CEITEC is a scientific centre in the fields of the life sciences, advanced materials and technologies which aims to establish itself as a recognized centre for basic as well as applied research. CEITEC offers a state-of-the-art infrastructure and excellent conditions for the employment of outstanding researchers. It is a consortium of partners that include the most prominent universities and research institutes in Brno, Czech Republic: Masaryk University, Brno University of Technology, Mendel University in Brno, Institute of Physics of Materials of the Academy of Sciences of the Czech Republic, University of Veterinary and Pharmaceutical Sciences Brno and the Veterinary Research Institute. CEITEC works closely with the Region of South Moravia and the City of Brno to help increase local innovative capacity.

**RESEARCH PROGRAMMES**

- **RP1** | Advanced Nanotechnologies and Microtechnologies
- **RP2** | Advanced Materials
- **RP3** | Structural Biology
- **RP4** | Genomics and Proteomics of Plant Systems
- **RP5** | Molecular Medicine
- **RP6** | Brain and Mind Research
- **RP7** | Molecular Veterinary Medicine

**BASIC OVERVIEW**

- 6 partners | 7 research programmes
- 61 research groups | 557 researchers (2015)
- 25,000 m² of new laboratories
- 10 core facilities

**www.ceitec.eu**
Abstracts of papers presented at the
CEITEC Conference: Nucleic Acids and Immunity
4-7 June, 2017

Scientific Organisers:
Mary O’Connell, CEITEC Masaryk University, Czech Republic
Liam Keegan, CEITEC Masaryk University, Czech Republic

Conference Organisers:
Dana Černošková, CEITEC Masaryk University, Czech Republic
Martin Smolík, CEITEC Masaryk University, Czech Republic

Funded under FP7 project, The ERA Chair Culture as a Catalyst to Maximize the Potential of CEITEC’ (contact no. 621368).
THANK YOU...

Conference is realized under the patronage of the Mayor of the City of Brno, Petr Vokřál and with support by the City of Brno.

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Silver Sponsors:

Other Sponsors:

Poster Session Award:
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Dear Colleagues,

As we say in Czech “Velmi vítáme ty, kdo zavítali na konferenci poprvé, a také Vás, se kterými jsme se už setkali na podzim na úplně první konferenci”.

You are all very welcome to the second ERA Chair Conference on: Nucleic Acids and Immunity. For those of you for whom this is your first visit, we hope you will enjoy the conference that is set in this historic city of Brno that will be forever linked to the great pioneer in genetics; Johann Gregor Mendel. We are also very happy and grateful that many of you have returned again to participate in this conference series.

This conference is second in a series of three funded by the EU under the ERA Chair program. The theme of these conferences is to explore the links between nucleic acid and immunity. The last conference which will be held September 2018 will expand on this theme and also include the topic of genome defense. We hope that this conference series will foster future research and collaborations in this exciting field.

During the conference you will get an opportunity to participate in a walking tour of Brno, a visit to Central European Institute of Technology (CEITEC) building at Masaryk University and a private tour of Mendel’s Abbey.

We would like to thank all speakers, members of the CEITEC team for organization, Masaryk University and of course our sponsors. We hope your stay in Brno will be memorable and that you will return again.

Best wishes

Mary O’Connell & Liam Keegan
GENERAL INFORMATION

- **Internet Facilities**
  Free Wi-Fi internet connection is available in all rooms free of charge. No password needed.

- **Time Zone**
  The local time in the Czech Republic at the time of the conference will be GMT +2 due to Summer Daylight Saving Time.

- **Electricity**
  The Czech Republic uses a 230 volt 50 Hz system.

- **Emergency Telephone Number**
  The emergency phone number is 112.

- **Insurance**
  The organizers of the conference do not accept liability for any injury, loss or damage, arising from accidents or other situations during the conference. Participants are therefore advised to arrange insurance for health and accident prior to travelling to the conference.

- **Taxi Service**
  We recommend using taxi service of the following reliable company:
  City taxi plus s. r. o. +420 542 321 321 or use hotel taxi.

- **Cloakroom**
  Cloakroom is located at the ground floor opposite of the registration desk. Service is provided free of charge during the official programme.

- **Venue**
  BEST WESTERN PREMIER
  Hotel International Brno ****
  phone: +420 542 122 111
  Address: Husova 16, 602 00 Brno, Czech Republic
  GPS: 49°11'41.55”N, 16°36'17.24”E

REGISTRATION AND INFORMATION DESK

All participants must be registered before attending the lectures.

**Opening hours**

<p>| | | |</p>
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<tr>
<td>Sunday</td>
<td>June 4, 2017</td>
<td>18:00 – 19:00</td>
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<tr>
<td>Monday</td>
<td>June 5, 2017</td>
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<tr>
<td>Wednesday</td>
<td>June 7, 2017</td>
<td>08:30 – 12:00</td>
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Badges
Participants and accompanying persons will receive a name badge upon registration. Everyone is kindly requested to wear their name badge when attending the conference. Only participants who are wearing their name badge will be admitted to the lecture halls.

Name badges have been colour-coded as follows:

<table>
<thead>
<tr>
<th>Colour</th>
<th>Description</th>
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<tr>
<td>Orange</td>
<td>Speakers</td>
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<td>Grey</td>
<td>Sponsors</td>
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<tr>
<td>Green</td>
<td>Participants</td>
</tr>
<tr>
<td>Blue</td>
<td>Organisers</td>
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</table>

Official Language
The official language of the conference is English.

Programme Changes
The organizers cannot assume liability for any changes in the programme due to external or unforeseen circumstances.

Mobile phones
Participants are kindly requested to keep their mobile phones in the off position in the meeting room while the session is being held.

Photograph
Photography is not allowed during the conference.

Social Events

Welcome Dinner, Hotel International – Siesta Lobby Bar
- Sunday, June 4
- Starts at 19:00

Guided city tour of Brno
- Sunday, June 4
- Starts at 15:00
- Meeting at hotel lobby at 14:50

Tour of CEITEC
- Tuesday, June 6
- Starts at 17:30
- Kamenice 5
- The shuttle will leave from the hotel at 17:15

Visit of Mendel Museum
- Tuesday, June 6
- Starts at 18:45
- Mendel Square 1a

Gala Dinner in Augustinian Abbey
- Tuesday, June 6
- Starts at 20:00
- Mendel Square 1a
POSTER SESSION

Posters will be displayed continuously Monday to Wednesday in the Poster session room C. One poster session have been scheduled to the conference programme. Scientific organizers have selected short talks for plenary sessions from submitted poster abstracts. The poster boards will be ready on Sunday June 4 from 18:00. When you bring the poster you will get the number at registration (the same as in book of abstract). The same number will be already displayed on the board. All posters must be displayed on Monday June 5 at 16:00 in the Poster session room C. Pins for hanging posters will be available at the hall.

Requirements
The participant is responsible for making sure that the poster display fits on the display board, and is completely responsible for attaching the individual elements to the display board according to our instructions.

Poster session programme and information for presenting authors
Poster session – Monday 5th June 18:00 – 19:30. Presenting authors are kindly requested to present throughout the official poster sessions time in order to explain their research and to answer the questions. There will be no guided formal discussion.

Book of Abstracts
Each participant receive the printed version.

RNA Society Award
All posters and short talks from junior delegates will be included in the poster competition. The two winners will be selected by scientific committee. The winner will be announced on Wednesday June 7th at 11:10.
PROGRAMME

Sunday 4th June

11:00  Bus leaving from Prague Convention Centre to Brno

15:00  Guided city tour of Brno

19:00  Welcome buffet dinner in Siesta Lobby Bar

Day 1, Monday 5th June

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<tr>
<th>Congress hall A</th>
<th>SESSION 1: Viruses and RNAi – Chair: Mary O’Connell</th>
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<tr>
<td>09:00 – 09:15</td>
<td>Opening Remarks</td>
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<tr>
<td>09:15 – 10:15</td>
<td>KEYNOTE SPEECH</td>
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<tr>
<td>09:15 – 10:15</td>
<td>Shizuo Akira – The control of inflammatory and immune responses by the endoribonuclease Regnase-1</td>
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<tr>
<td>10:15 – 10:45</td>
<td>Jean-Luc Imler – A dedicated siRNA pathway for antiviral immunity in Drosophila</td>
</tr>
<tr>
<td>10:45 – 11:15</td>
<td>Jörg Vogel – Dual RNA-seq and Single-cell RNA-seq approaches to studying Salmonella pathogenesis</td>
</tr>
<tr>
<td>11:15 – 11:45</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>11:45 – 12:15</td>
<td>Pierre Maillard – Unmasking the antiviral activity of RNA interference in mammalian cells</td>
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<tr>
<td>12:15 – 12:35</td>
<td>Sara Macias – INTERFERing with small RNAs</td>
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<tr>
<td>12:35 – 12:45</td>
<td>Group Photo</td>
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<tr>
<td>12:45 – 14:00</td>
<td>Lunch</td>
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<tr>
<th>Congress hall A</th>
<th>SESSION 2: RNA Modification – Chair: Vincent Kelly</th>
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<td>14:00 – 14:30</td>
<td>Kazuko Nishikura – ADAR1 controls apoptosis of stressed cells by inhibiting Staufen1-mediated mRNA decay</td>
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<tr>
<td>14:30 – 15:00</td>
<td>Yuri Motorin – High-throughput profiling of tRNA 2’-O-methylation in Bacteria</td>
</tr>
<tr>
<td>15:00 – 15:30</td>
<td>Yi-Tao Yu – RNA-guided RNA pseudouridylation and its applications</td>
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</tbody>
</table>
15:30 – 16:00 | Coffee Break

16:00 – 16:30 | Michaela Frye – Cytosine-5 RNA methylation in stem cells and disease

16:30 – 17:00 | Eric Miska – Attenuation of RNA viruses through 3’ terminal uridylation of the viral genome

17:00 – 17:20 | David Courtney – Epitranscriptomic enhancement of influenza A virus gene expression and replication

17:20 – 17:40 | Manuela Ye – RNA editing signature during myeloid leukemia cell differentiation

18:00 – 19:30 | Poster session and Refreshment (room C)

Day 2, Tuesday 6th June

Congress hall A | SESSION 3: Nucleic Acid Sensors – Chair: Jan Rehwinkel

09:00 – 09:30 | Emiko Uchikawa – Structural study of viral RNA detection by Rig-I- like helicases in innate immune system

09:30 – 10:00 | Liam Keegan – Evolutionarily conserved biological roles of ADAR RNA editing enzymes

10:00 – 10:20 | Ashish Dhir – Mitochondrial double stranded RNA triggers antiviral signalling in humans

