis. During this process, undifferentiated keratinocytes move to the apical side of the epidermis while running a complex cell differentiation program. LncRNAs such as LINC00941 were found to influence this process. Initially identified from full transcriptome sequencing of keratinocytes, LINC00941 was

shown to be highly expressed in undifferentiated keratinocytes and drastically repressed during differentiation. Preliminary LINC00941 studies revealed its role as a suppressor of premature keratinocyte differentiation since knock-down of LINC00941 leads to increased mRNA and protein levels of differentiation markers. Furthermore, chromatin isolation by RNA purification and mass spectrometry analyses were performed to identify LINC00941 associated chromatin and interacting proteins, respectively. These experiments showed that LINC00941 binds to MTA2 and to E2F4/E2F6 chromatin binding sites. To compare the phenotypes, knock-down studies of MTA2, E2F4 and E2F6 were performed and the influence on keratinocyte differentiation were examined. It was shown that MTA2 and E2F6 act as negative regulators of keratinocyte differentiation comparable to LINC00941 while E2F4 promotes this process. Further experiments will focus on chromatin occupancy to show dependency of the E2F4/E2F6 and MTA2 binding on the presence of LINC00941 to elucidate the LINC00941 mechanism.

O 28
Exploring the translation of Split-ORFs as cancer neoantigens

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Some PTC-containing NMD-isoforms encode two ORFs that divide the original reading frame, a feature termed Split-ORFs. Under specific cellular conditions Split-ORF-

containing transcripts are stable and translated giving rise to protein halves with different cellular functions.

To determine the prevalence of Split-ORFs we designed and benchmarked a computational pipeline to interrogate all annotated NMD-transcripts and transcripts with retained introns. We find that a large number of both transcript types encode Split-ORFs and hundreds are conserved between human and mouse. Characterizing the features of Split-ORFs we found that they are enriched among transcripts encoding RNA-binding proteins and often separate precisely the RNA-binding domains from the rest of the protein. This offers a simple way to increase protein diversity and generate protein isoforms with new functions and neoepitopes that could contribute to oncogenesis and cancer progression.

Defining RNA regions and peptide sequences that are unique to Split-ORFs we screened available Ribo-Seq and Proteomics data sets, and identified hundreds of translated Split-ORFs in specific conditions or cell types using stringent validation criteria. The expression of protein halves for some of these Split-ORFs was validated. Our pipeline can be used to identify translated Split-ORFs in cancer cells that might represent neoantigens, which could be used for cancer subtype identification or for the design of new treatments.

E 12
Deep assessment of human disease-associated ribosomal RNA modifications using Nanopore direct RNA sequencing

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The catalytically active component of ribosomes, rRNA, is long studied and heavily modified. However, little is known about functional and pathological consequences of changes in human rRNA modification status. Direct RNA sequencing on the Nanopore platform enables the direct assessment of rRNA modifications. We established a targeted Nanopore direct rRNA sequencing approach and applied it to CRISPR-Cas9 engineered HCT116 cells, lacking specific enzymatic activities required to establish defined rRNA base modifications. We analyzed these sequencing data along with wild type samples and in vitro transcribed reference sequences to specifically detect changes in modification status. We show for the first time that direct RNA-sequencing is feasible on smaller, i.e. Flongle, flow cells. Our targeted approach reduces RNA input requirements, making it accessible to the analysis of limited samples such as patient derived material. The analysis of rRNA modifications during cardiomyocyte differentiation of human induced pluripotent stem cells, and of heart biopsies from cardiomyopathy patients revealed altered modifications of specific sites, among them pseudouridines, 2'-O-methylation of riboses and acetylation of cytidines. Targeted direct rRNA-seq analysis with JACUSA2 opens up the possibility to analyze dynamic changes in rRNA modifications in a wide range of biological and clinical samples.

R 18
Functional characterization of a pair of KH

domain RNA-binding proteins in Campylobacter jejuni

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Despite the presence of several small RNAs, homologs of globally acting RNA-binding proteins, such as Hfq and ProQ, are missing in half of all bacterial species. To explore the RNA-binding protein (RBP) landscape in organisms lacking canonical RNA chaperones, our lab has developed a new method for unbiased capture of bacterial RBPs. Applying this approach to the food-borne pathogen Campylobacter jejuni did not only capture many known RBPs, but also revealed several new RBP candidates, including a largely unexplored KH-domain RBP, KhpB. Using reciprocal coIP, we demonstrate that KhpB interacts with another KH-domain protein, KhpA. To get insights into the cellular functions of these widespread proteins, we aim to phenotypically and functionally characterize KhpA and KhpB in C. jejuni. Deletion of khpA and khpB leads to colony size heterogeneity and also impacts sensitivity to certain stresses. Using co-immunoprecipitation combined with sequencing (RIP-seq) of epitope-tagged KhpA and KhpB we identified many mRNAs and small RNAs as potential direct RNA substrates of KhpA/B. RNA-seq of single deletion mutants revealed global transcript level changes, mostly of membrane-associated proteins. Our work highlights KhpA/B as global RBPs associated with unusual phenotypes in C. jejuni. Further investigation into the

functional roles of these KH-domain proteins will add molecular insights into RNA-based regulation in bacteria harboring this new class of global RNA-binding proteins.

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P 13
Analysis of miRNA-mediated gene silencing

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Gene expression can be post-transcriptionally regulated by miRNAs. In miRNA-mediated gene silencing, the miRNA is loaded into an Argonaute (AGO) protein and guides it to a partially complementary target RNA. To form the miRNA-induced silencing complex (miRISC) a TNRC6 protein is recruited which interacts with additional factors to mediate RNA degradation and translational repression. These factors include the deadenylase complexes PAN2-PAN3 and the CCR4-NOT complex as well as the poly-A-binding protein (PABP). Multiple other proteins have also been implicated in this process. However, clear evidence and detailed knowledge of their function is often missing.

We will analyze TNRC6 interactions by mass spectrometry and aim to validate and characterize further interaction partners. Furthermore, we will apply crosslinking-mass spectrometry (XL-MS) and generate an interaction map of the miRISC-complex. First crosslinking experiments have already been performed and the method will be optimized further. Additionally, we will analyze the regulation of miRNA-mediated gene silencing by post-translational modifications. Post-translational modifications of miRISC components have already been reported. We have

measured phosphorylation sites by mass spectrometry and will characterize them further.

Our experiments will contribute to a better understanding of miRNA-mediated gene silencing.

R 19
MicroRNA-dependent regulation of organismal proteostasis, longevity and stress resistance in Caenorhabditis elegans

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All aging organisms face the challenge of maintaining a functional proteome, able to adapt to physiological and environmental changes. MicroRNAs are associated with aging and stress resistance in Caenorhabditis elegans, but their role in proteostasis remains unknown. To elucidate this fundamental attunement, we screened a collection of ageing related miRNAs, using in vivo assays to monitor ubiquitin-mediated turnover of fluorescently labeled model substrates in C. elegans. Here we show that odor perception influences cell-type-specific miRNA–target interaction leading to an inter-tissue signaling. miR-71 regulates lifespan and promotes ubiquitin-dependent protein turnover, particularly in the intestine. Interestingly, miR-71 decreases the abundance of Toll-receptor-domain protein TIR-1 in AWC olfactory neurons and disruption of miR-71–tir-1 or loss of AWC neurons eliminates the

influence of food sources on proteostasis. We also show an increase in the stability of miR-71 during heat stress and a miR-71-dependent heat stress-resistance that leads to enhanced survival of the worms, possibly due to post-transcriptional repression of tir-1 mRNA. We anticipate that the proposed mechanism of food perception will stimulate further research on neuroendocrine brain-to-gut communication and may open the possibility for therapeutic interventions.

