NIVB Meeting 2022

30th November – 2nd December, 2022

Kutná Hora, Czech Republic

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

Organizers:

Institute of Organic Chemistry and Biochemistry of the CAS

Masaryk University

Charles University

University of Chemistry and Technology Prague

Palacký University Olomouc

Institute of Molecular Genetics of the CAS

Institute of Microbiology of the CAS

Biology Centre CAS

Book of abstracts

edited by

Šárka Šímová

National Institute of Virology and Bacteriology

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

Dear virologists and bacteriologists, dear colleagues,

The first annual meeting of the National Institute of Virology and Bacteriology (NIVB) in Kutná Hora on 30.11.–2.12. is an opportunity for participating teams to meet, discuss excellent science, establish collaborations, and introduce the project to the members of our newly established International Science Advisory Board. The primary objective of NIVB is to mediate the cooperation among the 28 participating research teams from 8 Czech research institutions: Institute of Organic Chemistry and Biochemistry, Institute of Molecular Genetic, and Biological Centre of the Czech Academy of Sciences, Charles University in Prague, Masaryk University in Brno, the University of Chemistry and Technology in Prague, and Palacký University in Olomouc. We are delighted that it was possible to arrange the meeting of all the participating research groups, which reflects the scientific breadth of NIVB. The research interests of the participating groups can be broadly assigned into the programs of (1) Pathogen-host interactions, (2) Immunity against viruses and bacteria, and (3) Treatments for viral and bacterial infections. We thank all participants for contributing 28 presentations and 65 posters to the meeting. This conference is a pioneering endeavor, the first meeting that will bridge the virology and bacteriology communities from the Czech Republic. While the research interests of all the participating groups are focused on virology and bacteriology, the contributing laboratories are very diverse in the research methodologies they employ. Therefore, the meeting has the potential to enable the establishment of new scientific collaborations among the participating teams, which is the main objective of NIVB.

NIVB Meeting 2022 has the ambition to launch a new series of annual meetings on virology and bacteriology and aims at informing wider scientific community about new development, trends and issues in these scientific fields.

We are looking forward to seeing you at the NIVB meeting.

Zdeněk Hostomský, Pavel Plevka, Iva Pichová, Nikola Kostlánová, and Šárka Šímová

INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY OF THE CAS

L-01
NOVEL ANTIBACTERIAL COMPOUND LIPOPHOSPHONOXINS: DESIGN, SYNTHESIS, EVALUATION, AND APPLICATIONS

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Most of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi.[1] Medicinal chemistry has played a key role in modifying natural products to optimize their pharmacological properties, while minimizing toxicity.[2] Nevertheless, bacterial diseases resistant to currently available drugs already cause at least 700,000 deaths globally a year, including 230,000 deaths from multidrug-resistant tuberculosis, a figure that could increase to 10 million deaths globally per year by 2050 under the most alarming scenario if no action is taken.

LPPOs are small amphiphilic molecules bearing positive charge(s). LPPO consist of four modules: (i) a nucleoside module NM, (ii) a polar module PM, (iii) a hydrophobic module HM (lipophilic alkyl chain), and (iv) a phosphonate connector module CM that holds together modules (i)-(iii) (Fig. 1). This first-generation LPPO (LPPO I)[1] demonstrated excellent bactericidal activity against various Gram-positive species, including multi-resistant strains such as vancomycin-resistant enterococci or methicillin-resistant *Staphylococcus aureus*. We have shown that at their bactericidal concentrations, LPPO act via disruption of the cytoplasmic membrane. By redesigning the iminosugar module so that it bears more positive charges, we developed the second generation of LPPO (LPPO II) with increased efficacy against Gram-positive species and an extended antibacterial activity range that now also includes serious Gram-negative pathogens.[2] LPPO II cause serious damage to the bacterial cell membrane, efflux of the bacterial cytosol and cell disintegration. Furthermore, LPPO II were shown to be well tolerated by live mice when administered orally and to cause no skin irritation in rabbits.

Importantly, using several of the most potent LPPO I and LPPO II we failed to select resistant strains of *Bacillus subtilis*, *Enterococcus faecalis*, *Streptococcus agalactiae* or *Pseudomonas aeruginosa*, while strains resistant to known conventional antibiotics (rifampicin and ciprofloxacin) readily emerged in control experiments. Recently, LPPO II were evaluated as additives in polymethylmethacrylate (PMMA) bone cements, preventing infections[3] and as an antibacterial component of polycaprolactone electrospun nanofiber dressing capable of reducing *S. aureus* induced wound infection in mice.[4]

Here we present the synthesis and evaluation of novel antibacterial compounds termed LEGO-LPPOs. LEGO-LPPOs are loosely based on LPPOs but with a dramatically altered modular architecture of the molecule. LEGO-LPPOs consist of a central linker module LM with two attached connector modules CM on either side. The connector modules are then decorated with polar PM and hydrophobic modules HM. We performed an extensive structure-activity relationship study by varying the length of the linker and hydrophobic modules, synthesizing >80 compounds. We identified the best compounds 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 further studies and possible therapeutic use.

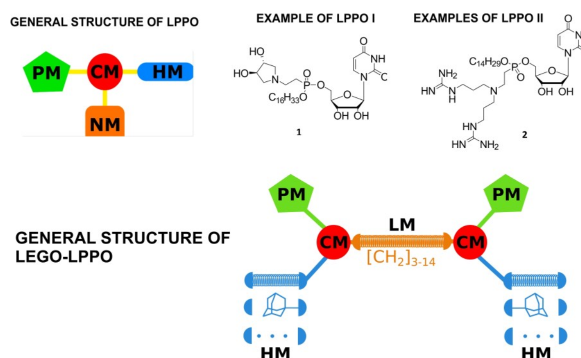

Fig. 1. **Structures of LPPO I, LPPO II, and LEGO-LPPO**

Acknowledgement
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L-02
PROTEIN INTERACTIONS CRITICAL FOR HEPATITIS B VIRUS REPLICATION AND MYCOBACTERIAL GROWTH

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Hepatitis B is a liver infection caused by the hepatitis B virus (HBV), a member of the family *Hepadnaviridae.* HBV can induce both acute and chronic disease and is a major global health problem. Although the chronic infection is mostly asymptomatic, it can lead to cirrhosis and hepatocellular carcinoma (HCC) development. Despite intensive research of HBV, little is known about the regulations of molecular mechanisms of HBV replication, cccDNA formation and degradation, HCC development, and about the reactivation of HBV. These mechanisms include a series of interactions of HBV proteins, genomic DNA, ccc DNA with host cell machineries. The genome of HBV consists of circular partially double-stranded DNA, which is approximately 3.2 kb and contains only four open reading frames (C, P, S, and X) that largely overlap and encode multiple proteins using different in-frame start codons[1]. The smallest intrinsically disordered protein HBx exerts its activities by interacting with a large number of cellular partners that are located either in the cytoplasm or in the nucleus. HBx is involved in multiple functions including gene transcription, intracellular signal transduction, cell proliferation, apoptotic cell death, and DNA repair. Currently, the main HBx role is attributed to activation of HBV transcription through interaction with the host DNA damage-binding protein (DDB1) E3 ubiquitin ligase and following targeting of the HBV transcription restriction Smc5/6 complex for degradation in proteasome[2,3]. Blocking of interactions between HBx-DDB1 and Smc5/6 represents thus promising target for specific inhibitor development. However, any details about these interactions are not currently known. Our project is focused on analysis of HBx protein interactions with components of Smc5/6 complex and on mechanism of HBx transcription regulation and viral replication. We also search for further cellular proteins interactiong with HBx and precore protein.

Mycobacterium tuberculosis (Mtb) is associated with millions of deaths per year. Tuberculosis is one of the top ten causes of death. Treatment of mycobacterial infections is complicated by increased prevalence of multidrug-resistant and extensively drug-resistant Mtb strains as well as by development of persistent infection. Despite global research efforts and investments, mechanisms underlying pathogenesis, virulence and persistence of *Mtb* infection remain poorly understood. Currently used drugs do not target latent infection and are less effective against drug resistant TB. The discovery of new types of small molecule inhibitors with new modes of action is increasingly urgent. Purines nucleotides, the substrates of nucleic acids, coenzymes, allosteric modulators and energy intermediate represent promising targets for development of novel inhibitor types. All enzymes from purine biosynthetic cascades are known in mycobacteria however, essentiality of particular enzymes and regulation of purine metabolism under normal and stress conditions are not completely understood. We investigate transcription regulation of the purine metabolism under normal growth conditions, hypoxia and nutrient starvation using transcriptome analysis, promoter reporter assay and other techniques. Project is further focused on possible formation of a purinosome, a complex formed from *de novo* purine biosynthetic enzymes, which can facilitate metabolic flux and minimize the escape of reactive and unstable intermediates in a model species *Mycobacterium smegmati*s. Identification and validation of essential enzymes from purine biosynthesis will identify new targets for inhibitor development.

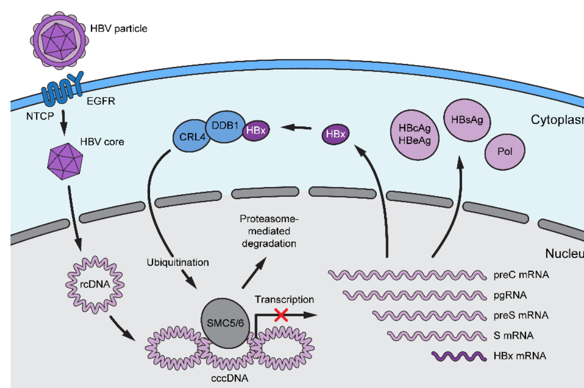

Fig. 1. **Hepatitis B virus evasion of cellular transcriptional inhibition.** After a hepatitis B virus (HBV) particle has entered a host cell (during which it becomes de-coated), its genome is converted to covalently closed circular DNA (cccDNA) that exists as a mini-chromosome in the nucleus and serves as the template for viral gene transcription. HBV proteins comprise of a core/precore proteins (HBcAg/HBeAg), a reverse transcriptase enzyme (Pol) and an envelope protein (HBsAg). HBx, viral regulatory protein, acts to degrade a cellular antiviral factor, the Smc5/6 protein complex. Smc5/6 binds to the HBV cccDNA and thus inhibits viral transcription. But HBx interacts with DDB1, an adaptor protein for the cell's CRL4 E3 ubiquitin ligase enzyme complex

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L-03

EXPLORING NON-TRADITIONAL TARGETS FOR ANTIVIRAL THERAPY – NOVEL INHIBITORS OF VIRAL METHYLTRANSFERASES

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The current COVID-19 pandemic caused by the SARS-CoV-2 virus clearly demonstrates the devastating effects on a significant portion of the world's population. This pandemic highlights our poor preparedness for such situations, both at the level of prevention and therapy. It is alarming that despite two clear signals of two coronavirus outbreaks, SARS and MERS,[1] there were no antivirals in our portfolio against these important and dangerous pathogens. The same is true for many other viral diseases. It can be assumed that many seemingly low-danger RNA viruses that normally cause only trivial illnesses have the potential to mutate, or viruses similar to them may emerge and cause a very significant danger to our society as a whole.

For several years, our group has been working on research of new antivirals specifically against selected RNA viruses, whether they are viruses that primarily cause respiratory diseases, such as coronaviruses, or RNA viruses transmitted by certain vector species, such as mosquitoes or ticks, e.g. Flaviviruses.

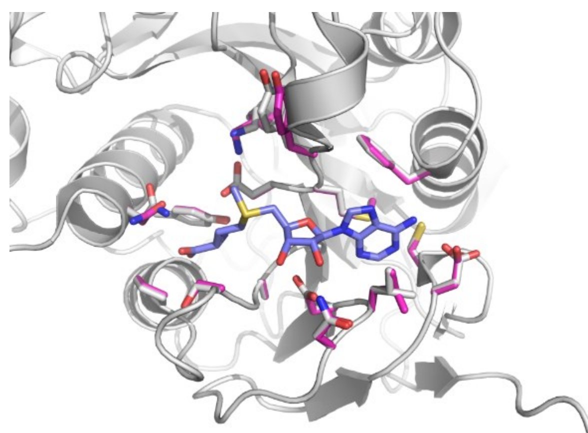

Fig. 1. **Structure of coronavirus MTase nsp16.** The comparison of the structures of MTase from SARS-CoV (magenta)[5] and SARS-CoV-2 (grey)[4] showing high conservation of the SAM binding site, which should enable the preparation of broad-spectrum inhibitors.

Although our group is also interested in traditional viral targets, e.g. viral RNA-dependent RNA polymerases[2], in recent years we have become interested in less conventional targets for antiviral therapy, in particular viral methyltransferases.[3] This talk will therefore focus primarily on these proteins, their structure[4], the design of inhibitors[5] and their biological activity (Figiure 1).

Acknowledgement
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L-04
MEDICINAL CHEMISTRY OF VIRAL POLYMERASES AND PROTEASES

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The influenza virus causes severe infectious diseases that represents a serious threat to public health. There is an urgent need for the development of new anti-influenza drugs effective against resistant viral strains and different viral subtypes. In this project we plan to study endonuclease and cap-binding small molecule inhibitors, as well as peptide inhibitors targeting protein-protein interaction in viral polymerase. Furthermore, due to the functional and presumed structural similarity between influenza A polymerase and L-protein of the emerging Rift Valley Fever virus (RVFV), we plan to utilize our prior experience[1–6] to develop and identify novel inhibitors of the endonuclease and polymerase activity of RVFV. This project will thus allow the development of active compounds against these important pathogens.

In the second part of the project, we focus on viral proteases which are key enzymes in virion maturation that contribute to viral pathogenesis. Targeting viral proteases represents a viable strategy of antiviral therapy. The main focus of this research objective involves proteases of HIV, SARS-CoV-2, Zika and Dengue viruses. Detailed understanding of regulatory steps during autoactivation of these enzymes, their role in virion maturation and pathogenesis is still lacking. We plan to combine biological, chemical and biophysical approaches to study proteases activation, regulation of maturation, intra and their interactions with low-molecular-weight ligands or other viral and host proteins.

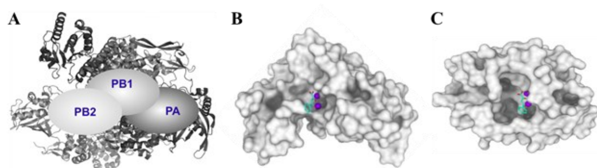

Fig. 1. **A) Schematic diagram of PB1, PB2 and PA subunits of the influenza A RNA polymerase heterotrimer using PDB 4WSB. Comparison of endonuclease domain of sequentially related Toscana virus (B) (PDB 6QW5), and the PA subunit of the influenza A virus (C) (PDB 4AWF),** Binding ligand in turquoise stick representation is 2-4-dioxo-4-phenylbutanoic acid (DPBA).

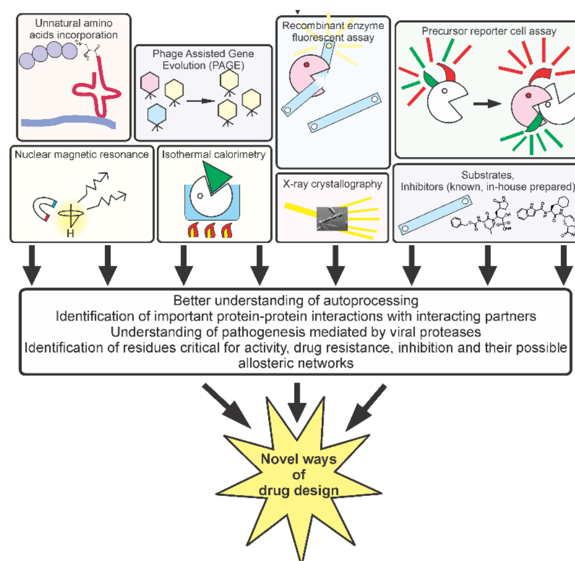

Fig. 2. **Schematic representation of the workflow and outcomes of the project.** We combine methods of chemical biology (expansion of genetic code enabling specific insertion of a light-activated amino acid), molecular biology (phage assisted gene evolution), in house developed cell-based and in vitro assays and biophysical methods (crystallography, NMR, solid-state NMR and others) to illuminate the process of proteases activation, inhibition, regulation and interaction with other viral and host proteins. Obtained results will help to understand various aspects of viral maturation and pathogenesis.

