MASARYK UNIVERSITY

CEITEC
Central European Institute of Technology
BRNO | CZECH REPUBLIC

Abstracts of papers presented at the

CEITEC Conference: Nucleic Acids and Immunity

7 -9 September 2016

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

Conference Organiser:
Dana Cernoskova, CEITEC Masaryk University, Czech Republic

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CONFERENCE

NUCLEIC ACIDS AND IMMUNITY

7–9 September, 2016 Brno, Czech Republic

BEST WESTERN PREMIER Hotel International Brno **

CONTENT

C O N F E R E N C E

NUCLEIC ACIDS AND IMMUNITY

4 – 6 June, 2017 Brno, Czech Republic

KEYNOTE SPEAKERS

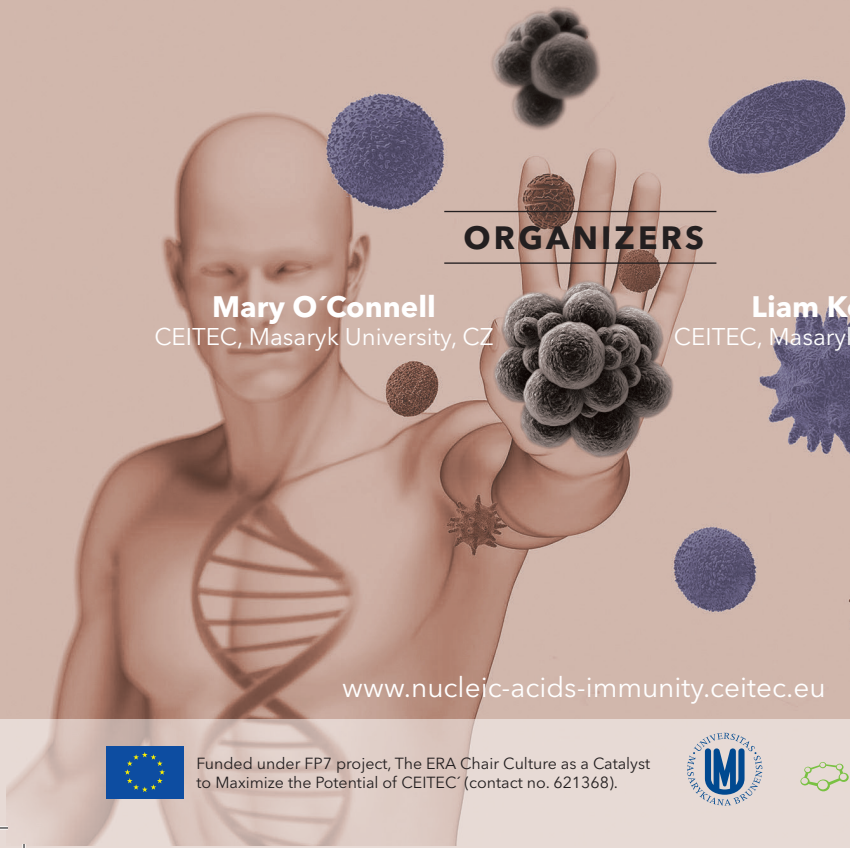
Shizuo Akira
Osaka University, JP

CONFIRMED SPEAKERS

Victoria Cowling
University of Dundee, UK

Erik Miska
University of Cambridge, UK

Jörg Vogel
Universität Würzburg, DE



ORGANIZERS

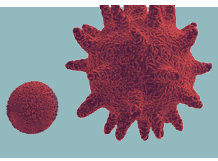
Mary O´Connell
CEITEC, Masaryk University, CZ

Liam Keegan
CEITEC, Masaryk University, CZ

www.nucleic-acids-immunity.ceitec.eu

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

CEITEC



WELCOME ADDRESS

Dear Colleagues,

As it is said in Czech 'Je mi velkou ctí Vás přivítat', it is our great honor and pleasure to welcome you to the ERA Chair Conference: Nucleic Acid and Immunity. We hope that you will find this conference not only scientifically stimulating but that you also have time to enjoy and absorb the atmosphere of Brno. It was here over 150 years ago that Johann Gregor Mendel performed his famous experiment on peas that established modern genetics. You can still visit his monastery and afterwards enjoy a beer next door in the brewery.

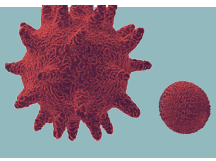
This conference is one in a series of three funded by the EU under the ERA Chair program. The theme of these conferences is to explore the links between nucleic acid and immunity. In particular the focus is on the role of DNA and RNA modification in immunity. This is a new and exciting area of research and we are hopeful that this conference series will foster future research and collaborations in this emerging field.

Brno is not only the second largest city in the Czech Republic but it is also a centre of education and science, with a student population of approximately 90,000. Recently the Central European Institute of Technology (CEITEC) which is an interdisciplinary institute was established here. This is a partnership between Masaryk University and other Institutes in Brno.

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 220 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
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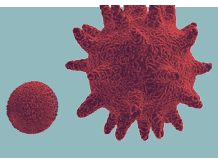
  Address: Husova 16, 602 00 Brno, Czech Republic
  GPS: 49°11'41.55"N, 16°36'17.24"E

REGISTRATION AND INFORMATION DESK

All participants must be registered before attending the lectures.

Opening hours

Tuesday	September 6, 2016	17:00 – 19:00
Wednesday	September 7, 2016	08:00 – 18:00
Thursday	September 8, 2016	08:00 – 18:00
Friday	September 9, 2016	08:00 – 14:00



CONFERENCE POLICY

• Badges

Participants and accompanying persons will receive a name badge upon registration. Everyone is kindly requested to wear their name badge when attending the meeting. Only participants who are wearing their name badge will be admitted to the lecture halls.

Name badges have been colour-coded as follows:

Orange: Speakers
Grey: Sponsor
Green: Participants
Blue: Organisers

• 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 workshop.

SOCIAL EVENTS

• Visit of Moravian Gallery, Welcome Dinner

- Tuesday, September 6
- Starts at 19:00
- Husova 14
- The meeting at the hotel lobby 18:50

• Visit of Mendel Museum, Gala Dinner in Augustian Abbey

- Thursday, September 8
- Starts at 19:15
- Mendel Square 1a
- The shuttles will leave from the hotel at 19:00
- Please bring the voucher

POSTER SESSION

Posters will be displayed continuously Wednesday to Friday in the lecture hall 2. Two poster sessions have been sheduled to the conference programme. Scientific organizers have selected short talks for plenary sessions for plenary sessions from submitted poster abstracts.
The poster boards will be ready on Tuesday September 6 from 17:00. 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 Wednesday September 7 at 17:00 in the lecture hall 2 Pins for hanging posters will be available at thehall.

Information for presenting authors

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.

There will be two groups :
Poster session 1 will present ONLY posters with odd numbers (1; 3; 5…)
Poster session 2 will present ONLY posters with even numbers (2; 4; 6…)

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 SESSIONS programme

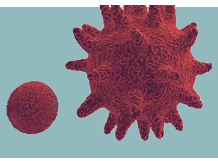
POSTER SESSION I – Wednesday 7th September 17:30 – 19:00
POSTER SESSION II – Thursday 8th September 17:30 – 18:30

BOOK OF ABSTRACT

Each participant receive the printed version.

RNA Society Award

All posters will be included to the poster competition. The posters will be selected by scientific committee. The winner will be announced on Friday September 9th at 12:30.



PROGRAMME

Tuesday, September 6

19:00	**Welcome dinner at Moravian Gallery**

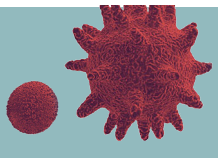
DAY 1 – Wednesday, September 7

09:00 – 09:30	**Opening Words**
09:30 – 10:30	**KEYNOTE SPEECH**
	Jean-Laurent Casanova - „Toward a genetic theory of childhood infectious diseases"
	SESSION 1: Nucleic Acid Sensors - Chair: **Gunther Hartmann**
10:30 – 11:00	**Lee Gehrke** - „RNAs containing modified nucleotides fail to trigger RIG-I conformational changes for innate immune signaling"
11:00 – 11:30	**Coffee break**
11:30 – 12:00	**Leonie Unterholzner** - „cGAS and IFI16: Sensing intracellular DNA as ‚stranger' and ‚danger' signal"
12:00 – 12:30	**Jean-Luc Imler** - „Sensing viral RNAs in the model organism *Drosophila*"
12:30 – 14:00	**Lunch**
	SESSION 2: RNA Modification - Chair: **Maria Öhman**
14:00 – 14:30	**Michael Jantsch** - „Understanding the effects of editing-deficiency"
14:30 – 15:00	**Mark Helm** - „RNA modifications affect recognition and immunestimulation by TLR7"
15:00 – 15:30	**Liam Keegan** - „Evolutionarily conserved immune effects of ADAR RNA editing, Conflict RNA Modifications and the RNA-DNA genome transition"
15:30 – 16:00	**Coffee break**

16:00 – 16:30	**Gideon Rechavi** - „mRNA modifications beyond RNA editing“
16:30 – 17:00	**Mashael Alqasem** - „Queuine Insertase tagging and selection in MDA-MB-231 cells via CRISPR and SmartFlare™“
17:00 – 17:30	**Nina Papavasiliou** - „RNA editing as a mechanism of diversification of cellular populations“
17:30 – 19:00	**Poster Session I**

DAY 2 – Thursday, September 8

	SESSION 3: Infectious Diseases - Chair: **Michael Jantsch**
09:00 – 09:30	**Mariano Garcia-Blanco** - „The RNA helicase DDX39B regulates IL7R alternative splicing reducing the risk of Multiple Sclerosis“
09:30 – 10:00	**Ann-Kristin Östlund Farrants** - „Transcriptome and DNA methylome analysis of two sympatric 1 ethic groups with 2 differential susceptibility to *Plasmodium falciparum* infection living in Burkina Faso“
10:00 – 10:30	**Yifat Ofir-Birin** - „Cell-cell communication between *Plasmodium* and host immune DNA sensing pathway via exosomes“
10:30 – 11:00	**Mayra Diosa-Toro** - „Dengue virus-induced ATF4 expression is independent of eiF2α phosphorylation and SG formation“
11: 00 – 11:30	**Coffee break**
	SESSION 4: Nucleic Acids Modification - Chair: **Michael Jantsch**
11:30 – 12:00	**Reuben Harris** - „Cancer Mutagenesis by APOBEC3B and Hypomorphic APOBEC3H“
12:00 – 12:30	**Marek Bartošovič** - „N6-methyladenosine demethylase FTO targets pre-mRNAs and plays a novel role in nuclear mRNA processing and expression“
12:30 – 14:00	**Group Photo and Lunch**



	SESSION 5: Inflammation - Chair: **Drew Weissman**
14:00 – 14:30	**Roth Shalom Hillel** - „A Computational Screen For RNA Editing Alterations In Autoimmune Diseases“
14:30 – 15:00	**Osamu Takeuchi** - „Posttranscriptional control of inflammatory responses by Regnase-1 and Roquin“
15:00 – 15:30	**Jan Rehwinkel** - „SAMHD1 limits activation of the cGAS-STING pathway“
15:30 – 16:00	**Coffee break**
	SESSION 6: Disease vectors - Chair: **Drew Weissman**
16:00 – 16:30	**Erika Girardi** - „Cross-species comparative analysis of Dicer proteins during *Sindbis* virus infection“
16:30 – 17:00	**Sara Cherry** - „RNA viruses are targeted by diverse RNA binding proteins“
17:00 – 17:30	**Susan Schuster** - „Using CRISPR/Cas9 to unravel the role of mammalian RNAi in antiviral immunity“
17:30 – 18:30	**Poster Session II**
19:00	**Departure to the Mendel Museum and Abbey**
20:00 – 22:00	**Gala Dinner**