O 29
The role of ribonuclease 6 on bacterial RNA processing for Toll Like Receptor 8 stimulation

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Mechanistic studies have revealed that Toll Like Receptor 8 requires simultaneous binding of uridine and di- or trinucleotides, respectively, on its apex, and concave pockets for RNA recognition. Thus, RNA has to be processed to become a ligand for TLR8. Meanwhile, ribonucleases (RNases) contribution on upstream RNA processing has been demonstrated as breakdown products generated by RNases T2 and 2 are sensed by TLR8. Other RNases may contribute on this process and RNase expression patterns differ in various immune cells. For instance, RNase 6, highly expressed in classical monocytes, and dendritic cells, is known to be involved in immune defense reactions, yet its function on RNA processing and TLR8 stimulation is unclear. To decipher the role and

cleavage mechanisms of RNase 6, BLaER1 cells lacking RNASE6 were infected with gram-positive and -negative bacteria or stimulated with the corresponding total bRNA. RNASE6-deficient cells were also stimulated with in vitro generated RNase 6 breakdown products. Supernatant was collected for cytokine measurements by ELISA. RNase 6 was required by certain tested bacterial strains for TNF and IL-6 production. On the same hand, significant cytokine reduction was observed upon stimulation with total bRNA. Also, preliminary data show that bRNA in vitro digested by RNase 6 can stimulate TLR8 in the absence of endogenous RNase 6. This data suggest the contribution of RNase 6 for bRNA processing for TLR8 activation in BLaER1 cells.

R 20
Specifying the Rules for Target Recognition and Regulation by Roquin Proteins

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Cis-regulatory elements in the untranslated regions of mRNAs are recognized by RNA-binding proteins (RBPs) to control posttranscriptional regulation. Most of the RNA-RBP interactions studied so far are sequence-based, involving short, single-stranded motifs. However, proteins can also recognize the three-dimensional structure of RNA. It is therefore important to consider both sequence composition and structure.

We use Roquin proteins as model system to study the recognition of structured RNA motifs. They bind to mRNA

to recruit the deadenylation machinery and/or decapping enzymes to initiate mRNA decay. Roquin proteins possess two RNA-binding domains: 1) The unique ROQ domain, which recognizes stem-loops by their shape. 2) A CCCH-type zinc finger, which binding preferences and contribution to mRNA recognition is still unknown.

High-throughput methods are necessary to characterize possible binding motifs and context specificities in detail. RNA Bind-n-Seq (RBNS) provides the ability to screen for binding motifs in vitro using randomized or partially randomized RNA pools by next generation sequencing. We used RBNS to identify the binding preferences of the zinc finger towards AU-rich single-stranded motifs. Further, we analyzed the ROQ domain preferences for the size and nucleotide composition of stem-loops. This detailed information on the binding preferences of both single domains is used to identify novel target genes and decipher complex binding sites in vivo.

O 30
Role of inhibitory 2'O-ribose methylation on RNA processing and TLR-mediated immune stimulation

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Recognition of foreign RNA by Toll-like-receptors (TLR) in endolysosomes of innate immune cells is a vital compound of the immune response against pathogenic bacteria. This process depends on nucleic acid modifications, which

enable the differentiation of non-self RNA to generate the necessary response. However, certain modifications, as the 2′-O-ribose methylation (2′-O-Me) on pathogenic RNA, can have an inhibitory effect on TLR stimulation. This can be explained by essential RNase-mediated processing events preceding TLR recognition, which are regulated by various modifications. As the stimulation of cytokine release by exogenous RNA was shown in monocytic cells with double-knockouts of the responsible RNases 2 and T2 the involvement of additional RNases can be concluded.

Therefore, the aim of this project is to establish the purification of endolysosomes from the human leukemia cell line BLaER1 to study the molecular pathways preceding TLR activation by RNA. For this, sucrose density gradients are prepared to separate cellular organelles during ultracentrifugation. Endolysosomal extracts obtained are used as substrate for pulldowns with various RNAs to identify bound interactors by quantitative proteomics with mass spectrometry. To study the effect of 2′-O-Me on binding affinities various oligoribonucleotides with defined modifications are used. Additionally, the use of RNase knockout cell lines support the identification of novel endosomal RNases involved.

R 21
Structural and molecular basis for Cardiovirus 2A protein as a viral gene expression switch

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Many RNA viruses contain regulatory elements in their genomes, which can induce alternative translation events, such as programmed –1 ribosomal frameshifting (–1PRF). –1PRF events are employed to increase the coding capacity of the genomes and to regulate the expression of structural and replicative proteins. Canonically, –1PRF is signaled by cis-acting stimulatory elements, a slippery sequence followed by a stimulatory RNA structure, such as a pseudoknot. Cardioviruses present an alternative to stimulatory signals, where the frameshifting is stimulated through the action of the viral 2A protein. 2A protein is also a primary virulence factor that has other crucial regulatory functions including inhibition of apoptosis and cap-dependent translation. Here we present the structure of 2A with the ribosomes and show how 2A potentially interferes with the binding of the elongation factors on the ribosome. Using single-molecule optical tweezers, we demonstrate that 2A stabilizes the cardioviral RNA element, which then forms an effective roadblock for the ribosomes during the translation of the slippery codons, likely explaining the increase in –1PRF efficiency in the presence of 2A. Together, these results define the structural basis for RNA recognition by 2A, show how 2A-mediated stabilization of an RNA pseudoknot promotes PRF, and reveal how 2A accumulation may shut down translation during virus infection.

R 22
The oxygen-induced small RNA FoxI provides the non-coding arm of the Sigma E response of the cancer-associated Fusobacterium nucleatum

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Global gene regulation by bacterial small noncoding RNAs (sRNAs) is often associated with a sRNA-binding proteins, i.e. Hfq. However, many bacteria including the cancer-associated species Fusobacterium nucleatum lack homologs of these proteins, raising the question if these bacteria also use sRNAs for global post-transcriptional control, and if so, for what physiological function and by what mechanisms.

Using differential RNA-seq, we have generate high-resolution global RNA maps for five clinically relevant fusobacterial strains. We have used these data sets to uncover fundamental aspects of fusobacterial gene expression architecture and a suite of non-coding RNAs including a conserved fusobacterial oxygen-induced small RNA, FoxI. To overcome the poor genetics in this phylum, we have developed different genetic tools to study the fusobacterial gene activity and the targetome of FoxI.

Collectively, our results uncover the fusobacterial homolog of the envelope stress sigma factor, σE, and reveal FoxI as its non-coding RNA arm that represses several abundant membrane proteins as well as additional targets. Interestingly, given that F. nucleatum is an early-branching species, our findings suggest that σE regulons with a coding arm and a non-coding arm might have independently evolved multiple times in bacterial evolution. In addition, we provide an experimental framework for the studying of coding and non-coding genes alike to understand F. nucleatum's role in human cancer.

P 14
Short pAgos systems can be repurposed for nucleic acid detection

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We have recently established that short prokaryotic Argonaute and associated TIR-APAZ (SPARTA) proteins degrade NAD(P)+ upon RNA-guided DNA detection. Here, we show that SPARTA systems can be reprogrammed with guide RNA sequences of choice to detect complementary DNA sequences. By using analogs of NAD+ or NADP+ that become fluorescent upon degradation by SPARTA, presence of specific DNA sequences can be monitored by fluorescence detection. This enables to repurpose SPARTA as a nucleic acid detection tool.