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-05
CHARACTERIZATION AND TARGETINT VIRUS-HOST INTERACTION

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Chronic hepatitis is a life-long liver disease caused by infection of Hepatitis B virus (HBV). Despite the existence of preventive vaccine, more than 250 million people worldwide suffer from chronic hepatitis. Current treatment, involving nucleos(t)ide analogs or pegylated interferon-alpha, has many adverse side effects and does not provide functional cure because it is unable to clear HBV from infected cells. HBV is a small enveloped virus that encodes only few viral genes. The replication of the virus entirely relies on host environment and the viral proteins evolved to perform multiple functions. The HBc protein is a perfect example of virus-host interaction that is critical for the establishment and maintenance of persistent infections. While HBc is the main structural component of the viral nucleocapsid, additional regulatory roles of HBc in the viral life cycle and pathogenesis have been described (Fig. 1).

The research of Jan Weber's group is focused on identification of novel core-host interactions and characterization of cellular pathways that play role in HBV-associated pathogenesis[1]. Another important aspect of the research is to elucidate how various posttranslational modifications, e.g. serine phosphorylation, arginine methylation, ubiquitination or sumoylation, affect the HBc protein stability, intracellular trafficking or interaction with other proteins[2,3]. Using affinity purification/mass spectrometry assay we identified a pool of potential HBc interacting proteins. Among them, we selected three groups of promising candidates with potential effect on HBV replication: i) host factors with E3 ubiquitin ligase or kinase activities that may regulate HBc posttranslational modifications, e.g. serine phosphorylation or ubiquitin-like modifications; ii) proteins involved in epigenetic modifications, transcriptional control and nucleosome assembly that may affect viral cccDNA formation, activity and maintenance; iii) host chaperone proteins with potential role in viral nucleocapsid formation, pgRNA encapsidation and reverse transcription. The results generated by proposed comprehensive analysis of HBc – host interaction pathways would bring novel knowledge into HBV field and eventually lead to development of new promising anti-HBV therapies.

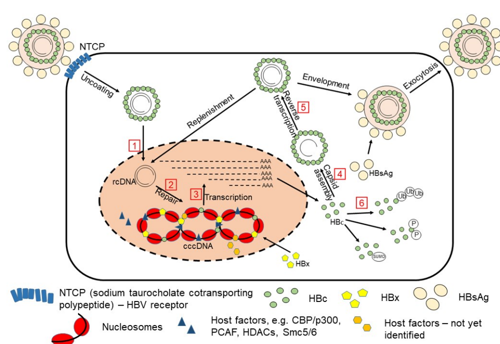

Fig. 1. **The graphical overview of the pluripotent role of HBc in the HBV life cycle.** Each number in red square (1–6) represents a specific HBc function that will be addressed in the proposed study: 1) HBc regulates transport and nuclear release of the viral genome. 2) HBc associates with cccDNA and regulates its activity. 3) HBc modulates viral gene expression. 4) HBc is required for nucleocapsid assembly and pgRNA encapsidation. 5) HBc is required for reverse transcription, and 6) HBc function is regulated by posttranslational modifications, e.g. phosphorylation and ubiquitin-like modifications.

In addition, our group is interested in characterization and targeting coronavirus attachment and entry. The emergence of third coronavirus causing severe acute respiratory syndrome in less than two decades transformed emerging coronaviruses in a new public health concern. Since SARS-CoV-2 detection in Wuhan in late 2019, the virus spread in almost every country, totaling so far 5 million deaths. It became obvious that we need a broad-spectrum intervention, that would halt this and future coronavirus pandemic. One of the best measures is to block virus before it establishes new infection in the cells, thus inhibition of viral attachment and entry are self-evident choices. Many viruses use abundant adhesion molecules such as heparan sulfate proteoglycan at surface for initial attachment to the cells[4].

We plan to characterize and target this interaction with sulfated nanoparticles that mimic heparan sulfate. Following successful attachment, coronaviruses can enter the cells via direct fusion or by endocytosis. Using mass-spectrometry-based proteomics, we will search for common host proteins involved in coronavirus entry and characterize their interactions with viral proteins. Our results will broaden the knowledge about coronavirus usage of heparan sulfate for attachment to the cells and characterize complex host cell network in coronavirus entry pathways. Furthermore, it can lead to development of heparan sulfate mimicking nanoparticles with virucidal activity and their application as a preventive measure to curb the coronavirus infection.

Acknowledgement
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L-06
STRUCTURAL AND FUNCTIONAL STUDIES OF CORONAVIRAL RNA-METHYLTRANSFERASES

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Viral RNA (vRNA) of single-stranded positive-sense RNA viruses (+RNA viruses) resembles mRNA. It's 5' end bears a cap, a structure that is chemically composed of guanine nucleotide connected to the rest of the RNA via a 5′ to 5′ triphosphate linkage. The guanine base is methylated at the 7 position; in the Coronaviridae family this function is performed by the nsp14 methyltransferase (MTase)[1]. The vRNA is also modified at the 2′-hydroxy group on the first ribose sugar which is performed by another coronaviral enzyme, the nsp16 MTase[1]. We have characterized these enzymes in cells[2] and solved crystal structures of both coronaviral enzymes (nsp14 and nsp16) in complex with their natural substrates (S-adenosyl methionine) and in complex with inhibitors[3–5]. The structural information is used to design a second generation of inhibitors with improved properties.

Acknowledgement
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P-01

INHIBITION OF NUCLEOTIDE BIOSYNTHESIS AS TARGET FOR NEW ANTIMICROBIAL COMPOUNDS

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The activity of hypoxanthine-guanine-(xanthine) phosphoribosyltransferase [HG(X)PRT] is essential for the survival of the two *Plasmodium* species, *falciparum* (*Pf*) and *vivax* (*Pv*). This is because these enzymes catalyze the synthesis of the 6-oxopurine nucleoside monophosphates, which are essential components for inclusion into the parasite's DNA. Therefore, inhibitors of these enzymes are recognized as potential drug leads.[1,2] Recently, prodrugs of inhibitors of *Mycobacterium tuberculosis* (*Mt*) hypoxanthine-guanine phosphoribosyltransferase (*Mt*HGPRT) were found to arrest the growth of *Mt* in cell culture[3,4]. Further support in validating *Mt*HGPRT as an anti-TB drug target comes from a random transposon mutagenesis study that showed that the expression of this enzyme is essential for the survival of *Mt*.[5] These two pieces of data confirm the importance of *Mt*HGPRT in the life-cycle of this pathogen.

Here we present design, synthesis and evaluation of pyrrolidine and hydroxymethylpyrrolidine bisphosphonate nucleotide analogues as inhibitors of HG(X)PRT as well as preparation of their prodrug forms for cell-based assays.

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P-02

CHARACTERIZATION OF STRINGENT RESPONSE IN MYCOBACTERIA

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Tuberculosis (TB), one of the leading causes of death worldwide, is a air droplet transmissible disease, caused by *Mycoacterium tuberculosis* (*Mtb*). About a quarter of the global population is estimated to be infected with Mtb, but only small minority of individuals develop the TB disease. In 2021, 10.6 million TB infections and 1.6 million deaths of which 450 000 deaths caused by drug-resistant TB were registered[1].

Mtb evolved various defense mechanisms to face unfavorable conditions during infection in human lungs such hypoxia, oxidative stress, acidosis and nutrient starvation which enable bacterial slow growth or long-term survival in dormant state[2]. Stringent response is a general mechanism of the bacterial adaptation to the nutrient starvation and is accompanied by the alarmone (p)ppGpp (guanosine-3'-di-phosphate-5'-tri- or diphosphate) accumulation. The Rel protein regulates the (p)ppGpp level. Rel is activated during amino-acid starvation, when cognate deacyl-tRNA binds to the ribosomal A (aminoacyl-tRNA) site[3]. However, the role of Rel activity in the mycobacterial physiology is not well understood yet.

To clarify the role of Rel protein in physiology of the model mycobacterial species *Mycobacterium smegmatis* (*Msm*), we have used a set of the *in vitro* and *in vivo* techniques. In agreement with previous reports, we confirmed that the Rel protein is not required for survival under carbon and nitrogen starvation conditions in *Msm*. However, our data rather show that it plays a crucial role in Msm adaptation during growth under nitrogen limitation. Further, we investigated different mechanisms of Rel activity regulation, such as free tRNAs accumulation in bacteria or ribosome binding.

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P-03

STRUCTURAL BASIS FOR ALLOSTERIC REGULATION OF MYCOBACTERIAL GUANOSINE-5´-MONOPHOSPATE REDUCTASE

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Guanosine 5´-monophosphate reductase (GMPR) catalyzes conversion of GMP to IMP, the hub metabolite for the biosynthesis of all purine nucleotides. This reaction enables mycobacteria and most other organisms to utilize guanine nucleotides in production of adenin nucleotides without the need of *de novo* synthesis.

In our studies of purine metabolism in mycobacteria, we use *Mycobacterium smegmatis* (Msm) as a model organism. Althougt GMPR is not essential for Msm or *Mycobacterium tuberculosis* (Mtb) under normal conditions, it may contribute to the regulation of the purine nucleotide pool by recycling GMP to IMP

In our recently published study[1] we showed that the enzymatic activity of Msm GMPR is allosterically regulated by ATP and GTP. While ATP inhibits the Msm GMPR enzymatic activity, GTP blocks this inhibition, and thus restores the activity of Msm GMPR.

Here, we present an explanation of allosteric regulation of Msm GMPR with ATP and GTP at the molecular level. It is based on crystal and cryoEM structures of Msm GMPR with ATP and GTP. Msm GMPR forms tetramers with four-fold axis which further assemble into octamers. The two tetramers in the octamer adopt either compressed or extended conformation[1]. The changes in conformation induced by the compression and extension are transferred through various loops to the active site. Our results show that the ligands trap the Msm GMPR octamer in either active or inhibited conformation.

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P-04

IDENTIFICATION OF SMC5/6 COMPLEX MEMBERS POTENTIALLY INVOLVED IN ITS PROTEASOMAL DEGRADATION VIA DDB1.

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Hepatitis B infection is still one of the most dangerous viral illnesses, affecting more than 250 million individuals worldwide and being a major risk factor for the development of hepatocellular carcinoma (HCC). Despite of the existence of effective vaccines and treatment strategies, hepatitis B virus (HBV) still causes more than a million of deaths per year. In hepatocyte nuclei, HBV genomes occur episomally as a covalently closed circular DNA (cccDNA). It has been previously described that the SMC5/6 complex acts as a host restriction factor that supresses the cccDNA transcription. HBV counteracts this restriction by expressing the X protein (HBx), which redirects the cellular DNA damage-binding protein (DDB1)-containing E3 ubiquitin ligase to target the SMC5/6 complex for proteasomal degradation. However, the details of how HBx modulates the interaction between DDB1 and SMC5/6 remains to be determined.

In the present study, we used biochemical and molecular biology approaches to identify possible interactions established between HBx, DDB1 and the SMC5/6 complex that could explain the mechanism by which HBx targets SMC5/6 for proteasomal degradation. Using yeast two-hybrid, immunoprecipitation and other approaches to study protein-protein interactions (PPI), we identified component of the SMC5/6 complex, which binds to DDB1 and provides basis for elucidation of SMC5/6 degradation. Further studies will focus on detailed description of SMC5/6 proteasomal degradation during HBV infection.

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P-05
MULTIPROTEIN ORGANIZATION OF PURINE BIOSYNTHETIC ENZYMES IN MYCOBACTERIA

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Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis, is the first bacteria per deaths worldwide. The increasing number of multidrug-resistant *Mtb* strains is currently a big threat for the public health and new therapeutic targets are urgently needed. The *de novo* purine biosynthesis pathway regulation in mycobacteria is far from being understood and a deeper view can unveil important details on their biology. The *de novo* pathway, involving tandem eleven enzyme reactions, produces essential molecules for the viability of bacteria and enzymes from this pathway are considered as suitable drug targets

Our aim is to investigate a possible presence of purinosome, a putative protein complex, that may prevent the dispersion and/or the degradation of highly reactive/instable metabolites, fastening the entire process. There are evidences of its presence in mammalians cells, suggesting that it is usually assembled when the cells undergo stress conditions as hypoxia, lack of nutrients, low pH, etc.

We examine possible formation of purinosome in the model species *Mycobacterium smegmatis*. Immuno-precipitation followed by mass spectrometry analysis revealed no interacting partners for the 1st and 4th enzyme of the pathway purF and purL, indicating the absence of strong non-covalent bonds between proteins. To examine possibility of weak non-covalent bond formations in bacteria, formaldehyde was used as a crosslinker. However, for effective MS analysis a de-crosslinking protocol must be used and it is currently developing. Further, we performed analysis of enzymes from *de novo* purine biosynthesis pathway localization by confocal microscopy.

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P-06
LOCALIZATION OF SARS-CoV-2 CAPPING ENZYMES REVEALED BY ANTIBODIES AGAINST THE NSP10 AND NSP14 SUBUNITS

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Key components of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication complex are the RNA-dependent RNA polymerase, helicase, nuclease and two RNA methyltransferases (MTases). Coronaviral methyltransferases, nsp10/16 and nsp14 catalyze the last two steps of viral RNA-cap creation[1]. This cap is essential for the stability of viral RNA and, most importantly, for the evasion of host immune system. Non-capped RNA is recognized by innate immunity, which leads to its degradation and the activation of antiviral pathways.

Recently, structures of the MTases have become available[2,3], however, their biological characterization within the infected cells remains largely elusive.

In this study, we generated a mouse monoclonal antibody against the SARS-CoV-2 nsp10 protein, a subunit of both 2'-O and N7 MTases[4]. We show that the antibody specifically recognizes the nsp10 subunit both in its native conformation and in its denatured form. Using this novel antibody, we investigated the cellular localization of nsp10 during cell culture infection with the SARS-CoV-2 virus. We show that the nsp10 protein is localized mainly in vesicular structures in the perinuclear region of the infected cells, where the virus is replicated. Characterization of mouse monoclonal antibody against SARS-CoV-2 nsp14 protein, the coronaviral N7-MTase, is now under investigation.

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P-07

DUAL INHIBITORS OF 2'-O- AND N7- METHYLTRANSFERASES NSP16 AND NSP14 INVOLVED IN RNA CAP FORMATION OF SARS-CoV-2

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the coronavirus disease-19 pandemic. One of the key components of the coronavirus replication complex are the RNA methyltransferases (MTases), RNA-modifying enzymes crucial for RNA cap formation enabling the virus to evade the immune system in the host cells[1]. In membranous replication organelles within infected cells[2], the viral RNA is methylated by N7 MTase consisting of viral non-structural proteins nsp10 and nsp14, and then by 2'-O MTase consisting of nsp10 and nsp16[3]. Identification of potent dual inhibitors targeting both nsp14 and nsp16 MTase activities[4] could lead to efficient prevention of coronavirus RNA cap formation and viral vulnerability to the host immune system.

Through cross-screening of the inhibitors that we previously reported for SARS-CoV-2 nsp14 MTase activity against nsp10-nsp16 complex, we identified two compounds (SS148 and WZ16) that also inhibited nsp16 MTase activity. To further enable the chemical optimization of these two compounds towards more potent and selective dual nsp14/ nsp16 MTase inhibitors, we determined the crystal structure of nsp10-nsp16 in complex with each of SS148 and WZ16. As expected, the structures revealed the binding of both compounds to SAM binding pocket of nsp16. However, our structural data along with the biochemical mechanism of action determination revealed an RNA-dependent SAM-competitive pattern of inhibition for WZ16, clearly suggesting that binding of the RNA first may help the binding of some SAM competitive inhibitors. Both compounds also showed some degree of selectivity against human protein MTases, an indication of great potential for chemical optimization towards more potent and selective inhibitors of coronavirus MTases.

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P-08

SCREENING FOR INHIBITORS OF METHYLTRANSFERASE ACTIVITY OF NSP14 FROM SARS-CoV-2

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Eukaryotic mRNAs contain a structure called cap attached on their 5' end. Viruses often also incorporate a cap structure at the 5′ end of their RNAs to mask them from the host innate immune system and enhance protein synthesis. Some viruses hijack the capping system of the host, others use their own RNA capping machinery. Coronaviruses encode unique proteins to cap their mRNAs. These include viral methyltransferases – N7-methyltransferase and 2′-*O*-methyltransferase, which could be used as possible drug targets within antiviral treatments[1]. To identify inhibitors of coronaviral methyltransferases, we need to use a robust system for testing the methyltransferase activity *in vitro*. Initially, we used radioactivity-based methyltransferase assay in collaborating laboratory to identify inhibitors of N7-methyltransferase nsp14 from SARS-CoV-2 (ref.[2]). Later we developed a method coupled with ECHO-MS system for measuring methyltransferase activity and used it to test activity of 2′-*O*-RNA methyltransferase from the OC43 coronavirus[3]. Now we are using this method to screen inhibitors of nsp14 from SARS-CoV-2.