DAY 3 – Friday, September 9

08:30 – 09:30	**KEYNOTE SPEECH**
	Caetano Reis e Sousa - „Sensing and restriction of RNA viruses“
	SESSION 7: - Towards Therapies Chair: Mark Helm
09:30 – 10:00	**Gunther Hartmann** - „Identification of the minimal ligand motif for the cytoplasmic DNA sensor cGAS“
10:00 – 10:30	**Gregory Heikel** - „Exploring the role of the RNA-binding activity of the E3 ubiquitin ligase Trim25 in antiviral innate immunity“
10:30 – 11:00	**Coffee break**
11:00 – 11:30	**Dmitriy Chudakov** - „Unique molecular barcoding in BCR and TCR repertoire profiling“
11:30 – 12:00	**Drew Weissman** - „Nucleoside-modified mRNA-lipid nanoparticle therapeutic protein delivery platform“
12:00 – 12:30	**Poster Award**
12:30 – 13:30	**Lunch**

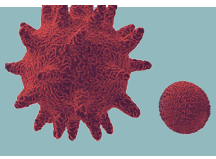
ABSTRACTS OF SPEAKERS

Keynote

K01 Toward a genetic theory of childhood infectious diseases

Jean-Laurent Casanova[1, 2, 3, 4]
[1] *The Rockefeller University*
[2] *Howard Hughes Medical Institute*
[3] *St. Giles Laboratory of Human Genetics of Infectious Diseases*
[4] *Necker Hospital and School of Medicine, University Paris Descartes*

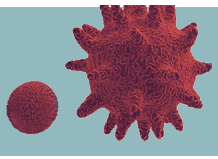
The hypothesis that inborn errors of immunity underlie infectious diseases is gaining experimental support. However, the apparent modes of inheritance of predisposition or resistance differ considerably between diseases and between studies. A coherent genetic architecture of infectious diseases is lacking. We suggest here that life-threatening infectious diseases in childhood, occurring in the course of primary infection, result mostly from individually rare but collectively diverse single-gene variations of variable clinical penetrance, whereas the genetic component of predisposition to secondary or reactivation infections in adults is more complex. This model is consistent with (i) the high incidence of most infectious diseases in early childhood, followed by a steady decline, (ii) theoretical modeling of the impact of monogenic or polygenic predisposition on the incidence distribution of infectious diseases before reproductive age, (iii) available molecular evidence from both monogenic and complex genetics of infectious diseases in children and adults, (iv) current knowledge of immunity to primary and secondary or latent infections, (v) the state of the art in the clinical genetics of non-infectious pediatric and adult diseases, and (vi) evolutionary data for the genes underlying single-gene and complex disease risk. With the recent advent of new-generation deep resequencing, this model of single-gene variations underlying severe pediatric infectious diseases is experimentally testable.



K02 Sensing and restriction of RNA viruses

Caetano Reis e Sousa [1]
[1] *The Francis Crick Insitute*

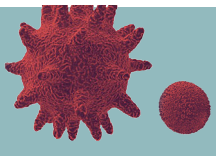
Direct sensing of pathogen components is a major trigger of dendritic cell (DC) activation, leading to innate and adaptive immunity. Over the years, we have studied multiple pattern-recognition pathways that mediate DC activation. One pathway for sensing infection by RNA viruses involves recognition of viral genomes or virally-infected cells in endosomal compartments and utilises members of the toll-like receptor (TLRs) family, including TLR9, 7, or 3. Viral genomes can additionally be recognised in the cytosol by DExD/H-box helicases such as RIG-I, which are activated by RNAs bearing 5′ tri- and di-phosphates. Finally, a distinct pathway involves cell surface and phagosomal recognition of fungi by C-type lectins, which signal via Syk kinase. Notably, we have recently found that sone Syk-coupled C-type lectin are involved in functions other than microbial recognition by DC. One, CLEC-2, allows DCs to relax lymph node stromal cells, permitting expansion of lymph nodes upon inflammation. Another, DNGR-1, allows DCs to detect dead cells by binding exposed F-actin and facilitates cross-presentation of dead cell-associated antigens. Interestingly, DNGR-1 marks CD8α+ DCs, a specialised subtype of DCs in mice and their human equivalents. DNGR-1 additionally can be used to fate map DC-committed precursors in mouse to define DCs by ontogeny. These studies help build a global picture of the receptors and signalling pathways that regulate DC activation and have applications in immunotherapy of cancer and infectious diseases.



S01 RNAs containing modified nucleotides fail to trigger RIG-I conformational changes for innate immune signaling

Ann Durbin[1], Lee Gehrke[1]
[1] Massachusetts Institute of Technology, Building E25-406, 77 Mass. Ave., Cambridge MA 02139

Pattern recognition receptors, both cytoplasmic (RIG-I like) and membrane-bound (Toll-like), bind to invading non-self nucleic acids, triggering the activation of innate immune signaling. Modified nucleotides, when present in RNA molecules, diminish the magnitude of these signaling responses. Although the presence of modified nucleotides in RNA correlates closely with diminished innate immune activation, mechanisms explaining the blunted signaling have not been elucidated. In this study, we used several independent biological assays, including inhibition of Dengue virus replication, RIG-I:RNA binding assays, and limited trypsin digestion of RIG-I:RNA complexes, to begin to understand how RNAs containing modified nucleotides avoid or suppress innate immune signaling. The experiments were based on a model innate immune activating RNA molecule, the polyU/UC RNA domain of hepatitis C virus, which was transcribed in vitro with canonical nucleotides or with one of eight modified nucleotides. The multiple assay approach revealed signature behaviors associated with individual modified nucleotides or classes of modified nucleotides. A striking result of the partial trypsin digest experiments was that RNAs containing one class of modified nucleotides were found to bind to RIG-I, but failed to trigger RIG-I conformational changes that activate innate immune signaling. These data advance our understanding of RNA-mediated innate immune signaling and inform the strategic use of nucleotide modifications for RNA therapeutics.



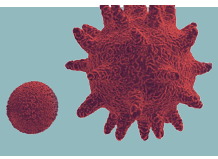
S02 cGAS and IFI16: Sensing intracellular DNA as ‚stranger' and ‚danger' signal

Leonie Unterholzner[1]

[1] *Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, LA1 4YQ, UK*

Intracellular DNA receptors play a key role in the detection of exogenous DNA as ‚stranger' signal during infection with intracellular pathogens. The body's own DNA can also be sensed as a ‚danger' signal, for instance when DNA is damaged or when DNA from dead cells is insufficiently cleared.

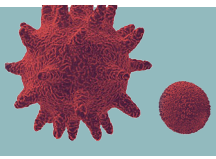
Several intracellular DNA sensors have been described. However, since the discovery of the cytosolic DNA receptor cyclic GMP-AMP synthase (cGAS), which plays an essential role in the sensing of intracellular DNA in a variety of cells, the function of other, previously identified DNA sensors has been called into question. We tested the function of IFI16, a DNA sensor that is predominantly nuclear at steady state, in human keratinocytes. We found that cGAS and IFI16 are not simply redundant, but instead perform distinct functions in the activation of the adaptor protein STING during the cell's response to viral DNA and to DNA damage.



S03 Sensing viral RNAs in the model organism *Drosophila*

Simona Paro[1], Joao Marques[1,2], Eric Aguiar[1,2], Carine Meignin[1], Jean-Luc Imler[1]
[1] *CNRS UPR9022, Institut de Biologie Moléculaire et Cellulaire, Université de Strasbourg, Strasbourg, France*
[2] *Dpt of Biochemistry and Immunology, UFMG, Belo Horizonte, Brazil*

RNA interference 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. A distinctive advantage of the fly antiviral immune system is that these siRNAs provide a footprint of its action. It has been unclear how RNAs from picornaviruses are sensed by the innate immune system. Here, we have developed a novel approach to analyse small RNA sequencing data and understand how Drosophila C virus (DCV), a member of the Picornavirales order, is sensed by Dcr-2. We show that a secondary structure on the 5' UTR of the viral RNA genome is recognized in a Dcr-2 dependent manner. From this entry point, the enzyme progresses onto and dices viral replication intermediates. Similar results were observed with two other viruses from this order, CrPV in drosophila and EMCV in mammalian ES cells suggesting a conserved mechanism. Dcr-2 shares with mammalian RIG-I-like receptors (RLRs) a Duplex RNA activated ATPase (DRA) domain. The ATPase activity of this conserved domain is not essential for viral RNA sensing, but is required for processive viral RNA cleavage and resistance to viral infection.



S04 Understanding the effects of editing-deficiency

Laura Cimatti[2], Cornelia Vesely[1], Prajakta Bajad[2], Michael Jantsch[1]

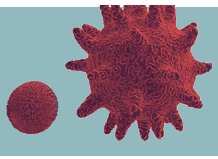
[1] *Center of Anatomy and Cell Biology, Department of Cell & Developmental Biology, Medical University of Vienna*

[2] *Department of Chromosome Biology, Max F Perutz Laboratories, Medical University of Vienna*

RNA editing by adenosine deaminases acting on RNA (ADAR) is an essential process. Mice lacking ADAR1 die embryonically and display elevated interferon signaling. Deficiency of ADAR2, in contrast, leads to lethality in young mice.

Conserved targets of ADAR2 encode the actin crosslinking proteins Filamin A and Filamin B which are edited at the same position. Lack of Filamin A editing leads to hypercontraction of smooth muscle cells but also to hypersensitivity to develop colitis which is accompanied by elevated inflammation markers. Currently we focus on deciphering the molecular mechanisms underlying these observations.

The embryonic lethality of ADAR1 deficiency can be rescued by depletion of the viral sensor protein MAVS. This suggests that endogenous RNAs may be triggering the embryonic lethality. Identifying candidate RNAs that trigger ADAR1 lethality is part of ongoing studies.



S05 RNA modifications affect recognition and immunestimulation by TLR7

Mark Helm[1], Alexander Dalpke[2], Yuri Motorin[3]
[1] *Johannes Gutenberg-Universität Mainz, Germany*
[2] *University of Heidelberg, Germany*
[3] *Université de Lorraine, Germany*

The innate immune system is equipped with a number of sensors, so called Pattern-Recognition Receptors (PRRs) destined to recognize pathogen-associated molecular patterns (PAMPs). TLR7 is an endosomal PRR 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 tRNAs to prokaryotic and eukaryotic tRNAs, it became clear that post-transcriptional modifications are the decisive parameter for tRNA mediated TLR7 response. In depth analysis identified a ribose-methylated guanosine, Gm18, as capable of silencing a TLR7 response, when placed in the correct dinucleotide context. This presentation will feature current insight into the scope of this modification in tRNA preparations from different sources, and means for its detection by high-throughput methods.