O 31
High-throughput RNA-based approaches to explore the interaction between Bacteroides thetaiotaomicron and its host

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Gram-negative, obligate anaerobic Bacteroides thetaiotaomicron is one of the predominant members of the intestinal microbiota of healthy western people. Many aspects of Bacteroides interaction with its native environment—the lower intestine—are unknown. Small noncoding RNAs (sRNAs), for example, are widely used by bacteria to adapt in response to external and intrinsic cues. We recently identified hundreds of sRNA candidates in B. thetaiotaomicron[1],[2],[3]. However, their possible role in the adaptation to the host niche has not yet been addressed.

In this work, we establish two orthogonal RNA-based approaches in B. thetaiotaomicron and use them to identify in vivo-relevant sRNAs. First, we performed a dual RNA-seq[4],[5] time-course of B. thetaiotaomicron colonizing a human gut epithelium, mucus-producing cell culture model. For the first time, this revealed Bacteroides sRNAs that are differentially regulated when the bacteria interact with the epithelial mucosa. Additionally, dual RNA-seq informed on the expression dynamics of bacterial coding genes and the corresponding host response to colonization. Second, we constructed a CRISPR interference (CRISPRi[6]) library of B. thetaiotaomicron intergenic sRNAs that allows us to screen for those noncoding RNAs that impact on Bacteroides fitness during colonization.

Together, these approaches are expected to predict Bacteroides sRNAs that act at the bacterial-host interface and that can be subjected to further functional characterization.

O 32
Deciphering mechanisms, function, and dynamics of RNA-protein interactions on multiple scales

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With its wealth of functional higher order structures and well-regulated interactions with proteins and other molecules, RNA has a long underestimated variety of biological functions that are in the focus of a growing number of research groups these days.

Understanding the relation between structure, conformational state, and function, as well as the functional role in membrane-less intracellular compartmentalization, is crucial for this journey towards a more complete picture of the diverse roles of RNA.

Here, we present fluorescence optical tweezers, a method that correlates the structural readout of mechanical single-molecule manipulation with fluorescence data (FRET, single-molecule localization). It has successfully been used to quantify the influence of sequence, protein binding and other factors on the stability of structures like quadruplexes or frameshifting elements.

In addition, processes of nucleic acid-based formation of membrane-less compartments can be investigated on scales ranging from the single molecule (condensation of proteins on DNA surfaces and stabilizing effect of condensates on higher order structures) to the macroscopic droplet level (droplet fusion and microrheology).

Taken together, we show how fluorescence optical tweezers allow to unravel not only structure-function relationships of RNA on the single molecule level, but also

to reveal the role of RNA in higher order intracellular organization.

O 33
NANOS1 is a negative regulator of TGF-ß signaling in human oral squamous cell carcinoma

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Oral squamous cell carcinoma (OSCC) is the most frequent type of cancer of the head and neck area accounting for approx. 275,000 new cancer cases worldwide every year. The 5-year survival rate is approx. 50%, which decreases to 20% for patients with advanced, metastatic disease. The epithelial-to-mesenchymal (EMT) program plays an important role in OSCC progression and metastasis. The transforming growth factor beta (TGF-ß) is a powerful inducer of EMT thereby increasing cancer cell migration and invasion by modulating gene expression programs. Here we aimed at identifying regulatory RNA-binding proteins (RBPs) that affect TGF-ß-induced EMT through post-transcriptional mechanisms. To this end we treated SAS cells with TGF-ß and performed RNA-sequencing analyses. We identified a total of 643 significantly regulated protein-coding genes in response to TGF-ß. Of note, 20 genes encoded RBPs and NANOS1 emerged as the most downregulated RBP. In subsequent cellular studies we observed a strong inhibitory effect of NANOS1 on migration and invasion of SAS cells. Importantly, NANOS1 overexpression efficiently blocked TGF-ß effects. Further

mechanistic studies revealed an interaction of NANOS1 and the mRNA encoding the TGF-ß-receptor 1 (TGFBR1) resulting in an enhanced decay of this transcript and a reduced cellular TGFBR1 expression thereby preventing downstream SMAD signaling. In summary, we identified a negative feedback loop between NANOS1 and TGF-ß signaling in human OSCC cells.

T 07
Artificial circular RNA sponges targeting miRNAs as a novel tool in molecular biology and medicine

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Natural circular RNAs have been found to sequester microRNAs and suppress their function. We have used this principle as a molecular tool, and produced artificial circular RNA sponges in a cell-free system by in vitro transcription and ligation. We were able to inhibit hepatitis C virus propagation by applying a circular RNA decoy strategy against microRNA-122, which is essential for the viral life cycle. In another proof-of-principle study, we used circular RNAs to sequester microRNA-21, an oncogenic and pro-proliferative microRNA. This strategy slowed tumor growth in a 3D cell culture model system, as well as in xenograft mice upon systemic delivery. In the wake of the global use of an in vitro transcribed RNA in Covid-19 vaccines, the question arose if therapeutic circular RNAs trigger cellular antiviral defense mechanisms. We present data on the

cellular innate immune response as a consequence of liposome-based transfection of the circular RNA sponges we previously used to inhibit microRNA function. We find that circular RNAs produced by the presented methodology do not trigger the antiviral response and do not activate innate immune signaling pathways. This allows a broad use of this type of circular RNAs as a therapeutic tool in molecular biology and medicine.

P 15
Functional characterization of P. oleovorans Type IV-A CRISPR-Cas activity in vivo

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CRISPR-Cas systems are widespread among prokaryotes and provide an adaptive immunity against foreign DNA. A diverse set of Cas proteins interacts with CRISPR RNA (crRNA) transcripts that guide a nuclease towards its target site, a protospacer, in foreign DNA or RNA. The largely uncharacterized Type IV CRISPR-Cas systems lack a discernible nuclease subunit and its mode of action is unknown. Here, we investigated the native activity of the Type IV-A CRISPR-Cas system in Pseudomonas oleovorans and constructed a recombinant system in Escherichia coli. We show that Type IV-A CRISPR-Cas activity of P. oleovorans depends on a protospacer-adjacent motif (PAM) and observed CRISPR interference (CRISPRi) against

phages and plasmids in the absence of DNA nuclease activity. However, deletion or mutations of the large subunit of the effector complex (Csf1), and a DinG helicase abolished CRISPRi, indicating that Csf1 and DinG are crucial for Type IV-A CRISPR-Cas activity. Target plasmids of different copy numbers affected CRISPR-Cas activity, suggesting competition of CRISPRi and plasmid replication. We introduced synthetic crRNAs to the native system, targeting specific host genes and observed clear phenotypes. The activity is comparable to methodologies using catalytically dead Cas9 (dCas9) or Type I effectors without the nuclease Cas3. Therefore, the Type IV-A CRISPR-Cas system acts as a natural CRISPRi system and enables manipulation of gene expression using engineered crRNAs.