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P-09
CELL PERMEABLE SYNTHETIC STING AGONISTS WITH ACTIVITY AGAINST CHRONIC HBV

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Hepatitis B virus is human hepatotropic DNA virus. About 5% of adults and more than 90% of children under 1 year develop chronic hepatitis B (CHB) after the acute infection. Thus, the host immunity significantly influences the clinical outcome of the acute infection. About 250 million people worldwide suffer from CHB and are under high risk of liver cirrhosis and hepatocellular carcinoma[1]. Sensing double-stranded DNA (dsDNA) in cytoplasm is one of the important innate immunity mechanisms that protects an organism from pathogens and cell damage. Several cytosolic dsDNA sensors have been identified, among them cGAS-STING pathway plays crucial role in cytosolic dsDNA-triggered signaling. Activation of the cGAS-STING pathway leads to production of interferons and proinflammatory cytokines with antiviral properties. STING can be also activated by bacterial and synthetic cyclic dinucleotides (CDNs)[2].

In our research, we focused on the design and synthesis of modified CDNs with STING activating properties. One of our major goal was the preparation of lipophilic prodrugs of CDNs that enable cellular uptake via free diffusion through the cell membrane[3,4]. Prepared synthetic CDNs and their prodrugs were studied for their potency to activate STING. Production of proinflammatory cytokines was measured in a physiologically relevant *in vitro* model, the human peripheral blood mononuclear cells (PBMCs). The prepared CDNs showed excellent potency in STING activation in nanomolar range and the prodrugs proved to greatly enhance the cellular uptake of CDNs. Moreover, the STING agonist-induced cytokines produced by PBMC had anti-HBV properties in HBV-infected primary human hepatocytes *in vitro*.

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P-10
ANALOGUES OF HeE1-2Tyr AS INHIBITORS OF SARS-CoV-2 RNA-DEPENDENT RNA POLYMERASE

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SARS-CoV-2 has caused an extensive pandemic of COVID-19 leading to more than 6 million deaths worldwide. In this context, the search for effective antiviral therapy is an absolute priority. RNA-dependent RNA polymerase (RdRp) is one of the prime targets among viral enzymes and disrupting it effectively sabotages the viral replication process. As a part of the large coronaviral replication complex it is responsible for copying the viral genetic information.

HeE1-2Tyr was originally identified as a potent inhibitor of RdRp of members of the genus *Flavivirus* by Tarantino[1]. In 2020 we reported HeE1-2Tyr and its derivatives as inhibitors of SARS-CoV-2 RdRp with significant potency to hinder viral replication in cell-based antiviral assays[2].

Here we present further series of the HeE1-2Tyr structural analogues. We focused on central core modification and truncation. Synthesis and biological evaluation for their ability to inhibit the SARS-CoV-2 RdRp will be discussed in detail. The inhibition effect of all prepared compound was determined in a PEX assay using fluorescently labeled RNA primer and an RNA template.

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P-11

SYNTHESIS AND BIOLOGICAL EVALUATION OF THE NOVEL C-NUCLEOSIDES AS POTENTIAL ANTIVIRALS

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Nucleosides and nucleotides have been playing an important role in the therapy of viral diseases for many decades. C-nucleosides are their subgroup, in which the nucleobase is connected to the ribose part by a C-C bond. This change dramatically increases the stability of these compounds in cells since the cleavage of the nucleoside bond is no longer possible. Recently, Remdesivir[1] became an important representative of this class of compounds. It has shown a broad spectrum of antiviral activity against paramyxoviruses, filoviruses, pneumoviruses, flaviruses and also against coronaviruses including SARS-CoV-2[2].

Here, we will present the design, synthesis and biological evaluation of the newly developed C-nucleosides. Their corresponding triphosphates and prodrugs were also prepared and investigated

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P-12

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

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Influenza virus and Rift Valley Fever virus (RVFV) are considered to be the priority viruses, most likely to cause future outbreaks or epidemics. 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 possible only in the case of Influenza, as no vaccine against RVFV is licensed for human use. Our project aims to develop and screen the small molecule inhibitors of the L-protein of the RVFV and heterotrimeric polymerase of the influenza virus. A remarkable functional similarity between the polymerases of these viruses was observed, although decisive data are still missing. Our scope of work includes the production of recombinant proteins in *E. coli* and baculovirus expression system, including production of full-length L-protein and complexes of influenza proteins with host factors. Furthermore, we are focused on characterization of the protein-protein interactions and protein-ligand interactions using the isothermal titration calorimetry, and X-ray crystallography[1–3]. 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-13
TARGETING PRECURSOR FORMS OF SARS-CoV-2 MAIN PROTEASE

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Viral proteases turn viral polyproteins into functional proteins. Morever, they are important factors of pathogenesis. The indispensable role of proteolysis in viral life cycle makes viral proteases first of choice drug targets.

We focused on studies of SARS-CoV-2 protease (Mpro). The polyprotein-embedded precursor form of Mpro (precMpro) may have a different inhibition susceptibility from the mature Mpro. Since precMpro autoprocessing is the first step in viral maturation, precMpro is a potential alternative drug target. An assay for the elusive precMpro has been lacking. We have developed a dual cell-based reporter assay that enables simultaneous evaluation of inhibitors of both Mpro and precMpro (used before on HIV protease[1]). The reporter consists of Mpro flanked by its natural autoprocessing sites (representing precMpro) and inserted between mCherry and EGFP in sufficient proximity to enable Förster resonance energy transfer (FRET). After precMpro autoproteolysis, fluorophores are released from the reporter and FRET disappears. The resulting intracellular Mpro is cytotoxic, which decreases the level of the reporter and fluorescence from both fluorophores. This reporter was characterized by fluorescence cross-correlation spectroscopy (FCCS), fluorescence lifetime imaging (FLIM) and flow cytometry. Screening of a small compound library has identified three established (11a, GC376 and nirmatrelvir) and two novel Mpro inhibitors (ketoamide and a trifluoromethyl-pyridinyl ester). Results were also evaluated with recombinant Mpro precursor and mature proteases and in viral inhibition assays. Our novel method can improve the knowledge of the earlier life cycle of the virus and reveal new inhibitors and also test them for cytotoxicity and cell membrane penetrance in one single HTS experiment.

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P-14
HOST RUVB LIKE AAA+ ATPASE 1 INTERACTS WITH HBV CORE PROTEIN AND REGULATES VIRAL REPLICATION

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Core protein is a small 21 kDa protein with indispensable role at almost every step in HBV life cycle. HBc contains the N-terminal (NTD) and the C-terminal (CTD) domains, connected by a flexible linker. To gain more complete understanding of the HBc-host protein-protein interactions, we performed liquid chromatography-tandem mass spectrometry analysis to identify candidate cellular interactors of HBc protein. The affinity-tagged HBc protein was over-expressed in HepG2-hNTCP cells and interacting host proteins were identified by LC-MS. The HBc candidate interactors selected for further investigation were RuvB Like AAA+ ATPase 1 and 2 (RUVBL1 and RUVBL2), which play a role in transcription regulation, DNA damage response and more. To validate RUVBL1/2-HBc interactions, HEK293T cells were co-transfected with RUVBL1/2 and HBc expression plasmids and the isolated protein lysates were analyzed by co-immunoprecipitation (co-IP). Our data showed that HBc interacted with RUVBL1 only. To search for the site of the interaction, we prepared several HBc deletion mutants and using co-IP we determined that RUVBL1 binds to the CTD of HBc. Next, the role of RUVBL1/2 in HBV lifecycle was investigated in HBV-infected HepG2-hNTCP cells with downregulated expression of both RUVBL1 and RUVBL2. Two days prior to HBV infection, the cells were transfected with RUVBL1/2 specific siRNAs and five days post-infection, the levels of HBV antigens secreted into media and levels of HBV RNAs were determined by ELISA and RT-qPCR, respectively. When compared to control cells (transfected with non-specific siRNA), the downregulation of both RUVBL1 and RUVBL2 led to decreased levels of HBV RNAs as well as decreased levels of HBeAg and HBsAg. These findings suggest that RUVBL1/2 enhances HBV replication.

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P-15
THE EXPRESSION CHANGES OF THE INDIVIDUAL AGPCRS IN LUNG ADENOCARCINOMA AND COLORECTAL CARCINOMA CELL LINES INFECTED WITH SARS-CoV-2

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Adhesion G protein-coupled receptors (aGPCRs), an intriguing class of seven-transmembrane proteins, play important roles in neurodevelopment, immune defense and cancer. However, their role during viral infections is mostly unexplored. Currently, we recognize 33 human aGPCRs with majority of them being orphan receptors with unknown functions. We search for specific aGPCRs involved in infection of mammalian cells and aim to characterize their interaction with viral proteins. At first, we determined expression profiles of all aGPCRs by RT-qPCR in eight human cell lines commonly used in viral research and two types of primary human cells isolated from different tissues. We evaluated individual aGPCRs expression levels and identified aGPCRs which are highly expressed across all tested cell lines, aGPCRs with overall low expression but also aGPCRs which expression levels vary significantly among tested cell lines. Next, we infected human epithelial cell line derived from lung adenocarcinoma (Calu-3) and cell line derived from colorectal carcinoma (Caco-2) with SARS-CoV-2. Subsequently, we analyzed changes in the mRNA level of individual aGPCRs at two time-points post-infection (namely 6 and 12 hours). Based on the significantly increased mRNA levels, we identified seven aGPCR candidates in Calu-3 cell line and three candidates in Caco-2 cell line. Two candidates, ADGRB3 and ADGRD1, were common for both cell lines, while the remaining were cell-line specific. The increase in mRNA levels was observable already 6 hours post-infection and was even more pronounced at 12 hours post-infection. Selected hits will be further evaluated to confirm the change of their expression on protein level (mass spectrometry and quantitative Western blots with protein specific antibodies) and to address their influence on the SARS-CoV-2 life cycle (specific aGPCR knock down).

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BIOLOGY CENTRE CAS

L-07

VIRUS RESEARCH IN CESKE BUDEJOVICE

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The Laboratory of Arbovirology (Fig. 1) is a joint research unit of the Institute of Parasitology, the Biology Centre of the Czech Academy of Sciences in Ceske Budejovice, and the Veterinary Research Institute in Brno, Czech Republic.

The laboratory has a long tradition in virus research and stores one of the largest collections of arboviruses in Europe. It studies the molecular basis of diseases caused by tick-borne encephalitis virus and other flaviviruses, with particular emphasis on the interface between pathogenesis and host immunity and on the development and testing of new antiviral agents and vaccines.

The laboratory contributed significantly to the characterization of tick-borne encephalitis virus structure[1] and the discovery of novel human monoclonal antibodies to tick-borne encephalitis virus with prophylactic and therapeutic potential[2].

Several key aspects of the pathogenesis of tick-borne encephalitis, including the immune response to infection[3,4], have been described by the laboratory, including changes in blood-brain barrier permeability during infection[5], interaction of the virus with cells that form the blood-brain barrier[6] and with primary human neurons and astrocytes[7,8], characterization of immunopathological features during disease, etc. Several new biomarkers of tick-borne encephalitis have been discovered in clinical samples from human patients, and novel genetic polymorphisms associated with severe tick-borne encephalitis were identified[9].

The laboratory also pioneered research on antiviral agents effective against tick-borne encephalitis virus[10] and other flaviviruses, and developed the first veterinary tick-borne encephalitis vaccine candidate[11].

Also molecular-epidemiological and virus discovery studies are performed by members of the laboratory. For example, the laboratory discovered Brno loanvirus, which is the very first bat-borne hantavirus identified in Europe[12].

More recently, the laboratory has begun research into the biology and pathogenesis of SARS-CoV-2 and has been involved in the development of a new mouse model for COVID-19. Using this model, the laboratory participated in the discovery of the first bispecific antibodies that neutralise SARS-CoV-2 (ref.[13]) and also contributed to the discovery of other unique monoclonal antibodies that have a potential to be used for postexposure prophylaxis or early therapy of COVID-19 (ref.[14]).

Fig. 1. **The Laboratory of Arbovirology has decent research facilities, including BSL-3 and ABSL-3 laboratories**

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P-16
PREVALENCE AND DIVERSITY OF ORTHOHNATAVIRUSES IN RODENTS AND SHREWS IN URBAN AREAS OF THE CZECH REPUBLIC

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Orthohantaviruses are globally distributed rodent- and insectivore-borne viruses. Some of the viruses are important human pathogens. Inhalation of virus-containing aerosols via excreta (urine, faeces etc.) of infected rodents represents the most common route of infection of human. Orthohantaviruses occurring in Europe cause hemorrhagic fever with renal syndrome or nephropathia epidemica, a milder disease caused by Puumala virus. Unlike the detected seroprevalence, the incidence of hantavirus infections in humans in the Czech Republic is lower than in neighboring countries. This might be caused by lower prevalence of pathogenic viruses in rodent populations, high occurrence of low pathogenic viruses/ strains or lower diagnostic efficiency. Therefore, we surveyed selected populations of free-living rodents to assess the prevalence and diversity of orthohantaviruses.

Collected tissue samples of the trapped rodents were screened by RT-PCR for the presence of orthohantavirus RNA. Positive samples were subsequently characterized by sequencing and analyzing partial nucleotide sequences of genomic segments L and M.

From the total of 153 trapped rodents, orthohantavirus RNA was detected in 37 individuals (24.2%). Based on phylogenetic analyses human pathogenic Kurkino virus (*Dobrava-Belgrade orthohantavirus*) was detected (7 individuals of *Apodemus agrarius*, 2 *A. sylvaticus*, 2 *Microtus arvalis*). Nevertheless, Tula virus (only potentially pathogenic to human) was the most frequently detected virus among the rodents. Moreover, we have tested 10 randomly found insectivores in which human non-pathogenic viruses Seewis (2 individuals of *Sorex araneus*) and Asikkala (1 *S. araneus*) were found. These results confirm the potential health risk for public health in the Czech Republic.

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P-17
TICK-BORNE ENCEPHALITIS VIRUS: DIFFERENCES AND SIMILARITIES OF VIRUS-CYTOSKELETON INTERACTIONS IN HUMAN AND TICK CELLS

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Tick-borne encephalitis virus (TBEV) is a flavivirus causing one of the most important tick-transmitted neurological diseases in humans. Due to the absence of specific antiviral treatment, a detailed knowledge of host molecular factors that take part in complex virus-host interactions is crucial. The cytoskeleton has been repeatedly mentioned in the context of flaviviral infections, however, its role seems to vary significantly, depending on the virus, virus strain, and/or cell type being used for the experiment. Thus, we investigated molecular interactions between TBEV and the cytoskeleton of human neuroblastoma cells as well as tick cells to address potential differences between these two important host/vector environments.

Using specific pharmacological inhibitors, we showed that in both cell lines, TBEV efficient replication relies on intact integrity and dynamics of both microtubules and actin filaments. Moreover, inhibition of motor proteins cytoplasmic dynein and myosin II led to a significant decrease in virus titre, suggesting their potential involvement in TBEV intracellular transport. Using immunocytochemistry, we revealed certain structural changes during a later stage of the infection in human neuroblastoma cells, but not in tick cells. In addition, we also examined the expression of various cytoskeletal and cytoskeleton-related genes upon the infection. TBEV infection in human neuroblastoma cells induced possibly compensatory up-regulation of genes for actin and spectrin, both essential for neurite structure. Interestingly, TBEV-infected tick cells showed the opposite trend in the down-regulation of actin and talin genes.

Our results offer a systematic comparison of TBEV-cytoskeleton interactions in human and tick cells, and suggest protein targets for future research of this important human pathogen.

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P-18
SUSCEPTIBILITY AND IMMUNE RESPONSE OF HUMAN MICROGLIA TO TICK-BORNE ENCEPHALITIS VIRUS INFECTION

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Tick-borne encephalitis (TBE) is a serious viral neuroinfection associated with compromised blood-brain barrier (BBB) integrity. Although the exact mechanism leading to BBB disruption remains elusive, there is evidence suggesting that this crucial event might be a result of host immune response to the infection. Thus, we focused on the role of microglia, CNS-resident macrophages, in TBEV infection.