Dalpke, A. & Helm, M. (2012). „RNA mediated Toll-like receptor stimulation in health and disease." RNA Biol 9(6): 828-842.
Gehrig, S., Eberle, M. E., Botschen, F., Rimbach, K., Eberle, F., Eigenbrod, T., Kaiser, S., Holmes, W. M., Erdmann, V. A., Sprinzl, M., Bec, G., Keith, G., Dalpke, A. H. & Helm, M. (2012). „Identification of modifications in microbial, native tRNA that suppress immunostimulatory activity." The Journal of Experimental Medicine, Feb 13., 209(2):225-33
Kaiser, S., Rimbach, K., Eigenbrod, T., Dalpke, A.H. & Helm, M. (2014). „A modified dinucleotide motif specifies tRNA recognition by TLR7." RNA Sep. 20(9):1351-5

S06 Evolutionarily conserved immune effects of ADAR RNA editing, Conflict RNA Modifications and the RNA-DNA genome transition

Simona Paro[1], Leeanne McGurk[1], Xianghua Li[1], Niamh M. Mannion[1,4], Marion C. Hogg[1], James Brindle[1], Robert Young[1], Rui Zhang[2], Ian R. Adams[1], Jin-Billy Li[2], Giuseppa Pennetta[3], Mary A. O'Connell[5], and Liam P. Keegan[5]

[1]MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine at the University of Edinburgh, Crewe Road, Edinburgh EH4 2XU, UK.

[2] Department of Genetics, Stanford University, 300 Pasteur Dr., Stanford, CA 94305, USA.

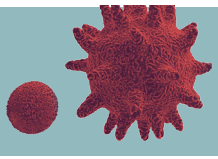
[3] Centre for Integrative Physiology, Euan MacDonald Centre for Motor Neurone Disease Research, Hugh Robson Building, University of Edinburgh, George Square, Edinburgh, EH8 9XD,

[4] Paul O'Gorman Leukaemia Research Centre, Institute of Cancer Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, 21 Shelley Road, Glasgow G12 0ZD, UK,

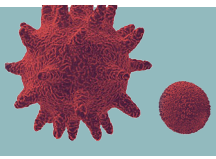
[5] CEITEC Masaryk University, Kamenice 753/5, A35/143 625 00 Brno Czech Republic

The most abundant base modification in mammalian mRNA and noncoding RNA is the hydrolytic deamination of adenosine to inosine, catalysed by members of the ADAR family of RNA editing enzymes (Adenosine deaminases acting on RNA). Editing occurs site-specifically within coding sequences at intron-exon hairpins as well as promiscuously within longer RNA duplexes formed by Alu and other repetitive elements in pre-mRNAs, in mature mRNA 3' UTR regions and in noncoding RNAs. Mutations in human ADAR1 cause Aicardi-Goutieres Syndrome, a rare genetic autoimmune encephalopathy in children that mimics congenital virus infection with increased levels of Type1 interferon (IFN).

Adar$^{1\Delta 3\text{-}13}$ null mutant mice die by embryonic day E12.5 with failure of fetal liver haematopoiesis and increased production of IFN. We have rescued *Adar*$^{1\Delta 3\text{-}13}$ mutant embryonic lethality to live birth in *Adar*$^{1\Delta 3\text{-}13}$*;Mavs* (mitochondrial antiviral signalling protein), double mutants in which the antiviral IFN response to cytoplasmic double-stranded RNA (dsRNA) is prevented. A mouse *Adar1*^{E861A}*;IfiH1* double mutant expressing catalytically inactive Adar^{1E861A} protein and lacking the MDA5 sensor for long dsRNA is fully rescued but *Adar1*$^{\Delta 3\text{-}13}$*;IfiH1* double mutants are not, indicating protective effects of the ADAR1 protein in addition to editing. We suppressed aberrant immune induction in *Adar1*$^{\Delta 3\text{-}13}$*;Trp53* mouse embryo fibroblasts with short blunt-ended dsRNA oligonucleotides containing inosines, suggesting that abberrant RIG-I is also being suppressed in this experiment. We proposed that I-U wobble base pairs and local helix destabilization at edited sites exert dominant suppressive effects by facilitating inactive dsRNA-bound conformations of RLRs.



Drosophila has a single *Adar* gene encoding the orthologue of vertebrate ADAR2 which edits nervous system transcripts; hundreds of CNS transcripts are edited site-specifically in *Drosophila*. *Adar* mutant flies show reduced viability, uncoordinated locomotion and age-dependent neurodegeneration. We have found that aberrant induction of innate immune transcripts also occurs in *Drosophila Adar* mutant heads. *Drosophila* and other arthropods have lost the inosine-sensitive RLRs during evolution. However the RNA cleavage activity of DICER proteins is also inhibited by inosine. DICER2 is a key player in insect defences against viruses and DICER2 has also been proposed to act as an innate immune RNA sensor in *Drosophila*. The *Drosophila* IMD innate immune signalling pathway conserves the nucleocytoplasmic part of the vertebrate RLR pathway.
Adar mutant phenotypes reveal an evolutionarily conserved role for inosine, and probably also other RNA modifications, in self versus non-self discrimination between host RNAs and those of viruses and other parasites. On the other hand ADAR RNA editing of adenosine to inosine may also mark as self very many host dsRNAs containing repetitive or parasitic sequences, helping the innate immune system to tolerate the presence of these. We propose that many types of enzymatic RNA modifications have roles as Conflict RNA Modifications, when they affect nucleic acids recognition in host-parasite interactions. We propose that double-stranded RNA was the first genomic nucleic acid and that DNA first evolved as a Conflict RNA Modification involved in defense against and tolerance of intragenomic parasites, i.e. DNA first arose by direct enzymatic modification of dsRNA by a ribonucleotide reductase that acted on RNA. The evolutionary biologist William D. Hamilton proposed that faster-evolving parasites maintain sexual reproduction in eukaryotes; Conflict RNA Modification extends this Parasite Red Queen concept to intragenomic parasites and to the RNA-DNA genome transition.



S07 mRNA modifications beyond RNA editing

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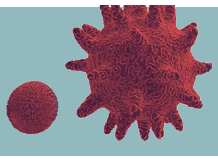
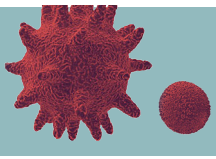
Messenger RNA editing, in particular of the Adenosine to Inosine type, affects gene expression significantly and emerges as a significant player in the regulation of the immune response. As editing results in nucleotide switch, it is easily detectable by reverse transcription and sequencing. An extensive repertoire of modifications is known to underlie the versatile coding, structural and catalytic functions of RNA, with methylation predominating. Recently methodologies based on immunocapturing and NGS enabled the identification of two major types of Adenosine modifications that decorate mRNA molecules. We mapped transcriptomic N6-methyladenosine (m6A), the most prevalent internal modification in mRNA in human and mouse cells and uncovered the features of the m6A methylome: m6A preferentially appears around stop codons and the 3' UTR as well as within long internal exons. This type of methylation is highly conserved, dynamically modulated and can affect the specific binding of reader proteins. Mettl3-null naïve embryonic stem cells are depleted of m6A in mRNAs, yet are viable. However, they fail to adequately terminate their naïve state and, subsequently, undergo aberrant and restricted lineage priming at the post-implantation stage, which leads to early embryonic lethality. Recently we identified another mRNA modification, N1-methyladenosine (m1A), and showed that it is enriched around the start codon upstream of the first splice site: it preferentially decorates more structured regions around canonical and alternative translation initiation sites, is dynamic in response to physiological conditions, and correlates positively with protein production. These unique features of the m1A methylome are highly conserved in mouse and human, strongly indicating a functional role for m1A in promoting translation of methylated mRNA. The study of the emerging field of RNA epigenetics, recently termed epitranscriptomics, is expected to increase our knowledge regarding regulation of gene expression.



S08 Queuine Insertase tagging and selection in MDA-MB-231 cells via CRISPR and SmartFlare™

Mashael Al-qasem[1], **Jill Browning**[1], **Vincent Kelly**[1]
[1] *Trinity College Dublin*

Queuine Insertase (QI) is a heteromeric complex in eukaryotic organisms that is responsible for incorporating queuine (a bacterial derived micronutrient) into the anticodon loop of transfer RNA for the amino acids tyrosine, histidine, asparagine and aspartic acid. Based on the results of early protein purification studies we suspect that the QI activity is part of a larger multifunctional complex. Proteins associated with QI activity from rabbit erythrocytes included immunophilin p59, human elongation factor 2 and the deubquitinating enzyme USP14 and our searches of supplied peptide sequences from bovine liver have identified asparaginyl tRNA synthetase and 2,4 dienoyl CoA reductase. In an effort to verify QI interacting partners under conditions of normal expression we have developed a novel and rapid procedure to tag and select endogenously labeled proteins in mammalian cells. Using this technique we have added a C-terminal Strep-His tag to the QI catalytic subunit in MDA-MB-231 cells and present preliminary data on the optimization of the strategy and identification of possible interacting partners.



S09 N6-methyladenosine demethylase FTO targets pre-mRNAs and plays a novel role in nuclear mRNA processing and expression

Marek Bartosovic[1], Helena Covelo-Moralez[1], Pavlina Gregorova[1], Grzegorz Kudla[2], Stepanka Vanacova[1]

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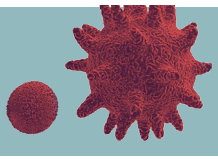
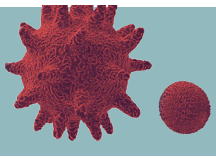
N6-methyladenosine (m6A) is an abundant modification present in a large fraction of mammalian mRNAs. Functional consequences of mRNA methylation can vary substantially and it has previously been implicated in diverse biological processes such as mRNA stability, splicing, translation or RNAi. Functionally, m6A plays a role during embryogenesis, circadian dynamics and cellular differentiation. M6A is subject to a dynamic removal by m6A demethylases FTO and AlkBH5 (erasers), which further underscores its regulatory potential. Whereas mechanism and function of the methylase complex has been studied to greater detail, substrate specificity and function of erasers still remains poorly understood. We present a transcriptome-wide characterization of FTO substrates and propose its molecular function. By using cross-linking and immunoprecipitation coupled to high-throughput sequencing (CLIPseq) we demonstrate that FTO binds preferentially to pre-mRNAs in intronic regions and in the proximity of polyA sites. The transcriptome analysis of the FTO depleted (KO) cell line by RNA-seq reveals correlation between mRNA expression and FTO binding. Furthermore we observe multiple effects of FTO depletion on nuclear mRNA processing steps. In summary we provide for the first time a global view on the function of an mRNA demethylase in gene expression and RNA metabolism.



S10 The RNA helicase DDX39B regulates IL7R alternative splicing reducing the risk of Multiple Sclerosis

Mariano Garcia-Blanco[1]
[1] *UTMB*

Multiple Sclerosis (MS) is an autoimmune disorder where rogue T cells attack CNS neurons leading to demyelination and neurological deficits. A driver of increased MS risk is the soluble form of the interleukin-7 receptor alpha chain gene (IL7R), produced by alternative splicing of IL7R exon 6. Analysis of factors required for regulation of exon 6 splicing led us to identify the RNA helicase DDX39B as a potent activator of this exon, and consequently a repressor of sIL7R. Further, we find a strong genetic association between DDX39B and MS risk, validating the significance of the functional studies. Indeed, we show that a genetic variant in the 5′ UTR of DDX39B reduces translation of DDX39B mRNAs and increases MS risk. This DDX39B variant showed strong genetic and functional interactions with allelic variants in IL7R exon 6. These studies provide a mechanism for the regulation of IL7R exon 6 splicing and its impact on MS risk.