O 34
Prediction of global RNA-binding proteins in Bacteroides thetaiotaomicron by RNase-sensitive gradient profiling

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Bacteroides thetaiotaomicron has emerged as a model organism in the field of microbiota research. Currently, little is known about post-transcriptional regulatory mechanisms that allow this bacterium to adapt to the dynamic environment associated with the human lower intestine. For example, despite encoding comparable numbers of small regulatory RNAs (sRNAs), the

Bacteroidetes lack homologs of the known global sRNA binders of other bacterial phyla, suggesting alternative RNA-binding proteins (RBPs) to exist in these organisms. Here, we set out to experimentally screen for proteins with RNA-binding potential in B. thetaiotaomicron type strain VPI-5482. To this end, we established the glycerol gradient sedimentation with RNase treatment and mass spectrometry (GradR) technique (Gerovac et al., 2020) in Bacteroides, which allows for the systematic identification of cellular proteins engaged in stable RNA-protein complexes. This way, we recorded in-gradient distributions for ~40 % of annotated B. thetaiotaomicron proteins and predicted a number of candidate RBPs, whose association with RNA is currently being validated. Combined with mechanistic follow-up characterization, this study is expected to extend the list of bacterial RBPs and provide insights into their functions in a predominant microbiota member.

R 23
Comprehensive transcriptome analysis and mutant fitness phenotypes reveal a cluster of small RNA paralogs that influence the adaptation of Bacteroides thetaiotaomicron to bile salts.

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Transcriptional plasticity allows bacteria to adapt to diverse environments and numerous stresses. However, despite

their presumed relevance in a niche as dynamic as the lower human intestine, transcriptional networks are largely unknown in bacterial microbiota species. Here, through genome-wide transcriptomics we map transcriptional start sites in the emerging microbiota model species, Bacteroides thetaiotaomicron, over 16 in vivo-relevant experimental conditions. This enabled us to infer carbon source- and stress-specific regulons, and expand the annotations of small regulatory RNAs (sRNAs) in this organism. Integrating our expression data with transposon mutant fitness data, we identify 14 sRNAs that are paralogs of GibS – a previously described GlcNAc induced intergenic sRNA that regulates transcripts of metabolic enzymes [1]. These sRNA paralogs of GibS (SpoGs) are highly expressed under in vivo-related stresses, including exposure to bile salts and, when inhibited, accelerate bacterial growth under this condition. Preliminary data suggest both, exclusive and shared functions between GibS and the SpoGs. Together, this study provides a rich resource of B. thetaiotaomicron expression profiles and mutant fitness data that will be implemented into 'Theta-Base' [1] and highlights the recurrent yet little understood theme of multicopy sRNAs.

[1] Ryan et al. (2020) Nat Commun. 11(1):3557.

R 24
Influence of novel lncRNA P4 on epidermal homeostasis and correct skin function

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The epidermis as the uppermost layer of the skin plays a critical part in fulfilling important protective functions and needs to be constantly renewed. This process termed epidermal homeostasis is tightly regulated by biomolecules such as proteins or non-coding RNAs.

To identify new regulators, we searched for long non-coding RNAs (lncRNAs) showing differentiation-dependent levels of abundance in keratinocytes, the main epidermal cell type. One of these candidates, a novel lncRNA hereinafter named P4, showed initially high expression in keratinocytes which decreased during differentiation.

In order to characterize the role of P4 in epidermal homeostasis, we are employing an organotypic skin model. By manipulating lncRNA levels of the cells used in this model, we can specifically investigate related changes in differentiation and identify histological phenotypes. Interestingly, P4 knockdown in organotypic tissue is associated with morphological features also present in known skin conditions. Furthermore, we are performing several assays to test for effects of P4 on different phenotypical aspects of keratinocytes. These analyses hint at P4-related changes in cell cycle state, apoptosis and proliferation.

Next up, we are applying several approaches in order to identify proteins which interact with P4. Once these mass spectrometry-based screenings are finished, we plan to validate putative interactors in order to get a better idea about the pathways that P4 might be involved in.

P 19
Structure of the human tRNA splicing endonuclease defines substrate recognition

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Transfer RNAs (tRNAs) rank among the most abundant RNA species in the cell playing a central role in protein biosynthesis and cellular homeostasis. tRNAs are transcribed as precursor molecules (pre-tRNAs) which need to undergo tightly controlled multistep processes for their maturation. A subset of pre-tRNAs contains intronic sequences, which are excised by the tRNA splicing endonuclease (TSEN). Mutations in TSEN were linked to the development of the neurodegenerative disease pontocerebellar hypoplasia (PCH). Here, we present two single-particle cryo-electron microscopy structures of human full-length and core TSEN with intron-containing pre-tRNA at 3.6 and 3.1Å resolution, respectively. Besides conserved endonuclease domains also found in Archaeal systems, the two structures reveal phylogenetically new elements which foster pre-tRNA recognition and positioning at the catalytic sites. Our data highlight the role of the TSEN54 subunit as a molecular ruler for splice site definition by key interactions with the mature tRNA body. Together with biochemical assays, we show that 40% of the TSEN polypeptides are intrinsically unstructured, dispensable for pre-tRNA cleavage, but necessary for association with the RNA kinase CLP1. Our work defines the molecular framework of pre-tRNA recognition and cleavage in humans and provides a structural basis to better understand PCH in the future.

E 13
Biological Roles of NAD-capped RNAs in Higher Eukaryotes

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The biological roles of NAD-capped RNAs in higher eukaryotes are currently unclear. There is evidence that NAD-capped RNAs are decapped and degraded; thus, having a shorter life span than m7G-capped RNAs. Moreover, NAD-capped RNAs were found not to be translated. However, these conclusions were largely drawn from transcripts that reside in the cytoplasm and nucleus. In this research, I aim to identify NAD-capped RNAs within the mitochondria. NAD levels and NAD capping efficiency in the mitochondria are higher, suggesting an higher proportion of NAD-capped RNAs. By examining the abundance and pattern of NAD-capping in mitochondria, I hope to shed light into the biological roles of NAD-capped RNAs.

O 35
Deciphering the cellular response to codon-specific translational defects

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The rate of protein synthesis is determined by the speed of mRNA decoding. Importantly, perturbation of local

translational dynamics can lead to protein homeostasis defects. However, the exact link between these processes remains unclear. While deletion of tRNA modification genes is a powerful tool to create defined perturbations of translation, this approach does not allow us to analyze the immediate cellular response to codon-specific translational defects.

Here we use two yeast toxins to induce codon-specific translational defects in a controlled manner: the γ-toxin and PaT cleave specific tRNA in eukaryotic cells allowing us to precisely induce the removal of these tRNAs. We have confirmed that the toxins are expressed and that they target the expected tRNA. When analyzing the effects of toxin expression on cellular translation by ribosome profiling, we observed a time-dependent and highly specific slowdown of the codon corresponding to the target tRNA of the toxin. Moreover, a plethora of genes changed their expression patterns in a time-dependent manner, providing us with a high-resolution dataset to decipher the underlying triggers and mechanisms of cellular stress response pathways. We are currently complementing our experiments with time-resolved proteomics.

This powerful approach will allow us to gain a mechanistic understanding of how perturbations of the translational machinery can trigger an imbalance in the cellular proteome.

