National Institute of Virology and Bacteriology

NIVB MEETING 2023

BOOK of ABSTRACTS

2nd – 5th OCTOBER 2023

Kutná Hora, Czech Republic

The second annual meeting of the National Institute of Virology and Bacteriology (NIVB)

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

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The project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union –Next Generation EU.

Funded by the European Union NextGenerationEU

MINISTRY OF EDUCATION, YOUTH AND SPORTS

CZECH RECOVERY PLAN

Dear virologists and bacteriologists, dear colleagues,

NIVB Meeting 2023 – the second meeting of the National Institute of Virology and Bacteriology (NIVB) in Kutná Hora on 2. 10. – 5. 10. 2023 will be again after one year an opportunity to meet the participating teams, discuss excellent science, establish collaborations, and present the progress of the project to the members of the International Scientific Advisory Board. Thanks to funding from the Czech Economic Recovery Plan, a number of important results have already been published in high impact journals. Several networking workshops have been organized and scientific collaborations have been established. The meeting in Kutná Hora is therefore a summary of all that has happened in the project over the past year. The main goal of the NIVB is to facilitate cooperation between 30 participating research teams from 8 Czech research institutions, and this goal is gradually being achieved. The NIVB acts as a common communication platform to discuss the desired cooperation of the NIVB research teams, especially those that have not had much contact so far due to their inter-institutional, interdisciplinary or inter-regional distance.

We thank all the participants who contributed to the meeting with 30 oral presentations and 67 posters. The NIVB 2022 meeting had the ambition to launch a new series of annual meetings dedicated to virology and bacteriology, and NIVB 2023 fulfils this ambition and continues to aim to inform the wider scientific community about new developments, trends and issues in these disciplines.

We are looking forward to seeing you at the NIVB Meeting 2023.

Zdeněk Hostomský, Robert Vácha, Iva Pichová, and Šárka Šímová

INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY OF THE CAS

L-01

DESIGN AND SYNTHESIS OF NEW MOLECULAR TOOLS TO PROBE CYTOSOLIC PROTEINS IN BACTERIA RESISTANT TO RIFAMYCIN ANTIBIOTICS

VIKTOR MOJR[a], PETRA SUDZINOVÁ[b], MARTIN HUBÁLEK[a], LIBOR KRÁSNÝ[b], DOMINIK REJMAN[a]*

[a] *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Praha 6, Czech Republic,* [b] *Institute of Microbiology, Czech Academy of Sciences,Vídeňská 1083, 142 20 Praha 4, Czech Republic. rejman@uochb.cas.cz*

Rifamycin-derived antibiotics are highly efficient inhibitors of prokaryotic DNA-dependent RNA polymerase (RNAP) used in treatment of many bacterial infections, including tuberculosis. During exposure, however, bacteria develop resistance to antibiotics, and several modifications of rifamycin skeleton that inhibit RNAP binding have already been discovered. A photo-crosslinking probe **1** (Fig. 1) with biotin handle based on rifamycin B was recently used for identifying specific protein that displaces antibiotic from RNAP in *Streptomyces venezulae*[1].

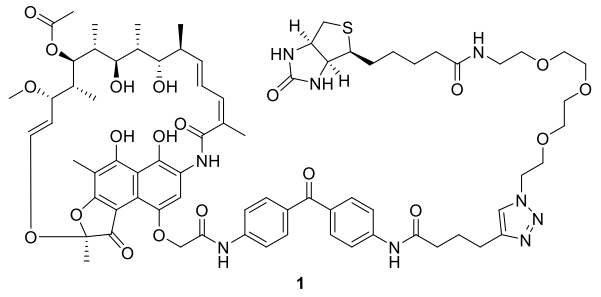

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Fig. 1. **Photo-crosslinking probe based on Rifamycin B**

Benzophenone photo-active moiety binds covalently to nearby molecules via highly reactive carbene generated upon UV irradiation, thus, increases the probe efficiency. Biotin handle allows detection/isolation of binding cytosolic proteins utilizing streptavidin-HRP conjugate.

Although rifamycin B has a free carboxylic group, which could potentially be utilized for derivatization through amide coupling, no commercially available antibiotic is based on it due to its poor activity and low stability on air and light[2]. Moreover, these reasons also probably affect the price and availability of rifamycin B on the market, leading to syntheses with small amounts of very expensive and hardly stable compound, which appeared to be challenging.

Rifampin and rifapentine are both derivatives of readily available 3-formylrifamycin SV, which is stable and relatively cheap chemical. Both drugs are derivatized on formyl group by *N*-alkyl-*N*'-aminopiperazines as hydrazones (Fig. 2).

In this work we present the newly designed rifamycin-biotin probes for identification of rifamycin-binding cytosolic proteins. The structure of the probes (Fig. 3) varies by utilizing (or not) at least two types (a–c) of photo-crosslinking group and employs a well-proven hydrazone-based linking moiety to rifamycin. We also decided to expand the study by variing the linker length (PEG4-, PEG5- and PEG6).

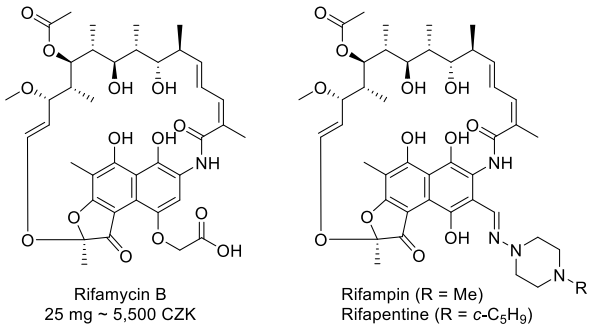

Fig. 2. **Rifamycin B and two commercially available antibiotics based on more stable 3-formylrifamycin SV**

The probes have the potential to increase knowledge of how bacterial resistance to rifamycin antibiotics works.

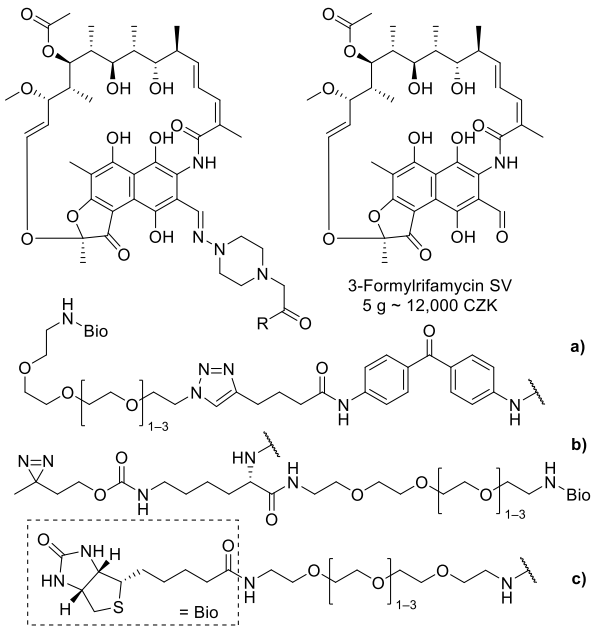

Fig. 3. **Designed probes based on 3-formylrifamycin**

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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L-02

DECIPHERING THE ALLOSTERIC REGULATION OF MYCOBACTERIAL INOSINE-5′-MONO-PHOSPHATE DEHYDROGENASE

ONDŘEJ BULVAS[a], ZDENĚK KNEJZLÍK[a], TOMÁŠ KOUBA[a], IVA PICHOVÁ[a]*

[a] *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 2, 166 10 Prague, Czech Republic*
ondrej.bulvas@uochb.cas.cz; iva.pichova@uochb.cas.cz

Inosine-5′-monophosphate dehydrogenase (IMPDH) is a crucial purine metabolism enzyme that is considered a promising drug target against mycobacterial infections[1]. IMPDH catalyzes the NAD-dependent oxidation of inosine-5′--monophosphate (IMP) to xanthosine 5′-monophosphate (XMP) a first committed step in the biosynthesis of guanine nucleotides. Regulation of IMPDH enzymatic activity is therefore crucial for cell survival. Despite recent advances in understanding the regulation in other bacteria[2], little is known about the allosteric regulation of mycobacterial IMPDH.

In this project, we describe the allosteric regulation of mycobacterial IMPDH and its underlying molecular mechanism. First, isolated recombinant IMPDH from model *Mycobacterium smegmatis* (*Msm*IMPDH) was used for *in vitro* biochemical characterization. The impact of selected purine nucleotide compounds on IMPDH activity was tested, showing the dramatic inhibitory effect of their combination at biologically relevant concentration ratios. Next, cryo-electron microscopy was used to determine a series of molecular structures of *Msm*IMPDH with combinations of substrates and nucleotide ligands bound to the regulatory domain. The *Msm*IMPDH forms octamers in compressed-inhibited or extended-active conformations. Purine inhibitors lock the octamer in the inactive 'compressed' conformation through specific interactions with residues in the flexible hinge region in between the catalytic and regulatory domains. This lock leads to extensive changes in the mobility of the *Msm*IMPDH catalytic core.

The described mechanism represents an example of how *Msm*IMPDH, an enzyme at the crossroad of two branches of purine metabolism, can integrate several allosteric signals to fine-tune its activity. This mechanism could potentially be exploited in the design of more selective antimycobacterial IMPDH-targeting drugs.

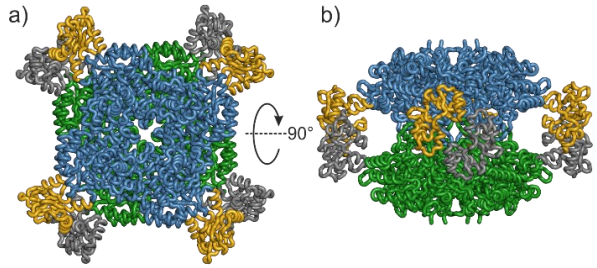

Fig. 1. **Structure of the octameric assembly of *Msm*IMPDH;** a) Top view of *Msm*IMPDH octamer. b) Side view. One of the tetramers is depicted in blue/gold, while the opposite one is in green/grey.

Acknowledgement
This work is supported by the project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103 funded by the European Union „Next Generation EU“. We acknowledge CF CF CryoEM of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127) and European Regional Development Fund-Project „UP CIISB“ (No.CZ.02.1.01/0.0/0.0/18_046/0015974).

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L-03
STRUCTURE AND INHIBITION OF MONKEYPOX VIRUS METHYLTRANSFERASE VP39

MARTIN KLIMA, JAN SILHAN, PETR SKVARA, DOMINIKA CHALUPSKA, TOMAS OTAVA, MICHALA ZGARBOVA, JAN WEBER, RADIM NENCKA*, EVZEN BOURA*

Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, v.v.i., 166 10 Prague 6, Czech Republic
nencka@uochb.cas.cz, boura@uochb.cas.cz

Monkeypox is a zoonotic disease with pandemic potential. It is caused by the monkeypox virus (MPXV), a double-stranded DNA virus from the *Poxviridae* family. MPXV replicates in cytoplasm and thus, it must encode for the DNA/RNA replication machinery. Besides the DNA-dependent DNA and RNA polymerases, it also encodes for the RNA capping enzymes. In poxviruses, the RNA cap is crucial for the translation and stability of viral RNA and for immune evasion[1]. One of the key components of the monkeypox virus RNA capping machinery is the RNA methyltransferase (MTase) VP39.

To gain insight into the molecular details of the enzymatic function of the MPXV VP39 protein, we co-crystalized it with the substrate of the methylation reaction, non-methylated capped RNA (also known as cap0-RNA), and the side product of the reaction, S-adenosylhomocysteine (SAH) (Fig 1). The structure explains the MPXV VP39 preference for a guanine base at the first position[2].

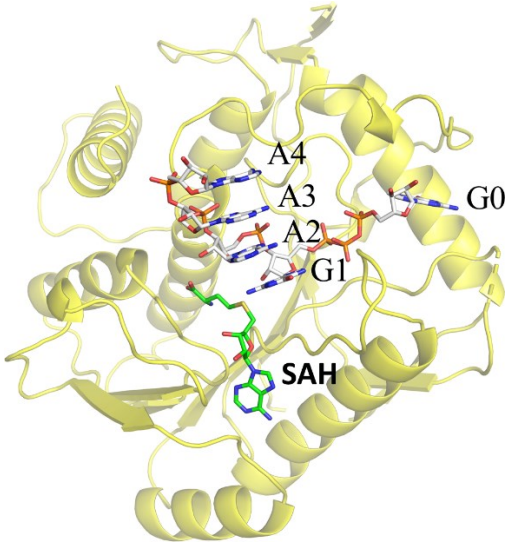

Fig. 1. **Crystal structure of the monkeypox virus VP39 methyltransferase in complex with SAH and cap0-RNA**

Inspection of the SAH binding site of MPXV VP39 revealed an unoccupied cavity adjacent to the adenine base of SAH, filled with a network of coordinated solvent molecules. We screened a small in-house library of SAH analogs bearing substituents at the 7-deaza position of the adenine base and identified several compounds that inhibited the VP39 enzyme significantly better than a pan-MTase inhibitor sinefungin[3]. Identified VP39 inhibitors were co-crystalized with the VP39 protein to reveal the binding mode of the inhibitors (Fig. 2). Finally, selected VP39 inhibitors were tested for their ability to inhibit MPXV replication in cells.

The discovery of this group of SAH-derived compounds can be the first step in the development of a completely new group of antivirals, aimed at blocking viral methyltransferases.

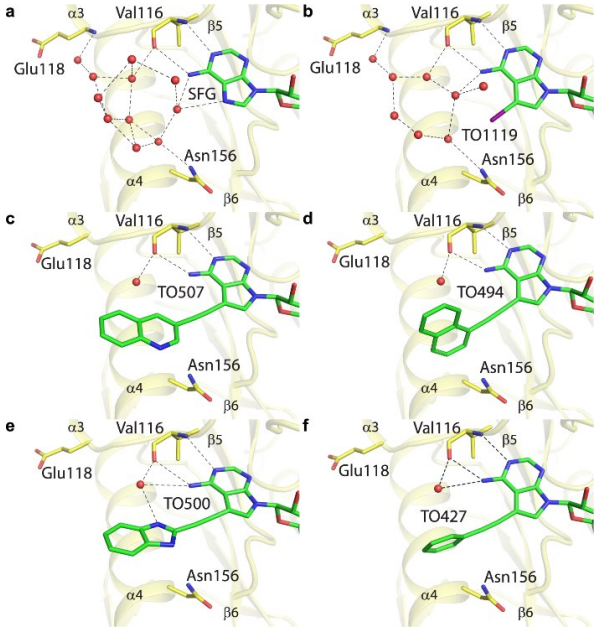

Fig. 2. **Crystal structures of the monkeypox virus VP39 methyltransferase in complex with SAH-derived inhibitors**

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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L-04
NOVEL ANTIVIRAL STRATEGIES TARGETING METHYLTRANSFERASES, RIPK2, AND STING PATHWAY

MILAN DEJMEK, MILAN ŠTEFEK, TOMÁŠ OTAVA, MBILO MISEHE, PETRA KRAFČÍKOVÁ, MARTIN KLÍMA, MICHAL ŠÁLA, PETRA BŘEHOVÁ, EVŽEN BOUŘA, RADIM NENCKA*

Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, v.v.i., Flemingovo nam. 2., 166 10 Prague 6, Czech Republic
nencka@uochb.cas.cz

Our group's research over the past year has been focused primarily on the medicinal chemistry of novel antivirals, with a major emphasis on three separate topics – inhibitors of viral methyltransferases, inhibitors of human RIPK2 as a host factor implicated in replication of various viruses, and STING agonists.

Inhibitors of Viral Methyltransferases, with Emphasis on SARS-CoV-2 and Mpox: Our group has dedicated a substantial effort to designing and synthesizing inhibitors targeting viral methyltransferases. Notably, our research has revolved around SARS-CoV-2, the virus responsible for the COVID-19 pandemic, and Mpox, a viral pathogen of significant concern. These inhibitors hold a promise as potential antiviral therapeutics acting by disrupting essential viral processes, i.e. capping of the viral RNA and thus preventing the recognition of viral RNA by the host immune system[1,2]. In this part of the work, we were able to design and prepare new nsp14 inhibitors of SARS-CoV-2 based on our previous work[3]. These compounds represent, without doubt, some of the most potent nsp14 inhibitors known to date (Fig. 1)[4].

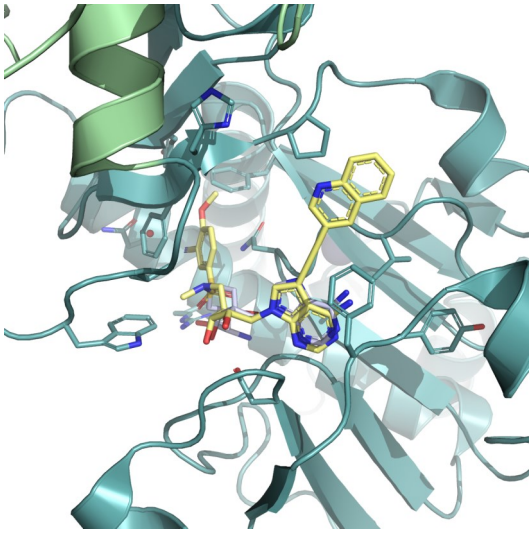

Fig. 1. **Docking of our nsp14 inhibitor (pale yellow) into SARS-CoV-2 MTase nsp14 structure (PDB ID: 7R2V) – comparison with SAH (light blue).**

RIPK2 Inhibitors as Novel Antiviral Agents: Another critical area of our research has been focused on the development of RIPK2 inhibitors. These molecules have the potential to combat various viral infections by disrupting the crucial role of the NOD2 in viral life cycles. We have prepared two series of novel RIPK2 inhibitors that significantly affect RIPK2-NOD1/2 signaling pathway. One of these series was based on a traditional quinazoline skeleton[5] and the other on a new thieno[2,3d]pyrimidine skeleton.

Detailed biological evaluation of these compounds and their antiviral activity will be discussed.

Exploration of Novel STING Agonists: The third major focus of our research has been the investigation of novel STING (Stimulator of Interferon Genes) agonists. STING agonists have garnered attention for their immunomodulatory properties, particularly in the context of antiviral and antitumor effect. Our studies aim to discover previously unexplored cyclic dinucleotides that can activate the pathway eliciting an innate and specific adaptive immune response and ultimately enhance the hosts ability to combat viral infections.

Our findings contribute to the development of potential therapies against a range of viral infections, including SARS-CoV-2, and offer exciting prospects for future research in this critical area of healthcare.

Acknowledgement
This work was supported by Czech Academy of Science (RVO, reg. No. 61388963) and by National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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L-05

IDENTIFICATION AND CHARACTERIZATION OF POLYMERASE INHIBITORS OF L-PROTEIN OF RIFT VALLEY FEVER VIRUS

MICHAL KRÁL'[a,b]*, TOMÁŠ KOTAČKA[a,b], KATEŘINA RADILOVÁ[a,b], JIŘÍ GREGOR[a,c], ALEŠ MACHARA[a,c], RÓBERT REIBERGER[a,c], MILAN KOŽÍŠEK[a]*

[a] *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo n. 2, 166 10 Prague 6, Czech Republic,* [b] *First Faculty of Medicine, Charles University, Kateřinská 1660/32, 121 08 Prague 2, Czech Republic,* [c] *Faculty of Science, Charles University, Hlavova 8, 128 00 Prague 2, Czech Republic*
michal.kral@uochb.cas.cz, milan.kozisek@uochb.cas.cz

Rift Valley fever virus (RVFV) is a mosquito borne, pathogenic phlebovirus of the order *Bunyavirales*, causing severe disease in both humans and domesticated animals. Outbreaks of the Rift Valley Fever can have devastating impact on the economy of the affected countries, as the virus can cause immense losses of livestock estimated in hundreds of millions USD[1]. Currently, no approved, specific treatment is available for the RVFV infections. Several vaccine types are available, although they are not widely used, and their actual efficiency and safety is questionable. Like other viruses of the *Bunyavirales* family, replication mechanism of the RVFV is mediated by the L protein. The 250 kDa large protein is responsible for most of the virus replication[2]; it contains the endonuclease domain, the RNA-dependent RNA-polymerase domain and the cap-binding domain. This organization corresponds to the linear composition of the heterotrimeric complex PA-PB1-PB2 of the influenza[3]. The process of virus replication is initiated by a cap-snatching mechanism, during which the host mRNA is cleaved by the L protein endonuclease domain[4]. The L protein is heavily conserved across the members of the virus family and, although sequentially different it is structurally and functionally closely similar to the RNA polymerase complex of the influenza A virus[5].

Currently, we are able to produce the full-size recombinant L-protein of the RVFV in baculovirus expression system. Our aim is to structurally characterize this viable drug target, using both X-ray crystallography and CryoEM microscopy in collaboration with Gabriel Demo's group at CEITEC. Furthermore, we have identified several polymerase inhibitors of the RVFV L-protein and characterized their efficacy against live virus *in vitro.* Several of these compounds were identified during screening of the library of the polymerase inhibitors from the Radim Nencka's group. We have generated resistant mutants of the available virus strains – non-pathogenic MP-12 strain as well as wild type ZH-548. These resistant variants have greatly increased or completed resistance to their respective inhibitors and the interplay between the resistant virus and its insect hosts will be further characterised by the research group of Petr Volf from the laboratory of vector biology.

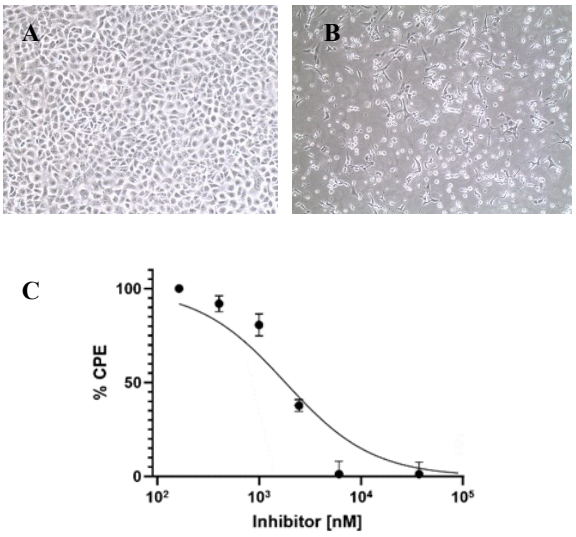

Fig. 1. **Polymerase inhibitors have potent effect on the Rift Valley fever virus *in vitro* (C). Treatment of VERO E6 cell culture with polymerase inhibitors (A) results in protection from the cytopathic effect caused by the virus, as opposed to the untreated cell cultures (B).**

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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L-06
ROLE OF E2/E3 UBIQUITIN LIGASE, UBE2O, IN HEPATITIS B VIRUS REPLICATION

BARBORA LUBYOVÁ[a], EVA TIKALOVÁ[a], VÁCLAV JANOVEC[a,b], KRISTÝNA KRULOVÁ[a], BORIS RYABCHENKO[b], SANDRA HUÉRFANO[b], IVAN HIRSCH[a,b], JAN WEBER[a]*

[a] *Institute of Organic Chemistry and Biochemistry of CAS, Flemingovo nám. 2, 166 10, Praha 6, Czech Republic,*
[b] *Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Vestec, Czech Republic*
jan.weber@uochb.cas.cz

Chronic hepatitis represents a life-long liver disease caused by infection of Hepatitis B virus (HBV) and affecting more than 250 million people worldwide. Since HBV replication is completely dependent upon host cell protein pathways, the study of virus-host interactions promises to reveal novel cellular targets for development of new therapies. Our strategy is to identify and characterize key cellular proteins and pathways that are essential for different steps of viral replication[1,2]. Using mass spectrometry, we identified a novel HBV Core (HBc) – interacting host protein, UBE2O[3]. UBE2O is an E2/E3 hybrid ubiquitin-protein ligase that displays both E2 and E3 ligase activities and mediates mono-ubiquitination of several chromatin-associated proteins, such as INO80, BAP1 and CXXC1, affecting their subcellular location[4]. Notably, UBE2O is also implicated in endosomal protein trafficking through its ubiquitination of the WASH regulatory complex[5].

Here, we confirmed the interaction between UBE2O and HBc protein using co-immunoprecipitation. Further analysis of various HBc deletion mutants demonstrated that HBc interacted with UBE2O via its C-terminal domain. Co-expression of HBc and UBE2O resulted in HBc protein mono-ubiquitination. Interestingly, single Ser-to-Ala mutations of two major phosphorylation sites involving serines at positions 164 and 172 of a 185-aa HBc variant resulted in increased UBE2O-mediated mono-ubiquitination, suggesting that HBc hypo-phosphorylation is vital for efficient ubiquitination.

The role of UBE2O in viral lifecycle was investigated in HBV-infected HepG2-NTCP cells as well as primary human hepatocytes (PHH) upon downregulation of endogenous UBE2O. Two days prior to HBV infection, the cells were transfected with UBE2O-specific siRNAs and five days post-infection, the levels of intracellular viral DNA/RNA, and extracellular HBe antigen were determined by (RT)qPCR and ELISA, respectively. In addition, the formation and stability of intracellular nucleocapsids and the secretion of viral particles was also examined. When compared to control cells (transfected with non-specific siRNAs), the downregulation of UBE2O led to decreased levels of both HBV DNA/RNA and HBeAg. Interestingly, the formation of intracellular viral nucleocapsids and secretion of virions was significantly impaired in cells with reduced expression of UBE2O. These data suggested that UBE2O may play important role in nucleocapsid maturation and viral secretion.

Recently, a compartment of the late endocytic pathway, the multivesicular body (MVB), has been shown to participate in the final stages of HBV maturation and release. The HBV transiently resides in the MVB and disruption of this compartment was shown to result in decreased virion secretion. Furthermore, the efficient HBV maturation and virion secretion was reported to employ the endosomal sorting complexes required for transport (ESCRT)-II, -III, and the AAA ATPase Vps4[6–8].

To link the cellular pathway involving UBE2O with HBV maturation and release, we analyzed the localization of UBE2O in HBV-infected HepG2-NTCP cells by immunofluorescence microscopy. As shown in Fig. 1A, the endogenous UBE2O protein resides in the cytosol in vesicular compartments that are also positive for the marker of MVBs, CD63. We also analyzed HBV core particles using an antibody that specifically recognized viral capsids. Interestingly, the co-staining of viral capsids and UBE2O (Fig. 1B) resulted in similar co-localization pattern, indicating the possible association of both UBE2O and core particles within MVBs.

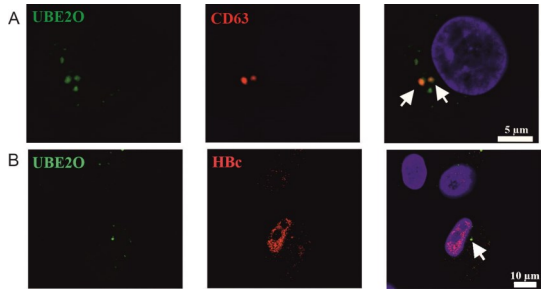

Fig. 1. **Association of UBE2O and viral capsids with multivesicular bodies (MVBs). HepG2-NTCP cells were infected with HBV (MOI of 1000 VGE/cell) and 5 days post-infection the cells were fixed and stained with antibodies to UBE2O and CD63 (A), or UBE2O and capsid/HBc (B). The nuclei were stained with DAPI, and the distribution of UBE2O, CD63 and capsids were visualized with a confocal fluorescence microscope.**

In conclusion, our results implicated UBE2O in host MVB functions and suggested that UBE2O is an important cellular regulator required for efficient maturation and release of enveloped HBV virions. Based on these findings, UBE2O may be a valuable target for HBV control.

Acknowledgement
This work was funded by the ERDF/ESF project ChemBioDrug CZ.02.1.01/0.0/0.0/16_019/0000729 and by the National Institute of Virology and Bacteriology (Programme EXCELES, Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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P-01
LIPOPHOSPHONOXINS – NOVEL MEMBRANE TARGETING ANTIMICROBIAL COMPOUNDS

DUY DINH DO PHAM[a], VIKTOR MOJR[a], GABRIELA MIKUŠOVÁ[a,b], RADOVAN FIŠER[b], EVA DÁVIDOVÁ[a], HANA ŠANDEROVÁ[c], JIŘÍ POSPÍŠIL[c], KATEŘINA BOGDANOVÁ[d], RENATA VEČEŘOVÁ[d], ALENA VLKOVÁ[c], ROBERT ZAJÍČEK[f], VĚRA JENČOVÁ[g], DAVID LUKÁŠ[g], MILAN KOLÁŘ[d], LIBOR KRÁSNÝ[c], DOMINIK REJMAN*[a]

[a] *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences v.v.i., Flemingovo nám. 2, 166 10 Prague 6, Czech Republic,* [b] *Department of Genetics and Microbiology, Faculty of Science, Charles University, Viničná 5, 128 43 Prague 2, Czech Republic,* [c] *Institute of Microbiology, Czech Academy of Sciences v.v.i., Vídeňská 1083, 142 20 Prague 4, Czech Republic,* [d] *Department of Microbiology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Hněvotínská 3, 775 15 Olomouc, Czech Republic,* [e] *Unit for Biomedicine and Welfare of Laboratory Animals, National Institute of Public Health, Šrobárova 49/48, 100 00, Prague 10, Czech Republic,* [f] *Prague Burn Centre, Third Faculty of Medicine and University Hospital Královské Vinohrady, Šrobárova 50, 100 34 Prague 10, Czech Republic,* [g] *Faculty of Science, Humanities and Education, Technical University of Liberec, Studentská 1402/2, Liberec 1, 461 17, Czech Republic.*
dominik.rejman@uochb.cas.cz

The alarming rise of bacterial antibiotic resistance demands development of new compounds. Such compounds, lipophosphonoxins (LPPOs), were previously reported by us to be active against numerous bacterial species but serum albumins abolished their activity[1,2].

Here we report synthesis and evaluation of novel antibacterial compounds termed LEGO-LPPOs, based on LPPOs, consisting of a central linker module with two attached connector modules on either side[3]. The connector modules are then decorated with polar and hydrophobic modules. An extensive structure-activity relationship study was performed, over 100 derivatives were produced differing in the length of linker module and in the composition of the hydrophobic modules. The best compounds were active against both Gram-negative and -positive species including multiresistant strains and persisters. LEGO-LPPOs act by first depleting the membrane potential and then creating pores in the cytoplasmic membrane. Importantly, their efficacy is not affected by the presence of serum albumins. Low cytotoxicity and low propensity for resistance development demonstrate their potential for therapeutic use.

Application of original LPPOs in an active wound dressing based on the polycaprolactone nanofiber scaffold (NANO) is also discussed[4]. We demonstrated in vitro that LPPO released from NANO exerted antibacterial activity while not impairing proliferation/differentiation of fibroblasts and keratinocytes. Secondly, using a mouse model we showed that NANO loaded with LPPO significantly reduced the *Staphylococcus aureus* counts in infected wounds as evaluated 7 days post-surgery. The rate of degradation and subsequent LPPO release in infected wounds was facilitated by lytic enzymes secreted by inoculated bacteria. Finally, LPPO displayed very little systemic absorption. In conclusion, the composite antibacterial NANO-LPPO-based dressing reduces the bacterial load and promotes skin repair, with the potential to treat wounds in clinical settings.

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P-02
INHIBITORS OF 6-OXOPURINE PHOSPHO-RIBOSYLTRANSFERASES BASED ON PROLINOL AND ISOPROLINOL BISPHOSPHONATE CORES

MAGDALENA PETROVÁ[a]*, RADEK POHL[a], DIANE T. KEOUGH[b], LUKE W. GUDDAT[b], DOMINIK REJMAN[a]

[a] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences v.v.i. Flemingovo nám. 2, 166 10 Praha 6, Czech Republic, [b] School of Chemistry and Molecular Biosciences, The University of Queensland Brisbane, 4072 QLD, Australia
petrova@uochb.cas.cz

6-Oxopurine phosphoribosyltransferases (PRTases) play a central role in the growth and cell proliferation of many organisms by synthesizing the respective nucleoside monophosphates required for the production of nucleic acids. In *Plasmodium species* they provide the only metabolic route as the parasites are incapable of synthesizing the purine ring *de novo,* and survive by its transport from the host cell[1–3]. Recently, the importance of *Mycobacterium tuberculosis* HGPRT as an anti-TB drug target was supported[4,5] as well as the crucial activity of 6-oxopurine PRTases for the growth and function of *Trypanosoma brucei* and *Helicobacter pylori*[6–8]. Generally, these inhibitors are non-toxic to human cells. The crystal structures of PRTases show two phosphate binding sites in the active site, one for the 5′-phosphate of the nucleotide and the second for pyrophosphate. Thus, bisphosphonate cores were suggested to be potent inhibitors of HG(X)PRT.

Here we present design, synthesis and evaluation of pyrrolidine, prolinol (2-hydroxymethylpyrrolidine), and isoprolinol (3-hydroxymethylpyrrolidine) bisphosphonate nucleotide analogues as inhibitors of HG(X)PRT as well as the comparison of the inhibitory activity thereof.