S11 Transcriptome and DNA methylome analysis of two sympatric 1 ethic groups with 2 differential susceptibility to *Plasmodium falciparum* infection living in Burkina Faso

Ann-Kristin Östlund Farrants[1,2,3,4]
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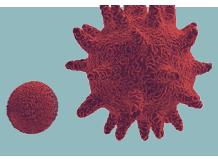
[2] *National de Recherche et de Formation sur le Paludisme, Ouagadougou,*
[3] *Central European Institute of Technology, Brno, Czech Republic*
[4] *Karolinska Institutet*

The Fulani ethnic group is known to be less susceptible to Plasmodium falciparum (P. falciparum) infection as compared to other sympatric ethnic groups, such as the Mossi in Burkina Faso. Numerous studies have been conducted in order to elucidate possible immunological and genetic factors involved in this protection. However, the underlying mechanisms for this relatively more protective response to P. falciparum infection in Fulani individuals remain largely unknown. In this study we hypothesize that transcriptome and DNA methylome analysis could help to establish the transcriptional and regulatory networks underlying the relatively better protection in the Fulani group. We aimed to characterize molecular events that occur during natural P. falciparum infection in Fulani individuals compared to Mossi individuals. Our findings revealed differential gene expression in monocytes of infected Fulani compared to uninfected Fulani. The difference between Fulani individuals upon P. falciparum infection concerned multiple classes of genes coding for proteins involved in signal transduction, the basal transcription machinery, transcription factors and immunological responses. DNA methylation analysis revealed that there were no major differences in DNA methylation patterns between the two ethic groups regardless of infection status. Instead we observed significant up-regulation of various genes coding for chromatin remodelling complexes and epigenetic enzymes that could contribute to the regulation of the protective response. Overall, the results presented in this study provide new knowledge on the molecules and regulatory mechanisms that are important for a relatively more protective response against P. falciparum, a causative agent of malaria.



S12 Cell-cell communication between *Plasmodium* and host immune DNA sensing pathway via exosomes

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Malaria, kills up to a million people each year, is caused by the protozoa of the genus Plasmodium falciparum (Pf). These vector-born parasites cycle between mosquitoes and humans and, in both contexts, are faced with an unstable and hostile environment. To ensure survival and transmission, the malaria parasite must infect and survive in the human host and differentiate into sexual forms that are competent for transmission to mosquitoes. We found, for the first time that Pf-infected red blood cells (iRBCs) directly exchange cargo between them using nanovesicles (exosomes). These tiny vesicles are capable of delivering protected genes to target cells.

Cell-cell communication is a critically important mechanism for information exchange that promotes cell survival. How Pf parasites sense their host environment and coordinate their actions, remain one of the greatest mysteries in malaria. Moreover, our understanding in the mechanism regulate human immune response to malaria infection is poor. Here, we found that malaria-derived exosomes carry remarkable cargo providing a secure and efficient mode for signal delivery. We developed an exosome tracking assay and could measure Pf exosome uptake by different cell types. Moreover, although early life-stages of Pf-iRBC are considered immunologically inert, our initial observations show that ring-stage derived exosomes are immunogenic. We show that exosomes can specifically activate and induce pro-inflammatory responses, resulting in interferon type I response. This is a new area of malaria research which may shed a light on the ability of malaria parasite to manipulate their host response.

S13 Dengue virus-induced ATF4 expression is independent of eiF2α phosphorylation and SG formation

Mayra Diosa-Toro[1, 3], Berit Troost[1], Alexander Martin Heberle[2], Kathrin Thedieck[2], Silvio Urcuqui-Inchima[3], Jolanda M. Smit[1]

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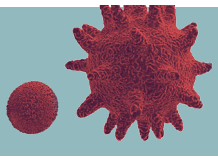
[3] *Grupo Inmunovirología, Sede de Investigación Universitaria, Universidad de Antioquia; Medellín, Colombia*

Despite the globally high burden of dengue virus (DENV) infections there are no specific antiviral therapies available. For the rational design of new therapies we need to better understand how the virus interacts with the host. In mammalian cells, various stresses, such as viral infections, cause phosphorylation of eiF2α. This results in the formation of cytoplasmic aggregates of stalled translational complexes, namely stress granules (SGs). The formation of SGs leads to a global translational arrest, yet allows selective expression of genes involved in stress recovery like the activating transcription factor 4 (ATF4). Accumulation of ATF4 induces the expression of DNA damage inducible 34 (GADD34), which mediates dephosphorylation of eiF2α. It has been reported that DENV induces nuclear accumulation of ATF4, but the mechanism and the consequences of it are not fully understood. Here, we investigated the expression levels of ATF4 upon DENV infection and whether it correlates with an increase in eiF2α phosphorylation. Upon DENV infection, an increase in ATF4 expression is seen at 18 to 30 hours post-infection. Yet, the phosphorylation status of eiF2α was not affected upon infection. In fact, sodium arsenite-induced eiF2α phosphorylation was abrogated by DENV independently of GADD34, suggesting an active mechanism by which the virus avoids to induce stress in the host cell. This is in line with the observation that DENV does not induce the formation of SGs at any time point, nevertheless was able to reduce the number of SGs per cell in chemically stressed cells. Currently, we are analyzing the expression levels of downstream targets of ATF4 to understand the function of this transcription factor during infection. Our results contribute to the poorly understood mechanism by which DENV modulates the cellular stress response, which is crucial in the development of safe and efficient therapies to counteract the burden of DENV infections.



S14 RNA editing as a mechanism of diversification of cellular populations

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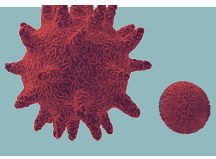
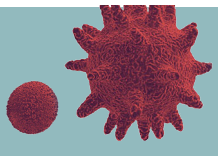
RNA editing is a mutational mechanism that specifically alters the nucleotide content in transcribed RNA. Editing rates of specific sites vary widely (though most frequently, these rates are low, <20%), and could result from equivalent editing amongst individual cells, or represent an average of variable editing within a population. Using statistical modeling and RNA-seq data from single cells and cognate bulk samples, we are able to distinguish between these possibilities. We find evidence for high variance in Apobec1 editing of specific sites in macrophages, as well as in dendritic cells, suggesting that RNA editing generates sequence diversity within cellular populations, with implications for the functional diversity of these groups of immune cells that are otherwise indistinguishable (genomically and with regard to chromatin state). We then turn our focus to the study of epitranscriptomic (sequence) heterogeneity imparted by RNA editing in the context of cancer (here, the APCmin model of intestinal cancer) and will discuss recent evidence that such heterogeneity aids cancer progression.



S15 Cancer Mutagenesis by APOBEC3B and Hypomorphic APOBEC3H

Reuben Harris[1,2]
[1] *Howard Hughes Medical Institute*
[2] *University of Minnesota*

Cytosine-focused "APOBEC" mutation signatures are prevalent in over half of human cancers and often account for the majority of mutations within a single tumor. The DNA cytosine deaminase APOBEC3B (A3B) explains this signature but, surprisingly, homozygosity of a natural A3B knockout allele failed to erase the signature from breast tumor sequences. Here, we show that another member of this antiviral family, APOBEC3H haplotype I (A3H-I), a variant previously deemed unstable and inactive, accounts for this residual signature in breast cancer and, importantly, also contributes broadly to cancer mutagenesis. First, A3B-null breast tumors with cytosine-biased mutational signatures, but not those without, have at least one copy of A3H-I. Second, genomic mutation, HIV-1 hypermutation, and biochemical data demonstrate that A3H-I is indeed catalytically active. Third, fluorescent microscopy images show that A3H-I has increased nuclear localization compared to stable A3H-II. Finally, A3H-I explains the majority of early-clonal APOBEC signature mutations in lung adenocarcinoma indicating broader relevance as a chronic source of mutation in cancer. These studies combine to reveal A3H-I as a significant and unanticipated source of somatic mutation in breast and lung cancer. A3H-I and A3B, together, account for the composite APOBEC signature reported across many human cancers. As dominant-acting DNA mutating enzymes, roles in tumor evolution, acquired resistance to targeted therapies, and chemo- and immune-therapy responsiveness are probable.



S16 A Computational Screen For RNA Editing Alterations In Autoimmune Diseases

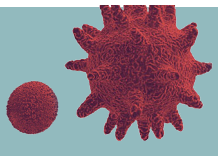
S. Hillel Roth[1], Miri Danan-Gotthold[1], Erez Y. Levanon[1]
[1] *Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University*

In most type I hypersensitivity autoimmune diseases, the immune system identifies ones own cells as malignant or infected and attacks them. Usually, MHCs are used to differ such cell from healthy ones on the cleansing of the host. However during autoimmune disease, peptides derived from normal self proteins are targeted as antigens by the body defenses. We suggest that some of the factors leading to inflammation and even to the body to mistaken self for non-self are be originated by the post transcriptional event of RNA editing. RNA editing usually occurs in introns, however part of the phenomena effects exons as well. By altering the sequence of the exon, miss-sense RNA mutation can occur, of whom some might be presented on the MHC marking the cell as defected. The magnitude of both editing and MHC loading is greatly enhanced by exposure to interferons, further increasing the putative antigenic properties of the cell and contributing to recognition of other self derived peptides as antigen, as they are presented next to the edited version, thus creating a putative positive feed back. We created a robust computational pipeline for the purpose of measuring the effect of the above from RNA seq data. We measure various patterns of A to I editing in the sequences to get a clean signal in order to measure the extent of editing in a sample, as well as deducing a list of putative edited sites. All This, in order to measure editing levels and editing derived peptides, as well of their affinity to the host HLAs, some of which might trigger the cascade described above.

S17 Posttranscriptional control of inflammatory responses by Regnase-1 and Roquin

Osamu Takeuchi[1]
[1] *Institute for Virus Research, Kyoto University*

Inflammation is initially evoked by the innate immune system in response to microbial infection and other cellular stresses. Proinflammatory cytokines produced by innate immune cells upon sensing of pathogens by Toll-like receptors (TLRs) are the mediators important for inflammation. Cytokine mRNAs tend to be degraded rapidly by a set of RNA binding proteins (RBPs) recognizing cis-elements such as AU rich elements and stem-loop structures present in the mRNA 3'-untranslated region (UTR). Among RBPs, Roquin recognizes stem-loop structures present in mRNAs encoding inflammatory proteins and degrades them by recruiting a CCR4-NOT deadenylase complex to its target mRNAs. Roquin-mutant mice spontaneously develop autoimmunity by elevated expression of ICOS on T cells and enhanced production of TNF in innate immune cells. We identified Regnase-1 (also known as Zc3h12a) as an RNA binding protein essential for degradation of inflammation-related mRNAs induced by TLR stimuli in innate immune cells. Regnase-1 is also critical for suppressing activation of T cells and maintenance of immune homeostasis in mice. Regnase-1 harbors an endonuclease activity to directly degrade immune response-related mRNAs such as IL-6. We found that Regnase-1 and Roquin regulate an overlapping set of mRNAs via a common stem-loop structure. However, Regnase-1 and Roquin function in distinct subcellular locations: ribosome/endoplasmic reticulum and processing-body/stress granules, respectively. Moreover, Regnase-1 specifically cleaves and degrades translationally active mRNAs and requires the helicase activity of UPF1, similar to the decay mechanisms of nonsense mRNAs. In contrast, Roquin controls translationally inactive mRNAs, independent of UPF1. Regnase-1 tends to control the early phase of inflammation when mRNAs are more actively translated. Taken together, our findings reveal that differential regulation of immune-related mRNAs by two RNA binding proteins, Regnase-1 and Roquin, depends on their translation status and enables elaborate control of inflammation.

