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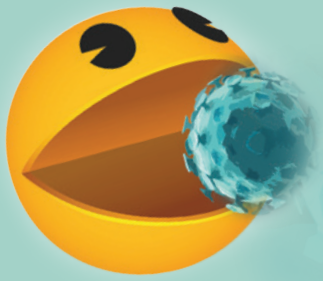

Eukaryotic RNA turnover and viral biology

20 – 23 June 2023 | Brno, Czech Republic

Abstract Book

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Workshop

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Abstract Book

Content

Welcome Speach

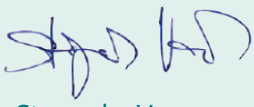
Dear Participants,

Welcome to the EMBO Workshop on Eukaryotic RNA Turnover and Viral Biology. Building upon the success of previous meetings focused on RNA stability, this workshop takes a profound step forward by exploring the captivating realm of RNA turnover and its intricate relationship with viral infections. By bringing together experts from both disciplines, we aim to uncover common themes that will enhance our understanding of viral infections and open new avenues for potential therapeutic targets.

We gather in Brno, a city steeped in scientific history as the home of Gregor Johann Mendel, the renowned Augustinian friar and founder of modern genetics. Hosted by the Central European Institute of Technology (CEITEC) at the Masaryk University, and city of Brno, this conference promises a remarkable experience. The gala dinner on Thursday evening will transport us to the Augustinian St. Thomas Abbey, a historical site in the heart of Brno where Mendel himself worked and resided. If Mendel had been aware of the profound impact of RNA and viruses, they would undoubtedly have been his favorite topics of exploration.

Let us embark on an exciting, intense, and ultimately rewarding meeting in the heart of Moravia. We eagerly await the exchange of knowledge, vibrant discussions, and collaborations that are destined to shape the future of this field. We extend our gratitude to all participants for their invaluable contributions, and with utmost confidence, we believe this conference will provide an unparalleled platform for fostering innovative ideas and forging lasting connections. Welcome to this captivating journey that will unravel the mysteries of RNA turnover and virology.

Warm regards on behalf of the organizing committee,



Stepanka Vanacova
CEITEC MU, Brno, Czech Republic

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Practical information

Download conference application via this QR code and get practical information about the conference and Brno.

Introducing our innovative conference application prepared in collaboration with the city of Brno! This app offers a comprehensive experience, combining EMBO workshop practical information with valuable insights into the cultural, historical, and entertainment offerings of Brno.

We would thanks to mayor of the Brno, Marketa Vankova, for the esteemed patronage of our EMBO workshop.

Programme

20 June 2023

16:00–19:30	Arrival & Registration
18:00–19:30	Welcome Reception and Light Dinner – Restaurant Siesta
Session 1: RNA in viral defense and immunity Chair: Stepanka Vanacova – Hall A	
19:30–19:40	**Organizers:** Welcome and introductory remarks
19:40–20:10	**Keynote lecture – Benjamin tenOever:** Decoding and recoding nature's software to build virus-based computers
20:10–20:40	**Mary O'Connell:** RNA modification and decay in innate immunity
20:40–21:00	**Oliver Mühlemann:** Universal features of Nsp1-mediated translational shutdown by beta-coronaviruses
21:00–22:00	**Poster Slam Session:** P05, P06, P08, P09, P11, P12, P16, P21, P23, P25, P26, P27, P31, P33, P35, P37, P38, P40, P44, P45, P47, P48, P52, Chair: Georg Stoecklin – Hall A
22:00	Socializing at the Bar

21 June 2023

Session 2: Virus regulated RNA decay, Chair: Volker Thiel – Hall A	
08:30–09:00	**Sara Macias:** Interactions of the RNAi and interferon pathways
09:00–09:30	**Anna-Lena Steckelberg:** Viral RNA structures as master manipulators of host RNA degradation machinery
09:30–10:00	**Jeff Wilusz:** RNA Binding Proteins as Key Targets for RNA Viruses to Dysregulate Cellular Gene Expression with Pathological Implications
10:00–10:30	Coffee Break – Foyer Hall A
Chair: Sarah Newbury	
10:30–10:50	**Boris Slobodin:** The molecular strategies employed by SARS-CoV-2 for inhibiting host mRNAs and promoting viral transcripts

10:50–11:10	**Khalid Khabar:** AU-rich elements-mediated mRNA turnover in coronaviral disease
11:10–11:30	**Vincenzo Ruscica:** Alphavirus replication is fed by XRN1-mediated cellular mRNA degradation
11:30–12:00	**Hannah Burgess:** Regulation of Human Cytomegalovirus Infection by the CCR4-NOT Deadenylase Complex
12:00–12:30	**Pierre Jalinot**: How the Human T-lymphotropic Virus type 1 protects its RNAs by hijacking key cellular factors
12:30–14:00	Lunch Break – Restaurant Lucullus
Session 3: Cellular RNA decay mechanisms, Chair: Jack Keene – Hall A	
14:00–14:30	**Eva Absmeier:** The CCR4-NOT deadenylation complex is a ribosome co-factor
14:30–15:00	**Alessia Ruggieri:** GADD34 mRNA turnover controls the molecular memory of the integrated cellular stress response
15:00–15:20	**Jernej Murn:** A paradigm for disordered region-driven translational silencing by RNA-binding proteins
15:20–15:40	**Veronika Rájecká:** The role of Trf4p in the biogenesis of protein-coding transcripts in Saccharomyces cerevisiae
15:40–16:00	**Ambro van Hoof:** Degradome sequencing reveals unexpected functions for well-characterized RNases
16:00–18:00	**Poster Session:** Odd Numbers Present including refreshments – Hall B+C
18:00–19:30	Light Dinner – Restaurant Siesta
Session 4: Advances in RNA methodology, Chair: Hana Cahova – Hall A	
19:30–20:00	**Jack Keene:** The Powers of RNA Regulons
20:00–20:30	**Marvin Tanenbaum:** Translation and replication dynamics of single RNA viruses
20:30–20:50	**Alicia Bicknell:** High ribosome loading is associated with rapid mRNA decay among coding sequence variants
20:50–21:10	**Jenny J. Seo:** Functional viromic screens uncover novel regulatory RNA elements

21:10–21:30	**Raecliffe Daly:** N-terminal acetylation of the influenza A virus ribonuclease PA-X separately controls its nuclear localization and host shutoff activity
21:30	Socializing at the Bar

22 June 2023

Session 5: RNA decay and modification in virus-host interactions Chair: Benjamin tenOever – Hall A	
08:30–09:00	**Volker Thiel:** Lost in translation – generation of attenuated SARS-CoV-2 vaccines by genome recoding
09:00–09:30	**Laura Lorenzo Orts:** Repression and storage of maternal mRNAs and ribosomes in the egg
09:30–10:00	**Sébastien Pfeffer:** Non-canonical roles of Dicer during viral infections
10:00–10:30	Coffee Break – Foyer Hall A
Chair: Jeff Wilusz	
10:30–10:50	**Lizandro R. Rivera-Rangel:** Hepatitis C virus RNA is 5′ capped with flavin adenine dinucleotide
10:50–11:10	**Callie Donahue:** Follow the Map: Ebola virus co-opts the mRNA decapping complex through the scaffold protein EDC4
11:10–11:30	**Megha Mallick:** Interplay between Nonsense Mediated mRNA Decay factors and SARS-CoV-2 Nucleocapsid protein and inhibition of NMD
11:30–12:00	**Hana Cahova:** Non-canonical RNA caps – from their discovery to their role
12:00–12:30	**Georg Stoecklin:** Control of mRNA turnover by the CCR4-NOT deadenylase complex
12:30–14:00	Lunch Break – Restaurant Lucullus
Session 6: RNA decay in development and disease Chair: Lena Steckelberg – Hall A	
14:00–14:30	**Imed Gallouzi:** Impact of mRNA turnover on Astrocyte involvement in neurodegenerative diseases
14:30–15:00	**Sarah Newbury:** Role of the exoribonucleases Pacman and Dis3L2 on miRNA and lncRNA stability in the context of tissue growth in Drosophila melanogaster

15:00–15:20	**Sakie Katsumura:** Deadenylase-dependent mRNA decay of hepatokines controls food intake and energy expenditure
15:20–15:40	**Maria Jose Lista:** Mechanism of viral mRNA restriction mediated by the ZAP-KHNYN complex
15:40–16:00	**Marcos Iuri Roos Kulmann:** Activation of antiviral RNAi in mammals
16:00–18:00	**Poster Session:** Even Numbers Present including refreshments – Hall B+C
19:00–20:00	Guided visit to the Mendel museum and refectory
19:30–23:00	Farewell Dinner – Augustinian Abbey

23 June 2023

Session 7: Translation, viral stress and phase separation Chair: Alessia Ruggieri – Hall A	
08:30–09:00	**Karel Říha:** P-bodies in plant reproduction and stress response
09:00–09:30	**Andrzej Dziembowski:** How mRNA vaccines hijack TENT5 cytoplasmic poly(A) polymerases to enhance stability
09:30–10:00	**Nicolas Locker:** Friends or Foes? The Many Routes Caliciviruses Use to Manipulate RNA Granules
10:00–10:30	Coffee Break – Foyer Hall A
Chair: Olivia Rissland	
10:30–10:50	**Luciana Castellano:** Codon-dependent regulation of gene expression during dengue virus infection
10:50–11:10	**Seyed Mehdi Jafarnejad:** SARS-CoV-2 protein NSP2 impairs interferon production and enhances microRNA-mediated translational repression
11:10–11:30	**Stefan Bresson:** The transcriptional and translational landscape of coronavirus OC43 infection
11:30–12:00	**Roy Parker:** Translation reprograming during viral infection
12:00–12:15	Organizers: Concluding Remarks
12:15–14:00	Coffee Break to go – Foyer Hall A

Abstracts of Speakers

01 Keynote lecture Decoding and recoding nature's software to build virus-based computers

Benjamin tenOever [1]
[1] *Virology Institute of NYU (The VIRION), New York University – Langone Health, Alexandria Center for Life Sciences, 430 East 29th Street, Room 540, New York, New York 10016*

RNA viruses employ adaptive strategies and ingenious tricks to optimize the efficiency and versatility of their genetic material, enabling them to exploit host cellular machinery and replicate with remarkable efficiency. Studying these viral tactics provides valuable insights into fundamental aspects of RNA biology, viral pathogenesis, and lays the groundwork for the development of RNA-based computers. By capitalizing on RNA's inherent properties, including its capacity to fold into intricate structures and perform diverse functions, one can engineer synthetic RNA sequences that serve as Boolean logic gates. These gates, inspired by the natural processes employed by RNA viruses, present a promising avenue for constructing versatile and programmable computational systems. The seminar will focus on the engineering and biological output that can be achieved through the exploitation of this RNA biology.

02

Mary Anne O´Connell
Research Group Leader Senior, Project Coordinator Manager
CEITEC, Masaryk University, CZ

03 Universal features of Nsp1-mediated translational shutdown by beta-coronaviruses

Katharina Schubert [1], Evangelos Karousis [2, 4], Ivo Ban [1], Christopher P. Lapointe [3], Marc Leibundgut [1], Emilie Bäumlin [2, 4], Eric Kummerant [1], Alain Scaiola [1], Tanja Schönhut [1], Jana Ziegelmüller [2], Joseph D. Puglisi [3], Oliver Mühlemann [2], Nenad Ban [1]

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[2] Department of Chemistry,Biochemistry and Pharmaceutical Sciences, University of Bern, Bern, Switzerland

[3] Department of Structural Biology, Stanford University School of Medicine, Stanford, CA, USA

[4] Multidisciplinary Center for Infectious Diseases, University of Bern, Bern, Switzerland

Nonstructural protein 1 (Nsp1) produced by coronaviruses shuts down host protein synthesis in infected cells. The C-terminal domain of SARS-CoV-2 Nsp1 was shown to bind to the small ribosomal subunit to inhibit translation, but it is not clear whether this mechanism is broadly used by coronaviruses, whether the N-terminal domain of Nsp1 binds the ribosome, or how Nsp1 specifically permits translation of viral mRNAs. Here, we investigated Nsp1 from three representative Betacoronaviruses – SARS-CoV-2, MERS-CoV, and Bat-Hp-CoV – using structural, biophysical, and biochemical assays.
We revealed a conserved mechanism of host translational shutdown across the three coronaviruses. We further demonstrated that the N-terminal domain of Bat-Hp-CoV Nsp1 binds to the decoding center of the 40S subunit, where it would prevent mRNA and eIF1A binding. Structure-based biochemical experiments identified a conserved role of these inhibitory interactions in all three coronaviruses and showed that the same regions of Nsp1 are responsible for the preferential translation of viral mRNAs. Our results provide a mechanistic framework to understand how beta-coronaviruses overcome translational inhibition to produce viral proteins.

04 Interactions of the RNAi and interferon pathways

Sara Macias[1]
[1] *University of Edinburgh, UK*

As part of the innate immune response against viruses, mammals activate the type I interferon pathway to prevent viral replication and dissemination. In specific contexts, an RNAi-based antiviral response can also be activated, suggesting that several antiviral mechanisms can co-occur in the same cell and that these pathways must interact to enable an optimal antiviral response. Here, I will discuss our most recent findings on how the RNAi and other small RNA biogenesis pathways interact with the type I interferon response in both mice and humans.

05 Viral RNA structures as master manipulators of host RNA degradation machinery

Anna-Lena Steckelberg [1]
[1] *Columbia University, US*

RNA viruses typically contain very small genomes and encode only a few proteins. As obligate intracellular parasites, many viruses have therefore evolved elegant RNA-based strategies to manipulate cellular machinery in order to enhance virus propagation and pathogenicity. Studying these RNA-centric viral mechanisms teaches us about important human pathogens, but also expands our understanding of the cellular machinery they employ. A particularly intriguing, yet poorly understood, example is the use of highly structured RNA elements to halt the degradation of viral RNAs by cellular 5'-3' exoribonuclease, such as the highly processive Xrn1. This mechanism was first discovered over a decade ago in flaviviruses, where the stalling of Xrn1 on viral genomes leads to the production of biologically active viral RNA degradation products with important functions in immune modulation and viral pathogenicity. More recently, we discovered that exoribonuclease-resistant RNA structures (called xrRNAs) are also found in many unrelated RNA viruses, including those of the plant-infecting Luteoviridae and Tombusviridae families. This finding established xrRNAs as an authentic functional class of structured RNAs and identified programmed exoribonuclease resistance as an important RNA maturation pathway in the viral world. Despite their widespread presence and continued discoveries of diverse roles, the structural basis of xrRNA function remains only partially understood. All xrRNAs discovered to date rely on the formation of a protective ring-like fold around the RNA, yet the sequences and three-dimensional folds that form these protective rings are highly diverse, thwarting any attempt to predict new xrRNAs from sequence data alone. We have solved the three-dimensional structure of several plant-virus xrRNAs by x-ray crystallography, and discovered a dynamic folding pathway that exploits Xrn1's helicase activity for co-degradational structure remodeling. Combining biochemical in vitro assays, viral infection studies, structural biology and single-molecule measurements of RNA dynamics to characterize diverse xrRNAs, we work towards predictive sequence-structure-function models of this new class of functional viral RNA.

06 RNA Binding Proteins as Key Targets for RNA Viruses to Dysregulate Cellular Gene Expression with Pathological Implications

Noelia Altina, David Maranon, Meghan Donaldson, Jeff Wilusz [1]
[1] *Colorado State University, Department of Microbiology, Immunology and Pathology, Fort Collins, CO 80523 USA*

The interface between viral RNAs and the RNA biology of the infected cell reveals numerous strategies that can lead to molecular explanations for pathogenesis and cytopathology. We have been investigating the functional implications of cellular RNA binding protein interactions with abundant viral transcripts generated during SARS-CoV-2 and Zika virus infections. The abundant ~70 base leader RNA found in the 5' UTR of all SARS-CoV-2 transcripts interacts with the cellular PTBP1 protein. PTBP1 knockdown events are mimicked during SARS-CoV-2 infection, suggesting that the cellular RBP is sequestered/inactivated. Along these lines, PTBP1 is progressively re-localized to the cytoplasm and the processing of pre-mRNAs that undergo PTBP1-mediated alternative splicing are dysregulated during infection. Coronaviral infection also disrupts other factors involved in cellular RNA biology. SARS-CoV-2, for example, also usurps the cellular PCIF1 protein to provide an m6Am modification to the adenosine residue adjacent to the 5' cap on all of its mRNAs. Finally, along these lines, we have identified 28 cellular RBPs
that interact in a conserved fashion with small flavivirus RNA (sfRNA) – a stable decay intermediate from the 3' UTR of Zika virus and other insect-borne flaviviruses. These RBPs are involved in cellular processes from splicing to RNA stability – and many of them are naturally antiviral as determined by knockdown/overexpression studies. Collectively, we present a model where RNA viruses have actively evolved to strategically usurp the functions of key cellular RNA binding proteins to dysregulate host cell gene expression at the post-transcriptional level. This RBP dysregulation is likely very important for the establishment of efficient viral replication and may have significant pathological implications.

07 The molecular strategies employed by SARS-CoV-2 for inhibiting host mRNAs and promoting viral transcripts

Boris Slobodin [1, 2], Urmila Sehrawat [1], Anastasia Lev [1], Daniel Hayat [1], Binyamin Zuckerman [1], Davide Fraticelli [1], Ariel Ogran [1], Amir Ben-Shmuel [3], Elad Bar-David [3], Haim Levy [3], Igor Ulitsky [1], Rivka Dikstein [1]

[1] Weizmann Institute of Science

[2] Technion Israel Institute of Technology

[3] Israel Institute for Biological Research

Upon entrance to the host cell, many pathogenic viruses need to escape the host's antiviral response and, at the same time, support the expression of their own genome. In the case of SARS-CoV-2, the non-structural protein 1 (NSP1) is particularly important for these tasks. In infected cells, NSP1 was reported to inhibit translation, lead to the degradation of host mRNAs, and interfere with the nuclear-cytosolic shuttling of mRNAs. Here, we show that the stand-alone ectopic expression of NSP1 leads to a massive destabilisation of mainly spliced cellular mRNAs. In contrast, the intron-less transcripts, a group including all viral and multiple anti-viral genes encoded by the host, remain less affected. Additionally, we found that NSP1 only partially reduces translation, mainly acting on the translation initiation step. Surprisingly, despite these deleterious effects on gene expression at multiple levels, cells expressing NSP1 are viable. We found that a conserved and precisely located cap-proximal guanosine-devoid RNA element confers resistance to NSP1-mediated inhibition. An artificial introduction of this element into 5'UTRs of random genes enables their enhanced co-expression with NSP1. This feature is present in all 5'UTRs of SARS-CoV-2 and conserved in multiple coronaviruses, indicating its strategic and evolutionarily conserved role. Interestingly, we observed that the long (genomic) version of the 5'UTR encoded by SARS-CoV-2 can mediate cap-independent translation initiation, ensuring the expression of multiple non-structural viral proteins even in conditions of prolonged inhibition of cap-dependent translation. Remarkably, this feature co-exists with the 5'cap structure decorating the mature viral transcript. The combination of these characteristics suggests a complex strategy enabling the virus to significantly limit the anti-viral host response at multiple levels and ensure a robust expression of the viral transcripts.