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P-03
THE INTERPLAY OF PURINE METABOLISM IN MYCOBACTERIA AND ITS IMPLICATIONS FOR BACTERIAL PHYSIOLOGY

ZDENĚK KNEJZLÍK[a], MATTEO DEDOLA[a], ONDŘEJ BULVAS[a], MICHAL DOLEŽAL[a], JIŘÍ POSPÍŠIL[b], LIBOR KRÁSNÝ[b], IVA PICHOVÁ[a,*]

[a] Institute of Organic Chemistry and Biochemistry of Czech Academy of Scicences, Flemingovo náměstí 542/2 160 00 Praha 6, [b] Institute of Microbiology of Czech Academy of Scicences, Vídeňská 1083, 142 00 Praha 4-Krč, Czech Republic
zdenek.knejzlik@uochb.cas.cz

Purine metabolism is a metabolic super-pathway involving branches of de novo synthesis, salvage and degradation of essential purine nucleotides and related compounds. As a supplier of nucleic acid building blocks, cofactors and signalling molecules, it is tightly regulated at many levels in the bacteria including transcription, enzyme activity and post-translational modification. However, organization and regulation of the purine metabolism in mycobacteria, involving also most spread pathogen *Mycobacterium tuberculosis*, is not well understood yet.

Using a combination of targeted deletion mutagenesis in the model bacteriium *Mycobacterium smegmatis*, biochemical and phenotypic analysis, super resolution microscopy and enzyme kinetics, we studied the *in vivo* and *in vitro* activity of a panel of enzymes from both the *de novo* and *salvage* pathways. Our data show that inactivation of the *de novo* pathway at different steps is associated with purine auxotrophy, but not for thiamine, dysregulated levels of purine nucleotides, growth defects in the late exponential phase and blockage of the biofilm formation. Localization studies revealed that the PurF enzyme, which catalyzes a fisrst step of the *de novo* pathway, forms membrane bound high-order structures that may be involved in the supramolecular organization of purine metabolism. *In vitro* structural studies and enzymological studies are currently under way. Next, our data show that the activity of hypoxanthine-guanine phosphoribosyltransferase, IMP dehydrogenase, GMP reductase and adenosine deaminase play important roles in the tight regulation of purine nucleotide levels under different growth conditions. The role of the free purine nucleobases in this process is currently being investigated.

Taken together, our observations suggest a complex regulation of mycobacterial purine metabolism via allosteric regulation and enzyme activity that differs from other bacteria. Future studies focused on a detailed understanding of the regulation of purine metabolism may help identify essential molecular targets for the development of selective compounds active against mycobacteria.

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P-04
INVESTIGATING THE ROLE OF SCAMP3 IN HEPATITIS B VIRUS INFECTION

KAROLÍNA ŠTAFLOVÁ, ALEŠ ZÁBRANSKÝ, IVA PICHOVÁ

[a] *Institute of Organic Chemistry and Biochemistry, Flemingovo náměstí 542/2 , 160 00 Prague, Czech Republic karolina.staflova@uochb.cas.cz*

Infection with hepatitis B virus (HBV) presents a global health problem and causes more than 800 000 deaths each year. HBV infects liver cells and causes acute and chronic liver disease. To unravel the mechanisms of viral replication and pathogenesis it is crucial to understand the interplay between HBV and the host cell. In this study, we focus on the possible role of the cellular protein SCAMP3 in HBV infection.

SCAMP3 (secretory carrier membrane protein 3) is a membrane protein involved in the regulation of membrane trafficking and vesicle fusion. It was previously described that SCAMP3 regulates also trafficking and recycling of EGFR (epidermal growth factor receptor)[1]. Interestingly, besides its roles in cellular signaling, EGFR also serves as a co-receptor for HBV infection[2]. Therefore, we hypothesize that SCAMP3 may be involved the regulation of HBV entry in the cell.

To test the possible effect of SCAMP3 on HBV infection, we have analyzed the effect of SCAMP3 silencing on HBV replication in HepG2-hNTCP cell line and in primary human hepatocytes. We have evaluated the markers of viral infection and observed that SCAMP3 silencing causes a decrease in the production of viral RNAs in the infected cells and a decrease in the secretion of viral HBe antigen.

We have also examined the effect of SCAMP3 knockdown on hepatitis delta virus (HDV) infection. HDV is a satellite virus of hepatitis B virus that requires envelope proteins produced by HBV for its infectious cycle. As a result, it utilizes the same pathway to enter the host cell as HBV. Similarly to HBV infection, we have observed that SCAMP3 knockdown reduces the production of hepatitis delta virus RNA.

Together, our results show that SCAMP3 is a host factor promoting hepatitis B virus and hepatitis delta virus infection.

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P-05
HEPATIDIS B VIRUS PRECORE PROTEIN INTRACELLULAR PATHWAY AND HOST INTERACTION

HELENA ZÁBRANSKÁ, IVA PICHOVÁ

Institute of Organic Chemistry and Biochemistry, Flemingovo náměstí 2, 160 00 Prague, Czech Republic helena.zabranska@uochb.cas.cz

The hepatitis B virus (HBV) can induce both acute and chronic hepatitis and is a major global health problem. HBV is a small enveloped DNA virus with a genome containing only four open reading frames that largely overlap and encode multiple proteins using different in-frame start codons. For example, the HBV preC-C gene gives rise to two different products translated from distinct mRNAs – core protein (HBc) and precore protein (HBe). HBc is a cytosolic protein responsible for the assembly of icosahedral viral particles and pre-genomic RNA encapsidation. On the other hand the HBe precursor (p25) is directed to the endoplasmic reticulum (ER) where cleavage of the signal peptide (sp) gives rise to the first processing product, p22. P22 can be retro-translocated back to the cytosol or enter the secretory pathway and undergo a second cleavage event resulting in secreted p17 (HBe).

We have previously decribed that the translocation of the precore precursor p25 to ER is promoted by the translocon-associated protein complex (TRAP). Within the p25 sp sequence we have identified three cysteine residues that control the efficiency of sp cleavage and appear to serve as an auto-regulating factor that influences the intracellular localisation of the precore.

In recent study we used biochemical and molecular biology approaches to follow the precore protein intracellular pathway in more details. Using the sensitive radioactive metabolic labeling we observed that the precore but not the core protein is translocated to mitochondria and we identified several mitochondrial proteins interacting with the precore. In ongoing experiments we also investigate the contribution of individual HBV proteins to the unfolded protein response activation.

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P-06
IN VITRO AND _IN VIVO_ TESTING OF SARS-COV-2 NSP14 METHYLTRANSFERASE INHIBITORS

VLADIMIRA HOROVA, DOMINIKA CHALUPSKA, KAREL CHALUPSKY, JANA TRYLCOVA, MARTIN KLIMA, JAN WEBER, RADIM NENCKA, EVZEN BOURA

Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, v.v.i., 166 10 Prague 6, Czech Republic

The replication of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) relies on key components, including the RNA-dependent RNA polymerase, helicase, nuclease, and two RNA methyltransferases (MTases). These methyltransferases, nsp16 and nsp14, play a critical role in viral RNA-cap formation[1]. The absence of an RNA cap exposes viral RNA to innate immunity, triggering RNA degradation and antiviral responses.

Viral methyltransferases offer promising targets for the development of antiviral drugs[2]. Therefore, the establishment of a robust screening methodology for identifying potential inhibitors is important. In our study, we employed a previously developed *in vitro* assay coupled with the ECHO-MS system to measure nsp14 methyltransferase activity[3] to identify promising compounds synthetized in collaborating laboratory. Next, we show results obtained from experiments conducted on infected Vero-6 and Calu-3 cell cultures. These experiments involved the treatment of cells with selected inhibitors to assess their impact on the methylation status of RNA and viral RNA replication.

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P-07
SYNTHESIS OF 5H-PYRAZINO[2',3':4,5]PYRROLO [3,2-D]PYRIMIDIN-4-AMINE AS A CORE STRUCTURE FOR POTENTIAL ANTIVIRALS

LUCA JULIANNA TÓTH[a,b], MILAN DEJMEK[a], RADIM NENCKA[a]*

[a] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 542/2, 166 10 Prague, Czech Republic, [b] Department of Organic Chemistry, Faculty of Science, Charles University, Hlavova 2030/8, 128 43 Prague, Czech Republic
luca.toth@uochb.cas.cz

Various compounds bearing 9-deazapurine core show a wide range of biological activity, such as anticancer[1] and antiviral[2] properties. Significant number of these bioactive molecules are tricyclic derivatives of this isosteric pyrrolo[3,2-d]pyrimidine[3], share identical core-geometry[4], or are nucleoside analogues bearing such heterocycles as nucleobase and found intriguing applications in the synthesis of oligonucleotides, that are utilized – for instance – in the antisense technology[5].

As the 9-deazapurine structure does not occur in nature, the search for its efficient synthesis and the preparation of its further derivatives through novel strategies is ongoing. One of the least explored structure is 5H-pyrazino[2',3':4,5]pyrrolo [3,2-d]pyrimidin-4-amine: only one direct derivative is known in the literature[6].

Herein, we present a successful synthesis of this crucial (pentaaza-fluoren-1-yl)-amine core molecule. Synthetic strategies, their challenges and further modifications of this novel structure will be discussed in detail.

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P-08
SYNTHESIS OF A UNIQUE CLASS OF C-NUCLEOSIDES AND THEIR ACTIVITY AS ANTIVIRALS

MICHAL ŠÁLA[a], SIMONA HORKELOVÁ[a], MILAN DEJMEK[a], PETRA BŘEHOVÁ[a], ELIŠKA PROCHÁZKOVÁ[a], LUDĚK EYER[b], EVZEN BOURA[a], JAN WEBER[a], DANIEL RŮŽEK[b], RADIM NENCKA[a,*]

[a] Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Flemingovo n. 2, 16010, Prague, Czech Republic, [b] Department of Virology, Veterinary Research Institute, Brno, Czech Republic
nencka@uochb.cas.cz

Chemistry of nucleosides and nucleotides indisputably represents a substantial part of medicinal chemistry. Nucleic acid components serve as a major pillar of antiviral and antimicrobial therapy as well as the treatment of various malignancies. C-nucleosides are nucleosides, in which the classical N-glycosidic bond between a nucleobase and a ribose part, is replaced by a C-C bond. This change has a dramatic impact on the stability of the molecules, as such bond is no longer cleaved by nucleoside phosphorylases. One of the most known, recent example of this group of compounds is Remdesivir[1], which has been originally developed against filoviruses (e. g. ebola virus), but then showed strong antiviral activity against various other viruses, such as paramyxoviruses, pneumoviruses, flaviviruses and coronaviruses[2].

This study introduces novel and unique class of C-nucleosides. Its design, synthesis and biological evaluation will be described in detail.

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P-09
IDENTIFICATION AND CHARACTERIZATION OF INHIBITORS OF THE BUNYAVIRIDAE VIRUSES

TOMÁŠ KOTAČKA[a,b]*, MICHAL KRÁĽ[a,b], KATEŘINA RADILOVÁ[a,b], JIŘÍ GREGOR[a,c], RÓBERT REIBERGER[a,c], ALEŠ MACHARA[a,c], MILAN KOŽÍŠEK[a]

[a] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo n. 2, 166 10 Prague 6, Czech Republic, [b] First Faculty of Medicine, Charles University, Kateřinská 1660/32, 12108, Prague 2, Czech Republic, [c] Faculty of Science, Charles University, Hlavova 8, 12800, Prague 2, Czech Republic
tomas.kotacka@uochb.cas.cz

Previously our work was focused mainly on polymerase of influenza virus from *Orthomyxoviridae*, for broaden our experetise we looked for other high priority viruses with a functional similarity between polymerases where we could use our previously gained experience from working on influenza virus. This description is suited to *Bunyaviriade* where there are many viruses with most likely causing future outbreaks or epidemics, such as Rift Valley fever virus (RVFV), Crimean-Congo Haemorrhagic Fever virus (CCHFV) and quite newly described Yezo virus (YEZV)[1,2]. Despite the growing burden of these viruses on both human and animal health, surprisingly small number of therapeutics are available for treatment of these potentially deadly viruses. Prevention is also still not possible since no vaccine against these viruses is licensed for human use.

Our project aims to develop and screen the small molecule inhibitors of the polymerases of the RVFV, CCHFV and YEZV. Our scope of work includes the production of recombinant proteins in *E. coli* and baculovirus expression system, including production of full-length L-protein of RVFV and endonuclease domain. Furthermore, we are focused on characterization of the protein-ligand interactions using the isothermal titration calorimetry, and X-ray crystallography, since there are still no structures of these proteins. Our expertise also includes the inhibitor testing against their respective targets using AlphaScreen technology, minigenome reverse genetics systems and work on living viruses.

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P-10
NEW INSIGHTS ON MATURATION OF THE SARS-CoV-2 MAIN PROTEASE

PAVEL NOVOTNÝ[a,b]*, ADÉLA MORAVCOVÁ[a,c], VERONIKA NOVÁKOVÁ[a,b], JAN WEBER[a], ROBIN KRYŠTŮFEK[a,b], JANA STARKOVÁ[a], MARTIN HRADÍLEK[a], JANA GÜNTEROVÁ[a], MICHALA ZGARBOVÁ[a], KATHRIN BACH[a], STANCHO STANCHEV[a], KVIDO STŘÍŠOVSKÝ[a], TAŤÁNA MAJEROVÁ[a]

[a] *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo n. 2, 166 10 Prague, Czech Republic,* [b] *Faculty of Science, Charles University, Hlavova 8, 128 00 Prague, Czech Republic,* [c] *Department of Biochemistry and Microbiology, University of Chemistry and Technology, 166 28 Prague, Czech Republic.*
pavel.novotny@uochb.cas.cz

SARS-CoV-2 3C-like protease (3CLPro), also known as the SARS-CoV-2 main protease (MPro), processes the viral polyproteins pp1a and pp1ab to release functional proteins. The mature protease is released from both the polyproteins pp1a and pp1ab by dimerization and self-cleavage. However, the precise orchestration of this process is still not fully understood.

We observed differences in in cis and in trans autoactivation of model MPro precursors using purified recombinant enzymes and in E. coli and HEK293T cells too. When the strictly conserved glutamine in the P1 position of the cleavage site (position –1 relative to the MPro residues) was mutated, in cis autoactivation was not blocked, even though this protease exhibits a strong preference for glutamine in this position for cleavage substrates in trans. However, we did identify a mutation blocking in cis autoprocessing. This enabled us to obtain and purify a model precursor form of MPro. We hypothesize that in cis (intradimeric) cleavage of MPro has different structural requirements than in trans (interdimeric) cleavage between the protease and other substrates.

We tested both established inhibitors (nirmatrelvir, GC376, bofutrelvir, ensitrelvir) and molecules prepared in-house for their inhibitory effects on mature MPro and the precursor form of MPro. We observed differences in the inhibition of MPro variants and precursor forms. Our experiments may contribute to a deeper understanding of the coronavirus life cycle and provide new insights for drug design.

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P-11
HELQUAT-BASED COMPOUNDS TARGETING G-QUADRUPLEXES AS A NEW CLASS OF ANTI-HIV-1 INHIBITORS

MARCELA PÁVOVÁ, PAUL EDUARDO REYES-GUTIÉRREZ, JAROSLAV KOZÁK, JURAJ DOBIÁŠ, YEVGEN YURENKO, MARTIN LEPŠÍK, FILIP TEPLÝ, JAN WEBER*

Institute of Organic Chemistry and Biochemistry of CAS, Flemingovo nám. 2, 166 10, Praha 6, Czech Republic
jan.weber@uochb.cas.cz

G-quadruplexes (G4s), the secondary structure of nucleic acids containing quartets of guanines, are known to regulate the transcription of many cellular genes and their misregulation is involved in various human diseases. Over the last few years, the presence of G4s in human pathogens, especially in viruses, and their involvement in many key steps of viral life cycles have attracted increasing interest[1]. The human immunodeficiency virus type 1 (HIV-1) establishes a persistent infection in cells of the human immune system. The presence of G4 structures has been described in both RNA and DNA forms of its genome, with implications throughout the HIV-1 life cycle[2]. Several G4s can be formed in the HIV-1 long terminal repeat promoter region and their stabilization results in the inhibition of HIV-1 replication[3].

Here we report the synthesis of a library of more than 500 substituted helquat-based compounds and screened them for anti-HIV-1 activity. We identified several candidates and by using Taq polymerase stop and FRET melting assays, we have demonstrated their ability to stabilize G-quadruplexes in the HIV-1 long-terminal repeat sequence. In cell-based assay we showed that these helquat-based compounds selectively inhibit HIV-1 replication at the stage of reverse transcription and provirus expression. Moreover, these compounds were not binding to the general G-rich region, but rather to specific G-quadruplexes in the HIV-1 promoter region. Finally, docking and molecular dynamics calculations indicate that the structure of the helquat core greatly affects the binding mode to the individual G-quadruplexes. Our findings can provide useful information for the further rational design of inhibitors targeting G-quadruplexes in HIV-1.

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P-12

ANTI-SARS-COV-2 VIRUCIDAL ACTIVITY OF TEXTILES COATED WITH MERCAPTOETHANE SULFONATED SILVER NANOPARTICLES

LUCIE ULRYCHOVÁ[a], ILANA PERELSHTEIN[b], NINA PERKAS[b], JAN HODEK[a], AHARON GEDANKEN[b], JAN WEBER[a]*

[a] *Institute of Organic Chemistry and Biochemistry of CAS, Flemingovo nám. 2, 166 10, Praha 6, Czech Republic,*
[b] *Department of Chemistry and BINA Center, Bar-Ilan University Ramat-Gan 5290002, Israel*
jan.weber@uochb.cas.cz

Recent SARS-CoV-2 pandemic emphasized the need for broad-spectrum intervention before effective vaccination or antivirals are available. Mimicking heparan sulfate by silver and gold nanoparticles (NPs) capped with mercaptoethane (AgMES) and mercaptoundecane sulfonate (AuMUS) molecules, thus inhibiting virus attachment to the cells, was successfully used against different viruses[1,2] and recently also against SARS-CoV-2 (ref.[3]). Here, we present antiviral effect of AgMES NPs and AgMES-coated fabrics against SARS-CoV-2 and common human coronavirus HCoV-OC43.

Cytotoxicity, antiviral and virucidal activity assays of powders and coated fabrics were performed against SARS-CoV-2 and HCoV-OC43 in Vero E6 cells. The AgMES NPs showed only moderate cytotoxicity in Vero E6 cells at a concentration 0.5 mg/mL, while they inhibited SARS-CoV-2 with EC_{50} of 60 μg/mL and HCoV-OC43 with EC_{50} of 1.3 μg/mL. Consequently, AgMES-coated cotton fabrics showed good virucidal activity against HCoV-OC43 but almost no activity against SARS-CoV-2. After optimization of coating conditions, we were able to improve the virucidal activity of fabrics against SARS-CoV-2. Fabrics coated with AgMES NPs, which were synthesized from 1g of silver and 1/0.5 ratio of Ag/MES, eradicated 97–100% of SARS-CoV-2 after 60 minutes contact time. In summary, the improved sonochemical method to deposit AgMES NPs onto fabrics, which lead to quick eradication of SARS-CoV-2, provides another step towards better preparedness in the case of future pandemics.

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BIOLOGY CENTRE CAS

L-07

A COMBINATION OF TWO RESISTANCE MECHANISMS IS CRITICAL FOR TICK-BORNE ENCEPHALITIS VIRUS ESCAPE OF BROADLY NEUTRALIZING HUMAN ANTIBODY

PAVEL SVOBODA[a,b,c,d], JAN HAVIERNIK[a,c], PETR BEDNAR[a,c,e], MILOS MATKOVIC[f], TOMÁS CERVANTES RINCÓN[f], JENNIFER KEEFFE[g], MARTIN PALUS[a,b], JIRI SALAT[a,b,c],MARIANNA AGUDELO[h], MICHEL C. NUSSENZWEIG[h,i], ANDREA CAVALLI[f,j], DAVIDE F. ROBBIANI[f], DANIEL RUZEK[a,b,c,k]*

[a] Veterinary Research Institute, Brno, Czech Republic, [b] Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic, [c] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic, [d] Department of Pharmacology and Pharmacy, Faculty of Veterinary Medicine, University of Veterinary Sciences; Brno, Czech Republic, [e] Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic, [f] Institute for Research in Biomedicine, Università della Svizzera italiana, Bellinzona, Switzerland, [g] California Institute of Technology, Pasadena, United States, [h] Laboratory of Molecular Immunology, The Rockefeller University, New York, NY, United States, [i] Howard Hughes Medical Institute, New York, United States, [j] Swiss Institute of Bioinformatics, Lausanne, Switzerland, [k] Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi City, Japan
ruzekd@paru.cas.cz

Tick-borne encephalitis (TBE) is a potentially lethal neuroinfection in humans, caused by TBE virus (TBEV), a member of genus *Orthoflavivirus*, family *Flaviviridae*. The disease is prevalent in forested areas of Europe and northeastern Asia. Specific anti-TBEV immunoglobulin is currently used with well-documented effectivity for post-exposure prophylaxis and TBE treatment in Russia and Kazakhstan, but the use of specific TBEV immunoglobulins has been discontinued in Europe due to concerns regarding antibody-dependent enhancement (ADE) of infection in naïve individuals.

Analysis of human antibody response to TBEV infection or vaccination revealed that expanded clones of memory B cells expressed closely related anti-envelope domain III (EDIII) antibodies in both cohorts, but the most potent neutralizing antibodies were found only in individuals who recovered from natural infection. These antibodies also neutralized other tick-borne flaviviruses. Structural analysis revealed a conserved epitope near the lateral ridge of EDIII adjoining the EDI-EDIII hinge region. Prophylactic or early therapeutic antibody administration was effective at low doses in mice lethally infected with TBEV.[1]

TBEV escape mutants, however, evolved rapidly *in vitro* in the presence of these mAbs, but had strikingly reduced pathogenicity. To delineate the underlying mechanism of antibody evasion, we determined the escape mutation profiles for one of these mAbs, T025. The escape of TBEV from T025 was associated with two mutations in EDII and EDIII (Fig. 1). The EDIII mutation affected the major epitope for the antibody and impaired the formation of a salt bridge critical for the interaction between the antibody and EDIII. This mutation also formed a predicted N-linked glycosylation consensus sequence, but the escape occurred independently of glycan occupancy. The EDII escape mutation was subject to the repulsion of positively charged residues in EDI within the adjacent E protein, resulting in a rearrangement of the quaternary structure of the envelope proteins on the viral surface. Remarkably, neither the EDIII nor EDII mutations, when used individually, were able to confer complete resistance to the antibody, but contributed only partially. Only mutants carrying both mutations were fully resistant.

This indicates that both direct and indirect resistance mechanisms must be involved simultaneously for TBEV to escape from T025. While T025 rapidly selected for TBEV escape mutants, a combination of T025 and another mAb targeting non-overlapping EDIII epitope had a synergistic effect in terms of viral neutralization efficiency and, most importantly, prevented TBEV escape.[2]

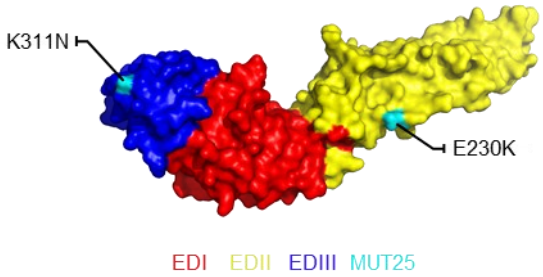

Fig. 1. **Surface model of the TBEV envelope protein with domains I, II, and III colored in red, yellow, and blue, respectively. The amino acid changes in TBEV-MUT25 are shown.**

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P-13
TICK-BORNE ENCEPHALITIS VIRUS IN PATIENTS AND IN TICKS FROM NATURAL ENDEMIC SITES IN SOUTH BOHEMIA

JAN KAMIŠ[a,b], MICHAL KŘÍHA[a,b,c], MARKÉTA DVOŘÁKOVÁ[a], JANA ELSTEROVÁ[a], ALEŠ CHRDLE[c,d,e], DANA TEISLEROVÁ[c], DANIEL RŮŽEK[a,f,g], MARTIN PALUS[a,g], VÁCLAV HÖNIG[a,g]*

[a] Biology Centre, Czech Academy of Science, Branišovská 31, Ceske Budejovice, 37005, Czechia, [b] Faculty of Science, University of South Bohemia, Branišovská 31, Ceske Budejovice, 37005, Czechia, [c] Infectious Diseases Department, Hospital Ceske Budejovice, B. Němcové 54, Ceske Budejovice, 37001, Czechia, [d] Royal Liverpool University Hospital, Prescot Street Liverpool Merseyside, L7 8XP, UK, [e] Faculty of Social and Health Sciences, University of South Bohemia, J. Boreckého 27, Ceske Budejovice, 37011, Czechia, [f] Department of Experimental Biology, Faculty of Science, Masaryk University, Kotlářská 267, Brno, 61137, Czechia, [g] Veterinary Research Institute, Hudcova 7, Brno, 62100, Czechia
honig@paru.cas.cz

Tick-borne encephalitis virus (TBEV) (*Flaviviridae, Orthoflavivirus*) causes infections of the central nervous system of varying severity. In the Czech Republic, an average of 643 human cases of tick-borne encephalitis are registered annually and a substantial proportion of the patients become infected in South Bohemia.

Using a sensitive one-step RT-qPCR method, we have screened patient serum samples collected at the Ceske Budejovice hospital and ticks (*Ixodes ricinus*) sampled in South Bohemia. Positive results were confirmed by sequencing. We also attempted to isolate the virus strains using cell cultures.

The initial patient set consisted of samples positive for anti-TBEV antibodies (at least IgM) by standard diagnostic ELISA. We detected TBEV RNA in 7.3% of patients (11 of 152). However, most positive samples were collected before seroconversion had occurred. Therefore, we also examined patient sera that were tested but negative for anti-TBEV antibodies and obtained a prevalence rate of 1.2% (3/248).

Of the total of 1200 ticks, three adult female ticks and a pool of ten nymphs were positive for TBEV RNA. Using cell culture, all four viral strains were successfully isolated from ticks and another strain from patient sera.

From these initial results, we can conclude that viremia is low and short in most cases. The detection of TBEV RNA in sera is negatively correlated with the antibody titers. Phylogenetic analyses revealed a high diversity of local strains as well as their remarkable historical genetic stability.

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P-14
HUMAN MICROGLIA AND TICK-BORNE ENCEPHALITIS VIRUS INFECTION

VERONIKA PRANCLOVA[a,b], VACLAV HÖNIG[a,c], LENKA NEDVEDOVA[a,b], MARIE VANCOVA[a,b], ELISKA KOTOUNOVA[a,b], DANIEL RUZEK[a,c,d], MARTIN PALUS*[a,c]

[a] Institute of Parasitology, Biology Centre CAS, Branisovska 1160/31, 370 05 Ceske Budejovice, Czech Republic, [b] Faculty of Science, University of South Bohemia (FSci USB), Branisovska 1760, 370 05 Ceske Budejovice, Czech Republic, [c] Department of Infectious Disease and Preventive Medicine, Veterinary Research Institute, CZ-62100 Brno, Czech Republic, [d] Department of Experimental Biology, Faculty of Science, Masaryk University, CZ-62500 Brno, Czech Republic
palus@paru.cas.cz

Tick-borne encephalitis (TBE) is a severe viral neuroinfection associated with impaired blood-brain barrier (BBB) integrity. Although the exact mechanism leading to BBB disruption remains unclear, there is evidence that this crucial event may be a consequence of the host immune response to infection[1]. Therefore, we focused on the role of microglia, the CNS resident macrophages, in TBEV infection.

Human microglial cell cultures were infected with three different TBEV strains. To characterise viral growth, infectious virus production was determined by plaque assay, while intracellular viral RNA was quantified by RT-PCR. A Luminex multiplex assay was performed to investigate changes in cytokine/chemokine (C/C) production. Ultrastructural changes after infection were investigated using electron microscopy. Moreover, an *in vitro* model of the BBB was assembled and co-cultured with either microglia, neuroblastoma cells or a combination of both. This model was then examined for viral growth and signs of cytopathic effect (CPE).

TBEV infection of microglia was found to be persistent and productive, with high viral yields and no detectable CPE. Infection was associated not only with ultrastructural changes but also with significantly increased expression of a broad spectrum of C/C, of which TNF-α, IL-6 and IL-17A are known to increase BBB permeability. When co-cultured with an *in vitro* model of the BBB, the presence of microglia resulted in a decrease in viral titer on the brain side in contrast to the model in which the BBB was co-cultured alone or only with neuroblastoma cells. Our results show that although TBEV-infected microglia produce a variety of inflammatory mediators, these do not appear to exacerbate the course of infection when co-cultured with the BBB model. On the contrary, the presence of microglia in the co-culture appears to reduce the infection. However, further studies are needed to clarify exactly what role microglia play and how they interact with other cells in the brain during TBE.

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UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

L-08
DHX15, A CELLULAR RNA HELICASE, STARRING IN THE MPMV REPLICATION CYCLE

IVANA KŘÍŽOVÁ[a,b], ALŽBĚTA DOSTÁLKOVÁ[a,b], PETRA JUNKOVÁ[c], JAN PRCHAL[d,b], LARISA ŠINKOVEC[a,b], MARINA KAPISHEVA[b], MICHAELA RUMLOVÁ[a,b]*

[a] *Department of Biotechnology, University of Chemistry and Technology, Prague, Prague 6, 16628, Czech Republic,* [b] *National Institute of Virology and Bacteriology, University of Chemistry and Technology, Prague, Prague 6, 16628, Czech Republic,* [c] *Institute of Organic Chemistry and Biochemistry of Czech Academy of Science, Prague 6, 16600, Czech Republic,* [d] *Department of Biochemistry and Microbiology, University of Chemistry and Technology, Prague, Prague 6, 16628, Czech Republic*
dostalkl@vscht.cz

The Mason-Pfizer Monkey Virus (M-PMV) is a member of the family *Retroviridae* and genus *Betaretrovirus*. As all viruses, M-PMV relies on the host-cell protein machinery. The retroviral genome is represented by two RNA molecules with positive polarity interacting with each other through the region situated at 5′end, resulting in a dimer formation. The dimer is coated by nucleocapsid (NC) protein molecules, facilitating the most thermodynamically suitable conformation inside the virion. The ribonucleoprotein complex together with enzymes is encapsulated in the core composed of the capsid protein. The core is enveloped by a bilayer of phospholipid membrane obtained during budding, and matrix protein bound in the inner layer of the membrane.

During the genome replication of RNA viruses, the dissociation of ribonucleoprotein complex and unfolding of RNA secondary structures are crucial for proper replication. These processes are often facilitated by RNA helicases. Some RNA viruses encode the RNA helicase in their genome; nevertheless, others, such as retroviruses, utilize host-cell RNA helicase to promote their replication. RNA helicases can participate in reshaping viral ribonucleoproteins, splicing, nuclear export of unspliced viral RNA, transcription, translation, and viral RNA packaging (reviewed in[1,2]).

As a simple betaretrovirus, M-PMV encodes its proteins only in three genes: *gag*, *pro*, *pol*. These are translated into three polyprotein precursors: Gag, Gag-Pro, Gag-Pro-Pol. At the C-terminus of Gag-Pro and N-terminus of Gag-Pro-Pol, a short glycine-rich peptide (G-patch, GP) is localized as a part of the protease (PR) and reverse transcriptase (RT), respectively. The GP-containing eukaryotic proteins are often involved in the recruitment and subsequent activation of DEAH/RHA RNA helicases, such as DHX15[3] (reviewed in[4,5]).

The MS analysis of the host-cell proteins in released M-PMV virions revealed the presence of RNA helicase DHX15 packaged into M-PMV particles but not into ΔGP M-PMV particles. Expectedly, during cell-based and *in vitro* experiments such as mutagenesis, mRNA silencing, photoactivatable ribonucleoside-enhanced crosslinking, immunoprecipitation assay, thermophoresis, and isothermal titration calorimetry, we observed that the DHX15 was involved in reverse transcription as the initial step of retroviral genome replication (Fig. 1). Surprisingly, it was also noticed that DHX15 acts during the packaging of retroviral genomic RNA (gRNA), although the Gag polyprotein was reported to be particularly responsible for that (Fig. 1).

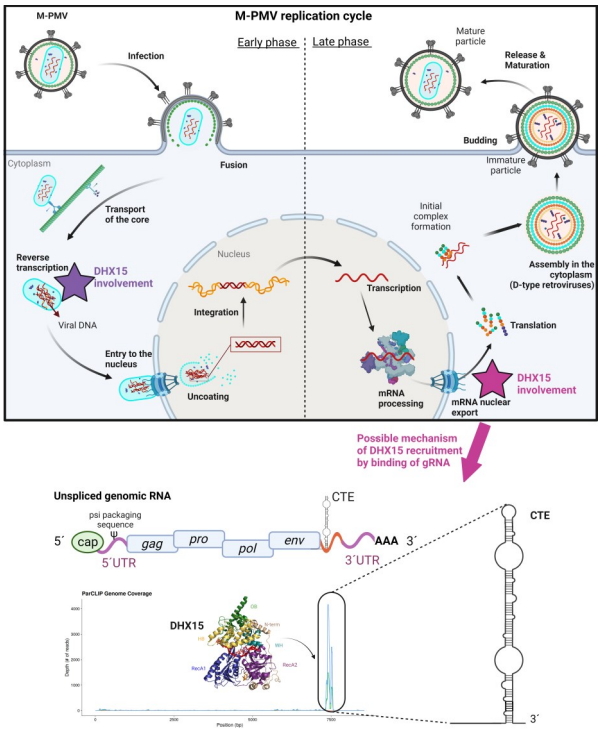

Fig. 1. **The schematic representation of the M-PMV replication cycle with depicted DHX15 involvement during the early (purple) and late (pink) phases. A possible region of gRNA (CTE) bound by DHX15 is depicted below.** The figure was created by BioRender.