10:20 – 10:40 | Rachel Ancar – Novel RNA end modifications detected by innate immune RNA sensors

10:40 – 11:10 | Coffee Break

11:10 – 11:40 | Gunther Hartmann – Direct RIG-I activation of NK cells triggers TRAIL-dependent cytotoxicity against autologous tumor cells

11:40 – 12:00 | Chun Kim – RIPK1 prevents ZBP1-mediated necroptosis

12:00 – 12:20 | Florian Ebner – The RNA-binding protein tristetraprolin schedules apoptosis of pathogen-engaged neutrophils during bacterial infection

12:20 – 12:40 | Mikhail Lebedin – Molecular barcoding as a tool for error-free antibody repertoire analysis
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<tbody>
<tr>
<td>12:40 – 14:00</td>
<td>Lunch</td>
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<tr>
<td></td>
<td><strong>SESSION 4: Nucleic Acid Modification – Chair: Kazuko Nishikura</strong></td>
</tr>
<tr>
<td>14:00 – 14:30</td>
<td>Mark Helm – How to shirk recognition of RNA by TLR7</td>
</tr>
<tr>
<td>14:30 – 15:00</td>
<td>Mariano Garcia-Blanco – Flaviviral RNAs and host innate immunity: a love-hate relationship</td>
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<tr>
<td>15:00 – 15:30</td>
<td>Vincent Kelly – A loaded GUN: artificial modification of transfer RNA to target autoimmune disease</td>
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<tr>
<td>15:30 – 16:00</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>16:00 – 16:30</td>
<td>Victoria Cowling – mRNA cap regulation during T cell activation</td>
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<tr>
<td>16:30 – 17:00</td>
<td>Anders Virtanen – Variable penetrance of PARN (poly(A)-specific ribonuclease) loss-of-function (LOF) variants in a three generation pedigree</td>
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<tr>
<td>17:30 – 18:30</td>
<td>Tour of CEITEC MU</td>
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<td>18:45 – 20:00</td>
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<td>20:00 – 23:00</td>
<td>Gala Dinner in Augustinian Abbey</td>
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**Day 3, Wednesday 7th June**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<tbody>
<tr>
<td></td>
<td><strong>SESSION 5: Infectious and Inflammatory Disease</strong></td>
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<tr>
<td></td>
<td>Chair: Eric Miska</td>
</tr>
<tr>
<td>09:00 – 09:30</td>
<td>Rayk Behrendt – The role of endogenous nucleic acids in the initiation of systemic autoimmunity</td>
</tr>
<tr>
<td>09:30 – 10:00</td>
<td>Mariano Garcia-Blanco – Flaviviral RNAs and host innate immunity: a love-hate relationship</td>
</tr>
<tr>
<td>10:00 – 10:20</td>
<td>Theopold Ulrich – Innate immune reactions against wounds and tumors – an insect model</td>
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<tr>
<td>10:20 – 10:40</td>
<td>Fujinaga Koh – Hili inhibits HIV replication in activated T cells</td>
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<tr>
<td>Time</td>
<td>Session</td>
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<tr>
<td>10:40 – 11:10</td>
<td>Coffee Break</td>
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<tr>
<td>11:10 – 11:25</td>
<td>RNA Society Award</td>
</tr>
<tr>
<td>11:25 – 11:55</td>
<td>Jan Rehwinkel – Z-RNA sensing by ZBP1/DAI induces necroptosis</td>
</tr>
<tr>
<td>11:55 – 12:15</td>
<td>Jaclyn Quin – Major transcriptional changes observed in the Fulani ethnic group less susceptible to malaria are associated with increased expression of non-coding RNAs</td>
</tr>
<tr>
<td>12:15 – 12:35</td>
<td>Pardi Norbert – Nucleoside-modified mRNA makes hemagglutinin into a potent universal influenza virus vaccine candidate</td>
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<tr>
<td>13:00 – 14:30</td>
<td>Lunch</td>
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Immune responses are accompanied by dynamic changes in gene expression. Gene expression is controlled at multiple points, including signal transduction, transcription and mRNA stability. So far, transcriptional regulation has been extensively studied. Many transcription factors including NF-kB and AP-1 are involved in induction of genes involved in inflammatory and immune responses. However, recent studies have revealed that control of gene expression at the mRNA level is as important as transcriptional control in the immune response. Gene expression profiles obtained from human Jurkat T cells stimulated with PMA plus ionomycin revealed that regulation of mRNA stability may account for as much as 50% of all measurements of changes in total cellular polyA mRNA. We have shown that Regnase-1 encoded by the Zc3h12a gene is an endoribonuclease involved in destabilization of a variety of mRNAs including IL-6, IL-12, and Regnase-1 itself mRNAs via the stem loop structure present in the 3’UTR of these genes. Although originally identified as LPS-inducible gene, Regnase-1 protein is present in unstimulated cells, and disappears in response to Toll-like receptor ligands via an IKK-dependent proteasome degradation pathway or in response to T cell receptor stimulation through the cleavage by Malt-1. Thus, Regnase-1 acts as a brake in unstimulated cells as well as a negative feedback regulator after cellular activation. I would like to discuss the role of Regnase-1 in the immune response.
S01 A dedicated siRNA pathway for antiviral immunity in Drosophila

F. Bergami1, L. Talide1, K. Majzoub1, Y. Verdier2, J. Vinh2, N. Martins1, C. Meignin1 & J.-L. Imler1

1 Université de Strasbourg- CNRS UPR9022, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France
2 École Supérieure de Physique et Chimie Industrielle, Laboratoire de Spectrométrie de Masse Biologique et Protéomique, Paris, France

RNA interference (RNAi) is a major pathway of antiviral host defence in insects. This mechanism relies on the RNase III enzyme Dicer-2 (Dcr-2), which processes viral double stranded (ds)RNAs into 21 nucleotides (nt) long small interfering (si)RNA duplexes. With the help of the dsRNA binding protein R2D2, these siRNAs are then loaded onto the enzyme Argonaute 2. There, one of the strand of the duplex will be discarded and the remaining siRNA will guide AGO2 towards target RNAs in a sequence specific manner. Other molecular players and interactants of this core pathway remain largely unknown.

To better understand the siRNA pathway, we have conducted a mass spectrometry analysis of the complexes that associate with Dicer-2, R2D2 and AGO2 in control and virus infected drosophila S2 cells. A targeted RNAi screen was then conducted to identify the functionally relevant interactions. These experiments identified novel co-factors of the core components of the siRNA pathway, which participate in the control of viral infections. Strikingly, some of the molecules identified are not required to silence gene expression triggered by exogenous long dsRNA. Our results point to the existence of a virus-derived siRNA pathway specifically dedicated to antiviral immunity, raising the question of the intrinsic features of viral RNA activating this pathway.
S02 Dual RNA-seq and Single-cell RNA-seq approaches to studying Salmonella pathogenesis

Vogel Jörg1,2
1 Institute for Molecular Infection Biology, University of Würzburg, Germany; 2 Helmholtz Institute for RNA-based Infection Research (HIRI), Würzburg, Germany

Understanding how bacteria cause disease requires knowledge of which genes are expressed and how they are regulated during infection. I will elaborate on two recent studies from the laboratory employing new approaches such as Dual RNA-seq and Single-cell RNA-seq to understand bacterial infections.

The first study looked for regulatory roles of small noncoding RNAs in Salmonella pathogenesis. Many of these small RNAs are conserved but hardly any of them gives a phenotype in standard virulence assays. To overcome this, we developed ‘dual RNA-seq’ to profile RNA expression simultaneously in pathogen and host during S. Typhimurium infection. This identified a PhoP-activated small RNA, PinT, which upon bacterial internalization temporally controls the expression of both invasion-associated effectors and virulence genes required for intracellular survival. This riboregulatory activity causes pervasive changes in coding and noncoding transcripts of the host. Interspecies correlation analysis linked PinT to host cell JAK-STAT signalling, and we also identified infection-specific alterations in multiple long noncoding RNAs. Dual RNA-seq provides a sensitive RNA-based analysis of intracellular bacterial pathogens and their hosts without physical separation, and lends itself as a new discovery route for hidden functions of pathogen genes.

In the other study, we used single-cell RNA-seq to understand how host cells respond to the large heterogeneity in intracellular growth rate displayed by Salmonella. The transcriptomes of individual infected macrophages revealed a spectrum of functional host response states to growing and non-growing bacteria. Intriguingly, macrophages harbouring non-growing Salmonella display hallmarks of the proinflammatory M1 polarization state and differ little from bystander cells. By contrast, macrophages containing growing bacteria have turned into an anti-inflammatory, M2-like state, as if fast-growing intracellular Salmonella overcome host defence by reprogramming macrophage polarization. Thus, gene expression variability in infected host cells shapes different cellular environments, some of which may favour a growth arrest of Salmonella facilitating immune evasion and the establishment of a long-term niche, while others allow Salmonella to escape intracellular antimicrobial activity and proliferate.
References:
*Dual RNA-seq unveils noncoding RNA functions in host-pathogen interactions*
*Nature* 529:496-501

*Resolving host-pathogen interactions by Dual RNA-seq*
*PLoS Pathogens* 13(2):e1006033

*Single cell RNA-seq ties macrophage polarization to growth rate of intracellular Salmonella*
*Nature Microbiology* 2:16206
S03 Unmasking the antiviral activity of RNA interference in mammalian cells

Maillard Pierre V.1, 2, Van der Veen Annemarthe G.1, Deddouche-Grass Safia1, Rogers Neil C.1, Merits Andres3, Reis e Sousa Caetano1

1 Immunobiology Laboratory, The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK
2 Present address: Division of Infection and Immunity, Medical Research Council Centre for Medical Molecular Virology, University College London, 90 Gower Street, London WC1E 6BT, UK
3 Institute of Technology, University of Tartu, 50411 Tartu, Estonia

Plants and invertebrates defend themselves against viral pathogens using a mechanism called RNA interference (RNAi), which is triggered by base-paired or long double-stranded (ds) RNA generated during virus infection. In contrast, it is debated whether this antiviral mechanism acts in vertebrates in which viral RNAs induce a distinct defence system known as the type 1 interferon (IFN) response. Our study demonstrates that long dsRNA-mediated RNAi (dsRNAi) is functionally active in mammalian differentiated cells, yet is inhibited or masked by the IFN pathway. Consistent with that notion, dsRNAi was revealed in IFN-defective cells and relied on canonical components of the RNAi pathway. Notably, dsRNAi specifically vaccinates cells against subsequent infection with viruses containing homologous sequences. Our data suggest that RNAi constitutes an ancient antiviral strategy that has been preserved during the evolution of vertebrate immunity.
**S04 ADAR1 controls apoptosis of stressed cells by inhibiting Staufen1-mediated mRNA decay**