08 AU-rich elements-mediated mRNA turnover in coronaviral disease

Khalid Khabar
King Faisal Specialist Hospital and Research Centre

AU-rich elements (AREs) exist in the 3'UTR of nearly 20% of the human mRNAs, including those induced during viral infections such as pro-inflammatory cytokines. The default functions of AREs are mRNA decay and translational inhibition, but these can be attenuated during acute infections and chronic diseases. Emerging viral infections, such as MERS-CoV and SARS-CoV, have significant morbidity and mortality and are associated with the „cytokine storm" characterized by over-production of pro-inflammatory cytokines. First, our bioinformatics analysis of RNAseq transcriptomics showed that ARE-mRNAs significantly enriched among over-expressed genes in both MERS-CoV and SARS-CoV-2 infections. Then, we sought to assess the role of accessory coronaviral proteins in ARE-mediated posttranscriptional control. We found that MERS-CoV ORF4A and ORF4B strongly increased cytokine and chemokine mRNA stability in 3´-UTR and ARE-mediated manner in lung cell line models. In addition, we observed increased phosphorylation of the RNA-binding protein, TTP (ZFP36), leading to the loss of its ability to promote AU-rich mRNA decay and, thus, increased cytokine abundance. Subsequently, we utilized the dsRNA as a viral mimic; it led to TTP phosphorylation in a dose- and time-dependent manner. It also activated the p38 MAPK signaling pathway and ARE-mRNA stabilization in lung and macrophage cells. We found that the basal and dsRNA-induced activation of ERK/MAPK and AKT/mTOR/70S6K pathways augmented ORF4A and ORFB-induced hyper-phosphorylation of TTP. Treatment of cells with the MEK inhibitors, trametinib, and selumetinib, potently reduced basal and ORF4-induced ARE-mediated aberrations. We also found that other kinase inhibitors that reduced ARE-mediated reporter activity can be further investigated with coronaviral infections. Overall, our studies pointed out that ARE-mediated control of gene expression is compromised in emerging coronaviral diseases leading to the prolonging of cytokine mRNAs and subsequently increased cytokine abundance. This suggests that targeting ARE pathway may be a viable option for treating coronaviral-induced acute inflammatory responses.

09 Alphavirus replication is fed by XRN1-mediated cellular mRNA degradation

Vincenzo Ruscica [1], Louisa Iselin [1], Ryan Hull [2], Honglin Chen [1], Quan Gu [1], Samyukta Narayanan [1], Natasha Palmalux [1], Azman Embarc-Buh [1], Zaydah Rolande De Laurent [1], Joseph Hugh [1], Marko Noerenberg [1], Wael Kamel [1], Catia Igreja [3], David Robertson [1], Shabaz Mohammed [4, 5], Vicent Pelechano [2], Ilan Davis [5], Alfredo Castello [1]

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[5] Department of Biochemistry, University of Oxford, South Parks Road, OX1 3QU, Oxford, UK.

XRN1 is a key regulator of mRNA degradation, with proposed roles in viral infection. However, the exact function of XRN1 in infection remains poorly understood due to antagonistic results. We recently showed that Sindbis virus (SINV, alphavirus genus) causes a global downregulation of host gene expression through a pervasive degradation of cellular mRNA. This is concomitant with an increased association of XRN1 with cellular RNA and the emergence of viral RNA. Nevertheless, the link between XRN1, cellular mRNA degradation, and SINV replication remains mysterious.

Here, we show that XRN1 and other members of the 5'-to-3' mRNA decay pathway, such as DCP1 and DCP2, are essential for alphaviruses replication. Rescue of XRN1 knock out cells with XRN1-WT, but not with a catalytically inactive mutant, partially restores infection, highlighting the importance of the exonuclease activity in the regulation of viral infection. iCLIP analysis revealed that XRN1 predominantly engages with the cellular transcripts that are unstable in infected cells, suggesting a link between its enzymatic activity and the degradation process. Furthermore, the RNA decay machinery localizes to viral replication centres by the interaction of XRN1 with the viral protein nsP1, physically bridging the fast degradation of cellular RNA with the viral replication machinery. This, together with the detection of nucleotide kinases accumulating at the viral replication factories, suggests that XRN1

may produce free nucleotides that are locally phosphorylated to nucleotides triphosphate to feed replication. Supporting this hypothesis, the addition of nucleosides to XRN1-KO rescued cells enhances SINV infection. In summary, our data support a model in which XRN1 sustains viral replication by degrading cellular mRNA to increase the local pool of free nucleotides.

10 Regulation of Human Cytomegalovirus Infection by the CCR4-NOT Deadenylase Complex

Hannah Burgess [1]
[1] *Department of Microbial Sciences, University of Surrey, Guildford, GU2 7XH, UK*

Unlike some RNA and DNA viruses that broadly stimulate mRNA decay and interfere with host gene expression, human cytomegalovirus (HCMV) extensively remodels the host translatome without producing a viral mRNA decay enzyme. Testing the hypothesis that HCMV instead relies on components of the cellular canonical mRNA turnover pathway we performed a targeted loss-of-function screen in primary human fibroblasts, identifying host CCR4-NOT deadenylase complex members CNOT1 and CNOT3 as unexpected pro-viral host factors that selectively regulate HCMV reproduction. We found scaffold subunit CNOT1 is specifically required for late viral gene expression and detect altered host responses in CCR4-NOT-disrupted cells. Profiling poly(A)-tail lengths of individual HCMV and host mRNAs using nanopore direct RNA sequencing revealed poly(A)-tails of viral messages to be markedly longer than those of cellular mRNAs and significantly less sensitive to CCR4-NOT disruption. Upregulation of select CCR4-NOT components during HCMV infection suggests active manipulation of complex activity by the virus to the benefit of replication. Our data establish mRNA deadenylation by host CCR4-NOT is critical for productive HCMV replication and defines a new mechanism whereby herpesvirus infection subverts cellular mRNA metabolism to remodel the infected cell gene expression landscape. Moreover, we expose an unanticipated host factor with potential to become a therapeutic anti-HCMV target.

11 How the Human T-lymphotropic Virus type 1 protects its RNAs by hijacking key cellular factors

Jalinot P. [1], **Prochasson L., Mghezzi-Habellah M., Robin J-P., Roisin A., Mocquet V.**

[1] ENS de Lyon, Laboratory of Biology and Modelling of the Cell, CNRS (UMR5239), INSERM (U1293), Lyon, France

The Human T-lymphotropic Virus type 1 (HTLV-1) is the etiological agent of several diseases including the Adult T-cell Leukemia/Lymphoma and Tropical Spastic Paraparesis. Several of the RNAs expressed by this retrovirus are sensitive to Nonsense Mediated mRNA Decay (NMD) as a consequence of specific features like intron persistence or long 3'UTR. However this RNA surveillance pathway, which acts as a defence barrier against viral infection, is counteracted by the action of two HTLV-1 proteins: Tax and Rex. The first one is a key activator of the transcription of the integrated provirus by the cellular RNA Pol II and also acts on many cellular factors to dysregulate important pathways. In particular Tax interacts with the eIF3e subunit of the eIF3 translation initiation factor, that is known to intervene in the NMD pathway, and also with UPF1, the cellular RNA helicase playing a central role in the execution of NMD. Rex interacts with viral RNAs via association to the Rex Responsive Element (RxRE) present at their 3' end to induce their export in the cytoplasm, thereby inhibiting their splicing and permitting their translation. Rex plays its role in RNA export by interacting with the CRM1 exportin and has also been reported to contact UPF1. In this presentation the interplay between these viral and cellular factors allowing protection of the viral RNAs with respect to NMD degradation will be described. Our studies also establish that the hijacking of UPF1 by Rex leads to a positive effect of this important RNA helicase in HTLV-1 replication, from viral RNA export to production of viral particles.

12 The CCR4-NOT deadenylation complex is a ribosome co-factor

Eva Absmeier [1], Viswanathan Chandrasekaran [1], Markus Höpfler [1], Francis J O'Reilly [2], James AW Stowell [1], Juri Rappsilber [2,3], Ramanujan S Hegde [1], Lori A Passmore [1]

[1] MRC Laboratory of Molecular Biology, Cambridge UK.

[2] Technische Universität Berlin, Chair of Bioanalytics, 10623 Berlin, Germany.

[3] Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh UK

mRNA translation and decay are intimately connected, and the translation and decay machinery physically interact. A key player at this interface is the multi-subunit CCR4-NOT deadenylation complex, which acts together with a plethora of interaction partners on various mRNA targets. One aspect of our work investigates the general role of mammalian CCR4-NOT during translational stalling. We use cryoEM and crosslinking mass spectrometry to show that mammalian CCR4-NOT specifically recognizes ribosomes that are stalled during translation elongation in an in vitro reconstituted system. Our cryoEM structure reveals that CCR4-NOT inserts a helical bundle of its CNOT3 subunit into the empty E site of the ribosome and locks the L1 stalk in an open conformation to inhibit further translation. In a second line of research, we investigate the role of CCR4-NOT in the targeted decay of tubulin mRNA in a process called tubulin-autoregulation. Our work reveals a novel ribosome interaction factor, SCAPER, which bridges a nascent chain-protein complex and CCR4-NOT for tubulin mRNA decay. Together our work reveals that CCR4-NOT recognizes specific ribosomal states to regulate the stability of various mRNAs.

13 GADD34 mRNA turnover controls the molecular memory of the integrated cellular stress response

Vera Magg [1], Alessandro Manetto [1], Nicolas Locker [2], Georg Stoecklin [3] and Alessia Ruggieri [1]

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The integrated stress response (ISR) is a key cellular signaling pathway activated by environmental alterations that represses protein synthesis to restore homeostasis. To prevent long-term damage, the ISR is counteracted by the upregulation of Growth Arrest and DNA Damage inducible 34 (GADD34), a stress-induced regulatory subunit of protein phosphatase 1 that mediates translation reactivation and stress recovery. We established that the 3' untranslated region of GADD34 mRNA contains an active AU-rich element recognized by TTP and Brf1, two proteins of the ZFP36 family, which promotes rapid mRNA turnover under normal conditions. Exposure to different types of stress simultaneously activates the p38-MAPK/MK2 and PI3K/Akt pathways, leading to downstream phosphorylation of TTP and Brf1 as well as to the rapid, transient stabilization of GADD34 mRNA for efficient expression of GADD34 protein in response to stress. Our findings reveal that regulation of GADD34 mRNA turnover is a key to the establishment of the ISR molecular memory, which helps to set the threshold for cellular responsiveness and mediate adaptation to stress.

14 A paradigm for disordered region-driven translational silencing by RNA-binding proteins

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RNA-binding proteins (RBPs) are key regulators of gene expression, but the mechanisms of their action remain largely obscure. Despite the increasingly better understanding of RNA sequence specificities and general functions of RBPs, little is still known about how individual RBPs convey their regulatory instructions to the core effectors of RNA processing. Here we dissect an RBP–effector junction responsible for translational control by a sequence-specific RBP. We find that a large, intrinsically disordered region (IDR) of an evolutionarily conserved RBP implicated in regulation of cell morphology, Unkempt, is exquisitely designed for translational repression of its targeted transcripts. Within the IDR, we identify an array of peptide motifs with which Unkempt directly interacts with and recruits two central effectors of mRNA stability and translational control, PABPC and the CCR4-NOT deadenylase complex. Spatial arrangement of the critical motifs as well as obligate homodimerization of Unkempt engender multiprong contacts with CCR4-NOT that are mandatory for precise RNA sequence recognition, translational repression, as well as the control of cell morphology by Unkempt. Remarkably, despite recruiting a major cellular deadenylase, Unkempt-bound mRNAs remain stable with intact poly(A) tails, but are translationally repressed within compact ribonucleoprotein particles. The RBP–effector nexus formed by Unkempt bears resemblance to existing models for miRNA/AGO-dependent translational silencing and sheds light on the regulatory principles of disordered regions of RBPs.

15 The role of Trf4p in the biogenesis of protein-coding transcripts in Saccharomyces cerevisiae

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RNA polymerase II (Pol II) transcribes protein-coding genes and several types of non-coding RNAs (ncRNA). One of the key steps in RNA synthesis by Pol II is proper termination of transcription. Transcription termination is tightly coupled to (m)RNA 3′-end processing. In Saccharomyces cerevisiae, mRNAs are terminated and processed by the cleavage and polyadenylation complexes while the 5′ to 3′ degradation of the downstream cleavage product by the Rat1-Rai1-Rtt103 complex torpedoes Pol II-DNA association and thus facilitates transcription termination. Transcription termination of Pol II ncRNAs involves Nab3-Nrd1-Sen1 (NNS) complex that further draws the polyadenylation Trf4-Air1/2-Mtr4 (TRAMP4) complex and the exosome. The TRAMP4 complex is a surveillance factor that in Saccharomyces cerevisiae mediates nuclear surveillance and processing of ncRNAs. The polyadenylation activity of Trf4p and helicase activity of Mtr4p facilitate exosome trimming and degradation of a number of RNA precursors and aberrant transcripts. Here we identify an additional role of TRAMP4 in maintaining the proper homeostasis of 3′ ends of protein-coding transcripts. We show that TRAMP4 mutant strains lead to Pol II read through phenotype and accumulation of 3′ extended forms of a subset of mRNAs. This feature is specific for TRAMP4 as Trf5p depletion does not show similar effect and its role is independent of the Trf4p polyadenylation activity but requires its C-terminal motif NIM. Transcriptome-wide analysis revealed that depletion of Trf4p leads to dysregulation of a number of protein-coding genes, but only some of these changes depend on Trf4p polyadenylation activity. In summary, these results present an additional role of TRAMP4 in maintaining the proper homeostasis of 3′ ends of protein-coding transcripts.

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16 Degradome sequencing reveals unexpected functions for well-characterized RNases

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Eukaryotic genomes encodes dozens of ribonucleases, but we have an incomplete understanding of what specific RNAs are matured or degraded by what RNases. For mRNA degradation, it is clear that two exoribonucleases, Xrn1 and the RNA exosome, carry out the bulk of cytoplasmic mRNA degradation. However, other RNases, including a variety of endonucleases also contribute by initiating the degradation of a subset of mRNAs. These additional RNases often target aberrantly processed mRNAs or a subset of normal mRNAs in response to a physiological signal. We are using degradome sequencing, or Parallel Analysis of RNA Ends, to determine the mRNA degrading roles of RNases. PARE is an unbiased transcriptome wide approach, but unlike many transcriptome wide approaches, it is relatively insensitive to indirect effects because it detects the direct product of RNases instead of assaying total mRNA level. Using PARE we have discovered unexpected roles for several well-characterized RNases and will present our ongoing progress.

17 The Powers of RNA Regulons

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My lab investigates mechanisms that coordinate genes of eukaryote cells. I started by sequencing and modification (6mA) of messenger RNAs of VS viruses and terminal 5' and 3' sequences of Rabies, Ebola, Marburg, and others. It became clear that RNA-binding proteins (RBP) were the future to understanding eukaryotic cells. Thus, in1985 my lab cloned the first RBP, La protein and then others. Our La RBP sequence made it possible for us to pinpoint the 90 amino acid motifs of La and U1-70K, that I named the RNA-Recognition Motif (RRM) (1988-89). RRMs are the largest group of RBPs in the human genome. Thus, driven by sequence-specific motifs of RBPs I conceived a hypothesis and demonstrated its importance for RBPs via multi-combinatorial targets of mRNAs with coherent functions that coordinate RNA stability, export, localization, and translation. This was possible because of my inventions of RIP/CLIPs that demonstrated individual mRNAs with cis-trans matched binding motifs could coordinate related proteins and functions. Using RBPs of the ELAV family from human brain mRNAs we used ultraviolet light to identify multiple AU-rich mRNAs using my novel selection method to demonstrate binding of 100 (1994) and then 650 (2000) specific functionally related brain mRNAs. Experiments by hundreds of labs supported our hypothesis and novel concepts and demonstrations of RBP-RNA interactions led to discovery of Post-Transcriptional-RNA-Operons/Regulons (PTROs). Therefore, I will present important PTRO subsets of diverse eukaryotic genes that can come together to generate dynamic functions that regulate cells and organs. I will discuss advances using PTROs that are beyond expectations by feeding back from mRNA cytoplasm to the nucleus and vice-versa. Indeed, genes can be coordinated on DNA chromatin in the nucleus and back to the PTRO cytoplasm. Overall, these paradigms led us to conceive combinatorial mechanisms that communicate and coordinate gene expression between transcription and translation.

18 Translation and replication dynamics of single RNA viruses

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RNA viruses are among the most prevalent pathogens and are a major burden on society. Upon viral entry into a host cell, viral proteins are rapidly synthesized, which is followed by viral replication. In response to virus infection, host cells can activate antiviral signaling pathways to restrict viral spread. Intriguingly, viral infection progresses at very different rates among infected cells. Similarly, cell-to-cell heterogeneity is often observed in the host cell response to viral infection, with only a (small) subset of infected cells successfully launching an antiviral response. To better understand heterogeneity in viral infection, we developed a new imaging assay called virus infection real-time imaging (VIRIM) to visualize infection and replication of individual RNA viruses in live cells. Applying VIRIM to picornaviruses resulted in a detailed characterization of viral replication dynamics, and identified the replication step of the incoming viral RNA as a major bottleneck for successful infection. To understand how such heterogeneity in viral replication dynamics affects host antiviral responses, we combined VIRIM with highly sensitive measurements of antiviral response activation, which revealed that cell-to-cell heterogeneity in viral replication rates early in infection determine the efficiency of antiviral response activation. Together, this work provides quantitative insights into the heterogenous outcomes of virus-host competition.

19 High ribosome loading is associated with rapid mRNA decay among coding sequence variants

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A common goal in therapeutic mRNA design is to maximize protein output per delivered mRNA molecule. Total protein output is a function of both protein production per unit time and the rate of mRNA decay. With the specific intent of understanding the principles that impact the therapeutic potential of mRNA drugs, we designed a set of mRNA sequences that all encode the same protein (GFP) and have identical UTRs, but differ in codon choice and predicted secondary structure. These mRNAs were transcribed in vitro and delivered to mammalian cells for characterization of RNA half-life, translation dynamics, and total protein output. Coding sequence variants differed in mRNA half-life by an order of magnitude, with translation-dependent decay mechanisms accounting for most of the observed half-life differences. GFP sequences with a 3' modification to block deadenylation showed a narrower range of mRNA half-lives, suggesting deadenylation as a main driver of half-life differences among sequence variants. Polysome profiling revealed a wide range of ribosome loads for GFP sequences, with differences surprisingly driven by differential initiation rates rather than elongation rates. mRNAs with middling initiation rates and ribosome loads had longer mRNA half-lives. Increasing ribosome load corresponded with a decrease in RNA half-life and a resulting decrease in total protein output. Our results suggest that for exogenous mRNAs, high levels of translation initiation lead to higher rates of translation-dependent mRNA decay, likely through deadenylation-dependent mechanisms. Optimal protein expression for therapeutic mRNAs is achieved by curbing initiation rates in order to prolong mRNA half-life and duration of protein expression.

20 Functional viromic screens uncover novel regulatory RNA elements

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Viruses harbor diverse regulatory modules, yet understanding of their functions lags behind the rapidly expanding universe of sequenced viral genomes. Here, we screen the activities of 30,367 viral segments from 143 species representing 96 genera and 36 families. These screens identify hundreds of RNA elements that enhance or suppress RNA abundance and/or translation. In a case study on a prominent positive element, K5, which is conserved within the genus Kobuvirus, we discover a previously uncharacterized protein, ZCCHC2, as a critical host factor for K5. ZCCHC2 recruits the terminal nucleotidyltransferase TENT4 to extend poly(A) tails with mixed sequences, delaying deadenylation. K5 potently enhances mRNA stability and translation in all contexts tested, including adeno-associated viral vectors and in vitro transcribed mRNAs. This study provides a unique resource for virus and RNA research and illustrates the potential of the virosphere for identifying novel regulatory mechanisms.