E 14
Structural and functional insights into human tRNA guanine transgylcosylase

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The eukaryotic tRNA guanine transglycosylase (TGT) is an RNA modifying enzyme incorporating queuine, a hypermodified guanine derivative, into the tRNAsAsp,Asn,His,Tyr. While both subunits of the functional heterodimer have been crystallized individually, much of our understanding of its dimer interface or recognition of a target RNA has been inferred from its more thoroughly studied bacterial homolog. However, since bacterial TGT, by incorporating queuine precursor preQ$_1$, deviates not only in function, but as a homodimer, also in its subunit architecture, any inferences regarding the subunit association of the eukaryotic heterodimer or the significance of its unique catalytically inactive subunit are based on unstable footing. Here, we report the crystal structure of human TGT in its heterodimeric form and in complex with a 25-mer stem loop RNA, enabling detailed analysis of its dimer interface and interaction with a minimal substrate RNA. Based on a model of bound tRNA, we addressed a potential functional role of the catalytically inactive subunit QTRT2 by UV-crosslinking and mutagenesis experiments, identifying the two-stranded βEβF-sheet of the QTRT2 subunit as an additional RNA-binding motif.

P 16
Dissecting the molecular functions of the RNA-binding, LCD-containing protein Rbfox1 in ovaries

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Drosophila Rbfox1 is homologous to human FOX proteins, deregulation of which is associated with multiple seemingly unrelated human diseases, such as autism, diabetes, obesity, epilepsy and spinocerebellar ataxia. We have previously found that Rbfox1 is promiscuously included in different stress-dependent RNA granules and its expression is regulated by a stress-related miR-980. Rbfox1 is an RNA-binding alternative splicing factor that contains a conserved RNA recognition motif (RRM). In addition, our data indicate that Rbfox1 has multiple low complexity domains (LCDs), which are important for liquid-liquid phase separation. To understand the role of these Rbfox1 domains, we have generated mutant flies that lack either the RRM or some of the LCDs using CRISPR/Cas9-mediated mutagenesis. We found that these mutants have different phenotypes, which means that different Rbfox1 domains have different functions. Since Rbfox1 deregulation leads to abnormal stress response, a further analysis of CRISPR/Cas9 mutants will help dissect the functional role of Rbfox1 domains. This knowledge should advance our understanding of how Rbfox1 contributes to deleterious symptoms, which comprise a vast spectrum of human disorders.

R 25
miRNA-mediated downregulation of Cyclase Associated Protein 1 is required for myoblast fusion

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Myoblast fusion is essential for the formation, growth, and regeneration of skeletal muscle, but the molecular mechanisms that govern fusion and myofiber formation remain poorly understood. Past studies have shown an important role of the actin and actin regulators in myoblast fusion. Here we report that Cyclase-associated protein 1 (CAP1), a regulator of actin dynamics, plays a critical role in cytoskeletal remodeling during myoblast fusion and formation of myotubes. Cap1 mRNA and protein are expressed in both murine C2C12 and human LHCN-M2 myoblasts, but their abundance decreases during myogenic differentiation. Perturbing the temporally controlled expression of CAP1 by overexpression or Crispr-Cas9 mediated knockout, impaired actin rearrangement, myoblast alignment, expression of profusion molecules, differentiation into multinucleated myotubes, and myosin heavy chain expression. Endogenous Cap1 expression is post transcriptionally downregulated during differentiation by canonical myomiRs miR-1, miR-133, and miR-206, which have conserved binding sites at the 3′ UTR of the Cap1 mRNA. Deletion of the endogenous 3′ UTR by Crispr-Cas9, in C2C12 cells phenocopies overexpression of CAP1 by inhibiting myotube formation. Our findings implicate Cap1 and its myomiR-mediated downregulation in the myoblast fusion process and the generation of skeletal muscle.

O 36
Guanidine- a secret player in the nitrogen cycle?

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Guanidine was first described in the 19th century by Adolf Strecker as a product of guanine pyrolysis. Due to its Y-aromaticity it is a very stable and strong basic molecule. Over the last century there were only rare reports of guanidine found in natural samples e.g. from animals and plants. Guanidine finally got greater attention, when riboswitches were discovered that sense the small molecule. Guanidine riboswitches are widespread in bacteria and suggest that guanidine might play a yet neglected role in nature[1,2,3]. Here, we present the discovery and characterization of a novel class of guanidine riboswitches that are found in different bacterial clades[4]. Guanidine riboswitches control the gene expression of a pleiotry of different genes, e.g. guanidine exporters that detoxify cells[5]. We add a new perspective to guanidines role in nature by presenting two independent, riboswitch-associated guanidine degradation pathways[6,7]. Both, the guanidine carboxylase and the guanidine hydrolase pathway enable bacteria to use guanidine as a sole source of nitrogen. The widespread occurrency and the divergent evolution of up to now four identified different riboswitch classes, together with detoxification and assimilation pathways for guanidine in bacteria suggests that guanidine is an underestimated player in the nitrogen cycle.

T 08
Antisense oligonucleotides against UMOD to treat autosomal dominant tubulointerstitial kidney disease

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Uromodulin is the most abundant urinary protein in humans and is expressed almost exclusively by cells lining the thick ascending limb (TAL) in nephrons. Hereditary gain-of-toxic-function mutations in the gene encoding uromodulin (UMOD) is the leading genetic cause of autosomal dominant tubulointerstitial kidney disease (ADTKD), a condition without viable treatment. To address this unmet medical need, the goal of this project is to develop an antisense oligonucleotide (ASO) therapeutic that targets the UMOD mRNA and prevents its translation. Chemically enhanced phosphorothioate and 2′-O-methoxyethyl (MOE-PS) gapmer ASOs that function through RNAse-H mediated degradation of the target RNA were designed against conserved wild type (wt) sequences and against a specific single nucleotide polymorphism (SNP), R185S. ASOs in the former "wt-targeting" library are able to reduce total uromodulin levels and have the potential to treat the entire ADTKD-UMOD patient population and may also slow progression of chronic kidney disease. The latter "SNP-targeting" library will explore structure-function relationships underlying SNP discrimination and allele-selective downregulation. Protein-binding ligands will be conjugated to the ASOs to increase their affinity to plasma proteins and thereby improve pharmacokinetic properties.

E 11
The RNA modification m6A affects RNA binding and functions of SRSF7

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The N^6-methyladenosine (m^6A) modification affects all steps of the mRNA life cycle, including splicing, polyadenylation and mRNA export. This pleiotropy is caused by different RNA-binding proteins (RBPs) that recognize m^6A and mediate various downstream effects. Besides 'reading' m^6A marks, RBPs also affect m^6A deposition or are repelled by it.

Recently, the RBP SRSF7 was shown to promote m^6A deposition, regulating the stability of a small set of mRNAs important for cell proliferation. SRSF7 plays essential roles in the regulation of alternative splicing, but it has also diverse non-splicing functions – it forms nuclear condensates, regulates mRNA export and modulates alternative polyadenylation (APA). SRSF7 binds upstream of proximal polyadenylation sites (pPASs) and enhances their usage generating mRNAs with short 3'UTRs. To test whether m^6A deposition affects APA we performed miCLIP2 followed by m^6Aboost and determined the methylome of P19 cells. Integration with SRSF7 iCLIP data revealed that m^6A sites are enriched at pPAS, overlap with SRSF7 binding sites and its binding motif (GAYGAY) is similar to the m^6A DRACH motif. To test whether SRSF7 affects m^6A deposition and APA, we generated P19 hGRAD cell lines for the rapid degradation of SRSF7 to minimize compensatory effects. We performed DART-Seq upon acute SRSF7 depletion and integrated changes in m^6A levels and 3'UTR length using DaPars. The data will illuminate the interplay of SRSF7, m^6A and the regulation of APA.