Three different human microglia cell cultures, including primary human microglia, were infected with three TBEV strains varying in virulence. To characterize viral growth in the cells, the infectious virus production was determined from cell-free supernatants by plaque assay, while the number of viral RNA from cell lysates was quantified by real-time qRT-PCR. Luminex multiplex assay was performed to investigate changes in cytokine/chemokine production. Additionally, electron microscopy was performed in order to investigate ultra-structural changes following the infection of human primary microglia.

TBEV infection of microglia showed to be persistent and productive with high viral yields and no observable CPE. Moreover, the infection was associated not only with ultra-structural changes, but also with significantly increased expression of multitude of cytokines/chemokines, of which TNF-α, IL-6 and IL-17A are known to increase BBB permeability.

Our results show that human microglia are susceptible to TBEV infection, producing a wide panel of inflammatory mediators. Further investigation will be needed to elucidate a role of microglia-secreted cytokines/chemokines in BBB disruption during TBE.

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UNIVERSITY OF CHEMISTRY AND TECHNOLOGY

L-08

SCIENTIFIC AIMS OF NIVB AT THE UVIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE

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The NIVB group at the University of Chemistry and Technology, Prague, is oriented on several aspects of the replication cycle of selected members of viruses from the Retroviridae, Flaviviridae and Coronaviridae as well as on the multidrug resistant bacteria. Mainly our current research is focused on: (i) the possibility of allosteric modulation of RNA-dependent RNA polymerase SARS-CoV-2; (ii) mechanisms of uncoating within the early phase of retroviral replication cycle and the interactions among viral and host cell proteins (and small molecules) needed by the virus to accomplish this process (iii) lipid membrane binding and RNA packaging process of flaviviral nucleocapsid-like structures formation, and (iv) and determination of antibiotic resistance mechanism in clinical isolates as well as the inhibitors of efflux pumps.

A novel coronavirus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in late 2019 in Wuhan China and has caused a global outbreak of acute respiratory disease associated with fatal pneumonia. Due to the proofreading activity of SARS-CoV-2 replication complex (RdRp), the usage of nucleoside analogues as antiviral drugs for COVID-19 treatment is limited. Therefore, we focused on the characterization of RdRp putative allosteric sites and testing of inhibitory activity of compounds binding into these allosteric pockets. We have pre-selected three hydrophobic pockets (Fig. 1) potentially involved in the allosteric regulation of RdRp activity and their characterization by a combination of mutagenesis and activity assay is currently in process. Furthermore, we are interested in *in vitro* analysis of naturally occurring mutations in the RdRp regions, whose occurrence relates to different SARS-CoV2 variants, which have been repeatedly reported in nsp7, nsp8, and nsp12 over the last three years. Another target of our SARS-CoV2 study is the exoribonuclease complex responsible for proofreading activity. The proofreading complex comprises the exoribonuclease domain (nsp14) and its co-factor nsp10. The intervention of proofreading during viral RNA replication is a promising approach to enhance the efficiency of active-site RdRp inhibitors.

Retroviral uncoating is an area of great scientific interest. The recent finding that HIV-1 mature core can enter the nuclear pore of the infected cells has shed new light on this process. By combining cryoEM, MS and iCLIP analysis, we investigate several aspects of the uncoating process, including the core structure and the involvement of host co-factor, for a member of D-type retroviruses, Mason-Pfizer monkey virus (M-PMV). Moreover, we identified a cellular factor, DEAH-box containing RNA helicase 15 (DHX15), that plays a critical role in M-PMV replication. Its role in both the early and the late phase of the M-PMV replication cycle is currently studied. We also aim to elucidate the relation between the plasma membrane interaction of retroviral polyproteins and regulation of their sequential processing.

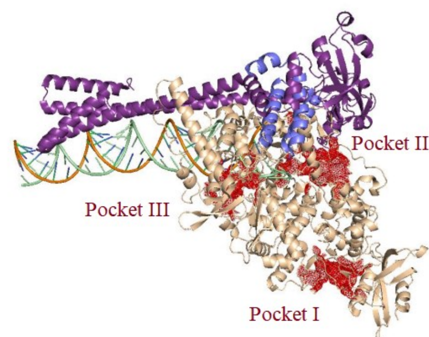

Fig. 1. **Structure of the SARS-CoV-2 RdRp-RNA complex (PDB ID: 7KRO).** Nsp12 (beige) with bound co-factors nsp7 (blue) and nsp8 (purple). The double-strand RNA consists of a template strand (orange) and a newly created strand (green). Predicted allosteric sites are pictured red, with pockets II and III located in the RdRp domain of nsp12 and pocket I found in NiRAN domain.

Flaviviruses such as Dengue virus (DENV), West Nile virus (WNV), Zika virus (ZIKV), Yellow fever virus (YFV), and Tick-borne encephalitis virus (TBEV) are important human pathogens belonging to the arthropod-borne viruses with a worldwide impact on human and animal health. Despite the availability of TBEV vaccine, there is still a need for tick-borne encephalitis treatment. Our research is focused on one of the promising targets for antivirals, a flaviviral capsid (C) protein. The C protein triggers the flaviviral assembly, most probably by simultaneous binding the viral genomic RNA and ER lipid bilayer. Based on our recent publication reporting the NMR structure of TBEV C protein (1), we focused on the identification of the regions/residues of TBEV C involved in lipid-membrane binding and viral genomic RNA interactions. The obtained data should significantly expand our knowledge of the mechanism and specificity of C protein in the process of vRNA packaging during TBEV infection and thus contribute to the development of targeted therapy.

Transmembrane efflux pumps export a wide range of structurally different antibiotics from drug-resistant bacterial cells, thereby reducing intracellular ATB concentration and bacterial sensitivity. Our research in this area is oriented on determination of mechanisms by which clinical isolates acquired their resistance and search for new efflux pumps inhibitors.

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P-19

ANTIBIOTIC RESISTANCE PLATFORM FOR HIGH-THROUGHPUT SCREENING OF ADJUVANTS FOR COMBINATION THERAPIES

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We are currently facing the post-antibiotic era, when most antibiotics will no longer be able to effectively treat bacterial infections. The introduction of new antibiotics into clinical practice does not keep up with the development of the resistance, and therefore adjuvant (combined) therapy appears to be a suitable alternative[1]. It is based on the simultaneous administration of an antibiotic and an inhibitor of the resistance mechanism that the bacteria use to eliminate the antibiotic. This therapy was first approved in 1981, when Augmentin, a preparation that contained clavulanic acid (β-lactamase inhibitor), and amoxicillin (β-lactam antibiotic)[2] was launched on the market.

The aim of this work is the identification of resistance mechanisms in clinical isolates of *Staphylococcus aureus* and their targeted inhibition using adjuvants. For this purpose, we are constructing a library of genetically modified *Escherichia coli* strains, carrying a single resistance determinant. This determinant usually represents the gene for destructase, an enzyme that modifies the structure of the antibiotic. *E. coli* strains prepared in this way have increased resistance to the action of the antibiotic. Subsequently, these strains are used for high-throughput screening of the library of substances capable of reverting the antibiotic-resistant phenotype back to the sensitive one. Promising compounds are subsequently tested for their ability to inhibit recombinant, purified destructase.

The *E. coli* DH5α/*aacA-aphD* expresses bifunctional enzymes that acetylate or phosphorylate the structure of gentamicin, which, after this modification, is no longer capable of binding to the ribosome and terminating proteosynthesis. This strain has a two-fold increase in resistance to gentamicin and a 96-fold increase in resistance to kanamycin compared to the wild-type strain. The set of flavonolignans was tested for its ability to modulate antibiotic resistance of the strain. Subsequently, the ability to modulate the gentamicin-resistant phenotype in clinical strains was also determined. 2,3-dehydrosilybin B demonstrated the most promising results of all flavonolignans tested.

Acknowledgement
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P-20

ALLOSTERIC SITES OF SARS-CoV-2 RNA-DEPENDENT RNA POLYMERASE AS A PROMISING TARGET FOR NEW ANTIVIROTICS

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Coronavirus disease (COVID-19) caused over six million deaths in the previous three years. Although the COVID-19 pandemic wave slowed down in 2021, a causative agent SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), is still present in the population. Moreover, due to the high mutation rate leading to continuous new variants emergence, SARS-CoV-2 has a high potential to persist in the population.

SARS-CoV-2 is an enveloped virus containing one strand of RNA with positive polarity. During the replication cycle, SARS-CoV-2 RNA is synthesized and processed by SARS-CoV-2 replication-transcription complex (RTC) composed of different non-structural proteins (nsp7 to nsp14). RNA-dependent RNA polymerase (RdRp) activity is mediated by a complex of three units: nsp7, nsp8, and nsp12. Nsp12 has enzymatic RdRp activity, while nsp7 and nsp8 function as cofactors. The active site of SARS-CoV-2 RdRp is a frequent target for current antivirotics. However, due to the proofreading activity of nsp14, many active inhibitors – nucleoside analogues – lose their efficiency. To overcome this problem, we have initiated a study focused on the allosteric modulation of RdRp activity.

Nsp12 consists of two major domains – nucleotidyl transferase domain (NiRAN) and polymerase domain, whereas the latter is further divided into fingers, palm, and thumb subdomains. In the structure of nsp12, three druggable allosteric sites were predicted, two of which are located in the polymerase domain. Our data showed that these regions contribute to the overall function and stability of RdRp complex. By mutational analysis, we found that a single amino acid substitution in proposed allosteric sites can significantly affect the polymerase activity. Moreover, our finding that the amino acid substitutions resulted in variation in the RdRp response to nucleotide analogues strongly supports the hypothesis that these allosteric pockets might be drugged to inhibit RdRp activity.

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P-21
MASON-PFIZER MONKEY VIRUS MATRIX PROTEIN MYRISTOYL SWITCH AND ITS POSSIBLE ROLE IN VIRUS MATURATION

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The final step of the retroviral life cycle is maturation of pre-assembled virus like particles. It is a stepwise event where viral Gag and Gag-Pol polyproteins are cleaved at specific sites by virus encoded protease. This precisely regulated process triggers structural changes accompanied with reorganization of the virus particle. Either incomplete proteolytic processing of viral polyproteins or their premature maturation lead to serious defects in virus infectivity. What triggers the activation of the protease and why it remains inactive until the budding is still unknown. In the C-type retroviruses, including HIV, where the particles assemble at the plasma membrane (PM) and then directly bud through PM, the trigger might include both the PM interaction and the assembly which brings protease monomers to proximity allowing its dimerization into active enzyme.

However, in the D-type retroviruses such as Mason-Pfizer monkey virus (M-PMV), the assembly takes place in the cytoplasm. The preassembled intracytoplasmic particles are transported unprocessed to PM where budding and maturation occurs. Therefore, in D-type retroviruses the maturation must be tightly regulated, and the dimerization of protease is not the only maturation-controlling mechanism. We have previously shown that M-PMV matrix (MA) domain lacking its natural N-terminal modification by myristoyl is readily cleaved by the M-PMV protease *in vitro* from the downstream phosphoprotein (PP) domain of Gag. However, when MA is myristoylated, the cleavage is significantly delayed. Our new structural data have indicated that the presence of myristoyl moiety in the hydrophobic pocket of MA induces formation of a short alpha helix at the cleavage site of the MA C-terminus preventing the cleavage. This suggests that the exposure of myristoyl from the MA molecule upon its interaction with the PM, so called myristoyl switch, affects the accessibility of the cleavage site at the MA C-terminus. This was further supported by the data showing that the interaction of MAPPHis with liposomes triggers the myristoyl switch. We suggest that the interaction of M-PMV MA with the PM and myristoyl switch significantly enhances MA cleavage from the rest of the Gag molecule and thus controls the sequential processing of Gag.

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MASARYK UNIVERSITY

L-09
STRUCTURAL CHARACTERISATION OF VIRUS REPLICATION *IN SITU*

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Research performed by the group of Pavel Plevka within the framework of the National Institute of Virology and Bacteriology will be focused on structural characterization of the replication cycles of human enteroviruses and of phages infecting pathogenic bacteria.

Viruses from the genus *Enterovirus*, including coxsackieviruses and rhinoviruses, cause billions of human infections every year. Diseases caused by enteroviruses include upper and lower respiratory tract infections, gastroenteritis, hand-foot-and-mouth-disease, and life-threatening encephalitis. Rhinoviruses are responsible for 40% of common cold cases, which result in a yearly cost of tens of billions of US$ in treatments and lost working hours worldwide. Despite the societal and economic impact of enterovirus infections, understanding of enterovirus replication cycle is incomplete. We will characterize enterovirus replication *in situ* using cryo-EM and tomography (cryo-ET) to determine: (i) how enteroviruses deliver their genomes into the cell cytoplasm[1,2]; (ii) the organization of “replication factories” that enteroviruses form in infected cells; and (iii) whether enterovirus capsids assemble around genomes or if the genomes are packaged into capsids (Fig. 1A). Results obtained within this aim will provide insight into the mechanism of cell membrane penetration by non-enveloped viruses, which is generally poorly understood. Furthermore, observing assembly intermediates in infected cells will enable characterization of the formation of enterovirus particles for which the genome-packaging mechanism is currently unknown. Because of the fast mutation rates enteroviruses exist as swarms of mutants called quasi-species that can evolve resistance to a treatment much faster than cellular organisms. Therefore, effective anti-enterovirus treatment will require the simultaneous use of at least three compounds targeting different steps in the enterovirus life cycle. Characterization of enterovirus replication, as planned in this proposal, will identify new targets for future anti-enterovirus therapeutics.

In 2017, the World Health Organization declared *Staphylococcus aureus* to be an antibiotic-resistant pathogen for which new therapeutics are urgently needed. Upon infection, *S. aureus* forms biofilms that can only be treated by the long-term application of several antibiotics in high doses or the surgical removal of the infected tissues. An alternative approach, phage therapy, has not been approved for clinical use, because the effects of phage infection of a biofilm are not sufficiently characterized. We propose to study the dynamics of the propagation of *Herelleviridae* phage phi812 in a *S. aureus* biofilm and molecular details of phi812 replication in a cell (Fig. 1B)[3]. We will study if and how sub-populations of metabolically dormant or phage-resistant cells in a biofilm provide herd immunity against phi812 infection. Structures of previously uncharacterized phi812 replication and assembly intermediates in *S. aureus* cells will be determined. Results obtained within this project will enable characterization of the function of bacterial membranes and macromolecular complexes in the initiation and completion of phage genome delivery, the assembly of phage portal complexes and heads, and the mechanisms of genome packaging and head-tail attachment. This aims’s biological significance lies in its focus on the as-yet uncharacterized interactions of phages and bacteria under biologically and clinically relevant conditions. Analyses of phage spread in a biofilm, herd immunity against phage infection, and phage replication in cells performed within this project may identify approaches for making phage therapy more effective.

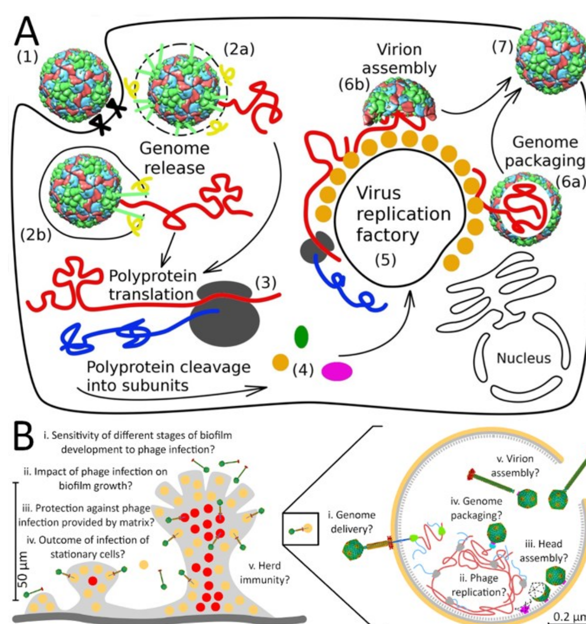

Fig. 1. **Overview of research questions directed towards characterizing enterovirus (A) and phage (B) replication cycles.**

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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., by Czech Ministry of Education, Youth and Sports ERC-CZ Consolidator grant LL1906, and by GACR-EXPRO grant GX19-25982X.