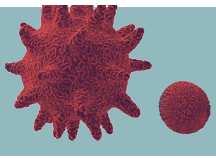


S18 SAMHD1 limits activation of the cGAS-STING pathway

Jan Rehwinkel[1]

[1] *Medical Research Council Human Immunology Unit, Medical Research Council Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford OX3 9DS, UK*

SAMHD1 is a restriction factor for HIV-1 infection. SAMHD1 mutations cause the autoinflammatory Aicardi-Goutières syndrome that is characterised by chronic type I interferon (IFN) secretion. We show that the spontaneous IFN response in SAMHD1-deficient cells and mice requires the cGAS-STING cytosolic DNA sensing pathway. Furthermore, we provide genetic evidence that cell-autonomous control of lentivirus infection in myeloid cells by SAMHD1 limits virus-induced production of IFNs and the induction co-stimulatory markers. This programme of myeloid cell activation required reverse transcription, cGAS and STING, and signalling through the type I IFN receptor. Furthermore, SAMHD1 reduced the induction of virus-specific cytotoxic T cells in vivo. Therefore, virus restriction by SAMHD1 limits the magnitude of IFN and T cell responses. This demonstrates a competition between cell-autonomous virus control and subsequent innate and adaptive immune responses, a concept with important implications for the treatment of infection.



S19 Cross-species comparative analysis of Dicer proteins during *Sindbis* virus infection

Erika Girardi[1], Mathieu Lefèvre[1], Béatrice Chane-Woon-Ming[1], Simona Paro[2], Bill Claydon[2], Jean-Luc Imler[2], Carine Meignin[2], Sébastien Pfeffer[1]

[1] *Architecture and Reactivity of RNA, Institut de Biologie Moléculaire et Cellulaire du CNRS, Université de Strasbourg, 15 rue René Descartes, 67084 Strasbourg, France*

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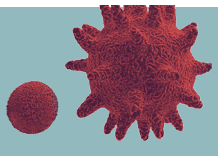
In plants and invertebrates RNA silencing is a major defense mechanism against virus infections. The first event in RNA silencing is dicing of long double stranded RNAs into small interfering RNAs (siRNAs) by Dicer proteins. In vertebrates, the predominant antiviral response is represented by the interferon (IFN) pathway, which is activated upon recognition of dsRNAs by pattern recognition receptor (PRRs). Vertebrate Dicer proteins might also participate to the innate immune response, but limited production of Dicer-dependent viral derived siRNAs has been observed in infected cells to date.

To gain insight on this restriction, we compared the dicing activity of human Dicer and fly Dicer-2 in the context of Sindbis virus (SINV) infection. Expression of human Dicer in flies inefficiently rescues the production of viral siRNAs but confers some protection against SINV. Conversely, expression of Dicer-2 in human cells allows the production of viral 21 nt small RNAs but, instead of conferring resistance to viral infection, results in stronger accumulation of viral RNA. We further showed that Dicer-2 expression in human cells perturbs interferon (IFN) signaling pathways and antagonizes protein kinase R (PKR)-mediated antiviral immunity. Overall, our data indicate a functional incompatibility between the Dicer and IFN pathways which might be explained by a competition for viral dsRNA substrate in mammalian somatic cells.



S20 RNA viruses are targeted by diverse RNA binding proteins

Sara Cherry[1]
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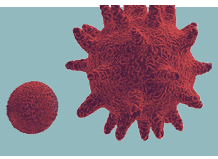
Viral pathogens are a common cause of morbidity and mortality in both the developing and developed worlds. Viruses have not only developed highly effective strategies to hijack cellular machinery and to subvert hosts' immune responses, but they have evolved broad host ranges, spanning arthropods and mammals. Little is known about the host factors required for the replication cycles of these viruses in either of their host genera, and less is understood about the hosts' innate immune pathways that act to restrict pathogenesis. We study the three families of arthropod-borne RNA viruses: the flaviviruses including West Nile virus, dengue virus and the newly emerging Zika virus, the alphaviruses including Sindbis and Chikungunya virus as well as the bunyaviruses Rift Valley Fever virus and La Crosse virus. We model the vector biology using the genetically tractable model organism Drosophila comparing host factor dependencies between the insect and human hosts. Combining forward genetics and functional genomics comparing and contrasting between viruses and hosts allows us to use these unbiased and global methodologies to identify many important and novel host factors that modulate virus-host interactions. Through these studies we have identified a large number of new factors and pathways involved converging on our discovery of new factors involved in viral nucleic acid sensing and restriction.

S21 Using CRISPR/Cas9 to unravel the role of mammalian RNAi in antiviral immunity

Susan Schuster[1,2], **Lotte Tholen**[1,2], **Ronald van Rij**[1,2]
[1] *Radboud University Medical Center, Nijmegen, The Netherlands*
[2] *Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands*

The RNA interference pathway (RNAi) is conserved amongst invertebrates and vertebrates and is crucial for antiviral immunity in plants, fungi and invertebrates. Recent evidence also suggests an antiviral role for RNAi in mammals, particularly in embryonic stem cells. In our study, we investigated whether RNAi contributes to antiviral immunity in differentiated cells. Our findings suggest that Ago2 deficient HeLa cells have higher viral loads and are stronger inducers of ISGs in response to poly (I:C) and positive sense RNA viruses. Our results suggest that RNAi might have an immune modulatory role in response to viral challenge.



S22 Identification of the minimal ligand motif for the cytoplasmic DNA sensor cGAS

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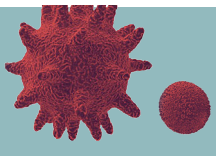
In vertebrates, nucleic acid sensing is the dominant antiviral defense pathway. Cytosolic long duplex DNA emerging during DNA- or retroviral infection can potently trigger innate antiviral immune responses via the cGAS-Sting pathway. We found that immunologically inactive short DNA duplexes are rendered highly immunostimulatory if flanked by unpaired guanosines. This specific G-ended and branched (Y-form) short DNA induced strong type-I-interferon secretion via the cGAS/STING pathway. Such Y-form DNA emerges within the highly structured early reverse transcripts (STRONG-STOP-DNA) of lentiviruses. Intriguingly, interferon induction by single-stranded DNA corresponding to HIV-1 STRONG-STOP-DNA was lost upon removal of unpaired guanosines in Y-form junctions. Thus, we identified a novel cGAS recognition motif that enables the detection of single-stranded DNA as present in early lentiviral replication.

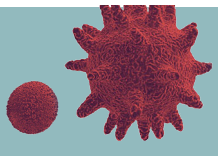


S23 Exploring the role of the RNA-binding activity of the E3 ubiquitin ligase Trim25 in antiviral innate immunity

Gregory Heikel[1], Gracjan Michlewski[1]
[1] *Wellcome Trust Centre for Cell Biology, University of Edinburgh*

Trim25 is an E3 ubiquitin ligase which is essential for activation of the type I interferon response after detection of viral 5'-triphosphate RNA (5'ppp-RNA) by the pattern recognition receptor RIG-I. Upon RIG-I binding to 5'ppp-RNA, Trim25 ubiquitylates the RIG-I 2CARD domains, allowing the recruitment of the downstream effector MAVS and progression of the signalling pathway, leading to interferon expression. Trim25 was identified as an RNA-binding protein in a genome-wide screen although the mechanism and any sequence specificity of Trim25 RNA binding has not yet been elucidated. It is not yet known whether Trim25 RNA-binding activity is necessary for its role in activating RIG-I, however there is evidence suggesting that Dengue virus subgenomic RNA can compete with RIG-I for Trim25 binding, reducing activation of RIG-I and increasing pathogenicity.

Our aim is to elucidate the mechanism of Trim25 binding to RNA and determine whether this activity is required for its role in the RIG-I signalling pathway.



S24 Unique molecular barcoding in bcr and tcr repertoire profiling

Mikhail Shugay[1,2,3], Alexey Davydov[1], Olga Britanova[1,2,3], Maria Turchaninova[2,3], Evgeniy Egorov[1,2,3], Mark Izraelson[1,2,3], Dmitriy Chudakov[1,2,3]

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Current challenges of high-throughput T- and B-cell receptor profiling include the lack of protocols that enable full-length, quantitative, unbiased and error-free analysis of antigen receptor repertoire. Unique Molecular Identifier (UMI) tagging techniques that allow assignment of sequencing reads to corresponding cDNA or gDNA molecules is a promising solution that can greatly improve the quality of immune repertoire sequencing. The efficiency of RNA or gDNA extraction and other antigen receptor library parameters can be substantially different from sample to sample and from experiment to experiment. UMI tagging technique allows to precisely estimate the number of molecules that enter the library preparation step and reveal possible sampling bottlenecks. Counting UMI-labeled molecules can be used for sample normalization and that is a pre-requisite for informative comparison of TCR repertoires using various metrics, most importantly, widely used repertoire diversity estimates, as well as other types of comparative analyses. Furthermore, grouping of sequencing reads that carry the same UMI and thus cover the same starting molecule dramatically improves sequencing quality even for poor-quality sequencing, allowing for the long range sequencing in MiSeq or HiSeq setting. As it was recently demonstrated for the case of BCR profiling, UMI-based analysis provides accurate long-read sequencing of the entire variable region, and efficiently eliminates PCR and sequencing errors without the need of applying complex contig assembly methods and empirical error correction techniques. Application of UMI-based protocols in routine TCR and BCR profiling studies solves a wide range of problems with current repertoire sequencing techniques thus promising a major boost to the entire immune repertoire sequencing field in the nearest years.

S25 Nucleoside-modified mRNA-lipid nanoparticle therapeutic protein delivery platform

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Nucleoside modified mRNA represents the newest frontier in protein therapeutics. The combination of nucleoside modification with removal of phage polymerase produced dsRNA contaminants results in an mRNA that is translated at very high levels in vivo and does not activate innate immune RNA sensors. This allows for repeated delivery of mRNA encoded proteins in a highly controlled manner. It also avoids the expense of therapeutic protein production and the risks of aberrant folding and modification. We have explored nucleoside modified mRNA for the delivery of host proteins (erythropoietin, cytokines, growth factors) and exogenous proteins (monoclonal antibodies, gene editing components). Nucleoside modified mRNA was also investigated as a vaccine delivery system with outstanding results. HIV-1 envelope and influenza hemagglutinin were used as immunogens and induced extremely potent neutralizing antibody responses. Hemagglutinin inhibition, the accepted measure of neutralization, after a single immunization was over 4 times greater than the gold standard, acute infection. Tier 2 neutralization of HIV was obtained after 2 immunizations. The central effect of this vaccine platform was the specific induction of T follicular helper (Tfh) cells. Half of the potent CD4+ T cell response were activated, antigen-specific Tfh cells that were associated with germinal center, plasma, and memory B cell induction resulting in potent, long-lived antibody responses that had characteristics associated with high levels of affinity maturation. The lack of adjuvant activity of the nucleoside-modified mRNA encoding the antigen was essential for the induction of the Tfh response. Thus, nucleoside modified mRNA has a wide range of uses that will impact medical research and therapy, which are further heightened by the ease and low cost of its production.

POSTERS

P01 "Z-RNA" fragments: Implications for folding, protein recognition and immune response

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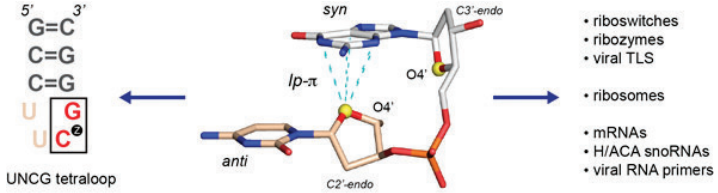
Since the work of Alexander Rich, who solved the first Z-DNA crystal structure, we have known that d(CpG) steps can adopt a particular structure that leads to forming left-handed helices and that these fragments are immunogenic. However, it is still largely unrecognized that other sequences can adopt 'left-handed' conformations in DNA and RNA, in double as well as single stranded contexts. These 'Z-like' steps involve the coexistence of several rare structural features: a C2'-endo puckering, a syn nucleotide and a lone pair–∏ stacking between a ribose O4' atom and a nucleobase. This particular arrangement induces a conformational stress in the RNA backbone, which limits the occurrence of Z-like steps to ≈ 0.1% of all dinucleotide steps in the PDB. Here, we report Z-like steps within r(UNCG) tetraloops but also within small and large RNAs including riboswitches, ribozymes and ribosomes. Proteins involved in immune response, like IFIT5, recognize/induce specifically these folds. Thus, characterizing the conformational features of these motifs could be a key to understanding some aspects of the immune response at a structural level. Besides, these dinucleotide steps represent a further example of the stunning diversity of motifs present in the assembly of RNA systems with unforeseen properties.