R 26
Long read DMS-Map-seq resolves the structures of spliced transcript isoforms

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HIV-1 genomic RNA is spliced into a myriad of transcripts, all of which share a common sequence in their 5′ untranslated region. Intriguingly, this common sequence contains a major packaging motif, yet only the unspliced RNA is efficiently packaged into virions. We hypothesize that interactions between the shared region and intronic sequences create a distinct RNA structure that is recognized by the viral packaging machinery.

Current RNA structural probing methods use short read sequencing that cannot be definitively mapped to shared regions of transcript isoforms. This intrinsic limitation blurs the structural landscape of RNA isoforms and is a major barrier to understanding RNA structure function relationships in cells.

Here, we used long read cDNA sequencing on nanopores to investigate HIV-1 transcripts in infected cells. We quantified and unambiguously determined the architecture of 16 spliced isoforms. Using dimethyl sulfide (DMS) coupled with a processive reverse transcriptase, we obtained structural information across each transcript isoform. Our results demonstrate that HIV-1 splice variants have distinct structures that cluster with exon use. We also identified subtle, but key, differences in the packaging

signal within spliced transcripts, that were previously hidden.

Our data suggest that in addition to increasing protein diversity, alternative splicing also results in the generation of transcripts with distinct functions through altered RNA structures.

O 37
Dissecting the binding behavior of the multi-RRM protein Rrm4 during endosomal mRNA transport

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Transport of mRNAs is crucial to regulate protein expression at the spatio-temporal level. The localization of mRNA is coupled to the intracellular transport machinery by RNA-binding proteins (RBPs). A well-studied translocation mechanism is the endosomal mRNA transport along microtubules in infectious hyphae of Ustilago maydis. The key component of endosomal mRNA transport is the RBP Rrm4, which is important for the unipolar. Rrm4 contains three RNA Recognition Motifs (RRM) and three MLLE domains, which are necessary to anchor the mRNP complex on endosomes. Rrm4 binds predominantly to the 3'UTR of target transcripts and the landmarks of translation - this supports the current hypthesis of local translation on endosomes. Furthermore, Rrm4 binds the sequence motif UAUG, which is present in one third of all identified binding sites. The motif occurs mostly in binding sites mapping to the ORF and start

codons and is bound by the third RRM domain (RRM3). Mutations within RRM3 do not interfere with hyphal growth, whereas mutating the first RRM (RRM1) domain leads to a loss of function of Rrm4 . This indicates that the RRM domains differentially contribute to RNA binding. However, the principles of RNA recognition by multi-domain RBPs are not well studied. Recently, we obtained a transcriptome-wide view of Rrm4 and Rrm4mR[1] by using a comparative iCLIP2 approach. This enables us to further characterize the roles of the different RRMs of Rrm4 during endosomal mRNA transport.

O 38
RBM47 is a tumor-suppressive RNA-binding protein in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is the most common malignancy of the pancreas with an extremely poor prognosis. With a 5-year survival rate of ~9 % it is currently the fourth leading cause of cancer death. Effectiveness of PDAC therapies is challenged by early and widespread metastasis and the epithelial-to-mesenchymal transition (EMT) is a major driver of cancer metastasis. Since RNA-binding proteins (RBPs) can modulate several cellular signaling pathways, we aimed to identify RBPs that affect EMT in PDAC cells. Using publicly available datasets we identified a positive correlation between E-Cadherin and RBM47 (RNA-binding motif 47) expression in PDAC. In line

with this, highly metastatic PaTu-T cells showed no expression of RBM47 whereas its epithelial-like sister cell line PaTu-S expressed high amounts. Intriguingly, RBM47 showed lower expression in primary tumor tissue compared to normal pancreas and the lowest level of RBM47 is found in the most aggressive subtype of PDAC suggesting a tumor-suppressive role of this RBP in PDAC. Indeed, overexpression of RBM47 in PaTu-T cells strongly inhibited cell motility and changed the expression of 1080 genes as analyzed by RNA-sequencing. On the other hand, CRISPR-mediated knock-out of RBM47 in PaTu-S cells induced an EMT-like gene expression profile. Our ongoing studies aim to map the molecular interactions of RBM47 in order to dissect the mode of action of this novel tumor suppressor in pancreatic cancer cells.

R 27
Functional characterisation of the RaiA noncoding RNA in Clostridioides difficile

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Noncoding RNAs (ncRNAs) represent key players of gene regulation in bacteria. They can fine-tune gene expression at the transcriptional or post-transcriptional level, taking roles in metabolism, stress adaptation, virulence and pathogenicity.

We have recently determined the global landscape of ncRNAs in Clostridiodes difficile (C. difficile), an opportunistic human pathogen that causes severe

inflammation of the gut. This revealed RaiA as one of the most abundant ncRNAs, suggesting regulation of important processes (1). RaiA was previously identified in silico as to be highly conserved in Actinobacteria and Firmicutes (2). Intriguingly, chromosomal deletion of RaiA leads to a distinct overgrowth phenotype in late exponential and stationary phase, particularly upon addition of monosaccharides. Additionally, their presence retains RaiA at high levels in stationary phase. Transcriptomic analysis of RaiA knockout revealed a global abundance of 5s rRNA and tRNAs at late stages of growth hinting towards a role of RaiA in regulating translation.

In the current study we aim to characterize the regulatory mechanism(s) of RaiA and its cellular RNA/protein partners C. difficile. In light of the strong expression and the high conservation, the elucidation of this ncRNAs' mode of action will be of interest beyond the field of C. difficile focused research.

(1) Weinberg et al., (2017) NAR
(2) Fuchs et al., (2021) PNAS

P 17
Description of secondary structures of RNA cis elements in full-length Ox40 3′-UTR and binding to the immune-regulatory protein Roquin

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Tight control of gene expression is crucial to maintain cellular homeostasis. During controlled mRNA decay cis-regulatory elements within 3′-untranslated regions (UTR) are recognized by trans-acting protein factors (RBPs). These ribonucleoprotein complexes (RNP) recruit degradation machineries and determine mRNA stability by integrating multiple signals. The RNPs and their complex cis-trans networks are often not well understood. The immune-regulatory protein Roquin binds constitutive and alternative decay elements (CDE, ADE) within target mRNAs. Complex formation is incompletely understood, majorly caused by the lack of information about target mRNA structure. We study the full-length 3′-UTR of the T-cell co-receptor Ox40 mRNA comprising both ADE and CDE. Guided by NMR spectroscopy we provide a full description of secondary structure elements of a 157 nt RNA. Small angle X-ray scattering (SAXS) and In-Line Probing support the modular architecture of the 3′-UTR. We provide insights into complex formation of Roquin with multiple independent elements. We dissect contributions of individual Roquin domains for RNA binding, give evidence for a ternary complex and we determine a sequential binding order. We show that RNA element-binding is depending on context. Our study highlights the impact of the structural depiction of regulatory RNPs and demonstrates the powerful combination of structural biology and RNP biochemistry as a strategy to obtain architectural information of RNPs.

O 39
Control of gene expression by light-driven RNA binding of the LOV photoreceptor PAL

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Sensory photoreceptors mediate numerous light-dependent adaptations across organisms. In optogenetics, photoreceptors are used as switches for the reversible, non-invasive and spatio-temporal precise control of different cellular processes. The light-oxygen-voltage (LOV) receptor PAL from the actinobacterium Nakamurella multipartita belongs to the ANTAR family and binds to small RNA aptamers with sequence specificity after blue light illumination. Here we present a one plasmid system that employs PAL to light-induce or light-repress gene expression in Escherichia coli. We also demonstrate that PAL can be used to control hammerhead ribozyme activity in a light-dependent manner, bringing new opportunities in optogenetics.