21 N-terminal acetylation of the influenza A virus ribonuclease PA-X separately controls its nuclear localization and host shutoff activity

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To counteract host anti-viral responses, influenza A virus triggers a global reduction of cellular gene expression, a process termed "host shutoff." A key effector of influenza A virus host shutoff is the viral ribonuclease PA-X, which degrades host mRNAs. As PA-X was discovered only recently, many of the molecular determinants of its activity remain unknown, including how specific sequence elements and protein modifications contribute to its function and regulation. It was recently reported that PA-X activity requires co-translational N-terminal acetylation. N-terminal acetylation is understudied, but can modulate a variety of processes, including protein synthesis, stability, folding, subcellular localization, and protein-protein interactions. It remains unclear how N-terminal acetylation influences the shutoff activity of PA-X. In experiments using ectopic expression of PA-X, outside of the context of infection, we have found that PA-X N-terminal acetylation has two functions that can be separated based on the position of the acetylation. N-terminal acetylation can occur either on the initiator methionine or on the second amino acid following initiator methionine removal. Either is sufficient to ensure PA-X localization to the nucleus, which is important for the host shutoff function. However, N-terminal acetylation of the initiator methionine specifically is required for PA-X RNA downregulation activity, independently of nuclear localization. Surprisingly, in infected cells, PA-X N-terminal acetylation at any position results in RNA downregulation. While this finding suggests that other viral proteins or cellular processes boost PA-X activity or its modification, the identity of these processes is unknown. We are currently investigating how infection supports host shutoff activity of N-terminally acetylated PA-X. These studies provide further insight into the regulation of PA-X activity and PA-X interactions with fundamental cellular processes, as well as new information on the functions of a poorly understood protein modification.

22 Lost in translation – generation of attenuated SARS-CoV-2 vaccines by genome recoding

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Live-attenuated virus vaccines (LAV) hold promise to provide protective immunity while not causing disease. We reverse engineered the SARS-CoV-2 genome to increase the likelihood to generate stop codons by introducing more than 600 synonymous nucleotide changes. In addition we inactivated several coronavirus-specific functions. We show the resulting LAV is fully attenuated in pre-clinical animal models, and induce B- and T-cell immune responses that protect against wild-type SARS-CoV-2 infection and against recent SARS-CoV-2 variants. Importantly, the SARS-CoV-2 LAV has increased vulnerability for antiviral treatment with mutagens, such as mulnupiravir, and spread of the vaccine candidate virus to contact animals is greatly reduced. Based on the exceptional safety profile the LAV has been classified for work under BSL2 conditions and clinical phase I/II trials are being prepared.

23 Repression and storage of maternal mRNAs and ribosomes in the egg

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In the absence of transcription, embryos rely on the proteins, RNAs, and macromolecular complexes deposited in the egg by the mother. Among these components, maternal ribosomes and mRNAs ensure translation during early embryogenesis. However, translation is repressed in the egg to maintain a state of quiescence. How eggs store maternal mRNAs and ribosomes was so far poorly understood. During my postdoc in the Pauli lab, we have identified a set of factors that associate with maternal ribosomes and contribute to their repression and stability (Leesch, Lorenzo-Orts et al. 2023). Translation of maternal mRNAs is also repressed by deadenylation. While deadenylated mRNAs are subject to decapping and degradation in somatic cells, maternal mRNAs with short polyA tails are stable during the first hours of embryogenesis. We have identified an essential role for an oocyte-specific paralog of the translational factor eIF4E, named eIF4E1b, in repressing and storing maternal mRNAs with short polyA tails. Canonical eIF4E proteins recognize the mRNA cap and interact with eIF4G to form a complex required for cap-dependent translation. Our data show that while eIF4E1b binds to the mRNA cap, it does not interact with eIF4G and thus cannot initiate cap-dependent translation. Instead, eIF4E1b interacts with eIF4ENIF1, which directs eIF4E1b localization to P-bodies in zebrafish embryos. Consistent with an important role for eIF4E1b in maternal mRNA storage, loss of eIF4E1b in zebrafish impairs female germline development. Taken together, our studies advance our understanding of key mechanisms that maintain a quiescent state in the egg.

24 Non-canonical roles of Dicer during viral infections

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One of the first layer of defense against viral infections is based on the sensing of the invading nucleic acid and/or replication intermediates by the cell. Among the mechanisms that have appeared throughout evolution is RNA interference (RNAi), a very potent antiviral pathway in plants, arthropods or nematods. RNAi is triggered by the type III ribonuclease Dicer, which senses and cleaves viral double-stranded (ds) RNA into small interfering (si) RNAs. Those small RNAs serve as guides for Argonaute proteins, which act in a sequence-specific manner to restrict the infection. In mammals, the importance of RNAi in antiviral innate immunity appears to be restricted to certain cell types, such as pluripotent stem cells, and seems to be in conflict with other innate immune pathways that also rely on dsRNA sensing. We previously showed that the human Dicer protein could interact with a number of dsRNA binding proteins via its helicase domain, and that cells expressing a helicase-truncated version of Dicer displayed a strong antiviral phenotype. Here, I will present our latest results that confirm that the helicase-deleted mutant Dicer protein is indeed antiviral against several (+) RNA viruses. Interestingly, this property does not depend on RNAi, since a catalytically inactive Dicer retains its antiviral phenotype. We analyzed the transcriptome of cells expressing the helicase-mutant Dicer and found that they display important changes compared to control cells. Interestingly, it seems that the NF-kB pathway and non-canonical functions of PKR are implicated in these alterations. I will elaborate on these results indicating that Dicer is involved in the regulation of antiviral innate immunity in unexpected ways.

25 Hepatitis C virus RNA is 5’ capped with flavin adenine dinucleotide

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Background and Aims: RNA viruses have evolved elaborate strategies for protection of their genomes, including 5’ capping. However, so far no RNA 5’ cap was identified for hepatitis C virus (HCV). Based on previous observations that flavin adenine dinucleotide (FAD) is required for HCV replication, the conservation across HCV isolates of 5’ A on the negative strand, and partially on the positive strand, we hypothesized that HCV RNA is 5’ capped with FAD.
Methods: To probe RNA FAD capping, we modified the CapZyme-seq methodology using the FAD specific decapping enzyme AtNUDX23, developed an RT-qPCR reduction assay for analysis of in vivo samples, and optimized mass-spectrometry methodology for FAD detection. HCV replicons were used to assess FAD dependency of replication, and in vitro replication initiation assays were used to study NS5B RNA-dependent RNA polymerase de novo initiation.
Results: We demonstrate that FAD is used as noncanonical initiating nucleotide by the viral NS5B polymerase resulting in a 5’ FAD cap on the HCV RNA. The HCV FAD capping frequency is ~75%, which is the highest observed for any RNA metabolite cap across all kingdoms of life. FAD capping is conserved among HCV isolates for the negative strand and partially for the positive strand. It is also observed in vivo on HCV RNA isolated from patient sera and the liver and serum of the uPA-SCID human liver chimeric mouse model. HCV replication is abrogated in the absence of the FAD precursor, riboflavin, for highly FAD-capped isolates. Furthermore, we show that 5’ FAD capping protects RNA from RIG-I-mediated innate immune recognition but has no effect on HCV RNA stability.
Conclusions: These results establish capping with cellular metabolites as a novel viral RNA capping and innate immune evasion strategy, which could be used by other viruses and affect viral treatment outcomes and persistence of infection.

26 Follow the Map: Ebola virus co-opts the mRNA decapping complex through the scaffold protein EDC4

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Ebola virus (EBOV) causes devastating disease, and frequent outbreaks of EBOV and other filoviruses demonstrate an increasing burden on public health. Only few therapeutic options exist to treat disease, and further drug development is needed. Cellular proteins co-opted by the virus during infection can serve as ideal targets for therapeutic development and may even provide opportunities to develop pan-filo inhibitors. We recently conducted a protein-protein interaction screen for 6 EBOV proteins using the BioID labeling technique. Host protein hits were mapped to a human protein-protein interaction network using the Prize Collecting Steiner Forest Algorithm, and virus protein interactors were overlayed on the resulting network map. From this modeling, we identified several clusters of host proteins labeled by the same viral protein, suggesting that the virus interacted with these proteins by binding the putative protein complex. Within these clusters, we identified the EBOV RNA synthesis cofactor VP35 binding 5 members of the host mRNA decapping complex. We confirmed an interaction between VP35 and the decapping scaffold protein EDC4 through coimmunoprecipitation and proximity-ligation analysis experiments. Depletion of EDC4 and other decapping factors with siRNA resulted in a significant decrease in EBOV infection, suggesting a dependence on the decapping complex for successful viral replication. EDC4 knockdown (KD) cells yielded reduced levels of both viral RNA and protein, suggesting

that the decapping complex participates in EBOV infection at an early stage in the virus's replication cycle. Overall, our work suggests that EBOV co-opts the mRNA decapping complex for infection by interacting with the complex through EDC4. We predict that the virus modulates processes associated with the decapping complex through VP35 for the benefit of infection. From this work, we have identified a novel host-pathogen interaction that assists in successful EBOV replication.

27 Interplay between Nonsense Mediated mRNA Decay factors and SARS-CoV-2 Nucleocapsid protein and inhibition of NMD

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SARS-CoV2, the terror behind the recent pandemic is a positive-sense RNA betacoronavirus of the Coronaviridae family. Although a lot of work has been done on the molecular mechanisms of SARS-CoV2, particularly on virus entry, replication and propagation, there remain many unanswered questions on host-pathogen protein-protein interactions.
A high throughput study in 2020 identified several networks of interactions between the SARS-CoV2 and human proteomes (Gordon et al., 2020).
One such cluster of interactions is that of the viral nucleocapsid (N) protein with the RNA processing factors UPF1 and MOV10, that are the RNA helicases involved in the nonsense-mediated mRNA decay pathway (NMD).
The N protein is one of the major structural proteins, besides Envelope (E), Membrane (M) and Spike (S) protein and is highly conserved with very few mutations among all other viruses of the Coronaviridae family (MERS and SARS-CoV) and involved in genome packaging, viral replication, viral assembly and immune regulation. The 30 Kb SARS-CoV RNA genome is a potential target for NMD as it has multiple ORFs each ending in a stop codon, which in turn lead to long 3'-UTRs in the mRNA transcript. Using a combination of biochemical and biophysical methods, we investigated the interaction of the SARS-CoV2 N-protein with major NMD factors at a molecular level.
In addition to UPF1 and MOV10, we found that the N-protein engages other core NMD factors in multi-partite interactions. These interactions result in modulation of UPF1 catalytic activity by the N-protein.
Our data suggests that N inhibits NMD by indirectly interfering with UPF1 catalytic activity and has implications for a broader impact on viral genome replication as well as post-transcriptional gene regulation in host cells.

28 Non-canonical RNA caps – from their discovery to their role

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The 5′ termini of the RNA are critical structures and are the least characterized among RNA modifications. Until recently only canonical structures, NAD or CoA have been known as 5′ RNA caps. We discovered an entirely new class of 5′RNA caps – dinucleoside polyphosphates (NpnN) in prokaryotic cells. The role of free NpnNs, identified fifty years ago, is yet to be elucidated. NpnNs are called alarmones, as their concentration increases under stress conditions. The mechanism by which the alarm is recognized in cells is unknown. We presume that their cellular effects are mediated by the RNA, where they serve as RNA caps. As such, they become an important part of RNA metabolism and can be recognized by various RNA interacting proteins, triggering additional effects in cellular metabolism. To prove the existence of NpnN-RNA caps in vivo, we developed an LC-MS technique for the detection of NpnNs in isolated and digested RNA. We detected nine previously unknown NpnN in fractions of short RNA from E. coli harvested in the exponential phase and in the late stationary phase. We identified two enzymes (RppH from the NudiX family and ApaH) as decapping enzymes of NpnN-RNA. Besides,these new caps, we also studied a role of NAD RNA cap in viral infected cells. We found that subset of snRNA and snoRNA lost NAD cap upon HIV-1 infection and that the NAD capping is undesirable for the viral infection. Our work introduces a different perspective on the chemical structure of RNA and on the role of RNA caps. We present evidence that small molecules such as NpnNs and NAD are incorporated into RNA and may thus influence the cellular metabolism and RNA turnover.

29 Control of mRNA turnover by the CCR4-NOT deadenylase complex

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With its two exoribonucleases CAF1 and CCR4, the CCR4-NOT complex acts as a major deadenylase in eukaryotic cells responsible for shorting of poly(A) tails, thereby inducing mRNA degradation. In previous work, we discovered that bulk mRNA decay is strongly accelerated by HDAC inhibition, in part through acetylation of CAF1 by p300/CBP acetyltransferases. We now explored acetylation-induced changes in the composition of the CCR4-NOT complex by purification of the endogenously tagged scaffold subunit NOT1, and identified the E3 ubiquitin ligase RNF219 as an acetylation-regulated cofactor. We demonstrate that RNF219 is an active RING-type E3 ligase that associates with the CCR4-NOT complex via the NOT9 module through a short linear motif embedded within the C-terminal low-complexity region of RNF219. By using both in vitro deadenylation assays as well as transcriptome-wide mRNA half-life measurements, we find that RNF219 acts an inhibitor of deadenylation and mRNA decay.
In a parallel approach, poly(A) capture analysis identified CPEB4 as a RNA-binding protein induced upon HDAC inhibition. Photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation revealed that CPEB4 preferentially binds to the 3′UTR of immediate early gene (IEG) mRNAs, at G-containing variants of the canonical CPE motif. Notably, our transcriptome-wide mRNA decay measurements and biochemical analysis showed that CPEB4 binding causes degradation of IEG mRNAs by recruitment of the CCR4-NOT complex. Taken together, our work describes a range of mechanisms whereby protein acetylation accelerates mRNA turnover via the CCR4-NOT deadenylase complex.

30 Impact of mRNA turnover on Astrocyte involvement in neurodegenerative diseases

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With the rise of global life expectancy, age-related neurodegenerative diseases like Alzheimer's Disease (AD) increasingly contribute to limiting health and lifespan whilst burdening healthcare systems. Specifically, AD manifests
as a progressive cognitive decline, leading to dementia and premature death. While familial AD (fAD) is caused by known mutations, the vast majority are sporadic, multifaceted, with few known genetic risk factors. Nonetheless most AD patients present the accumulation and aggregation of the Amyloid-β (Aβ) protein in their brains, yet it is not clear whether Aβ accumulation in neurons is the cause or consequence of AD. It is well-established that the disfunction of other types of brain cells, such as glia, specifically astrocytes, play an important role in the onset of neurodegenerative diseases like AD. Indeed, it has been shown that the induction of a senescence-like phenotype (a state of permanent cell-cycle arrest induced by DNA Damage Response (DDR) telomere attrition, irradiation, reactive oxygen species and oncogene activation) in astrocytes is associated with the onset of neurodegenerative diseases such as AD. However, whether and how senescence play a direct role in these conditions remains unknown. Unexpectedly, in this work we show that triggering senescence in primary human astrocytes causes them to accumulate Aβ aggregates. This AD-associated phenomenon is due to a significant increase in the expression levels of mRNA encoding Amyloid precursor protein (APP) and BACE1 (β-site APP cleaving enzyme I) proteins. This increase is not due to transcriptional up-regulation of these genes, but rather to a stabilization of their mRNAs. We identified the RNA binding protein Ras-Gap-SH3 Binding Protein 1 (G3BP1) as a factor that binds and destabilizes

APP and BACE1 mRNA in proliferative astrocytes, keeping their levels low. The expression of G3BP1 protein decreases during the senescence of astrocytes, explaining the increased stability of the APP and BACE1 mRNA. Collectively, our findings describe a novel mechanism that link cellular aging to AD through the G3BP1-mediated upregulation of Aβ biogenesis. Our results provide evidence supporting that clearance of brain senescent cells might be a therapeutic avenue for AD prevention.

31 Role of the exoribonucleases Pacman and Dis3L2 on miRNA and lncRNA stability in the context of tissue growth in Drosophila melanogaster

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Ribonucleases are critically important in many cellular and developmental processes with defects in their expression being associated with human disease. Pacman/XRN1 is a conserved cytoplasmic exoribonuclease which degrades RNAs in a 5' - 3' direction after they have been decapped. The cytoplasmic 3'-5' exoribonuclease Dis3L2 is also highly conserved and has a preference for uridylated RNAs. In Drosophila melanogaster, null mutations in pacman cause an increase in apoptosis and smaller wing imaginal discs whereas null mutations in dis3L2 result in larger wings and wing imaginal discs. To understand the role of Pacman and Dis3L2 in the degradation of microRNAs and lncRNAs in the context of cell apoptosis as well as tissue growth, we have used RNA-seq on dissected Drosophila imaginal discs to identify transcripts which change in levels upon Pacman or Dis3L2 deletion. Our results show that the pacman null mutation affects the levels of more mature miRNAs than the dis3L2 null mutation, suggesting that Pacman plays a key role in regulating miRNA levels in this tissue. Our work also provides insights into the regulation of the conserved microRNA, let-7, which is harboured within the lncRNA let-7-C. In pacman mutants, let-7-C and the three highly conserved miRNAs contained within it, decrease in levels, presumably due to an indirect effect. Downregulation of let-7-C has previously been shown to have severe effects on metamorphosis and developmental transitions, which is consistent with the phenotypes we see in pacman mutants. We have integrated our results with our previous data to generate a model to understand the role of Pacman in apoptosis. We are also exploring the regulation of long-non-coding RNAs by Pacman and Dis3L2. As in human cells, long-non-coding RNAs appear to be more sensitive to Pacman (XRN1) than Dis3L2. We have also identified a potential role for translation in regulating lncRNA decay.

32 Deadenylase-dependent mRNA decay of hepatokines controls food intake and energy expenditure

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mRNA turnover is controlled by mRNA synthesis and degradation. The internal and external stimuli alter mRNA expression. The CCR4-NOT complex is one of the major deadenylase machinery and plays an important role in the rate-limiting step of mRNA decay. Organokines, such as hepatokines from the liver, indicate the group of secretory proteins that mediate inter-organelle crosstalk and maintain whole-body metabolism, such as food intake and energy expenditure. The imbalance of inter-organelle communication by dysregulation of organokines leads to metabolic disorders that increase the risk of type 2 diabetes and atherosclerosis. Although transcriptional regulation to induce organokines has been well-studied, the mechanisms underlying how organokine expression is switched off by mRNA decay are largely unknown. We demonstrated that hepatokines are post-transcriptionally turned off by CNOT6L deadenylase in the CCR4-NOT complex to maintain metabolic balance (Katsumura et al., Cell Metab, 2022). To identify the switched-off hepatokines by CNOT6L, we performed RNA-immunoprecipitation transcriptomics. The genome-wide screening of the CNOT6L targets discovered the mRNAs encoding GDF15 and FGF21, which are attractive as anti-obese targets. To determine the molecular mechanism, we identified an inhibitor of CNOT6L deadenylase by in silico and high-throughput screenings. The inhibitor administration in mice increases hepatic Gdf15 and Fgf21 mRNAs by stabilizing these mRNAs, leading to increasing the corresponding serum protein levels. The increases of GDF15 and FGF21 dramatically suppress food intake through the hindbrain and stimulate

energy expenditure and fat utilization via communication with white and brown adipose tissues and the hypothalamus. The CNOT6L inhibition further shows the therapeutic potential for the treatment of metabolic disorders by ameliorating hyperglycemia, hyperlipidemia, and insulin resistance in mice. To identify more specific inhibitors against CNOT6L, further drug screening and the modification of the compounds are underway. Our discovery will lay the foundation for an unprecedented strategy to treat metabolic disorders.