D'Ascenzo L., Leonarski F., Vicens Q. and Auffinger P. (2016). *"Z-DNA like " fragments in RNA: a recurring structural motif with implications for folding, protein recognition and immune response*, Nucleic Acids Res.



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Keywords: Z-RNA; lone pair-π stacking ; RNA-protein recognition

P02 DNase II-derived 3´ monophosphate DNA resists cytosolic exonuclease TREX1 degradation and potently activates cGAMP synthase

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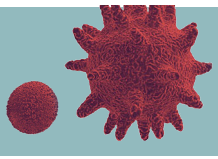
cGAMP synthase (cGAS) mediated sensing of cytosolic dsDNA initiates the immune response against many pathogens, yet its inappropriate activation can also precipitate autoinflammation. 3‘ repair exonuclease 1 (TREX1) efficiently degrades cytosolic DNA depleting potential cGAS ligands. We previously described that DNA modified by oxidative damage became resistant to TREX1-mediated degradation, promoting autoimmune pathology. However, a physiological role for TREX1 resistant DNA forms has not yet been defined. Here we report that the 3‘ monophosphate (3‘P) modification of DNA completely abrogated TREX1 degradation, and we identify lysosomal DNase II degradation products as an important source of these 3‘P modifications. DNA exposed to DNase II during phagocytic uptake or pyroptotic cell death incurred 3‘P, and thus functioned as a damage-associated molecular pattern (DAMP) boosting cGAS activation. Moreover, introduction of 3‘P robustly enhanced the immunogenicity of synthetic oligodeoxynucleotides, a promising approach for the development of therapeutic cGAS agonists.

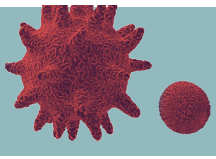


P03 Dealing with dsRNA in the bidirectional transcribed circular genome of human mitochondria

Ashish Dhir[1], Somdutta Dhir[1], Roman Szczęsny[2], Andrzej Dziembowski[2], Nicholas Proudfoot[1]

[1] Sir William Dunn School of Pathology, South Parks Road, University of Oxford, U.K.
[2] Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Poland

The mammalian mitochondrial genome, unlike the complex nuclear genome, is a compact, circular double-stranded DNA encoding 37 genes. These RNAs derive from both DNA strands and are transcribed as large polycistronic transcripts from separate promoters in the D-loop region. Such bidirectional transcription inevitably generates transcripts with extensively complementarity to each other which poses a severe challenge to the cell. We have identified the presence of a highly unstable long double stranded RNA (dsRNA) generated as a result of transcription of the mitochondrial genome. We further uncover a key role for mitochondrial RNA helicase SUV3 and an exoribonuclease PNPase in suppressing this dsRNA accumulation. Significantly loss of PNPase causes dsRNA accumulation and triggers interferon-β response primarily via MDA5 receptor as dsRNA escapes from mitochondria into the cytoplasm. This results in massive cell death. In effect we have uncovered a novel pathway that guards the cells by suppressing accumulation of deleterious dsRNA formed as a consequence of bidirectional transcription of the mitochondrial genome.



P04 Targeted DNase gene deletions suggest a cooperative mechanism of DNA degradation during terminal differentiation of epidermal keratinocytes

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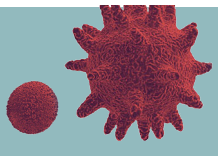
Cornification of epidermal keratinocytes and programmed cell death of sebaceous gland keratinocytes (sebocytes) during holocrine secretion of sebum involve the breakdown of nuclear DNA. To investigate the underlying mechanisms of DNA degradation, we deleted three deoxyribonuclease (DNase) genes (Dnase1, Dnase1l2, Dnase2) in mice and determined the resulting skin phenotypes. Ablation of Dnase1 suppressed neutral DNase activity in the sebum but did not block nuclear DNA degradation in keratinocytes and sebocytes. Likewise, inactivation of Dnase1l2 was compatible with DNA breakdown in sebocytes and interfollicular keratinocytes whereas cornifying hair and nail keratinocytes of DNase1L2-deficient mice retained DNA. Deletion of Dnase2 specifically in K14-positive keratinocytes and sebocytes abrogated acid DNase activity in the stratum corneum and in sebum. Both nuclear and mitochondrial DNA was significantly increased in the sebum of DNase2-deficient mice. In situ labeling of DNA showed that terminally differentiated DNase2-deficient sebocytes failed to remove nuclear DNA. Co-deletion of DNases 2 and 1L2, but not deletion of any single DNase, caused aberrant retention of nuclear DNA in the stratum corneum, known as parakeratosis. These findings suggest that DNase 2 is essential for DNA degradation in terminally differentiating sebocytes and that the cooperative activities of DNase 1L2 and DNase 2 are required for cell-autonomous DNA degradation during stratum corneum formation.



P05 Role of calcineurin nfat signaling in mouse myeloid cells and human peripheral blood monocytes

Kamila Bendíčková[1], Federico Tidu[1], Jose Shyam Sushama[1], Jan Frič[1]
[1] Cellular and Molecular Immunoregulation Group (CMI) Center for Translational Medicine (CTM) International Clinical Research Center (ICRC) St. Anne's University Hospital Brno

The calcineurin-NFAT pathway has been recently identified as an important player in the innate immune response and there is increasing evidence that transcription factor NFAT is functional in various subset of myeloid cells (Fric et al., 2012). Recently we have shown that calcineurin/NFAT signaling in myeloid cells is essential for better survival of mice with aspergillosis (Zelante et al., 2016). Upon trigger with TLR4 and Dectin-1 ligands we identified number of calcineurin NFAT dependent genes driving direct innate responses and genes regulating renewal of myeloid compartment. These findings remain to be confirmed in human myeloid cells.
Monocytes from peripheral blood, which serve as a systemic reservoir of myeloid cells, were analyzed in order to understand the involvement of calcineurin NFAT signaling in pattern recognition receptors (PRRs) driven responses. Calcineurin NFAT pathway is activated through PRRs signaling during tissue damage or infection. To study the role of PRRs signaling in human myeloid cells, we used monocytes isolated from blood of healthy volunteers or patients with sepsis. We sorted out 3 monocyte subpopulations CD14hiCD16lo, CD14loCD16hi, and CD14hiCD16hi and stimulated them with broad range of PRRs ligands (LPS, zymozan, β-glucan) under selective inhibition of NFAT signaling by addition of immunosuppressive drug cyclosporine A or tacrolimus. Changes in gene expression upon the triggers were analysed using qPCR and this outcome was used to validate the results obtained in global gene analysis of mouse myeloid cells conditionally deficient in calcineurin.
Observed changes in calcineurin-NFAT dependent gene expression upon PRRs triggers in mouse CD11c+ myeloid cells and in human monocytes showed importance of NFAT pathway during innate immune response. Need of these results is especially pertinent for immunosuppressed patients treated with calcineurin/NFAT inhibitors where such data can explain part of patients' susceptibility to infections.

P06 Structural study of Tick-borne encephalitis virus using cryo-electron microscopy

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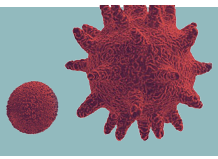
[2] Department of Virology, Veterinary Research Institute, Hudcova 70, Brno, Czech Republic

Tick-borne encephalitis virus (TBEV) is an enveloped virus belonging to the family Flaviviridae. It is mainly transmitted by ticks and causes severe disease of central nervous system in humans. Virion surface is covered by envelope proteins (E-protein), that are together with the membrane proteins (M-protein) anchored in virus lipid bilayer. The arrangement of these proteins in the virion is unknown, therefore, detailed structural study of the virus is needed.
We determined structure of mature TBEV virions grown in tissue culture and of complexes of virions with Fab fragments of neutralizing antibodies. Because of fragileness and non-homogeneity of the virions, we used cryo-electron microscopy to determine the structures. The observed particles (~50 nm in diameter) were suitable for reconstruction of the virus envelope. To obtain high-resolution electron density maps, single particle reconstruction techniques were employed, using programs from image-processing packages EMAN2, XMIPP, and RELION. The final reconstructed volume revealed structure in accordance with general structural organization of other flaviviruses including dengue and West Nile viruses. The reconstructions of TBEV particles in complexes with neutralizing antibodies showed attachment of the antibodies to specific sites on the viral surface.
Further improvement of the reconstructions may provide electron density maps of resolutions suitable for de novo model building of structural proteins, detailed structural studies of the virus shell and identification of virus residues constituting the binding site of the neutralizing antibodies. These structural studies may help to get better insight into TBEV particle organization as well as to obtain therapeutic anti-TBEV antibodies.



P07 Sensitivity of retroviruses to APOBEC3 innate immunity factors is governed by reverse transcriptase

Benedikt Hagen[1], **Stanislav Indik**[1]
[1] *University of Veterinary Medicine Vienna*

APOBEC3 innate immunity factors (APOBEC3s) restrict infection of retroviruses by hypermutation of the viral genome during reverse transcription. Retroviruses have evolved several mechanisms to evade the deleterious effect. The known examples include: HIV-1 Vif-mediated degradation of APOBEC3s in proteasomes, foamyvirus Bet-directed sequestration of APOBEC3s and HTLV-1 nucleocapsid-mediated APOBEC3 avoidance. For the vast majority of retroviruses remains the counteraction mechanism obscure. We show that the prototypic betaretrovirus (MMTV) has evolved reverse transcriptase (RT) to impede the access of APOBEC3s to its substrate, single-stranded DNA (-ssDNA), thereby neutralizing APOBEC3s. During reverse transcription the DNA polymerase domain of RT synthesizes –ssDNA using viral RNA as a template. Simultaneously, another domain of RT, RNase H, degrades the RNA template behind the polymerizing DNA polymerase. Because the DNA polymerase activity is generally greater than the activity of the RNase H domain, the RNA template is not completely degraded and small RNA fragments remain annealed to the nascent minus DNA strand. Size of the RNA/DNA hybrids depends on a balance between the DNA polymerase and RNase H enzymatic activities. The size of hybrids is of especial interest, because these regions cannot be mutated by APOBEC3s. The MMTV RT has the balance between the two RT domains noticeably shifted in favor of the DNA polymerase activity. Therefore, long RNA/DNA heteroduplexes are produced and these regions are resistant to the mutagenic activity of APOBEC3s. When the DNA polymerase activity is reduced and the balance tipped in favor of the RNaseH, the mutant synthetizes shorter RNA/DNA hybrids and becomes more sensitive to inhibition by APOBECs than the wild-type virus. Conversely, inhibited RNase H activity leads to the synthesis of longer heteroduplexes and the RNase H mutant becomes more sensitive to inhibition by APOBEC3s. The novel mechanism described here may be used by other retroviruses to evade deleterious mutagenesis.