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L-10
HOW LARGE MOLECULES CAN ENTER CELL

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Various endocytic pathways have evolved to tightly regulate the vital internalization of large molecules into cells (see Figure 1). However, viruses can hijack these processes to enter their hosts. After the interaction between the virus and membrane receptors, the plasma membrane is bent and wrapped around the virus. Once the wrapping is completed, the virus is internalized in the endosome. We have shown that such wrapping could be a spontaneous process, i.e., not requiring ATP, and its efficacy depends on the virus size, shape, and coverage of binding sites[1,2]. This pathway is not limited to viruses and could be utilized by nanoparticles and other drug carriers. Later in the cell, viruses need to release their content into the cell. This release was previously assumed to occur via tiny pores/openings observed in non-enveloped RNA virus structures. However, such a release would be slow, requiring the unwinding of putative double-stranded segments and enabling genome degradation. We have recently combined cryo-electron microscopy and computer simulations to demonstrate an alternative release mechanism in which the capsid cracks open, and the genome rapidly releases via a large opening[3,4]. This release was triggered by decreased pH *in vitro*, and self-reassembled capsids were found to occasionally miss one or few capsid-protein pentamers after the release. The shape and extent of the opening were determined to depend primarily on the interaction range between the pentamers[5]. These findings uncover molecular details of virus entry and genome release that could be utilized in the development of antiviral drugs or nanoparticles for drug delivery.

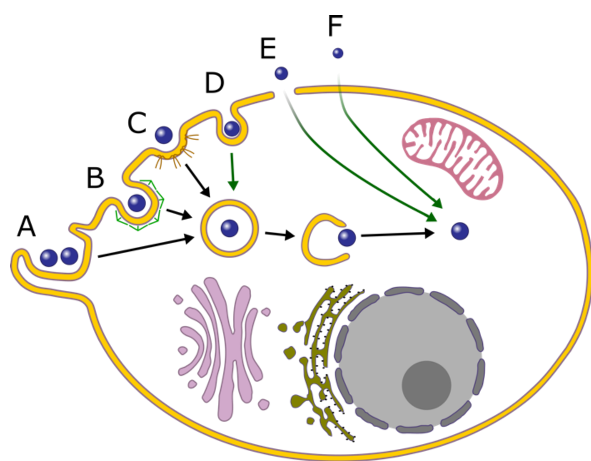

Fig. 1. **Illustration of internalization pathways of large molecules in cells.** A-C) endocytosis including phagocytosis and clathrin and calveolin mediated endocytosis D-F) passive internalization via D) clathrin and calveolin independent path, E) transport via membrane pores, and F) direct permeation.

An alternative pathway for large molecules to enter the cell is via large transmembrane pores (see Fig. 1E). Such pores do not occur spontaneously but could be formed and stabilized by proteins/peptides. We developed a computational approach to formulate the design guidelines and generate de novo peptides able to form such transmembrane pores with few nanometers in diameter[6]. The guidelines for pore-forming peptides were verified on several examples by fluorescent dye leakage experiments using lipid vesicles and atomic force microscopy with a supported lipid membrane. The advantage of our approach is the identified role of each residue, which could be used for fine-tuning the peptides/pores to specific applications. We demonstrated this fine-tuning on the generation of antimicrobial peptides that are able to kill even antibiotic-resistant bacteria while having low toxicity for human cells. Such peptides could be a good starting point for developing new antibiotics. Similar fine-tuning can be performed for other medical and biotechnological applications.

Acknowledgment
This work was supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 101001470) and by 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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L-11
THE CLOSE INTERPLAY OF TWO GENE EXPRESSION MACHINERIES

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In *Bacteria*, the transcribed messenger RNA (mRNA) can be directly attached to the first translating ribosome by creating a physical interaction between the ribosome and RNA polymerase (RNAP)[1]. The lead ribosome, in this transcription-translation complex, regulates the progression of transcription and protects the transcribed mRNA from premature transcription termination or mRNA degradation[2]. In the coupled system of transcription-translation the lead ribosome closely trails the RNAP, with rates of translation matching the transcription to support the efficient gene expression[2]. The direct interactions between RNAP and the translational machinery allow them to co-localize in bacterial cell and maintain the genome stability[2,3].

The recent cryo-electron microscopy structures of RNAP-ribosome derived from an *in vitro* transcription-translation reaction suggest a key role for accessory transcription factors[4,5]. Here, the *E. coli* transcription factors NusG and NusA can interact with RNAP and ribosome, supporting a bridged mode of coupling (Fig. 1). Perhaps the transcription factors interact with 30S small ribosomal subunit during the transition from translation initiation to elongation and help direct RNAP toward the mRNA tunnel entry[6].

However, it is not yet known how transcription-translation is controlled in bacteria. Perhaps, structured mRNAs during cellular stress can play a major role in coupled systems in terms of RNAP pausing and allowing the leading ribosome to rescue the transcription.

Cytoplasmic viruses transcribe and translate their mRNAs in the cytoplasm of the infected cells[7]. These viruses coordinate viral genome replication and viral assembly within the viral factories in the cytoplasm of the host cell. The viral mRNAs transcribed by viral RNAPs within the viral factories closely associate with host translation initiation and elongation factors[7]. Considering the co-localization of host translation machinery with viral factories, the viruses can tightly coordinate viral genome replication and viral assembly by indirect coupling of the viral transcription and host translation.

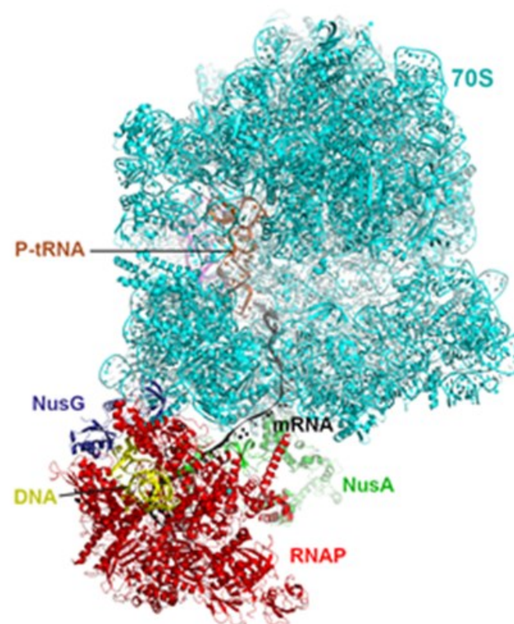

Fig. 1. **Structural representation of NusG-NusA mediated (PDB 6X7Y)[5] coupled system between bacterial RNAP and ribosome.**

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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.

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L-12
ALTERNATIVE ANTIBACTERIAL THERAPY USING BACTERIOPHAGES: PHAGE HOST INTERACTIONS AND PHAGE THERAPY SAFETY

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Bacterial viruses (bacteriophages) have significant impact on the evolution of bacteria and regulation of bacterial populations, including the microflora of the human body. Due to the global antibiotic crisis, interest in the use of bacterial viruses, phage-derived enzybiotics and chemical compopunds inspired by structures and functions of phage proteins to combat bacterial infections has increased worldwide. Historical clinical data on the effective use of bacteriophages in patients exists from Eastern Europe, but no evidence of it has been validated under recent European Regulatory standards. Discussions in professional societies such as ESCMID Study Group for Non-traditional Antibacterial Therapy – ESGNTA are currently underway on setting the rules for production and safety of the use of bacteriophage preparations. The regulatory authorities also communicate an increased interest in the therapeutic use of bacteriophages. In July 2021, the European Pharmacopoeia Commission launched work on new general chapter on phage therapy active substances and medicinal products for human and veterinary use, establishing harmonised quality standards for phage therapy products and providing a framework for their safe use in European countries.

Our research focuses on the molecular biology and genomics of clinically important pathogens from the genus *Staphylococcus* and its bacteriophages (Fig. 1). In particular, emergence of multidrug-resistant virulent methicillin-resistant *Staphylococcus aureus* (MRSA) is a remarkable public health problem. Therefore, the major part of our research deals with role of temperate bacteriophages in the dissemination of antibiotic resistance and virulence and the molecular mechanisms that lead to the evolution, adaptation and success of these pathogens[1,2].

Besides temperate staphylococcal siphoviruses that have an impact on virulence, toxin production, mobilization of variable genetic elements, there are the strictly lytic staphylococcal podoviruses[3] and myoviruses naturally characterized by a wide host range[4] and that are empirically used for treatment over a long period. The aim of the ongoing projects is to describe interactions between bacteria and these lytic therapeutic phages, trace how phages contribute to horizontal gene transfer, describe the interactions of phages with bacterial cell and the macroorganism (human), and discover new synergistic effects of phages with other antimicrobials. To fully demonstrate the safety of the use of phage therapy and exclude of any risk effects on human, it is necessary to elucidate the relationship between phage-bacteria and the human immune system at the multi-omics levels[5].

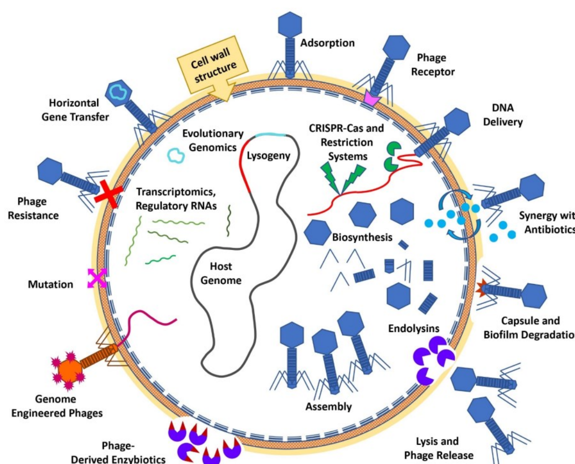

Fig. 1. **Trending topics in research on bacteriophages in medicine and biotechnology.**

Numerous case studies based on magistral phage preparations show the phage therapy as very promising, but there is currently no detailed guidance for quality, safety and requirements. The proposed main specific requirements are as follows: i) choose the suitable bacteriophage strain against the disease-causing bacterial strain on a case by-case basis with the efficacy of treatment linked to the phage lytic activity; ii) bacteriophages and their propagating host bacteria shall be manufactured based on a master seed system; iii) the absence of genes coding for resistance and virulence (including inducible prophages) shall be shown in both phage and production strain master seeds; iv) detailed description of genetic modifications in phages and/or bacterial strains used for phage amplification.

Without detailed basic research focused on molecular mechanisms of interactions between bacteriophages and bacterial hosts, the safety of phage therapy cannot be guaranteed. Last but not least, European regulators should define a Regulatory Framework for both the industrial and personalized approaches and authorisation procedures.

Acknowledgement
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L-13
SYPHILIS CAUSING STRAINS: DIFFERENCES IN GROWTH RATES AND PATIENT INFECTION CHARACTERISTICS

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Treponema pallidum subsp. *pallidum* (TPA) is the causative agent of syphilis, a sexually transmitted disease with increasing incidence in many areas of the world including USA, China, and Europe[1].

Previous genetic analyses of TPA reference strains and human clinical isolates have revealed that there are two genetically distinct groups of TPA strains[2,3], one group related to the reference strain TPA Nichols (Nichols-like strains) and the latter to the reference strain TPA SS14 (SS14-like strains).

In this study, we have compared growth characteristics of several TPA strains during experimental co-infections of single rabbits and revealed that the generation time for individual TPA strains varies between 30 and 46.7 hours. Interestingly, similar findings were found also when TPA were grown under *in vitro* cultivation system[4,5].

As revealed by analysis of metadata of patients infected with TPA, Nichols-like strains appear to be more frequently isolated from older males, more frequently from patients in the secondary syphilis stage, more often from blood samples when compared to ulcer swabs. Moreover, patients with higher RPR titres are more often infected with Nichols-like strains forming more often single primary ulcers compared to multiple ulcers caused by SS14-like strains. All these differences suggest important differences in the syphilis pathogenesis.

It has been shown previously that the majority (over 90%)[3] of recent human infections have been caused by SS14-like TPA strains, while the majority of laboratory propagated TPA strains belong to the Nichols-like group. This can be explained by this study results that show a higher growth rate for the Nichols-like strains during experimental rabbit infection. However, it is not clear if strains of the SS14-like group are better adapted for human infection when compared to the Nichols-like strains. Further studies are needed to answer these questions.

This study clearly showed important physiological growth differences between the two groups of TPA strains, both causing human syphilis. Although these groups of strains differ by less than 0.1% of their total chromosomal genetic information, there appear to be important differences in pathogenicity to humans, and also to experimental animal hosts.

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-22

STRUCTURAL ANALYSIS OF TWO DIVERSE TICK-BORNE ENCEPHALITIS VIRUS STRAINS USING CRYO-ELECTRON MICROSCOPY

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Flaviviruses, such as Tick-borne encephalitis virus (TBEV), Zika or Dengue fever virus, are a family of mostly human pathogens transmitted by arthropods[1]. Their importance is growing as the incidence of Flaviviral infections is on the rise and new areas of occurrence keep emerging – this may be further aggravated by climate change in the foreseeable future[2].

Tick-borne encephalitis (TBE) is a disease which causes severe inflammation in the brain and may lead to death. Although vaccination is available, only 23% of people in Czechia are vaccinated[3]. No cure for TBE is currently available and knowledge about the biology of TBEV is limited.

Structure of the TBEV 50nm virion has only been solved in full in 2018, showing that the virus consists of a layer of envelope (E) proteins embedded in a phospholipid bilayer together with the membrane (M) proteins[4].

Our work focuses on two TBEV strains – Neudörfl, a prototypic TBEV strain, and Hypr, a strain with strong neuro-invasiveness and higher virulence[5]. We have separately purified the two strains from infected tissue culture cells and used cryo-electron microscopy to visualise the purified viral particles. We have solved structures of the two strains to high resolution using single particle analysis.

Studying the structure in higher molecular detail provides insights into structural features which could aid with development of specific antiviral drugs or more advanced vaccines against TBEV.

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P-23

STRUCTURAL INSIGHT INTO A REPLICATION CYCLE OF VIRUS INFECTING CLIMATE-MODULATING MICROALGA

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Emiliania huxleyi is the most abundant marine alga whose cells are covered by calcite disks. By reflecting light, the calcite disks reduce the absorption of sunlight in ocean water, which impacts planetary climate. Large nucleocytoplasmic DNA viruses, including *E. huxleyi* virus 201 (EhV-201), are a major factor limiting the population density of *E. huxleyi*. Despite the impact of EhVs on the Earth's climate, there is limited information about their structure and replication.

Here we show that EhV-201 virions are covered by the inner membrane, capsid, and outer membrane. Upon infection EhV capsids remain attached to cell surface whereas the genomes are delivered into the cytoplasm. Replication of EhV-201 results in the formation of cytoplasmic virus factories, which are the sites of virion formation. Capsid assembly is initiated at the surface of endoplasmic reticulum-derived membrane segments. The assembling capsid bends and engulfs the membrane, resulting in the formation of a membrane sack. The virus genome is packaged through an opening in incompletely assembled capsid. EhV-201 capsids are uniform in size and assemble according to the rules of quasi-icosahedral symmetry; however, contacts between the capsid proteins are flexible, and most of the particles become deformed. Genome-filled particles acquire the outer membrane by budding into intracellular vesicles. Most EhV-infected cells lose their polysaccharide envelopes, which enables the release of virions by exocytosis or lysis of the infected alga. Structural characterization of EhV infection, a process that impacts the Earth's climate, may inspire means to influence it.

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P-24

STRUCTURE OF TICK-BORNE ENCEPHALITIS VIRUS IMMATURE PARTICLE SOLVED BY CRYO-ELECTRON MICROSCOPY AND SUB-TOMOGRAM AVERAGING

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Tick-borne encephalitis virus (TBEV) is an enveloped virus belonging to the family *Flaviviridae*. It is mainly transmitted by ticks and causes severe disease of central nervous system in humans. Virion surface is covered by envelope proteins (E-protein), that are together with the membrane proteins (M-protein) anchored in virus lipid bilayer. During the viral life cycle, the immature non-infectious virus undergoes a maturation process. This process includes proteolytic cleavage of prM and 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. In comparison with smooth mature TBEV particles, the immature particles have "spiky" surface formed by the E-protein—prM-protein complex. Because of non-icosahedral arrangement of the immature virus surface, single particle analysis methods did not lead to high resolution electrostatic potential maps. To improve the quality of the maps, we employed cryo-electron tomography and sub-tomogram averaging of single "spikes" from the immature particle surface, combined with extensive 3D-classification of the sub-tomograms.

The results show more detailed insight in the viral maturation process which may be targeted by specific antiviral drugs.