*Kazuko Nishikura¹*

¹The Wistar Institute, 3601 Spruce Street, Philadelphia, Pennsylvania 19104, USA

Both p150 and p110 isoforms of ADAR1 convert adenosine to inosine in double-stranded RNA (dsRNA). ADAR1p150 suppresses the dsRNA sensing mechanism that activates MDA5-MAVS-IFN signaling in the cytoplasm. In contrast, the biological function of the ADAR1p110 isoform, usually located in the nucleus, remains largely unknown. Here we show that stress-activated phosphorylation of ADAR1p110 by MKK6-p38-MSK MAP kinases promotes its binding to Exportin-5 and export from the nucleus. Once translocated to the cytoplasm, ADAR1p110 suppresses apoptosis of stressed cells by protecting many anti-apoptotic gene transcripts that contain 3’UTR dsRNA structures primarily made from inverted Alu repeats. ADAR1p110 competitively inhibits binding of Staufen1 to the 3’UTR dsRNAs and antagonizes the Staufen1-mediated mRNA decay. Our studies revealed a new stress response mechanism, in which human ADAR1p110 and Staufen1 regulate surveillance of a set of mRNAs required for survival of stressed cells. This newly discovered ADAR1 function will be discussed in relation to its function in the dsRNA sensing mechanism mediated by the MDA5-MAVS-IFN pathway.
S05 High-throughput profiling of tRNA 2′-O-methylation in Bacteria

Virginie Marchand¹, Adeline Galvanin², Alexander Dalpke³, Mark Helm⁴, Yuri Motorin²

¹ Next-Generation Core Facility, FR3209 CNRS-UL, Biopole Lorraine University, 54505 Vandoeuvre-les-Nancy, France;  
² IMoPA UMR7365 CNRS-UL, Biopole Lorraine University, 54505 Vandoeuvre-les-Nancy, France;  
³ Department of Infectious Diseases, Medical Microbiology and Hygiene, Ruprecht-Karls University Heidelberg, 69120 Heidelberg, Germany  
⁴ Institute of Pharmacy and Biochemistry, Johannes Gutenberg University Mainz, 55128 Mainz, Germany

Analysis of RNA modifications by traditional physico-chemical approaches is labor intensive, requires substantial amounts of input material and only allows site-by-site measurements. The recent development of qualitative and quantitative approaches based on next-generation sequencing (NGS) opens new perspectives for the analysis of various cellular RNA species. The Illumina sequencing-based RiboMethSeq protocol was initially developed and successfully applied for mapping of ribosomal RNA (rRNA) 2′-O-methylations. This method also gives excellent results in the quantitative analysis of rRNA modifications in different species and under varying growth conditions. However, until now, RiboMethSeq was only employed for rRNA, and the whole sequencing and analysis pipeline was only adapted to this long and rather conserved RNA species. A deep understanding of RNA modification functions requires large and global analysis datasets for other important RNA species, namely for transfer RNAs (tRNAs), which are well known to contain a great variety of functionally-important modified residues. We evaluated the application of the RiboMethSeq protocol for the analysis of tRNA 2′-O-methylation in Escherichia coli, Saccharomyces cerevisiae and in human pathogen. After a careful optimization of the bioinformatic pipeline, RiboMethSeq proved to be suitable for relative quantification of methylation rates for known modified positions in different tRNA species.
**S06 RNA-guided RNA pseudouridylation and its applications**

**Yu Yi-Tao**

1 University of Rochester Medical Center

Pseudouridylation and 2’-O-methylation are the most abundant modifications found in RNAs. These modifications are catalyzed largely by RNA-guided mechanisms. By changing the guide sequences within the guide RNA, we can re-direct modifications to new sites. Importantly, our work indicates that modifications can profoundly alter the chemical properties of an RNA, thus influencing the contributions of the RNA to cellular process in which it participates.
Many of the hundreds of known chemical modifications in RNA were discovered over forty years ago but then forgotten because suitable, sensitive tools to detect the modifications at high resolution were lacking. Through the development of novel biochemical, functional and genomics tools we are only now beginning to understand the whole breadth and extensive functional roles of RNA modifications in higher organisms. I will present some of the first mechanistic examples how RNA modifications shape normal tissue homeostasis, and how aberrant formation of RNA modifications contributes to tumour development, progression and chemotherapy resistance. Together, our work demonstrates that by understanding the role of RNA modifications in physiology and pathology, novel and powerful therapeutic drug targets for human diseases and cancer can be identified and further optimized for clinical studies.
S08 Attenuation of RNA viruses through 3’ terminal uridylation of the viral genome

Miska Eric¹

¹ University of Cambridge, Great Britain

RNA viruses are a major threat to human, animal and plant health. RNA interference (RNAi) and the interferon response (IR) are the major innate antiviral defence systems. The Orsay RNA virus (OrV) and its natural host, the nematode Caenorhabditis elegans, are a new model to study host-virus interaction in a whole animal. Here we have performed the first large-scale genetic antiviral immunity screen in any animal and identified cde-1 as a gene essential for antiviral defence. CDE-1 is a homologue of the mammalian TUT4/7 terminal uridyl transferases. We show that the catalytic activity of CDE-1 is required for its antiviral function. Surprisingly, we find that CDE-1 acts independently of the RNAi pathway. Instead, we demonstrate that CDE-1 directly uridylates the 3’ end of the OrV RNA genome itself, which directs its degradation. In mammalian cells infected by the Influenza H1N1 virus, we find that TUT4/7 similarly uridylates the flu mRNAs and act as a first barrier to reduce the production of viral proteins. In summary, we define 3’ terminal uridylation of viral RNAs as a new, likely general, antiviral defence mechanism.
The innate immune response is the first defense line against the pathogens in the cell. This response plays a key role in infections by pathogens. The Rig-I-like helicases RIG-I, MDA5, and LGP2 are the primary innate immune pattern recognition receptors for sensing the negative stranded viral RNAs. Each of the RIG-I-like helicases functions differently. RIG-I and MDA5 are activated by the binding of the short 5' triphosphate blunt end of dsRNAs and long dsRNAs, respectively, resulting in downstream signaling and interferon production. LGP2, which lacks signaling domains, is proposed to be a positive regulator of MDA5 and a negative regulator of RIG-I. All Rig-I-like helicases hydrolyze ATP upon dsRNA binding, however, the role of ATP hydrolysis remain controversial.

We present several high-resolution crystal structures of full-length chicken (ch) LGP2 that uniquely depict the motions occurring upon ATP hydrolysis. The analysis of RNA binding and functional studies of ATPase mutants of Rig-I and MDA5 suggest that the Rig-I-like helicases could discriminate between self and non-self RNA using a kinetic mechanism that depends on ATP hydrolysis. Furthermore, we show that MDA5 activation can be enhanced by LGP2 in a dsRNA-binding-dependent manner. Using electron microscopy, we show a synergistic filament formation by chLGP2 and chMDA5 mixtures. As a future goal, we would like to resolve the molecular details of these cooperative processes by visualizing their complexes.
S10 Evolutionarily conserved biological roles of ADAR RNA editing enzymes

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ADAR RNA editing enzymes deaminate adenosine bases to inosines in RNA. During translation inosine is read as guanosine and this editing diversifies the proteins present in the organism. In vertebrates, the editing by ADAR1 protein also helps to differentiate between self and non-self dsRNA. ADAR editing generates dsRNA containing I-U base pairs and recent published work from our lab on the mouse Adar1 mutant demonstrated that ADAR1 prevents cellular dsRNA from aberrantly activating cellular innate immune responses. A mutation in human Adar1 leads to an extreme condition known as Aicardi-Goutieres Syndrome in which children with a defective ADAR1 aberrantly express antiviral interferon and die with encephalitis. The vertebrate ADAR2 protein is involved primarily in editing transcripts encoding ion channels subunits and other proteins, particularly in CNS, but also in pancreatic β-cells, where ADAR2 facilitates insulin secretion.

Drosophila has one Adar gene which is an orthologue of vertebrate Adar2. Hundreds of transcripts encoding ion channel subunits, membrane proteins and secretory and neurotransmitter vesicle-associated proteins are edited in Drosophila. AdarSG1 null mutant flies show locomotion defects, are male sterile and develop age-dependent neurodegeneration. There is also aberrant upregulation of transcripts encoding Anti-Microbial Peptides (AMPs), in AdarSG1 flies. This suggesting a possible role of ADAR2-type proteins in immunity; a role not identified in vertebrates. Drosophila Adar mutant phenotypes are rescued by human Adar2 expression. Deciphering the role of Adar in Drosophila will help us to understand the evolutionarily conserved roles of ADAR2-type RNA editing enzymes. The main vertebrate innate immune signalling are at least partially conserved in Drosophila melanogaster and preliminary results indicate a potential role of CNS-associated Toll signalling in rescuing AdarSG1 mutant phenotypes.

We previously performed a genetic screen for suppressors of the reduced viability associated with the AdarSG1 null mutant. This screen identified a strong rescue by reduced Tor gene dosage of all tested Adar mutant phenotypes, except male infertility. Reduced Tor gene dosage leads to increased autophagy, which rescues AdarSG1 mutant phenotypes. Increased expression of the autophagy regulators Atg1 and Atg5 mimics Tor mutant rescue of AdarSG1 mutant phenotypes. We propose that AdarSG1 mutant cells aberrantly activate autophagy to clear virus RNA or aberrant intracellular dsRNA. Our current work aims to identify interactions between innate immune signalling pathways and insulin /tor signaling pathways in the AdarSG1 mutant.