P 18
Structural basis of branch site recognition by the human spliceosome

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Recognition of the intron branch site (BS) by the U2 snRNP is a critical event during spliceosome assembly. In mammals, BS sequences are poorly conserved and unambiguous intron recognition cannot be achieved via a base-pairing mechanism. We isolated human 17S U2 snRNP and reconstituted in vitro its ATP-dependent remodelling and binding to the pre-mRNA BS. We

determined a series of high-resolution (2.0-2.3 Å) structures of the reconstituted complexes. Our data reveal a critical role of HTATSF1 in stabilising the branch-interacting stem-loop (BSL) and facilitating engagement with the BS. SF3B6 stabilises the BS:U2 snRNA, which could aid binding of introns with poor sequence complementarity. ATP-dependent remodelling uncoupled from substrate binding results in an autoinhibited U2 snRNA conformation that can no longer bind pre-mRNA. Based on these results, we have developed a model for how the spliceosome selects branchpoint sequences.

Reference: Tholen J, ..., Galej WP, 2021. Structural basis of branch site recognition by the human spliceosome. Science. https://doi.org/10.1126/science.abm4245

R 05
Sibling Team Work – Interplay of a family of regulatory RNAs with an RNA sponge in Caulobacter crescentus

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Small RNAs (sRNAs) contribute significantly to the post-transcriptional control of gene expression in bacteria. By engaging in direct base-pairing interactions with target transcripts, sRNAs may influence mRNA translation and stability. In some cases, a given bacterium expresses multiple, seemingly homologous sRNAs, so-called sibling

sRNAs. It is generally not well understood why the cell has several copies of nearly identical regulators, how it integrates the activity of multiple sRNA homologs into established regulatory circuits, and whether the siblings act redundantly.

The alpha-proteobacterium Caulobacter crescentus encodes a family of four αr8 sibling sRNAs, which share conserved sequence elements and a characteristic structure. In contrast to previously investigated sibling RNAs, the αr8 sRNAs are expressed from independent genomic loci under different environmental conditions (including carbon starvation and iron deficiency), indicating sibling-specific transcriptional input. We have performed comparative transcriptome analyses to determine the target spectra of each family member, and have identified both conserved as well as individual interactions. Intriguingly, our data also revealed the presence of a stress-responsive sponge RNA that binds to and facilitates αr8 turn-over. Using both in vivo as well as in vitro approaches, we have untangled a complex post-transcriptional network orchestrating carbon metabolism when Caulobacter switches between feast and famine.

O 40
Thorough Data Analysis for RNA-RNA Interactomics

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RNAs form complex regulatory networks by direct RNA-RNA interactions (RRI). Several experimental methods have been developed to analyse these interactions in vivo and in high-throughput. The common scheme of all these Direct Duplex Detection (DDD) methods is that RRIs are fixed in vivo via direct RNA-RNA crosslinking, followed by trimming and enrichment of crosslinked RNA. Finally, interacting strands are merged into a chimeric RNA via Proximity Ligation. These chimeras get sequenced using RNA-seq and provide information avout individual RRIs. Of course, none of these steps is perfect, such that the resulting data is biased and noisy. But even more important, chimeric sequencing reads are atypical input data and require special methods for their analysis. We have designed RNAnue, a tool for an unbiased, sensitive and holistic analysis of DDD data. It combines sensitive split read mapping with thorough filtering by complementarity and thermodynamic stability, and performs a statistical assessment for ligation artefacts. Furthermore, a newly designed clustering procedure that is independent of the geneome annotation, allows to find RRis of previously unknown RNAs. Altogether this results in a higher prediction quality of RNAnue compared to other analysis pipelines while the runtime remains comparable.

O 41
Pan-species analysis of RNA binding domains in RNA-binding proteins

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RNA-protein complexes are involved in key cellular processes, often linked to health and disease. Thus, a lot of effort has been put in the last decade to identify RNA-binding proteins (RBPs) in high throughput assays. Recently, we assembled the RBP2GO database (https://RBP2GO.dkfz.de), compiling all the 105 proteome-wide datasets for RBPs across 13 species in one user-friendly website, associating a score to each protein reflecting its potential as an RBP candidate (Caudron-Herger et al., Nucleic Acids Res 2021). In this study, we used this pan-species dataset and analyzed the domain structures of the proteins. We uncovered that only about 25% of the previously suggested RBP candidates contain an annotated RNA-binding domain (RBD), while many proteins not yet identified as RBPs include well-characterized RBDs - especially in those species for which very few proteome-wide studies are available. We also showed that the RBP2GO score correlated with the expression level of the proteins, suggesting that proteome-wide screens could not detect some RBPs due to their low expression level. Moreover, RBPs with high scores but without an RBD are explored to identify novel RBDs. Overall, the presence of an RBD can be exploited in two ways: first to identify new RBP candidates especially in species with no or few proteome-wide studies available - second to fine-tune the RNA-binding potential of RBP candidates in highly covered species to further increase their likelihood to be true RBPs.

E 15
Metabolic labeling indicates internal RNA glycosylation in E. coli K12

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In contrast to biomolecules like proteins and lipids which are frequently glycosylated in nature, only a few studies report carbohydrate modifications of RNA. Besides modifications of RNA nucleobases, sugar nucleotides might also decorate the 5′ end of RNA molecules in a cap like fashion.

This work aims to identify RNA sequences modified with the abundant hexosamine N-acetylglucosamine (GlcNAc). Therefore, metabolic labeling with tetraacetylated N-azidoacetylglucosamine (Ac_4GlcNAz) in E. coli K12 was combined with an RNA capture and sequencing protocol.

In a test experiment, metabolically labeled total RNA from E. coli K12 was conjugated to a fluorophore via azide-alkyne cycloaddition (AAC). PAGE analysis revealed a fluorescent band whose intensity depended on the Ac_4GlcNAz concentration in the growth media. Illumina sequencing of a prepared library showed enrichment of one RNA sequence in particular. The length of this RNA sequence matches the length of the fluorescently labeled band in the PAGE analysis.

This RNA sequence was isolated from metabolically labeled total RNA in a pull-down experiment and the presence of an azide label was confirmed via AAC with a fluorophore. Sequence-specific DNAzyme cleavage narrowed down the location of the modification to eight nucleotides at an internal position.

The identified RNA sequence would be the first reported RNA sequence decorated with a GlcNAc moiety as well as the first reported glycosylated RNA in prokaryotes.

P 20
Widespread RNA structures promote immune prioritization across CRISPR-Cas9 systems

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CRISPR-Cas systems provide adaptive immunity against plasmids and phages. They store small fragments of invaders' genetic material as "spacers" in a CRISPR array. Each spacer is located between fixed repeats. Each spacer-repeat pair gives rise to a CRISPR RNA (crRNA) that directs a nuclease to cleave nucleic-acid sequences matching the spacer, thus defending against the corresponding invader. With repeats on both sides of each spacer, there is one more repeat than spacer, and the extra repeat is not paired with a spacer. While this extra repeat is often used to acquire new spacers, it would yield a crRNA whose spacer originates outside of the array. This "extraneous" crRNA (ecrRNA) would not target an invader, and could interfere with crRNA biogenesis. We investigated type II-A systems, whose extra repeats are proximal to the array leader. With multiple lines of experimental evidence in three organisms, we showed that the leader base pairs with the extra repeat to inhibit its processing. This conference submission focusses on work to determine bioinformatically if this mechanism is widespread within type II-A systems. We designed a statistical test to computationally analyze pairing potential between leaders and the extra repeat in multiple type II-A systems, and calculated an aggregate p-value on an independent test set of 3×10^{-6}. This significant p-value suggests that many type II-A systems use this

strategy to silence their ecrRNAs, amplifying the experimental results.