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P-25

TESTING ANTIMICROBIAL PEPTIDES

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Antimicrobial peptides (AMPs) are promising candidates for the development of a new class of therapeutics against resistant bacteria. The main limitation of the further development is a full understanding of the peptide kill mechanism and the role of individual residues/patterns in the mechanism. Large portfolio of mechanisms have been reported including the disruption of the pathogen membrane, inhibition of vital processes in its cytoplasm or at the membrane, and the modulation of host immunity. We use a combination of different techniques/assays to systematically determine the peptide mechanism of action with focus on membrane activity.

In the first step, we determine peptide minimal inhibitory concentration on both Gram-negative (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus carnosus)*. Secondly, we determine the membrane permeabilization ability of AMP by peptide-induced leakage of self-quenching fluorescent dye calcein from the vesicles. The pore forming mechanisms can be more directly confirmed by atomic force microscopy and quartz crystal microbalance experiments on surface supported membranes. To determine the secondary structure of the peptides we use circular dichroism. Finally, we also evaluate peptide toxicity by hemolytic assay on human erythrocytes.

The obtained findings from the combination of our experiments could be used in further optimization of the peptides to be more efficient and less toxic AMP.

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P-26
ALL-ATOM MODEL OF PHOSPHOLIPIDS WITH ENHANCED MEMBRANE DIFFUSION

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Due to the comparatively slow lateral diffusion of lipids in membranes, in silico determination of the equilibration state is demanding for heterogeneous membranes. Therefore, several methodologies have been suggested to accelerate the diffusion in lipid membranes. In 2012, Tajkhorshid *et al.* introduced the highly mobile membrane-mimetic (HMMM) model to overcome the slow dynamics of membranes[1]. In 2020, Tieleman and Elber proposed using alchemical steps and Monte Carlo to enhance the sampling of lipid mobility[2,3]. Recently, Tajkhorshid *et al.* developed a toolkit for the efficient shuffling of lipids in heterogeneous membranes[4], which could be used to generate multiple replicas with different spatial and conformational configurations of lipids. Despite all this progress, a simple way to speed up investigations of lipid redistributions in membranes at all-atom level is still lacking.

We introduced a novel, simple-to-use all-atom model, which accelerates lipid diffusion in phospholipid bilayers more than ten times. Each phospholipid thus could traverse the membrane patch of 20×20 nm^2 in less than 1.5 µs. We tested our model with pure POPC, binary POPC:POPS, and mammalian plasma membrane mimics. The mimic was mixed from POPC, POPE, POPS, POPA, and PSM lipids in different ratios. The interactions of the DEP domain Dishevelled protein with our model membranes are compatible with previous findings showing stronger interaction with negatively charged POPS than zwitterionic POPC[5]. The developed model could be extended to other lipid types in a straightforward way enabling the study of complex lipid mixtures.

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P-27
MECHANISMS OF PORE FORMATION IN BACTERIAL MODEL MEMBRANES

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Pore-forming antimicrobial peptides (AMPs) are being extensively studied since they are believed to be an alternative therapeutic class to classic antibiotics which are facing severe loss of efficiency due to the rise in multiresistant bacterial strains[1]. However, the mechanisms of pore formation induced by these amphipathic helical peptides are still not fully understood at the atomic and molecular scale. To create new guidelines for peptide design to improve their efficiency and selectivity to specific targets (bacterial strains, cancer cells, *etc.*), we are developing *in silico* methods to describe the process of pore formation.

Based on all-atom and coarse-grained molecular dynamics (MD) simulations of spontaneous pore closure, we developed a new collective variable (CV) which describes accurately the process of pore formation and closure in a model lipid membrane and permits to obtain a quantitative estimation of the free energy of pore formation. Several former attempts, including the work by Tolpekina *et al.*[2] and the CV developed by Hub and coworkers[3,4], pointed out that the process of pore formation should be split in – at least – two subprocesses, *i.e.* the nucleation of a so-called defect, or pre-pore, and the expansion of the pore. Our work confirms that two distinct mechanisms are at stake in the formation of toroidal pores. Moreover, we addressed the pore mobility in the membrane and the presence of metastable pores. Our work gives a better understanding in the process of pore formation and will help to design and optimize selective and efficient AMPs.

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P-28

BIOCHEMICAL ANALYSIS OF COMPLEX FORMATION BETWEEN RNA POLYMERASE AND RIBOSOME

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Transcription of messenger RNAs and translation of proteins, mechanisms necessary for gene expression, can be physically coupled and coordinated in some bacterial species[1,2]. The interaction between the leading ribosome and RNA polymerase (RNAP) forms a transcription-translation coupling complex (CTT)[2–4] modulated by transcription elongation factor NusG[3,4]. The leading ribosome also regulates the propagation of the RNAP during transcription[1] and protects the RNAP from backtracking. Moreover, the transcription pausing and possible premature termination can be modulated by cis-acting RNA structures (riboswitches) which may play a crucial role in formation of coupled transcription-translation.

Here, we investigate the coordination of translation initiation and elongation with RNAP pausing by forming CTT complexes in sucrose gradients. For translation initiation we use specific riboswitch to form and stabilize the initiation complex during early steps of CTT formation. For translation elongation we load the aminoacylated-tRNA into the CTT in the form of ternary complex.

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P-29

CELLULAR COLOCALIZATION OF TRANSCRIPTION AND TRANSLATION

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Free-living bacteria live in competitive and fast-changing natural habitats with non-optimal growing conditions. To survive in response to a variety of stress, they need to have a fast adaptation mechanism for gene expression. The coupling of transcription and translation coordinates optimal gene expression in bacteria. Thus, it could provide a rapid response to the stress induced unstable environment[1]. The occurrence of coupled transcription-translation (CTT) in bacteria is broadly accepted due to *in vitro* structures describing CTT complexes[2], but *in vivo* visualization of CTT in living cells is still missing. The bacterial cellular space is very dynamic but highly organized. Recent *in vivo* single-particle fluorescent studies were focused on the spatial organization of RNA polymerase (RNAP) or ribosomes in the context of nucleoid localization[3,4,5]. However, these studies monitor separately RNAP or ribosomes and not the whole CTT in bacterial cells.

We developed *in vivo* fluorescent system to monitor CTT in *Escherichia coli* through a set of strains with fluorescence labelling of proteins (no interference with the CTT function). The system will allow us to monitor the dynamics of this complex system in a single cell in response to different stress conditions and determine the conditions of CTT formation.

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P-30
CHARACTERIZATION OF *ESCHERICHIA COLI* ISOLATES FROM PATIENTS WITH COLON CANCER

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Escherichia coli is a one of the most important human pathogens. Besides diarrheal and extraintestinal infections, *E. coli* has been associated with pathologic conditions such as inflammatory bowel diseases and colorectal cancer[1,2].

In this study, we characterized mucosal *E. coli* isolates from 63 patients with colon cancer. Using PCR screening, we classified *E. coli* isolates to four main phylogenetic groups (i.e., A, B1, B2, and D) and analyzed prevalence of encoded virulence factors in *E. coli* genomes.

We foud that mucosal *E. coli* (n = 200) from patients with colorectal neoplasia most frequently belonged to phylogroup B2 (38.0%), followed by phylogroups A (28.5%) and D (26.0%), while phylogroup B1 was not very common (7.5%). Isolates of phylogroup B2 encoded the most of analyzed virulence factors.

Mucosal *E. coli* from cancer patients rarely (<1%) harboured determinants for toxicity (i.e., *lt*, *st*, *stx1*, and *stx2*) and adhesion (i.e., *bfpA* and *ial*), which are typical for diarrheal *E. coli* pathotypes such as EHEC, ETEC, and EPEC. In contrast, these *E. coli* isolates frequently encoded virulence factors typical for extraintestinal pathogenic *E. coli* (ExPEC).

High prevalence was observed for fimbriae (i.e., P-fimbriae (*pap*, 31.5%) and S-fimbriae (*sfa*, 28.0%)), several various iron-acquisition systems (i.e., yersiniabactin (*fyuA*, 68.5%), enterobactin (*fepC*, 63.0%), and salmochelin (*iroN*, 45%)), and toxins such as colibactin (*pks*, 25.5%), UPEC-specific protein (*usp*, 45%); and hemolysin (*α-hly*, 13.0%).

In conclusion, *E. coli* from patiens with colorectal cancer harbored virulence factors, which allow *E. coli* to bind to eukaryotic cells, survive in low-iron conditions, and damage cells and tissues. As a result, specific *E. coli* strains could contribute to the development of neoplastic processes in the large intestine.

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P-31
MINION AS A TOOL FOR SEQUENCING OF VARIABLE AND PARALOGOUS GENOMIC LOCI OF *TREPONEMA PALLIDUM*

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Genus *Treponema* includes number of human and animal pathogenic species and subspecies, e.g., *Treponema pallidum* subspecies *pallidum* (TPA) causing syphilis, *Treponema pallidum* subspecies *pertenue* (TPE) causing yaws. Genome similarity among TPA genomes (99.95%)[1] and TPA and TPE subspecies (99.8%)[2] indicates that small differences in the genome sequence play big roles in pathogenicity. Regions that show high sequence variability among strains, repetitive regions, paralogous genes and regions of intragenomic recombination are key for understanding pathogenicity of treponemes and host immune response and many of them code for surface proteins. Those regions are important candidates for vaccine development[3]. Determination of sequences of described genomic regions by Illumina sequencing is often problematic reflecting their paralogous character, thus new approaches need to be developed to determine reliable complete chromosome sequences.

Specific primers were designed to amplify regions of interest (ROI, n=36) with unique 200 nt tags at the start and end of each gene. All PCR products of each clinical sample were equimolarly pooled and barcoded and multiple samples were sequenced together using long-read sequencing technology (Oxford Nanopore, MinION). Unique tags were used for identification of reads origin by mapping to masked reference genome. *De novo* assembly with filtered reads was performed followed by quality assesment of consensus for each ROI. Clinical samples of TPA and TPE with different sequence profiles were selected, sequenced and consensus sequences of ROIs were analyzed.

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P-32

DEVELOPMENT OF DIRECT DETECTION METHODS FOR MONITORING OF THERAPEUTIC STAPHYLOCOCCAL BACTERIOPHAGES IN CLINICAL SAMPLES

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The spread of drug resistant bacterial pathogens and their increased ability to survive in the environment combined with the lack of new classes of antibiotics causes the need to look for new ways to treat bacterial infections. One suitable alternative is phage therapy. For the effective treatment of infections caused by *Staphylococcus aureus* strains, which belong to ESKAPE group[1], the most promising is the application of polyvalent Twort-like bacteriophages with broad-host range[2] and similarly podoviruses. Although, the genomic properties of phages are well-studied, new findings are emerging on the pharmacokinetic profiling of phage drugs[3]. The major factors relevant for the therapeutic use of phages include: i) optimal dosing, ii) duration of exposure to phages, iii) absence of genes for toxins and virulence factors in phage genome, and iv) selection of suitable phages depending on the infection agent.

Currently, there is also lack of methods for capturing and monitoring bacteriophages in clinical material. The aim of our study is to develop a sufficiently sensitive method for the detection and quantification of phage particles in different types of clinical material during phage therapy based on qPCR, specially designed luminescent (upconversion, UCNPs) and metal nanoparticles[4] and magnetic particles[5].

Our results show comparison of the bacteriophage detection methods based on the sensitivity and present novel approaches to phage detection in a variety of clinical material.

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P-33

RATHER DIE THAN BE TAKEN BY THE PHAGE: *STAPHYLOCOCCUS AUREUS* PROPHAGE IMMUNITY PROTEIN PROTECTS POPULATION AGAINST KAYVIRUSES

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Bacteriophages are crucial in shaping population of pathogens, such as *Staphylococcus aureus*. Prophages play an important role in the virulence, pathogenesis or host preference, as well as in horizontal gene transfer. On the other hand, broad host range staphylococcal bacteriophages of the genus Kayvirus are promising agents for therapeutic applications. The lysogens become immune to infection by closely related phages, but the interactions between temperate and lytic staphylococcal phages are not understood.

We describe a resistance mechanism towards lytic phages of the genus Kayvirus, mediated by *S. aureus* prophage accessory gene. The responsible membrane-anchored protein shows the presence of a putative membrane-binding α-helix in its N-terminal part and a cytoplasmic positively charged C-terminus. We demonstrated that the mechanism of action does not prevent the infecting Kayvirus to adsorb onto the host cell, deliver its genome into the cell but, phage replication is halted. Changes in the cell membrane polarity and permeability, which lead to prophage-activated cell death were observed from 10 min after the infection. Furthermore, we describe a mechanism of overcoming resistance in a spontaneous host-range Kayvirus mutant in which a gene fusion has emerged, and which was selected on *S. aureus* strain harbouring a prophage encoding the immunity protein.

We assume that this first case of staphylococcal inter-family phage-phage competition is analogous to some other abortive infection defense systems ensuring the survival of the host population without producing phage progeny.

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INTERACTION OF THERAPEUTIC KAYVIRUS PHAGE WITH STAPHYLOCOCCUS AUREUS ON TRANSCRIPTOMIC LEVEL

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The treatment of infections caused by human and veterinary pathogen *Staphylococcus aureus* is becoming worldwide healthcare concern due to the increasing resistance of the bacterium to antibiotics. A promising alternative to currently used drugs is represented by lytic phages from genus *Kayvirus*. However, the implementation of rational phage therapy into medicine requires to understand the interactions between bacteriophages and pathogens. To address this issue, we analysed RNA seqeuncing data of two *Staphylococcus aureus* strains infected by type phage K of the *Kayvirus* genus K with potential in phage therapy. The temporal transcriptional profile of phage K was similar in both analysed strains except for a few genes. Analysis of the RNA-Seq data also revealed antisense transcription and transcription from non-coding DNA with potential role in the regulation of phage and host gene expression.

The transcription response of *S. aureus* to phage K infection firstly corresponded to a general response to stress and DNA damage[1]. Bacterial differentially expressed genes were involved in nucleotide biosynthesis, amino acid and energy metabolism and cell wall synthesis genes. Furthermore, we detected during the late phase of phage K infection slightly increased transcription of staphylococcal virulence genes functioning in adhesion and immune system evasion. Our results clarify the global transcriptional interaction between phage and bacterial host, which will ensure safer usage of phage therapeutics and may also serve as a basis for development of new antibacterial strategies.

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L-14 MECHANISMS UNDERLYING *BORDETELLA PERTUSSIS* VIRULENCE AND TRANSMISSION

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Bordetella pertussis is a Gram-negative coccobacillus and an exclusively human pathogen that causes the highly contagious respiratory infectious illness known as pertussis, or whooping cough[1]. Pertussis-related pneumonia used to be the leading cause of infant mortality in the pre-vaccination era and despite massive global vaccination against pertussis for almost six decades, whooping cough persists globally as one of the least-controlled vaccine-preventable infectious diseases. It is estimated that about 20 million whooping cough cases, yielding up to 200,000 infant deaths due to pertussis-related pneumonia occurr annually, mostly in developing countries with problematic access to medical care[2]. Recently, *Bordetella pertussis* infections massively re-emerged in the highly vaccinated populations of the wealthiest industrialized countries, which switched from the reactogenic whole bacterial cell-based pertussis vaccines to the use of less reactogenic and less efficient subunit acellular pertussis vaccines in the last two decades.

Bordetella pertussis is an exquisitely equipped bacterial pathogen that produces numerous virulence factors capable to suppress and hijack host immune defences on the mucosa of the upper airways. It produces several protein toxins secreted by various pathways, including an adenylate cyclase toxin-hemolysin and the notoriously known pertussis toxin. These two immunosuppressive toxins hijack the host immune cell signaling pathways by different mechanisms that manipulate cellular cAMP levels and downstream signaling cascades. This paralyzes in particular the bactericidal activities of the sentinel myeloid phagocytic cells of host innate immunity, such as neutrophils, macrophages, eosinophils and granulocytes. The bacterium further produces several potent adhesins enabling bacterial attachment to cilliated airway epithelial cells on which the bacteria grow in form of microcolonies and form a biofilm. Bacterial colonization of the upper airways is enabled by production of several efficient complement resistance factors and by the excreted exopolysaccharide that plays a role both in resistance to antimicrobial peptides and complement, as well as in biofilm formation on ciliated epithelia of the nasal septum and of the upper airway[1].