Abstracts of Speakers
S11 Direct RIG-I activation of NK cells triggers TRAIL-dependent cytotoxicity against autologous tumor cells

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Activation of the innate immune receptor retinoic acid-inducible gene I (RIG-I) by its specific ligand 5′-triphosphate RNA (3pRNA) triggers anti-tumor immunity dependent on natural killer (NK) cell activation and cytokine induction. However, to date RIG-I expression and functional consequences of RIG-I activation in NK cells have not been examined. Here, we show for the first time the expression of RIG-I in human NK cells and their activation upon RIG-I ligand (3pRNA) transfection. 3pRNA activated NK cells killed melanoma cells more efficiently than NK cells activated by type I Interferon. Whereas expression levels of different activating NK cell receptors were not affected, activation of RIG-I with 3pRNA increased the surface expression of TNF-related apoptosis-inducing ligand (TRAIL). 3pRNA-induced TRAIL expression initiated death receptor pathway mediated apoptosis not only in allogeneic but also in autologous HLA class I-positive and HLA class I-negative melanoma cells. Thus, our data unravel a novel mechanism how RIG-I activation in NK cells leads to enhanced killing of autologous tumor cells. This highlights RIG-I activation as promising drug target for the treatment of tumors.
S12 How to shirk recognition of RNA by TLR7

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Among the various sensors of the innate immune system that recognize nucleic acids, TLR7 is an endosomal Pattern-Recognition Receptor (PRR) destined to recognize hypomodified RNA as a pathogen-associated molecular patterns (PAMPs). TLR7 is tasked to discriminate “self” from “foreign” RNA, and the molecular details of the RNA that allow such discrimination are in the centre of our research efforts. From comparison synthetic unmodified RNAs to prokaryotic and eukaryotic RNAs, it became clear that chemical alterations such as native post-transcriptional, or synthetic modifications are the decisive parameter that governs RNA mediated TLR7 response. Using a combination of what is commonly known as RNA “microsurgery” and chemical synthesis, we have engineered several series of differentially modified RNAs. Testing the interferon response of dendritic cells to these RNAs has revealed different modifications that are capable of attenuating the TLR7-mediated response. Foremost among these are different ribose methylations, which can now be mapped in the epitranscriptome by RNA Seq approaches. A possible conjecture of these findings implies ribose methylation as an inducible means for immune evasion by microbial pathogens.
S13 m6A mRNA methylation regulates alternative splicing in Drosophila sex determination

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Methylation of adenosine to N6-methyladenosine (m6A) is the most common internal modification of eukaryotic messenger RNA (mRNA) and decoded by YTH (YT521-B homology) domain proteins. The mammalian mRNA m6A methylosome is formed by a complex of nuclear proteins that include METTL3 (Methyltransferase-like 3), related METTL14, WTAP (Wilms tumour 1 associated protein) and KIAA1429, and Drosophila has corresponding homologues named IME4 and METTL14, and Female-lethal(2)d (Fl(2)d) and Virilizer (Vir). In Drosophila, fl(2)d and vir are required for sex-dependent regulation of alternative splicing of the sex determination factor Sex-lethal (Sxl), an ELAV/Hu related RNA binding protein. However, the functions of m6A in alternative splicing regulation remain uncertain. Here we show that Drosophila lacking IME4 do not have m6A in mRNA. In contrast to mouse and plant knock-out models, Drosophila IME4 null mutants remain viable, though flightless and show a sex bias towards maleness. This is because m6A is required for female-specific alternative splicing of Sxl, which determines female physiognomy, but also translationally represses male-specific lethal2 (msl-2) to prevent dosage compensation normally occurring in males. We further show that the m6A reader protein YT521-B decodes m6A in the sex-specifically spliced intron of Sxl and phenocopies lack of dIME4. Loss of m6A also affects alternative splicing of a number of other genes, predominantly in the 5'UTR, and has global impacts on the expression of metabolic genes. Requirement of m6A and its reader YT521-B for female-specific Sxl alternative splicing reveal this hitherto enigmatic mRNA modification as constituting an ancient and specific mechanism to adjusts levels of gene expression.
Autoimmune diseases such as multiple sclerosis are characterised by the rapid expansion of T cells that are directed to self-antigens. Governing this immune process are numerous gene networks that, in addition to coordinating the uptake and utilisation of metabolic precursors, switch-on mRNA production for the provision of immune-relevant proteins. Protein synthesis is not exclusively controlled by mRNA abundance however, and additional regulatory steps act after genes have been expressed to control mRNA translation. Pivotal to the translation process are transfer RNA molecules that function as the decoding adapters for the genetic message.

Recently, we demonstrated that it is possible to control transfer RNA function for therapeutic purposes (Vargheese et al., 2017). This involves replacing a naturally occurring modification on transfer RNA, known as queuosine, with an artificial nucleobase, which we termed NPPDAG (a de novo designed small molecule). Queuosine itself is an extremely ancient transfer RNA modification found in almost all eukaryotic species in the anticodon loop of tRNA of the GUN family (Boland et al., 2011). Surprisingly, given its widespread occurrence in eukaryotic life, queuosine is synthesised exclusively by bacteria and in the case of humans must be salvaged from the gut flora or ingested from food as a nucleobase molecule (Rakovich et al, 2011; Fergus et al., 2015).

Administration of NPPDAG to spleen cell cultures was shown to result in its irreversible incorporation into RNA. When mice that were induced to succumb to experimental autoimmune encephalomyelitis, a established mouse model of multiple sclerosis, NPPDAG treatment resulted in an unprecedented reversal of clinical symptoms to baseline after only five daily doses. Furthermore, there was a dramatic reduction of markers associated with immune hyperactivation and neuronal damage even after one dose. Our data indicate that NPPDAG can selectively limit the expansion of activated T-cells, sparing the naïve T-cell population. This selectivity for activated immune cells is partly ensured by the fact that only transfer RNA of rapidly-proliferating, non-differentiated cells are under-modified with queuosine and are therefore receptive for NPPDAG incorporation.

A deeper understanding of how transfer RNA modifications influence the translation process may offer the opportunity for the therapeutic targeting of a wide range of immunological conditions.


S15 mRNA cap regulation during T cell activation

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The mRNA cap is a collection of structures which form on the 5’ end of RNA pol II transcripts. Capping enzymes catalyse modification of the first few nucleotides of mRNA to form the cap. Most of the capping enzymes are recruited to phosphorylated RNA pol II at the early stages of transcription, thus capping the transcript as it is being synthesised. Our research investigates how the capping enzymes function biochemically and how they influence gene expression. We look at how cellular signalling pathways influence the expression or activity of the capping enzymes to change gene expression, cell function and cell physiology. We have found that different signals which the cell encounters (developmental, immunological, oncogenic) can alter the rate and extent to which the mRNA cap forms, either across the transcriptome or on specific transcripts. Thus the mRNA cap is an integrator of cellular signalling information, which directs reshaping of the cellular proteome in response to external and internal signals. In the talk, I will discuss how T cell activation upregulates the capping enzymes and the impact that this has on gene expression and the response to activation.
Aicardi-Goutières syndrome (AGS) is rare genetic Lupus-like condition in which chronic stimulation of cytoplasmic nucleic acid sensors results in production of pathogenic amounts of type I interferon (IFN) and causes autoimmunity. In addition to mutations in five other genes, loss of function mutations in the Three Prime Repair Exonuclease 1 (TREX1, DNaseIII) and in SAM Domain and HD Domain 1 (SAMHD1) cause AGS. Loss of Trex1 in mice triggers a cGAS-dependent type I interferon (IFN) response and systemic autoimmunity. Products of reverse transcription originating from endogenous retroelements have been suggested to be a major substrate for Trex1 and reverse transcriptase inhibitors (RTIs) were proposed as a therapeutic option in autoimmunity ensuing from defects of Trex1. On the other hand it was suggested that the enzyme degrades genomic “waste”, which is produced during its replication or repair. Nucleotide excision repair (NER) is a major DNA repair pathway, which ultimately releases DNA flaps from the genome that could be a substrate for Trex1. Interestingly, neither treatment of Trex1-/- mice with RTI nor inactivation of NER had any effect on the activation of the IFN system or systemic autoimmunity, raising the question of the actual origin of the pathogenic DNA in TREX1-deficient humans and mice.

Samhd1-deficient mice are devoid of any autoimmune pathology but feature the spontaneous transcriptional ISG signature found in the patients suggesting ongoing chronic innate immune activation. In these mice, we found that Samhd1-deficient macrophages spontaneously produce IFNβ that subsequently activates transcription of ISGs. Surprisingly, additional inactivation of both, the intracellular RNA and DNA sensing machinery, by knocking out Mavs or Sting in Samhd1-deficient mice, respectively, suppressed the spontaneous IFN production. This finding is in sharp contrast to the situation in Trex1-/- and Adar-/- mice, in which either a functional RLR or cGas-Sting pathway is required to spontaneously activate the type I IFN system. Our findings might point to a so far unrecognized function of Samhd1 in the suppression of a spontaneous cell-intrinsic immune response against an aberrant immunogenic RNA species.
S17 Flaviviral RNAs and host innate immunity: a love-hate relationship

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The subgenomic flaviviral RNA (sfRNA) is a non-coding RNA that interacts with host RNA binding proteins to cripple the innate immune system for the human host and the mosquito vector. We will present several examples that elucidate the mechanisms by which this non-coding RNA interferes with immune mechanisms, and enhances viral fitness and epidemic potential.
S18 Z-RNA sensing by ZBP1/DAI induces necroptosis

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Nucleic acids are potent triggers for innate immunity. Double stranded DNA and RNA adopt different helical conformations, including the unusual Z-conformation. Z-DNA/RNA is recognised by Z-binding domains (ZBDs), which are present in proteins implicated in antiviral immunity. These include ZBP1 (also known as DAI or DLM-1), which induces necroptosis, an inflammatory form of cell death. Using reconstitution and knock-in models, we report that mutation of key amino acids involved in Z-DNA/RNA binding in ZBP1’s ZBDs prevented necroptosis upon infection with mouse cytomegalovirus. Induction of cell death was cell-autonomous and required RNA synthesis but not viral DNA replication. Accordingly, ZBP1 directly bound to RNA via its ZBDs. Intact ZBP1-ZBDs were also required for necroptosis triggered by ectopic expression of ZBP1 and caspase blockade, and ZBP1 cross-linked to endogenous RNA. These observations show that Z-RNA constitutes a molecular pattern that induces inflammatory cell death upon sensing by ZBP1.
Innate immune sensors must respond rapidly to a variety of pathogens while avoiding aberrant detection of normal cellular molecules. RIG-I-like receptors (RLRs) are cytosolic dsRNA sensors that bind viral RNA transcripts. RLRs such as retinoic acid inducible gene I (RIG-I) and Melanoma Differentiation-Associated protein 5 (MDA5) couple detection of viral RNAs to the expression of type 1 interferon and pro-inflammatory cytokine genes. Structural and biochemical studies previously identified short viral dsRNAs with 5´-triphosphate moieties as RIG-I ligands. The 5´-triphosphate is a unique feature of viral replication, suggesting a mechanism by which RIG-I would specifically identify viral RNAs while avoiding recognition of self-RNAs. Recent studies show that RNA cleavage products of the endoribonucleases Ire1 and RNase L can activate a RIG-I-dependent type I interferon response. However, these endoribonucleases produce RNA products with 5´-OH and 2´,3´-cyclic phosphate termini, so it is unclear how these RNA cleavage products activate RIG-I. We hypothesize that RNA end modification enzymes remodel the termini of these RNA cleavage products to promote their recognition by RIG-I. We use genetic and biochemical approaches to determine the impact of RNA modification enzymes on RNA detection by RIG-I and define the chemical requirements for RIG-I mediated detection of RNA cleavage products. Our preliminary studies suggest that the 5´ and 3´-ends of RNA are differentially detected by RIG-I, possibly by 3´-end RNA modification enzymatic activity. We show that synthetic dsRNAs with 3´-monophosphates are capable of activating interferon expression, while 5´-monophosphates do not activate the antiviral response. Furthermore, interferon expression is dependent on the position of the phosphate at the 3´-end of the RNA. These studies suggest that host RNA end modification enzymes modulate detection of intracellular RNA by RIG-I.
P02 Epitranscriptomic enhancement of influenza A virus gene expression and replication