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P-15
THE INTRODUCTION OF A TRIFLUOROMETHYL GROUP INTO FLAVONOIDS INCREASES THEIR SYNERGY WITH ANTIBIOTICS

MARTINA HURTOVÁ[a,b], DANIELA BRDOVÁ[b], BÁRA KŘÍŽKOVSKÁ[b], KATEŘINA VALENTOVÁ[a], VLADIMÍR KŘEN[a], JITKA VIKTOROVÁ[a,c,*]

[a] Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 3, 166 28 Prague, [b] Department of Biotechnology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague, [c] NIVB at the University of Chemistry and Technology, Prague, Technická 5, Prague 6, 166 28, Czech Republic
prokesoj@vscht.cz

The development of new antibiotics has not been enough to keep up with the development of bacterial resistance for quite some time. New antibiotics are developed only very slowly and often are only modifications of existing structures to which bacteria have already developed resistance. However, with the development of new molecular biological methods, we have the possibility of more effectively and significantly faster identifying the mechanisms that bacteria use to eliminate antibiotics.

The aim of this work is to prepare a platform for the search for inhibitors of antibiotic resistance. The clinical isolates of *Staphylococcus aureus* were phenotypically and genotypically characterised from the point of view of sensitivity to antibiotics.

This work contains a detailed study of the reactivity of C-8 halogenated flavonoids in cross-coupling reactions to prepare a new C-N bond. A library of 14 new quercetin and luteolin derivatives substituted with various amines at C-8 was prepared. The biological activity of the prepared derivatives was evaluated with special focus on modulating antibiotic resistance.

Although the ability of quercetin and luteolin to act synergistically with some antibiotics has been published several times in the past, we are the first to publish that quercetin and luteolin modulate resistance to erythromycin by inhibiting resistance to ermB (ribosomal methylase B) and gentamicin by inhibiting aadD (4′,4″ adenyltransferase). Furthermore, we prepared the quercetin derivative by introducing a trifluoromethyl group, which is roughly 2× more effective than the parent compound and has a higher therapeutic index.

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P-16
PRODUCING THE NON-INFECTIOUS MASON-PFIZER MONKEY VIRUS PARTICLES TO STUDY THE Llate PHASE PROCESSES

KAROLÍNA ŠTRBOVÁ[a,c], IVANA KŘÍŽOVÁ[b,c] MARKÉTA ČASTORÁLOVÁ[a,c], TOMÁŠ RUML[a,c,*]

[a] Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 3, 166 28 Prague, [b] Department of Biotechnology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague, [c] NIVB at the University of Chemistry and Technology, Prague, Technická 5, Prague 6, 166 28, Czech Republic
strbovak@vscht.cz

The retroviral life cycle comprises two phases. The early phase involves host cell infection, reverse transcription, and integration into the host genome. The late phase begins with the production of retroviral polyprotein precursors, forming immature viral particles that bud through the cell membrane. These particles undergo maturation through proteolytic cleavage, becoming infectious.

The assembly of Mason-Pfizer monkey virus (M-PMV) immature particles takes place in cytoplasm of the host cell, and they are transported towards the cytoplasmic membrane before budding occurs. Studying the late phase of the M-PMV life cycle in our laboratory requires a safe, non-infectious virus which has that specific life cycle stage undisturbed. For this reason, two amino acid substitutions were proposed in the enzymes reverse transcriptase (RT) and integrase (IN). Substitutions D188A in RT and D65V in IN were introduced based on the similarity of the active site of these enzymes between M-PMV and human immunodeficiency virus type 1 (HIV-1). The prepared vectors were transfected into tissue culture cells and single round infectivity assay demonstrated that these vectors yield viruses capable of late phase but are non-infectious.

The prepared non-infectious vectors were subjected to additional mutations to introduce amino acid substitutions presumably affecting myristoyl binding pocket of matrix protein (MA). HEK 293 tissue cultures were transfected, and cell media samples were collected 48 h post transfection. Our results showed proteasomal degradation of polyprotein Gag in “myr OUT” variants, which prevented yield of viral particles. On the other hand, the “myr IN” variant caused accumulation of Gag precursors in the cytoplasm with delayed viral particle release. Additionally, mutation of the junction at the C-terminus of MA prevented its cleavage by viral protease which is in accordance with our *in vitro* data. However, it did not significantly affect further maturation steps 48 h post transfection. These data were obtained using western blot, and transmission electron microscopy.

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P-17
IMPACT OF NATURALLY OCCURRING AMINO ACID EXCHANGES IN THE PROTEINS OF SARS-COV-2 RNA-DEPENDENT RNA POLYMERASE COMPLEX

MATĚJ DANDA, JAN PRCHAL, ANNA KLIMEŠOVÁ, ANETA PAGÁČOVÁ, MICHAELA RUMLOVÁ*

NIVB at University of Chemistry and Technology, Technická 5, Prague 6, 160 28, Czech Republic
dandam@vscht.cz

Since the first appearance of SARS-CoV-2 back in 2019, there has been immense effort in drug research and development. One of the most favorable drug targets is the RNA-dependent RNA polymerase (RdRp). The RdRp is a tertiary complex of three different proteins – the main enzymatic unit NSP12, the NSP7 subunit, and two NSP8 subunits. Although SARS-CoV-2 possesses a powerful proofreading mechanism, similar to other RNA viruses its genome is liable to mutations, and mutations in the RdRp gene sequences have been reported as early as the beginning of 2020. Missense mutations result in amino acid substitutions that have the potential to alter the activity of the RdRp complex. Nevertheless, the experimental information on such substitutions has so far been limited.

In the presented work, we have selected several mutations in the genes encoding NSP7 and NSP8 based on their reported frequency and localization of the respective substituted amino acid. Subsequently, we analyzed their impact on the protein stability, protein-protein interactions during the RdRp complex assembly, and the overall RNA polymerase activity. Our results indicate, that even a single amino acid exchange can not only affect the formation of the RdRp complex but also enhance its relative activity significantly. Missense mutations in the RdRp genes should therefore be of greater concern in the development of drugs targeted at SARS-CoV-2 RdRp.

Acknowledgement
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MASARYK UNIVERSITY BRNO

L-09
STRUCTURE AND REPLICATION CYCLE OF A VIRUS INFECTING CLIMATE-MODULATING ALGA EMILIANIA HUXLEYI

MIROSLAV HOMOLA[a], CARINA R. BÜTTNER[a], TIBOR FÜZIK[a], PAVEL KŘEPELKA[a], RADKA HOLBOVÁ[a], JIŘÍ NOVÁČEK[a], MARTEN CHAILLET[b], FRIEDRICH FÖRSTER[b], WILLIE WILSON[c], DECLAN C. SCHROEDER[d,e], PAVEL PLEVKA[a]*

[a] Central European Institute of Technology, Masaryk University, Brno, Czech Republic, [b] Bijvoet Centre for Biomolecular Research, Utrecht University, Utrecht, Netherlands, [c] Marine Biological Association of the UK, Plymouth, United Kingdom, [d] Veterinary Population Medicine, The University of Minnesota, St Paul, USA, [e] School of Biological Sciences, University of Reading, Reading, United Kingdom
pavel.plevka@ceitec.muni.cz

The globally distributed marine alga *Emiliania huxleyi* produces reflective calcite disks (coccoliths) that increase the albedo of ocean water and thus reduce the heat absorption in the ocean, which cools the Earth's climate[1]. The population density of *E. huxleyi* is restricted by nucleocytoplasmic large DNA viruses, including *E. huxleyi* virus 201 (EhV-201)[2]. Despite the impact of *E. huxleyi* viruses on the climate, there is limited information about their structure and replication.

Here we show that the dsDNA genome inside the EhV-201 virion is protected by an inner membrane, capsid, and outer membrane decorated with numerous transmembrane proteins. The virions are prone to deformation, and parts of their capsids deviate from the icosahedral arrangement. EhV-201 virions infect *E. huxleyi* by using their fivefold vertex to bind to a host cell and fuse the virus's inner membrane with the plasma membrane. Whereas the replication of EhV-201 probably occurs in the nucleus, virions assemble in the cytoplasm at the surface of endoplasmic reticulum-derived membrane segments. Genome packaging initiates synchronously with the capsid assembly and completes through an aperture in the forming capsid. Upon the completion of genome packaging, the capsids change conformation, which enables them to acquire an outer membrane by budding into intracellular vesicles. EhV-201 infection induces a loss of surface protective layers from *E. huxleyi* cells, which allows the continuous release of virions by exocytosis. Our results provide insight into how EhVs bypass the surface protective layers of *E. huxleyi* and exploit the organelles of an infected cell for progeny assembly.

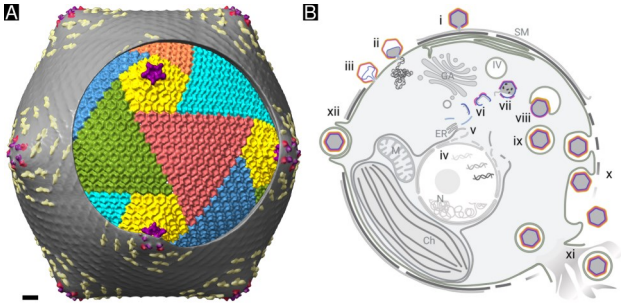

Fig. 1. **EhV-201 virion structure and its lifecycle. (A)** Composite map of EhV-201 virion. The surface of the virion is covered with the outer membrane (grey) with central (magenta) and peripheral (red) vertex proteins and dimers of ridge proteins (light yellow). A circular region of the outer membrane was removed to reveal the arrangement of the major capsid proteins forming the capsid. Pentamers of capsid proteins are shown in magenta. Pseudo-hexamers of major capsid proteins belonging to the penta-symmetrons are shown in yellow, whereas those forming tri-symmetrons are in various other colors. Scale bar 10 nm. **(B)** Infection cycle of EhV-201. Abortive infection: (i) Surface membrane and cell envelope protect *E. huxleyi* from EhV-201 infection. Productive infection: (ii) EhV-201 virion fuses its inner membrane with plasma membrane to deliver its genome into the cytoplasm. (iii) Empty capsid containing the collapsed inner membrane sack remains attached to the cell surface. (iv) EhV-201 genome probably replicates in the cell nucleus. (v) EhV-201 infection induces segmentation of the endoplasmic reticulum and outer nuclear membrane to form a virus factory. (vi) Genome packaging and capsid assembly initiate on opposite surfaces of membrane segments. (vii) Genome is packaged into a particle through an aperture in the forming capsid. (viii) The completion of the genome packaging induces a conformational change in the capsid, which enables it to bud into intracellular vesicles. (ix) Virion inside an intracellular vesicle. (x) EhV-201 infection causes the loss of surface protective layers from *E. huxleyi* cells, which enables the continuous release of virions by exocytosis. (xi) The EhV-201 replication cycle is terminated by cell lysis, which results in the release of virions inside vesicles. (xii) Alternative infection pathway utilizing phagocytosis of EhV virions inside vesicles. Created using BioRender.com.

Acknowledgement
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L-10
MULTIPLE FACTORS MODULATING THE FORMATION OF TOROIDAL MEMBRANE PORES

DENYS BIRIUKOV[a], TIMOTHÉE RIVEL[a], ROBERT VÁCHA[a,b,c,*]

[a] CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic, [b] Department of Condensed Matter Physics, Faculty of Science, Masaryk University, Kotlářská 267/2, 611 37 Brno, Czech Republic, [c] National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic
robert.vacha@muni.cz

Pore formation and stability in cell membranes play a pivotal role in drug delivery into cells such as bacteria[1]. Particularly, the stability of toroidal pores — pores with a doughnut-shaped geometry where lipid headgroups cover the pore rim — is primarily regulated by the rim-associated line tension. While molecular simulations are a known tool for screening drug candidates, they can also forecast the potential of new therapeutics to modulate line tension. However, not all models (known as force fields) tailored for biological computer simulations accurately capture the line tension of lipid membrane compositions characteristic of mammalian and bacterial cells. This deficiency eventually hinders the drug design.

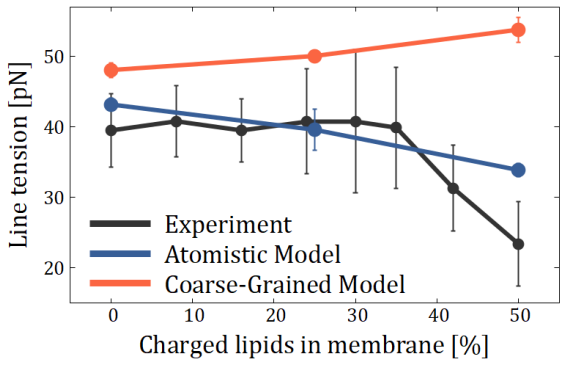

Fig. 1. **The line tension of a 1-palmitoyl-2-oleoyl-*sn*-glycero-3--phosphocholine membrane upon increasing the amount of negatively charged 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-glycerol lipids in its composition.** The experimental data obtained from electroporation measurements[3] (black line) are compared to the results from molecular dynamics simulations with atomistic (prosECCo75, blue line) and coarse-grained (MARTINI 3, red line) models.

In this work, we investigate the use of molecular dynamics simulations for the rational design of novel molecules, including antimicrobial peptides known to form pores and translocate across cell membranes[3]. We first delve into factors like lipid composition, which varies between mammalian and bacterial cells, and the unique solution environment, elaborating on their impact on the line tension. Furthermore, we describe the complete process of toroidal pore formation, its molecular origins, and showcase methods to assess the pore formation by computer simulations using available simulation models.

Figure 1 illustrates that while widely-used atomistic models, e.g., prosECCo75, can adequately reproduce the decrease in line tension upon adding negatively charged lipids such as phosphatidylglycerols[3], coarse-grained models – especially the highly favored in biological fields MARTINI model – lack this capability. The failure to reproduce line tension both qualitatively and quantitatively originates from the loss in the chemical specificity coming as a price to enable biological simulations of enhanced spatio-temporal resolution. Therefore, coarse-grained models should be cautiously used for line tension predictions unless specific modifications are incorporated, for instance, those related to polarization effects.

Having identified appropriate molecular models, we show the impact of the solution environment (e.g., ions and their concentration) on the line tension behavior and discuss strategies for the systematic design of organic molecules that can modulate line tension and thereby regulate the membrane pore stability. In conclusion, we will underscore the future potential of our methodology, which, in tandem with experimental work, can significantly enhance the efficient design of new antiviral and antibacterial therapeutics.

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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L-11
INTERPLAY BETWEEN VIRAL TRANSCRIPTION AND HOST TRANSLATION

AMIYARANJAN DAS[a,b], JULIA BARTULI[c], CLEMENS GRIMM[c], UTZ FISCHER[c], GABRIEL DEMO[a]

[a] Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic, [b] National Centre for Biomolecular Research (NCBR), Faculty of Science, Masaryk University, Brno, Czech Republic, [c] Department of Biochemistry and Cancer Therapy Research Center, Theodor-Boveri Institute, University of Wurzburg, Wurzburg, Germany
gabriel.demo@ceitec.muni.cz

The coupling of transcription and translation (CTT) is considered as one of the main gene regulation mechanisms in bacteria and plays an important role in genome stability[1]. Recent cryo-electron microscopy (cryo-EM) studies showed the physical coupling of these two biological machineries *in vitro*[2,3] and *in vivo*[4]. Although this mechanism cannot exist in eukaryotes due to the physical separation of the nucleus and cytoplasm by a membrane, it is possible that some cytoplasmic viruses use the coupling mechanism to optimize the viral gene expression. The promising candidate is the double-strand DNA Vaccinia virus (VACV). Interestingly, VACV performs the viral genome replication, transcription and translation in infected mammalian cells within discrete cytoplasmic foci called viral factories[5]. The initial round of viral transcription and translation during the early phase of infection occurs inside the host cytoplasm. However, in intermediate and late stage of infection the viral gene expression is carried out inside the viral factories in close association with host ribosomes[6]. The co-localization of viral RNA polymerase with host ribosomes increases the probablity of physical interaction in a highly dense enviroment of viral factories. Additionally, in late stage of infection VACV is capable to phosporylate the RACK1 protein on small ribosomal subunit to enable the translation of 5′- polyA leader viral mRNAs[7]. These mRNAs mostly encode viral capsid proteins. Therefore, the recent findings show that VACV has probably developed another mechanism to control the production of viral proteins by direct modification of the structure of human ribosome.

Here, we present intial *in vitro* experiments (binding assays) to evaluate the potential interaction of VACV RNA polymerase with mammalian ribosomes mediated by specifically designed mRNAs. The primary goal is to reconstitute the CTT *in vitro* and use single particle cryo-EM to uncover the detailed view of the structural architecture of the viral-host CTT. Moreover, our structural analysis of human ribosomes from infected (late stage) cells indicate that roughly 70 % of ribosomes do not carry RACK1 protein (Figure 1). This observation indicates, that perhaps the phoshorylation of RACK1 is a signal for protein depletion in order to take a full control of host translation machinery to enable the viral protein synthesis.

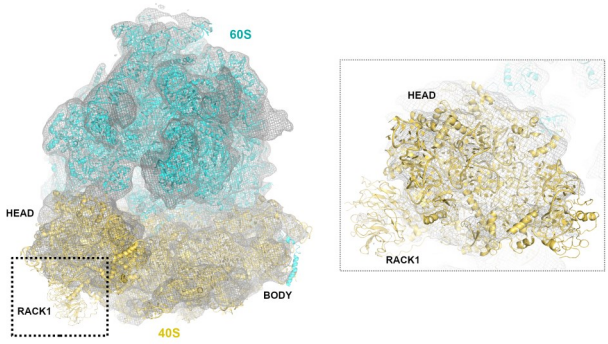

Fig. 1. **Overall view of the human 80S ribosome structure isolated from infected cells (late stage of infection), left panel. The close-up view of the head region of 40S subunit, right panel.** The map shows low occupancy for RACK1 protein. The cryo-EM density map is highlighted in grey mesh. Large ribosomal subunit (60S) colored in cyan and small ribosomal subunit (40S) colored in yellow.

Acknowledgement
This study was supported by the project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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L-12

IN VITRO CULTIVATION OF *TREPONEMA PALLIDUM*: NEW TOOL TO STUDY PHYSIOLOGY AND GENETICS OF THE SYPHILIS AGENT

JURAJ BOSÁK, MATĚJ HRALA, ELIŠKA VRBOVÁ, MONICA MEDAPPA, PETRA POSPÍŠILOVÁ, DAVID ŠMAJS*

Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
dsmajs@med.muni.cz

Treponema pallidum subsp. *pallidum* is the causative agent of syphilis, a sexually transmitted human disease with over 6 million new syphilis cases per year worldwide[1]. There are two genetically distinct groups of strains denoted as Nichols-like strains and SS14-like strains[2,3].

T. pallidum is an obligatory human pathogen, which was considered to be uncultivable for decades[4]. Handful of treponemal strains has been isolated and maintained *in vivo* using passages in rabbits[5]. In 2018, Edmondson and colleagues published a revolutionary technique allowing *in vitro* cultivation of *T. pallidum* in laboratory conditions[6]. *T. pallidum* multiplies in a presence of rabbit epithellial cells and modified complete medium at 34 °C in microaerobic atmosphere (5% CO_2 and 1.5% O_2). Long-term cultivation requires regular subcultures every 7 days[7].

In our study, seven different *T. pallidum* strains (out of 16 known) from Nichols-like as well as from SS14-like group (n=3 and n=4, respectively) have been successfully adapted for *in vitro* cultivation system. Now, these strains are continually cultivated for more than two years.

Results obtained from *in vitro* cultivation revealed that average generation time ranges between 42.9 and 67.4 hours and significantly differs among various *T. pallidum* strains. Strain DAL-1 (from Nichols-like group) showed the fastest multiplication compared to all six other strains. Based on the growth patrameters and genetic differences of different strains, *in vitro* genetic manipulation was performed[8] and two genes/genetic regions involved in faster multiplication of DAL-1 strain has been identified.

Besides growth rates, set of *T. pallidum* strains has been characterized with respect to *in vitro* susceptibility to two clinically relevant antibiotics – penicillin and ceftriaxone. The average minimal inhibition concentration (>90% of growth inhibition) has been established as 0.0005 μg/mL and 0.005 μg/mL for penicilin and ceftriaxone, respectively.

Using *in vitro* cultivation, proteome for six different *T. pallidum* strains has been obtained and analyzed. Proteomic analysis determined more than 80% of predicted proteins from previous genomic studies. In addition, several abundant flagellar and outer membrane proteins were found among all six strains. These proteins represent candidates for syphilis vaccine design.

Taken together, we identified several physiological characteristics of *T. pallidum* using *in vitro* cultivation. This technique represents an important tool for better understanding of evolution and infection strategies of treponemal pathogens.

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L-13
BACTERIOPHAGES: AN ALTERNATIVE OR A POWERFUL COMPLEMENT TO ANTIBIOTICS?

ELIŠKA KUČEROVÁ*, TIBOR BOTKA, IVANA MAŠLAŇOVÁ, LUCIE KUNTOVÁ, ADÉLA FINSTRLOVÁ, JIŘÍ DOŠKAŘ, ROMAN PANTŮČEK

Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
kucerovae@sci.muni.cz

Bacterial viruses, or bacteriophages, are the most abundant entity in the world and form an everyday part of our lives. However, despite more than one hundred years of human interest in using them to fight bacterial infections, their interactions with the bacterial host and the implications of these interactions considering the treatment are still poorly understood. Even so, phages are either regularly used in some Eastern countries to treat bacterial infections or offered as an experimental treatment where antibiotics are failing today (Poland, USA, Australia, etc.)[1]. Also, pilot clinical trials further confirm that side or negative effects on patients' health during treatment are none or minimal, especially when compared to antibiotic treatment. Unfortunately, it is impossible to skip antibiotics or other treatments entirely, so it is also essential to be aware of the interactions of bacteriophages with other drugs, primarily antibiotics.

The first promising report on phage-antibiotic interaction was a study by Comeau *et al.*, which described a phenomenon called Phage-Antibiotic Synergy (PAS)[2]. Basically, it is a situation in which the lysis of a bacterium by a phage is enhanced. The manifestations attributed to synergy vary according to the method used. Most commonly, an increase in phage plaque on Petri dishes, an increase in phage yield, or an absence of bacterial regrowth during spectrophotometric measurement of optical density at 600 nm is observed (Fig 1). Phage-antibiotic interactions also include other possible scenarios, such as antagonistic, additive, or no effect.

One of the research directions of our team is a project that aims to capture the current situation of phage-antibiotic interactions in selected clinical strains of *Staphylococcus aureus* in cooperation with St. Anne's University Hospital Brno. An equally important goal of the project is to propose a treatment approach using a combination of phage and antibiotics based on the identified characteristics of these bacterial strains (susceptibility to selected antibiotics and phages). In this work, we tested over 20 *S. aureus* strains for susceptibility to 3 potentially therapeutic phages (P68[3], 812h1[4], and 812 K1/420[4]) and 10 antibiotics with different mechanisms of action. Antibiotic-phage interactions were then verified in laboratory prophage-less strains. Further tested antibiotics were selected according to the increase in phage plaque size. The plaque enlargement effect was mainly observed for antibiotics targeting the bacterial cell wall (fosfomycin, amoxicillin, nafcillin). These antibiotics also accelerated the onset of lysis of the laboratory strains.

One of the pitfalls of verifying phage-antibiotic-bacteria interactions is the methodology for their quantitative evaluation. Due to the simplicity, availability, and cost-effectiveness of spectrophotometry, we decided to use the Local Virulence methodology described by Storms *et al.*[5] and apply it to the course of action of the antibiotics in addition to the phages. The criterion to evaluate synergy was the calculation of the Combination Effect, which follows the Bliss Independence assumption[6].

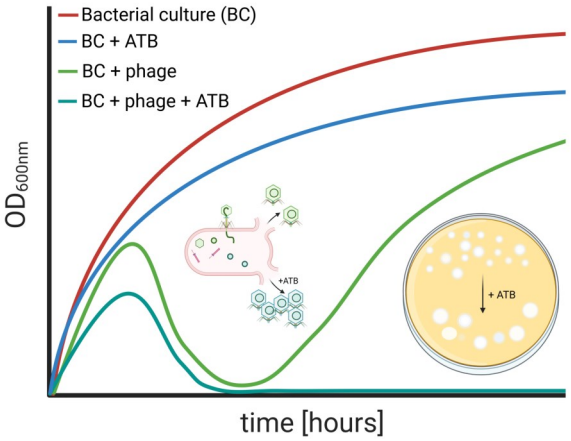

Fig. 1. **Bacterial growth/lysis and tendency to resistance after the treatment with antibiotic/phage alone or with its combination.**

Finally, these findings will further serve as the basis for an *in vivo* study of combination therapy, which is a promising next step in the introduction of phage therapy into medical practice.

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology project (Programme EXCELES, ID Project No. LX22NPO5103), funded by the European Union – Next Generation EU.

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L-14
TRANSLATION CONTROL AND CO-TRANSLATIONAL PROCESSES IN HEALTH AND DISEASE

PETR TĚŠINA

CEITEC MU, Kamenice 753/5, Brno, 625 00, Czech Republic
petr.tesina@ceitec.muni.cz

Co-translational quality control is triggered as a response to translational stalling events. Yet, different molecular mechanisms are employed for the recognition of these stalls and to trigger downstream rescue and quality control pathways. While the recognition of individual stalled ribosomes is poorly understood, the use of collided ribosomes as a proxy for the recognition of translation problems in the cell is conserved from bacteria to humans[1–3]. In eukaryotes, co-translational quality-control processes triggered by ribosome collisions accomplish several tasks and eventually trigger stress response signalling pathways[4]. These tasks include the degradation of aberrant mRNAs, the degradation of potentially deleterious nascent peptides, the ribosomal subunit recycling and tRNA recycling. Collided eukaryotic ribosomes are cleared via subunit dissociation by the ribosome quality control trigger complex (RQT/ASCC)[5,6]. Subsequently, the ribosome-associated quality control takes place on the released large ribosomal subunit and ensures the degradation of the potentially toxic nascent peptide[7].

Scheme 1. **An illustration representing the pure gorgeousness and gorgeosity of the eukaryotic ribosomal collision.** For an animated version see: https://youtu.be/eaT5hVeHZ2A
Structural information from PDB ID 6I7O [1] was visualized using Chimera X.

We mainly use structural analysis by cryo-EM to gain mechanistic understanding of these co-translational quality control events. To that end, we employ cell-free *in vitro* translation systems derived from bacteria, yeast and human cells in order to recapitulate ribosomal stalls and to isolate collided ribosomes. On this basis, we can reconstitute recognition, rescue and other processes *in vitro*. These processes include ubiquitination by Hel2 and ribosome dissociation by RQT in the yeast system or mRNA cleavage by the endonuclease SmrB in *E. coli*. Moreover, we use genomically tagged quality control factors as bait proteins for *in vivo* expression and subsequent isolation of corresponding quality control intermediates after triggering ribosomal stalls. Resulting complexes are then used for structural characterization by single particle cryo-EM, which usually yields ensembles of structures representing distinct functional intermediates with specific conformation and/or composition[8]. The most recent findings elucidating the molecular mechanisms underlying co-translational quality control will be presented along with future plans in research of host-pathogen interactions involved in translation control.

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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P-18
CONFORMATIONAL CHANGES IN BASEPLATE REQUIRED FOR GENOME DELIVERY OF *S. AUREUS* PHAGE PHI812

JÁN BÍŇOVSKÝ[a], MARTA ŠIBOROVÁ[a], JIŘÍ NOVÁČEK[a], KAREL ŠKUBNÍK[a], TIBOR BOTKA[b], MARTIN BENEŠÍK[b], ROMAN PANTŮČEK[b], MARK VAN RAAIJ[c], PAVEL PLEVKA[a]*

[a] Central European Institute of Technology – Masaryk University, Kamenice 5, 62500, Brno, Czech Republic, [b] Faculty of Science, Masaryk University, Kamenice 5, 62500, Brno, Czech Republic, [c] Department of Macromolecular Structures, National Department of Biotechnology, Consejo Superior de Investigaciones Cientificas, E-28049, Madrid, Spain
jan.binovsky@ceitec.muni.cz

Molecular machines such as bacteriophages with contractile tails and contractile injection systems employ a syringe-like mechanism to penetrate the host cell envelope. While most structurally characterized contractile machines aim at Gram-negative bacteria or eukaryotic cells, detailed structures of those targeting Gram-positive bacteria are lacking.

Here we present a structural analysis of the baseplate and tail of contractile-tailed phage phi812, which infects Gram-positive *Staphylococcus aureus*. We show that instead of fibers, phi812 binds to the cell wall using six rigid baseplate arms with two types of primary receptor-binding complexes and twelve robust pyramidal complexes. Using an integrative approach, we determined the protein structures in the tail tip complex, which orchestrates the degradation of the host cell wall. We further show that the distinct tail sheath structure allows for the flexibility of the contractile tail and that interaction between the sheath and baseplate is reflected in the conformational adaptation of the baseplate-proximal sheath. Finally, comparing the pre-attachment (native) and post-attachment (contracted) states of the baseplate and tail of phi812 provides mechanistic insights into the cascade of conformational changes within the baseplate, leading to tail sheath contraction and penetration of the host cell.

This study represents the first detailed structural characterization of a bacteriophage with a contractile tail infecting a Gram-positive bacterium and sheds light on the initial stages of bacteriophage infection on the molecular level. As the host-range mutants of phage phi812 exhibit exceptional efficacy in eradicating *S. aureus*, our findings provide a solid foundation for engineering phage particles to combat *S. aureus* infections in humans and pave the way for novel therapeutic applications.

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P-19
ASYMMETRIC RECONSTRUCTIONS OF IMMATURE TICK-BORNE ENCEPHALITIS VIRUS PARTICLES REVEAL DEFECTS CAUSED BY THE ASSEMBLY PROCESS

TIBOR FÜZIK[a], LENKA ŠMERDOVÁ[a], PETRA POKORNÁ FORMANOVÁ[b], PETRA STRAKOVÁ[b], JIŘÍ NOVÁČEK[c], DANIEL RŮŽEK[b], PAVEL PLEVKA[a]*

[a] Structural Biology, Central European Institute of Technology, Masaryk University, Kamenice 5, Brno, Czech Republic, [b] Department of Virology, Veterinary Research Institute, Hudcova 70, Brno, Czech Republic, [c] Cryo-electron Microscopy and Tomography Core Facility, Central European Institute of Technology, Masaryk University, Kamenice 5, Brno, Czech Republic
tibor.fuzik@ceitec.muni.cz

Tick-borne encephalitis virus (TBEV) is an enveloped virus belonging to the family *Flaviviridae*, which causes severe disease of central nervous system in humans. The smooth virion surface is covered by envelope proteins (E-protein), that are together with the membrane proteins (M-protein) anchored in the virus lipid bilayer. During the viral life cycle, the immature non-infectious virus undergoes a maturation process. This process includes proteolytic cleavage of prM and a major reorganization of the envelope proteins on the viral surface.

To determine the structure of immature TBEV particles, we purified them from infected tissue culture cells and used cryo-electron microscopy for visualization. The immature particles have “spiky” surface formed by the E-protein-prM-protein complex. We performed single-particle analysis and cryo-electron tomography to reveal the asymmetric nature of the TBEV immature particles. The symmetric, icosahedral, organization of the E-protein-prM-protein spikes on the particle surface is often disrupted by defects introduced during the assembly process of the immature particle. However, these irregularities do not hinder the subsequent maturation process and instead result in mature particles with empty patches in the “herring bone” organization of the mature viral surface.

The results provide further insight into the viral maturation process which could be targeted in the future by specific antiviral drugs.

Acknowledgement
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P-20
BACTERIOPHAGE PHI812 HEAD ASSEMBLY IN THE CELLS OF *STAPHYLOCOCCUS AUREUS*

MARYNA ZLATOHURSKA[a,b,*], MICHAELA PROCHÁZKOVÁ[a], TIBOR FÜZIK[a], PAVEL PLEVKA[a*]

[a] Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic, [b] Zabolotny Institute of Microbiology and Virology of the National Academy of Science of Ukraine, Zabolotny 154, 03143 Kyiv, Ukraine
maryna.zlatohurska@ceitec.muni.cz, pavel.plevka@ceitec.muni.cz

Staphylococcus aureus is an opportunistic pathogen causing a diverse range of human illnesses. It possesses a wide potential to develop resistance against antimicrobial agents. Additionally, biofilm-forming strains of S. aureus contribute to the contamination of medical equipment and the establishment of persistent infections. The lytic bacteriophage phi812, a member of the *Herelleviridae* family, possesses a wide host range, including antibiotics-resistant and biofilm-forming *S. aureus* strains, which makes it a suitable candidate for the treatment of staphylococcal infections. Nonetheless, it has not been approved for general clinical application because of the insufficient understanding of many aspects of phage biology.

To understand the assembly mechanism of phage phi812, we conducted an examination of infected cells using cryo-electron microscopy. We used focused ion beam milling for the preparation of electron-transparent lamellas from infected *S. aureus* cells and cryo-electron tomography for three-dimensional reconstructions of the cell content, followed by sub-tomogram averaging of the phage assembly intermediates.