O 42
Transcriptome-wide analysis of self-cleaving ribozyme activity

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Ribozymes are RNAs that catalyze chemical reactions. Some ribozymes, so-called self-cleaving ribozymes, cut their own phosphate backbone at a specific position to implement a variety of biological functions, such as RNA replication or gene regulation. To date, self-cleaving ribozymes have been mostly discovered by chance. We developed a targeted, transcriptome-wide method, called cyPhyRNA-seq, to analyze ribozyme cleavage activity in total RNA extracts. cyPhyRNA-seq enriches for both products of active ribozymes by specifically targeting their characteristic end groups with high-throughput RNA-seq. With this strategy, we detected the activity of, in total, nine different self-cleaving ribozymes from three structural classes in four bacterial strains. cyPhyRNA-seq proved especially useful for monitoring ribozyme activity in organisms that carry several self-cleaving RNAs and we currently apply it to Schistosoma mansoni, a human parasite predicted to contain thousands of self-cleaving RNAs. Moreover, cyPhyRNA-seq facilitates the investigation of self-cleavage activity under different conditions, such as stress, and it bears the potential to be used to discover novel self-cleaving ribozymes in diverse

organisms. Lastly, due to the detection of specific RNA ends by cyPhyRNA-seq, this method could be applied to the investigation of other cellular processes involving RNAs with identical end groups, for example, products generated by Cas6, MazF or IRE1α nucleolytic activities.

R 28
Natural antisense transcripts play different roles in soma and male germ cells

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Natural antisense transcripts (NATs) are generally considered as regulatory, long noncoding RNAs. NATs are most prominently expressed in testis but also found in other organs. They are transcribed at low levels and co-expressed with the related protein coding sense transcripts. We have investigated NATs from a genomic perspective in mouse testis and researched mechanistic details of the bi-directional SLC34A/PFN3pseudogene locus in cell lines.

We isolated dsRNA from testis using the J2 antibody and sequenced the samples. Comparison with published data from liver revealed that in testis, dsRNA derives predominantly from mRNAs, whereas in liver it originates more from introns/intergenic transcription. dsRNA is enriched in pachytene spermatocytes. We also found that in testis (but not in liver) NATs contribute significantly to the dsRNA transcriptome and that endo-siRNAs associate with dsRNA forming genes.

The genome wide studies in testis contrast the results with HKC-8 and HEK293 cells. We induced sense/antisense transcription of SLC34A1/PFN3 using dexamethasone and CRISPR. Expression of the sense transcript was paralleled by reduced sense promoter methylation and activating histone marks.

Our findings suggest different mechanisms/biological roles for NATs in soma and germ cells. We propose that in somatic cells, NATs display hallmarks of lncRNAs. In germ cells, however, our findings point to a testis-specific mechanism involving dsRNA formation and endo-siRNAs.

P 21
Single-molecule FRET measurements uncover an unexpected conformation of hAgo2-RNA complexes during target-directed miRNA degradation

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Human Argonaute 2 (hAgo2) is a key player of RNA interference. Target mRNAs are recognized by complementarity to a hAgo2-bound miRNA-derived guide RNA. Downregulation of targets occurs either by translational inhibition or hAgo2-mediated cleavage depending on the degree of complementarity between guide and target RNA. Tight control of the amounts of cellular miRNAs prevents dysregulation. Target-directed miRNA degradation (TDMD) was described as a cellular

pathway for the regulation of miRNA levels. In the context of TDMD, miRNA guides as well as the hAgo2 protein itself is subject to degradation.

The varying fates of hAgo2 and hAgo2-bound nucleic acids are probably determined by structural rearrangements enabling e. g. interactions with other proteins. We site-specifically engineered fluorophores into native hAgo2 and the bound nucleic acids to uncover dynamics and hidden conformational states using single-molecule FRET. The analysis of conformations along the catalytic cycle enabled insights in an unexpected dynamic behavior of hAgo2 even in complex with a guide and a target RNA with full base pairing potential. Furthermore, we found that complexes composed of hAgo2-guide RNA and a TDMD target lead to a so far unobserved conformation in which the guide RNA moves significantly out of its binding channel within the protein. In summary, our study underscores the importance of conformational flexibility of hAgo2 and hAgo2-nucleic acid complexes as a pre-requisite to function.

O 43
Probing the effect of ribosome-interacting proteins on co-translational quality control

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Ribosome stalling during translation elicits different translation quality control responses: Ribosome-associated quality control (RQC) degrades truncated nascent peptides, no-go decay (NGD) degrades the affected mRNAs.

We recently characterized the RNA interactome of key RQC trigger factor Hel2 in Saccharomyces cerevisiae, which recognizes and ubiquitinates stalled ribosomes (Winz et al. Nat Commun, 2019). Our crosslinking and analysis of cDNA (CRAC) studies revealed interactions with ribosomal RNA close to Hel2 ubiquitination targets and with mRNA, on both sides of the termination codon.

To identify additional factors that mediate translation quality control, we characterized ribosome-associated proteins. Loss of Zuo1, a ribosome-associated chaperone component, leads to preferential recruitment of Hel2 to the coding sequence. In contrast, loss of ATPase New1, causes Hel2 recruitment to the 3′-untranslated region (UTR) of some mRNAs. Notably, Hel2 enrichment on 3′-UTRs is seen on mRNAs with specific 3′-terminal codons; Lys(AAA), Arg(AGG/CGU), but not Lys(AAG) or Arg(AGA). A recent publication (Kasari et al. Nucleic Acids Res, 2019) showed that ribosomes queue on such terminal codons. Our data suggest that ribosome queueing facilitates stop codon readthrough and elicits quality control. Downregulation of New1 under stress and modulation of New1 through its prion-inducing domain would potentially represent mechanisms for regulating expression of the translation machinery.

P 22
Decoupling the bridge helix of Cas12a results in a reduced trimming activity, increased mismatch sensitivity and impaired conformational transitions

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The widespread and versatile prokaryotic CRISPR-Cas systems (clustered regularly interspaced short palindromic repeats and associated Cas proteins) constitute powerful weapons against foreign nucleic acids. Recently, the single-effector nuclease Cas12a that belongs to the type V CRISPR–Cas system was added to the Cas enzymes repertoire employed for gene editing purposes. Cas12a is a bilobal enzyme composed of the REC and Nuc lobe connected by the wedge, REC1 domain and bridge helix (BH). We generated BH variants and integrated biochemical and single-molecule FRET (smFRET) studies to elucidate the role of the BH for the enzymatic activity and conformational flexibility of Francisella novicida Cas12a. We demonstrate that the BH impacts the trimming activity and mismatch sensitivity of Cas12a resulting in Cas12a variants with improved cleavage accuracy. SmFRET measurements reveal the hitherto unknown open and closed state of apo Cas12a. BH variants preferentially adopt the open state. Transition to the closed state of the Cas12a-crRNA complex is inefficient in BH variants but the semi-closed state of the ternary complex can be adopted even if the BH is deleted in its entirety. Taken together, these insights reveal that the BH is a structural element that influences the catalytic activity and impacts conformational transitions of FnCas12a.