33 Mechanism of viral mRNA restriction mediated by the ZAP-KHNYN complex

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The zinc finger antiviral protein (ZAP) restricts viral replication by targeting viral RNA for degradation and/or inhibiting its translation. ZAP directly binds CpG dinucleotides in RNA and restricts HIV-1 containing a ZAP-response element enriched in CpGs (HIV-CpG) by promoting RNA decay. We previously identified KHNYN as a ZAP-interacting protein through a yeast two-hybrid screen. Overexpression of KHNYN inhibits HIV-CpG, while its depletion abolishes ZAP-mediated restriction. KHNYN depletion does not affect ZAP binding to HIV-CpG RNA and we hypothesise that ZAP recruits KHNYN to viral RNA to mediate RNA decay. KHNYN contains a putative di-KH domain, a PIN endoribonuclease domain and a CUBAN domain that binds ubiquitin-like proteins. We have characterized the role of each domain for its contribution to antiviral activity. First, we mutated potential catalytic residues in the PIN domain and showed that they are required for KHNYN to restrict HIV-CpG. Second, we showed that the CUBAN domain is required for full KHNYN antiviral activity. This domain regulates KHNYN homeostatic turnover, likely through its interaction with NEDD8, and also contains a nuclear export signal required for KHNYN cytoplasmic localization and interaction with ZAP. Third, we showed that deleting the di-KH domain reduces KHNYN antiviral activity and solved its crystal structure. While KH domains often bind RNA, KH1 of KHNYN lacks the GxxG motif, usually required for RNA binding and mutation of the GxxG motif in KH2 does not inhibit antiviral activity. Further, the di-KH domain displays no in vitro RNA-binding and we propose that it required as a protein interaction domain. We are now investigating proteins that interact with the di-KH domain and their role in HIV-CpG RNA degradation. Taken together, our work provides new insights into the mechanism of ZAP-KHNYN-mediated viral RNA decay.

34 Activation of antiviral RNAi in mammals

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RNA interference (RNAi) mediates sequence-specific degradation of cognate RNAs. For that, long double-stranded RNAs (dsRNAs) are processed by Dicer into small interfering RNAs (siRNAs) that guide cleavage of perfect complementary targets. Besides regulating gene expression and suppressing mobile elements, RNAi is an ancestral antiviral response in plants and invertebrates. However, the relevance of antiviral RNAi in mammals remains controversial because: i) mammals have evolved an Interferon-based innate immunity; and ii) mammalian Dicer is structurally adapted to process microRNA precursors, but not long dsRNAs. Here, we show that enhancing Dicer activity is sufficient for activation of antiviral RNAi in mammals. We found that expression of DicerΔHEL1, known to facilitate dsRNA processing, increases viral siRNA production and confers antiviral protection in cells. For testing a similar principle in vivo, we developed a mouse model with transgenic expression of DicerΔHEL1 from the Rosa26 locus. Remarkably, DicerΔHEL1 led to activation of antiviral RNAi in vivo, protecting animals during Lymphocytic Choriomeningitis Virus (LCMV) infection. We present a proof of concept for functional in vivo antiviral RNAi in adult mammals and show the key limiting factor is having sufficient Dicer activity. The activation of antiviral RNAi provides an additional layer of immunity in mammals.

35 P-bodies in plant reproduction and stress response

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Gene expression is a multilayer process that can be regulated at the level of RNA production, processing, transport, translation and RNA stability. Many of these processes are associated with membraneless organelles, dynamic structures formed by multivalent interactions of proteins and nucleic acids that may exhibit properties of liquid-liquid phase condensates. In my presentation I will explore the role of P-bodies, cytoplasmic biocondensates sequestering translationally repressed mRNA and RNA decay proteins, in plant germline differentiation and stress response. I will show how dynamic behavior of P-bodies contributes to regulation of meiosis and discuss a broader function of these structures in other plant physiological processes.

36 How mRNA vaccines hijack TENT5 cytoplasmic poly(A) polymerases to enhance stability

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Though mRNA vaccines against COVID-19 have revolutionized vaccinology and have been administered in billions of doses, we know incredibly little about how mRNA vaccines are metabolized in vivo. Here we implemented enhanced nanopore Direct RNA sequencing (eDRS), to enable the analysis of single Moderna's mRNA-1273 molecules, giving in vivo information about the sequence and poly(A) tails.

We show that mRNA-1273, with all uridines replaced by N1-methylpseudouridine (mΨ), is terminated by a long poly(A) tail (~100 nucleotides) followed by an mΨCmΨAG sequence. In model cell lines, mRNA-1273 is swiftly degraded in a process initiated by the removal of mΨCmΨAG, followed by CCR4-NOT-mediated deadenylation. However, when injected intramuscularly, complex modifications occur. Notably, mRNA-1273 is re-adenylated after mΨCmΨAG removal.

In macrophages, which are the primary target of the vaccine, mRNA-1273

is very efficiently re-adenylated, and poly(A) tails can extend up to 200 nucleotides. We further show that TENT5 poly(A) polymerases mediate the enhancement of mRNA stability in macrophages. Without TENT5s antigen production decreases, and specific immunoglobulin production is compromised following mRNA vaccination, but not with protein-based vaccination.

Together, our findings provide an unexpected principle for the high efficacy of mRNA vaccines and open new possibilities for their improvement. They also emphasize that, in addition to targeting a protein of interest, the design of mRNA therapeutics should be customized to its cellular destination.

37 Friends or Foes? The Many Routes Caliciviruses Use to Manipulate RNA Granules

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To rapidly adapt to stresses such as viral infections, cells have evolved several mechanisms, which include the activation of stress response pathways and the innate immune response. These stress responses result in the rapid inhibition of translation and condensation of stalled mRNAs with RNA-binding proteins and signalling components into cytoplasmic biocondensates called stress granules (SGs). Increasing evidence suggests that SGs contribute to antiviral defence and thus viruses need to evade these threatening responses to propagate.
Caliciviruses are responsible for gastroenteritis outbreaks worldwide. We have examined how different caliciviruses interacts with stress pathways. We show that human norovirus infection represses host cell translation and induces a metabolic stress that is uncoupled from eIF2a signalling. Infection also results in a redistribution of the SG-scaffolding G3BP1 protein interactome, to prevent SG assembly. In contrast, feline calicivirus impairs SGs assembly by cleaving the scaffolding protein G3BP1. We also observed that uninfected bystander cells assembled G3BP1-positive granules, suggesting a paracrine response triggered by infection. We show that paracrine signalling from infected cells induces the formation of SG-like foci, that we named paracrine granules. They are linked to antiviral activity and exhibit specific kinetics of assembly-disassembly, and protein and RNA composition that are different from canonical SGs. We propose that this paracrine induction reflects a novel cellular defence mechanism to limit viral propagation and promote stress responses in bystander cells.
Overall, these results also illustrate novel and different strategies that related viruses have developed to avoid and manipulate the host stress response, promoting efficient replication.

38 Codon-dependent regulation of gene expression during dengue virus infection

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Control of translation is crucial during virus-host interaction because viral mRNA translation is strictly dependent on the host cell machinery. Recent work indicates that codon composition can have a great impact on mRNA stability and translation efficiency, through a phenomenon known as codon optimality. 'Optimal' codons lead to increased mRNA and protein levels, while 'non-optimal' codons have the opposite effect. However, whether this codon-mediated regulation, different from codon usage bias, is exploited during viral infections remains unknown. We focused our work on dengue infection, the most prevalent mosquito-borne viral disease in the world. To study whether dengue virus uses optimal or non-optimal codons relative to its hosts, we first defined the codon optimality code in mosquito cells through blocking transcription and studying the correlation between stability and codon composition of endogenous genes. Surprisingly, analysis of codon choice in thousands of worldwide isolated dengue genomes revealed that dengue uses non-optimal codons more frequently than most human and mosquito genes, suggesting the existence of an evolutionary pressure to select non-optimal codons. This preference for non-optimal codons is conserved in over 300 human viruses, including Zika and SARS-CoV-2. Thus, we hypothesized that dengue infection affects host gene expression in a codon-dependent manner. RNA-seq analysis indicated that human genes up-regulated during infection are enriched in codons preferred by dengue. Strikingly, relative quantification of tRNA abundance using qPCR revealed that tRNAs decoding non-optimal Arginine codons are up-regulated during dengue infection in human cells, suggesting that changes in the tRNA pool might remodel the host transcriptome in a codon-biased manner during infection. Interestingly, analysis of the fitness effect of mutations in the dengue genome revealed that mutations toward dengue's preferred codons tend to be beneficial. Altogether, our findings uncover a novel mechanism underlying virus-host adaptation and underscore codon optimality as a driving force of virus evolution.

39 SARS-CoV-2 protein NSP2 impairs interferon production and enhances microRNA-mediated translational repression

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Viruses utilise microRNAs (miRNAs) to impair the host antiviral immune system and facilitate viral infection by expressing their own miRNAs or co-opting cellular miRNAs. miRNAs inhibit translation initiation of their target mRNAs by recruiting the GIGYF2/4EHP translation repressor complex to the mRNA 5′ cap structure. We previously reported the 4EHP-mediated, miR-34a–directed translational repression of Ifnb1 mRNA and showed that this mechanism limits IFN-β production upon vesicular stomatitis virus infection, likely to prohibit prolonged inflammatory responses.
Here we document a mechanism by which the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) encoded non-structural protein 2 (NSP2) protein impedes IFN-β expression through translational repression of Ifnb1 mRNA by coopting the GIGYF2/4EHP complex, leading to evasion of a cellular innate immune response and enhanced viral replication. Furthermore, we demonstrate the pervasive augmentation of the miRNA-mediated translational repression of cellular mRNAs by NSP2. We show that NSP2 interacts with Argonaute 2, the core component of the miRNA-Induced Silencing Complex (miRISC) and enhances the translational repression mediated by natural miRNA binding sites in the 3′ UTR of cellular mRNAs. Our data reveal an additional layer of the complex mechanism by which SARS-CoV-2 and likely other coronaviruses manipulate the host gene expression program through co-opting the host miRNA-mediated silencing machinery.

40 The transcriptional and translational landscape of coronavirus OC43 infection

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The present COVID-19 pandemic provides a grim illustration of the threat posed by novel coronaviruses. The relatively understudied coronavirus OC43 circulates continuously in the human population and is a frequent cause of the common cold. We generated a high-resolution atlas of the transcriptional and translational landscape of OC43 infection at various timepoints following infection of human lung fibroblasts. We show that the OC43 transcriptome consists of a full-length genomic RNA and seven major subgenomic RNAs generated through discontinuous transcription events. Using ribosome profiling, we quantified the relative expression of the canonical open reading frames (ORFs) and identified several unannotated ORFs, including three short upstream ORFs with a potential regulatory function, and two candidate ORFs nested inside larger genes. In parallel, we analyzed the cellular response to infection. Endoplasmic reticulum (ER) stress response genes are transcriptionally and translationally induced beginning ~12 hours post infection and comprise the majority of differentially expressed genes. A smaller number of cellular genes are regulated predominantly at the translational level. The most prominent example is EIF4A2, encoding a rate-limiting factor involved in translation initiation. Notably, infected cells fail to induce interferon mRNAs, suggesting that OC43, like SARS-CoV-2, efficiently antagonizes cellular antiviral signaling. Taken together, our work provides a genomic resource for further study of OC43 and the cellular response to infection.

41 Translation reprograming during viral infection

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The formation of RNP granules and the control of RNA levels by RNA degradation can affect the outcome and process of viral infection. One area of research is to study the function of poly(A) specific deadenylases such as Usb1, PARN, and TOE1, which remove short oligo(A) tails added by non-cannonical poly(A) polymerases, which can otherwise promote 3' to 5' exonuclease decay. We have focused on mutations in the 3' to 5' RNA exonuclease USB1 known to cause hematopoietic failure in poikiloderma with neutropenia (PN). While USB1 is known to regulate U6 snRNA maturation, the molecular mechanism of PN remains unknown, as pre-mRNA splicing is unaffected in patients. We generated human embryonic stem cells harboring the PN-associated mutation c.531_delA in USB1 and show that this mutation impairs human hematopoiesis. Dysregulated miRNA levels in USB1 mutants contribute to hematopoietic failure, due to a failure to remove 3' end adenylated tails added by PAPD5/7. Modulation of miRNA 3' end adenylation through genetic or chemical inhibition of PAPD5/7 rescues hematopoiesis in USB1 mutants. This work shows that USB1 can act as a miRNA deadenylase and suggests PAPD5/7 inhibition as a potential therapy for PN.
Additional experiments examine how RNP granules such as stress granules, dsRNA induced foci (DRIFs) and RNaseL Bodies (RLBs) affect both the innate immune response and the outcome of viral infections.

List of Posters

Posters

P01 Glycolysis is enhanced under chronic hypoxia via RNA stabilization

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It has been well studied that the altered gene expression under hypoxia is mainly regulated by HIF-1, a hypoxia specific transcription factor. However, recent researches propose that HIF-1 independent gene regulation plays an important role in adaptation of cells against hypoxia, especially chronic hypoxia. Gene expression is decided by the balance between transcription and RNA degradation. To approach the effect of RNA degradation on adaptation of cells to chronic hypoxia, we determined transcription rates and RNA degradation rates comprehensively in HCT116 cells. Moreover, we quantified the contribution of degradation and transcription to differential gene expression based on these rates. As a result, we identified 1,164 differentially expressed RNAs under chronic hypoxia, and 102 mRNAs of which mainly the degradation contributes to differential expression. GO term analysis revealed that mRNAs encoding the glycolytic enzymes were significantly enriched in the 102 mRNAs. It is well known that glycolysis is enhanced in response to hypoxic stress for anaerobic respiration. Here, we hypothesize that chronic hypoxia stabilizes mRNAs encoding enzymes involved in glycolysis, evoking enhanced glycolysis pathway.

P02 Molecular study of the role of the SKI complex during Sindbis virus infection

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RNA decay pathways regulate transcript abundance and ensure quality control. Deadenylation of mRNAs is either followed by recruitment of the decapping complex leading to exonucleolytic degradation by Xrn1 in a 5'-3' direction or alternatively, to degradation by the exosome complex in a 3'-5' direction. The SKI complex acts as a co-factor to regulate specific degradation mediated by the exosome. Mutations of some genes encoding human SKI complex subunits are associated with trichohepatoenteric congenital syndrome and recent studies suggest a potential role for the SKI complex in viral infection, but to date, no molecular mechanism has been elucidated. To investigate the role of the SKI complex during viral infections, we used the single-stranded positive RNA Sindbis virus (SINV) as a model.
We generated HEK293 knockout (KO) cell lines lacking SKIV2L or SKIC3 proteins, two of the three subunits of the SKI complex to determine the importance of these factors during infection. Infection was monitored by flow cytometry and titration. At the early and late stages of the infection, both SKIV2L and SKIC3 KO-lines showed a significantly reduced viral multiplication. Similar results were obtained in cells infected with another alphavirus, the Semliki Forest virus, but not in cells infected with the vesicular stomatitis virus (from the rhabdovirus family), suggesting that the complex is specifically required for certain viruses. The proviral activity of the SKI complex was further confirmed by complementation of the KO cells. Proteomic analysis by mass spectrometry to determine the interactome of a tagged version of SKIV2L expressed in mock or SINV-infected cells is currently underway. These should facilitate an understanding of the molecular mechanisms played by the SKI complex during an alphavirus infection.

P03 Cytoplasmic polyadenylation by Tent5a is essential for teeth formation

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Tooth enamel formation, known as amelogenesis, is a complex process orchestrated by specialized cells called ameloblasts. These cells secrete enamel matrix proteins (EMPs), including Amelogenin (Amelx) and Ameloblastin (Ambn), which play a crucial role in depositing hydroxyapatite crystals for mineralization. In this study, we utilized a mouse model with a Tent5a knock-out (KO) show that Tent5a non-canonical poly-A polymerase is crucial for EMPs synthesis, secretion and enamel mineralization. Tent5a belongs to the Terminal nucleotidyl transferases (TENTs) superfamily and is responsible for protecting mRNA from degradation, ensuring stability, and promoting mRNA translation.

Our findings using micro-computed tomography revealed that mice lacking Tent5a exhibited teeth hypomineralization, thinner enamel layers, and disrupted enamel patterning. Through nanopore direct mRNA sequencing, we identified that Tent5a polyadenylates Amelx and other mRNAs encoding secreted proteins, thereby increasing their expression during amelogenesis. Tent5a is predominantly localized in the cytoplasm and the endoplasmic reticulum, where it regulates the synthesis of Amelx. Moreover, our results demonstrated that the self-assembly of Amelx in the extracellular organic matrix was impaired in Tent5a KO mice, which is essential for directing hydroxyapatite deposition during enamel formation.

In conclusion, this study establishes the critical role of Tent5a-mediated cytoplasmic polyadenylation in the biomineralization of teeth. Our findings enhance our understanding of the new posttranscriptional regulation mechanisms underlying tooth formation.

P04 A scalable method to map the protein interactomes of individual RNAs by quantitative mass spectrometry

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RNA-binding proteins play a crucial role in determining the fate of RNAs, making the study of RNA-protein interactions important for understanding gene regulation and the function of non-coding RNAs. To obtain a quantitative and unbiased understanding of these interactions in live cells, hybridization-capture approaches in combination with UV or chemical crosslinking procedures are frequently employed. However, current methods require large amounts of biological material and costly reagents, limiting their scalability and applicability.

To overcome these limitations, we developed a new approach based on the purification of UV-crosslinked RNA-protein complexes using phenol-chloroform extractions and a sequence-specific hybridization strategy. Our method requires 10- to 20-fold less input material compared to state-of-the-art methods such as RNA antisense purification coupled with mass spectrometry (RAP-MS), allowing for higher scalability and reducing costs. In a proof-of-principle experiments we targeted two non-coding RNAs with well-characterized core interaction partners: the U1 snRNA, a core component of the spliceosome, and the transcriptional regulator RNA 7SK. Compared to RAP-MS, our new method achieved near comprehensive capture of all known U1 and 7SK core proteins from 20-fold less input material, while virtually no background proteins were detected. We are currently exploring the suitability of this technique to target defined sequence regions within larger RNAs to enable region-resolved capture of RNA-protein interactions from endogenous transcripts without the need for genetic manipulation. This work has the potential to make RNA interactome studies more scalable and enable the systematic dissection of regulatory RNA-protein complexes.

P05 N6-methyladenosine modification is not a general trait of viral RNA genomes

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N6-methyladenosine (m6A), the most common internal RNA modification in eukaryotic mRNAs, is described to be abundantly present in the genomes of cytoplasmic-replicating RNA viruses. Yet, how the host nuclear m6A writer has access to the viral RNAs in the cytoplasm and what are the associated biological consequences remain fundamental questions.
Here, we comprehensively combine antibody-dependent (m6A-seq) with antibody-independent SELECT and nanopore direct sequencing on the cytoplasmic-replicating Chikungunya virus (CHIKV) RNA, and found no evidence of m6A modifications. In addition, neither depletion of components of the m6A machinery affected CHIKV infection, nor CHIKV infection altered their cellular location. Consistently, no significant m6A modification levels were found in the RNA genome of dengue virus (DENV), another cytoplasmic-replicating virus. Our results challenge the idea that m6A modification is a general trait of cytoplasmic-replicating RNA viruses and highlight the need of confirming antibody-dependent detection of m6A modifications with orthogonal antibody-independent methods.