The phage head assembly starts 15 minutes post-infection at the inner surface of the cytoplasmic membrane. In a short time (30 min) the cell is fully packed with phage assembly intermediates structures, empty, filling, and genome-containing heads and tails connected to fully packed heads. Sub-tomogram averaging of the assembly intermediates revealed distinct classes of phage heads that differ in size and surface features. Our findings offer structural insights into the assembly of the phage phi812 head in near-native conditions.

Acknowledgement
This project has received funding through the MSCA4Ukraine project, which is funded by the European Union, and was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union –Next Generation EU.

P-21
DESIGNING NANOPARTICLES FOR MEMBRANE FUSION

SOFÍA BLASCO[a,c], LUKÁŠ SUKENÍK[a,b], ROBERT VÁCHA[a,b,c]

[a] CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic, [b] Department of Condensed Matter Physics, Faculty of Science, Masaryk University, Kotlářská 267/2, 611 37 Brno, Czech Republic, [c] National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

Membrane fusion is a key process involved in many cellular processes including infection of membrane enveloped viruses. Artificially induced membrane fusion is also important for drug delivery via lipid vesicles called liposomes. Fusogenic nanoparticles can induce or control desired fusion of membranes. However, the underlying mechanisms of nanoparticle-induced fusion and the ideal properties of such nanoparticles remain largely unknown. Here, we used molecular dynamics simulations to investigate the ability of spheroidal nanoparticles of varying size, prolateness, and ligand interaction strength, to promote fusion between two vesicles. We identified the range of nanoparticle parameters that most efficiently promote fusion, and we show how the variation of each parameter affects the fusion process. Our findings provide insights into fusion mechanisms which could be used in the design of fusogenic nanoparticles with biotechnological and biomedical applications.

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This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

P-22
ANTIMICROBIAL PEPTIDE BUFORIN II TRANSLOCATION ACROSS PEPTIDE-CROWDING-INDUCED ASYMMETRIC MEMBRANE

MEHRNOOSH KHODAM HAZRATI, ROBERT VÁCHA*

CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic
robert.vacha@muni.cz

Buforin II (BF2) is a well-known antimicrobial peptide that accumulates predominantly in the cytoplasm and can kill bacterial cells without rupturing their membranes.[1,2] However, the molecular mechanism of peptide translocation into the cell remains unclear. We calculated the free energy (FE) of BF2 translocation across model bilayers consisting of POPE:POPG (3:1 mol:mol), which mimics the plasma membrane of E. coli. Apart from the peptide-free membrane, we investigated non-equilibrium conditions, where peptides predominantly adsorb from one side creating the peptide-induced asymmetric membrane. According to the FE profiles, the preferred mechanism of BF2 insertion into both membranes is via its C-terminus. However, the asymmetric adsorption of peptides reduced the translocation barrier by about 25 kJ/mol. In addition, the free energy minimum at about 2 nm from the membrane center was lost due to the peptide saturation of that membrane leaflet. The transmembrane state was stabilized compared to the peptide-free membrane. In conclusion, peptide asymmetric adsorption could destabilize the adsorbed state and promote peptide translocation across the membrane. Our results are expected to be helpful in the design of new antimicrobial and cell-penetrating peptides by shedding light on the crowding effect.

Acknowledgment
This work was supported by the Czech Science Foundation (grant GA20-20152S), the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 101001470), and the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU. Computational resources were provided by the CESNET LM2015042 and the CERIT Scientific Cloud LM2015085 provided under the program Projects of Large Research, Development, and Innovations Infrastructures. Additional computational resources were obtained from IT4 Innovations National Supercomputing Center – LM2015070 project supported by MEYS CR from the Large Infrastructures for Research, Experimental Development and Innovations.

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P-23
IMPACT OF INTERACTION DISTRIBUTION BETWEEN CAPSID BUILDING BLOCKS ON GENOME RELEASE PATHWAYS IN PICORNAVIRUSES

LUKÁŠ SUKENÍK[a,b], ROBERT VÁCHA[a,b,c]

[a] CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic, [b] Department of Condensed Matter Physics, Faculty of Science, Masaryk University, Kotlářská 267/2, 611 37 Brno, Czech Republic, [c] National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

Genome release process is a pivotal stage in the life cycle of picornaviruses. As these viruses are transported intracellularly via endosomes, exposure to acidic pH triggers a conformational change in the capsid structure, a prerequisite for the release of the viral genome. Consequently, certain viruses form openings along their symmetry axes, which are theorised to allow the slow release of the genome. However, recent observations from cryo-electron microscopy reveal that viral capsids can crack open, leading to a rapid genome release. As a result, the mechanism of genome release remains elusive. We combined *in vitro* cryo-EM data of genome release intermediates from four viruses with simulations of a mesoscopic virion model to study the genome release pathways and stability of virions. Here we show how the distribution of interactions between capsid building blocks influences virion stability and the genome release pathway. Slow release through transient pores is more likely for capsids whose interaction sites are located towards the inside of the capsid or grouped around the 2-fold or 3-fold axis of capsid symmetry. Conversely, rapid release is favoured when capsid interactions are located towards the outside of the capsid. These findings elucidate the genome release dynamics of picornaviruses and propose a strategy for designing virus-like nanoparticles for drug delivery.

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P-24
DECIPHERING THE FUNCTIONALITY OF THE UNSTRUCTURED N-TERMINAL DOMAIN OF THE BACTERIAL INITIATION FACTOR 2

HASSAN ZAFAR[a], LUDMILA VESELA[a], XUELIANG GE[b], SUPARNA SANYAL[b], GABRIEL DEMO[a]

[a] Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic, [b] Department of Cell and Molecular Biology, Biomedical Center, Uppsala University, Uppsala, Sweden
hassan.zafar@ceitec.muni.cz

Compartmentalization is a hallmark of living cells that allows them to perform complex tasks by dynamically coordinating matter and energy fluxes in space and time[1]. This compartmentalization of membrane-less organelles in prokaryotes is driven by Liquid-Liquid Phase Separation (LLPS)[2]. Studies have shown LLPS to be a major driving force in the subcellular organization of bacterial cells[3]. These "biomolecular condensates" are comprised of proteins that are generally rich in intrinsically disordered regions (IDRs)[4]. In prokaryotes, translation Initiation Factor 2 (IF-2) is a GTPase that binds the initiator tRNA and catalyzes the ribosomal subunit joining to form the elongation competent 70S complex[5]. A large portion of the N-terminal domain of IF-2 contains IDRs, making the protein a favorable candidate to form LLPS. Additionally, due to the dynamic behaviour of the N-terminus of IF-2 the structure of the full-length protein is still missing. Therefore, the mechanistic understanding of its functional role is quite elusive, especially in ribosomal subunit joining.

Here, we present biochemical evidence that IF-2 can phase separate under specific conditions. The IF-2 LLPS formation can provide deeper insight into compartmentalized translation machinery in bacterial cells. We also use time-resolved cryo-EM to capture intermediate states of the initiation process using native GTP and its non-hydrolysable analogue GDPCP. Here, the main goal is to gain structural insight about the disorded N-terminus of IF-2 and its role in ribosomal subunit joining.

Acknowledgement
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P-25
RNAseq OF *TREPONEMA PALLIDUM* DURING EXPERIMENTAL RABBIT INFECTION

PETRA POSPÍŠILOVÁ, PAVLA FEDROVÁ, NIKOLA TOM[a], DAVID ŠMAJS*

Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
dsmajs@med.muni.cz

Treponema pallidum subspecies *pallidum* (TPA), causative agent of venereal syphilis, has a genome of size 1.14 Mbp comprising 1039 open reading frames out of which 55% are proteins of unkown function[1]. Research of syphilis has lagged behind other bacterial diseases due to the long-term absence of robust *in vitro* cultivation system and our knowledge of physiology and pathogenesis was mostly based on *in silico* analysis of genome sequences. Recently published cocultivation of *T. pallidum* with rabbit epithelial cells[2] enables genetic manipulations leading to functional analysis of proteins and proteomic analyses. Finding immunogenic proteins leading to protective immunity is a key to select candidates for future vaccine against syphilis. Hundred of genomic sequences were obtained from contemporary clinical samples but transcriptomic studies are scarce[3].

RNA isolation protocol from treponemes harvested from rabbit testes was optimized to achieve a high quality RNA from a mixture of treponemal and rabbit cells from experimental infection.

Transcriptomes of laboratory strains Nichols and Haiti B were assessed using Illumina sequencing with the aim to discover which genes are expressed during experimental rabbit infection. Expression levels of all annotated genes were assessed with the aim to identify gene coexpression and confirmation of expression of outer membrane protein vaccine candidates.

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P-26
THE MICROBIOLOGICAL QUALITY OF BACTERIOPHAGE PRODUCT AND ITS INFLUENCE ON ANTIBACTERIAL PHAGE EFFECT

MARIE KOMÁRKOVÁ[a,b], MARTIN BENEŠÍK[a,b], TEREZA ŠOPÍKOVÁ[b], KATEŘINA PLACHÁ[b], MONIKA LAICHMANOVÁ[c], ROMAN PANTŮČEK[a,*]

[a] *Department of Experimental Biology, Faculty of Science, Masaryk University, 611 37 Brno, Czech Republic,*
[b] *FAGOFARMA s.r.o., 120 00 Prague 2, Czech Republic,*
[c] *Czech Collection of Microorganisms, Department of Experimental Biology, Faculty of Science, Masaryk University, 625 00 Brno, Czech Republic*
pantucek@sci.muni.cz

Developing a drug form containing bacteriophages is an essential step of phage therapy implementation in practice. A crucial criterion of the final phage product is phage stability in variant conditions, in which applied phages could be exposed during manipulation with the product (i.e., contaminations by microorganisms and drug additives). This work concerns phage titer stability during exposition to preservatives, yeast, and mold.

We assessed the stability of myovirus MB501 against *Pseudomonas aeruginosa* and myovirus MB402 against *Staphylococcus aureus*. These purified phages were inoculated by *Candida albicans* CCM 8215 or *Aspergillus niger* CMM 8222 and stored at room temperature in six variant preservatives, including organic acids salts (sodium propionate, potassium sorbate, sodium benzoate) and alcohols (m-cresol, phenoxyethanol, phenylethyl alcohol). After 24 hours, the titer of phages was determined by a standard drop test.

In the case of pseudomonal phage MB501, the titer decrease was detected after exposition to organic acid salts (i.e., potassium sorbate and sodium benzoate), in contrast with staphylococcal phage MB402, when the decrease was after exposition to all alcohols (i.e., m-cresol, phenoxyethanol, phenylethyl alcohol). Any influence of yeast or mold on phage titer was not determined. The organic acid salts had minimal influence on the titer of *C. albicans* and *A. niger*, in contrast with alcohols, which caused *C. albicans* titer loss, particularly *A. niger* titer loss. The antifungal effect did not differ between samples with phages and controls without a phage. This study points out that optimizing the preparation composition individually for every phage is essential and includes variant previously proposed criteria[1].

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P-27
ROLE OF *STAPHYLOCOCCUS EPIDERMIDIS* BACTERIOPHAGES IN PATHOGENESIS AND HORIZONTAL GENE TRANSFER

ADAM VINCO, TIBOR BOTKA, KRISTÍNA ROVŇÁKOVÁ, IVANA MAŠLAŇOVÁ, ROMAN PANTŮČEK*

Department of Experimental Biology, Faculty of Science, Masaryk University, 611 37 Brno, Czech Republic
pantucek@sci.muni.cz

Staphylococcus epidermidis is a commensal bacterium that can act as an opportunistic pathogen and cause diseases that are difficult to treat due to biofilm formation and widespread antibiotic resistance. These bacterial species also serve as a reservoir for horizontally transferable genes for other pathogenic species, mainly *Staphylococcus aureus*. Temperate phages play a crucial role in horizontal gene transfer in staphylococci, thus impacting their properties, such as virulence or resistance. Many of these genes are encoded by mobile genetic elements, including phage-inducible chromosomal islands (PICIs).

Here, we focus on *S. epidermidis* phage-inducible chromosomal islands that are molecular parasites exploiting temperate phages as helpers using various strategies to manipulate the phage life cycle and promote their spread. To this date, only one case of successful mobilization of *S. epidermidis* PICI has been reported[1].

This PICI, designated $SeCI_{SE48}$, does not encode resistance or virulence genes. Nevertheless, we showed that it affects the transfer of genes located on the bacterial chromosome. $SeCI_{SE48,}$ together with helper and non-helper phages, facilitates packaging the bacterial genome into capsids. Increased packaging potentially leads to higher transduction frequency and, thus spread of genes located on the bacterial chromosome.

As $SeCI_{SE48}$ interferes with the phage life cycle, it prevents plaque formation by phages E72 and 459. We isolated spontaneous phage mutants that overcome PICI-induced resistance and identified changes in their genomes using Illumina sequencing. Observed mutations affect a non-coding region in phage E72 and four genes of phages 459.

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P-28
IDENTIFICATION OF PHAGE DEFENCE SYSTEMS TARGETING OTHER VIRUSES INFECTING BACTERIAL HOSTS

ADÉLA FINSTRLOVÁ[a], CEDRIC LOOD[b], BAS E. DUTILH[c], IVANA MAŠLAŇOVÁ[a,*]

[a] Masaryk University, Faculty of Science, Department of Experimental Biology, Kamenice 5, 625 00 Brno, Czech Republic, [b] University of Leuven, Microbial and Plant Genetics (CMPG), Kasteelpark Arenberg 20, 3001 Leuven, Belgium, [c] Friedrich Schiller University, Viral Ecology and Omics, Rosalind Franklin Straße 1, 07745 Jena, Germany maslanova@sci.muni.cz

Bacteriophages are highly diverse and ubiquitous viruses that infect and replicate within bacteria. Virulent phages are increasingly used in phage therapy, an alternative approach for antibiotic treatment of infectious diseases caused by multidrug-resistant bacteria, and in biocontrol against plant and veterinary pathogens.

However, phage infection can be inhibited by phage defense systems that are frequently found on bacterial genomes.[1] While novel phage defense mechanisms are described regularly, little is known about the abundance of phage defense systems in various environmental samples. Of particular interest, some defense systems have also been found within viral genomes, where they can mediate inter-phage competition.

In this study, we conducted a large-scale investigation of the presence of phage defense systems in viral genome sequences from metagenomic samples. Phage defense systems were predicted in 257,066 viral genomes obtained from IMG/VR database[2]. The following exploratory analysis of the analysed viral genomes showed heterogenous distribution of phage defense systems across various biomes and link their presence with that of bacteria at various taxonomic ranks. As the use of phage for biocontrol and therapy has significantly increased in recent years, this investigation contributes to a better understanding of host-virus interactions and the ecological consequences of the use of phages on complex communities.

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P-29
DIRECT DETECTION METHODS FOR MONITORING OF THERAPEUTIC STAPHYLOCOCCAL BACTERIOPHAGES IN CLINICAL SAMPLES

LUCIE KUNTOVÁ[a], RADKA OBOŘILOVÁ[b], VILÉM SVOJANOVSKÝ[c], ZDENĚK FARKA[d], JAN PREISLER[c], ROMAN PANTŮČEK[a], IVANA MAŠLAŇOVÁ[a,*]

[a] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic, [b] Central European Institute of Technology, Masaryk University, Brno, Czech Republic, [c] Department of Chemistry, Masaryk University, Brno, Czech Republic, [d] Department of Biochemistry, Masaryk University, Brno, Czech Republic maslanova@mail.muni.cz

The most promising alternative for effectively treating infections caused by *Staphylococcus aureus* is the application of therapeutic bacteriophages of the genus Kayvirus. Although the genomic properties of phages are well-studied, new findings are emerging on the pharmacokinetic profiling of phage drugs. The significant factors relevant to the therapeutic use of phages include i) optimal dosing, ii) duration of exposure to phages, iii) absence of phage genes for toxins and virulence factors, and iv) selection of phages suitable for the infectious agent[1]. Currently, there is a lack of methods for capturing and monitoring phages in clinical material.

Our study aims to develop a technique for a sufficiently sensitive method for the detection and quantification of phage particles in different types of clinical material during phage therapy and clinical trials.

The double-layer agar method was used for the stability assay of phage therapeutics in clinical material. Direct detection of virions was performed by qPCR.

The lowest stability of therapeutic phages was observed in serum, where after seven days, there was a decrease by one order of magnitude of PFU/mL. Direct detection and quantification by qPCR was optimized with a detection limit 10^2 to 10^3 PFU/mL in whole blood sample.

Our study also included a stability study, where it is evident that bacteriophages survive long-term in blood samples, but their amount in serum decreases significantly.

The availability of effective diagnostic techniques for detecting bacteriophages in clinical samples is a critical factor for introducing phage therapy into broader medical practice. The most appropriate technique is the direct quantification of phages by qPCR.

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L-15
DECIPHERING THE EARLY INNATE IMMUNE RESPONSE TO BORDETELLA PERTUSSIS INFECTION

LUDMILA BRÁZDILOVÁ[a,b], JANA HOLUBOVÁ[a], ONDŘEJ STANĚK[a], LADISLAV BUMBA[a], VERONIKA NIEDERLOVÁ[b,c], ALEŠ NEUWIRTH[c], JURAJ MICHALÍK[c], ONDŘEJ ŠTĚPÁNEK[c], PETER ŠEBO[a,*]

[a] Institute of Microbiology of the Czech Academy of Sciences, Czech Republic, [b] Faculty of Science, Charles University, Czech Republic, [c] Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic

Bordetella pertussis, a strictly human pathogen, elicits a highly contagious respiratory disease known as pertussis, or whooping cough. Current mouse models enabled identification of many bacterial virulence factors and development of pertussis vaccines, but mechanisms underlying the process of *B. pertussis* transmission during the catarrhal phase of pertussis disease remain largely unexplored due to lack of a convenient animal model. Recently, we have used immunodeficient MyD88 knock-out mice to achieve a human-like high level of nasal mucosa infection that triggers rhinitis and catarrhal shedding of bacteria from mouse nasal cavity, yielding transmission of the infection onto co-housed adult animals.

Here, we characterized the early innate immune response of the conventional C57BL/6 mice, compared to the MyD88KO mice, following intranasal challenge with *B. pertussis*. Flow cytometry analysis of cells from nasal tissue shows how multiple immune cell populations infiltrate the nasal mucosa upon infection. Single-cell RNA sequencing revealed that nasal mucosa response of conventional C57BL/6 mice infected with B. pertussis is characterized by the expansion of a highly activated neutrophil subset that is characterized by an interferon-stimulated gene signature and that infection leads to an increased expression of genes encoding antimicrobial peptides and chemoattractant molecules, as confirmed by qPCR analysis.

These results open the way for a detailed understanding of *B. pertussis* clearance from nasal mucosa of the host and highlight the differences in early immune responses between conventional and immunocompromised animals.

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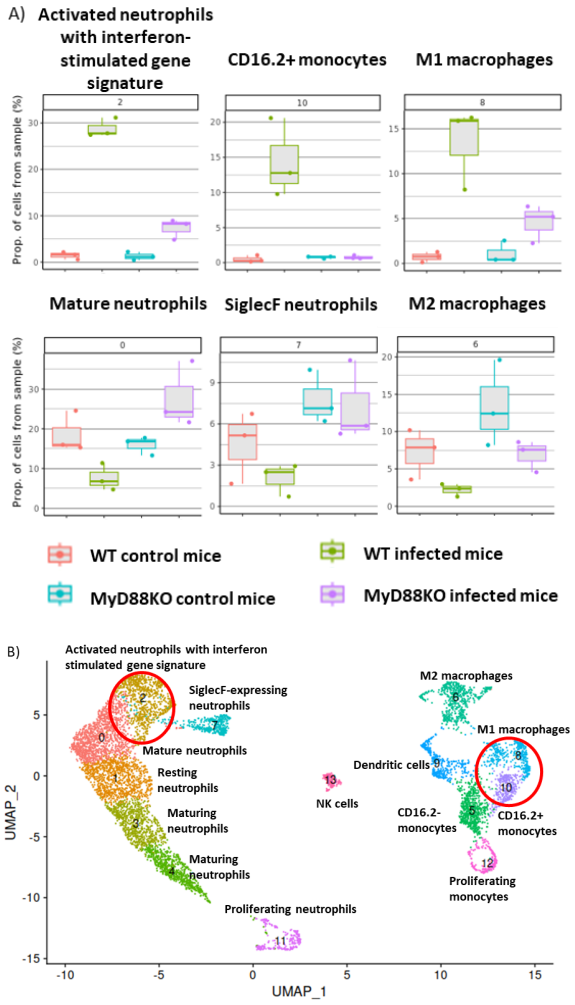

Fig. 1. **Relative abundance of CD11b$^+$ cell subsets in the nasal cavity of mice.** Animals were intranasally infected by 10^7CFU in a 5 µl volume. Mice were anesthetized, bled to reduce the content of circulating immune cells in the periphery and sacrificed. Nasal cavities were isolated and digested by Collagenase D and DNAse I. Cells were stained by antiCD11b antibody and Calcein green and positive cells were sorted. Libraries were prepared by Chromium Next GEM Single Cell 5′ Kit v2 and sequenced. Data were further analyzed in R version 4.2.1, Seurat version 4.1., first annotated using SingleR package (version 1.10.0) and ImmGen and further annotated manually.
Cell subpopulations expanded in infected WT mice are highlighted among all subpopulations.

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L-16
A NEW ROLE OF SIGMA FACTORS IN REGULATION OF BACTERIAL TRANSCRIPTION

JIŘÍ POSPÍŠIL[a]*, MAREK SCHWARZ[a], ALICE ZIKOVÁ[a], DRAGANA VÍTOVSKÁ[a], MILUŠE HRADILOVÁ[b], MICHAL KOLÁŘ[b], ALENA KŘENKOVÁ[c], MARTIN HUBÁLEK[c], LIBOR KRÁSNÝ[a]*, JIŘÍ VOHRADSKÝ[a]*

[a] Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic, [b] Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic, [c] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nam. 542/2, 160 00 Prague 6, Czech Republic
jiri.pospisil@biomed.cas.cz, krasny@biomed.cas.cz

Bacterial gene expression is controlled by sigma (σ) factors, that bind to RNA polymerase (RNAP), recognize specific promoter sequences in DNA, and mediate transcription initiation. Bacteria always contain one primary or so called "housekeeping" σ factor. In the gram-positive antibiotic producing bacterium, *Streptomyces coelicolor*, 65 genes encoding different σ factors are found. HrdB is the main sigma factor in *S. coelicolor* and is responsible for expression of essential genes. On the other hand, genes that are required in different stresses or environmental conditions are regulated by alternative σ factors. Alternative σ factors are typically about half the size (~30 kDa) of HrdB and lack some of its domains. These factors recognize promoter consensus sequences different from HrdB. They are responsible for transcription of gene subsets with specific functions[1,2].

The extracytoplasmic function (ECF) σ factors are a subclass of alternative σ factors that control genes that provide bacteria with mechanisms that allow them to respond to extracellular threats, such as antimicrobials. In *S. coelicolor*, σ^E is an intensively studied ECF σ factor. It was reported that σ^E is important for resistance against cell envelope damaging agents such as vancomycin, lysozyme and other muramidases [47]. Another ECF σ factor of *S. coelicolor*, σ^R, also plays a critical role in resistance against translation-inhibiting antibiotics such as erythromycin, lincomycin or tetracycline. Consistently in *Mycobacterium tuberculosis*, *S. coelicolor* σ^R homologues (Mtb-σ^E and Mtb-σ^H) are induced upon erythromycin or tetracycline treatment[3–5].

ECF σ factors are mostly regulated by anti-σ factors and in some cases also by anti-anti-σ factors. Anti-σ factors, mostly localized in the cytoplasmatic membrane, bind to their respective σ factor in the absence of extracellular stress stimuli. The σ/anti-σ complex is highly stable and incompatible with RNAP binding. Environmental stimuli lead to conformational changes of anti-σ factor or its proteolysis, resulting in liberation of σ factor and transcription of its regulon (subset of genes regulated by one σ factor)[3].

Here, using a panel of molecular biology methods and computational modeling of transcriptomic data, we analyzed the activity and chromosomal binding of σ^E from *Streptomyces coelicolor* and identified a new regulatory role of this s factor in gene expression. The mechanism will be presented and discussed.

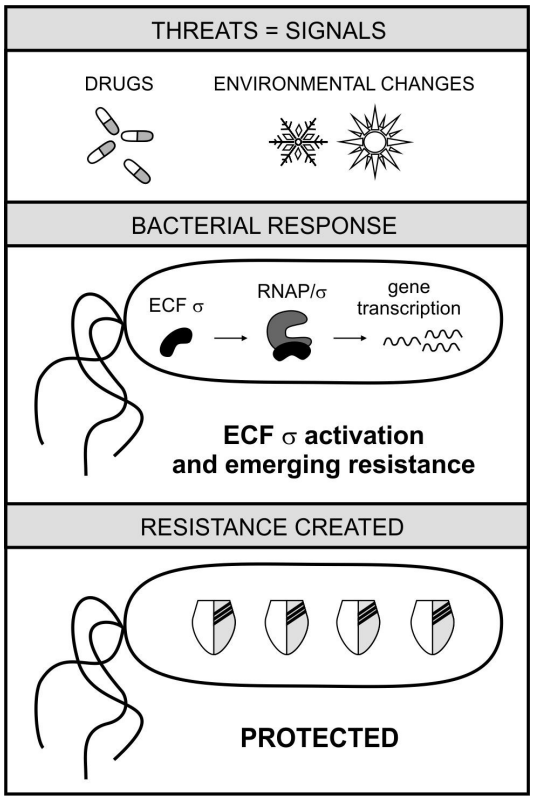

Fig. 1. **Bacterial extracellular stress response.** Upper panel – stressors (antibiotics, changing environmental conditions – cold or heat shock). Middle panel – Activation of ECF s factors upon stress. Binding of s to RNAP and initiation of resistance gene expression. Bottom panel – The bacterium is protected against the cause of stress.

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L-17
ABCF ATPASES IN ANTIBIOTIC RESISTANCE AND REGULATION OF BACTERIAL TRANSLATION

MARKÉTA KOBĚRSKÁ, DURGA MAHOR, FANNY DEMAY, MICHAELA NOVOTNÁ, PAMELA OMENA PETRAVICIUS, ZDENĚK KAMENÍK, JIŘÍ JANATA, GABRIELA BALÍKOVÁ NOVOTNÁ*

Institute of Microbiology of the CAS; BIOCEV, Průmyslová 595, Vestec, Czech Republic
gnovotna@biomed.cas.cz

Antibiotic resistance is a serious public health problem, complicating the treatment of infectious diseases and reducing the effectiveness of therapeutic interventions. To address this threat, efforts are underway to develop new antibiotics that are effective against resistant strains. However, the efficacy of new agents may be compromised by emerging or insufficiently explored resistance mechanisms.

The ATP-binding cassette transporters (ABC) of the F family (ABCF) form a group of poorly studied cytosolic proteins that interact with the ribosome. A substantial fraction of these proteins confer resistance to antibiotics that bind to the large subunit of the bacterial ribosome[1]. These proteins are called antibiotic resistance elements (ARE). Their expression is triggered by the presence of antibiotics bound to the large subunit of the ribosome[2–4], and they protect the ribosome by displacing the bound antibiotics[5]. The antibiotic displacement catalyzed by the ARE ABCF proteins leads not only to antibiotic resistance, but it can also regulate gene expression. This ability we have demonstrated in variants of the resistance protein VgaA in *Staphylococcus aureus*, which fine-tunes its own expression depending on whether it confers resistance to a particular antibiotic from the group of lincosamides, streptogramins A, and pleuromutilins (LSaP)[2]. However, our main discovery is the antibiotic signaling function of the ARE5 ABCF protein, LmrC, from the soil bacterium *Streptomyces lincolnensis*. LmrC is encoded within the biosynthetic gene cluster for lincomycin (BGC). It responds to the presence of lincosamides by synchronizing lincomycin production by activating transcription of the transcriptional regulator gene *lmbU*[4].

The ABCF family proteins comprise at least 10 taxon-specific subfamilies that are predominantly found in Firmicutes (ARE1-ARE3, ARE6-ARE8) and Actinobacteria (ARE4-5, AAF1 and AAF4). These subfamilies differ in the spectrum of antibiotics to which they respond. Many other taxon-specific proteins encoded in nearly half of all known bacterial genomes remain largely unexplored[1]. Nevertheless, it is likely that these subfamilies also belong to the ARE protein groups.

Our new findings show that within a single ABCF protein subfamily and the same microorganism, highly specialized resistance-associated proteins coexist with signaling proteins that fine-tune the response to antibiotic stress. These results suggest that ABCF proteins play a central role in innate antibiotic resistance of many bacteria, including important pathogens, and that these signaling proteins may evolve into specialized resistance determinants that could influence the efficacy of newly developed antibiotics. A comprehensive understanding of the mechanisms and resistance specialization of these proteins could pave the way for new strategies in the fight against resistant microorganisms.

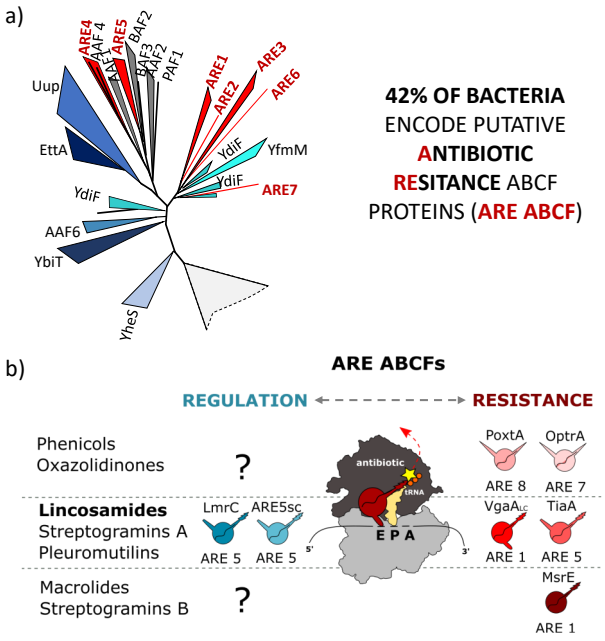

Fig. 1. **(a) Phylogenetically diverse and largely undescribed ABCF-ATPases include proteins that confer resistance to antibiotics targeting the large ribosomal subunit (ARE). (b) The mechanism of resistance involves displacement of the antibiotic from the ribosome.** This mechanism probably originally served to transfer the antibiotic signal to gene expression, which then evolved to protect the ribosome from the high concentrations of antibiotics.

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P-30
PREPARATION OF *BORDETELLA PERTUSSIS* MINICELLS FOR VISUALIZATION OF CELL SURFACE STRUCTURES BY CRYO-ELECTRON MICROSCOPY

PETRA KAŠPAROVÁ[a]*, LADISLAV BUMBA[a], MONIKA ČÍŽKOVÁ[a], JANA KAMANOVÁ[b], PETER ŠEBO[a]

[a] Laboratory of Molecular Biology of Bacterial Pathogens, Institute of Microbiology of the CAS v.v.i., Vídeňská 1083, Prague 4 – Krč, 142 20B, [b]Laboratory of Infection Biology, Institute of Microbiology of the CAS v.v.i., Vídeňská 1083, Prague 4 – Krč, 142 20, Czech Republic
petra.kasparova@biomed.cas.cz

Minicells are nano-sized, non-living, non-dividing particles resembling small cell-like membrane vesicles. They are formed by abnormal defective cell division due to deletion in the Min system (*minC*, *minD* and *minE*) associated with cell division and localization of mid-cell site for septum formation[1]. Another approach to minicell creation could be overexpression of *ftsZ* and overformation of diving septa without proper placement thus creating asymmetric cell division[2]. Minicells are a useful tool for visualization using advance miscroscopy due to their stability, inactivity, and easier handling for microscopic sample prepatation[3]. In addition, due to lack of chromosomal DNA, minicells do not possess pathogenic determinants, which is beneficial when working with otherwise pathogenic microorganisms, such as *Bordetella pertussis* – the causative agent of whooping cough[4].

In the present work, we created a strain of *Bordetella pertussis* Tohama I with deletion of *minD* and a point substitution (A126V) in the MreB protein, which lead to stable production of round minicells of about ~300 nm in diameter. The multi-step separation from parental "non-minicell" cells consisted of high speed and gradient centrifugation with 5–20% OptiPrep®. Separated minicells were plunge-freezed in liquid ethene and stored in liquid nitrogen for cryo-EM visualization.

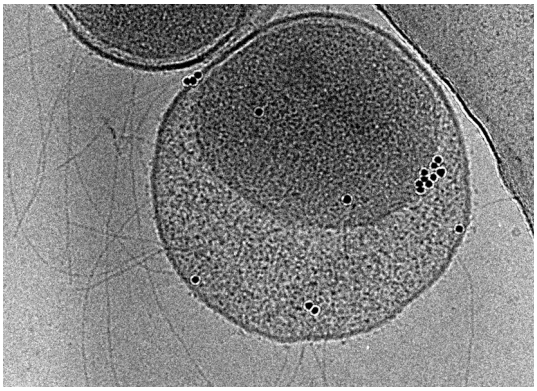

Fig. 1. ***Bordetella pertussis* Tohama I MreB A126V Δ*minD* minicell with visible fimbriae attached to the surface** (cryo-EM 80000×, Cu 200 mesh R2.1 grid). The black dots are golden nanoparticles used for tomography.