P08 An investigation of damaged transcriptome in the human neuronal cells during oxidative stress

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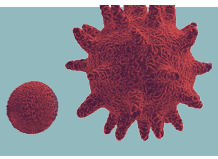
Reactive oxygen species (ROS) are produced inside the cells and can cause oxidative damage to the biomolecules. In normal physiological conditions ROS are used in cellular homeostasis and cell signaling but during stress condition they are overproduced. The over production of the ROS is associated with age and neurological disorders like Alzheimer's disease, Parkinson's disease and multiple sclerosis. The weakened antioxidant defense mechanism in the brain of the patients with the neurological disorders makes the neurons susceptible to oxidative damage. The ROS can damage every major class of biomolecules including DNA, RNA and proteins. In the past the focus was mainly on the study of DNA and protein damages, while the detrimental effects of RNA damages are being highlighted in recent years. 8-Hydroxyguanosine (8-OHG) is the most common oxidative modification found in the RNA. By identifying the modified mRNA molecules during oxidative stress in the neuronal cells (SH-SY5Y), we aim to deconvolute the relationship between specific mRNA oxidation and neurodegeneration. We have isolated the modified mRNA from the stressed SH-SY5Y cells by immunoprecipitation with anti 8-OHG antibody. Using RT-qPCR assay we have shown the differential mRNA expression during the oxidative stress (high expression of superoxide dismutase 1 and carbonyl reductase mRNAs). We further aim to establish the identity of the modified mRNA molecules by using iLumina® RNA sequencing platform. This study aims investigate the link of mRNA oxidation to neurodegeneration.



P09 Toll-like receptors expressing hek293 cells – a unique detection system

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Innate immunity is the first line defence system of the organism targeted against microbial pathogen invasion or other possible dangers. A part of this system is a family of pattern recognition receptors (PRR) activated by different pathogen-associated molecular patterns (PAMP) particularly nucleic acids typical for viruses, bacteria, and parasites or by endogenous danger-associated molecular patterns (DAMP). Activation of PRR results in inflammatory response including cytokines/chemokines release, antimicrobial peptides induction, cell death and phagocytes activation.
Toll-like receptors (TLR) were the first identified family of PRR. They are 10 (in humans) membrane-bound receptors activated by different PAMP and DAMP. Except TLR3, 7, and 9 located in endosomes, they are found in cytoplasmic membrane.
The goal is to prepare a unique detection system of cells expressing different TLR receptors. For this purpose, HEK293 cells were chosen. Using electroporation, stabile clones expressing TLR3, TLR2/CD14, TLR6/2 and TLR1/2 were prepared. This detection system could be used to detect pro-inflammatory activity of, for example, nucleic acids, to study mechanisms of compounds with suspected pro-inflammatory effects or, on the other hand, compounds prepared or chosen to serve as anti-inflammatory drugs as it is known that these receptors play a role in chronic inflammatory disorders like IBD, arthritis etc.

P10 Evolution, functions and pathogenesis of the Nse3 subunit of the SMC5/6 complex

Jan J. Paleček[1], Lucie Vondrová[1], Marek Adamus[1], Kateřina Zábrady[1], Lenka Jurčišinová[1], Lucie Bozděchová[1], Saskia N. van der Crabben[2], Marije P. Hennus[2], Grant McGregor[3], Deborah I. Ritter[4], Alan R. Lehmann[3], Antony W. Oliver[3], Sharon E. Plon[4], Johanne M. Murray[3], Gijs van Haaften[2]

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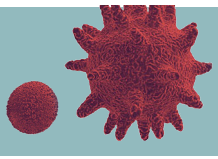
The SMC (structure maintenance of chromosome) complexes are conserved from bacteria to humans. They are key components of higher-order chromatin structures and play important roles in genome stability. The SMC5/6 complex is involved in the homologous recombination-based DNA repair, in replication fork stability and processing, and in cohesin regulation. Eight subunits compose the SMC5/6 complex and contribute to its functions and dynamics. Recently, we discovered several important features of the Nse3 subunit: its role in structural organization of the SMC5/6 complex, its DNA-binding ability, its evolution from bacteria to novel mammalian protein family. In addition, we described new chromosome breakage syndrome associated with Nse3 mutations.

We will present the new chromosome breakage syndrome associated with severe lung disease in early childhood. Four children from two unrelated kindreds died during infancy of severe pulmonary disease following viral pneumonia with evidence of combined T- and B-cell immunodeficiency. Whole exome sequencing revealed biallelic missense mutations in *NSE3*, which disrupt NSE3 interactions within the SMC5/6 complex, leading to destabilization of the complex. Patient cells showed chromosome rearrangements, micronuclei, sensitivity to replication stress and DNA damage, and defective homologous recombination.

Funding: Czech Science Foundation (GACR: P305/13/00774S)



P11 An overview of regulation of plant defense response by nonsense mediated RNA decay

Vivek Kumar Raxwal[1], Jiradet Gloggnitzer[2], Karel Riha[1]

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The first line of plant defense response begins with the recognition of pathogen produced elicitors, known as pathogen/microbe associated molecular patterns (PAMP/MAMP). Membrane bound PAMP receptors identify these pathogen signatures and initiate transcriptional reprogramming, known as PAMP triggered immunity. However, pathogens continuously evolve to breach this barrier and inject host cells with effector molecules that interfere with defense mechanisms. To counteract such pathogens, plants possess another layer of defense that relies on the activation of intracellular nucleotide-binding/leucine-rich repeat receptors (NLRs) initiating effector triggered immunity (ETI). The activity of NLRs must be very tightly regulated as their misfiring can lead to an autoimmune response that is associated with severe growth retardation and a substantial fitness cost. Nonsense mediated RNA decay (NMD) has been identified as one of the mechanisms restricting activation of NLRs in Arabidopsis. Impaired function of key NMD protein SMG7, UPF1 or UPF3 triggers autoimmunity resulting in severe development defect and necrosis. Genetic abrogation of ETI triggered immunity fully rescued the growth retardation in Arabidopsis *smg7 (smg7pad4)* mutants and lethality in *upf1 (upf1pad4)*. Transcriptome wide studies revealed that the autoimmunity is triggered by deregulation of NLR receptors containing N-terminal Toll/interleukin 1 domain (TNLs). Interestingly, infection of plants with a pathogenic bacteria led to host-programmed inhibition of NMD and stabilization of NMD-regulated TNL mRNAs suggesting pathogen-induced NMD downregulation is a physiologically relevant mechanism potentiating plant innate immunity. Here we present an overview of NMD regulation of plant defense response and its impact on evolution of plant defense genes.

This work was supported by the Czech Science Foundation (16-18578S).

P12 Resolving inflammation by regulation of mRNA stability

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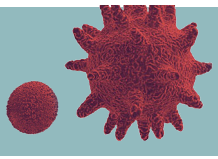
Precise regulation of mRNA decay is fundamental for robust yet not exaggerated inflammatory responses to pathogens. The mRNA-destabilising protein tristetraprolin (TTP) is crucial for such regulation, as it selectively destabilises inflammation-associated mRNAs, most notably mRNAs of cytokines and chemokines. The significance of TTP is obvious from the phenotype of TTP knockout mice, who develop normally but display progressive inflammation in several organs, eventually leading to their death. TTP exhibits complex and yet poorly understood regulation. For instance, data from our laboratory revealed that TTP can bind many mRNAs without causing their destabilisation. To provide a better insight into the behaviour of TTP, we established a global model integrating the transcriptome-wide binding of TTP at single-nucleotide resolution to its mRNA-destabilising function in LPS-stimulated bone marrow derived macrophages. To allow easy navigation through this functionally annotated database of TTP-binding sites, we developed the "TTP-binding atlas" (http://ttp-atlas.univie.ac.at). This work identified an essential function of TTP in the transition from the inflammatory to the resolution phase of the immune response (Sedlyarov et al., Mol Syst Bio 2016). Ongoing work in our lab continues to expand this global model and to elucidate the regulation of the immune homeostasis by TTP in animal models. These studies indicate that TTP regulates mRNA stability in cell type-specific ways which results in distinct functions of TTP in different immune cells and/or under different inflammatory conditions.



P13 Pattern Recognition Receptors signalling in maintenance and differentiation of Mesenchymal Stem Cells

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Mesenchymal Stem Cells (MSCs) have important role in control of immune response including crosstalk with dendritic cells or natural killer cells. MSCs can sense the environment through own expressed Pattern Recognition Receptors (PRRs). PRR triggers recruiting from the inflammatory milieu can affect MSC cytokine profile, switching to a pro-inflammatory or immunosupressory phenotype. The aim of the project is to investigate whether inflammatory stimuli affect MSCs differentiation and how the sensing of microenvironment elicits or suppresses these processes. We show changes of transcriptional control of MSCs functions upon PRRs trigger with a special focus to the interplay between various transcription factors including NFkB, HIF1a and NFAT. Our results show that PRR ligands treatment such as LPS or zymozan leads to intracellular flux of calcium followed by NFAT translocation. Reporter assay experiments were used to validate translocation of main transcription factors controlling inflammation, including NFkB, HIF1a and NFAT. Especially the link of PRRs signaling with NFAT activation has not been fully described in MSCs, therefore detailed analysis of downstream signalling is needed. Understanding how the inflammatory processes and PRRs signaling specifically influence MSC differentiation will provide new candidates to target immune disorders and tissue regeneration related inflammation in more specific manner.

P14 Osmium tetroxide - bipyridine as a potent sensor for detection of 5-methyl uridine

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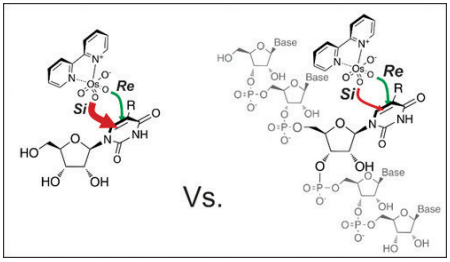
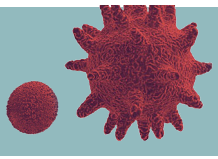


In this study we investigated the applicability of osmium tetroxide-bipyridine (os-bipy) as a labeling agent for detection of 5-methylated pyrimidines. On a nucleoside level we showed that os-bipy was about 10 times more selective for 5-methyl uridine than its non-methylated equivalent. This preference remained almost preserved when reaction was performed on a short oligonucleotide. Additionally, we determined the absolute configuration of the preferred diastereomer that builds after the reaction of os-bipy with 5-methyl uridine. Furthermore, we investigated the diastereoselectivity of the labeling agent towards uridine, 5-methyl uridine and 5-methyl cytosine as nucleosides or incorporated in a short RNA chain. We found the diastereoselectivity strongly depended on the relative position of the corresponding pyrimidine inside the chain. This allows the usage of os-bipy not only as labeling agent for 5-methyl uridine, but also in the field of structure probing for sensitive mapping of pyrimidines in different chemical environments.



P15 Importance of direct binding of Ku heterodimer to viral genomes for innate immunity

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Virus infection elicits strong responses in cells. Pattern recognition receptors (PRRs) sense viral infection via binding to molecular patterns (PAMPs) and couple recognition of viruses with induction of viral interference. Direct recognition of viral DNA and RNA by PRRs leads to activation of innate immunity and it is crucial for viral defense. DNA-PK is a heterotrimeric complex containing the highly conserved Ku70/Ku80 heterodimer (Ku). Ku binds with high affinity to DNA and RNA and it is involved in DNA double strand break repair as well as protection and maintenance of telomeres. Furthermore, DNA-PK was shown to elicit cytokine responses to VACV and HSV-1 DNA virus. DNA binding of Ku is necessary to activate the immune response to VACV. However, due to sequence independent mode of binding, Ku was also shown to enhance the rAAV replication, it has a role in HIV-1 transcription and it inhibits the geminivirus multiplication in plants. Whether direct binding of DNA-PK is required for innate processes for all viruses remains unclear. To understand biological function of Ku in different cellular processes, we generated a battery of mutant Ku complexes that have altered affinity to DNA. These mutant Ku complexes will be instrumental in understanding functional interactions between Ku and viruses.