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Many viral RNAs are modified by methylation of the N6 position of adenosine (m6A), yet the effect of m6A on RNA function remains unclear. Influenza A virus (IAV) was the first virus reported to encode extensively m6A-modified RNAs and we demonstrate that global inhibition of m6A addition strongly inhibits IAV gene expression and replication. In contrast, overexpression of YTHDF2, a key cellular m6A “reader” protein, increases IAV gene expression and replication by ≥10-fold. To address whether m6A residues modulate IAV RNA function in cis, we mapped the m6A residues located on the IAV plus (mRNA) and minus (vRNA) strands and then used synonymous mutations to ablate m6A on both strands of the IAV HA gene. These mutations selectively inhibited HA mRNA and protein expression while leaving other IAV mRNAs and proteins unaffected. We conclude that IAV uses m6A residues on its transcripts to enhance viral gene expression.
Mitochondria as descendants of endosymbiotic bacteria retain essential prokaryotic features such as a compact circular genome. Consequently, in mammals, the mitochondrial DNA (mtDNA) is subjected to bidirectional transcription that inadvertently generates overlapping transcripts capable of forming long double-stranded (dsRNA) structures. Nevertheless, mitochondrial (mt) dsRNA has not been previously characterized in vivo. Here, we describe the presence of a highly unstable native mtdsRNA species at single cell level and identify the key roles for the degradosome components, mitochondrial dsRNA helicase SUV3 and exoribonuclease PNPase in restricting mt dsRNA levels. Loss of either enzyme results in massive accumulation of mt dsRNA that escapes into the cytoplasm in a PNPase dependent manner. This engages cytosolic antiviral signalling that triggers the type I interferon response. Consequently, loss of PNPase leads to cell death which is reversible upon loss of the cytosolic dsRNA receptor MDA5. The localisation of PNPase to the mitochondrial inter-membrane space (IMS) argues for its role as a barrier to the release of mt dsRNA into the cytoplasm. This in turn acts to prevent the engagement of potent innate immune defence mechanisms which have evolved to protect eukaryotic cells against microbial and viral attack.
HIV is the retrovirus that causes AIDS. Piwil proteins restrict the replication of mobile genetic elements in the germline. They are also expressed in many transformed cell lines. In this report, we discovered that the human piwil 2 (hili) can also inhibit HIV replication, especially in activated CD4+ T cells that are the preferred target cells for this virus in the infected host. Although resting cells do not express hili, it is rapidly induced following T cell activation. In these cells and transformed cell lines, depletion of hili increased levels of viral proteins and new viral particles. Further studies revealed that hili binds to tRNA. Some of them represent rare tRNA species, whose codons are over-represented in the viral genome. Targeting tRNAArg(TCT) with an antisense oligonucleotide replicated effects of hili and also inhibited HIV replication. Thus, hili joins a list of host proteins that inhibit the replication of HIV.
N6-methyladenosine demethylase FTO targets pre-mRNAs and plays a role in nuclear mRNA processing

**P05**

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Nucleotide modifications of mRNA, recently termed as the “epitranscriptome” (e.g. m6A, m6Am, m5C, pseudouridylation or A to I editing) represent an important mechanism for regulation of gene expression. In mammalian mRNAs, the most prevalent is the internal N6-methyladenosine (m6A). M6A was implicated in the regulation of diverse processes of mRNA metabolism, ranging from pre-mRNA splicing, nuclear export, stability and translation. Interestingly, the epitranscriptome is not static in nature and can be actively shaped by RNA demethylases. Human cells express at least two m6A demethylases, ALKBH5 and FTO. Whereas ALKBH5 has been implied in spermatogenesis and nuclear export of polyA RNAs, the function of FTO remains largely unexplored. By using the combination of CLIP-seq and RNA-seq strategies, we uncovered that FTO preferentially binds to pre-mRNAs in intronic as well as exonic regions and acts as a potential regulator of pre-mRNA processing. We demonstrate, that FTO activity promotes inclusion of a subset of alternative exons and is most likely involved in the regulation of 3’ end processing.
Abstract

Discovering altered expression of microRNAs (miRNAs) in many disease states, including obese and diabetes mellitus, and increasing relevance of miRNAs in biofluids in different pathologies has prompted the study of their potential application in metabolic disorders diseases as biomarkers in order to identify new therapeutic targets. The aim of this study was to identify and characterize microRNAs that are differentially expressed in obese, diabetic and control C57BL/6 mice by using small RNA sequencing. Thirty male C57BL/6NTac mice were used to identify differentially expressed microRNA in obese and diabetic animals. Total RNAs were extracted from the serum of ten obese, diabetic and control mice, respectively, and pooled for each of the target group. Next, the small RNAs were sequenced using the TruSeq small RNA Library Prep Kit in a MiSeq Illumina sequencer. Individual validation of the twenty microRNAs selected as favourable biomarkers was carried out using RT-qPCR. A bioinformatics-pipeline was established to distinguish 1,915 from the large pool of sequencing data. After a comparison of the log2 fold change of obese and diabetic mice (with normal mice as control; FC ≥ 2) only 127 mature miRNAs were subsequently used for the downstream statistical analysis, while the remaining 1,788 were excluded. Interestingly, 17 miRNAs were found specifically in the obese group in and 26 miRNAs in particular diabetic group. From the obese and diabetic groups, miR-144, miR-181, miR-27, miR-191, miR-128, miR-221 showed up-regulated expressions. From the diabetic group, miR-144-3p, miR-451, miR-328 and miR-142 displayed down-regulated expressions, whereas, the obese group did not show any significant down-regulated miRNAs. These were then confirmed through RT-qPCR analysis. This finding will help better understand the mechanism of metabolic disorders and may influence future approaches for the diagnosis and treatment of obesity and diabetes. Keywords: MicroRNA, Obesity, Diabetes, Gene expression, C57BL/6N.
P07 KINETICS OF REVERSE TRANSCRIPTION INFLUENCES SENSITIVITY OF THE PROTOTYPIC BETA-RETROVIRUS TO INHIBITION BY APOBEC3 PROTEINS

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Introduction
Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (APOBEC3G) is an interferon-inducible innate immunity factor which catalyzes deamination of deoxycytosine to deoxyuridine in single-stranded DNA (ssDNA). The enzyme effectively inhibits the replication of various retroviruses by targeting an intermediate product of reverse transcription - minus sense ssDNA – and causing its extensive mutation. Several counter mechanisms allowing replication of retroviruses in the presence of APOBEC3G have been described. This includes proteosomal degradation of APOBEC3G directed by viral accessory proteins (Vif of HIV-1), impaired cytoplasmic solubility of APOBEC3G (Bet of Foamyviruses) and preclusion of A3G packaging to virions (HTLV). For the majority of retroviruses, however, the underlying mechanism allowing their replication in APOBEC3G-expressing cells remains unknown. One of these viruses is the prototypic beta-retrovirus mouse mammary tumor virus (MMTV). The virus replicates in cell of myeloid and lymphoid origin expressing a mouse orthologue of the human APOBEC3 (mA3). However, viral DNA does not carry a signature of the cytidine deaminase-directed hypermutation. Thus, MMTV has evolved a mechanism alleviating the deamination capacity of APOBEC3 proteins including human APOBEC3G and mouse APOBEC3.

Experimental
The viral vectors were prepared by co-transfection of a packaging construct, a gfp gene-carrying vector, a helper plasmid supporting nuclear RNA export and finally a plasmid encoding VSV-G envelope. An APOBEC3 protein-expression construct (up to 200 ng/6 well plate) was added to the transfection mix when needed. The level of deamination was determined by MiSeq using PCR amplified proviral regions. Mutagenesis was performed using standard techniques. The ribonucleotide reductase was inhibited by hydroxyurea. AZT was used as a nucleoside RT inhibitor.
Results and Discussion

First, we tested the ability of MMTV to withstand restriction by various APOBEC3 proteins in a dose-response analysis using a EGFP-expressing MMTV-based vector. The sensitivity of MMTV to inhibition by APOBEC3 proteins was directly compared with the sensitivity of a Vif-deficient HIV-1, which is potently inhibited by APOBEC3 proteins. We found that MMTV is less potently (three to five-fold difference) inhibited by APOBEC3 proteins compared to HIV-1 vector. A trans-complementation assay aiming to rescue the infectivity of Vif-deficient HIV-1 produced in the presence of APOBEC3G did not reveal the presence of a factor capable of counteracting APOBEC3G antiviral effect. Neither a complete molecular clone of MMTV nor the packaging construct, co-transfected together with the plasmids required for the generation of infectious HIV-1, enhanced the infectivity of HIV-1ΔVif.

Furthermore, we found that the cytidine deaminase activity was required for the inhibition. This stems from the fact that physiologically relevant levels of a deaminase deficient variant of APOBEC3G(E259Q), in contrast to its enzymatically active counterpart, did not inhibit MMTV infectivity.

Inhibition of the MMTV infectivity with APOBEC3G was accompanied by approximately three-fold lower frequency of deamination events in MMTV compared to HIV-1 sequences. Importantly, both viruses packaged the same amounts of APOBEC3G proteins, therefore the decreased level of C-to-U mutagenesis could not be attributed to a difference in intra-virion APOBEC3G levels. Taken together, these results support the concept that MMTV has evolved so far unrecognized APOBEC3G-antagonizing mechanism that alleviates the deaminase activity of the innate immunity factor.

APOBEC3 proteins exert their antiviral effect during reverse transcription. More specifically, they deaminate minus DNA strand that become transiently single-stranded following the RNaseH domain of RT-mediated degradation of viral RNA template. Therefore, we investigated whether the kinetics of reverse transcription has an effect on the rate of inhibition. First, we generated a MMTV variant carrying F120L mutation in the DNA polymerase domain of RT. The mutant showed a delayed kinetics of reverse transcription, a slower replication speed and an increase in recombination frequency relative to the wild-type virus. Importantly, the mutant was markedly more sensitive to inhibition by APOBEC3G. Next, we inhibited the DNA polymerase activity of RT by decreasing the intracellular dNTPs levels (inhibition of ribonucleotide reductase) or by using nucleoside RT inhibitors. Analogously to the genetic approach, we detected an increased sensitivity to inhibition by APOBEC3G.