Due to its vigorous innate immune defence suppressing mechanisms, *Bordetella pertussis* is able to proliferate to high densities in the nasopharynx of naïve infants, where it can reach up to 10^8 colony forming units per 1 mL of undiluted nasal aspirate. The massively shed bacterial cell wall components harness the innate immune signaling mechanisms of the mucosal layer and trigger a nasopharyngeal catarrh. This initially mild disease is characterized by the absence of fever and its early symptoms resemble common cold. It manifests by heavy uncontrollably running nose, a massive rhinorrhea that by postnasal drip irritates the larynx and enforces sneezing and cough that makes the subject to aerosolize the exudate accumulated in the nasopharynx and loaded by the proliferating bacteria, which ensures their efficient transmission to new hosts. This highly contagious phase of the catarrhal disease peaks at about two weeks form occurrence of the first symptoms and precedes the paroxysmal whooping cough disease phase, characterized by vigorous coughing fits with inspiratory whoops that can last for up to three months. Eventually, the infection descends into the lungs, provoking a severe pneumonia in infants that is often complicated by viral or bacterial superinfection and is often fatal[1,2].

For long time no suitable animal models of the exclusively human catarrhal infection by *B. pertussis* were available. Therefore, the bacterial virulence factors and host mucosal physiological mechanisms underlying the catarrhal disease and transmission of the pathogen remained largely undefined. Tackling them remains a major task of pertussis research, as better understanding of *B. pertussis* virulence factors crucial for bacterial transmission is needed for development of improved vaccines that will not only save infant lives, as the current parentheral vaccines do, but will also enable restriction of circulation of the pathogen by conferring protection of human nasopharyngeal mucosa from infection and *B. pertussis* proliferation. Using the immunodeficient MyD88 knock-out mouse we were recently able to develop a mouse model of human catarrhal pertussis infection in which human-like high bacterial loads in mouse nasal cavity could be reached[3]. This allowed to demonstrate nasal shedding and transmission of *B. pertussis* in adult mice. Using a set of isogenic mutants deficient in production of various known virulence factors of *B. pertussis* we were able to narrow down those required for high-level infection of the nasal cavity, bacterial shedding and transmission to new hosts. These comprise the adhesins FhaB and fimbriae[3]. We are now about to characterize the synergic contributions of the two adhesive systems and their role in immunosuppressive signaling into ciliated airway epithelial cells and in protection of bacteria from the attack of antimicrobial peptides and complement. Establishment of a mouse model of human nasopharyngeal catarrhal pertussis disease and *B. pertussis* transmission opens the way to exploitation of the wealth and power of mouse genetics tools for dissection of the host mechanism involved in pathogen transmission. Use of bacterial mutants then enables to identify and characterize bacterial virulence factors that harness host physiology and account for the capacity of the pathogen to infect the nasopharynx and transmit to new hosts.

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L-15
BACTERIAL RESISTANCE TO RIFAMPICIN BY ITS MODIFICATIONS

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Rifamycins are antibacterial compounds that target RNA polymerase (RNAP)[1]. 3D structures of these compounds resemble "baskets" composed of an aliphatic chain (reminding a handle) attached to a naphthalene aromatic core (bottom part of the basket). Rifampicin (syn. rifampin), a semisynthetic compound[2], is the most clinically relevant rifamycin, used against Gram-positive bacteria, perhaps most notably against mycobacteria that contain serious pathogens such as *Mycobacterium tuberculosis*[3]. Resistance to rifampicin arises due to mutations in the binding site in RNAP[4] and to various other mechanisms. An important class of rifampicin resistance mechanisms is mediated by four types of modifications of the compound (Fig. 1).

First, rifampicin can be phosphorylated. This is mediated by a homolog of a phosphoenolpyruvate synthase named rifampicin phosphotransferase (RPH). RPH phosphorylates the hydroxyl group on C21 of the aliphatic chain of rifampicin, disturbing its interaction with the b subunit of RNAP[5].

Second, rifampicin can be ADP-ribosylated. This is mediated by the enzyme ADP-ribosyltransferase (Arr). Rifampicin binds to Arr through main chain atoms of the protein, not utilizing the side chains of the amino acids. For *Mycobacterium smegmatis*, it was shown that Arr2 binds NAD+ and transfers its ADP-ribose to C23 of rifampicin, replacing the hydroxyl group[6]. This hydroxyl group is important for binding of rifampicin to RNAP and its modification abolishes one of the contacts of rifampicin to the b subunit of the enzyme, providing resistance.

Third, rifampicin can be glycosylated. Glycosylation is performed by the enzyme glycosyltransferase (Rgt), the sugar moiety is transplanted onto rifampicin from UDP-glucose. The modification occurs on C23, as in the previous case[7].

Fourth, the closed "basket" structure of rifampicin can be linearized by rifampicin monooxygenase (Rox). Rox transfers a hydroxyl group to C2 of the aromatic core of rifampicin. C-N bond cleavage at C2 ensues, breaking the aliphatic chain[8].

Of these mechanisms, rifampicin glycosylation is perhaps the most abundant mechanism of rifampicin inactivation, at least among soil microorganisms[9].

Modifications of rifampicin by bacteria inactivate/ decrease efficiency of the drug. Conversely, rifampicin is being modified by researchers to overcome bacterial defences against this compound[10]. It is an arms race and novel modifications are urgently needed, as well as deeper insights into the mechanisms of rifampicin resistance. We will discuss the strategies employed by both bacteria and researchers, describe emerging mechanisms of rifampicin resistance, relevant protein factors involved, such as the RNAP interacting factor HelD (HelR)[11,12], and present results about genetic regulatory circuits governing bacterial response to this antibiotic.

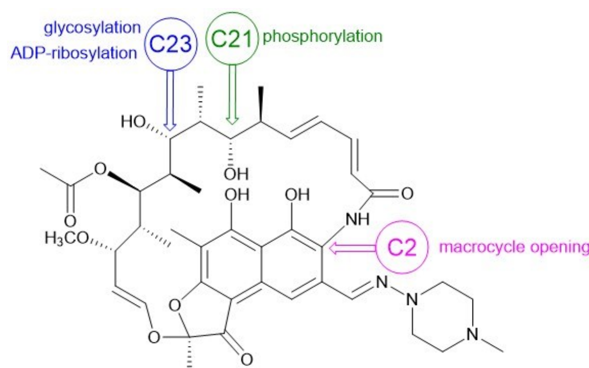

Fig. 1. **Structure of rifampicin with indicated positions where bacteria modify this antibiotic to inactivate it.**

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L-16
BREAKING ANTIBIOTIC RESISTANCE IN BACTERIA

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Our efforts are directed to novel ribosome-targeting antibiotics overcoming the clinically most problematic ribosome-operating resistance mechanism: Erm methyltransferase conferring cross-resistance to MLS antibiotics (Macrolides-Lincosamides-$Streptogramins_B$). For this, we capitalized on the experience gained from almost two decades of our research on molecular mechanisms of ribosomal operating antibiotics, principles of molecular evolution of their biosynthesis[1,2] an also on function of relevant resistance-mechanisms, in particular on ABCF-mediated resistance[3,4,]. We found that the resistance conferred by ABCF family ATPases has some surprising and unique features sharing charateristics of resistance and signaling function[4]. Despite their ubiquity and flexibility in antibiotic-specificity and thus the risk potential, the knowledge on this protein family is fragmentary only. Two poster presentations (G. Balikova Novotna and M. Koberska) are thus presenting our results on ABCF proteins in antibiotic-producing strains as well as in pathogenic bacteria.

Regarding our summarized knowledge, we postulated lincosamides as the most promising development group of ribosome-targeting molecules: There is a low potential risk for development of ABCF-mediated resistance if compared to macrolides, the prolonged shape of lincosamide molecule is open for improving modifications at its both ends and existing natural lincosamides (lincomycin and celesticetin) provided direct inspiration for starting modification attempt. Celin is a hybrid lincosamide compounds designed based on the detailed knowledge of the biosynthesis of natural lincosamides, celesticetin and lincomycin, and their ribosomal binding site[5]. As expected, hybrid Celin was found to be a more effective antibiotic if compared to natural lincomycin, probably due to its extended molecular surface for interaction with ribosome. In parallel to lincomycin and its more potential chlorinated derivative clindamycin, also chlorination of Celin molecule (resulting in ClinCelin) improved antibiotic activity and even more dramatically. The *in vitro* MIC testing against a panel of characterized pathogenic strains proved usually 8-16x better efficiency when compared to clindamycin, the most efficient lincosamide antibiotic on the market. Also, the effect against *Clostridioides difficile* was documented, what is important to decrease the risk of preudomembraneous colitis, a frequent and dangerous infective complication after extensive antibiotic treatment.

In vivo testing of ClinCelin on mice and rats also provided some promising outputs. The maximal tolerated dose (MTD) testing revealed the extremely low overall toxicity for per os administration (MTD > 2000 μg/kg), similar or even better value, when compared to less efficient clindamycin. Further *in vivo* testing on the rat model revealed partial degradation of the antibiotic molecule, mainly the cleavage of the ester bond connecting a salicylate moiety to the amino sugar unit. However, after a single dose per oral administration, the intact molecule prevailed in tested organs like the liver, heart and lungs, but in the feces and urine the metabolic degradation products dominated as well as in the blood serum.

MIC testing of ClinCelin against MLS resistant *Staohylococcus* and *Streptococcus* strains resulted in even more dramatic MIC decrease (even by two orders of magnitude for some tested strains), however, still clinically insufficient for many of them. In cooperation with the small company Santiago chemicals within the synergic project, dozens of Celin and ClinCelin derivatives were prepared. Notably, this „knowledge based approach“ led to remarkably high portion of the compounds exhibiting MIC values comparable to ClinCelin and several were effective even to MLS resistant strains. This project synergy considerably accelerated the preparedness for following testing on the infection *in vivo* models in NIVB project.

Last but not least, we are developing a bioinformatic tool for sophisticated search of functionally related groups of genes (see poster of Z. Kamenik). The complex natural products are encoded by comprehensive biosynthetic gene clusters, exhibiting “mosaic” pattern. Mosaic clusters consist of several gene subclusters coding or for biosynthesis of precursors of complex final product, or their connecting elements or even for some functionally related proteins not reflected in the final structure. Example of such functional gene subcluster is a “regulatory-resistance unit” in lincomycin cluster, coding for ABCF protein, pathway specific regulator, antibiotic efflux protein and for the methyltransferase conferring MLS resistance. We found that subclusters of similar composition are frequently present in biosynthetic gene clusters for ribosome-targeting antibiotics. Also, this bioinformatic tool can be used specifically for search of yet unkown groups of ribosome-targeting antibiotics.

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P-35

THE AAA[+] ATPASE RAVA AND ITS BINDING PARTNER VIAA MODULATE *E. COLI* AMINOGLYCOSIDE SENSITIVITY THROUGH INTERACTION WITH THE INNER MEMBRANE

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Enteric bacteria have to adapt to environmental stresses in the human gastrointestinal tract such as acid and nutrient stress, oxygen limitation and exposure to antibiotics. Membrane lipid composition has recently emerged as a key factor for stress adaptation. The *E. coli ravA-viaA* operon is essential for aminoglycoside bactericidal activity under anaerobiosis but its mechanism of action is unclear. Here we characterise the VWA domain-protein ViaA and its interaction with the AAA[+] ATPase RavA, and find that both proteins localise at the inner cell membrane. We demonstrate that RavA and ViaA target specific phospholipids and subsequently identify their lipid-binding sites. We further show that mutations abolishing interaction with lipids restore induced changes in cell membrane morphology and lipid composition. Finally we reveal that these mutations render *E. coli* gentamicin-resistant under fumarate respiration conditions. Our work thus uncovers a *ravA-viaA*-based pathway which is mobilised in response to aminoglycosides under anaerobiosis and engaged in cell membrane regulation[1].

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

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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.

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THE FIM AND FHA B ADHESINS PLAY A CRUCIAL ROLE IN NASAL CAVITY INFECTION AND BORDETELLA PERTUSSIS TRANSMISSION IN A NOVEL MOUSE CATARRHAL INFECTION MODEL

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Pulmonary infections caused by *Bordetella pertussis* used to be the prime cause of infant mortality in the pre-vaccine era and mouse models of pertussis pneumonia served in characterization of *B. pertussis* virulence mechanisms. However, the biologically most relevant catarrhal disease stage and *B. pertussis* transmission has not been adequately reproduced in adult mice due to limited proliferation of the human-adapted pathogen on murine nasopharyngeal mucosa. We used immunodeficient C57BL/6J MyD88 KO mice to achieve *B. pertussis* proliferation to human-like high counts of 10^8 viable bacteria per nasal cavity to elicit rhinosinusitis accompanied by robust shedding and transmission of *B. pertussis* bacteria to adult co-housed MyD88 KO mice. Experiments with a comprehensive set of *B. pertussis* mutants revealed that pertussis toxin, adenylate cyclase toxin-hemolysin, the T3SS effector BteA/BopC and several other known virulence factors were dispensable for nasal cavity infection and *B. pertussis* transmission in the immunocompromised MyD88 KO mice. In contrast, mutants lacking the filamentous hemagglutinin (FhaB) or fimbriae (Fim) adhesins infected the nasal cavity poorly, shed at low levels and failed to productively infect co-housed MyD88 KO or C57BL/6J mice. FhaB and fimbriae thus appear to play a critical role in *B. pertussis* transmission. The here-described novel murine model of *B. pertussis*-induced nasal catarrh opens the way to genetic dissection of host mechanisms involved in *B. pertussis* shedding and to validation of key bacterial transmission factors that ought to be targeted by future pertussis vaccines.

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EFFECTS OF SELECTED PROTEINS ASSOCIATED WITH BACTERIAL RNA POLYMERASE ON THE CELL'S RESISTANCE TO ENVIRONMENTAL STRESS

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Bacterial RNA polymerase (RNAP) is a multisubunit enzyme. The core RNAP consists of a dimer of α subunits, the small subunit ω, and two large subunits β and β' that form the active site. This core is capable of transcription elongation and termination but not initiation. To localize and bind to the initiation site, RNAP must associate with a σ factor to form the holoenzyme. σ factors allow RNAP to recognize DNA sequences called promoters where the transcription bubble is formed and transcription initiates with nucleoside triphosphates or alternative substrates[1]. In addition to the mentioned subunits, RNAP from gram-positive Firmicutes (industrially and medicinally important microorganisms) contain other subunits such as δ and ε. δ is important for promoter selectivity and the transcription initiation process[2,3]. The role of ε is still poorly defined[4,5].

We have performed a panel of experiments with *Bacillus subtilis* (a species of the Firmicutes phylum) strains lacking various non-essential subunits of RNAP. We compared these strains with wt and determined phenotypic effects after environmental stress (nutritional downshift, antibiotic challenge), defined the transcriptomes of the strains, and characterized mechanistic aspects of the absence of these subunits in transcription by *in vitro* experiments with purified components. The results will be presented and discussed.

Acknowledgement
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P-39

BACTERIAL WARS: IDENTIFICATION OF NEW TOXINS IN *BACILLUS SUBTILIS*

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Different bacterial species use extracellular toxins to fight against each other. The aim of this project is to identify and characterize so far unknown extracellular toxic proteins/ lipids produced by gram-positive *Bacillus subtilis*. The laboratory strain of *B. subtilis*, used in our studies, produces known toxins such as sublancin or WapA. Sublancin is an example of how bacteria can obtain beneficial genes from bacteriophages. Sublancin is encoded by the *sunA* gene and together with its antitoxin gene *sunI* is localized on the SPβ prophage region. Sublancin itself is an highly stable toxin that displays antimicrobial activity against various gram-positive bacteria (*e.g. Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus cereus*)[1]. Despite years of sublancin research, the exact mechanism of its action is unknown. WapA was then identified as a contact-dependent growth inhibition protein with tRNAse activity[2,3]. Its mode of transfer between bacteria by nanotubes was recently challenged[4].

In this work, genes encoding the two toxins (*sunA* and *wapA*) were deleted. Subsequently, using culture filtrates of the Δ*sunA*Δ*wapA* strain, we observed that the strain was still able to kill target bacteria and this did not require direct cell-to-cell contact of the toxin producer with the target cell. Next, by systematical deletions, we localized genes for this toxin(s) to specific DNA region. The identity of the toxin(s) will be discussed. This project thus reveals new weapons used by *B. subtilis* in interspecies wars.