We were able to visualized *Bordetella* virulence factors like fimbriae and possibly FHA (filamentous hemagglutinin) still linked to cell surface. For further elucidation, deletion mutants of fimbriae (Δfim) and/or fhaB (gene for FHA, ΔfhaB) and/or sphB1 (serine protease partly responsible for FHA maturation) were prepared. It was proven to be a great tool for visualization and will be rapidly used also for other proteins.

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P-31
ROBOLECTOR XL® – FUTURE OF OPTIMIZATION AND AUTOMATIZATION OF MICROFERMENTATIVE PROCESSES FOR ACCELERATED SCALE-UP AND STREAMLINED RESEARCH APPLICATIONS

PETRA KAŠPAROVÁ*, GABRIELA BALÍKOVÁ-NOVOTNÁ, PETER ŠEBO

Institute of Microbiology of the CAS v.v.i., Vídeňská 1083, Prague 4 – Krč, 142 20, Czech Republic
petra.kasparova@biomed.cas.cz

Optimization and scale-up of a bioprocess is an important step on the way from research lab scale to industrial practice and it can be very time-consuming and expensive. It often requires returning to smaller scale to improve important variables while maintaining some of them constant[1].

Biolector (Robolector when merged with robotic instrument assisting measurement and preparation of media/solutions) is an elaborate robotic microcultivation device with continuous real-time sampling and/or supplementation of the cultures and with advanced optical sensor-based monitoring of biomass, pH, DO_2, monitoring of atmosphere above plate or fluorescence, which can be performed in parallel for 36–48 samples at 5 to 50 °C (ref.[2]). In addition, microfluidic plates can be used for in-process nutrient/ inductor feeding and pH control, mimicking a bioreactor process. Numerous studies confirmed great reproducibility of Biolector-based results in greater volumes[3–5].

Biolector XL is suitable for bacterial and yeast cultivations and can be used also for anaerobic processes or even cultivation of filamentous microorganisms, thus enabling highly efficient metabolite sampling, activity testing of compounds and process development[6,7]. The instrument will be purchased within NIVB grant (as listed below) to accomplish cultivations with purpose of high reproducibility and need for proper growth parameters monitoring or cultivations of demanding microorganisms such as *Streptomyces* spp., *Clostridium* spp., *Mycobacterium* spp. and *Bordetella* spp. by labs of Institute of Microbiology CAS. Address collaboration requests to the authors.

Acknowledgement
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P-32
NEUROTOXIC ACTIVITY OF *BORDETELLA* DERMONECROTIC TOXIN AT SUB-PICOMOLAR CONCENTRATIONS

ONDŘEJ STANĚK[a]*, IRENA LINHARTOVA[a], JANA HOLUBOVÁ[a], ZUZANA NICHTOVA[b], PETR MACEK[b], ATTILA JUHASZ[b], MILOSLAV KORINEK[c], TEREZA SMEJKALOVA[c], PETER ŠEBO[a]*

[a] Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, Prague, 142 20, Czech Republic, [b] Biocev – Institute of Molecular Genetics of the Czech Academy of Sciences, Průmyslová 595, Vestec, 252 50, Czech Republic, [c] Institute of Physiology of the Czech Academy of Sciences, Vídeňská 1083, Prague, 142 20, Czech Republic
stanek@biomed.cas.cz

Pathogenic *Bordetella* bacteria release upon lysis a neurotropic dermonecrotic toxin (DNT) that is endocytosed into certain animal cells and permanently activates the Rho family GTPases by polyamination or deamidation of the glutamine residues in their switch II regions (e.g. Gln63 of RhoA). DNT was found to enable high level colonization of the nasal cavity of pigs by B. bronchiseptica and the capacity of DNT to inhibit differentiation of nasal turbinate bone osteoblasts accounts for atrophic rhinitis in infected pigs. However, it remains unknown whether DNT plays any role in virulence of the human pathogen B. pertussis and in pathogenesis of the whooping cough disease.

We report a procedure for purification of large amounts of LPS-free recombinant DNT that exhibits a high biological activity on cells expressing DNT receptors, the Cav3.1 and Cav3.2 low voltage T-type calcium channels. These channels are highly expressed on neuronal cells – astrocytes and neurons. We show that low doses of DNT (≈fM) affect the function of primary rat neurons sub-cultured *in vitro*. The toxin destroys the long astrocyte protrusions, which subsequently stop feeding the neurons, leading to death of neurons and loos of their action potential transmission capacity over time. Intraperitoneal or intravenous administration of as little as 3 ng (18 fmol) of DNT causes weight loss and neurological symptoms leading to death of mice. Progress in deciphering of the molecular basis of toxin action at the extremely low concentrations and the mapping of its cell-binding domains is reported.

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P-33
HEID PROTEINS: PROTECTING BACTERIAL RNA POLYMERASE AGAINST THE ANTIBIOTIC RIFAMPICIN

NABAJYOTI BORAH[a]*, HANA ŠANDEROVÁ[a], PETRA SUDZINOVÁ[a], TOMÁŠ KOVAL[b], JAN DOHNÁLEK[b], MARTIN HUBÁLEK[c], TOMÁŠ KOUBA[c], LIBOR KRÁSNÝ[a]*

[a] Institute of Microbiology of the Czech Academhy of Sciences, Vídeňská 1083, 142 20 Prague, Czech Republic, [b]Institute of Biotechnology of the Czech Academy of Sciences, Centre BIOCEV, 252 50 Vestec, Czech Republic, [c] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences v. v. i., Flemingovo nám. 2, 166 10 Prague 6, Czech Republic
nabajyoti.borah@biomed.cas.cz, krasny@biomed.cas.cz

Rifampicin is an ansamycin antibiotic used to treat several types of bacterial infections[1]. Rifampicin binds to the DNA/RNA channel of bacterial RNA polymerase (RNAP), interacting with its b subunit. Rifampicin (and its variants) blocks extension of RNA beyond 2–3 nucleotides during transcripition initiation, thereby shutting off this key cellular process[2]. Recently, new proteins and mechanisms are being identified that contribute to the ability of various bacteria to resist this antibiotic. One such a protein is HelD (alternative name HelR)[3]. HelD is an ATP/GTPase that binds to RNAP, penetrating both its primary and secondary channels[4,5]. By doing so, it interacts with the active site of the enzyme and affects the conformation of the rifampicin bindig pocket. This not only decreases the ability of rifampicin to bind to RNAP but it also causes dissociation of bound rifampicin. Under rifampicin nonsaturating condition, this mechanism, called target protection, regenerates RNAP and allows the bacterium to function in the presence of this antibiotic. Here, we will present and discuss results of biochemical and structural studies focusing on rifampicin resistance by the HelD proteins and the role and mechanistic functioning of HelD during the transcriptional cycle.

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P-34
WHERE SUGARS MEET ANTIBIOTICS: EFFECT OF THE PHOSPHOTRANSFERASE SYSTEM ON ANTIBIOTIC RESISTANCE

JANA WIEDERMANNOVÁ*, PETRA SUDZINOVÁ, TAMARA BALGOVÁ, LIBOR KRÁSNÝ*

Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague, Czech Republic
jana.wiedermannova@biomed.cas.cz, krasny@biomed.cas.cz

The phosphoenolpyruvate phosphotransferase system (PTS) is a pivotal metabolic pathway ubiquitous in eubacteria and absent from eukaryotes, which makes it an ideal candidate for targeting antimicrobial agents. It consists of a cascade of proteins being subsequently phosphorylated to catalyze the concomitant uptake and phosphorylation of various sugars.

PTS proteins and their structural homologs were repeatedly implicated in sensitivity or resistance to different bactericidal compounds[1–4]. An active PTS is a prerequisite for the activity of some antimicrobial agents while the phosphate transfer could induce a deleterious gain-of-function. PTS proteins can also transport some agents or even phage DNA into the cell[5–7]. Therefore, overexpression of some components of the PTS renders cells more sensitive to some anti-microbials[2]. Additionally, PTS was suggested to provide the means to control outbreaks of *Enterococcus faecium*, the leading cause of multi-drug resistant enterococcal infections in hospitals[3].

Although PTS proteins impact the cell response to a number of antimicrobial agents, a detailed systematic study addressing their contribution to antibiotic resistance is missing.

This project aims to elucidate the unknown functions of PTS proteins in antibiotic resistance and define their contribution to the natural antibiotic resistance of bacteria.

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P-35
WHY IS THE N-TERMINAL DOMAIN OF BACTERIAL σ FACTORS ATTRACTIVE?

HANA ŠANDEROVÁ[a]*, DÁVID TUŽINČIN[b,c], PAVEL KADEŘÁVEK[b,c], LUKÁŽ ŽÍDEK[b,c], LIBOR KRÁSNÝ[a]*

[a] Laboratory of Microbial Genetics and Gene Expression, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic, [b] National Centre for Biomolecular Research (NCBR), Faculty of Science, Masaryk University, Brno, Czech Republic, [c] Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic
sanderova@biomed.cas.cz, krasny@biomed.cas.cz

σ factors are essential players in bacterial transcription as they allow the RNA polymerase (RNAP) holoenzyme to recognize promotor sequences and initiate transcription. The number of different σ factors varies between species. σ factors are divided according to their structure into two main groups σ^{70} (further subdivided into subgroups 1–4) and σ^{54}. The subgroup 1 σ^{70}-like factors contains four domains: domain 1.1, domain 2 (regions 1.2–2.4), domain 3 (regions 3.0–3.2), and domain 4 (regions 4.1–4.2)[1]. Domain 1.1 has a specific, self-regulatory function as it inhibits the binding of σ factor itself to DNA in the absence of RNAP. Furthermore, when σ is bound to RNAP, domain 1.1 occupies the DNA binding channel and must be displaced from this area to allow entry of promoter DNA that subsequetnly forms the trasncription bubble [2,3].

Here, we have performed a set of experiments with full-lenght σ and σ lacking domain 1.1, revealing an important role of this domain in transcription at elevated temperatures[4]. Additionally, the differences in domain 1.1 structures in different bacterial species were studied, revealing at least two principally distinct architectures of this domain. The results will be presented and discussed.

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P-36
STRUCTURE-FUNCTION STUDY OF CARRIER PROTEIN-DEPENDENT AMIDE BOND FORMING PROTEIN IN THE BIOSYNTHESIS OF LINCOSAMIDE ANTIBIOTICS

TAKAHIRO MORI[a], STANISLAV KADLCIK[a,b], SHUANG LYU[a], ZDENEK KAMENIK[b], KOSUKE SAKURADA[a], ANINDA MAZUMDAR[b], HUIBIN WANG[a], JIRI JANATA[b], IKURO ABE[a]

[a] Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan, [b] Institute of Microbiology, Czech Academy of Sciences, Videnska 1083, 142 20 Praha 4, Czech Republic
kadlcik@biomed.cas.cz

Lincosamides, including natural compounds lincomycin and celesticetin, are antibacterial compounds effective against Gram-positive bacteria. In biosynthesis of lincomycin and celesticetin, the condensation enzyme LmbD and CcbD, respectively, generates the lincosamide core structure by forming an amide bond between the carrier protein (CP)-tethered amino acid (proline or proline derivative) and ergothioneine-conjugated thiooctose. Although the function of CcbD has been investigated, structure and catalytic mechanism remained unclear.

We revealed that CcbD exhibits promiscuous substrate specificity. Furthermore, structural analyses indicated that CcbD possesses an unusual overall fold, while the N-terminal region shows weak similarity to cysteine proteases. Like cysteine proteases, CcbD utilizes the Cys-His-Glu catalytic triad to form amide bond in a CP-dependent manner, which is significantly different from other known amide bond-forming enzymes. The structures of the CcbD/thiooctose complex and the cross-linked CcbD/CcbZ-CP complex, as well as structure-based site-directed mutagenesis, revealed the structural details of the CP-dependent amide bond formation reaction[1].

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P-37
DECIPHERING THE SIGNALING FUNCTION OF ANTIBIOTIC RESPONSIVE ABCF ATPase LmrC

DURGA MAHOR, MARKÉTA KOBĚRSKÁ, JAKUB MUSIL, MICHAELA NOVOTNÁ, ZDENĚK KAMENÍK, JIŘÍ JANATA, GABRIELA BALÍKOVÁ NOVOTNÁ*

Institute of Microbiology of the CAS; BIOCEV, Průmyslová 595, Vestec, Czech Republic
gnovotna@biomed.cas.cz

Antibiotics are essential for the treatment, control, and prevention of infections. However, the use of antibiotics is limited by increasing multidrug resistance. ABC Family F ATPases (ABCFs) are ribosome-binding proteins that regulate translation or confer antibiotic resistance to all clinically significant groups of antibiotics that bind the large ribosomal subunit (50S) *via* a ribosome protection mechanism[1]. LmrC is a member of antibiotic resistance subfamily 5 (ARE5) from the lincomycin biosynthesis gene cluster. Recently, we reported that ARE-ABCFs LmrC has an antibiotic-responsive transduction function that activates transcription of the transcriptional regulator[2]. However, the mechanisms by which ribosomally bound LmrC transduces the signal to transcription are not known.

Our goal is to unravel the mechanism of antibiotic signalling mediated by LmrC. We are using biochemical, structural, and genetic approaches, including single-molecule cryo-electron microscopy, ribosome toeprinting[3], *in vitro* coupled transcription and translation assays[4], and *in vivo* reporter assays, to test the hypothesis that LmrC regulates transcription *via* the 5′untranslated region of messenger RNA. Preliminary results supporting our hypothesis are presented and discussed.

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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P-38
CLUSEEK:
BIOINFORMATICS TOOL TO IDENTIFY AND ANALYZE GENE CLUSTERS

ONDREJ HREBICEK*, STANISLAV KADLCIK, JIRI JANATA, JANA KAMANOVA, ZDENEK KAMENIK*

Institute of Microbiology, Czech Academy of Sciences, Videnska 1083, 142 20 Praha 4, Czech Republic
kamenik@biomed.cas.cz

Gene clusters, representing functional and structural units within bacterial genomes, play a pivotal role eg. in the biosynthesis of specialized metabolites and the assembly of bacterial virulence factors. The surge in publicly available sequencing data underscores the need for advanced bioinformatics tools capable of efficiently identifying, analyzing, and visualizing these gene clusters.

In response to this demand, we will introduce CluSeek, a Python-based tool designed for the exploration of co-localized genes and their genomic neighborhoods within bacterial genomes. Unlike existing tools such as antiSMASH, PRISM, or SecReT6, CluSeek operates on a distinctive principle. It does not rely on predefined libraries of known gene clusters; instead, it systematically searches all GenBank data, retrieving gene clusters containing user-specified genes, irrespective of the encoded phenotype.

To showcase CluSeek's capabilities, we will present at the conference two illustrative case studies: (i) genome mining of specialized metabolites featuring a 4-alkyl-L-proline motif and (ii) the analysis of type III secretion systems.

CluSeek offers an open-source solution, accessible at https://cluseek.com, with a user-friendly graphical interface.

Acknowledgement
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P-39

THE REGULATION OF ABC-F RESISTANCE GENES EXPRESSION GUIDES NEW INSIGHTS INTO THE MODE OF LINCOSAMIDES, STREPTOGRAMINS A AND PLEUROMUTILINS ANTIBIOTICS ACTION

MICHAELA NOVOTNÁ[a], MARKÉTA KOBĚRSKÁ[a], FANNY BOISSIER[b], C. AXEL INNIS[b], GABRIELA BALÍKOVÁ NOVOTNÁ[a]*

[a] Institute of Microbiology of the CAS, BIOCEV, Vestec, Czech Republic, [b] INSERM U1212 (ARNA), Institut Européen de Chimie et Biologie, Université de Bordeaux, Pessac, France
gnovotna@biomed.cas.cz

Bacterial Antibiotic REsistance ABC ATPases F (ARE ABC-F) are cytosolic proteins conferring resistance to 50S ribosomal subunit binding antibiotics[1,2]. Expression of all previously characterised ARE ABC-F genes that confer resistance to lincosamides, streptogramins A, and pleuromutilins (LS_AP), antibiotics that target the peptidyl transferase centre, is regulated by ribosome-mediated attenuation. This regulatory mechanism requires amino acid sequence-dependent translational arrest induced by the respective antibiotic at a short upstream open reading frame encoded by the attenuator. This means that knowledge of the precise mechanism of antibiotic translational inhibition is necessary to understand the induction processes. However, in the case of LS_AP antibiotics, a detailed mechanism of action is lacking, leaving LS_AP-driven gene regulation unclear.

Using *in vivo* fluorescence assays and *in vitro* ribosome profiling techniques such as toeprinting and iTP-seq[3], we uncover the mechanism of how LSAP antibiotics inhibit protein synthesis and the impact this has on the regulation of resistance genes. In addition, we demonstrate that our newly developed hybrid lincosamide derivative Cl-ODCELIN[4] acts in a similar manner, although it exhibits superior antimicrobial activity against Gram-positive pathogens. Using single-particle cryo-electron microscopy, we demonstrated that this improved activity is likely due to the compound's enhanced binding capabilities to the ribosome.

Acknowledgement
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P-40

DISTINCT ROLES OF ANTIBIOTIC RESPONSIVE ABC-F ATPASES IN STREPTOMYCES COELICOLOR: ANTIBIOTIC RESISTANCE AND STRESS RESPONSE

MARKÉTA KOBĚRSKÁ, LUDMILA VESELÁ, PAMELA OMENA PETRAVICIUS, DURGA MAHOR, MICHAELA NOVOTNÁ, ANINDA MAZUMDAR, ZDENĚK KAMENÍK, JIŘÍ JANATA, GABRIELA BALÍKOVÁ NOVOTNÁ*

Institute of Microbiology of the CAS; BIOCEV, Průmyslová 595, Vestec, Czech Republic
gnovotna@biomed.cas.cz

The bacterial ribosome is one of the main targets on which many small bioactive molecules act. We study the ubiquitous family of antibiotic-responsive (ARE) ABC-F ATPases, which catalyze the displacement of these molecules from binding sites on the large ribosomal subunit. The action of displacement may confer resistance to ribosome-bound antibiotics but also fine-tune gene expression to ensure bacterial survival under stress conditions depending on the specificity of the ARE ABC-F protein[1,2]. ARE ABC-F comprises seven groups that differ in both resistance phenotype and taxonomic origin. Groups ARE1-3, ARE6 and ARE7 include clinically relevant and characterized resistance proteins (e.g., VgaA, LsaA, MsrA or OptrA) that are found predominantly in Firmicutes, whereas proteins in groups ARE4 and ARE5 are found almost exclusively in Actinobacteria, including Streptomyces, the most prolific antibiotic producers.

Our functional analysis of two *Streptomyces coelicolor* ABC-F AREs from the ARE5 subfamily shows that protein ARE5sc has a regulatory function fine-tuning gene expression in response to lincosamide, streptogramin A, and pleuromutilin antibiotics, while TiaA has become a highly specialized resistance protein that allows *S. coelicolor* to grow in close proximity to pleuromutilin-producing basidiomycetes. Phylogenetic analysis suggests specialization of one of the two genome-encoded ARE5 proteins is common in Actinomycetes.

Assuming that ARE ABC-F genes are encoded in the genomes of many clinically important bacterial species, they constitute a reservoir of new potential resistance determinants. Understanding the molecular basis of the evolution from antibiotic-responsive functions to antibiotic resistance specialization may therefore be essential for preventing the emergence of new resistant strains.

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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CHARLES UNIVERSITY

L-18
STING AGONISTS ACTIVATE MULTIPLE REGULATED CELL DEATH MECHANISMS IN MONOCYTES

MARKETA PIMKOVA POLIDAROVA[a,b], IVAN HIRSCH[a,b], ANDREA BRAZDOVA[a,b], KLARA GRANTZ SASKOVA[a,b]

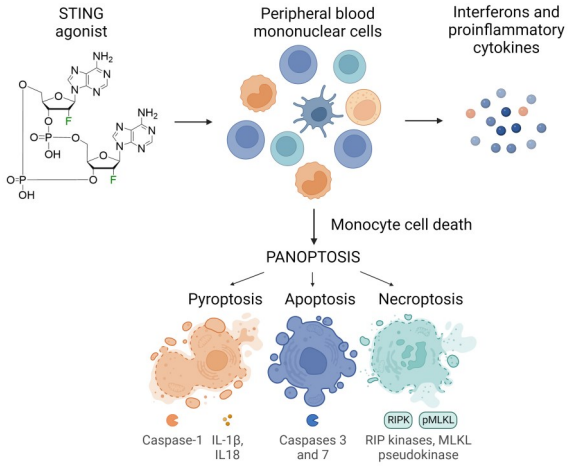
[a] Faculty of Science, Charles University, Albertov 6, 128 00 Praha, [b]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo namesti 2, 160 00 Praha, Czech Republic
marketa.polidarova@uochb.cas.cz

Stimulation of the innate immune responses using agonist of pattern recognition receptors is one of the investigated therapeutic approaches for treatment of chronic diseases, such as cancer or chronic infections. The cyclic-GMP-AMP synthase – stimulator of interferon genes (cGAS-STING) pathway is one potential targetable pathway, as it naturally recognizes double-stranded DNA in the cytoplasm, and in turn triggers secretion of interferons and other proinflammatory cytokines[1]. The secreted cytokines further mediate various innate immune processes, some of which also induce adaptive immune responses against viral infection or tumors[1,2]. Importantly, the cGAS-STING pathway can be activated also by small molecules, STING agonists[2,3].

However, the cGAS-STING pathway activation can also lead to cell death via multiple regulated cell death pathways[4]. Indeed, we discovered, that STING agonists induced cell death of monocytes within the population of peripheral blood mononuclear cells[3]. The phenotype of dying cells had apoptotic characteristics but we could not fully exclude the involvment of other regulated cell death pathways.

Therefore, we further investigate the mechanisms of STING agonist-indcued cell death of monocytes. We focus on the phenomenon called PANoptosis, a regulated cell death combining features of pyroptosis, apoptosis and/or necroptosis[5]. So far, we detected activated caspases 3 and 7, which characterize active apoptosis. We also demonstrated involvement of pyroptosis by showing activation of caspase 1 as well as secretion of interleukin 1β (IL1β) and IL18, which require processing by inflammasome. Eventhough the activation of necroptosis is yet to be determined, we claim that the STING agonist-induced cell death of monocytes involves at least two pathways (apoptosis and pyroptosis).

As monocytes are supposedly the main producers of proinflammatory cytokines induced by the STING agonists, rapid negative feedback may be needed to tightly regulate the accute inflammation in organism to prevent the potential life-threatening cytokine storm. Moreover, acute inflammation inhibits the subsequent adaptive immune processes. As such, the monocyte cell death via apoptosis and pyropotosis (possibly PANoptosis) could be a natural immunoregulatory mechanism inhibiting the primary proiflammatory cytokine secretion while being immunogenic for further activation of adaptive immune responses.



Scheme 1. **In peripheral blood mononuclear cells (PBMCs), the STING agonists not only trigger secretion of a broad portfolio of proinflammatory cytokines, but they also induce monocyte cell death with characteristics of multiple regulated cell death pathways.** (Created with BioRender.com)

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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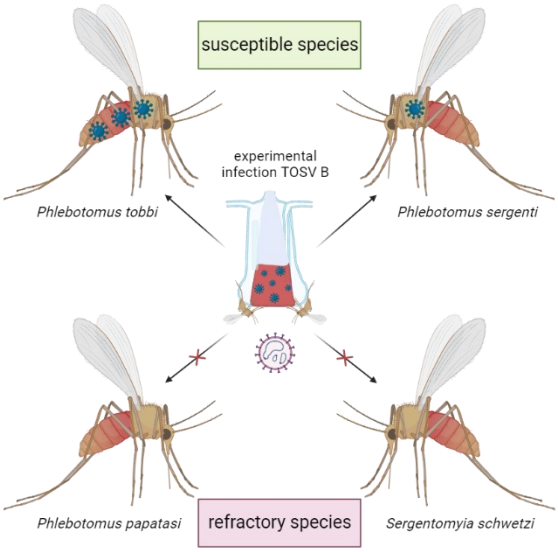
L-19
SAND FLIES AND TOSCANA VIRUS

MAGDALÉNA JANČÁŘOVÁ[a], NIKOLA POLANSKÁ[a], MAXIME RATINIER[b], FREDERICK ARNAUD[b], MARKÉTA STEJSKALOVÁ[a], ADRIEN THIESSON[b], MARKÉTA REHBERGEROVÁ[a], ALAIN KOHL[c], PETR VOLF[a]

[a] Charles University, Viničná 7, Prague 2, 128 00, Czech Republic, [b] IVPC UMR754, INRAE, Univ Lyon, Université Claude Bernard Lyon 1, EPHE, PSL University, F-69007 Lyon, France; [c] LSTM, Pembroke Place, Liverpool L3 5QA
volf@cesnet.cz



Phlebovirus toscanaense (Toscana virus, TOSV) from the *Phenuiviridae* family is an emerging but still neglected human pathogen. Infections vary from non-symptomatic forms through febrile illness to CNS disease, rarely death. The virus circulates in the Mediterranean area, where it is transmitted by sand flies (Diptera, Phlebotominae); however, there are consequently many knowledge gaps about its biology and its persistence in nature; no reservoir hosts of TOSV have been found (despite thorough examinations of various vertebrates), and vertical and sexual transmission between sand flies is not sufficiently effective to sustain the virus cycle in nature[1,2,3,4].

Based on the nucleotide sequences, TOSV is currently divided into three lineages A, B and C; for the last one no isolate and only a partial sequence has been obtained[1,5]. No differences have been observed in the nature of the host, the clinical picture, or disease severity associated with virus lineages, but they seem to a certain extent to differ in geographical distribution[2,6,7].

The only proven vectors are *Phlebotomus perniciosus* and *Phlebotomus perfiliewi*. However, repeated findings of human cases or anti-TOSV antibodies in humans and animals in regions outside of the range of distribution of these two vectors suggests the involvement of other species of sand flies[1]. We tested the vector competence of four sand fly species: *Phlebotomus tobbi, Phlebotomus sergenti, Phlebotomus papatasi and Sergentomyia schwetzi* to two TOSV strains belonging to TOSV lineages A and B.

The strain 1500590, representing TOSV lineage A, infected vertebrate cells *in vitro*, but failed to develop in all four sand fly species tested. The strain MRS20104319501, representing lineage B, developed in *P. tobbi*, with an infection and dissemination rate of 60% and 46%, respectively. Another species that appears to be sensitive to infection, although less so than the previous species is *P. sergenti* with a total infection rate of 5.2% but a high dissemination rate of 100%. It seems that *P. sergenti* possesses an efficient midgut barrier; nevertheless, when the virus overcomes it successfully, it disseminates and establishes vector infection. Interestingly, two tested sand fly species, *P. papatasi* and *S. schwetzi,* were resistant to TOSV. For further testing, a colony of *Sergentomyia minuta*, a common species in the Mediterranean region was established[8]. We possess a reverse genetic system which will allow us to focus our research on parts of the genome that play an important role in virus-vector interactions[9].

In addition, we studied antiviral immunity in sand flies using *Phlebotomus papatasi*-derived cell lines. Following TOSV infection, distinctive 21 nucleotide virus-derived small interfering RNAs were detected. Silencing the exo-siRNA effector Ago rendered the exo-siRNA pathway inactive. Thus, our data show that this immune pathway is active in sand flies as an antiviral response against TOSV[10].

Acknowledgement
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L-20
EXPLORING THE MOLECULAR MECHANISMS USED BY HUMAN BK POLYOMAVIRUS TO ACTIVATE IMMUNE RESPONSES IN "RESERVOIR CELLS"

KATEŘINA BRUŠTÍKOVÁ, BORIS RYABCHENKO, JITKA FORSTOVA, SANDRA HUÉRFANO*

Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 25250 Vestec, Czech Republic
huerfano@natur.cuni.cz

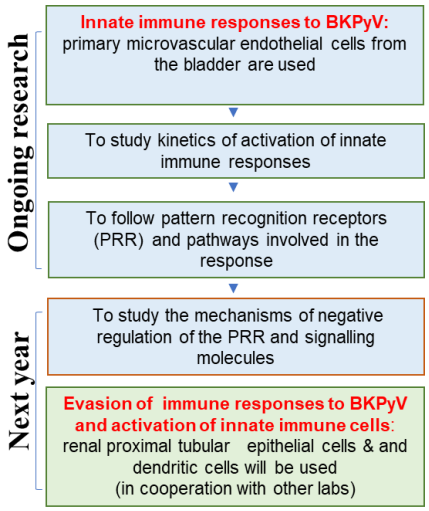
At present, the Polyomaviridae family contains 14 human polyomaviruses (PyVs), among the most important are Merkel cell polyomavirus (MCPyV), the etiological agent of 80% of Merkel cell carcinomas, JCPyV and BKPyV causing progressive multifocal leukoencephalopathy and nephropathy, respectively, in immunocompromised individuals. Primary infection by polyomaviruses occurs in early childhood and is usually asymptomatic.[1,2] After initial primary infection and dissemination, PVs persist in the organism by a not well-known mechanism. In fact, it has been suggested that innate immune responses in the reservoir cells may contribute to PyV persistence.[3,4] Although two cellular models have emerged recently to study the mechanism of BKPyV infection, there are still many gaps in the understanding of these models. One of the models is human microvascular endothelial cells (HMEVC) from the bladder (bd) or lung, which respond to BKPyV by producing interferon (IFN), and the other is renal proximal tubular epithelial cells (RPTEC), which do not respond immunologically to the virus. Cells that respond to BKPyV with production of IFN have been postulated as reservoirs of the BKPyV. In our group, a part of our research focuses on understanding the mechanisms of innate immune response activation and modulation in response to BKPyV infection.

Since our previous studies showed that mouse polyomavirus (MPyV) induces a moderate IFN response in host cells as a results of DNA sensing via cGAS-STING pathway[5], we hypothesized that: i) in the 'reservoir cells' (HMVECs bd) BKPyV activates cGAS-STING pathway to produce IFN responses that lead to lower levels in viral progeny, thereby contributing to viral persistency and that ii) cGAS-STING pathway is heavily regulated either by post-translational modifications of cGAS and/or STING and/or possibly by interactions between viral proteins and proteins of the innate immune system.

To follow our hypotheses, we first investigated the life cycle of BKPyV in primary HMVECs bd during 96 hours post infection (hpi). We have found that the viral early antigen LT can be detected after 12hpi, while the production of the late antigen VP1 and massive replication of viral DNA started between 24–36hpi. Low levels of virion release occur from 48hpi (whether or not these virions are new progeny needs to be determined) with a marked increase at 60hpi. Signs of cell toxicity are apparent at 72hpi. Additional experiments to better understand the BKPyV life cycle in HMVECs bd will be carried out.

Next, we had shown that HMVECs bd launch an IFN response at late times, around 72hpi. The response is characterized by upregulation of IFNB, ISG56, CXCL10, and CLCL20. Further, we followed the possible involvement of cGAS sensor by observing mutual colocalization of viral DNA (stained by FISH) and cGAS in the cytosol and found that at early times post-infection (24h), cGAS colocalized with spot pattern clusters of viral DNAs from the incoming virus. Later, at 62hpi, we also found cGAS colocalizing with viral DNA leaked from the nucleus into the cytosol. We suggest that the clustering of cGAS with incoming viral DNA corresponds to sorting the virus to autophagosomes. In fact, colocalization of viral particles and LC3B, the central protein in autophagy, was detected at 24hpi. Further studies will be carried out to understand the role of cGAS mediated autophagy in BKPyV infection. On the other hand, the colocalization of cGAS with viral DNA at 62hpi very likely results in the activation of cGAS. In agreement, activation of STING was detected at high levels at 62 and 72hpi. Ongoing experiments are being carried out to confirm the cGAS activation by determining the levels of cGAMP.

Finally, we have prepared cell lines expressing tagged STING and cGAS proteins, plasmids coding for the tagged proteins, and viral mutants to study the regulation of the IFN responses in RPTE and HMVECs bd. In the table below, a summary of our research is presented.



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L-21
CREATING A CELL LINE SUITABLE FOR INVESTIGATION INTO THE ADAR1 ROLE IN HEPATITIS C VIRUS REPLICATION

KRISTINA ROUČOVÁ[a], VÁCLAV VOPÁLENSKÝ[a], TOMÁŠ MAŠEK[a], EDGAR DEL LLANO[a,b], JAN PROVAZNÍK[c], JONATHAN J. M. LANDRY[c], NAYARA AZEVEDO[c], EDVARD EHLER[d], MARTIN KUBŮ[a], VLADIMÍR BENEŠ[c], MARTIN POSPÍŠEK[a]*

[a] *Laboratory of RNA Biochemistry, Dpt. Of Genetics and Microbiology, Faculty of Science, Charles University, Viničná 5, Prague, 128 00, Czech Republic,* [b] *Laboratory of Biochemistry and Molecular Biology of Germ Cells, Institute of Animal Physiology and Genetics, CAS, Rumburská 89, Liběchov, 277 21, Czech Republic,* [c] *GeneCore Facility, EMBL, Meyerhofstraße 1, Heidelberg, 69117, Germany* [d] *Dpt. of Biology and Environmental Studies, Faculty of Education, Charles University, Magdalény Rettigové 4, Prague, 116 39, Czech Republic*
martin.pospisek@natur.cuni.cz

Adenosine deaminases acting on RNA (ADAR) perform the adenosine to inosine (A-to-I) type of editing. Out of the three human ADAR proteins, ADAR1 is responsible for the majority of A-to-I editing of dsRNA outside the brain. By introducing I into the RNA sequence, thereby altering the base pairing in the region, or by its sheer dsRNA binding activity, ADAR1 can influence miRNA processing, alternative splicing, nuclear export, degradation or protection of RNA molecules (as reviewed in 1). On top of the variety of effects ADAR1 can have on a particular RNA, ADAR1 editing itself has been shown to be influenced heavily by the cell type. In recent years, studies on particular ADAR1 effects have relied mainly on RNA-seq experiments and knock-down cell line assays.