P16 Comparison of the mirna expression profiles in fresh frozen and formalin fixed paraffin embedded tonsillar

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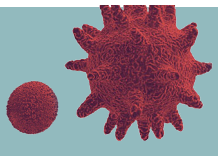
Objectives: MicroRNAs are small non coding RNAs involved in the gene expression regulation. Characteristic miRNA expression profiles define different tumor types and miRNAs are considered as promising prognostic and diagnostic biomarkers of human cancer. Most of the miRNA profiling studies of tumors were at first performed on the fresh frozen samples (FF) where the RNA is well preserved however this type of clinical material is rarely available. Therefore, the use of the archived material, formalin-fixed paraffin-embedded tissue samples (FFPE), should be better applicable and allow to do retrospective large studies. Here we aimed to compare the miRNA expression profiles in a set of FF tonsillar tumor samples and in a set of macrodissected FFPE tonsillar tumors.

Methods: Ten tonsillar tumors and five non-malignant tonsillar tissues of both types of clinical material were analyzed. The FFPE samples were enriched for the tumor cell fraction by macrodissection. The determination of miRNA expression profiles was performed using TaqMan Low Density Array system (Life Technologies). The data were processed by different software programs and we applied two types of normalization methods for comparison. The selected differentially expressed miRNAs in FFPE samples revealed by microarrays were confirmed by individual RT qPCR system.

Results: The correlation between miRNA expression profiles of paired FF and FFPE samples was relatively good however the differentially deregulated miRNAs overlap in only 27 38 %. The comparison of the results with regard to the used data normalization method revealed the overlap in 58-67 %. The selection of software program doesn´t influence the results. The expression of selected differentially expressed miRNAs in FFPE tumors was confirmed in 64 % of comparisons.



Conclusions: Our study demonstrates the comparison of miRNA expression profiles in tonsillar tumors detected in FF and FFPE tonsillar tumor samples. We pointed to the fact that for the accurate comparison of miRNA expression profiles of published studies it is important to use the same type of clinical material and to consider the right selection of normalization method for the data evaluation.

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P17 Solution structure of the double stranded RNA binding protein Staufen1 bound to its natural RNA target involved in Staufen-mediated mRNA decay

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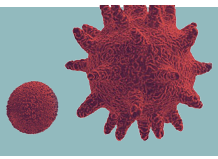
Double-stranded RNA (dsRNA) binding proteins recognize their cellular dsRNA targets through a combination of structure- and sequence-specific recognition to regulate many posttranscriptional processes. The human dsRNA binding protein Staufen binds dsRNA elements in certain mRNAs to control their cytoplasmic mRNA transport, translation and decay, in a pathway called staufen-mediated mRNA decay (SMD). The two homologues of human Staufen protein, Staufen1 and Staufen2 consist of multiple dsRNA binding domains (dsRBD) but mainly dsRBD3 and dsRBD4 are known to bind dsRNA. In SMD pathway, Staufen binds specific dsRNA elements such as a stem-loop within human ADP-ribosylation factor1 (hARF1) mRNA and thereby regulates its cytoplasmic levels. However, which features of this dsRNA as well as other targets are specifically recognized by Staufen remained mysterious.

To reveal the dsRNA target specificity of Staufen1, we are determining the solution structure of the dsRNA - Staufen1 complex by nuclear magnetic resonance spectroscopy. We designed the dsRBD3 and 4 of human Staufen1 protein for expression in E. coli and purified the protein by affinity, anion-exchange and size-exclusion chromatography. The hARF1 dsRNA was prepared by in vitro transcription and purified by anion-exchange and size-exclusion chromatography. Electrophoretic mobility shift assay and fluorescent anisotropy measurement show that the interaction of hARF1 dsRNA with dsRBD34 results in a single complex with affinity in the nanomolar range. A complete set of 2D and 3D NMR experiments was measured for the assignment of the dsRNA - Staufen1 complex. The NMR data show that both dsRBDs are well folded in free form and presence of the interaction partner hARF1 dsRNA. We will present the preliminary structure of the hARF1 dsRNA - Staufen complex which reveals that Staufen is indeed a sequence-specific dsRNA binding protein which specifically binds dsRNA targets by sequence readout in the minor groove. The biological implications will be discussed.



P18 TDS (TUT-DIS3L2 Surveillance) targets aberrant ncRNAs and short transcripts of protein-coding genes in human cytoplasm

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The 3'-terminal RNA uridylation is a posttranscriptional modification present in most eukaryotic organisms. In mammals, terminal uridyltransferases (TUTases) catalyze oligo(U) addition to various types of coding and noncoding RNAs mediating their degradation[1]. Terminal uridylation however has also opposing role, in which it can promote ncRNA processing, such as for pre-miRNAs or U6 snRNA[2,3]. In mammalian cells, two oligo(U) specific exonucleases have been identified to date; ERI1 and DIS3L2[4,5,6]. Whereas ERI1 targets histone mRNAs for degradation[4], DIS3L2 is involved in decay of uridylated precursors of let-7 miRNAs, tRNAs and cleaved mRNAs[5,6,7]. DIS3L2 has a medical importance as it mutations have been associated to the Perlman syndrome development and Wilms tumor progression[8,9]. Moreover, disregulation of certain TUTases and DIS3L2, respectively causes severe cell cycle defects in mammalian cell cultures. Currently, the link between uridylation and the involvement of DIS3L2 in disease and cell phenotype remains largely unknown.

By using catalytical mutant of DIS3L2 as a bait, we have identified uridylated aberrant forms of multiple types of coding and noncoding RNAs. We demonstrate, that extended and aberrantly processed forms of ncRNAs, such as snRNAs, rRNA, tRNAs, YRNAs, and also transcripts originating from pseudogenes are uridylated, and then bound and degraded by DIS3L2. Most interestingly, we uncovered a fraction of reads mapping to 5' termini of protein coding genes. The uridylation positions overlaps with the position of stalled RNA polymerase II indicating, that these fragments originate from RNA Pol II stalling. Next, we show, that uridylated 5' fragments of mRNAs are exported to cytoplasm, where they are removed by the activity of DIS3L2. In summary, our results demonstrate, that TUT-DIS3L2 surveillance (TDS) is a general cytoplasmic RNA mechanism assuring the removal of aberrant transcripts.

1. Lee M., et al. (2014), Cell 158:980-987
2. Heo I., et al. (2009), Cell 138:696-708
3. Heo I., et al. (2012), Cell 151:521-532.
4. Hoefig K.P., et al. (2013), Nat Struct Mol Biol, 20:73-81.
5. Ustianenko D., et al. (2013), RNA, 19:1632-1638.
6. Chang H.M., et al. (2013), Nature, doi: 10.1038/nature12119.
7. Lubas M., et al., (2013), EMBO 32:1855-1868.
8. Astuti D., et al., (2012), Nat Genet, 44:277-284.
9. Morris M.R., et al., (2013), Am J Med Genet C Semin Med Genet, 163C:106-113.

P19 Molecular aspects of tdp-43 – ssrna interactions

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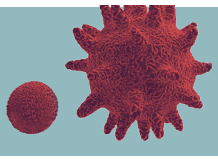
TAR DNA-binding protein (TDP-43), a member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family is involved in multiple stages of RNA processing and regulation of innate immune response to RNA virus sensing. The involvement of TDP-43 in alternative splicing of cystic fibrosis transmembrane conductance regulator (CFTR) gene results in severe forms of cystic fibrosis. TDP-43 binds with high affinity to a mutated, extended UG-rich region with a shortened polypyrimidine tract upstream of the 3' splice site (3'ss) of CFTR exon 9 and recruits another splicing regulator, hnRNP A1. 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. Functional implications of RNA recognition and binding by TDP-43 alone and in complex with other hnRNPs are vast, nevertheless, little is still known about molecular details of these interactions.
Our structural and biophysical studies of TDP-43 - CFTR exon 9 3'ss RNA (3'ss RNA) interactions reveal that two copies of TDP-43 bind to the extended, UG-rich sequence not independently, but create a new protein-protein interface upon dimerization. Binding affinity of TDP-43 to the RNA sequence, at the same time, increases as a function of UG-repeat length reaching an optimum at 10 repeats. Fluorescent anisotropy binding assays further show that not only the TDP-43 RRM domains drive the interaction with 3'ss RNA, since the binding affinity of the full-length TDP-43 to 3'ss RNA is much higher than for the RRM domains alone. We further reveal that two copies of hnRNP A1 which is recruited by TDP-43 to the intron 8 - exon 9 junction are involved in interaction with 3'ss RNA via their RRM2 domains while RRM1 domains presumably participate in protein dimerization.
How does the ternary TDP-43 – hnRNP A1 - 3'ss RNA complex prevent the formation of the canonical splicing complex (U2AF65/35) at the 3'ss? NMR monitoring of the TDP-43 - hnRNP A1 complex assembly confirmed that their interaction with 3'ss RNA is not masking the polypyrimidine tract on the pre-mRNA for binding of the canonical splicing factor U2AF65. Shortening of the polypyrimidine tract, on the other hand, lowers the affinity of U2AF65 and binding of hnRNP A1 at the 3'ss blocks the interaction of U2AF35. Thus a network of RNA-protein and protein-protein interactions which competes with the formation of the canonical splicing complex drives CFTR exon 9 skipping.



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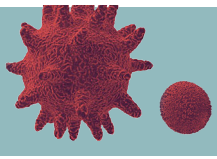

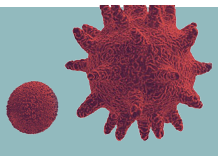

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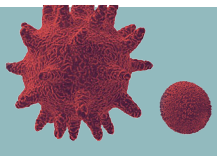

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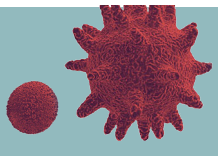

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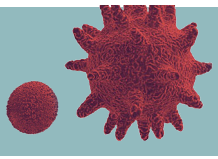
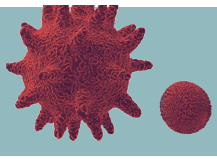
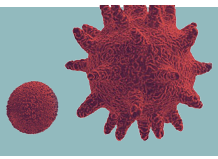
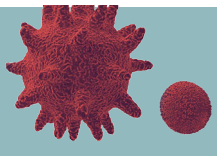
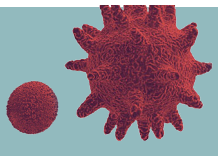
NOTES

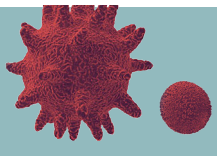


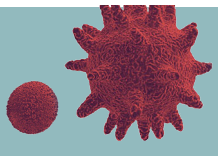


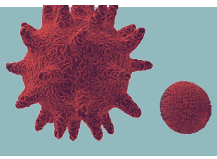


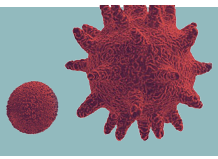


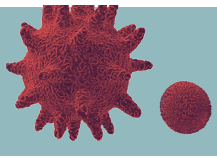


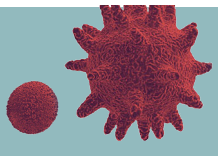


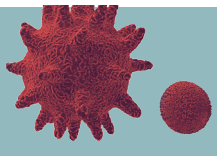












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