In conclusion, we found an inverse correlation between the rate of polymerization during reverse transcription and the deaminase-dependent inhibition of MMTV infectivity by APOBEC3G.

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References:


P08 RIPK1 prevents ZBP1-mediated necroptosis

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Receptor-interacting protein kinase 1 (RIPK1) plays an important role in regulating cell death and inflammation. While the kinase function of RIPK1 is utilized to induce caspase-8-dependent apoptosis and RIPK3 dependent necroptosis, its kinase independent scaffolding function can prevent apoptosis and necroptosis in different physiological contexts. Currently, it is unclear how RIPK1 counteracts RIPK3 mediated necroptosis. Here we present that RIP homotypic interaction motif (RHIM) of RIPK1 inhibits cell death and inflammation by blocking Z-DNA binding protein 1 (ZBP1, also known as DAI or DLM1)-dependent necroptosis. The perinatal lethality of RIPK1mRHIM mice, which have the core amino acids IQIG mutated to IAAA in RHIM, was abolished by RIPK3, MLKL or ZBP1 deficiency. Furthermore, the spontaneous skin inflammation developed in mice expressing RIPK1mRHIM mutant protein in keratinocytes was abrogated by MLKL or ZBP1 deficiency. Mechanistically, the RHIM of RIPK1 prevents ZBP1 from binding and activating RIPK3. Taken together, these results demonstrated that RIPK1 prevents perinatal death and inflammation in the skin of adult mice by inhibiting ZBP1-induced necroptosis. Furthermore, these findings identify ZBP1 as a critical mediator of inflammation beyond its previously proposed role in antiviral defense.
**P09 BIOCEV GeneCore – More than just gene expression profiling**

**Langerová Lucie**

We present services of BIOCEV Gene Core - the best equipped core facility and service provider in the field of gene expression in Central Europe. We have broad experience in quality control (QC e.g. Fragment Analyser) in a single cell analysis (automated cell picking ALS Cellcelector), high-throughput and digital PCR (Fluidigm Biomark, BioRad QX200 Droplet Digital PCR System) and NGS library preparation.

We emphasise quality control, which is often neglected. Effective QC is based on the use of molecular tools to control contamination (RNA/DNA spikes), genomic background (ValidPrime) and quality of RNA (ΔAmp, RIN). We also take part in development of these methods to facilitate analysis of gene expression starting from bulk samples, down to the level of individual cells (direct lysis).

In addition to conventional qPCR analysis, we focus on single cells expression profiling and multi-analyte approach. Analysis of DNA/RNA/protein in parallel in one sample even on the single cell level provides comprehensive tool to map gene expression and characterizes types of cells and to determine the degree of differentiation and to study the pathological condition.

Currently we offer assistance with library preparations and experimental design of RNA-Seq experiments, which are key preconditions for a successful project.
P10 MOLECULAR BARCODING AS A TOOL FOR ERROR-FREE ANTIBODY REPertoire ANALYSIS

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Summary
High-throughput analysis of antibody repertoires is a widely used method for immune system profiling. Existing methods lack accuracy because of accumulation of PCR and sequencing errors and variation of antibodies expression level between B-cells. Here we describe a method for the production of the barcoded antibodies libraries which uses the genomic DNA of plasma cells as a template. The developed approach could be used for error-free antibody repertoire profiling due to barcoding-mediated error correction. Moreover, the technique presented is insensitive to differences in expression level as it uses genomic DNA instead of RNA and outputs the high-quality sequences covering the whole variable region. The method, including DNA extraction, sequencing and data analysis, takes 5 days to complete.

Introduction
Humoral immunity represents one of the defensive mechanisms against various diseases. This aspect of immunity is mediated by complement proteins, antimicrobial peptides and antibodies. Antibodies are secreted by B-cells and are widely diverse due to recombination of gene segments occurring in developing lymphocytes. Most of the organism’s reaction to different diseases is determined by antibody repertoire. Antibody repertoire sequencing is a powerful diagnostic tool allowing to estimate the features of B-cell adaptive responses, monitor the patient immune status and reaction to infectious, oncological and autoimmune diseases and vaccination1,2,3. High-throughput sequencing of antibody repertoires is still a challenging task due to the accumulation of the polymerase errors, skewing of amplification, incorrect base calling during a sequencing run. Apart from these technical problems, antibodies genes undergo somatic hypermutation, further complicating the analysis. Finally, antibodies expression level substantially varies between B-cell populations (naive, memory and plasma cells) as well as between cells belonging to the same population4. All these factors hinder the accurate quantitative evaluation of B-cell clonal diversity.

The antibody repertoires parsing became more reliable with the developing of the unique molecular identifiers (UMI) or barcoding technique5. This approach exploits the introduction of the random nucleotide sequences prior to amplification and allows to correct almost all PCR and sequencing errors and compensate the amplification bias6.
Experimental
The approach was tested on the genomic DNA, extracted by a column-based method from FACS-sorted plasma cells. Blood samples were collected from the patients nine days after their immunization by the yellow fever vaccine with donor’s informed consent. The developed method of library construction consists of several steps. First, we tagged the immunoglobulin genes by linear amplification of genomic DNA with barcoding primer binding to the J segments. The products were then purified by the magnetic beads separation and used as a template in the exponential amplification step. During this PCR we use step-out primer homologous to the 5’-sequence of the barcoding primer (step-out primer) and multiplex primers binding to the variable segments of the immunoglobulin gene. The obtained amplicons were purified by the gel extraction. To enable the sequencing amplicons were ligated with Illumina adapters, amplified and purified by the magnetic beads separation. Finally, the library was sequenced by means of 2 x 300 Illumina MiSeq kit. The resulting reads were assembled and grouped by barcodes using MIGEC software (Shugay 2014). Grouped data were analyzed by means of MiXCR and VDJtools software (LINKS).

Results and Discussion
In order to assess the quality of the developed approach, we constructed the libraries by the method described above and by previously used RNA-based approach (Turchaninova). We then compared the B-cell clones abundance by means of VDJtools software. We demonstrated the increased correlation of B-cell clonal diversity between samples in replicas compared to RNA-based libraries. Therefore, the developed method outputs more reproducible results and allows us to determine the B-cell clonal diversity with higher accuracy. Moreover, the simplicity of obtaining, processing and storage make the genomic DNA highly convenient for the analysis of antibody repertoires.

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References:


P11 INTERFERing with small RNAs

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The presence of dsRNA in the cytoplasm of cells is a hallmark of viral infections. Generally, double-stranded RNA is not produced by mammalian cells, but produced by viruses during their replication cycle in the host. For this reason, cells have developed a sensing mechanism to trigger an antiviral response upon detection of cytoplasmic dsRNA. Generally, this antiviral response consists of the production and secretion of type-I interferon and other cytokines, and second, a translational shut-off mediated by PKR. MiRNAs, which are 21-23nt small RNAs, have an essential role in controlling the activation of the type-I interferon response in mammalian cells (Witteveldt J, unpublished). Interestingly, this regulation seems to work in a feedback mechanism. In a cellular setting where type-I interferon can be robustly activated by the presence of cytoplasmic dsRNA, we observed a rapid and transient global downregulation of miRNA biogenesis which leads to a decrease in miRNA levels. Since miRNAs are negative regulators of type-I IFN response, our hypothesis is that miRNA biogenesis is transiently inhibited to allow a strong and robust type-I interferon activation. Accordingly, when cells cannot regulate miRNA biogenesis, we observed a significant reduction in the production of type-I interferon. All these together suggest that the Interferon response apart from INTERFERing with viral replication, INTERFERes with other endogenous processes in cells, such as miRNA biogenesis, and this appears to be an essential mechanism to mount a robust antiviral response.
P12 Investigation of the possible presence of homologs of Ten Eleven Translocation (TET) enzymes within plants

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Variation in patterns of gene expression can result from modifications in the genome that occur without a change in the sequence of the DNA; such modifications include methylation of cytosine to generate 5-methylcytosine (5mC) resulting in the generation of heritable epimutation and novel epialleles. This type of non-sequence variation is called epigenetics. The enzymes responsible for generation of such DNA modifications are named Ten Eleven Translocation (TET) proteins, which belong to the 2-oxoglutarate dependent dioxygenase family. In various mammalian cells/tissues including embryonic stem cells, cancer cells and brain tissues, it has been confirmed that these proteins are able to induce the stepwise oxidization of 5-methyl cytosine to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5faC), and finally 5-carboxylcytosine (5caC). Each stage from initial methylation until the end of the DNA demethylation process is reconsidered as a specific epigenetic mark that may regulate gene expression. Different methods with various sensitivities and specifications have been used to evaluate whether the presence of oxidation products of 5-methyl cytosine results from TET-like activities in plant DNA. This review discusses controversial evidence for the presence of oxidative products, particularly 5hmC, in various plants and tissues. Whereas some reports suggest no enzymatic DNA demethylation, other reports suggest that the presence of oxidative products is followed by the active demethylation and indicate the contribution of possible TET-like proteins in the regulation of gene expression in plants.

Keywords: Epigenetics, 5-methyle cytosine, ten eleven translocation, biotic and abiotic stress.