P06 Identification of Myoferlin as a novel cofactor in viral RNP trafficking

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Influenza A virus (IAV) infections are a major global health and economic burden, while also posing a significant pandemic threat. Thus, elucidating the molecular determinants that underlie Influenza infection is essential to better understand the disease and its spread, and develop new antivirals. IAV is a segmented, negative-sense RNA virus, and research is underway to determine how the eight separate RNA genome segments are packaged together after replication and assembled into new virions. Genome copies are generated by the Influenza RNA-dependent RNA-polymerase (FluPol) in the nucleus. Upon transcription, single segments are immediately encapsidated with the FluPol and nucleoprotein, forming the vRNP. To investigate vRNP trafficking, we generated a FluPol-FLAG IAV, that we employed to immunoprecipitate and characterise the late-infection vRNP interactome. This analysis produced a robust dataset comprising known FluPol-interacting complexes and novel targets. Knockdown of most of the hits resulted in severely diminished IAV gene expression and replication. Among the targets, Myoferlin is a transmembrane protein with potential implications in vRNP trafficking. Myoferlin is involved in various cellular processes, including membrane fusion, endocytosis, vesicle trafficking, and endosomal recycling of receptors. Knockdown of Myoferlin significantly affected viral transcription, and the replication of different IAV strains. Moreover, the interaction of FluPol and MYOF was confirmed by co-immunoprecipitation. Using a combination of immunofluorescence and single-molecule FISH we found that Myoferlin colocalized extensively with vRNPs during late infection, forming perinuclear accumulations at the MTOC. There, vRNPs are known to interact with the Rab11 endocytic recycling compartment for bundling and trafficking to the plasma membrane. Recent evidence from other labs also points towards a role of MYOF in this compartment. These findings suggest a role for Myoferlin in orchestrating the transport of vRNPs across the cell, from uptake to release.

P07 ZAP subcellular localisation regulates its antiviral activity

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The zinc finger antiviral protein (ZAP) is the central player in an antiviral system that restricts a broad range of viruses including retroviruses, SARS-CoV-2, Ebola virus and human cytomegalovirus by targeting viral RNA for degradation or inhibiting translation. It has multiple isoforms with the large (ZAP-L) and short (ZAP-S) being the best characterised. While both isoforms have a N-terminal domain that binds CpG dinucleotides in viral RNA, ZAP-L differs from ZAP-S by the presence of an ART-like domain and a S-farnesylation motif at its C-terminus.
Using isoform-specific ZAP knock-out cells, we show that ZAP-L is primarily responsible for the restriction of HIV-1 containing a CpG-rich ZAP response element (HIV-CpG) with ZAP-S having minimal activity. Consistent with this, ectopic expression of ZAP-L but not ZAP-S in ZAP knockout cells restricts HIV-CpG. TRIM25 and the putative endoribonuclease KHNYN are essential for ZAP-mediated restriction of HIV-CpG. We found that while TRIM25 co-immunoprecipitated with both isoforms, KHNYN preferentially co-immunoprecipitated with ZAP-L. ZAP-L antiviral activity correlates with its intracellular localisation since mutation of the S-farnesylation motif eliminated its localisation to the endomembrane system and its ability to restrict HIV-CpG. Conversely, introducing the S-farnesylation motif into ZAP-S rendered it partly antiviral. Polysome fractionation in uninfected cells showed that ZAP-L co-fractionated mainly with free mRNPs. ZAP may not require active translation of the viral RNA to target it for degradation since we found that UPF1 is not required for antiviral activity. Translationally inactive mRNAs can be sequestered in cytoplasmic membraneless organelles such as stress granules and P bodies. Interestingly, our preliminary data point towards a role for several stress granules components in the ZAP antiviral pathway. We are currently investigating whether these individual proteins are ZAP co-factors or whether stress granule compartments are required for ZAP-mediated restriction of HIV-CpG.

P08 SMG7 is a master regulator of P-bodies modulating their function and dynamics

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Biological condensates provide the cell with a dynamic tool to compartmentalize biomolecules and activities. Among them, P-bodies (PBs) constitute the main hub for translationally arrested mRNAs and proteins involved in RNA quality control and decay.
Here we present SMG7 as a master regulator of PBs. SMG7 is known for its role in nonsense mediated RNA decay (NMD) where it recruits phosphorylated UPF1 bound to mRNAs to be degraded to PBs.
In our recent work, we demonstrate that SMG7 plays a role beyond NMD. We show that at the transition from meiosis to mitosis, SMG7 bestows PBs with the capacity to inhibit protein translation facilitating cell fate reprograming (Cairo et al., 2022 Science). In this process, SMG7 recruits the meiotic specific protein TDM1, which sequesters the translation initiation complex eIF4F into PBs. Interestingly, the eIF4F subunit eIF4A, which acts as a helicase in the translation initiation, is also recruited to PBs directly through SMG7 interaction. Our data indicate that eIF4A shapes the size and dynamics of PBs by modulating the RNA landscape of the biocondensate. Without eIF4A activity, PBs become less dynamic and bigger, which is reflected in the plant development and reproduction.
These results reveal the functional plasticity of PBs, positioning SMG7 as a central component which mediates recruitment and interaction with additional factors that affect their structure and functionality.

This work is supported by the Czech Science Foundation (project 23-07969X).

P09 Codon-dependent regulation of gene expression during dengue virus infection

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Control of translation is crucial during virus-host interaction because viral mRNA translation is strictly dependent on the host cell machinery. Recent work indicates that codon composition can have a great impact on mRNA stability and translation efficiency, through a phenomenon known as codon optimality. 'Optimal' codons lead to increased mRNA and protein levels, while 'non-optimal' codons have the opposite effect. However, whether this codon-mediated regulation, different from codon usage bias, is exploited during viral infections remains unknown. We focused our work on dengue infection, the most prevalent mosquito-borne viral disease in the world. To study whether dengue virus uses optimal or non-optimal codons relative to its hosts, we first defined the codon optimality code in mosquito cells through blocking transcription and studying the correlation between stability and codon composition of endogenous genes. Surprisingly, analysis of codon choice in thousands of worldwide isolated dengue genomes revealed that dengue uses non-optimal codons more frequently than most human and mosquito genes, suggesting the existence of an evolutionary pressure to select non-optimal codons. This preference for non-optimal codons is conserved in over 300 human viruses, including Zika and SARS-CoV-2. Thus, we hypothesized that dengue infection affects host gene expression in a codon-dependent manner. RNA-seq analysis indicated that human genes up-regulated during infection are enriched in codons preferred by dengue. Strikingly, relative quantification of tRNA abundance using qPCR revealed that tRNAs decoding non-optimal Arginine codons are up-regulated during dengue infection in human cells, suggesting that changes in the tRNA pool might remodel the host transcriptome in a codon-biased manner during infection. Interestingly, analysis of the fitness effect of mutations in the dengue genome revealed that mutations toward dengue's preferred codons tend to be beneficial. Altogether, our findings uncover a novel mechanism underlying virus-host adaptation and underscore codon optimality as a driving force of virus evolution.

P10 Mechanisms of αSynuclein-mediated coordination of RNA metabolism

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Parkinson's disease (PD) is a severe neurodegenerative disorder. The disease exhibits neuroinflammatory phenotype and is linked to the aggregation of a small amyloid protein, alpha-synuclein (aSyn), which is implicated in synaptic vesicle trafficking and neurotransmitter release, with an as yet undefined role. In PD, aSyn is found in brain inclusions in neurites and in Lewy bodies. A well characterized mutation of aSyn (G209A), encodes for A53TαSyn protein that exhibits faster aggregation kinetics and is directly linked to the familiar type of PD. In this study, we use a toolkit of neuronal cell line with stable expression of A53TαSyn, primary hippocampal neurons from transgenic A53T mice and patient derived hiPSC-neurons. Our transciptomics and proteomics analysis of hiPSC derived neurons revealed altered expression levels of core molecules involved in RNA metabolism linked to A53T mutation. Combining "-omics" approaches with high end microscopy and single molecule RNA FISH, we aim in investigating how the expression of A53TαSyn affects RNA dynamics in neurons. Initial data bridge aSyn biology to RNA granule organization and imbalanced metabolism of RNA machinery triggered by the presence of A53T aSyn in cellular models of PD.

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P11 Modelling cellular lifecycle of +RNA viruses suggests strategies for limiting productive cellular infection

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Positive-strand RNA (+RNA) viruses have been responsible for many emerging and re-emerging infectious diseases, like the current SARS-COVID19, Zika, Hepatitis C and Poliomyelitis. Despite differences in the strategies they employ, life cycle processes of +RNA viruses inside a host cell are broadly conserved. For example, +RNA viruses induce alteration to intracellular membranes of the host cell to form compartments conducive for viral replication. A quantitative and unified analysis of the lifecycle of +RNA viruses can, thus, highlight bottlenecks common to them.
In this study, we develop a generalized dynamical model to monitor cellular levels of viral molecules. We computationally explore the parameter space and estimate lifecycle determinants for several +RNA viruses. We find that replication and virus assembly associated to poliovirus is very fast, whereas the Japanese encephalitis virus has a higher rate of translation and efficient cellular reorganization compared to the hepatitis C virus. For a particular virus, the model also parses the effects of viral mutations, drugs and differences in host cell permissivity, on viral lifecycle dynamics.
Stochastic simulations of the model demonstrate infection extinction if all the viral genome (+RNA) entering a cell, degrade before establishing a robust replication machinery. We evaluate how the probability of establishment of productive infection, or 'cellular infectivity', is affected by virus–host processes and viral seeding. For example, an increase in cytoplasmic RNA degradation and delay in intracellular rearrangements (facilitating viral replication) reduces cellular infectivity, more so when combined. Synergy among these parameters in limiting +RNA virus infection as predicted by our model suggests new avenues for inhibiting infections by targeting the early lifecycle bottlenecks.

P12 PCID2 regulates HIV-1 transcription and viral RNA processing during latency

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HIV-1 latency is tightly enforced by distinct blocks to gene expression at multiple transcriptional and post-transcriptional levels. Recent reports have showed that blocks to HIV-1 gene expression in CD4+ T cells obtained from people living with HIV-1 occur mainly at post-transcriptional levels. Still, the distinct mechanisms that regulate HIV-1 viral RNA (vRNA) processing and viral protein production during latency are yet largely unknown. An LTR locus-specific chromatin immunoprecipitation coupled with mass spectrometry identified PCID2, a member of the transcription and export complex 2 (TREX2) as a putative LTR-bound regulator of HIV-1 latency. In this study, we further characterize PCID2 as a novel regulator of HIV-1 gene expression during latency. First, we confirm that PCID2 is bound to the latent HIV-1 LTR and is removed upon transcriptional activation. In our system, PCID2 acts as a repressor of HIV-1 gene expression that acts both at transcription initiation and post-transcriptional steps of latency regulation. Specifically, we found that absence of PCID2 leads to overall de-repression of the HIV-1 LTR locus by deposition of activation histone marks and recruitment of transcription factors. At a post-transcriptional level, we show by immunoprecipitation coupled with mass spectrometry that PCID2 interacts with members of the spliceosome complex and its absence alters the ratios of HIV-1 vRNA splicing variants leading to increased abundance of completely spliced vRNAs, suggesting that PCID2 has a role in inhibiting vRNA splicing. In summary, we describe and characterize PCID2 as a previously unidentified factor involved in HIV-1 latency regulation that has a dual role in blocking HIV-1 gene expression by acting on transcription initiation and viral RNA processing.

P13 Structural basis for the cleavage of human tRNA methyltransferase TRMT1 and downregulation of m2,2G tRNA modification during SARS-CoV-2 infection

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Like many viruses, SARS-CoV-2 employs diverse strategies to manipulate host biochemical pathways and downregulate host translation. Here we show that the human tRNA methyltransferase TRMT1, which installs tRNA m2,2G modifications important for global cellular protein synthesis, can be recognized and cleaved by the SARS-CoV-2 main protease (Mpro). Mpro-mediated TRMT1 cleavage results in removal of the TRMT1 C-terminal zinc finger domain, which is required for tRNA binding and efficient m2,2G modification in cells. Our kinetic and evolutionary analyses show that the TRMT1 cleavage sequence is proteolyzed with comparable efficiencies to known viral polypeptide cleavage sites and is highly conserved in mammals, with the exception of some rodents, where a single substitution may confer resistance to Mpro-directed proteolysis and loss of m2,2G modification. We determined the co-crystal structure of the human TRMT1 cleavage sequence in complex with Mpro that shows how TRMT1 engages the viral protease active site in an uncommon binding conformation. Together, our results show at high resolution how SARS-CoV-2 Mpro can recognize and cleave human TRMT1 to impair cellular tRNA modification during viral infection. This raises the possibility that dysregulation of m2,2G tRNA modification during SARS-CoV-2 infection may contribute to disruption of host translation, RNA stability, and viral pathogenesis or phenotypes.

P14 Non-coding flavivirus RNA as key target to engineer live-attenuated vaccines that are 'safe-by-design'

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Viruses in the Flavivirus genus, such as dengue, Zika, West Nile (WNV) and tick-borne encephalitis (TBEV) , can cause severe illness in humans. Due to their extensive spread in recent years, they pose a significant public health threat. In particular, WNV and TBEV are on the rise in Europe. For few flaviviruses effective vaccines are available. We have identified a key target that enables the 'safe-by-design' engineering of novel flavivirus vaccines. Research has demonstrated that all flaviviruses produce abundant quantities of small non-coding RNA required for induction of cytopathic effect in cells and for viral pathogenicity in vivo. This so-called subgenomic flavivirus RNA (sfRNA), is the product of incomplete viral genomic RNA degradation by the cellular exoribonuclease XRN1 that stalls at conserved 3D RNA structures in the 3' untranslated region (UTR). Despite the increasing global risk of flavivirus infections, for many flaviviruses no safe and effective vaccines have been developed. Therefore, this research aims to engineer live-attenuated flavivirus vaccines based on the elimination of sfRNA production. During this workshop I would like to present a strategy for generation of WNV and TBEV vaccine candidates by construction of sfRNA-deficient mutant viruses. This strategy includes mutagenesis of defined three-dimensional RNA structures within the 3'UTR that are essential for sfRNA biogenesis. An innovative deep mutational scanning approach will be applied to generate a library of mutants, which will be phenotypically selected in a high-throughput manner. Selection is based on the ability of the mutant viruses to efficiently replicate yet fail to induce CPE in cell culture.

P15 Structural and functional characterization of the human exoribonuclease DIS3L2 in concert with its recruiting RNA binding protein complex

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Regulation of gene expression by microRNAs (miRNAs) is an important process in humans. In this pathway, a 20-24 nucleotides long miRNA guides an Argonaute protein to specific target mRNAs and with the contribution of additional factors, post-transcriptional gene silencing is initiated. The maturation of mature miRNAs from precursor molecules requires several steps and is tightly regulated itself. After transcription by RNA polymerase II, primary miRNA transcripts are cleaved by Drosha in the nucleus to generate pre-miRNAs that are subsequently exported to the cytoplasm for final processing by Dicer to yield mature miRNAs. The let-7 miRNA family plays an important role in development and cancer and the two processing steps are tightly regulated by the highly similar RNA binding proteins LIN28A and LIN28B. LIN28B binds pri-let-7 in the nucleus and sequesters it from processing by Drosha. LIN28A acts in the cytoplasm of stem cells and recruits TUT4/7, which oligo-uridylates pre-let-7 at its 3' end. This prevents cleavage by Dicer and makes the oligo-uridylated RNA an ideal substrate for the exoribonuclease DIS3L2, which subsequently degrades pre-let-7 miRNAs. DIS3L2 shows high similarities with the human exonucleases DIS3 and DIS3L, which function as catalytic subunits of the nuclear and cytoplasmic exosome complex. We plan to investigate whether a stable complex of the pre-let-7 miRNA, LIN28A (and potentially also LIN28B) and DIS3L2 can be formed in vitro, which would allow for structural studies. We could already confirm the interaction of DIS3L2, LIN28 and pre-let-7d in vivo and could show that this interaction is specific for DIS3L2 compared to the other DIS3 exoribonucleases. Currently, we are extending these findings towards a comprehensive functional and structural characterization of this complex.

P16 Investigating the effects of G-quadruplex stabilising ligand GQC-05 on alternative splicing of Mcl-1 pre-mRNA

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Mcl-1 regulate apoptosis. In cancer Mcl-1 overexpression is linked to a poor prognosis. The gene expresses two antagonistic isoforms by inclusion or exclusion of exon 2. The larger and generally more abundant isoform, Mcl-1L, inhibits apoptosis, whereas the smaller isoform, Mcl-1S, favours apoptosis. We recently found a compound called GQC-05 that shifting the splicing to pro-apoptotic isoform in cancer cells. GQC-05 is known to bind RNA G-quadruplexes (G4s), which are four-stranded structures formed by the interaction of such sequences. G4s may affect various RNA processing reactions, including splicing. The aim of this project is to determine the mechanism by which GQC-05 affect alternative splicing of Mcl-1.
RNase H cleavage assays were performed to detect the accessibility of RNA in splicing conditions. In pre-splicing complexes, GQC-05 increases the accessibility of the sequences on the downstream of 5'ss. In the splicing conditions of 7-deaza RNA, GQC-05 reduce the accessibility at the 3'ss and downstream of 5'ss. Moreover, primer extension assays showed existence of stable RNA structures on downstream of 5'ss. These results are consistent that GQC-05 affects splicing by binding to G4's.
In vitro splicing assays have shown that splicing to exon 2 depends on ESE's (exonic splicing enhancers) and it is inhibited by sequences just downstream of 5'ss. Sequences determine response to GQC-05, lying on exon 2 and upstream intron 2 of Mcl-1.
We have identified some critical groups in GQC-05 that are required to affect splicing. This will help us to design new and proved variants of drugs.

P17 Impact of mRNA turnover on Astrocyte involvement in neurodegenerative diseases

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With the rise of global life expectancy, age-related neurodegenerative diseases like Alzheimer's Disease (AD) increasingly contribute to limiting health and lifespan whilst burdening healthcare systems. Specifically, AD manifests as a progressive cognitive decline, leading to dementia and premature death. While familial AD (fAD) is caused by known mutations, the vast majority are sporadic, multifaceted, with few known genetic risk factors. Nonetheless most AD patients present the accumulation and aggregation of the Amyloid-β (Aβ) protein in their brains, yet it is not clear whether Aβ accumulation in neurons is the cause or consequence of AD. It is well-established that the disfunction of other types of brain cells, such as glia, specifically astrocytes, play an important role in the onset of neurodegenerative diseases like AD. Indeed, it has been shown that the induction of a senescence-like phenotype (a state of permanent cell-cycle arrest induced by DNA Damage Response (DDR) telomere attrition, irradiation, reactive oxygen species and oncogene activation) in astrocytes is associated with the onset of neurodegenerative diseases such as AD. However, whether and how senescence play a direct role in these conditions remains unknown. Unexpectedly, in this work we show that triggering senescence in primary human astrocytes causes them to accumulate Aβ aggregates. This AD-associated phenomenon is due to a significant increase in the expression levels of mRNA encoding Amyloid precursor protein (APP) and BACE1 (β-site APP cleaving enzyme I) proteins. This increase is not due to transcriptional up-regulation of these genes, but rather to a stabilization of their mRNAs. We identified the RNA binding protein Ras-Gap-SH3 Binding Protein 1 (G3BP1) as a factor that binds and destabilizes APP and BACE1 mRNA in proliferative astrocytes, keeping their levels low.