Not only cellular RNAs, but also viral RNAs can be targeted by ADAR1, where the effect of ADAR1 protein binding or editing can have a proviral or antiviral effect (as reviewed in 2). Identifying the ADAR1 effect on a particular virus faces the same difficulties as in the case of cellular RNAs – cell tropism and possible artifacts of the knock-down method used. Hepatitis C virus (HCV) exhibits a high level of tropism for hepatocytes and is almost exclusively studied in cell lines derived from hepatocellular carcinoma, Huh7.5 (3).

In order to avoid knock-down effects in the identification of ADAR1 effect in further virological experiments, we decided to use CRIPSR/Cas9 system to establish an Huh7.5 ADAR1 KO cell line. While the Huh7.5 ADAR1 KO cell line exhibits similar morphology as the parental Huh7.5 wt, we observed an increase of cell area of Huh7.5 ADAR1 KO. The ADAR1 KO cell line also exhibited increased sensitivity to IFN-α and IFN-β treatment at concentrations as low as 0.1 nM. The interferon treatment induced a rapid growth and translation arrest in the Huh7.5 ADAR1 KO cell line.

For further characterization of the Huh7.5 ADAR1 KO cell line, we performed RNA sequencing of poly(A) RNA from total RNA and polysomal profile fractions originating from the Huh7.5 wt and Huh7.5 ADAR1 KO cell lines. The poly(A) RNA sequencing data from total RNA was used for transcriptome analysis, which revealed hundreds of genes with altered transcript abundance. The poly(A) RNA sequencing data from separate polysome profile RNA fractions was used for a new method of translatome analysis. We found that both transcriptome and translatome of the Huh7.5 ADAR1 KO cells were significantly changed. Among the RNAs and processes that were significantly changed in the Huh7.5 ADAR1 KO cell line, there were the deregulations of snoRNA and Y RNA levels and negatively affected transcription by RNA polymerase III. Furthermore, we observed that polysomal fraction in Huh7.5 ADAR1 KO was enriched in mRNAs coding for proteins pivotal role in a wide range of biological processes such as RNA processing and RNA localization, whereas the unbound fraction was enriched mainly in mRNAs coding for translational factors and ribosomal proteins. This indicates that ADAR1 possibly plays a more relevant role in small RNA metabolism and ribosome biogenesis.

With the cell line characterized, we are now ready to move onto the virological experiments. We already performed pilot experiments with HCV infection of Huh7.5 wt and Huh7.5 ADAR1 KO aimed at the investigation into ADAR1 role in HCV replication in hepatocytes.

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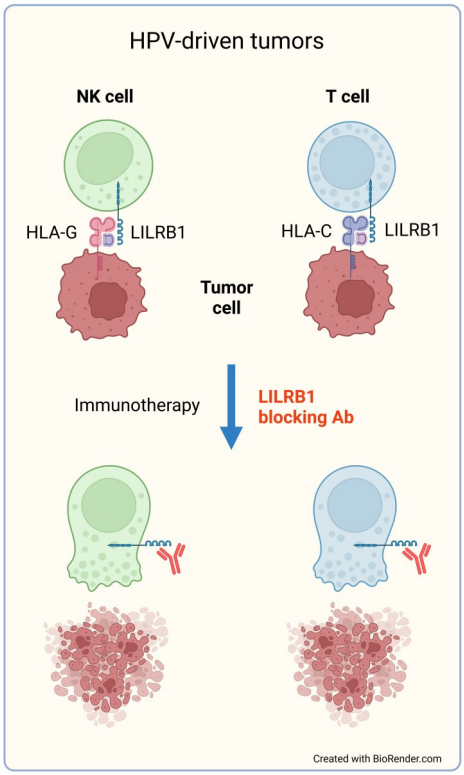
L-22
CAN THE TYPE OF ONCOGENIC TRANSFORMATION BY HUMAN PAPILLOMAVIRUSES INFLUENCE THE CHOICE OF CANCER IMMUNOTHERAPY?

MICHAL ŠMAHEL*, JAROSLAV NUNVÁŘ

Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 252 50 Vestec, Czech Republic
smahelm@natur.cuni.cz

High-risk human papillomaviruses can induce malignant transformation of cells resulting in anogenital cancers, particularly cervical cancer (CC), and a proportion of head and neck squamous cell carcinomas (HNSCC). The viral E6 and E7 oncoproteins are required for the induction and maintenance of transformation and the E5 protein contributes to tumorigenesis (1). The E6 and E7 expression is usually increased after the integration of viral DNA into the host genome which is associated with inactivation of the viral E2 gene (2). In HNSCC and CC cases driven by HPV infection, an alternative tumorigenesis pathway has been described with extrachromosomal persistence of the HPV genome and higher expression of the viral E2, E4, and E5 genes (3). In addition to affecting cellular genes associated with proliferation, survival, and differentiation of infected cells, the E2, E5, E6, and E7 proteins have been shown to modify the expression of immune-related genes (4). Since antitumor immunity is a critical factor in the development of HPV-associated cancers which may also influence the efficacy of cancer immunotherapy, we analyzed the expression of immune-related genes in HPV-associated tumors with respect to the types of tumorigenesis. Transcriptomic HNSCC and CC datasets from The Cancer Genome Atlas were used for this analysis. Clustering with immune-related genes resulted in two clusters of HPV16-positive squamous cell carcinomas in both tumor types: cluster 1 had higher activation of immune responses, including stimulation of the antigen processing and presentation pathway, which was associated with higher immune cell infiltration and better overall survival, and cluster 2 was characterized by keratinization. In CC, the distribution of tumor samples into clusters 1 and 2 did not depend on the level of E2/E5 expression, but in HNSCC, most E2/E5-high tumors were localized in cluster 1 and E2/E5-low tumors in cluster 2. Further analysis did not reveal any association between the E2/E5 levels and the expression of immune-related genes. Nevertheless, it confirmed the role of components of the antigen processing and presentation pathway in HNSCC and CC survival. In addition, high expression of 11 leukocyte immunoglobulin-like receptor (LILR) genes was found in tumors with high immune cell infiltration. Since some of these receptors, that are expressed on different subsets of immune cells, are inhibitory (LILRB) and bind both classical and non-classical MHC class I molecules (5), their blockade could be used for immunotherapy of tumors with various MHC class I expression.



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L-23

THE INCREASED OCCURRENCE AND VIRULENCE OF *STREPTOCOCCUS PYOGENES*: CURRENT EPIDEMIOLOGY IN THE CZECH REPUBLIC

MARCELA KRŮTOVÁ[a]*, MARIE BRAJEROVÁ[a], MICHAL VANČURA[a], MAREK ŠTEFAN[b], PAVEL DŘEVÍNEK[a]

[a] Department of Medical Microbiology, 2nd Faculty of Medicine Charles University and Motol University Hospital, [b] Department of infectious diseases and travel medicine, 2nd Faculty of Medicine Charles University and Motol University Hospital. V Úvalu 84, Praha 5, 150 06, Czech Republic marcela.krutova@lfmotol.cuni.cz

Introduction: In 2023, a significant increase in *Streptococcus pyogenes* culture positivity was observed at microbiological laboratories in the Czech Republic. This trend has also been reported in other European countries, where this increase was attributed to the spread of the epidemic *S. pyogenes* M1 lineage from the UK. For the Czech Republic, data on circulating strains of *S. pyogenes* in the population are lacking, as this pathogen is not an infection, subject to mandatory reporting.

The aim: The multicentric study had two aims: i) to obtain epidemiological data on the current incidence of infections caused by *S. pyogenes* with a characterisation of circulating strains and ii) to evaluate the discriminatory power of Fourier transform infrared spectroscopy (FT-IR) for typing clinical isolates of *S. pyogenes*.

Methods: i) 12 microbiology departments, well representing the geographic area of the Czech Republic, provided retrospective anonymized data on the unduplicated culture of *S. pyogenes* from the period of January 1, 2017 to May 31, 2023. In addition, 10 consecutive unduplicated *S. pyogenes* isolates from each site were sent for characterization by whole-genome sequencing (Illumina) and susceptibility testing to an expanded range of antibiotics (n=16) using a disk diffusion method. ii) 24 clinical isolates of *S. pyogenes,* of different emm types (differences in surface M-protein sequence) and 11 clinical isolates of epidemic emm type 1 were selected from the above-mentioned collection (aim i) representing strains currently circulating in the Czech Republic. The bacterial suspension (after 24 h culture on blood agar at 37 °C in a 5% CO2 atmosphere) was plated on a silicon plate, dried and measured with an IR-Biotyper (Bruker Daltonics). The spectra for lipids (3000–2800 and 1500–1400/cm), proteins and carbohydrates (1800–900/cm) as well as the default spectra (1300–800/cm) were analysed using the software (v 3.1). Each isolate was analyzed in technical triplicate or quadruplicate.

Results: i) When analysing retrospective data, there was a threefold increase in the average monthly culture capture of *S. pyogenes* in the first 5 months of 2023 compared to the pre-SARS-CoV-2 pandemic period and a fourfold increase compared to 2022. The percentage of positive blood cultures remains the same. In 120 *S. pyogenes* isolates sent from 12 hospitals, resistance was detected to the following antibiotics: erythromycin 14.2%, clindamycin 5.8%, norfloxacin 2.5%, and tetracycline 9.2%. Twelve different emm types and 16 sequence types (ST) were identified with the predominance of 35.0% for emm type 1 (ST28) and 32.5% for emm type 12 (STs 36, 101, 242, 1366). The resistance genes found were: *ermB* (5%, macrolides, lincosamides), *ermA* (3.3%, macrolides), *msrD* and *mefA* (5%, macrolides), *tetM*, *tetO*, *tetL* (12.5%, 1.6%, 0.8%, tetracyclines), *catQ* (4.2%, chloramphenicol).

ii) Using FT-IR, a cluster of emm type 1 *S. pyogenes* isolates was reliably distinguished from other emm types using linear discriminant analysis (LDA) of protein and carbohydrate spectra (Figure 1). Other emm types are indistinguishable from each other.

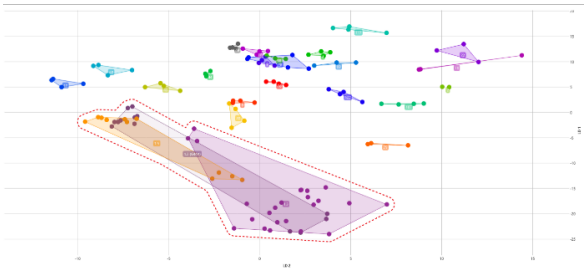


Fig. 1. **Linear discriminant analysis (LDA) of clinical *S. pyogenes* isolates belonging to different emm types (differences in surface M protein sequences) with an identified cluster of epidemic emm type 1 isolates highlighted by hatching.**

Conclusions: i) Laboratory data from microbiology departments across the country confirmed a significant increase in *S. pyogenes* culture positivity. The dominant emm types are 1 and 12. Due to penicillin withdrawals and the associated suboptimal therapy of streptococcal infections, it is necessary to monitor the development of resistance especially to macrolides and lincosamides in the coming years.
ii) FT-IR is not able to distinguish all currently circulating emm types, but it reliably identifies the epidemic *S. pyogenes* emm type 1, which may represent important information for the physician from the perspective of patient care due to its described increased virulence.

Acknowledgement
This work was supported by the National Institute for Virology and Bacteriology project no. LX22NPO5103 (Program Exceles, NextGenerationEU).
We would like to thank the microbiology laboratories which provided S. pyogenes isolates and data: Ivana Vítková – Faculty Hospital Brno; Elka Nyčová – Bulovka Hospital, Praha; Lenka Ryšková – Faculty Hospital Hradec Králové; Pavla Kučová – Faculty Hospital Olomouc; Lenka Geigerová – Faculty Hospital Plzeň; Denisa Veselá – Jindřichův Hradec Hospital; Magda Balejová – České Budějovice Hospital; Nataša Bartoníková – Tomáš Baťa Hospital, Zlín; Lenka Havlínová – Liberec Hospital; Erika Czyzová – Šumperk Hospital; Jan Kubele – Homolka Hospital, Prague. Great thanks for the technical support to Barbora Dratvová, Milena Antušková, Jaroslava Zíková and Jan Tkadlec.

L-24
ANALYSIS OF BACTERIAL LIPOPOLYSACCHARIDES ("SEROTYPING") BY MALDI-TOF MASS SPECTROMETRY: MISSION IMPOSSIBLE?

LUCIA ĎAĎOVSKÁ, JAROSLAV HRABAK[a,b]

[a] *Biomedical Center, Faculty of Medicine, Charles University, Pilsen, Czech Republic,* [b] *Department of Microbiology, Faculty of Medicine, University Hospital in Pilsen, Charles University, Pilsen, Czech Republic*
Jaroslav.Hrabak@lfp.cuni.cz

In the last decade, the use of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) revolutionary changed clinical microbiology. The technology allowed significant shortening of turnaround time needed for taxonomical identification of bacteria and fungi, as well as rapid identification of microbes from blood cultures and other clinical specimens, e.g., urine (1, 2, 3). Similarly, applications for antibiotic resistance determination have been also developed and validated for the use in clinical diagnostics (4). Among them, the routinely used is beta-lactamase activity determination by a detection of the changes of molecular mass of indicator beta-lactams, or detection of polymyxin resistance using analysis of lipid A of lipopolysaccharides (5).

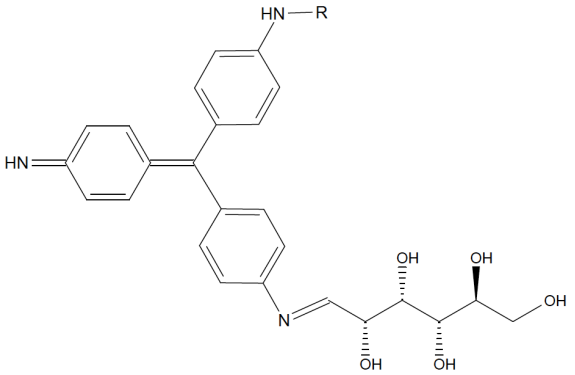
As MALDI-TOF MS provides efficient and rapid species identification, there is a key issue whether the method can be used for epidemiological typing directly from obtained spectra. So far, no general typing algorithm was proposed but only specific peaks representing significant epidemiological markers have been identified in some species. Very recently, artificial intelligence for spectra analysis has been described as a promising tool for prediction of antibiotic resistance and epidemiological typing (6).

Despite the use of artificial intelligence methods for analysis of big data, MALDI-TOF MS should be considered as a biochemical tool allowing precise analysis of molecules based on their molecular weight and fragmentation characteristics. Thus, we believe that scientific community should not resign ourselves to exact identification of detected molecules/peaks. For such an analysis, it is usually insufficient to analyze crude bacterial extract without further processing, i.e., specific extraction and enhancement of MALDI-TOF MS-based ionization (1).

Recently, MALDI-TOF MS-based analysis using cell-wall lipid fingerprinting was developed not only for the detection of colistin resistance, but also for identification and typing of some bacteria with the cell-walls rich for lipids, i.e., *Mycobacterium* spp. Similarly, periplasmic proteins, e.g., beta-lactamases, can be specifically isolated and detected via MALDI-TOF MS (7).

Surface structures of bacterial cell wall play an important role in antibiotic resistance, typing, and in vaccination strategy as most of them are common targets of immune response to the infection. The most important surface structures are lipopolysaccharides (e.g., *Enterobacterales* including *Escherichia coli* and *Salmonella* spp., *Pseudomonas aeruginosa*), polysaccharides (e.g., *Haemophilus influenzae*, *Neisseria meningitidis*, *Staphylococcus* spp., and *Streptococcus* spp.), and proteins including flagella and outer membrane proteins (e.g., *E. coli*, *Neisseria meningitidis*, *Streptococcus pyogenes*) (8). Analysis of polysaccharides is hindered by a poor ionization ability, especially in comparison to detection of proteins or lipids where there are many protocols available. On the contrary, in the field of polysaccharide detection there is a need for innovative approaches.

We present here a novel approach for saccharide derivatization and polysaccharide fingerprinting that allows detection of those structures by MALDI-TOF MS as well as LC/MS. The method can be used not only for bacterial typing but also for identification of bacteria and fungi directly from clinical specimens.



Scheme 1. **Glucose derivatization for MALDI-TOF MS measurement.**

Acknowledgment
This work was supported by the project National Institute of Virology and Bacteriology (Program EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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L-25
PYRAZINAMIDE DERIVATIVES: ANTIMICROBIAL ACTIVITY AND BEYOND

JAN ZITKO[a,*], MAREK KERDA[a], MARTIN JUHÁS[a], JAROSLAV ROH[b]

[a] Dpt. of Pharmaceutical Chemistry and Pharmaceutical Analysis, [b] Dpt. of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Ak. Heyrovského 1203/8, 500 05, Hradec Králové, Czech Republic
jan.zitko@faf.cuni.cz

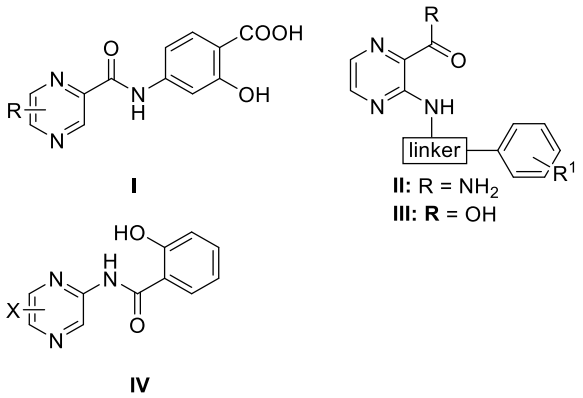
In the opening parts of the lecture, we will recapitulate the latest theories of the mechanism of action of the first-line antitubercular pyrazinamide (pyrazine-2-carboxamide, PZA) and a second-line antimycobacterial agent, 4-aminosalicylic acid (PASA).

We will present and discuss our latest and most promising results in the research of new derivatives of PZA as potential antimicrobial agents. A project focusing on hybrid compounds consisting of PZA and PASA fragment (**I**)[1] led to compounds with selective antimycobacterial activity and low toxicity for human cells. The acquired candidate compounds were thoroughly studied *in silico*, *in vitro* and *in vivo*. The compounds retained their activity in drug-resistant mycobacterial strains, were non-toxic *in vivo* (an invertebrate model of *Galleria mellonella* and vertebrate zebrafish model), and proved their efficacy in a mouse model of tuberculosis. Biochemical studies showed that the compounds target mycobacterial dihydrofolate reductases (DHFR). An *in silico* docking study combined with molecular dynamics identified a viable binding mode in mycobacterial DHFR. In the hit-2-lead optimization, we prepared prodrugs and salts with improved pharmacokinetic profile and studied their metabolization by human liver microsomes.

The other project is focused on simple structural derivatives of 3-aminopyrazinamide and 3-aminopyrazinoic acid, respectively. Compounds of general structure **II** were designed as inhibitors of mycobacterial prolyl-tRNA synthetase (mtProRS). The design was based on a confirmed inhibitor of human ProRS[2]. In this project, we designed new simple structural derivatives of this inhibitor and were able to shift the activity from eukaryotic to mycobacterial cells. *In silico* simulations proved that the new compounds of general structure **II** bind to mtProRS[3]. Currently, we are in the process of confirmation of the target by crystallographic studies. Related 3-substituted pyrazinoic acids (**III**) were designed and evaluated as inhibitors of mycobacterial aspartate decarboxylase (PanD), which is one of the most widely recognized target of PZA[4].

We will also present the results of our hit-expansion study[5] on antistaphylococcal compound **IV**, which might be considered a PZA derivative with an inversed carboxamide linker.

In silico methods (molecular docking, molecular dynamics, quantum mechanics-based methods) are employed in our drug design and hit-2-lead optimization workflows, and we will discuss how these can be helpful – but sometimes also misleading – for medicinal chemists.



I **II:** R = NH_2 **III:** **R** = OH **IV**

Fig. 1. **General structures**

Acknowledgement
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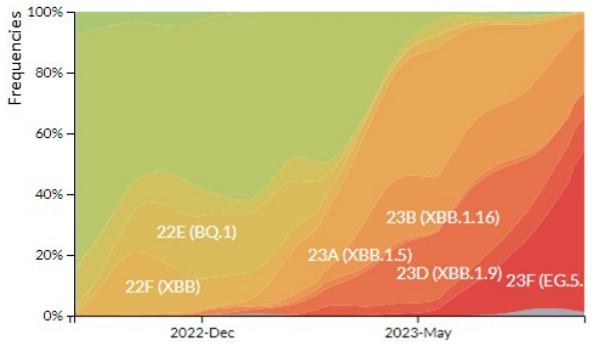
L-26
DYNAMIC EVOLUTION OF SARS-CoV-2 VARIANTS: AFFINITY-ENHANCING MUTATIONS IN SPIKE PROTEIN AND RECEPTOR BINDING DOMAIN

JIRI ZAHRADNIK*

First Faculty of Medicine at Biocev, Charles University, Prague, Czechia
jiri.zahradnik@lf1.cuni.cz

Viruses undergo rapid evolution to stay ahead of the immune system's responses. While every SARS-CoV-2 variant exhibits mutations across its entire genome, a substantial portion of the observed phenotype can be attributed to mutations within the Spike protein, notably the Receptor Binding Domain (RBD), a hotspot for mutational activity. When looking at the evolution of the RBD domain, it becomes evident that the SARS-CoV-2 population's effective size acts as a counterbalance, mitigating genetic drift and the stochastic accumulation of degenerative mutations. Instead, a well-balanced evolution where an affinity-enhancing mutation is acquired first, allowing for subsequent incorporation of immune escape mutations with a usually negative impact on binding, is observed. The tight SARS-CoV-2 ACE2 receptor interaction is crucial for high infectivity and competitive advantage among viral strains.

The interaction between the SARS-CoV-2 Spike protein and the ACE2 receptor holds pivotal importance for infectivity, escape from neutralizing antibodies and as a result the virus's competitive edge among different strains. Notably, the XBB variant emerged as a recombinant of two Omicron subvariants and established itself in the population in late 2022 [1]. XBB's initially lower affinity relative to the prevalent BA.2.75 strain confined its dissemination and did not allow it to outcompete BA.2.75 and other circulating strains. However, the acquisition of a single nucleotide mutation in position 486 causing S486P substitution significantly enhancing XBB's affinity prompted a nearly complete population sweep with new XBB.1.5 strain quickly dominating the population (Scheme 1)[2]. The surplus of affinity coupled with an evolutionary pressure exerted by the neutralizing antibodies in the population caused the rapid acquisition of additional immune escape mutations. Consequently, the lineage gave rise to the XBB.1.16 strain[3], followed by the more recent EG.5.1 strain. Yet, both strains display higher affinity than their parental XBB.



Scheme 1. **Frequencies of SARS-CoV-2 lineages in the population. Adapted from www. https://nextstrain.org/**

We expect a similar trajectory for the emerging and highly mutated BA.2.86 strain. Its spread in the human population is gradual and relatively slow, which mirrors the initial phases of XBB strain evolution. As with XBB, the trajectory of this strain can be drastically altered by the acquisition of an affinity-enhancing mutation. Our insight gained from previous experiments mimicking viral evolution by yeast display *in vitro* evolution showed that a good candidate for such an affinity-enhancing mutation is H445K[4].

Detailed monitoring of viral evolution and the development at the interface helps us, not only understand the fundamental outlining mechanisms in host-pathogen evolution but also allows us to anticipate future trends. These insights hold the potential to inform the design of vaccines and antibody therapies, thus improving our pandemic mitigation strategies.

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU and by the PRIMUS UK 23/ MED/002 grant.

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P-41

MiRNA146a IS A KEY COMPONENT OF IMMUNNOSUPRESSIVE ENVIRONMENT OF HEPATOCYTES CHRONICALLY INFECTED WITH HBV AND MELANOMA CELLS

OLENA BEREHOVSKA[a,b], MARKETA PIMKOVA POLIDAROVA[a,b], VACLAV JANOVEC[a,b], IVAN HIRSCH[a,b], KLARA GRANTZ SASKOVA[a,b]*

[a] *Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo namesti 542/2, 160 00, Prague, Czech Republic,* [b] *Charles University, Faculty of Science, Albertov 6, 128 00 Prague, Czech Republic*
olena.berehovska@uochb.cas.

Interferon α (IFNα) is a pro-inflammatory cytokine with antiviral properties. Our project focuses on Toll-like receptors (TLR)7/9, which upon activation lead to a massive production of INFα.[1] Therefore, the process needs to be strictly regulated. One of the negative regulators of TLR7/9 signaling is a micro RNA (miRNA) miRNA146a.[2]

miRNAs are small non-coding RNAs, part of the RISC complexes, which regulate translation of target protein by silencing corresponding mRNA.[3] RISC complexes can be sorted to multivesicular bodies (MVBs), and then to extracellular vesicles (EVs). Once exported, the RISC complex stays functional and can affect different cells upon uptake of RISC complex-containing EVs.[4]

We focus on miRNA146a, which has immunosuppressive properties. It is the most abundant miRNA in hepatocytes. Furthermore, hepatocytes persistently producing HBV virus have significantly higher level of miRNA146a than HBV-negative hepatocytes. Moreover, increased levels of miRNA146a were detected in melanoma cells that correlated with immunosuppressive effects.[5]

We investigate whether miRNA146a is one of the key players in immunosuppression duringHBV infection and melanomas. We speculate that miRNA146a could be be packaged into EVs in the form of active RISC complex, and in turn, contribute to the immunosuppresive microenvironment.

We performed a series of experiments which show, that the miRNA146a intracellular and extracellular levels are increased in both HBV-producing cells and melanoma cells lines. Moreover, the supernatant from HBV-producing cells and melanoma cell lines reduces the IFNα secretion by Gen2.2 cells (model of plasmacytoid dendritic cells) upon TLR7/9 agonist treatment. Importantly, the inhibition of miRNA146a in HBV-producing cells restored the Gen2.2 cell response to TLR7/9 agonist, suggesting that miRNA146a could be an immunosupressive factor transfered from HBV-infected cells to immune cells via EVs.

We will further analyze the EVs composition by fractionation to determine the miRNA146a origin and the mechanism of transport. Regulation of the circulating levels of miRNA146a can be a new target in the clinical research as it can reimpose the immune response of the host.

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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P-42
PML NUCLEAR BODIES ORCHESTRATE ANTIVIRAL RESPONSE IN HBV-INFECTED HEPATOCYTES

VACLAV JANOVEC, BORIS RYABCHENKO, BARBORA LUBYOVA, SANDRA HUÉRFANO, JAN WEBER, IVAN HIRSCH, KLÁRA GRANTZ ŠAŠKOVÁ

Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, 25250 Vestec, Czech Republic
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 16000 Prague, Czech Republic
janovecv@natur.cuni.cz

Promyelocytic leukaemia nuclear bodies (PML-NBs) play roles in various cellular functions and represent a key component of antiviral defense. In the case of hepatitis B virus (HBV), the antiviral activity of PML-NBs has been associated with the presence of SMC5/6 and epigenetic silencing of cccDNA. However, PML-NBs can have a pleiotropic effect on the life cycle of HBV and the role of PML-NBs needs to be further elucidated.

HBV infection does not induce formation of typical "antiviral" PML-NBs probably due to the missing IFN-signalling. Thus, we analyzed the antiviral role of IFN-α induced PML-NBs in HBV-infected hepatocytes. We demonstrated that IFN-α treatment upregulates PML in HepG2-NTCP cells and in primary human hepatocytes. Furthermore, induction of the antiviral state in HepG2-NTCP cells by IFN-α led to inhibition of the formation of cccDNA in the early step of infection and the secretion of HBV antigens. We also analyzed the formation and composition of PML-NBs by confocal microscopy. We found, that a long IFN-α treatment (>24 hours) led to the reorganization of PML-NBs that was probably dependent on sumoylation. We also observed the association of HBV DNA with PML-NBs by FISH. Moreover, PML-NBs formed ring-shaped structures during the late phase of infection (5 dpi) that directly entrapped HBV capsids. Thus, we demonstrated that PML-NBs inhibits various steps of HBV lifecycle. Collectively, our results demonstrated that PML-NBs positionally orchestrate antiviral response in HBV-infected hepatocytes. These experiments have clinical relevance because of IFN-α therapy in chronically infected patients with HBV.

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P-43
SAND FLIES AS A VECTORS OF VIRUSES

NIKOLA POLANSKÁ, PETR VOLF, VÍT DVOŘÁK, MAGDALÉNA JANČAŘOVÁ

Charles University, Viničná 7, Prague 2, 128 00, Czech Republic
nikola.polanska@natur.cuni.cz

Sand flies (Diptera: Phlebotominae) are mostly known for their role in the transmission of parasitic protists of the genus *Leishmania* that cause leishmaniases, while their involvement in the transmission of many arboviruses of medical and veterinary importance is neglected[1]. The World Health Organization (WHO) established four main criteria to define a vector of a virus: (i) the virus must be isolated from wild-caught arthropods free from visible blood in the alimentary tract; (ii) arthropods get infected by feeding on viraemic vertebrate host or by artificial substitution; (iii) arthropods must be able to transmit the virus biologically by bite; and (iv) field evidence confirming association between arthropods and appropriate vertebrate host must exist. All four criteria must be met to describe an arthropod as a proven vector. If only a single criterion is satisfied, the arthropod is considered as a 'suspected vector'. If the conditions of natural infection and experimental transmission are met (criteria ii and iii), we use the term 'potential vector'[2].

Unfortunately, our understanding of the involvement of sand flies in arbovirus transmission cycles remains fragmentary and poorly understood. The reasons are manifold, ranging from highly variable methodology to the unavailability of laboratory colonies and difficulties of experimental infections in the laboratory. We reviewed the literature to summarize the so far published information on viruses isolated from phlebotomine sand flies; sand fly-borne viruses from the *Phenuiviridae*, *Rhabdoviridae*, *Flaviviridae*, *Sedoreoviridae* and *Peribunyaviridae* families are reviewed for the first time with regarding to their distribution in nature, host and vector specificity, and potential natural transmission cycles to show knowledge gaps, potential epidemiological risks and future research prospects[3].

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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P-44
MOUSE PML PROTEIN ISOFORMS AND THEIR ROLE IN MOUSE POLYOMAVIRUS INFECTION

KAROLÍNA ANDEROVÁ*, BORIS RYABCHENKO, VOJTĚCH ŠROLLER, LENKA HORNÍKOVÁ, JITKA FORSTOVÁ, SANDRA HUERFANO*

[a] *Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 25250 Vestec, Czech Republic*
karolina.anderova@natur.cuni.cz

Promyelocytic leukaemia nuclear bodies (PML NBs) are dynamic, spherical, membrane-less structures composed of the main scaffold PML protein and a variety of stable or transient partner proteins. Apart from many endogenous functions, PML NBs play an important role in antiviral defence, both as direct restriction factors and as regulators of the interferon responses. Hence, many viruses developed effective mechanisms to counteract this restriction[1–3]. This project uses Mouse polyomavirus (MPyV) as a model for studying interactions of PML and viral components. The mouse PML (mPML) protein occurs in three confirmed (mPML1-3) and six predicted (mPMLX1-X6) isoforms. Individual isoforms may affect the composition and functions of PML NBs and mediate antiviral effects[3]. Our data showed, that during MPyV infection, mPML NBs appeared in close proximity to viral replication centres. In *Pml* KO cells, the transcription of MPyV regulatory genes was significantly increased and the amount of viral progeny was approximately two times higher. These results indicate a potential restriction function of mPML NBs and/or mPML protein in MPyV infection. Therefore, here we focused on the role of the individual mPML isoforms. Their expression has been examined in different mouse tissues – liver, spleen, kidney, lung, heart, femoral muscle, brain and thymus and the detected isoforms have been subsequently tested for PML NBs formation. The expression of all, confirmed and predicted isoforms was proved and, in addition, a novel isoform (named by us mPMLXK) was detected in all tested samples. All confirmed mPML1, mPML2 and mPML3 isoforms formed speckles when expressed in *Pml* KO cells and in WT cells, they incorporated into endogenous mPML NBs. The activity of individual isoforms in IFN signalling and their proposed antiviral role are currently under investigation.

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P-45
ROLE OF THE PML NUCLEAR BODIES AND ITS ASSOCIATED HISTONES CHAPERONES IN THE REMODELING OG MOUSE POLYOMAVIRUS MINICHROMOSOMES

BORIS RYABCHENKO*, CHRISTOS STRATZEMIS, JITKA FORSTOVA, SANDRA HUÉRFANO

Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 25250 Vestec, Czech Republic
boris.ryabchenko@natur.cuni.cz

Polyomaviruses (PyVs) are small non-enveloped viruses replicating in the host cell nucleus. Viral replication centers (VRCs) share features with cellular euchromatic or heterochromatic regions, depending on the stage of infection. In the mouse polyomavirus (MPyV) virions, the circular dsDNA genome and cellular histones are present in the form of a condensed minichromosome arranged into 24 nucleosomes. Here we studied the contribution of PML NBs and their chaperone complexes HIRA and ATRX/DAXX in remodeling MPyV chromatin.