References
**P13 Single-cell transcriptome analysis of metabolic stress response in macrophage**

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Obesity-induced chronic inflammation developed by metabolically activated adipose tissue macrophages and other immune cells has a major impact on the progression of insulin resistance and type 2 diabetes. The key role of macrophages in these pathological processes has been widely studied using conventional methods, however standard approaches averaging measurements from thousands of cells are unable to capture cell heterogeneity typical for macrophages. We used single-cell transcriptome analysis of palmitate stressed THP-1 isogenic cells to identify key regulatory pathways underlying obesity-induced metabolic stress response and to describe the spectrum of macrophage molecular phenotypes. Based on the whole transcriptome data from individual palmitate-stressed macrophages we observed three distinct transcriptional states corresponding to M1-like, M2-like and M1-like low responsive cells. Using weighted gene correlation network analysis, we identified gene clusters and regulatory factors specific for each macrophage transcriptional state. M1-like state featured increased expression of pro-inflammatory genes regulated by TLR signalling pathway (IL1B, IL8) as well as ER stress markers (DDIT3), whereas M2-like cell were characterized by high expression of lipid metabolism regulators. We observed that key pro- and anti-inflammatory hub genes, such as IL1B and ATF3 feature mutually exclusive expression in distinct cell subpopulations. These results demonstrate that the response to metabolic stress is controlled by the ratio of cells characterized by different metabolic and inflammatory state. Our findings were approved by immunofluorescence detection of key regulatory proteins. Our results prove that even under mild metabolic stress conditions macrophages form distinct groups balancing the response and maintaining homeostasis on cell population level rather than within a cell. Systems analysis of gene regulatory networks underlying different macrophage subpopulations represents a valuable resource for further functional experimental approaches. Overall, our study shed new light on the analysis of macrophage role in obesity and chronic inflammatory disease.
P14 Set up of an high throughput screening assay for the identification of small molecules targeting the YTH domain

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N6-methyladenosine (m6A) is a reversible RNA modification, conserved in all eukaryotes, from yeast to humans. It is mainly observed in the 3’UTR, the 5’UTR and near the Transcription Start Sites (TSS), inside a consensus sequence identified as [G/A/U][A/G][m6A-C][U/A/C]. Even if the biological role of this modification is still poorly understood, the players of the methylation machinery have been identified. The reversible methylation process is regulated by two class of proteins: methyltransferases METTL-3 and METTL-14, in complex with WTAP, are responsible for the methylation of the adenosine on target mRNAs, while the demethylases FTO and ALKBH5 act together to revert this modification. m6A-binding proteins belong to the YTH-domain family, are divided in three cytoplasmic isoforms (YTHDF1-3) and two nuclear isoforms (YTHDC1-2) and recognize the N6-methyladenosine modification. We aim to identify an inhibitor of YTH activity and evaluate its effect in eukaryotic cells to help clarifying the functional role of YTH protein family. Exploiting the spectrophotometric properties of the YTH domain, we developed a biochemical assay amenable for high throughput screening.
P15 Mutation detection and analysis from Malaysian breast cancer patients by Denaturing High Performance Liquid Chromatography (DHPLC)

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1 Hadi JN

Introduction: Breast cancer is a major health problem affecting women globally. In Malaysia, breast cancer constitutes of 33% of the newly diagnosed female cancer. Women who carry mutations in BRCA1 have about 80% for developing breast cancer, and 40% to 60% for developing ovarian cancer during their lifetime. As direct sequencing is costly and time-consuming, selection of method with high accuracy and sensitive is one the challenges for early diagnosis and treatment. In this report we carried out screening for the possible germline mutations from exon 11 of BRCA1 by Denaturing High Performance Liquid Chromatography (DHPLC) technique. The sensitivity of the method was further evaluated by direct sequencing. Method: 40 peripheral blood samples from west Malaysia were collected from women with either early-onset (≤ 45) breast cancer or a positive family history of breast cancer and controls. DNA from the target samples were extraction and the target genomic region was amplified by PCR using reported primers. Mutations were detected in exon 11 of BRCA1 gene by the method of DHPLC. Further sequencing was carried out to double confirm the results. Results: Mutations were detected in 12.5% (5/40) of the total number of the patients. Three of them were Chinese, one Indian and one Malay. All were under age of 45y with family history of breast cancer. The procedure repeated two times with the same result. All the five mutations detected here were reported previously. We could not report any novel mutation in this study. Conclusion: The result indicated that DHPLC method is quite sensitive and accurate for mutation detection and molecular diagnosis. This is a preliminary report and further studies are required to assess the novelty of this method.
P16 The translocation event generates specificity for adenine in poly(A)-specific ribonuclease (PARN)

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The deadenylase PARN is a poly(A)-specific, processive and cap-interacting 3’-5’ exoribonuclease that efficiently degrades poly(A) tails of eukaryotic RNAs. We have developed a working model for PARN action (Crit. Rev. Biochem. Mol. Biol., 48, 192-209) that defines two reaction steps, a translocation event followed by a hydrolytic event that releases AMP. The active site of PARN consists of three binding sites for adenine base (sites “-1”, “-2” and “-3”). The hydrolytic site is located between the two sites “-1” and “-2”. We find that two translocation events are required to position the first scissile bond into the hydrolytic site. Subsequently, each translocation step will position the next phosphodiester bond into the hydrolytic site. Thus, the 3’ end located adenine base of a poly(A) tail binds first to site “-3”, then translocates into site “-2” at the same time as the penultimate adenine base enters site “-3”. Subsequently, the 3’ end located adenine base and the penultimate adenine base translocates from sites “-2” and “-3” into sites “-1” and “-2”, respectively, at the same time as the next adenine base enters into site “-3”. At the end of these two translocation steps the first phosphodiester bond to be cleaved has been positioned in the hydrolytic site of PARN. Our analysis demonstrates that adenosine recognition is primarily linked to the translocation event where the adenine base moves from site “-3” to “-2”. Structural studies (EMBO J., 24, 4082-4093) identify a rotational event of the base when it moves from site “-3” to “-2”. Thus, our study suggests that coordinated rearrangements of the poly(A) substrate and the active site are critical for providing the specificity of PARN for degrading poly(A). Taken together, we conclude that the translocation event generates specificity for the adenine base in PARN.
P17 Nucleoside-modified mRNA makes hemagglutinin into a potent universal influenza virus vaccine candidate

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Despite the significant progress in influenza research, an effective universal vaccine has not been developed, thus, requiring yearly revaccination. In recent years, numerous studies have demonstrated the outstanding abilities of mRNA to elicit potent immune responses against pathogens, making it a viable new platform for vaccine development. We have generated a potent vaccine platform where nucleoside-modified mRNAs were encapsulated into lipid nanoparticles (LNPs) that have recently proved to be safe and efficient tools for in vivo nucleic acid delivery. Mice were intradermally immunized with a single dose of influenza hemagglutinin (HA) encoding mRNA-LNPs and immune responses were followed. Compared to the current human inactivated influenza virus vaccine, potent antigen-specific CD4+ T cells were generated, where half of the response were T follicular helper (Tfh) cells that play a crucial role in eliciting broadly neutralizing antibodies. Increased numbers of germinal center and memory B cells were associated with extremely high titers of neutralizing antibodies. Most interestingly, over a third of the antibody responses was directed at the stalk, which is conserved across groups and represents the target of “Universal” Flu vaccines. Our results demonstrate that antigen-encoding nucleoside-modified mRNA in LNPs induces highly potent and broad influenza-specific immune responses and has great potential for vaccination against infectious diseases.
P18 Major transcriptional changes observed in the Fulani ethnic group less susceptible to malaria are associated with increased expression of non-coding RNAs

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The Fulani ethnic group has relatively better protection from Plasmodium falciparum malaria, as reflected by fewer symptomatic cases of malaria and lower infection rates compared to sympatric ethnic groups. We have performed a pilot study to examine global transcription patterns in specific immune cell populations in the Fulani, to elucidate the mechanisms that confer this lower susceptibility. When we compared uninfected and infected Fulani individuals, in contrast to uninfected and infected individuals from the sympatric ethnic group Mossi, a strong transcriptional response was only detected in the monocyte fraction of the Fulani. Fulani monocytes had higher baseline levels of expression in uninfected individuals, with the majority of DE genes downregulated in the infected Fulani (1095/1239). Interestingly, the few DE genes upregulated in the infected Fulani (144/1239) were enriched in non-coding RNA genes. It is unclear whether increased expression of ncRNAs in the Fulani is involved in regulating chromatin states and gene expression, or in triggering the innate immune response. We will present our ongoing studies addressing whether the differences in rates of non-coding RNA transcription mediate the heightened response of these innate immune cells to P.falciparum malaria.
P19 Innate immune reactions against wounds and tumors – an insect model

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We are interested in exploring how the innate immune system reacts towards larger microbial intruders and against aberrant cells including tissues at early stages of tumor progression. For studying infections, we use insect-pathogenic nematodes (EPNs) and the workhorse of genetics, Drosophila melanogaster as host 1,2. The nematodes force their way into the body cavity thus creating wounds. We could show that clotting factors, which help to seal wounds, protect against nematode infections 1. Our recent results indicate that wound-healing processes are also activated during nematode infections and we are presently testing their protective role 2. Epistatis analysis in the fly model will allow us to dissect the differences between properly healed wounds and chronic fibrotic lesions. In our model for early stages of tumorigenesis we induce a tumorigenic state in non-immune tissues in fly larvae. We find that this leads to the activation of both a cellular and a humoral immune response 3. The lethality of the tumor phenotype is rescued by modulating one of the pathways we found induced (retinoic acid signaling). Our findings are in line with the idea that tumors are “wounds that fail to heal” 4.

New regulatory proteins suppress infertility of Arabidopsis smg7 mutants

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Nonsense-mediated mRNA decay (NMD) is an evolutionary conserved mRNA quality control mechanism that detects and degrades aberrant mRNAs containing premature translation termination codons (PTC). Nevertheless, some NMD factors appear to participate in additional cellular processes. In our previous work we discovered that SMG7 is required for completion of meiosis in Arabidopsis thaliana. The smg7-1 null mutant shows arrest in anaphase II that is associated with delayed chromosome decondensation and aberrant rearrangement of the spindle, while a weak smg7-6 allele causes meiocytes to undergo third meiotic division characterized by formation of four spindles and retention of chromosome compaction. The meiotic function of SMG7 protein does not seem to be directly related to its NMD function, but rather represents a novel gene function. To dissect the non-canonical function of SMG7, we performed a forward genetic screen for suppressor mutations that rescue the fertility of smg7-6 mutants. We have obtained 90 mutant lines with increased fertility and candidate causative mutations were identified in selected lines by next generation sequencing. One of the candidate genes encodes for a translation initiation factor that operates as a critical scaffold required for recruiting 40S ribosomal subunit on mRNA. Functional analysis of this gene in meiotic progression is under investigation. The project is supported by the Czech Science Foundation (14-223465) and the program SoMoPro II(3SGA5833) co-financed by EU and the South-Moravian Region.
P21 Variable penetrance of PARN (poly(A)-specific ribonuclease) loss-of-function (LOF) variants in a three generation pedigree