P18 Structural basis for exoribonuclease resistance in virus-associated RNA

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Viruses require a host cell to replicate, and they have consequently evolved mechanisms not only to evade, but also to co-opt, host defense machinery. One such mechanism is the use of highly structured RNA elements in the viral genome that resist degradation by the processive 5'-3' RNA exonuclease Xrn1. Known as Xrn1-resistant RNAs (xrRNAs), these stable sub-genomic degradation products are implicated in multiple roles during infection. xrRNAs were first identified in flaviviruses, then later in several plant-infecting viruses of the Luteoviridae and Tombusviridae families. Although the sequence and structure of the two xrRNA classes are highly divergent, both rely on a ring-like structure facing Xrn1 that mechanically confounds the exonuclease. The diversity of known xrRNA classes led us to hypothesize that other xrRNA classes might exist.
Recent studies have identified potential new xrRNAs of unknown structure, some of which exist in subviral, or virus-associated RNA elements. Such RNAs, known as Tombusvirus-like associated RNAs (tlaRNAs), encode their own polymerases, but they require co-infection with an associated plant virus to transmit. We identified the minimal element of the tlaRNA xrRNA and determined the structural characteristics allowing the RNA to resist degradation. Mutational and structural results indicate a new xrRNA subclass that is related to previously characterized xrRNAs but possesses some strikingly distinct molecular properties. The comparison of divergent xrRNAs will contribute to the fundamental understanding of how exonuclease-resistant rings are formed. We propose that the detailed structural characterization of this new xrRNA subclass will inform computational and experimental "mining" for more xrRNAs – in viruses, in viral-like elements, and potentially outside of the viral world, as Xrn1 is a conserved eukaryotic enzyme.

P19 Discovering host protein interactions specific for SARS-CoV-2 RNA genome

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SARS-CoV-2 is a positive single-stranded RNA virus that interacts with proteins of infected cells at different stages of its life cycle. These interactions are necessary for the host to recognize and hinder the replication of the virus. Yet, if cells fail to block SARS-CoV-2, host proteins are recruited to translate, transcribe and replicate the genetic material of the virus. To identify the host proteins that bind to SARS-CoV-2 RNA, we adopted the RNA-Protein Interaction Detection coupled to Mass Spectrometry (RaPID-MS) technology, which enables, by MS-based proteomics, the purification and identification of the proteins associated with a specific RNA of interest expressed in mammalian cells. We investigated proteins associated with the 5′ and 3′

end regions of SARS-CoV-2 RNA, known to be highly structured and to attract a considerable number of proteins. As the detection of associations might be influenced by experimental conditions, we confirmed these results with the predictions of protein-RNA interactions computed by the catRAPID algorithm, which assesses the binding potential of proteins to a given RNA region. Among the specific SARS-CoV-2 RNA interactors, we identified the pseudouridine synthase PUS7 that binds to both 5′ and 3′ ends of viral RNA, which harbor the canonical consensus sequence modified by this protein. We corroborated our results through SARS-CoV-2 RNA analysis by nanopore direct RNA sequencing. We confirmed that these PUS7 consensus regions were found modified on viral RNAs, as demonstrated by ionic current features that differ significantly from the unmodified in vitro transcribed RNA. Among them, we identified a modified site in the Transcription Regulatory Sequence - Leader (TRS-L) of three SARS-CoV-2 subgenomic RNAs. Overall, our data map the specific host protein interactions of the SARS-CoV-2 RNA UTR regions and point to a putative functional role of cellular pseudouridine synthases and post-transcriptional pseudouridine modifications in the viral life cycle.

P20 Dissecting the role of RNA decay factors during respiratory syncytial virus infection

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The globally prevalent Human Respiratory Syncytial Virus (RSV) is a common cause of severe lower respiratory tract infections in vulnerable groups but there are still no approved vaccines or effective medications. RSV is an enveloped virus with a non-segmented, negative-sense RNA genome that induces formation of cytosolic inclusion bodies, which are sites of viral transcription and replication. Although the viral particle comprises the core viral transcription-replication complex, RSV replication relies in substantial parts on host factors. In particular, host RNA-binding proteins (RBPs) play an essential role by regulating the function and fate of cellular RNA at multiple levels, including transcription, transport, storage, translation, and degradation. Recently, we adopted RNA antisense purification coupled with mass spectrometry (RAP-MS) to identify proteins that directly and specifically bind the positive or negative-strand RNAs of RSV in human cells undergoing authentic infection. Quantitative comparison of resulting RNA-protein interactomes confirmed that components of the RSV ribonucleoprotein particle were strongly associated with negative-strand RSV genome, while translation factors and ribosomal proteins bound exclusively to the mRNAs of RSV. Several proteins involved in RNA surveillance and decay pathways were overrepresented among the factors bound to RSV mRNA, particularly those involved in mRNA decapping and deadenylation. We employed a CRISPR/Cas9-based screening approach to functionally dissect a subset of RSV RNA binders and observed a significant impact on RSV virus growth when depleting several cellular RNA decay factors in human cells. In a next step, we will confirm the direct engangement of these RBPs with viral RNA and elucidate structural features and sequence elements responsible for mediating these interactions. This study will deepen our understanding of virus-host interactions and provide valuable insights for improving the development of novel antivirals.

P21 Using transcriptomics for the identification of novel DIS3L2-sensitive transcripts that play a role in the cellular stress response

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The regulation of RNA stability functions to maintain the fine balance that exists between RNA synthesis and decay which is crucial for key processes involved in cell survival, including the cellular stress response and maintaining cellular homeostasis. Exoribonucleases are enzymes which are key in controlling targeted RNA decay with their dysregulation leading to severe phenotypic defects. For example, DIS3L2 is a highly conserved 3'-5' exoribonuclease with human mutations resulting in Perlman syndrome and Wilms' tumour. Our lab has previously shown that loss of DIS3L2 enhances cell proliferation and results in an increase in the PI3-K/AKT/mTOR signalling pathway within human HEK-293T cells and Drosophila melanogaster. Interestingly, our more recent work suggests a potential regulatory role for DIS3L2 within the stress response by increasing the survival and resistance of Drosophila melanogaster and HEK-293T cells in response to nutrient deprivation. Using CRISPR-Cas9 gene editing we have generated DIS3L2 knockout HEK-293T cell lines along with an isogenic control and exposed these to optimised nutrient deprivation and Endoplasmic Reticulum (ER) stress over a range of time points. Subsequent, RNA sequencing analysis is allowing us to investigate the effect of DIS3L2 activity on the interplay between RNA stability and translation of specific transcripts in response to stress. This analysis has identified a greater number of transcripts that show sensitivity to DIS3L2 specifically under nutrient deprivation or ER stress in comparison to normal conditions. Further characterisation of these potential targets will allow us to elucidate the key cellular mechanisms that are aiding cell survival during the cellular stress response and to unpick the regulatory role that DIS3L2 plays within this. Our findings will further our understanding of disease pathologies associated with DIS3L2 in addition to those associated with cellular stress, such as neurodegeneration and cancer.

P22 Airway MIWI2+ Multiciliated Cells and Host Susceptibility to Influenza A Infection

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Influenza is a respiratory infection that represents a severe burden to public health. During early influenza A viral (IAV) infection, airway multiciliated cells are predominantly targeted. Previously believed to be a homogeneous population, we found that 5% of multiciliated cells express MIWI2, an Argonaute family protein. In the germline, MIWI2 binds to piRNAs and maintains genomic integrity by suppressing retrotransposable elements such as LINE-1 and endogenous retroviral RNA. However, the function of MIWI2 in somatic cells and its impact on exogenous viral RNAs is unclear. We therefore employed a model of murine A/Puerto Rico/8/1934 H1N1 (PR8) infection in Miwi2+/+ (wild-type), Miwi2+/tom (haplosufficient), and a Miwi2tom/tom (deficient) knock-in reporter mice. We found MIWI2 deficient mice exhibited significantly decreased viral burden compared to wild-type and haplosufficient mice. To understand these observations further, we performed small and bulk RNA sequencing of uninfected sorted MIWI2 haplosufficient and deficient multiciliated cells. A limited number of mRNAs and no miRNAs were differentially expressed in a MIWI2-dependent manner. In addition, we found no MIWI2-dependent changes in expression of the LINE-1 derived protein, ORF1p. In contrast, reductions in levels of tRNA fragments and piRNAs in MIWI2 expressing cells were observed. These data suggest a potential role for MIWI2 in the biogenesis and/or turnover of specific classes of small RNAs during homeostasis. Future studies will determine whether PR8 infection modifies host and viral genomic expression, including small RNAs, within MIWI2+ and MIWI2- multiciliated cells. We anticipate these studies will provide novel information regarding the lung host response to viral infections, and the role of Argonaute family proteins and small RNAs in immune regulation.

P23 Investigating antiviral defenses protecting plant stem cells and germline

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Stem cells are essential for the development and organ regeneration of multicellular organisms, so their infection by pathogenic viruses must be prevented. Accordingly, mammalian stem cells are highly resistant to viral infection due to dedicated antiviral pathways including RNA interference (RNAi). In plants, a small group of stem cells harbored within the shoot apical meristem (SAM) generates all postembryonic above-ground tissues, including the germline cells. Many viruses do not proliferate in these cells, yet the molecular bases of this exclusion remain only partially understood.
In our recent main body of work, we show that a plant-encoded RNA-dependent RNA polymerase, after activation by the plant hormone salicylic acid, amplifies antiviral RNAi in infected tissues. This provides stem cells with RNA-based virus sequence information, which prevents virus proliferation. Furthermore, we find RNAi to be necessary for stem cell exclusion of several unrelated RNA viruses, despite their ability to efficiently suppress RNAi in the rest of the plant. In parallel lines of research we developed cutting-edge live imaging techniques to track virus movement through meristems and flowers in three dimensions and over time, which allow unprecedented insight into the dynamics of virus infection. Finally, we developed biological tools to investigate the factors protecting plant germlines from infection, which block the vertical transmission of infection by many viruses through plant generations yet remain largely unknown.

P24 The role of DHX9 and ADAR1 in resolving RNA structures

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Half of the human genome consists of transposable elements of which 38% locate within the introns of protein-coding genes. During transcription, structured RNAs can be formed when these repetitive elements pair. Long dsRNA can interrupt proper splicing and lead to an immune response. We inspect two of the most abundant dsRNA binding proteins in the nucleus, DHX9 and ADAR1, and their role in resolving secondary structures. DHX9 interacts directly with the interferon-induced isoform of ADAR1p150 and in a RNA-mediated way with the prominent isoform ADAR1p110. Using multi-omics approaches we aim to understand the function of this interaction. For that, we established an inducible double degron system targeting DHX9 and/or ADAR1 in a human cell line. Our large RNA-seq dataset after single and double degradation revealed effects on splicing, termination and a pro-inflammatory response. Accumulation of dsRNA could lead to such an outcome and we are currently investigating their origins in the human genome by dsRNA-sequencing. From here we will extract the type of transposable elements that are transcribed, confirm their structure by comparison to in situ prediction and match this information to observed splicing defects in the RNA-seq data. The cooperative action of DHX9 and ADAR1 decreases the dsRNA accumulation in the nucleus. Therefore, we additionally inspect DHX9 binding in an ADAR1-independent context to further explore rules for dsRNA binding of these abundant RNA binding proteins.

P25 Charecterization of Transcription Mediator Complex in Trypanosomatids

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Transcription Mediator (MED) is an evolutionary conserved multi-subunit protein complex (comprising 25 subunits in budding yeast, up to 30 subunits in humans, and 35 subunits in A. thaliana), structurally organized into the head, middle and tail modules and a dissociable Cyclin-Dependent Kinase 8 (CDK8) module. Evidence suggests that Mediator is a key complex in transcription initiation and modulates gene expression by multi-level checkpoint function as a bridge between DNA binding transcription factors and pol II. The role of MED is now well established in various diseases and so many physiological functions.
5 subunits of MED are discovered in T. brucei as Yeast homolog, three of these subunits are conserved in the Leishmania genus. However, there is no single study till date to validate its role in this organism. We found at RNA level, all known MED subunits are differentially expressed in different life cycle stages of T. brucei, Leishmania Mexicana and L. donovani. In fact some subunits have multi-fold RNA expression change between Procyclic to Bloodstream form of Trypanosoma brucei as well as Promastigote to Amastigote stages of Leishmania mexicana. Downregulation of MED subunits by siRNAs reduced cell proliferation as well as lethal effects. However non-targeting siRNA had no effect. We also mapped novel putative noncoding RNAs in the vicinity of these genes suggesting multifactorial “direct or indirect role” of MED in Trypanosomatids. We also tagged these MED subunits with V5 tag and performed pull-down/I.P. assays to discover other subunits of MED as well as it’s interactome. This would be pioneer study of mapping MED subunits in Trypanosomatids.

P26 mRNA decay of hepatokines orchestrates inter-organ communication to regulate food intake and energy expenditure

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Hepatokines, secretory proteins from the liver, mediate inter-organ communication to maintain a metabolic balance between food intake and energy expenditure. However, molecular mechanisms by which hepatokine levels are rapidly adjusted following stimuli are largely unknown. Here, we unravel CNOT6L deadenylase switches off hepatokine expression after responding to the stimuli (e.g., exercise and food) to orchestrate energy intake and expenditure. Mechanistically, CNOT6L inhibition stabilizes hepatic Gdf15 and Fgf21 mRNAs, increasing corresponding serum protein levels. The resulting up-regulation of GDF15 stimulates the hindbrain to suppress appetite, while increased FGF21 affects the liver and adipose tissues to induce energy expenditure and lipid consumption. Despite the potential of hepatokines to treat metabolic disorders, their administration therapies have been challenging. Using small-molecule screening, we identified a CNOT6L inhibitor enhancing GDF15 and FGF21 hepatokine levels, which dramatically improves diet-induced metabolic syndrome. Our discovery, therefore, lays the foundation for an unprecedented strategy to treat metabolic syndrome. In this presentation, we will introduce our recent data and discuss the physiological functions and therapeutic potentials of the CCR4-NOT deadenylase for the treatment of metabolic syndrome.

P27 How the SARS-CoV-2 main protease targets human TRMT1 to disrupt tRNA modification and translation during infection

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Like many viruses, SARS-CoV-2 employs diverse strategies to manipulate host biochemical pathways and downregulate host translation. Here we show that the human tRNA methyltransferase TRMT1, which installs tRNA m2,2G modifications important for global cellular protein synthesis, can be recognized and cleaved by the SARS-CoV-2 main protease (Mpro). Mpro-mediated TRMT1 cleavage results in removal of the TRMT1 C-terminal zinc finger domain, which is required for tRNA binding and efficient m2,2G modification in cells. Our kinetic and evolutionary analyses show that the TRMT1 cleavage sequence is proteolyzed with comparable efficiencies to known viral polypeptide cleavage sites and is highly conserved in mammals, with the exception of some rodents, where a single substitution may confer resistance to Mpro-directed proteolysis and loss of m2,2G modification. We determined the co-crystal structure of the human TRMT1 cleavage sequence in complex with Mpro that shows how TRMT1 engages the viral protease active site in an uncommon binding conformation. Together, our results show at high resolution how SARS-CoV-2 Mpro can recognize and cleave human TRMT1 to impair cellular tRNA modification during viral infection. This raises the possibility that dysregulation of m2,2G tRNA modification during SARS-CoV-2 infection may contribute to disruption of host translation, RNA stability, and viral pathogenesis or phenotypes.

P28 Investigating the relationship between processing bodies and coronavirus replication

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Processing bodies (PBs) are cytoplasmic membraneless ribonucleoprotein granules that contain cellular proteins involved in mRNA decay and translational suppression. PBs are enriched with mRNAs encoding potent regulatory molecules, suggesting they serve to post-transcriptionally regulate gene expression of important biological processes. mRNAs are addressed to PBs via features, such as the presence of short AU-rich elements (AREs), long 3'UTRs, and low GC content. Once localized to PBs, mRNAs are removed from the translation pool and either deadenylated, decapped, and decayed or translationally repressed. Although a diversity of viruses trigger PB disassembly during infection, including multiple human coronaviruses (CoVs), the impact of PB disassembly on virus replication remains unknown. CoVs have a ~30 kb RNA genome and produce multiple subgenomic messenger RNAs (sgmRNAs), which all possess a 5'm7G cap and a 3'polyA tail to allow the virus to access cellular translation machinery; however, these characteristics also make viral RNAs subject to post-transcriptional regulation by PBs. CoV genomic and sgmRNAs contain multiple features that may promote their localization to PBs during infection, including multiple AREs, long 3'UTRs, and low GC content. Here, we identified that at early SARS-CoV-2 infection times, PBs remain present. Using RNA-FISH/IF we find that these residual PBs co-localize with SARS-CoV-2 genomic and sgmRNAs. SARS-CoV-2 sgmRNA but not genomic RNA was enriched in residual PBs at early infection times. Ectopic expression of individual sgmRNAs recapitulated sgmRNA-PB enrichment, suggesting that viral sgmRNAs contain features in cis that promote their recruitment to PBs. These data suggest that PBs have the potential to negatively impact SARS-CoV-2 replication. Consistent with this, our preliminary data showed that knockdown of the PB scaffolding protein Lsm14a increased CoV replication. Taken together, these data suggest that PBs may sequester and repress viral RNA during infection, reconciling why CoVs promote P-body disassembly during infection.

P29 Inhibition of nonsense-mediated mRNA decay reduces the tumorigenicity of human fibrosarcoma cells

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Nonsense-mediated mRNA decay (NMD) is a eukaryotic RNA degradation pathway that targets for degradation faulty mRNAs with premature termination codons as well as many physiological mRNAs that encode full-length proteins. Consequently, NMD is conceived not only as a quality control mechanism but also as a post-transcriptional regulator of gene expression, and it has been implicated in a wide range of biological processes like cellular differentiation, stress response, viral defense and cancer progression.
To investigate the role of NMD in cancer, we knocked out SMG7 (SMG7KO) in the HT1080 human fibrosarcoma cell line. SMG7 is involved in the deadenylation-coupled exonucleolytic pathway, one of the two main degradation pathways in mammalian NMD. Genome-wide proteomic and transcriptomic analysis confirmed that NMD is severely compromised in SMG7KO cells. We compared the oncogenic properties between parental HT1080, SMG7KO and a rescue cell line in which we re-introduced both isoforms of SMG7. In parallel, we tested the effect of a drug inhibiting the NMD factor SMG1 on the HT1080 cells to distinguish NMD-dependent effects from putative NMD-independent functions of SMG7. Using cell-based assays as well as a mouse xenograft tumor model, we show that the oncogenic properties of the parental HT1080 cells are severely compromised when NMD is inhibited. Molecular pathway analysis revealed a strong reduction of the matrix metalloprotease 9 (MMP9) gene expression in NMD-suppressed cells. Since MMP9 expression promotes cancer cell migration and invasion, metastasis and angiogenesis, its downregulation in NMD-suppressed cells explains, at least partially, their reduced tumorigenicity. Collectively, our findings emphasize the therapeutic potential of NMD inhibition for the treatment of certain types of cancer.