We demonstrated that, in addition to canonical histones, PyV minichromosome contains the non-canonical histone, H3.3, Accumulation of H3.3 in VRCs occurs as early as 24 hours post infection (hpi) when viral genomes undergo massive replication. Furthermore, we showed that PML NBs surround VRCs and increase in number and size as the infection progresses. The proximity of PML NBs to VRCs is retained even after inhibition of viral DNA replication which indicates that PML NBs could directly recognize viral chromatin. Next, although we found that DAXX and ATRX, appear in the VRCs 24 hpi, the absence of DAXX did not prevent H3.3 incorporation into virions, suggesting that other chaperones, e.g. HIRA contribute to the deposition of the H3.3.

Surprisingly, we observed that knockout of *PML* gene leads to increased accumulation of H3.3 in viral minichromosomes and is beneficial for viral transcription. Thus, the PML NBs limit or 'buffer' the accumulation of H3.3 in PyV minichromosomes.

Our results highlight the possibility that HIRA is the chaperone responsible for H3.3 deposition into MPyV genomes and its function is controled by PML NBs. Next, this hypothesis will be investigated. Furthermore, the distribution of H.3.3 in the genomes, its post-translational modifications and the impact of H.3.3 in the transcription of genomes will be studied.

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P-46
UNDERSTANDING THE INTERPLAY BETWEEN MPYV INFECTION AND NUCLEAR LAMINA

KATEŘINA BRUŠTÍKOVÁ, BORIS RJABCHENKO, JITKA FORSTOVÁ, LENKA HORNÍKOVÁ*

Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 25250 Vestec, Czech Republic
Hornikova@natur.cuni.cz

The nuclear lamina (NL) is a dense meshwork of intermediate filaments V, type A lamins (lamin A/C), type B lamins and membrane associated proteins. It is located below the inner nuclear membrane. NL maintains the shape and structural integrity of the nucleus and plays an important role in fine-tuning of DNA related processes, e. g. replication or transcription. Also, it has been suggested to play a role in cell defence against pathogens. DNA viruses evolved mechanisms to exploit NL for their own purposes. In this study, we followed the changes of NL in cells infected with mouse polyomavirus (MPyV) and a possible role of lamins in MPyV replication. We examine the structure and integrity of the NL late times post infection (40h) and after transient expression of capsid proteins. Under both conditions, the major capsid protein VP1 significantly and non-randomly accumulates in close proximity of NL. Despite the irregularities in NL staining observed by confocal microscopy, and detected partial lamin A/C degradation, we proved that nuclear envelope remains intact. After in situ fractionation of infected cells (40h), MPyV DNA genomes, VP1 and LT (large T antigen necessary for viral genome replication) were found together with lamin A/C and lamin B1 in the last insoluble fraction, indicating possible complex formation. This phenomenon was later shown to be independent of the presence of lamin A/C. Further, we found that lamin A/C was solubilized during the infection progress probably due to its detected hyperphosphorylation. Given that lamin A/C was detected in virus transcription/replication centres and that in the absence of lamin A/C, slight reduction of LT andVP1 gene transcription were observed (24 hpi), it seems that lamin A/C supports viral gene transcription at early stage of infection. Despite that, the level of LT and VP1 proteins finally increased in cells lacking lamin A/C, probably by their stabilization caused by stronger binding to lamin B. This assumption is supported by observation, that VP1 together with lamin B1, was found in the insoluble fraction after in situ fractionation of cells with lamin A/C knockdown.

Altogether, these data suggest that lamins are affected by MPyV replication and are involved in the formation of the viral DNA replication/transcription centres and/or virus assembly. Mechanisms and importance of these processes have to be further elucidated.

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P-47
ABSENCE OF 5' METHYLGUANOSINE CAP IN POSTREPLICATIVE GENES' TRANSCRIPTS OF VACCINIA VIRUS

VÁCLAV VOPÁLENSKÝ[a]*, MICHAL SÝKORA[a], ZORA MĚLKOVÁ[b], IVAN BARVÍK[c], TOMÁŠ MAŠEK[a], KAMILA HORÁČKOVÁ[a], MARTIN POSPÍŠEK[a]*

[a] Department of Genetics and Microbiology, Faculty of Science, Charles University, Viničná 5, Prague, 12800,
[b] Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Studničkova 7, Prague, 12800,
[c] Institute of Physics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 3, Prague, 12000, Czech Republic
vaclav.vopalensky@natur.cuni.cz
martin.pospisek@ natur.cuni.cz

The Vaccinia virus (VACV) played a pivotal role in the discovery of the 5' mRNA. Over the past decades, VACV investigation has contributed extensively to understanding the mechanism behind 5' mRNA cap synthesis. In this study, we conducted a comprehensive analysis of VACV transcripts at the level of individual mRNA molecules. Surprisingly, our findings reveal that postreplicative VACV mRNAs, which incorporate nontemplated 5' poly(A) leaders, lack the conventional 5' cap structure *in vivo*.

Remarkably, our research uncovers a notable trend wherein the occurrence of 5' caps in viral mRNAs gradually diminishes across successive gene time classes of VACV. This stands in contrast to the concurrent increase observed in the lengths of 5' poly(A) leaders. Strikingly, these two variables exhibit a mutually inverse correlation. Further exploration demonstrates a direct or indirect influence of the initiator region element (INR) on both the frequency of 5' mRNA capping and the presence of 5' poly(A) leaders, encompassing their varied lengths in postreplicative VACV mRNAs.

By amalgamating our observations, we posit a hypothesis suggesting a potential link between the extent of 5' mRNA polyadenylation and the synthesis of the 5' cap, mediated through an as-yet-undisclosed mechanism. This notion gains support from our identification that 5' poly(A) leaders in VACV late transcripts containing m^7G caps are notably shorter than their counterparts, whose lengths were computed from an unbiased collection of all VACV late mRNAs.

In concert, our results underscore a compelling proposition: the regulatory framework governing VACV transcription orchestrates a gradual transition in viral mRNA translation initiation, shifting from a reliance on cap-dependent mechanisms to cap-independent ones. This intriguing phenomenon coincides with the virus-induced alteration of the host's translation machinery.

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P-48
TORQUE TENO VIRUS INFECTION AMONG PSORIASIS PATIENTS ON BIOLOGIC THERAPY

MARTINA SALÁKOVÁ[a], JANA ŠMAHELOVÁ[a], DOMINIKA KADLEČKOVÁ[a], RUTH TACHEZY[a]

[a] Department of Genetics and Microbiology; Faculty of Science, Charles University, Prague, Czech Republic, BIOCEV, Průmyslová 595, 252 50 Vestec, Czech Republic martina.salakova @natur.cuni.cz

Torque teno virus (TTV) is a ubiquitous member of the healthy human virome with broad tissue tropism. TTV can induce chronic active infection without clinical manifestations. Although little is known about the life cycle of the virus, it is clear that infection and replication of the virus are controlled by the host immune system. The plasma viral load reflects the immune status, and the viral load is higher in patients with immune dysfunction compared to healthy individuals. For this reason, monitoring TTV viremia by qPCR is widely accepted as a marker of the immune function in infected subjects.

The aim of this study was to evaluate the effect of different biologic treatments with specific immune targets on TTV prevalence and load in blood and oral lavage in psoriasis patients.

Blood samples and oral lavages from 246 psoriasis patients aged 18–65 years were tested. Of these, 105 (42.7 %) were treated with topical therapy, 71 (28.9 %) with anti-TNF-α, 28 (11.4 %) with anti-IL-12/23, and 42 (17.1 %) with anti-IL-17 therapy for at least 6 months. TTV was detected and quantified by qPCR.

TTV in blood was present in 73.6% (181/246) of samples with a mean DNA load of 3.7 log copies/mL. The differences between groups of patients with different psoriasis treatments were detected; TTV infection was present in 74.3% of patients receiving topical therapy, 80.3% of patients on anti-TNF-α, 60.7% of patients on anti-IL-12/23, and 69.0% of patients on anti-IL-17 therapy. TTV load was significantly higher in patients receiving anti-TNF-α therapy compared to individuals with topical therapy (p=0.023). Compared to blood, in oral lavages, TTV was more prevalent in patients receiving topical therapy (80.0%), in patients on anti-TNF-α (93.0%), and on anti-IL-17 therapy (88.1%). While in patients on anti-IL-12/23 therapy, the prevalence in oral lavages was comparable in both types of samples. Viral load, as measured by viral copy number to the amount of DNA, was significantly higher in patients who received anti-TNF-α (p=0,041) and anti-IL-17 therapy (p=0.031).

The study demonstrated the differences between the prevalence and mean value of TTV DNA in blood and oral lavages. Increased TTV viremia and oral viral load were found in psoriasis patients treated with anti-TNF-α, providing a basis for prospective investigation of the potential value of TTV load as a pharmacodynamic biomarker.

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P-49
THE DETECTION OF HYPOXIA MARKERS IN NON-HPV AND HPV-ASSOCIATED HEAD AND NECK CANCERS: IMPLICATIONS TO PATIENT SURVIVAL

JANA ŠMAHELOVÁ[a]*, BARBORA POKRÝVKOVÁ[a], ELIŠKA ŠTOVÍČKOVÁ[a], ONDŘEJ VENCÁLEK[b], MICHAL ŠMAHEL[a], MAREK GREGA[c], JAN KLOZAR[d], RUTH TACHEZY[a]

[a] Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 252 50 Vestec, Czech Republic, [b] Department of Mathematical Analysis and Applications of Mathematics, Faculty of Science, Palacky University in Olomouc, 771 46 Olomouc, Czech Republic, [c] Department of Pathology and Molecular Medicine, 2nd Faculty of Medicine, Charles University, 150 06 Prague, Czech Republic, [d] Department of Otorhinolaryngology and Head and Neck Surgery, 1st Faculty of Medicine, Charles University, University Hospital Motol, 150 06 Prague, Czech Republic smahelovaj@natur.cuni.cz

In the microenvironment of growing solid tumors, oxygen level often decreases and a hypoxic state is induced. This can lead to a worse treatment response and poor patient prognosis. One of the hypoxia-responsive genes is aspartate-β-hydroxylase (*ASPH*), whose activity promotes the growth, invasiveness, and metastasis of many solid tumors[1]. Head and neck cancers (HNC) are highly heterogeneous. A proportion of HNC is induced by high-risk human papillomavirus (HPV) infections and is associated with better patient outcomes compared to patients with tumors linked to tobacco and alcohol abuse.

In our study, we analyzed 93 HNC specimens. ASPH and selected endogenous hypoxia markers (HIF1A, HIF2, VEGFA, GLUT1, P4HA1, CA9, MMP9, and MMP13) were detected by multiplex fluorescent immunohistochemistry. The results were correlated with tumor etiology, clinical and pathological characteristics of the patients. The Cox proportional hazards model was used to evaluate the prognostic value of the analyzed markers.

Statistically significant higher protein expressions of ASPH, HIF1A, GLUT1, and MMP13 were detected in the HPV-positive tumor group compared to the HPV-negative group. Except for MMP9/13, higher expression of the markers was detected in the tumor parenchyma compared to the stroma. Increased protein expression of GLUT1 and HIF1A had a positive impact on 5-year overall and disease-free survival in HNC patients, independently of HPV tumor status.

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P-50
WHOLE GENOME SEQUENCING OF CZECH VANCOMYCIN-RESISTANT ENTEROCOCCI, A MULTICENTRIC SURVEILLANCE STUDY, 2022

MARIE BRAJEROVA, PAVEL DREVINEK, MARCELA KRUTOVA*

2nd Medical Faculty, Charles University and Motol University Hospital, Prague, V Úvalu 84, 150 06 Praha 5, Czech Republic
marcela.krutova@lfmotol.cuni.cz

Aim: In order to obtain data on the epidemiology of vancomycin-resistant enterococci (VRE) in the Czech Republic, we performed a surveillance study with a detailed characterization of VRE isolates in a large network of hospitals. *Methods:* Between September and December 2022, 10 consecutive VRE isolates were sent for whole genome sequencing and antimicrobial susceptibility testing. *Results:* A total of 159 non-duplicated vancomycin-resistant *Enterococcus faecium* isolates from different biological samples were collected from 20 Czech hospitals. The average age of the patients was 67.03 years and 47.2% were female. With the newly published multi-locus sequence typing (MLST) scheme of Bezdicek et al (Microbiol Spectr. 2023: e0510722), 29 sequence types (ST) were detected among 159 isolates. The most prevalent STs were 123 (n=44, 27.16%), 17 (n=20, 12.35%) and 327 (n=17, 10.5%). Whole-genome MLST detected clonal relatedness between isolates of the same ST within and between hospitals. Three isolates were resistant to linezolid by G2576T in 23S rDNA, one isolate carried the *poxt*A gene and three isolates carried the *cfr*B gene, both genes are associated with linezolid resistance. In seven isolates, only tigecycline remained available for possible patient treatment. *Conclusion:* The epidemiology situation of vancomycin-resistant *E. faecium* in the Czech Republic is driven by STs 123, 17 and 327. Importantly, clinical isolates with very limited treatment options have been identified.

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P-51
MICROBIOME CHANGES FOLLOWING FAECAL MICROBIOTA TRANSPLANTATION IN IRRITABLE BOWEL SYNDROME PATIENTS

ILIYA KWOJI[a], JAKUB HURYCH[a], JIRI VEJMELKA[b], LUCIE HLINAKOVA[a], MICHAELA CERMAKOVA[a], KLARA HUBACKOVA[a], PAVEL KOHOUT[b], ONDREJ CINEK[a]*

[a] Dept of Medical Microbiology, 2nd Faculty of Medicine, Charles University, V Úvalu 84/5, 15006 Prague, Czechia,
[b] Dept. of Internal Medicine, 3rd Faculty of Medicine, Charles University, Vídeňská 800, 14059 Prague, Czechia
ondrej.cinek@ lfmotol.cuni.cz;

Irritable bowel syndrome (IBS) is a chronic functional disease of unknown aetiology with a contribution from the distorted gut microbiota. An effective treatment is unknown. The aim of the study was to test whether faecal microbiota transplantation (FMT) improves the clinical symptom score of IBS patients. Here we present interim results of microbiome investigation: 16S rDNA profiling of the bacteriome from regularly collected stool.

The study is designed as a three-group (20 each), double-blind, randomised, cross-over, placebo-controlled study of two pairs of gut microbiota transfer[1]. Group A first receives two enemas of a microbiota mixture (deep-frozen stored stool microbiota mixed from eight healthy donors); after 8 weeks receives two enemas with a placebo (autoclaved microbiota mixture), whereas group B will first receive the placebo, then the microbiota mixture. Finally, group C receives the placebos only. Patients are followed using regular clinical visits, standardized questionnaires and stool samples. Recruitment has been finalized, but ¼ of the patients are still being followed up.

The alpha (within-sample) diversity of the patient's faecal bacteriome increased after active FMT ($p<0.006$) in multiple alpha diversity indices but not the Simpson index. The distance of stool sample profiles from the active substance significantly decreased following active FMT ($p<0.006$) compared to placebo ($p=0.06$). The order of interventions had no effect on the changes in the faecal bacteriome composition described above. Acquisition of strains from the FMT and evaluation of their persistence using strain tracking by shotgun metagenomics will be reported in further analysis.

In conclusion, significant changes in the faecal bacteriome of IBS patients have been noted after FMT. Later shotgun metagenomics analysis will reveal the degree of this modulation of strain acquisition and persistence.

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P-52
CLONAL DISSEMINATION OF NDM-5 PRODUCING ESCHERICHIA COLI ST38 IN THE CZECH REPUBLIC; FIRST REPORT OF THE BRNO OUTBREAK

TSOLAIRE SOURENIAN[a], KATERINA CHUDEJOVA[a,b], MONIKA DOLEJSKA[a,b,c,d], JAROSLAV HRABAK[a,b], IBRAHIM BITAR[a,b]*

[a] Biomedical Center, Faculty of Medicine, Charles University, Pilsen, Czech Republic, [b] Department of Microbiology, Faculty of Medicine, University Hospital in Pilsen, Charles University, Pilsen, Czech Republic, [c]Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno, Brno, Czech Republic, [d]Department of Clinical Microbiology and Immunology, Institute of Laboratory Medicine, The University Hospital Brno, Brno, Czech Republic
ibrahimbitar5@gmail.com

Carbapenem-resistant *Enterobacterales* (CREs) are of particular concern due to the increased incidence of resistance to several essential antibiotics. Throughout a routine genomic screening of carbapenemase producers in the Czech Republic, a relatively high number of NDM-5-producing *Escherichia coli* ST38 have been reported in Brno. *Enterobacterales* with a chromosome-located carbapenemase gene are rarely reported and its occurrence in such ST is scarce. The aim of the study is to perform an in-depth regional investigation to unveil the evolutionary aspects and genomic characteristics of these isolates driving the outbreak in this region. During the period of 2020–2022, from the clinical surveillance samples collected in the Czech Republic, 42 *E. coli* were isolated from Brno from 7 different hospitals. The isolates were examined for ST38 using a multilocus sequence typing (MLST) scheme. Twenty-six representative isolates were subjected to whole-genome sequencing (WGS) to analyze the genetic elements and the presence of carbapenemase-encoding genes using HiSeq7 Illumina. Based on Illumina results, 14 isolates were sequenced using PacBio Sequel I platform to generate a complete genome and circular plasmids. Both the resistome and the virulome of ST38 were analyzed *in silico* using online databases: ResFinder and VirulenceFinder (VFDB). Biofilm formation assay was performed using a 96-well microtiter plate and evaluated using one-way ANOVA (α= 0.05). The clonality of the circulating isolates was examined by detecting single nucleotide polymorphisms (SNPs) using Snippy v4.6.0. SNPs-based phylogeny was generated using 2,322 ST38 genomes retrieved from the EnteroBase repository to demonstrate the genomic correlation with other ST38 isolates. The analysis of WGS data confirmed that *E. coli* belonged to the ST38 lineage. It revealed the presence of $bla_{\text{NDM-5}}$ in all isolates (n=42), and the latter was localized on the chromosome. All isolates were able to form Biofilm which was significantly associated with time (p-*value* < 0.05). The virulence factor encoding genes including *fimH* and *afa/draBC* were mainly related to the expression of phenotypic traits. Thus, all isolates could adhere and form biofilm after 24 h, 3 days, and 6 days. 15 % of the *E. coli* were classified as weak, 35% as moderate, and 50% as strong. SNP analysis performed on the collected isolates revealed a range of 2 to 20 SNPs only. In addition, phylogenetic analysis clustered our isolates on the same clade with several isolates from Australia (n=4), Norway (n=1), and Vietnam (n=2) harboring $bla_{\text{NDM-5}}$ as well. Based on our findings, the chromosomal integration of $bla_{\text{NDM-5}}$ gene in *E. coli* isolates of ST38 appears to be circulating in different hospitals in Brno in the Czech Republic. The possible cause of this prevalence could be the dissemination of this successful virulent clone or even the same isolate circulating within the same hospital settings. The virulence features suggest the possible emergence of a regional and subsequent national clonal outbreak.

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P-53
A NOVEL PLASMID-ENCODING MCR-10 IN A CLINICAL ENTEROBACTER LUDWIGII STRAIN ISOLATED FROM A TERTIARY HOSPITAL IN CZECH REPUBLIC

TSOLAIRE SOURENIAN[a], JAROSLAV HRABAK[a,b], IBRAHIM BITAR[a,b]*

[a] *Biomedical Center, Faculty of Medicine, Charles University, Pilsen, Czech Republic,* [b] *Department of Microbiology, Faculty of Medicine, University Hospital in Pilsen, Charles University, Pilsen, Czech Republic*
Sourenian.Tsolaire@lfp.cuni.cz

The emergence of multidrug-resistant pathogens in clinical settings is a persistent challenge. *Enterobacter ludwigii*, a prominent pathogen within the *Enterobacter* genus and a member of the *Enterobacter cloacae* complex (ECC) has become clinically significant. Furthermore, the onset of the genetic elements (*mcr* genes) has evoked universal concern, as their dissemination may compromise the effectiveness of colistin in therapeutic approaches. Here, we identify a novel IncFIA plasmid harboring *mcr-10* in a clinical *Enterobacter ludwigii* strain from a University Hospital in the Czech Republic. The isolate was selected from an ongoing survey of *mcr* genes in the Czech Republic and retrieved from a clinical sample at the University Hospital in Pilsen in 2021. The isolate was subjected to antibiotic susceptibility testing and was assessed using a broth microdilution assay based on EUCAST 2023 breakpoints. To assess the genomic elements of our isolate, short-read sequencing (HiSeq 4000 Illumina) and long-read sequencing on the MinION platform were used to generate complete and circular nucleotide sequence of the chromosome and plasmids. Comparative genomic alignment was utilized by Mauve v.2.3.1 and Blast Ring Image Generator (BRIG). Species identification, sequence types (ST), plasmid replicons, antibiotic resistance genes, and virulence-associated genes were also determined by uploading the assembled data and analysing via *in silico* databases. Retaining WGS, we confirmed the taxonomic classification of I9455333cz as *E. ludwigii* of ST20 through MLST analysis. The genomic examination of the strain highlighted the presence of intrinsic antibiotic resistance genes on the chromosome (5,247,533 bp). Additionally, a distinctive plasmid of IncFIA(HI1)/IncFII(Yp) type (129,863 bp), harboring the *mcr-10* gene, was identified. Importantly, this plasmid carried genes responsible for replication, transfer, maintenance, and a toxin-antitoxin system. Further genomic exploration underscored the virulence attributes of I9455333cz, including genes associated with curli fibers, adhesin type 3 fimbriae, and siderophore biosynthesis pathways. Regulatory genes, such as *rpoS* and *phoP/Q*, pivotal for stress responses and survival under environmental challenges, were also observed. This study extends our understanding of the genetic milieu in which I9455333cz functions, providing insights into mechanisms governing *mcr* gene dispersal, antibiotic resistance, and pathogenicity. We revealed a novel plasmid harboring the *mcr-10* gene within a clinical *Enterobacter ludwigii* strain, a first in the Czech Republic. Despite conferring limited resistance to colistin, the gene is carried on a stable element with the potential for dissemination, underscoring the necessity for ongoing surveillance and further investigation.

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P-54
THE INTESTINAL CARRIAGE OF PLASMID-MEDIATED ANTIMICROBIAL RESISTANCE IN CZECH TRAVELLERS

SUHANYA PRASAD, MARIE BRAJEROVA, JAN TKADLEC, PAVEL DREVINEK, MARCELA KRUTOVA*

Department of Medical Microbiology, Charles University, 2nd Faculty of Medicine and Motol University Hospital, Prague, V Úvalu 84, 150 06 Praha 5, Czech Republic
marcela.krutova@lfmotol.cuni.cz

Background: Plasmid-mediated resistance to colistin, carbapenems and new-generation beta-lactams poses a serious concern to health care. Little is known about its prevalence in the community, especially in citizens with a high risk of colonisation by multidrug-resistant (MDR) bacteria such as travellers[1,2].

Methods: Stool samples of travellers were cultured after enrichment on a selective medium for detection of extended-spectrum β-lactamase producing (ESBLs), carbapenem-resistant (CRE), and/or colistin-resistant Enterobacterales. Whole genome sequencing was used to characterise the strains. A total of 165 stool samples were collected between September 2019 and October 2021, 90 travellers (54.5 %) were males and 72 (43.6 %) were females; the average age was 41 years (median 40 years). The most visited regions of travel included Southeast Asia (Thailand, Philippines, Indonesia), the Indian subcontinent (India, Nepal), Africa (Ghana, Tanzania, Rwanda, Nigeria), Europe (Greece, Croatia, Germany), Northern America (Mexico, Cuba, Dominican Republic), and Russia. Results: Overall 35 travellers (21.2 %) were found to be carriers of ESBL-producing Enterobacterales. The $bla_{\mathbf{CTX\text{-}M\text{-}15}}$ (48.5 %) and $bla_{\mathbf{TEM\text{-}1B}}$ (31.4 %) were the most prevalent causative genes followed by $bla_{\mathbf{OXA\text{-}1}}$ (22.8 %). Additionally, three *E. coli* strains carried *mcr-1* gene (1.8 %). No CRE strain was cultured.

Conclusion: Czech travellers can be more likely colonised by ESBL-producing than by carbapenem or colistin-resistant Enterobacterales. The sporadic plasmid-mediated colistin resistance is in line with the previous study (1).

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P-55
JOURNEY FROM ANTIMYCOBACTERIAL TO ANTISTAPHYLOCOCCAL ACTIVITIES – HOW TO APPROACH RATIONALLY, WHEN MECHANISM OF ACTION IS UNKNOWN

MAREK KERDA, JAN ZITKO

Dpt. of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Ak. Heyrovského 1203/8, 500 05, Hradec Králové, Czech Republic
kerdam@faf.cuni.cz

The development of new antibacterial agents evolves rapidly and simultaneously with the advancement of technologies. Currently, a rational approach is preferred in the development of new drugs over random derivatization of the known active candidate. We wanted to meet these current trends, to widen the spectrum of antimycobacterial compounds usually prepared in our group, and to extend their activity to Staphylococcus aureus. The mechanism of action of those compounds[1] is unknown, making the goal even more challenging. The first step was to synthesize various derivatives, determine those with the best antistaphylococcal activity, and evaluate SAR. The series showed significant antimycobacterial activities (MIC = 3.91 µg/mL), but not so high antibacterial activities (MIC = 62.5 µg/mL)[2]. The second step is the determination of a specific molecular target using a biochemical approach called 'target fishing'[2] with the use of biotin-labeling. After this determination, we can process the results in silico and modify the design of the compound rationally.

Acknowledgement
This research was supported by the Ministry of Health of the Czech Republic, grant nr. NU21-05-00482, and by „The project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.“

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PALACKÝ UNIVERSITY OLOMOUC

L-27
INTRODUCTION OF DIFFERENT MODELS OF ANIMAL INFECTIONS

MIROSLAV POPPER[a], ZBYNĚK NOVÝ[a], MARIÁN HAJDÚCH[a], KATEŘINA DVOŘÁKOVÁ-BENDOVÁ[a], KRISTÝNA KRASULOVÁ[a], BARBORA NEUŽILOVÁ[a], KATEŘINA FRANTÍKOVÁ[a], TEREZA SCHNEIDEROVÁ[a], KATARÍNA HAJDUOVÁ[a], MILOŠ PETŘÍK[a]*

[a] *Institute of Molecular and Translational Medicine Faculty of Medicine and Dentistry of Palacky University, Olomouc, Czech Republic*

The animal facility of Institute of Molecular and Translational Medicine of Palacky University (IMTM) provides preclinical *in vivo* efficacy models for numerous diseases including those caused by infectious agents.

What is an animal model? An animal model is a non-human species used in biomedical research because it can mimic aspects of a biological process or disease found in humans. By using animal models, researchers can perform experiments that would be impractical or ethically prohibited with humans.

Why are animal models used? Animal models have proven useful to design and minimize the risk of subsequent clinical trials on antimicrobial drugs. The study of pharmacokinetics/pharmacodynamics in specific, well-standardized models such as the mouse thigh infection model has led to the formulation of indices and targets that determine optimal drug exposures. These results can be translated from the *in vivo* model to humans because the antimicrobial target is in the microorganism and not the host. Exposure relative to the MIC provides a normalization across host species[1]. An animal model may be used to predict the likely effectiveness of a novel treatment before it progresses to studies in patients. Animal models play a critical role in studying a variety of infectious diseases.

What types of animal models for studying infection are used at IMTM? At IMTM, we used to work with topical infection animal models: full-thickness skin model, skin abscess infection model, myositis-induced model of infection, lung infection model, and stereotactic brain infection model.

Brief description of infectious animal models used at IMTM:

Skin and muscle infection models are essential for new skin and deep wound infection treatment strategies. Injury or surgery often leads to persistent chronic infections, which are challenging to treat due to multi-drug resistance and the formation of biofilms.

Infections in the lung leading to pneumonia constitute a significant cause of death in the young, elderly, immunocompromised, and cystic fibrosis patients. The development of multi-drug resistant strains makes effective treatment complicated.

Stereotactic (stereotaxic) surgery is a minimally invasive form of surgery in which three-dimensional systems of coordinates are used to chart the locations of precise spots in the body so surgeons can perform actions on them such as injections, stimulations, biopsies, or radiosurgery[2]. The main applications of stereotactic surgery in rodents are introducing fluids directly to the brain or implanting cannulae and microdialysis probes[3].

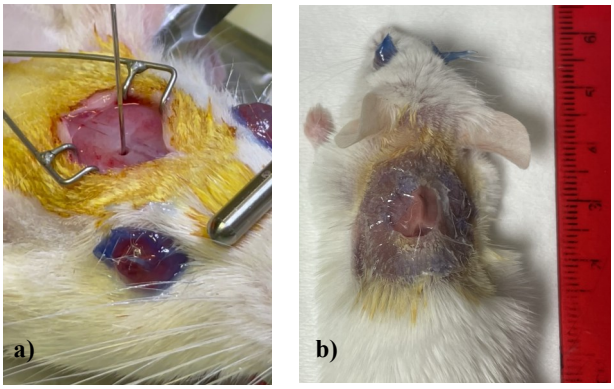

Fig. 1**. a) Stereotactic brain infection; b) Full-thickness skin model**

We use stereotactic surgery to create rodent brain infection models. The reason for creating these models is the research of brain infection therapies focused on developing and progressing related pathophysiological changes, including inflammation and loss of adequate response or function to stimulus.

The sensitivity and specificity of infection agent imaging improved with the synthesis and use of radiotracers with better translational potential. However, radiotracers used for these methods still lack the capability of measuring symptoms of microbial infections. There is progress in identifying infections caused by infection agents (e.g., bacteria, molds, yeasts) with the use of computed tomography, positron emission tomography, and magnetic resonance. We test and develop these imaging methods with the assumption they should be capable of measuring the presence and number of different infection agents.

All aspects of animal studies meet the acceptability criteria for the care of laboratory animals and experimental use as described in Act 246/1992 Coll. as amended.

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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L-28

SURVEILLANCE AND MOLECULAR CHARACTERIZATION OF MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA ISOLATES FROM PATIENTS IN OLOMOUC UNIVERSITY HOSPITAL AND OLOMOUC MILITARY HOSPITAL, 2020–2022

KRISTÝNA NOVÁKOVÁ[a], IVA SUKKAR[b], IVA VÁGNEROVÁ[a], VENDULA PUDOVÁ[a]

[a] Department of Microbiology, Faculty of Medicine and Dentistry, Palacký University Olomouc and University Hospital Olomouc, Czech Republic, Hněvotínská 3, 775 15 Olomouc, [b] Central European Institute of Technology, University of Veterinary Sciences Brno, Czech Republic Palackého tř. 1946/1, 612 42 Brno-Královo Pole, Czech Republic
kristyna.novakova@fnol.cz

Multidrug-resistant (MDR) strains of *Pseudomonas aeruginosa* (PSAE) represent a large-scale problem in healthcare due to low effectiveness of antimicrobial therapy. In addition to the significant PSAE resistance, the increasing prevalence of MDR PSAE strains is also becoming dangerous. Therefore, it is necessary to focus on the molecular characterization and surveillance analysis of MDR PSAE isolates, based on which it will be possible to identify and subsequently monitor dangerous epidemiological sequence types (STs) associated with MDR phenotype.

Whole-genome sequencing of selected 127 PSAE isolates showing the MDR phenotype was performed. These isolates were recovered from 104 patients hospitalized mainly in the Intensive Care Department of the Olomouc Military Hospital (MHO) and the Department of Anaesthesiology, Resuscitation and Intensive Care (DARIC) of the Olomouc University Hospital (UHO) in 2020–2022. Research scheme can be seen in Figure 1.

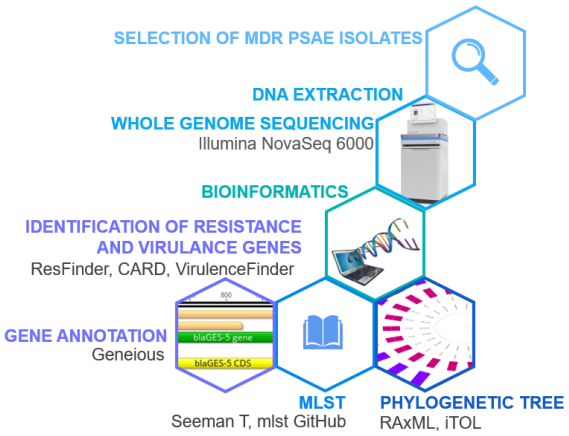

Fig. 1. **Research scheme**

Whole-genome sequence data were analyzed using various bioinformatic tools. The bioinformatic analysis provided information on the presence of resistance genes[1,2], genes encoding virulence factors[3] and sequence types (ST)[4]. Phylogenetic trees were created based on the difference in the number of single nucleotide polymorphisms for those isolates that were assigned to the most represented STs[5]. Due to the risk of the spread of resistant determinants in a horizontal way through mobile genetic elements, the genetic surroundings of genes encoding production of metallo-beta-lactamases (MBL) were identified.

From the analysis of 127 MDR PSAE isolates, ST175 was the most frequently represented (n = 38/127 isolates). PSAEs of this ST were isolated from 29 patients, 19 of whom were hospitalized at DARIC UHO. However, if we assume the number of patients from whom the isolates were obtained, the most frequent ST was 357 (n = 31/104 patients), corresponding to 35 isolates.