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Dyskeratosis congenita (DC) and Hoyeraal-Hreidarsson (HH) syndrome are disorders of short telomeres that can be caused by biallelic pathogenic PARN variants. Telomere maintenance requires telomerase protein and RNA (hTR). Adenylation targets hTR for degradation. PARN deadenylates and stabilizes hTR. PARN loss-of-function (LOF) reduces hTR level and telomerase activity. Monoallelic LOF PARN variants cause pulmonary fibrosis, however, penetrance is incomplete. To further investigate PARN LOF effect on health and telomere length, we studied a kinship with three children in the 3rd generation, two with HH and one unaffected. The proband had bone marrow failure, while the younger affected child did not. A rare PARN missense variant (PARN-Y91C) was transmitted to all children. A novel LOF variant (PARN-ins) was transmitted to the two affected children. PARN-Y91C deadenylation activity in vitro was reduced 30-fold compared to wild type. PARN-ins transcript underwent nonsense-mediated decay. Therefore, hypofunctional PARN-Y91C was presumably the predominant protein in the affected siblings. In lymphoblastoid cell lines (LCLs) and compared to controls, PARN protein was reduced in proband and mother but similar in the affected sibling. In lymphoblastoid cell lines (LCLs) and compared to controls, PARN mRNA level in all family members was similar to controls. Together, these results indicate that additional factors modify PARN protein level in LCLs and differ between the proband and affected sibling. Also consistent with modifier presence, levels of multiple oligoadenylated H/ACA-box small nucleolar RNAs were increased in the proband, but not affected sibling, compared to controls. Telomere flow FISH showed the mean B lymphocyte telomere length (B-TL) of the affected siblings was <1st percentile; father and unaffected sibling (carried PARN-Y91C) were ~10th percentile. The B-TL of the mother and her mother (carried PARN-ins) were ~50th percentile, maintaining telomere length despite reduced PARN level. This tolerance may reflect previously observed incomplete penetrance.
P22 How does human Staufen1 recognize its natural dsRNA target involved in Staufen-mediated mRNA decay? Solution structure of RNA-Staufen1 complex

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Several dsRNA binding proteins (dsRBP) are involved in posttranscriptional regulation of gene expression and function based on recognition of their dsRNA targets. In general, dsRBPs interact with their cellular dsRNA targets through a combination of structure- and sequence-specific recognition. Staufen1 is a dsRBP involved in mRNA transport and localization, regulation of mRNA stability, translational efficiency and mRNA decay by a staufen-mediated mRNA decay (SMD) pathway. Staufen1 contains multiple domains and RNA binding domains (dsRBD) 3 and 4 bind to dsRNA targets. The recognition of dsRNA targets in 3'UTRs by Staufen1 has been shown to depend on enrichment of GC-content and secondary structure. The stem-loop within the 3'UTR of human ADP-ribosylation factor1 (hARF1) is one such dsRNA target and Staufen1 binding regulates cytoplasmic ARF1 mRNA levels by the SMD pathway. However, how Staufen proteins recognize specific mRNA targets is still unknown. To reveal how Staufen1 binds specific dsRNA targets, we are determining the solution structure of the ARF1 dsRNA-Staufen1 complex by nuclear magnetic resonance (NMR) spectroscopy. Electrophoretic mobility shift assay and fluorescent anisotropy measurement show that the interaction of ARF1 dsRNA with dsRBD3 and 4 results in a single complex with affinity in the nanomolar range. Our preliminary structure of the ARF1 dsRNA-Staufen1 dsRBD4 complex reveals that Staufen1 is indeed a sequence-specific dsRNA binding protein which specifically binds dsRNA targets by sequence readout in the minor groove. Staufen1 dsRBD4 interacts with dsRNA by three distinct binding regions, namely helix α1 which recognizes consecutive pyrimidines via the minor groove, β1β2 loop which anchors the dsRBD at the end of the dsRNA in the minor groove as well as lysines in helix α2 which bind to the phosphodiester backbone from the major groove side. Mutagenesis data and the biological implications will be discussed.
RNA editing is an important post-transcriptional process able to increase transcriptome and proteome. In humans, the most common type of RNA editing is mediated by ADAR enzymes, which convert Adenosine into Inosine within double-stranded RNA (dsRNA). It is well known that the ADARs are key proteins for hematopoietic stem cell self-renewal and for survival of differentiating progenitor cells. However, their specific role in myeloid cell maturation has been poorly investigated. Here we show that the two ADARs, ADAR1 and ADAR2, are differently regulated during myeloid differentiation: ADAR1 is present at the basal level in the primary myeloid leukemia cells obtained from patients at diagnosis as well as in myeloid U-937 and THP1 cell lines and its expression correlates with the editing levels. Remarkably, ADAR2 is absent in the undifferentiated cell stage, being strongly up-regulated at the end of the differentiation process. Of note, peripheral blood monocytes display editing events at the selected targets similar to those found in differentiated cell lines. Taken together, the data indicate that ADAR enzymes play important and distinct roles during myeloid cell differentiation; suggesting ADAR2 as a novel marker for myeloid blasts cell differentiation.
The majority of human genes can produce multiple protein isoforms with distinct functions by alternative splicing. This process is highly regulated and even small malfunction causes diseases. TAR DNA-binding protein 43 (TDP-43) inhibits splicing of exon 9 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which in turn results in severe forms of cystic fibrosis. Mutation in the 3’ splice site (3’ss) of CFTR exon 9 causing extension of a UG-rich region and polypyrimididine tract shortening, creates a high affinity binding site for TDP-43. After RNA binding TDP-43 recruits hnRNPA1 and thus formed complex prevents the recognition of the 3’ss of exon 9 by the spliceosomal machinery and causes exon 9 skipping resulting in a non-functional CFTR protein. Although RNA recognition and binding by TDP-43 alone and in complex with other hnRNPs has numerous functional implications, molecular details of such interactions remained elusive. Our structural studies combined with biophysical approaches reveal that two copies of TDP-43 RBD create a new protein-protein interface with a salt bridge upon binding to the extended UG-rich sequence. Site-directed mutagenesis of amino acids involved in salt bridge formation reveals the functional significance of this protein-protein interface. Mutation at the interaction site of the two TDP-43 RBD copies reduces exon 9 skipping almost to the same extent as completely abolishing UG-rich RNA binding. This functional complex recruits two copies of hnRNPA1 to the intron 8 - exon 9 junction where they interact with 3’ss RNA via their RRM2 domains while RRM1 domains presumably participate in protein dimerization. NMR monitoring of the TDP-43 - hnRNPA1 complex assembly shows that their interaction with CFTR exon 9 3’ss RNA is not masking the polypyrimididine tract on the pre-mRNA for binding of the canonical splicing factor U2AF65. Shortening of the polypyrimididine tract, on the other hand, lowers the binding affinity of U2AF65 twofold. At the same time, hnRNPA1 binding at the 3’ss blocks access of U2AF35. Thus CFTR exon 9 skipping is driven by a network of interactions formed by TDP-43 and hnRNPA1 at the CFTR exon 9 3’ss which competes for the formation of the canonical splicing complex.
P25 The RNA-binding protein tristetraprolin schedules apoptosis of pathogen-engaged neutrophils during bacterial infection

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Protective responses against pathogens require a rapid mobilization of resting neutrophils and the timely removal of activated ones. Neutrophils are exceptionally short-lived leukocytes, yet it remains unclear whether the lifespan of pathogen-engaged neutrophils is regulated differently from that in the circulating steady-state pool. Here, we have found that under homeostatic conditions, the mRNA-destabilizing protein tristetraprolin (TTP) regulates apoptosis and the numbers of activated infiltrating murine neutrophils but not neutrophil cellularity. Activated TTP-deficient neutrophils exhibited decreased apoptosis and enhanced accumulation at the infection site. In the context of myeloid-specific deletion of Ttp, the potentiation of neutrophil deployment protected mice against lethal soft tissue infection with Streptococcus pyogenes and prevented bacterial dissemination. Neutrophil transcriptome analysis revealed that decreased apoptosis of TTP-deficient neutrophils was specifically associated with elevated expression of myeloid cell leukemia 1 (Mcl1) but not other antiapoptotic B cell leukemia/lymphoma 2 (Bcl2) family members. Higher Mcl1 expression resulted from stabilization of Mcl1 mRNA in the absence of TTP. The low apoptosis rate of infiltrating TTP-deficient neutrophils was comparable to that of transgenic Mcl1-overexpressing neutrophils. Our study demonstrates that posttranscriptional gene regulation by TTP schedules the termination of the antimicrobial engagement of neutrophils. The balancing role of TTP comes at the cost of an increased risk of bacterial infections.
P26 B CELL RECEPTOR SIGNALING ACTIVITY IS ASSOCIATED WITH GENOMIC DEFECTS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Summary:
Our findings provide further evidence for functional crosslink between BCR signaling activity and selected genomic defects in CLL.

Introduction:
Chronic lymphocytic leukemia (CLL) is the most common leukemia type of elderly people characterized by expansion of pathological B-lymphocytes. Survival and proliferation of these leukemic cells are crucially driven by B cell receptor (BCR) signaling pathway. CLL has heterogeneous genetic background with recurrence of certain chromosomal aberrations and gene mutations. Such heterogeneity is also reflected in highly variable disease course. We aimed to understand if and how BCR signaling is involved in accumulation of genomic defects in CLL.

Experimental:
Fresh frozen CLL samples, separated from peripheral blood of 78 patients, were selected from the collection of the University Hospital Brno. BCR signaling was stimulated using specific antibody. Samples were fixed, permeabilized and labeled with antibodies against phosphorylated components of BCR signaling and measured by flow cytometry. BCR signaling activation in stimulated cells was compared to the basal phosphorylation in control unstimulated cells. To determine possible associations, the obtained data were correlated with genetic and cytogenetic findings and other clinical parameters.

Results and Discussion:
We identified a number of associations of BCR signaling activity with patients’ clinical and laboratory characteristics on the level of basal and also activated phosphorylation. Generally, genomic defects are predominantly associated with lower phosphorylation of several BCR signaling components. In particular, phosphorylation of upstream kinases ZAP and SYK was most prominently associated with del11q (Figure 1), complex karyotype and mutations in NOTCH1 and TP53.
Figure 1: The association of del(11q) and BCR signaling activity in CLL.

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P27 Effect of adenosine to inosine substitution on stability of canonical RNA duplexes studied by molecular modelling approach

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The most widespread type of RNA editing in the higher eukaryotes is the conversion of adenosine to inosine in double-stranded RNA. This reaction is catalysed by the family of adenosine deaminase acting on RNA (ADAR) proteins. While inosine in DNA is regarded as damage, inosine in RNA is a normal and essential modification which changes the informational content of the RNA molecule, as inosine preferentially base pairs with cytidine (and is therefore interpreted as guanosine). In addition, inosine-uracil base pairs introduced by ADAR editing also assist innate immune discrimination between self and non-self RNAs [1].

To study the impact of adenosine to inosine substitution on the structure, dynamical behavior and thermodynamical characteristics, we have carried out a series of 0.5 μs molecular dynamics simulations of the RNA sequence 5'GCAAUUAACCAAGGAAAAGC3' which was studied experimentally by Lehmann and Bass [2]. An effect of the adenosine to inosine substitutions in a different sequential context has been tested and properly analysed to estimate an impact of the mutation on the double-helical structure and dynamics of the RNA molecule. Molecular dynamics data have been complemented by intramolecular entropy calculations. In addition, to determine opening of IU pairs in RNA duplexes free energy computations using adaptive biasing method were conducted.

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