P30 Reading m^{7}Gp4Gm-RNA in *E. coli*: Development of Selective CaptureSeq Method

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Bacterial adaptation to stress is essential for their survival and RNA turnover control plays a pivotal role in the process. Increasing evidence suggests that RNA 5'-end modifications represent additional tuning level of their metabolism. We have recently reported that *E. coli* small (s)RNA contains an assortment of new noncanonical dinucleoside polyphosphate caps. Among them, m^{7}Gp$_4$Gm, substantially increases in response to stress conditions[1]. m^{7}Gp$_4$Gm-RNA showed resistance to RNA 5'-pyrophosphohydrolase, enzyme decapping non-methylated RNA caps, indicating an important role of m^{7}Gp$_4$Gm cap in RNA faith. To understand the purpose of m^{7}Gp$_4$Gm cap in broader biological context, new techniques to identify m^{7}Gp$_4$Gm-capped RNA sequences are needed.
Here, we present newly developed immunoprecipitation-based method targeting m^{7}Gp$_4$Gm-RNA employing m^{7}G-specific monoclonal antibody. The method was applied on sRNA isolated from *E. coli* harvested at exponential and stationary phase of growth. Captured RNA was reverse transcribed into cDNA and sequenced on Illumina. sRNA treated with a decapping enzyme prior immunoprecipitation was used as negative control. Bioinformatic analysis of sequencing data from six biological replicates revealed several RNAs significantly enriched compared to controls. In order to validate the method, we optimized a pull-down protocol for selected RNAs to confirm presence of m^{7}Gp$_4$Gm as the RNA cap by LC-MS. We believe that revealing m^{7}Gp$_4$Gm-capped RNA sequences will help us to understand how *E. coli* respond to stress by selective RNA capping.

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P31 Modulation of nucleotide metabolism by CVB3

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The family Picornaviridae includes many well-known pathogens of relevance to the health of human and animals such as enteroviruses (e.g. poliovirus, rhinoviruses and coxsackieviruses; CVB) and cardioviruses (e.g. encephalomyocarditis virus; EMCV). These viruses cause a variety of diseases in ranging from (hand-)foot-and-mouth disease, myocarditis, and conjunctivitis to aseptic meningitis and acute flaccid paralysis. Upon infection of their host, these viruses modulate several cellular processes for efficient replication and spreading, such as host cell gene expression, intracellular protein and membrane transport, and cell death pathways. While it is known that viruses also actively reprogram the metabolism of the host, little is known about the effects of picornaviruses on cellular metabolism. Here, we studied the modulation of host metabolism by coxsackievirus B3 (CVB3), a member of the enterovirus genus using steady-state and isotope tracing metabolomics. We demonstrate that CVB3 increases the levels of pyrimidine and purine metabolites and provide evidence that this increase is mediated through degradation of nucleic acids and nucleotide recycling, rather than upregulation of de novo synthesis. Furthermore, we studied the role of 2Apro, a non-structural proteins of CVB3 that induces translational host shut-off, causes nucleocytoplasmic trafficking disorder (NCTD) and suppresses anti-viral responses, in modifying the host-metabolome during CVB3 infection. Interestingly, it was observed that 2Apro inhibits de novo nucleotide synthesis during CVB3 infection, but does not influence the levels of nucleic acid degradation. Insight into picornaviral modulation of cellular metabolism is important to increase our understanding of picornavirus-host interactions and may uncover novel therapeutic strategies.

P32 Universal requirements for RNA promoter activity for RNA synthesis in dual and single host flaviviruses

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The genus Flavivirus contains over 100 members including many important emerging and re-emerging human pathogens such as dengue virus, Zika virus, yellow fever virus and Japanese encephalitis virus among others. Collectively, these viruses infect hundreds of millions of humans and cause thousands of deaths annually.
Their genome is a single positive-sense RNA molecule of ~11 kb that, in addition of coding the viral polyprotein, contains signals in RNA structures that contribute to viral RNA replication, pathogenesis and host adaptation. A mechanism of RNA synthesis for all flaviviruses includes an RNA element present at the 5' end of the genome called stem-loop A (SLA), which binds the polymerase and promotes RNA synthesis. In order to identify functional common elements in the SLA, we constructed a library of viruses by replacing subunits of SLA with sequences selected from different flaviviruses or from rationally design subunits into a replicating ZIKV infectious clone. Viral RNA synthesis and viral fitness in mosquito and mammal cells were assessed for the complete library. This analysis, together with structural and sequence RNA alignments, allowed us to identify conserved essential elements in the SLA. Interestingly, some key sub-elements of the SLA were found to be specific to dual-host human-insect virus, while others seem to be specific for insect viruses, indicating a possible co-evolution with the viral polymerase NS5 in distinct ecological environments. In this regard, we are dissecting specific requirements for each SLA-NS5 pair by searching spontaneous mutations in cell culture using non-functional SLA-NS5 partners. Understanding the similarities and differences between flaviruses that infect dual hosts, only invertebrates or only vertebrates will help to define restriction factors for replication in different conditions and for the emergence of new human pathogens. Finally, identification of common pathways among flaviviruses will be also important for designing universal countermeasures.

P33 The Zika virus regulates mRNA translation in human hosts by activating the Integrated Stress Response pathway

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The integrated stress response (ISR) is a signaling pathway that suppresses protein synthesis in response to various stimuli, such as the accumulation of unfolded proteins, hypoxia, amino acid deprivation, and viral infection. Although it is known that virus replication can activate the ISR, its role during viral infection is still unclear. In some instances, the ISR serves as a protective mechanism for host cells against viral infection, while viruses may hijack the ISR to facilitate their replication.

The purpose of this study was to investigate how the ISR regulates Flavivirus replication in human cells, using a Zika virus (ZIKV) strain that we constructed from a cDNA clone isolated from Argentina. ZIKV is an enveloped RNA virus belonging to the Flaviviridae family, along with other significant human pathogens transmitted by mosquitoes, such as dengue and yellow fever viruses. Our findings revealed that ZIKV infection activates the ISR pathway, leading to the arrest of host translation by phosphorylation of the eIF2α factor. Activation of the ISR during ZIKV infection resulted in the assembly of stress granules, suggesting that this mechanism can silence the translation of several cellular mRNAs.

We performed ribosome profiling in infected human cells and confirmed that ZIKV causes a shutdown of mRNA translation, decreasing the levels of new protein synthesis. Surprisingly, we found that suppressing the activation of the ISR also reduced ZIKV replication and virus secretion, providing evidence for the proviral role of the ISR. This study presents novel insights into the impact of the ISR on flavivirus replication. It is likely that the activation of the ISR during infection leads to the inhibition of translation of cellular factors, resulting in a more favorable environment for viral propagation.

P34 Chandipura virus increases the expression of miR-21-5p to activate NF-κBp65 in human microglial cells.

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Chandipura virus (CHPV) infection is one of the most neglected types of viral infection. CHPV, a part of Rhabdoviridae family, is a vesiculovirus which causes outbreaks of fatal encephalitis in central and southern India. Neuroinflammation is the hallmark of CHPV infection. Several studies have pointed that CHPV infection causes neuronal apoptosis and the microglial activation. Various studies on encephalitis-causing viruses have demonstrated that microglia can respond to viral PAMPs and play a pivotal role in viral encephalitis. During neuro-viral infections, the microRNAs (miRNAs) are important regulators of microglial activation. MicroRNAs are non-coding RNAs that are 22–25 nt long and found in all eukaryotic cells. To understand the role of miRNAs in CHPV-mediated neuroinflammation, human microglial cells were infected with CHPV and miR-21-5p expression was quantified through qPCR. Expression of PTEN and the downstream genes were studied using qPCR and immunoblotting and NF-κB promoter activity was studied through luciferase assay. Additionally, microRNA target validation was done by overexpression and knockdown of miR-21. CHPV infection elevated the expression of miR-21-5p, which targeted and suppressed the expression of PTEN in human microglial cells. PTEN inhibition increased the phosphorylation of AKT and NF-κBp65 proteins which resulted in the production of IL-6 and TNF-α in CHPV infected human microglial cells, resulting in the induction of pro-inflammatory responses. Overexpression and knockdown of miR-21-5p expression affected the PTEN expression and thereby affecting the downstream genes. Therefore, we concluded that CHPV infection mediate the pro-inflammatory responses via miR-21-5p and contribute to CHPV-mediated neuroinflammation. Our findings highlight the role of miR-21-5p in the activation and regulation of the inflammatory responses in CHPV-infected human microglial cells. This is the first report on the perturbed miRNA expression in CHPV-infected human microglial cells. The findings of this study may help in the development of microRNA-based therapeutics against CHPV in future.

P35 The landscape of the nucleocapsid-associated-regions of SARS-CoV-2 reveals viral packaging signals

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The recognition and packaging of the viral genome into virions is a crucial step in the viral life cycle. Yet, the packaging signal of the SARS-CoV-2 genome remains elusive. Here, we employed two different CLIP-seq techniques to map the RNA regions that interact with the SARS-CoV-2 nucleocapsid (N) protein. Using a virus-like particle system to assess functionality, we identified two prominent packaging elements within the genomic RNA. Mutagenesis experiments showed that these elements operate in a sequence- and structure-dependent manner. We also discovered that when mutations were introduced into the packaging signals of wild type SARS-CoV-2, the mutated viruses were defective in propagation and packaging. Consistently, under high multiplicity-of-infection conditions, partial viral RNAs retain the packaging signals and are successfully incorporated into the virions. These packaging signals are conserved across all coronaviruses. Our findings shed light on the coronaviral assembly process and offer a strategy to inhibit the coronaviral life cycle.

P36 Epitranscriptomic regulation of cellular lncRNAs involved in neuroinflammation induced in microglia infected by HIV-1

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HIV-1 is the causal agent of AIDS pandemic. Viral infection of the Central Nervous Systems cells, like microglia, induces the release of HIV proteins (such as Tat) and pro-inflammatory cytokines, responsible for neurocognitive dysfunctions and neuronal tissue inflammation identified as HAND. The molecular mechanism involved in these inflammatory processes on microglia-infected are still unknown. It has been described that lncRNAs are relevant molecules in HIV infection and inflammatory response. Interestingly, it was reported that the presence of m6A on lncRNAs plays a role on the metabolism of cytokines in pro-inflammatory microglia in a context-independent of infection. This evidence makes lncRNAs and their m6A as a potential regulator of the immune response. In this work, we are evaluating five lncRNAs (showing changes in the m6A status), that may be relevant in the inflammatory response in microglia-HIV infected, to better understand the molecular mechanism associated. Through a MeRIP-seq we observed that 175 lncRNAs change their m6A status in infected microglia (186 up-methylated and 68 down-methylated). Additionally, we analyzed the m6A profiles of these lncRNAs, concluding that in addition to changing the methylation states, the sites that are methylated also change. Subsequently, we predict lncRNAs interaction with a software predictor and we selected five lncRNAs that are possibly interacting with factors involved in immune response and m6A machinery (NEAT1, GASAL1, ZBTB11-AS1, LINC01410, and OTUD6B). Using MeRIP-RTqPCR we corroborate the previously observed differences in the m6A status of these 5 lncRNAs. Interestingly, using RT-qPCR we observed that only some of these lncRNAs changes their expression levels, despite the differences we observed in the methylation levels. At present, we are evaluating the knockout of these lncRNAs (CRISPR/CAS9 systems) to understand their role in the inflammatory response in microglia infected and then be able to evaluate the impact of m6A in these lncRNAs associated with HAND.

P37 Evolutionary insights into the impact of viral infections on the metabolism of mRNAs by the CCR4-NOT complex and associated proteins

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Due to their nature as intracellular obligatory parasites, viruses are dependent on their ability to hijack the host cellular machinery for their replication. This involves numerous interactions with host cellular proteins, leading to either pro-viral or antiviral effects. Both types of interaction can lead, over evolutionary time, to the establishment of a genetic conflict between the two interactors. These genetic conflicts can be detected on either host or viral genes through the presence of evolutionary marks such as positive selection, gene duplication and gene family expansion, recombination, etc...
CCR4-NOT is a major cellular complex, which role and functions are broadly conserved from yeast to humans. One of its main functions is mRNA metabolism through the deadenylation of the poly(A) tail, which in turns destabilizes the mRNA and causes its degradation. Given this central role in mRNA homeostasis, we hypothesized that viruses have, over time, evolved different ways to evade this deadenylation-mediated degradation of their mRNAs. In this study, we propose to use evolutionary analyses to look for the presence of hallmarks of genetic conflicts on CCR4-NOT genes and associated genes, which might potentially have arisen from selective pressure by viruses.
We will here highlight the results obtained from looking at the eleven genes involved in the CCR4-NOT complex, as well as two sets of genes related to two mechanisms which intersect with CCR4-NOT and are involved in some viral infections: the methyl-6-adenosine (m6a) machinery, which interact with CCR4-NOT through the reader YTHDF2, and the ZCCHC14-TENT4B complex, which has been shown to protect mRNAs from CCR4-NOT deadenylation by introducing guanosines into the poly(A) tail.
We hope that these results will provide new avenues for studying the intersection between mRNA metabolism and viral infection, by providing insight in the evolutionary history of those mechanisms and the potential impact of viruses on them.

P38 Discovery of novel trans-factors directly and indirectly affecting –1 programmed ribosomal frameshifting by interactome capture and CRISPR interference screens

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Programmed ribosomal frameshifting is an alternative translation event, also known as recoding, that ensures the translation of the polycistronic genomic RNA of many RNA viruses including the human immunodeficiency virus 1 (HIV-1) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and consequently influences the virus replication capacity. Two conserved RNA elements cause the ribosomes to frameshift: a) a secondary structure formed by the RNA that builds a barrier or roadblock for the ribosome and thus stalls it on b) the upstream heptanucleotide long slippery sequence. RNA secondary structures have been known to be crucial for viral frameshifting and thus viral replication - for a long time. Whereas only a few trans-factors modulating frameshifting are identified or characterized yet, such as ZAP-S for SARS-CoV-2. Here, a genome-wide, unbiased CRISPR interference screen in combination with a dual-fluorescence reporter assay was performed to identify novel host modulators that directly or indirectly regulate programmed ribosomal frameshifting. Among those hits we identified proteins which are reported to be involved in RNA-binding, pre-mRNA splicing and translation. Yet, most potent host-encoded effectors of frameshifting are poorly characterized proteins and therefore require in-depth investigation for their RNA binding activities and co-associated factors. Interestingly, few of the hits in the CRISPR interference screen were commonly identified in our RNA interactome capture pointing to a direct interaction with the frameshift RNA. We now seek to identify their role in mRNA translation, their binding sites as well as their role in viral replication using functional and biochemical assays.

P39 tRNA synthetases as potential RNA capping enzymes

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In our laboratory, we have discovered a novel type of 5‘ RNA cap in bacteria known as dinucleoside polyphosphate (NpnNs) caps. However, the role of these caps in cellular processes remains unclear and requires further investigation. To unravel their function, it is essential to understand their formation mechanism. One potential mechanism involves the incorporation of dinucleoside polyphosphate caps into RNA during transcription as non-canonical initiation nucleotides (NCINs). This mechanism has been demonstrated in vitro. Nevertheless, some NpnNs attached to RNA have not been previously detected in the free form in cells, suggesting the existence of an RNA capping enzyme. A compelling hypothesis is that tRNA synthetases, enzymes responsible for tRNA aminoacylation, could also function as 5‘ RNA capping enzymes in bacteria. These enzymes zare excellent candidates due to their capability to produce free diadenosine polyphosphates. Instead of using ATP as a substrate for Ap4A formation, tRNA synthetases might accept triphosphate RNA as a substrate, leading to the formation of Ap4A RNA caps. In this research project, we aim to explore the potential of tRNA synthetases as 5‘ RNA capping enzymes. To investigate this hypothesis, we selected and subcloned five tRNA synthetases from an *E. coli* cDNA library and expressed them in *E. coli*. His-tagged tRNA synthetases were then purified using immobilized metal affinity chromatography (IMAC, HisTrap), followed by size exclusion chromatography (SEC). Subsequently, we examined the production of free dinucleoside polyphosphates using high-performance liquid chromatography (HPLC) analysis. Additionally, we will assess the capping potential of tRNA synthetases by conducting experiments with radioactively labelled RNA, followed by analysis on boronate gel electrophoresis.

P40 Poly-ribo-seq reveals a link between novel translation events and Pacman mediated degradation highlighting new roles for long non-coding RNAs.

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The control of gene expression is fundamental for the correct growth and development of all living organisms. RNA degradation is a vital mechanism through which control of gene expression is maintained, with errors in these processes involved in many human diseases, including cancers and neurodevelopmental disorders. Here, we set out to characterise the role of two cytoplasmic ribonucleases, Pacman (acting 5' to 3') and Dis3L2 (acting 3' to 5'), in shaping the in vivo RNA landscape of Drosophila melanogaster wandering 3rd instar larvae.

Using Poly-ribo-seq, we show that loss of Pacman or Dis3L2 result in specific changes to both the transcriptome and the translatome, and demonstrate their roles in regulating RNA transcripts involved in specific cellular pathways. We identify 26192 translating open reading frames and, strikingly, detect novel translation events in 36 long non-coding RNAs (lncRNA) in vivo. Furthermore, secondary analysis with increased bioinformatic stringency to remove isoform duplicates and nested ORFs refines these to 8566 unique translating open reading frames, and identifies 47 confidently translated novel open reading frames across 32 lncRNAs.

Subsequent molecular analysis confirms the translation of these lncRNAs, and bioinformatic analysis shows specific codon usage, framing for novel ORFs, and a broader effect on translating lncRNAs in the absence of Pacman than seen in the absence of DIS3L2. Ongoing work indicates a trend of translating lncRNAs having a shorter half-life than non-translating lncRNAs suggesting specific regulatory mechanisms. Together, these experiments reveal a novel role for Pacman in controlling lncRNA biology, and further demonstrates the requirement for in depth characterisation and reconsideration of the protein coding potential of lncRNAs.

P41 Unravelling the molecular function of CDM1 zinc-finger protein in meiotic progression in *Arabidopsis thaliana*

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Plant meiosis, in contrast to animals, is followed by several mitotic divisions to produce functional gametes. The transition from meiosis to post meiotic development is poorly understood. We discovered that in Arabidopsis, this transition during male gametogenesis is governed by unknown mechanism that involves SUPPRESSOR WITH MORPHOGENETIC EFFECTS ON GENITALIA7 (SMG7), THREE DIVISION MUTANT1 (TDM1) genes. Mutations of these genes leads to third meiotic division and fail to produce microspores, which results in male sterility. To decipher this mechanism, we performed a forward genetic screen to identify genes that rescue fertility of smg7 mutants. We found two recessive and one dominant mutations in a gene coding for CALLOSE DEFECTIVE- MICROSPORE1(CDM1). CDM1 is a transcription factor required for the formation and dissolution of callose in male meiosis and secondary cell wall formation. In my Ph.D. project, I aim at unraveling the molecular function of CDM1 during meiosis.

We confirmed through both genetic association studies and complementation experiments that mutations in CDM1 restore fertility and microspore formation of smg7 and tdm1 mutants. CDM1 encodes 308 amino acids protein containing two Tandem Zinc Finger (TZF) motifs separated by a linker at the C-terminus. Reporter lines lacking TZF motifs could not complement the null mutation, demonstrating that TZF motifs are crucial for molecular functions of CDM1. CDM1-GFP reporter lines showed that CDM1 forms distinct cytoplasmic foci specifically in cells undergoing meiosis. Ectopic co-expression analysis in leaf protoplasts revealed that CDM1 co-localizes with DCP1, a marker of P-bodies, which are the hubs for RNA processing. Further in-vitro experiments indicated that, the CDM1-GFP has the propensity to form Liquid-Liquid Phase Separation (LLPS) condensates in the presence of RNA.

It implies a role of CDM1 in RNA metabolism during meiosis. Localization of CDM1 to P-bodies and presence of TZF motifs indicate direct binding of CDM1 to RNA and its metabolism. Our further research is aimed at identification of transcripts that associate with CDM1 and subsequent elucidation of CDM1 function in meiosis exit.