All ST357 isolates showed phenotypic resistance to meropenem. This ST is associated with the presence of the bla_{IMP-7} gene encoding production of MBL, which was detected in 31 of 35 isolates. Three isolates of the ST 357 harboured bla_{VIM-2} gene whose expression is also associated with the phenotypic resistance to carbapenems. One isolate of ST357 did not harbour any MBL gene. Our data regarding the dominant occurrence of ST357 correlates with data from previous publication. The clonal spread of this ST was documented in the Czech Republic in 2015[6]. According to our results, this epidemiologically significant ST is still circulating in the healthcare facilities, and its monitoring should be the subject of ongoing epidemiological studies.

Acknowledgement
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P-56
POSITRON EMISSION TOMOGRAPHY IMAGING OF KLEBSIELLA PNEUMONIAE INFECTION

KATEŘINA DVOŘÁKOVÁ BENDOVÁ[a], MICHAELA ZAPPEOVÁ[a], KRISTÝNA KRASULOVÁ[a], BARBORA NEUŽILOVÁ[a], PATRIK MLYNÁRČIK[b], MILOŠ PETŘÍK[a]

[a] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Hněvotínská 5, Olomouc 779 00, [b] Department of Microbiology, Faculty of Medicine and Dentistry, Palacky University, Hněvotínská 3, Olomouc 775 15, Czech Republic
katerina.dvorakovabendova@upol.cz

Bacterial infections are progressively more threatening due to the growing antimicrobial resistance. The situation is even more dangerous in hospital setting, where quick diagnosis is crucial to prevent the spread of the pathogen through the medical environment. This study is focused on *Klebsiella pneumoniae* (*KP)*, bacterial pathogen that is increasingly more challenging to treat due to the scarcity of effective treatment.

Here we present the use of radiolabelled siderophores, low molecular weight chelators produced by bacteria, for diagnosis of *KP* infection by positron emission tomography. The aim of this study is to explore siderophores selected based on previous *in vitro* experiments (salmochelin S4, enterobactin, coprogen and ferrirubin) and evaluate the most perspective one for infection imaging.

Selected siderophores were radiolabelled with gallium-68. The radiochemical purity of resulting complexes was assessed on radio-iTLC. *In vitro* uptake was compared in *KP* growth in minimal and complete media. PET/CT imaging was performed in healthy mice and in two infectious models induced by *KP:* murine model of acute myositis and rat model of acute pneumonia.

All selected siderophores were radiolabelled with high radiochemical purity. We observed significant uptake in *KP* cultures cultured in rich medium and the highest uptake overall was achieved with [^{68}Ga]Ga-Ferrirubin. *In vivo* biodistribution showed renal excretion and low activity in blood 90 min p. i. for most siderophores. In addition, [^{68}Ga] Ga-Salmochelin S4 showed low activity in the liver and [^{68}Ga]Ga-Enterobactin was heavily accumulating in the gall bladder and intestine. In mouse model of acute myositis, the highest signal in the site of infection was observed with [^{68}Ga] Ga-Ferrirubin. In rat model of acute pneumonia, we observed a signal accumulation in the infected tissue.

We have demonstrated that radiolabelled siderophores can be used for *KP* infection imaging. Furthermore, from the selected siderophores, [^{68}Ga]Ga-Ferrirubin appears to be the most perspective one.

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P-57
INFLUENCE OF SIDEROPHORE CHIRALITY ON THEIR USE FOR MOLECULAR IMAGING

KRISTYNA KRASULOVA, BARBORA NEUZILOVA, KATERINA DVORAKOVA-BENDOVA, ZBYNEK NOVY, MIROSLAV POPPER, MILOS PETRIK*

Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, 779 00 Olomouc, Czech Republic
kristyna.krasulova@upol.cz

Siderophores are iron chelators produced by fungi and bacteria. Iron is an essential element for microbial physiological processes, including virulence. Iron can be replaced by 68-gallium in the siderophore complex without loss of activity and subsequently used for *in vivo* positron emission tomography (PET) imaging of infection. Ferrirhodin (FRH) and ferrirubin (FR) are microbial ferrichrome-type *cis-trans* isomeric siderophores. It is known that the specific three-dimensional structure of the iron-siderophore complex is responsible for recognition to receptors, transporters and/or iron utilization, and moreover, the stereospecificity of the compounds may affect their pharmacokinetic profile. Here we report on the influence of siderophore chirality on their use for molecular imaging of infections.

FR and FRH were labeled with 68-gallium using acetate buffer. Its radiochemical purity was measured on RP-HPLC. The basic *in vitro* characteristics of ^{68}Ga-FR and of ^{68}Ga-FRH were determined. *Ex vivo* biodistribution studies were performed at 30 and 90min after injection, as well as *in vivo* PET/CT imaging in healthy Balb/c mice. *In vitro* uptake of both ^{68}Ga-siderophores was evaluated in different microbial pathogens and specific uptake was also investigated.

Tested siderophores were radiolabeled with high (> 95%) radiochemical purity. ^{68}Ga-FRH showed less hydrophilic properties (log P = –1.91 vs. –2.72) and higher protein binding values (~ 50 % vs. 6 % by 120 min incubation) than ^{68}Ga-FR. ^{68}Ga-FR displayed rapid renal clearance with low retention in blood (1.59 ± 0.06 %ID/g 30 min) and fast clearance from examined organs, whereas ^{68}Ga-FRH showed moderate retention in blood (5.98 ± 0.35 %ID/g 90 min) and in perfused organs. These results were in accordance with PET/CT imaging studies. Specific uptake of ^{68}Ga-FR and ^{68}Ga-FRH into *S. aureus*, *P. aeruginosa* and *K. pneumoniae* was proven *in vitro*. Uptake could be inhibited by use of heat-inactivated bacteria and incubation in an iron-sufficient media.

Both studied isomers were readily radiolabeled with ^{68}Ga. Their pharmacokinetic profile was different. However, the *in vitro* uptake of both ^{68}Ga-siderophores was comparable. In this case, chirality played a minor role in microbial recognition. In a certain case both can be used for PET/CT imaging of infection, however ^{68}Ga-FR has better properties. The effect of the chirality on the properties of siderophores for molecular imaging application was demonstrated.

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P-58
CIRCULATING AND SALIVARY DNA-BASED BIOMARKERS FOR EARLY DIAGNOSIS AND RECURRENCE MONITORING OF OPSCC

ONDREJ BOUSKA[a], VLADIMIRA KOUDELAKOVA[a,b,*]

[a] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, 779 00 Olomouc, [b] Laboratory of Experimental Medicine, University Hospital Olomouc, 779 00 Olomouc, Czech Republic
vladimira.koudelakova@upol.cz

Oropharyngeal squamous cell carcinoma (OPSCC) incidence has significantly increased over the last decades. OPSCCs are etiologically divided into two distinct groups: human papillomavirus (HPV) related OPSCCs and non-HPV OPSCCs. The overall number of annually diagnosed OPCs has more than tripled during the last 30 years in the Czech Republic. Almost 800 OPC cases were newly diagnosed in 2021[1], making OPSCCs more prevalent than etiologically related cervical cancers. As well as in cervical cancer, risk stratification, early diagnosis, and eventually locoregional recurrence monitoring methods are needed. Liquid biopsies, including blood and saliva, represent a promising approach for OPSCC management. This study aims to validate the applicability of liquid biopsies and DNA-based biomarkers for early OPC diagnosis and its recurrence in clinical practice.

In this study, newly diagnosed OPSCC patients (prospective group) and patients in remission (retrospective group) were enrolled. HPV tumor status was determined by combined high-risk HPV detection and p16 immunohistochemistry. Pre & post-treatment HPV testing in gargle lavage (GL), oropharyngeal swabs (OPS), and plasma samples were performed, followed by regular testing according to the standard follow-up protocol.

In total, 85 OPSCC patients have been enrolled. HPV-related OPSCC was diagnosed in 87% (65/75) of cases, while the HPV16 genotype was detected in 100 % of cases. GL and OPS's sensitivity (SE) and specificity (SP) for newly diagnosed HPV-related OPSCCs were 80%, 90.5%, and 100%, 100%, respectively. Detection of circulating tumor HPV DNA showed 92% SE and 100% SP. Post-treatment/ follow-up HPV infection remained persistent in 8 OPSCC patients. In conclusion, these preliminary data show a predominant incidence of HPV-related OPSCCs compared to non-HPV OPSCCs. At diagnosis, combined HPV testing in saliva and plasma showed excellent sensitivity.

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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P-59
ANALYSIS OF BETA-LACTAMASES IN BACTERIA

PATRIK MLYNÁRČIK[a], VERONIKA ŽĎÁRSKÁ[a], JIŘÍ SCHMIDT[b], KRISTÝNA HRICOVÁ[a], KRISTÝNA MEZEROVÁ[a], JAN BARDOŇ[a,c], PETRA PROCHÁZKOVÁ[a], MILAN KOLÁŘ[a]

[a] Department of Microbiology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Hněvotínská 3, 775 15 Olomouc, Czech Republic, [b] Department of Biotechnology, Faculty of Science, Palacky University Olomouc, 17. Listopadu 1192/12, 779 00 Olomouc, Czech Republic, [c] State Veterinary Institute Olomouc, Jakoubka ze Stříbra 462/1, Nové Sady, 779 00 Olomouc, Czech Republic
patrik.mlynarcik@upol.cz

In our research, we are looking at bacterial resistance to antibiotics, which is a large and growing problem today. Since 1929, antibiotics were first introduced to the public, and resistant bacteria have emerged. Since then, resistant strains have been increasing, and we are running out of ways to deal with them. It is predicted that by 2050, bacteria will be resistant to all known antibiotics, and people will be at risk of dying from trivial infections. Bacteria have evolved different mechanisms to resist antibiotics. We focus on their ability to produce inactivating enzymes, specifically beta-lactamases. We are identifying and analyzing genes encoding these enzymes, such as the AmpC type EC beta-lactamases, and designing primers to search for them. In this way, it is possible to detect the presence of resistant strains in a particular environment, discover new resistance genes in specific strains, or confirm their presence.

Regarding the EC-type AmpC beta-lactamases, our results suggest that the primers tested could be used for PCR to detect and monitor the spread of these genes to other bacterial species. This study also provides more information on point mutations in these enzymes and EC variants with an extended spectrum of action on higher-generation cephalosporins.

In another scientific area, we focused on determining resistance to beta-lactams in bacteria isolated from a chicken farm in Moravia. One hundred twenty-three strains of beta-lactam-resistant bacteria were analysed, and 16 different beta-lactamase classes were identified. At the same time, new putative beta-lactamases were also identified.

Our other results on beta-lactamases provide comprehensive information on the occurrence of these enzymes in different bacterial genera. In addition, the distribution of putative beta-lactamases in many clinical and environmental isolates was provided. In addition, although we could not determine precisely which factors regulate the presence of beta-lactamases in specific bacteria, we found that these factors do not include the proportion of regulatory genes, genome size, or other factors.

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P-60

EVALUATION OF THE MODIFIED IN-HOUSE METHOD FOR DIRECT IDENTIFICATION OF POSITIVE BLOOD CULTURES BY MALDI-TOF MS TECHNOLOGY AND RAPID ANTIMICROBIAL SUSCEPTIBILITY TESTING (RAST) ACCORDING TO THE PROTOCOL

KATEŘINA BOGDANOVÁ, IVA VÁGNEROVÁ, MIROSLAVA HTOUTOU SEDLÁKOVÁ*, MILAN KOLÁŘ

Department of Microbiology, University Hospital Olomouc, Zdravotníků 248/7, 779 00, Olomouc, Czech Republic and Faculty of Medicine and Dentistry, Palacky University Olomouc, Hnevotinska 3, 779 00, Olomouc, Czech Republic
MiroslavaHtoutouSedlakova@fnol.cz

The standard blood-culture examination is dependent on blood cultivation, and it takes more than 48 hours to get a definitive result including an antibiogram. However, in patients with sepsis, mortality increases by 7% with each hour of delay in the application of adequate antibiotic therapy[1]. At the Department of Microbiology, University Hospital Olomouc, Czech Republic, a modified rapid in-house method for the identification of agents by MALDI-TOF MS directly from blood cultivation bottles was introduced and recently certified[2]. The sensitivity of this method is esspecially high in gram-negative bacilli and enterococci (97% in Enterobacterales, resp. 100% in enterococci). The high specificity of this method for all species (97%) suggests that it is a reliable method, which should be additional to standard microbiological blood-culture examination. Thanks to the in-house MALDI-TOF MS method the identification procedure was shortened by 3–24 hours[3].

In addition to identifying the bacterial agent, it is important to determine susceptibility/resistance to antibiotics as soon as possible. Therefore, at the Department, the Rapid Antimicrobial Susceptibility Testing (RAST) according to the EUCAST protocol is used[4]. This method allows the antibiogram to be obtained in 4, 6, and 8 hours, i.e. on the same day that the blood-culture is detected as positive.

The introduction of the new methods into the algorithm of the blood-culture examination enabled to shorten the time from blood collection to the result and to speed up the administration of adequate antibiotic therapy. The results and experience with the new algorithm including the in-house MALDI-TOF MS method and RAST will be presented.

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INSTITUTE OF MOLECULAR GENETICS OF THE CAS

L-29

RESOLVING THE CONFUSION OVER THE TWO SPECIFIC CELLULAR RECEPTORS FOR SYNCYTIN-1 AND OTHER ENDOGENOUS RETROVIRUSES

KRYŠTOF ŠTAFL[a,b], MARTIN TRÁVNÍČEK[a,b], JIŘÍ HEJNAR[a,b]*, KATEŘINA TREJBALOVÁ[a,b]*

[a] *Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 14220 Prague 4, Czech Republic,*
[b] *National Institute of Virology and Bacteriology*
katerina.trejbalova@img.cas.cz

syncytin-1 is a human protein-coding gene of retroviral origin. During human placenta morphogenesis, the interaction of Syncytin-1 with the transmembrane protein ASCT2 (Alanine, Serine, Cysteine Transporter 2) triggers cell-to-cell fusion of trophoblasts, resulting in the formation of a syncytiotrophoblast, a large multinucleated syncytium[1,2]. The syncytiotrophoblast is the outermost surface of the human placenta. It facilitates the transport of nutrients and waste products across the maternal-fetal interface, produces hormones and cytokines, and protects the fetus from pathogens and the mother's immune system. Altered behavior of Syncytin-1 may contribute to human placenta pathologies, including preeclampsia, low platelet syndrome/intrauterine growth restriction, and gestational trophoblastic diseases, e.g., mole and choriocarcinoma[3].

ASCT1, a sodium-dependent neutral amino acid transporter with a related structure to ASCT2, was postulated as an alternative Syncytin-1 cellular receptor[1,4]. Furthermore, both ASCT1 and ASCT2 have been reported to be receptors for the largest interference group, the RD114-and D-type (RDR) retroviruses. The contribution of both receptors to Syncytin-1-induced cell-cell fusion, as well as the receptor preference of RDR retroviruses, remain to be understood.

In this study, we investigated the individual involvement of each receptor in the interaction with Syncytin-1 using three quantitative molecular assays (Fig. 1). We measured the infection with the Syncytin-1-pseudotyped virus triggered by the ASCT2 or ASCT1 receptors independently. Further, we assessed the binding of Syncytin-1 to ASCT2 vs. ASCT1 on the cell surface. Finally, we determined the Syncytin-1 fusogenic activity induced by the interaction with ASCT2 or ASCT1. Our results demonstrate that ASCT1 is at least two orders of magnitude less active as a receptor than ASCT2, which casts doubt on the importance of ASCT1 for syncytiotrophoblast differentiation.

Interestingly, certain members of the RDR group are able to use both receptors efficiently. These findings demonstrate the variability of the interaction between homologous envelope glycoproteins and a particular receptor molecule.

Our results highlight the function of ASCT2 during placental development and challenge the physiological role of ASCT1 during Syncytin-1-induced fusion.

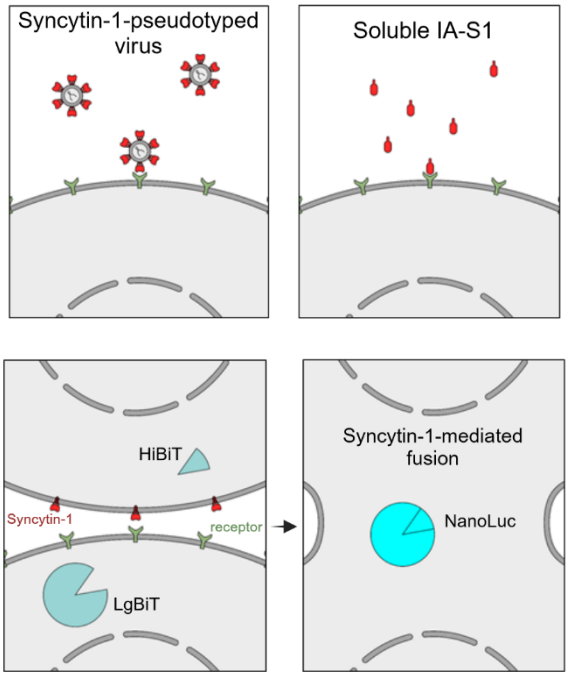

Fig. 1. **Schematic depiction of the analytic tools: infection with the Syncytin-1-psedotyped virus (upper left scheme), Syncytin-1 binding to the receptor (upper right scheme), Syncytin-1-induced fusion (lower scheme).**

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L-30
REGULATION OF T-CELL ACTIVATION DURING THE IMMUNE RESPONSE AGAINST INFECTIONS

SARKA JANUSOVA, ONDREJ STEPANEK*

Institute of Molecular Genetics of the Czech Academy of Science, 142 20 Prague, Czech Republic
sarka.janusova@img.cas.cz

T cells play a critical role in the immune system. Their activation and effector functions are tightly regulated. For the full activation, T cells require the antigenic signal as well as costimulatory signals through CD28 and TNF-receptor superfamily receptors, such as GITR, OX40, and CD137, which trigger downstream signaling events, including NF-κB or MAPK pathways to prolong T-cell survival, proliferation, and effector functions[1].

Using mass spectrometry, we identified novel signaling components of the GITR and OX40 proximal signaling complexes, including A20-binding inhibitor of NF-κB1 (ABIN1), a polyubiquitin binding protein that serves as a negative regulator of TNFRI and MyD88 signaling. However, very little was known about its role in T cells[2].

We addressed the role of ABIN1 in T cells using *Abin1*$^{-/-}$ mice. Antigenic stimulation of *Abin1*$^{-/-}$ CD8^{+} T cells showed more robust proliferation and increased expression of major effector molecules Granzyme B and IFNγ than WT control cells *ex vivo*. As *Abin1*$^{-/-}$ T cells showed higher phosphorylation of p38 kinase and the p38 inhibitor reduced the *ex vivo* proliferation and Granzyme B and IFNγ expression upon activation, we propose that *Abin1*$^{-/-}$ is a negative regulator of p38 activation.

The phenotypic analysis of the lymphocyte compartment of *Abin1*$^{-/-}$ mice showed increased regulatory T cell and decrease in CD8^{+} T cell populations. The analysis of WT + *Abin1*$^{-/-}$ mixed bone marrow chimeras showed that these effects are intrinsic. We crossed the *Abin1*$^{-/-}$ mice with OVA-specific OT-I TCR transgenic *Rag2*$^{-/-}$ mice to prevent the overt inflammation observed in the mice and to study the intrinsic role of ABIN1 in CD8^{+} T cells, which were adoptively transferred to polyclonal congenic Ly5.1 mice. *Abin1*$^{-/-}$ OT-I T cells were hyperresponsive to cognate transgenic Listeria monocytogenes-OVA and LCMV-OVA infections and formed more short-lived effector cells than WT OT-I cells. They also showed higher infiltration of the cognate MC-38-OVA tumors than the control cells.

In conclusion, we uncovered that ABIN1 is a negative regulator of T-cell activation *ex vivo* and *in vivo*. As such, it is a potentially target for future immunomodulatory therapies.

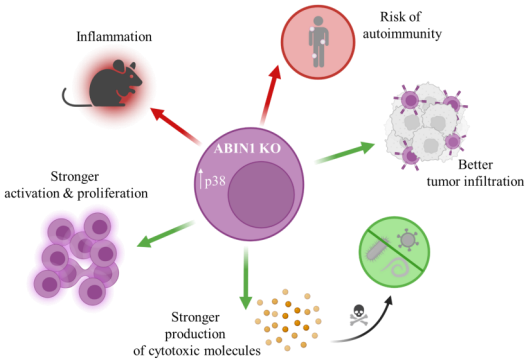

Fig. 1. **Scheme of altered properties of T cells after depletion of ABIN1.**

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P-61
DISCOVERY OF NOVEL AVIAN INTERFERON REGULATORY FACTORS

LENKA UNGROVÁ[a], JIŘÍ NEHYBA[a], VERONIKA KRCHLIKOVÁ[b], TOMÁŠ HRON[a], JIŘÍ HEJNAR[a], DANIEL ELLEDER[a]*

[a] Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic,
[b] Institute for Medical Virology and Epidemiology of Viral Diseases, Elfriede-Aulhorn-Str. 6, 72076 Tuebingen, Germany
daniel.elleder@img.cas.cz

Interferon regulatory factors (IRFs) are transcription factors that play a key role in anti-viral immunity response of vertebrates. After pathogen recognition by RIG-I-like receptors (RLR), IRF3 and IRF7 activate expression of IFN-β which is together with other type I interferons responsible for expression of interferon-stimulated genes (ISGs) through JAK-STAT1-IRF9 pathway[1,2]. These pathways are well described in mammals and some other vertebrates but there is little information available in birds. Moreover, IRF9 and IRF3 are thought to be missing in birds[4,5] even though both of the pathways are functional.

Using *in silico* comparative analysis and de novo assembly, we were able to annotate IRF9 in multiple avian species across most avian orders, and IRF3 in paleognath birds. We used duck fibroblast cell line as a model system for characterization of avian IRF9. For the first time, we show the role of duck IRF9 (dIRF9) in type I IFN pathway and its role in regulation of ISGs expression after interferon stimulation. Our results suggest time-dependent decrease in the induction of ISGs in the absence of dIRF9 that is partially restored in cells with stable expression of endogenous dIRF9 as well as in cells transiently transfected with endogenous dIRF9. We also focused on a general effect of the dIRF9 absence in cells with or without interferon stimulation using RNAseq data. In summary, we provide the first characterization of previously unknown avian IRF genes.

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P-62
FUNCTIONAL RECONSTRUCTION OF SYNCYTIN-2 ENVELOPE GLYCOPROTEIN

KRYŠTOF ŠTAFL[a,b], LUKÁŠ JECH[a,b], MARTIN TRÁVNÍČEK[a,b], VERONIKA KRCHLÍKOVÁ[c], JIŘÍ HEJNAR[a,b], KATEŘINA TREJBALOVÁ[a,b]

[a] Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 14220 Prague 4, Czech Republic,
[b] National Institute of Virology and Bacteriology,
[c] Institute for Medical Virology and Epidemiology of Viral Diseases, University Hospital Tübingen, Elfriede-Aulhorn-Straße 6, 72076 Tübingen, Germany
krystof.stafl@img.cas.cz

Syncytin-2 is a membrane protein expressed in the human placenta[1]. After interaction with its partner, MFSD2a, Syncytin-2 facilitates membrane fusion of cytotrophoblast cells into a multinucleated syncytiotrophoblast[2]. This layer acts as a fetomaternal barrier, facilitating the selective transport of nutrients and metabolites and protecting the fetus against the mother's immune system. The fusogenic function of Syncytin-2 relates to its origin. A retrovirus enveloped with Syncytin-2 infected a human ancestor 40 million years ago and integrated into its DNA as HERV-FRD[1]. Since then, Syncytin-2 has kept its ability to recognize MFSD2a receptor and mediate membrane fusion. However, it has lost the ability to envelop an infectious virus.

We aim to identify and revert the mutations in Syncytin-2 that restrict the original envelope function. Namely, we address its expression at the level of splicing and signal peptide recognition, and we are especially interested in its cytoplasmic tail, which probably modulates the fusogenic activity and blocks its incorporation into budding virions. Modifications of these regulatory regions may help release infectious viral particles.

Such an infectious virus in non-replicative settings will be exploited in future research of MFSD2a-Syncytin-2 interactions and mechanisms of antiviral innate immune response. Moreover, we would like to investigate the molecular link between human diseases and Syncytin-2 function.

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P-63
ENLIGHTENING THE QUIETNESS – THE SEARCH FOR EARLY SILENCED PROVIRUSES

JAKUB KAŇKA, DALIBOR MIKLÍK, JIŘÍ HEJNAR

Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Praha 4, Czech Republic
jakub.kanka@img.cas.cz

Integration of retroviral DNA into the genome of an infected cell is a hallmark of retroviral infection. Genomic and epigenomic features at the site of integration may determine the expression of retroviral genes. While some proviruses (integrated retroviral genome) are stably expressed for several months, other proviruses are silenced shortly after integration. In the case of HIV infection, provirus silencing is the first step leading to the establishment of the latent reservoir *in vivo*, the main hurdle in curing HIV infection. Thus, understanding the mechanisms involved in early proviral silencing is crucial for the functional cure of HIV.

Currently, it is possible to distinguish between transcriptionally active and inactive proviruses 2–3 days after transduction. However, whether silenced proviruses were ever transiently active after integration remains to be known.

To determine this, we developed a novel detection system. First, sensor cells were generated using the transposon-mediated insertion of a detection cassette. After the sensor cells were established, a replication-defective retroviral vector transducing a recombinase gene was used as an activator. In this system, any cell in which the recombination is detected without the expression of the proviral marker (GFP) contains an early silenced provirus. These cells could then be obtained and further studied.

Our data from initial experiments demonstrate that the sensor cells can be prepared within 2–3 weeks using the *piggyBac* transposon system. Contrary to published data, the use of *piggyBac* transposon in our system showed that most human embryonic kidney cells (293T) transduced by avian sarcoma leukosis virus (ASLV) contained proviruses that were transiently active after integration. This suggests that early proviral silencing predominantly does not occur immediately after, but within several hours after integration.

Our approach can be further applied to virtually any model of retroviral infection, helping to elucidate conditions under which a temporal proviral expression occurs and processes integral to it.

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P-64
RAPID ADAPTIVE EVOLUTION OF AVIAN LEUKOSIS VIRUS TO BIOTECHNOLOGICALLY INDUCED HOST RESISTANCE

MAGDA MATOUŠKOVÁ, JIŘÍ PLACHÝ, DANA KUČEROVÁ, LUBOMÍRA PECNOVÁ, VÍT KARAFIÁT, JIŘÍ HEJNAR*

Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic
magda.matouskova@img.cas.cz

Recently, chickens resistant to avian leucosis virus subgroup J (ALV-J) were developed by deleting a single amino acid, W38, in the ALV-J receptor NHE1 using the CRISPR/Cas9 technique[1]. This resistance was confirmed both *in vitro* and *in vivo*, and W38$^{-/-}$ chicken embryonic fibroblasts (CEF) displayed resistance to all tested virus strains *in vitro*. However, viruses evolve very rapidly, and the capacity of ALV-J adaptation to the resistant receptor warrants further investigation.

We established reporter virus-based assay to select for adapted ALV-J variants. We assumed that escape mutations within the envelope protein could overcome cellular resistance *in vitro*. In accordance with this assumption, we isolated numerous escape virus variants and sequencing of their *env* genes revealed eight single nucleotide substitution mutations. To confirm the adaptive capacity of these substitutions, we introduced these mutations into a retroviral vector RCASBP(J)GFP and tested the virus entry into cells with modified receptor. All eight variants replicated effectively *in vitro* in W38$^{-/-}$ CEFs. Notably, two of these variants successfully induced tumors in W38$^{-/-}$ chickens.

Our results illustrate that cellular resistance, ensured by minor receptor modifications, can be overcome by a single nucleotide mutation that evolves rapidly in ALV-J.

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P-65
LET THERE BE LIGHT: A SENSITIVE LUCIFERASE ASSAY FOR QUANTIFYING CELL-CELL FUSION

MARTIN TRÁVNÍČEK[a,b], KRYŠTOF ŠTAFL[a,b], LUKÁŠ JECH[a,b], JIŘÍ HEJNAR[a,b], KATEŘINA TREJBALOVÁ[a,b]

[a]Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 14220 Prague 4, [b] National Institute of Virology and Bacteriology, Czech Republic
martin.travnicek@img.cas.cz

The successful formation of the mammalian placenta depends on the expression of genes of retroviral origin. Among these genes are human *syncytin-1* and *syncytin-2*, which encode retroviral envelope glycoproteins capable of triggering the fusion of phospholipid membranes. During evolution, both Syncytins were repurposed to facilitate the fusion of placental cells, introducing a novel physiological role. Within the placental cytotrophoblast, Syncytin-1 employs the neutral amino acid transporter ASCT2 as its specific membrane receptor[1], while Syncytin-2 engages with the lysophosphatidylcholine transporter MFSD2a[2]. These interactions induce a fusion of cytotrophoblast cells, leading to the creation of a multinucleated syncytiotrophoblast–a tissue that coordinates the exchange of nutrients, waste metabolites and hormones between the fetus and the mother. The principal function of the human placenta is thus dependent on the fusogenic activity of Syncytin proteins.

Our work focused on the development of a reliable and sensitive assay for cell-cell fusion assessment. In previous studies on Syncytins, various methods were used to quantify fusogenic activity. To detect fusion events, these methods often relied on manual nuclei counting within fused cells or utilized the split GFP complementation system. However, these approaches demanded extensive image analysis, which was time-consuming and sometimes not very accurate. To address this, we have created a new assay based on the complementation of two luciferase fragments, which reconstitute into a functional enzyme after cell-cell fusion is induced. We show that our method can be used to explore the fusogenic features of several retroviral proteins, their interaction with receptors, and even inhibitors. A deeper understanding of these interactions could shed light on the mechanisms contributing to the development of syncytiotrophoblast-related diseases.

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P-66
THE ROLE OF LCK IN THE FORMATION OF EFFECTOR AND MEMORY T CELLS

VALERIA ULERI, ONDREJ STEPANEK*

Institute of Molecular Genetics of the Czech Academy of Science, Videnska 1083, 142 20, Czech Republic
valeria.uleri@img.cas.cz

T cells play a critical role in the immune response, defending the host against a wide range of pathogens, viruses, cancer, and allergies.

To ensure protection, T cells use their unique T cell receptor (TCR) in combination with co-receptors to recognize their cognate antigen presented on the antigen-presenting cells[1]. Upon antigen recognition, T cells trigger a signaling cascade that turns into transcriptional changes and T cell activation.

The first biochemical event after the antigen recognition is the phosphorylation of the immunoreceptor tyrosine-based activation motifs in the CD3 chains by Src-family kinases (SFK), primarily by LCK and, to a lesser extent, by FYN[2].

Previous studies demonstrated the importance of LCK in T cell development, still its role in periphery is not fully understood.

Therefore, the main aim of this study is to define the role of LCK and co-receptors in T cell signaling in peripheral T cells and investigate their importance in T cell differentiation into effector or memory T cells. Our preliminary results, obtained using CD8+ T cells isolated from LCK KO and LCK WT OT-I mice and adoptively transferred into congenic Ly5.1 hosts, show that LCK KO OT-I cells are able to expand despite producing weaker immune responses than their WT counterparts. We expected that LCK KO OT-I cells would receive a lower activation signaling and, therefore, resemble the immunological response produced after the encounter of T cells with a low affinity antigen. Instead, FACS analysis conducted 6 and 30 days after infection showed that LCK KO OT-I form more KLRG1+ CD127- cells in comparison to WT. These results suggest that the recruitment of the LCK to the immunological synapse might be important in ensuring proper cell polarization during division. Consequently, we believe that LCK KO T cells might be committed toward the effector lineage rather than memory. This study will contribute to a better understanding of T cell biology and could have potentially important implications for the development of novel immunotherapies.

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UNCOVERING THE DIFFERENCES BETWEEN RESPIRATORY PATHOGEN-INDUCED T-CELLS

ANNA KRATOCHVÍLOVÁ[a], JANA HOLUBOVÁ[b], ONDŘEJ STANĚK[b], PETR ŠEBO[b], ONDŘEJ ŠTĚPÁNEK[a]

[a] *Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic,* [b]*Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic*
anna.kratochvilova@img.cas.cz

The upper respiratory tract (URT) is a site of entry for many public health-concerning pathogens such as SARS-CoV-2 (ref.[1]), the Influenza virus[2], or *Bordetella pertussis*[3]. Although the URT represents an important site for initiating and transmitting infection, understanding the site-specific immunity in the nasal tissue during different infections is limited. So far, most works focused on respiratory infections have been concerned only with the lower respiratory tract (LRT)[2]. The adaptive immune system of URT consists of nasal-associated lymphoid tissue, which is a highly organized lymphoid structure with T and B cell areas and dispersed Tissue-resident memory T cells (Trm). Trm cells are a subset of memory T cells, which reside in non-lymphoid tissues, where they can act as alarm sensors in the immune surveillance network or as cytotoxic cells. Due to their advantageous location, they can be part of the first line of defense against many infections. The mechanism of development and the diversity and function of Trm cells are not yet completely understood[4]. We hypothesize that different infections such as intracellular viral infection or extracellular bacterial infection give rise to phenotypically and functionally distinct T-cell subsets. Moreover, $CD4^+$ and $CD8^+$ Trm cells induced by these infections might differ in their ability to persist in URT or LRT and respond to re-infection. To address this hypothesis we utilized two infection models: viral infection (Influenza A) and bacterial infection (*Bordetella pertussis*). We were able to identify various key differences between these two infections which will be further studied.

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