Acknowledgments
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P42 Live cell imaging approaches for the detection of viral RNA in enterovirus-infected cells

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Enteroviruses (EVs) are a genus of positive-sense single-stranded RNA viruses within the family Picornaviridae. Aside from the prototypical EV poliovirus, the cause of poliomyelitis, several other EVs impose a significant health or socio-economic burden. We currently lack a profound understanding of the earliest phases of viral infection, which are critical to determining the outcome of infection. Many conventional techniques/methodologies are inadequate to dissect these dynamic processes in live cells with the required sensitivity.

To improve on this, our group previously established VIRIM, an imaging approach that allows for live cell visualization of active translation on viral RNAs. This for the first-time enabled elucidation of the real-time dynamics of infection in single infected cells. Expanding our imaging capabilities, we set out to develop ways to visualize viral RNA molecules directly in live infected cells. To this end we made use of the BiRhoBAST aptamer system. Due to an avidity-effect BiRhoBAST has extremely high affinity for the cell-permeable dye tetramethylrhodamine-2 (TMR2), which fluoresces in the orange spectrum. Via genetic engineering we successfully obtained Coxsackievirus-B3 (CVB3) containing a 4x-BiRhoBAST cassette in the 3'-UTR of the genome. This was stably maintained over continued passage of the virus and allowed us to visualize viral RNA at single molecule resolution in fixed cells. We are currently trying to optimize this further to achieve similar resolution in live cells. Moreover, to increase the number of repeats we can stably maintain in the genome, and thereby enhance the signal-noise ratio, we made use of CVB3-replicons that lack the P1 structural region. Upon further optimization the BiRhoBAST system, in combination with VIRIM and other live cell imaging approaches, will enable further elucidation of the early viral life cycle of CVB3. Moreover, these tools could potentially be employed for the study of a wide range of other RNA viruses.

P43 INHAT subunit SET/TAF-Iβ regulates PRC1 independent H2AK119 mono-ubiquitination via E3 ligase MIB1 in colon cancer

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SET/TAF-Iβ, a subunit of the inhibitor of acetyltransferases (INHAT) complex, exhibits transcriptional repression activity by inhibiting histone acetylation. We find that SET/TAF-Iβ regulates ubiquitination of histone H2A at lysine 119 (H2AK119), which is involved in polycomb-mediated transcriptional repression in HCT116 cells. In this report, we demonstrate that SET/TAF-Iβ acts as an E2 ubiquitin-conjugating enzyme for PRC1 independent H2AK119 ubiquitination. Furthermore, we identify that MIB1 is the E3 ligase partner for SET/TAF-Iβ using LC-MS/MS and ubiquitination assays. Transcriptome analysis reveals that SET/TAF-Iβ and MIB1 regulate the expression of genes related to DNA replication and cell cycle progression in HCT116 cells, and knockdown of either protein reduces proliferation of HCT116 cells by impeding cell cycle progression. Together, our study reveals another epigenetic regulatory mechanism for H2AK119 ubiquitination by SET/TAF-Iβ-MIB1 ubiquitinating complex and following transcriptome reprograming in colon cancer.

P44 SARS-CoV-2 Nsp1 mediated mRNA degradation requires mRNA interaction with the ribosome

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Nsp1 is a SARS-CoV-2 host shutoff factor that both represses cellular translation and promotes host RNA decay. However, it is unclear how these two activities are connected or what assemblies Nsp1 forms in cells. Here, we performed mutational analyses of Nsp1, and these revealed that both the N and C terminal domains of Nsp1 are important for translational repression. Furthermore, we demonstrate that specific residues in the N terminal domain are required for cellular RNA degradation but not bulk translation shutoff of host mRNAs, thereby separating RNA degradation from translation repression. We also present evidence that Nsp1 mediated RNA degradation requires engagement of the ribosome with mRNA. First, we observe that cytosolic lncRNAs, which are not translated, escape Nsp1 mediated degradation. Second, inhibition of translation elongation with emetine does not prevent Nsp1 mediated degradation, while blocking translation initiation before 48S ribosome loading reduces mRNA degradation. Taken together, we suggest that Nsp1 represses translation and promotes mRNA degradation only after ribosome engagement with the mRNA. This raises the possibility that Nsp1 may trigger RNA degradation through pathways that recognize stalled ribosomes.

P45 Modifications of mRNA 5'-end defining transcripts as ‚self' for innate immune

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A key feature of eukaryotic mRNA is the presence of a 5' cap structure, which is indispensable for several biological processes such as pre-mRNA splicing, mRNA export, and 5' cap-translation. The mRNA cap consists of 7-methylguanosine (m7G) linked by a 5',5'-triphosphate bridge to the first transcribed nucleotide. In mammals, m7G-adjacent nucleotides undergo extensive modifications. Ribose of the first or the first and the second transcribed nucleotide can be subjected to 2'-O-methylation to form cap1 or cap2, respectively. When the first transcribed nucleotide is 2'-O-methylated adenosine, it can be additionally modified to N6,2'-O-dimethyladenosine (m6Am). Recently, the crucial role of cap1 in distinguishing between 'self' and 'non-self' transcripts in mammalian cells during viral infection was revealed. Here, we attempted to understand the impact of cap methylations on RNA-related processes. Therefore, we synthesized tetranucleotide cap analogues and used them for RNA capping during in vitro transcription. Using this tool, we found that 2'-O-methylation of the second transcribed nucleotide within the mRNA 5' cap influences protein production levels in a cell-specific manner. This modification can strongly hamper protein biosynthesis or do not influence protein production levels. Interestingly, 2'-O-methylation of the second transcribed nucleotide and the presence of m6Am as the first transcribed nucleotide serve as determinants that define transcripts as 'self' and contribute to mRNA escape from the host innate immune response. Additionally, cap methylation status does not influence transcript affinity towards translation initiation factor eIF4E or susceptibility to decapping by DCP2 in vitro; however we observe the resistance of cap2-RNA to DXO-mediated decapping and degradation.

P46 A 2.7Kb virally encoded lncRNA blocks cell cycle progression

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Human cytomegalovirus (HCMV) is a prototypical herpesvirus which infects the majority of the world's population, leading to severe disease in newborns and immunocompromised adults. The HCMV genome contains ~240kb, making it the largest known human virus. Although viruses are known for their compact genomes and HCMV hardly contains any intergenic regions, HCMV encodes four lncRNAs which are expressed to high levels during infection. Of these, the lncRNA RNA2.7 is the most abundant viral transcript. It accumulates in the cytosol early in infection and amazingly accounts for 13% of the non-ribosomal RNA in infected cells. Despite its extremely high abundance, the functions of RNA2.7 are still poorly understood. Using a deletion mutant virus lacking RNA2.7, we show that RNA2.7 is needed for viral propagation specifically in cycling cells. This phenotype is due to an RNA2.7 mediated inhibition of the G1-S cell cycle transition, and involves the inactivation of the CDK4-cyclin D1 complex. Given RNA2.7's extreme abundance, our leading hypothesis is that this cell cycle arrest is driven by sequestration of RNA binding proteins leading to changes in cellular genes in infected cells. In order to elucidate the molecular function of RNA2.7, we performed a pull-down assay to characterize proteins that bind to RNA2.7 which revealed its binding to proteins related to RNA stability. In parallel we carried out genome-wide measurements of mRNA translation and stability revealing broad changes in cellular gene expression mediated by RNA2.7, most of which are driven by changes in mRNA stability. These results support a mechanism by which RNA2.7 sequesters RNA binding proteins leading to changes in cellular gene expression which drive the cell cycle arrest. Overall, our results elucidate the enigmatic function of RNA2.7 in HCMV infection and demonstrate how a viral cytosolic lncRNA can mediate substantial changes in gene expression, interfering with cellular processes to promote vial propagation.

P47 Characterization of the ZAP-mediated viral RNA decay pathway

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The zinc finger antiviral protein (ZAP) modulates viral and cellular gene expression through several mechanisms including directly binding RNA and targeting it for cytoplasmic degradation, inhibiting translation initiation and programmed ribosomal frameshifting, inhibiting miRNA activity and modulating m6A-mediated degradation. Consistent with this diverse set of activities, ZAP has been shown to interact with many cellular proteins, several of which are implicated in RNA degradation. To determine which proteins in the ZAP interactome are required for targeted viral RNA decay, we have screened a portion of the interactome using HIV-1 containing defined ZAP-response elements (ZREs).

ZAP-interacting proteins could act as cofactors by regulating ZAP binding to the ZRE or by targeting the RNA to specific degradation pathways. The RNA binding E3 ubiquitin ligase TRIM25 is required for ZAP to inhibit viral replication and genomic RNA abundance for HIV containing ZREs located in different regions of the viral genome. RNA-immunoprecipitation experiments show that TRIM25 promotes ZAP binding to HIV RNA containing a ZRE. Interestingly, iCLIP experiments indicate that ZAP and TRIM25 have different binding profiles on HIV RNA and TRIM25 appears to bind non-specifically, indicating that TRIM25 binding sites are not part of the ZRE. ZAP has been reported to utilize 5'-3' and 3'-5' exonucleolytic as well as endonucleolytic RNA degradation mechanisms. Depleting core proteins in the 5'-3' and 3'-5' degradation pathways does not specifically affect the replication or RNA abundance for HIV containing a ZRE. However, depleting the putative endoribonuclease KHNYN specifically increases viral replication and RNA abundance for HIV containing a ZRE and its paralog N4BP1 may contribute a small amount of activity. Overall, we propose that the major pathway for ZAP-mediated decay of retroviral RNA is that TRIM25 promotes ZAP binding to viral RNA and ZAP then recruits KHNYN to mediate endonucleolytic cleavage of the transcript.

P48 Genome-wide assessment of RNA turnover reveals stress-specific changes in RNA dynamics

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Control of RNA degradation is critical to maintain cellular homeostasis, with its dysfunction related to an array of diseases. DIS3L2 is a highly conserved 3'-5' exoribonuclease responsible for specific and targeted RNA decay and is mutated in the human overgrowth disorders Perlman syndrome and Wilms' tumour of the kidney. In previous work we used Drosophila melanogaster and human HEK-293T cells to demonstrate that the loss of DIS3L2 results in increased cell proliferation and tissue growth in a PI3-K/mTORC1 dependent manner. More recently, we have uncovered a conserved role for DIS3L2 during the stress response. Loss of DIS3L2 in both HEK-293T cells and Drosophila melanogaster results in a significant resistance to nutrient deprivation stress. Despite the fundamental role of stress response mechanisms in facilitating cell survival during environmental stress, the role of RNA decay within these processes has been largely overlooked. Here, we used SLAM-seq in an unbiased approach to directly characterise genome-wide RNA decay rates in both wild-type and DIS3L2 deficient HEK-293T cells under normal, nutrient depleted and endoplasmic reticulum stress conditions. Crucially, our approach does not require transcriptional inhibition, and therefore allows a full endogenous stress response whilst providing the simultaneous measurement of changes in transcription, RNA decay and the resulting effect on steady state levels. Using this approach, we have begun to unpick the stress-specific transcriptional and post-transcriptional contributions to changes in gene expression, reveal widespread changes in the regulation of RNA degradation in response to stress, and show that DIS3L2 may play a fundamental regulatory role. We show that DIS3L2 targeting shows drastic changes in response cellular stress, with many transcripts showing stress-specific sensitivity and are now beginning to the unlock the molecular mechanisms that may govern these processes.

P49 Identification of m^{6}A modification in viroid RNA using LC-MS and Nanopore direct RNA sequencing

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Viroids are small circular non-coding RNAs that act as infectious pathogens in higher plants causing serious damage in agriculture. Despite viroid RNAs do not code for any proteins, viroids can autonomously replicate in plant cells. The replication can occur either in the nucleus (family Pospiviroidae), or in the chloroplasts (family Avsunviroidae). The structural features of viroid RNA determining the subcellular sorting into these two organelles are not well understood, however, RNA modifications were suggested to play a role. Here, we set out to identify the m6A RNA modifications in the Citrus exocortis viroid (CEVd, Pospiviroidae) and the Avocado sunblotch viroid (ASBV, Avsunviroidae) using LC-MS and Oxford Nanopore Technology (ONT) direct RNA sequencing approaches. Using LC-MS analysis we detected m6A and ribose methylated nucleosides in viroid RNA. While we were not able to detect m6A modification in the ASBV RNA, the CEVd (Pospiviroidae) RNA contained one m6A modification per molecule of RNA. To precisely allocate the m6A modification into the structural context of the CEVd viroid RNA, we have implemented a protocol for ONT direct RNA sequencing of viroid RNA. Using several available algorithms for m6A detection in direct RNA reads, we identified candidate m6A sites and we are currently confirming these sites by biochemical methods. Once confirmed, we plan to perform in vivo functional studies to reveal the role of m6A modification in CEVd viroid biology.

P50 Understanding ADARs role in innate immune response via protein-protein interactions

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Inosine is one of the most abundant RNA modifications present in mammalian cells. It is generated via hydrolytic deamination of adenosine in double-stranded (ds)RNA by the family of an enzymes; adenosine deaminases acting on a dsRNA (ADARs). The presence of inosine in dsRNA is an important mark of cellular "self" dsRNA and is critical for preventing aberrant activation of the innate immune response. In mammals, ADAR1 and ADAR2 have enzymatic activity with the former having two isoforms; a constitutively expressed, predominantly nuclear isoform (p110) and an interferon (IFN)-inducible cytoplasmic isoform (p150). There are also editing-independent functions of ADAR1 that are important in the regulation of the immune response.

To further understand this editing independent roles of ADARs, as well as to find regulators of RNA editing activity, we investigated ADAR protein-protein interactions under different conditions. Network of proteins interacting with endogenous ADAR1, under non-treated and IFN-induced conditions was obtained. To determine the isoform-specific role of ADAR1, inducible Flp-In-293 stable cell line, overexpressing both ADAR1p150 and ADAR1p110, as well as ADAR2 were generated. Enrichment of the more transient interactions was obtained with the proximity labelling technique, BioID, as well as affinity tag-based immunoprecipitation. Multiple common complexes were identified across all datasets, ranging from rRNA, mRNA processing factors, and miRNA biogenesis components. Taken together, we have a comprehensive data set of ADAR1 protein complexes with and without induction of the immune response. Furthermore, our results are consistent between all datasets and in agreement with published interacting proteins of ADARs.

P51 Role of RNA binding in the antiviral activity of TRIM25

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TRIM25, an E3-ubiquitin ligase, is involved in antiviral mechanisms against several viruses (HIV-1, Ebola virus, Sindbis virus and influenza), however whether this is due to direct antiviral activity or activation/ recruitment of associated proteins is still unclear. One such associated protein is ZAP (Zinc finger antiviral protein), which acts by binding RNA regions rich in CpGs, targeting them for degradation. Previously, we were able to modulate antiviral activity by altering CpGs present in the viral genome of an Ebola transcription and replication competent virus like particle system (EBOV trVLP). Furthermore, the antiviral activities of both ZAP and TRIM25 on EBOV trVLPs were co-dependent on each other, however the exact mechanism of this is unknown. But overexpression of either led to a reduction in cellular RNA.

TRIM25 comprises a RING domain, two Bbox domains, a coiled-coil domain and a SPRY domain, which contains the RNA binding domain (RBD). Previously, we have shown that not only E3 ubiquitin ligase activity and RING dimerisation but also the RNA binding ability are important for the antiviral activity of TRIM25 against EBOV trVLPs. For this observation we employed a mutant carrying the deletion of the RBD domain (ΔRBD) but it was unclear whether attenuation of TRIM25-mediated antiviral activity was due to the absence of RNA binding or whether it was due to a potential protein misfolding caused by the removal of a significant region in the SPRY region.

In this study we attempt to further explore the role of RNA binding in the antiviral activity of TRIM25 on EBOV trVLPs. Here we utilised targeted point mutations at critical residues in the RBD of TRIM25, to inhibit RNA binding without affecting SPRY domain structure. Additionally, we investigated the impact of these RNA binding mutants in the context of TRIM25 association with ZAP.

P52 LINE-1 mRNA 3’ end dynamics shape its biology and retrotransposition potential

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LINE-1 (L1) retrotransposons are mobile genetic elements posing a mutational danger to modern human genomes and underlying self-immune reactivity. L1s are tightly regulated by multi-layered transcriptional and post-transcriptional processes. To learn about the role of L1 mRNA 5’ end in L1 biology and retrotransposition we comprehensively analyzed the effects on L1 of XRN1, the major cytoplasmic 5’→3’ exoribonuclease. We hypothesized that in the absence of XRN1 there would be more L1 mRNA and more L1 retrotransposition. Conversely, we observed that loss of XRN1 substantially decreased L1 retrotransposition despite stabilization of L1 mRNA. By analyzing the effects on L1 of depletion of DCP2, the catalytic subunit of the decapping complex, we excluded major contributions of 5’ cap and L1 proteins’ translation on L1 retrotransposition. Instead, our observations support the crucial role of L1 3’ ends, whereby in both XRN1 and DCP2 depletion conditions we observe shortening of the poly(A) tails and their increased uridylation. We thus nail down the observed reduction of L1 retrotransposition to the quality of the L1 mRNA 3’ ends demonstrating the unappreciated role of the 3’ end dynamics in L1 biology.

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Additional Posters

P53 Single-molecule imaging reveals translation- dependent destabilization of mRNAs

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The relationship between mRNA translation and decay is incompletely understood, with conflicting reports suggesting that translation can either promote decay or stabilize mRNAs. The effect of translation on mRNA decay has mainly been studied using ensemble measurements and global transcription and transla- tion inhibitors, which can have pleiotropic effects. We developed a single-molecule imaging approach to con- trol the translation of a specific transcript that enabled simultaneous measurement of translation and mRNA decay. Our results demonstrate that mRNA translation reduces mRNA stability, and mathematical modeling suggests that this process is dependent on ribosome flux. Furthermore, our results indicate that miRNAs mediate efficient degradation of both translating and non-translating target mRNAs and reveal a predominant role for mRNA degradation in miRNA-mediated regulation. Simultaneous observation of translation and decay of single mRNAs provides a framework to directly study how these processes are interconnected in cells.

P54 The HelZ2 factor implicated in viral infections is a new human 3'-5' exoribonuclease of the RNB family

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To date, 3 human proteins with 3'-5' exonucleolytic RNB domain have been described and characterized: DIS3, DIS3L1 and DIS3L2. Database searches indicate that HelZ2 (Helicase with Zinc Finger 2) also possesses an RNB domain, albeit with a substitution of a key conserved amino acid. Interestingly, HelZ2 has recently been shown to be interferon-induced and to modulate positively or negatively infection by SARS-CoV2, Dengue and hepatitis C viruses.
Analysis of the human HelZ2 sequence stored in databases reveals that the central RNB domain in its center flanked at its N-terminus by 2 Zn fingers and a SF1 helicase domain while a second SF1 domain is present at its C-terminal side. Both helicase domains belong to the UPF1 family. Intriguingly, the described human protein is significantly shorter than its metazoan counterparts. Gene organization, protein conservation, capping and Ribo-seq data suggested that human HelZ2 expression relies on non-canonical translation initiation, adding several putative Zn fingers to the protein. This peculiarity was validated experimentally and may be related to control of its expression by interferon as well as during viral infections. Activity tests demonstrate that HelZ2 is endowed with 3'-5' exoribonuclease activity despites its divergence from the consensus. HelZ2 substrate characteristics were determined. In the presence of nucleic acid, HelZ2 also possesses ATPase activity steming from its helicase domains. Our analysis demonstrate that HelZ2 is the 4th RNB exoribonuclease in human. Information on its molecular function of HelZ2 will be key to decipher its physiological role.

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