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P-40
RIFAMPICIN RESISTOME OF *BACILLUS SUBTILLIS*

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Nowadays, antibiotic resistance is a serious global problem. Rifampicin is a potent inhibitor of bacterial RNA polymerase (RNAP) of clinical importance and it is used to treat severe bacterial infections. It binds to RNAP and blocks transcription of newly synthetised RNA at the stage of 2–3 nucleotides made[1]. Resistance to rifampicin can be mediated by mutations in its binding pocket on RNAP, by active efflux or enzymatic modification of rifampicin[2].

We are interested in novel mechanisms of rifampicin resistance in *Bacillus subtilis*. First, by a proteomic approach we identified proteins with altered levels after rifampicin treatment (in sub-inhibitory concentration) compared to an untreated sample. Subsequently, we focused on upregulated proteins as they could play roles in rifampicin resistance. Second, we examined by RT-qPCR whether the changes in protein levels were at the transcriptional or translational level. Third, we performed phenotypic assays, challenging knock-outs in genes encoding the upregulated proteins with sub-inhibitory concentration of rifampicin. Some of the knock-outs became more sensitive to rifampicin. The roles of selected rifampicin-upregulated proteins in rifampicin resistance as well as regulation of their gene expression will be presented and discussed

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P-41
ANTIBIOTIC RESISTANCE ABC-F PROTEINS: MECHANISM OF RIBOSOME-MEDIATED ATTENUATION DRIVEN BY LINCOSAMIDES, STREPTOGRAMINS A AND PLEUROMUTILINS

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Bacterial Antibiotic REsponsive ABC Family F ATPases (ARE ABC-F) are cytosolic proteins that confer resistance to antibiotics targeting the large ribosomal subunit via a ribosome protection mechanism[1,2] or transduce an antibiotic signal on gene expression[3]. We have shown that the production of two ARE ABC-F proteins, VgaA, which mediates resistance to the lincosamide, streptogramin A, and pleuromutilin (LSaP) antibiotics in staphylococci, and LmrC, which activates lincomycin production in response to lincosamide in *Streptomyces lincolnensis*, is regulated in response to LSaP antibiotics[3,4]. Induction of expression of the both, *vgaA* and *lmrC,* genes requires binding of the antibiotic to the ribosome preventing the formation of a premature transcriptional terminator in the 5' untranslated region (5'UTR). However, the two attenuators differ in the mechanism of premature termination controled by the leader peptide synthesis. Whereas in the case of *vgaA*, disruption of the leader peptide by mutation of the start codon of the corresponding uORF prevents induction, the same mutation in *lmrC* 5′UTR results in constitutive production. The two attenuators differ in the length and sequence of the leader peptides, suggesting that the mechanism of ribosome arrest induced by LSaP antibiotics may be different. Understanding the molecular details of how the ribosomal arrest complex is formed in response to LSaP antibiotics in different attentuators will facilitate the development of new derivatives with improved activities against mutli-resistant pathogens.

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P-42

ANTIBIOTIC RESISTANCE OR REGULATION? THE ROLE OF RIBOSOME-BINDING ABCF-ATPASES

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Bacterial Antibiotic REsistance ABC-F family ATPases (ARE ABC-F) confer resistance to antibiotics by displacement from their binding sites on the 50S ribosomal subunit. ARE ABC-F form seven groups that differ in both resistance phenotype and taxonomic origin. Protein groups ARE1-3, ARE6, and ARE7 include clinically important and characterized resistance proteins (e.g., VgaA, LsaA, MsrA, or OptrA) found predominantly in Firmicutes, whereas proteins in groups ARE4 and ARE5 are found almost exclusively in Actinobacteria, including Streptomyces, the most prolific producers of antibiotics. Streptomycete-derived antibiotics interfere with proteosynthesis and inhibit bacterial cell growth, but also act as triggers for secondary metabolism at subinhibitory concentrations. We found that the ARE5 ABC-F protein, LmrC, from the lincomycin biosynthesis gene cluster of *Streptomyces lincolnensis* activates transcription of the cluster-specific transcriptional regulator LmbU in response to lincosamides, enabling high-level lincomycin production. Construction of transcriptional reporters and mutagenesis of the 5'-untranslated region of the *lmbU* gene suggest that LmrC regulates transcription of *lmbU* by arresting ribosomes at a regulatory upstream uORF, a mechanism consistent with ribosome-mediated transcriptional attenuation regulating antibiotic resistance genes in response to antibiotics[1]. The presence of ARE ABC-Fs in known biosynthetic clusters for ribosome-targeting antibiotics suggests that ABC-F-mediated signaling is widespread in antibiotic-producing actinomycetes and therefore can be used to identify novel biosynthetic pathways for these drugs.

Furthermore, functional analysis of the two ARE5 ABC-Fs from *Streptomyces coelicolor*, which are not encoded in any known biosynthetic gene cluster, shows that the functions of antibiotic resistance and antibiotic signal transduction can be assigned to the members of the same protein subfamily. Considering that the ARE ABC-F genes are encoded in the genomes of many clinically relevant bacterial species, it is important to determine the molecular determinants of antibiotic resistance and antibiotic signaling functions and understand to what extent the ARE ABC-Fs pose a threat to newly developed ribosome-targeting drugs.

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P-43

BIOINFORMATICS TOOL FOR GENE COLOCALIZATION – GENOME MINING TOWARDS NEW BIOACTIVE COMPOUNDS

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Microbial specialized metabolites represent a great reservoir of bioactive compounds including antibiotics. Current strategies to discover novel drug-leads of microbial origin take advantage of the enormously increasing number of genome sequences of microorganisms. These publicly available data include information on biosynthetic gene clusters (BGCs), i.e., sets of biosynthetic, regulation, and (self)resistance genes associated with the production of a specialized metabolite.

Genome mining facilitates analysis of genome sequences focused on BGCs. Top-down tools such as MIBiG, antiSMASH, or PRISM, which are dependent on databases of known BGCs, are suitable for dereplication of known BGCs and BGC prioritization. However, targeted search for BGCs of new classes of metabolites, metabolites with exotic structural motifs, or metabolites of a specific function requires bottom-up approach independent of databases of known BGCs.

We have developed a bottom-up online tool, which searches the GenBank data and retrieves gene sequences with two (or more) marker genes co-localized within certain distance in a genome. The main output displays the genetic neighborhoods of the marker genes and allows their further analysis. The tool is based on a Python code and includes a general-purpose graphical user interface (GUI) for users with no bioinformatics background.

We have applied this tool to discover (on the genomics level) new metabolites with a structural motif of 4-alkyl-L-proline, which has been known to be incorporated only in three classes of microbial metabolites including antibiotics and anticancer agents. Biosynthesis of 4-alkyl-L-proline motif is encoded by a gene subcluster of 2–6 genes. Using these genes as a query, we mined >100 new putative BGCs with the subcluster for 4-alkyl-L-proline biosynthesis. It includes dozens of BGCs of metabolites, into which 4-alkyl-L-proline is incorporated in a new structural/biosynthetic context.

The tool was primarily designed to search for specific structural motives via specific biosynthetic gene subclusters. However, it can be utilized more generally including search for metabolites of certain function via regulation/(self) resistance genes. And even more generally – to point to colocalization of any two or more genes within genomes in the GenBank.

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P-44

DEVELOPMENT AND TESTING OF MORE EFFICIENT AND SAFER LINCOSAMIDE HYBRID ANTIBIOTICS ACTIVE AGAINST MULTIDRUG-RESISTANT BACTERIA

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Celin is a hybrid lincosamide (LS) antibiotic proposed based on the detailed knowledge of the biosynthesis of natural lincosamides and their ribosomal binding site. It combines the properties of two naturally biosynthesized lincosamides celesticetin and lincomycin. Similarly to a more efficient clinically used clindamycin, prepared by chlorination of lincomycin molecule, the Celin chlorinated variant, Clincelin, of was also synthesized. The initial hypothesis supposed improved antimicrobial activity of new hybrid lincosamides and possibly also overcoming of MLS resistence due to more extensive interaction with the ribosomal target. Indeed, the *in vitro* MIC testing against a panel of well characterized pathogenic strains proved ~8–16x better efficiency when compated to clindamycin, the most efficient LS antibiotic on the market. Also the effect against *Clostridioides difficile* was documented, what is important to decrease the risk of preudomembraneous colitis, a dangerous possible consequence of extensive antibiotic treatment. MIC testing against MLS resistant *Staohylococcus* and *Streptococcus* strains showed remarkable MIC decrease, however, still insufficient for clinical use. The maximal tolerated dose (MTD) of Clincelin was tested on the mice model showing extremely low overall toxicity for per os administration (MTD > 2000 µg/kg). Further *in vivo* testing on the rat model revealed partial degradation of the antibiotic molecule, mainly the cleavage of the ester bond connecting a salicylate moiety to the aminosuggar. After a single dose per oral administration, the intact molecule prevailed in tested organs like the liver, heart and lungs, but in the feces and urine the metabolic degradation products dominated as well as in the blood serum, where, however, the overall compounds concentration was low. In cooperation with the start-up company Santiago chemicals within the synergical project, dozens of Celin and Clincelin derivatives were prepared. Notably, this „knowledge based approach“ led to remarkably high portion of the compounds exhibiting MIC values comparable to Clincelin and several were effective even to MLS resistant strains. This project synergy accelerated the preparedness for testing on the infecion *in vivo* models.

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CHARLES UNIVERSITY

L-17
DISCOVERY OF NEW ANTIVIRAL DEFENSE MECHANISMS

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Chronic infection with hepatitis B virus (HBV) is a major public health problem that affects approximately 250 million people worldwide. Treatment of chronic hepatitis B with nucleot(s)ide analogues inhibits the formation of new infectious viral particles but does not eliminate stable covalently closed circular DNA (cccDNA) in hepatocytes, and pegylated interferon α (IFN-α) monotherapy leads to functional cure in less than 8% of people with chronic hepatitis B. To target the mechanisms by which HBV escapes antiviral IFN-α effects, we propose (i) to study the interplay between promyelocytic leukemia protein nuclear bodies (PML-NBs) and HBV in the cytoplasm and nucleus of infected hepatocytes[1], (ii) to identify and characterize interactions of the HBV core protein (HBc) with host proteins and to inhibit virus replication by targeting this mechanism[2], and (iii) to investigate the effects of microenvironment of HBV-infected hepatocyte on plasmacytoid dendritic cells (pDCs), which are important cellular component of the innate immunity system and producers of IFN-α[3]. Our goal is to uncover the antiviral effect of PML NB in the cytoplasm, as well as that of the IFN-α-induced PML NBs on the conversion of HBc-mediated rcDNA to cccDNA and the role of histone, such as H3.3, loading and cccDNA chromatinization and transcription. The early steps of HBV infection were followed by confocal microscopy combining 3D-fluorescence in situ hybridization (FISH) with immunostaining to visualize the location of HBV DNA in the cytoplasm and in the nucleus. Investigation of the regulation of HBV transcription by the histone variant H3.3 and its chaperones HIRA and DAXX/ATRX, and the dynamics of viral chromatin in an antiviral state could allow the discovery of new therapeutic targets. Another approach to achieve functional HBV cure is to target the interface between viral and cellular proteins of HBV cccDNA. We are using proximity-dependent biotinylation proteomic technique (BioID2) to identify novel proteins interacting with HBc and cccDNA. The goal is to identify host proteins that affect viral replication and select new target candidates for possible therapeutic intervention. We used the in vitro high-throughput screening assay to identify small compounds that modulate the respective protein-protein interactions. Among the HBc BioID interactome hits (P<0.01), we characterized some in more details, such as CCDC88A (GIV, GIRDIN) protein, an interacting partner of EGFR and coreceptor of HBV entry; HBV restriction SMC5-SMC6 complex localization factor 2 (SLF2); and the exon-junction complex (EJC) recycling factor PYM1, which binds to the capsid of flavivirus and elicits antiviral activity against these viruses. In addition, we aim to validate whether small molecules modulating NRF1 (nuclear factor erythroid 2-related factor 2; NFE2L1) pathway, responsible for proteasome synthesis and heat shock protein expression, discovered in our group affect HBV replication[4]. Finally, our goal is to target and counteract the inhibitory mechanism by which the microenvironment of HBV-infected hepatocytes suppress production of IFN-α by pDCs. Our aim is to decipher which component of conditioned medium (CM), including infectious HBV particles, subviral particles (SVPs), HBV proteins or extracellular vesicles (EVs), or alternatively a close cell-to-cell contact of HBV-infected hepatocytes co-cultured with pDCs, is responsible for the inhibitory effect[3]. We investigate the effect of the neutral sphingomyelinase-2 inhibitor GW4869, which blocks the export of EVs and HBV particles from hepatocytes on the restoration of the activity of pDC exposed to HBV-infected hepatocytes. We also study a possible inhibitory effect of microRNA, namely the miR-122, miR-146a, and miR155, present as a cargo in EVs from HBV-infected hepatocytes, transferred into pDCs. Collectively, we propose to study mechanisms of HBV escape from intrinsic and innate immunity for discovery of new antiviral therapeutic targets to develop drugs for the treatment of chronically infected HBV patients.

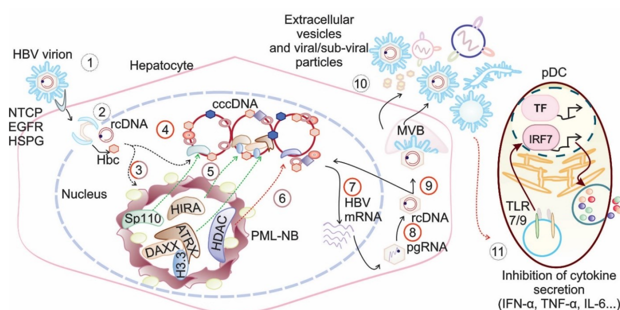

Fig. 1. **Overview of research questions directed towards early steps of HBV replication and interaction with pDCs.**

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L-18
PHLEBOVIRUS TRANSMISSION BY SAND FLIES

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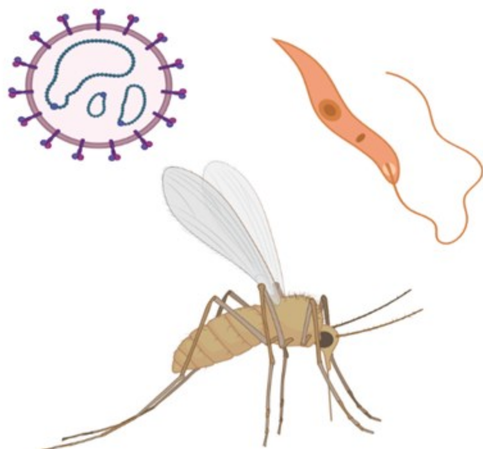

Arboviruses of the genus *Phlebovirus* are currently represented by 67 species found in Eurasia, Africa, and the Americas[1]. Although they have a great impact on human and animal health, information about their biology and transmission is mostly missing. The predicted or confirmed vectors of most phleboviruses are sand flies (Diptera: Psychodidae), tiny bloodsucking insects occurring in latitudes between 50°N and 40°S. Both males and females feed on a natural sugar source, females need also the blood as a source of proteins to develop eggs. Our laboratory has more than 25 years of experience in studies on sand flies and *Leishmania*, maintains the largest collection of sand fly colonies and is considered as a leading team in this area. Here we decided to focus on the development, life cycle, and transmission of two most important phlebovirus species: *Toscana phlebovirus* (TOSV) and *Rift Valley Fever phlebovirus* (RVFV).

TOSV is a human pathogen with symptoms that varies from febrile illness to CNS disease. It is transmitted by sand flies in the Mediterranean area and North Africa[2–5]. Despite a significant effort and examination of various vertebrates, no reservoir hosts of TOSV were confirmed and it is apparent that transovarial or sexual transmission between sand flies is not effective enough to maintain the virus cycle in nature[2,4,6]. Two species, *Phlebotomus perniciosus* and *Phlebotomus perfiliewi,* are considered as proven vectors but the seroprevalence of TOSV in humans and animals in areas where these vectors are not present suggests the involvement of other sand fly species[2]. Our experimental infections of various sand flies revealed high susceptibility of *Phlebotomus tobbi* (infection and dissemination rates of 59.5% and 46% respectively). Moreover, our previous experiments on closely-related Massilia virus suggest the role of sugar meal as a source of sand fly infection[7].

RVFV is endemic in sub-Saharan Africa and the Arabian Peninsula. It affects mainly domestic animals, causing haemorrhagic fever accompanied by anorexia, abortions, and a high rate of death in young animals. RVFV also affects humans, occasionally causing encephalitis or haemorrhagic fever with potential mortality. Mosquitoes are considered as main RVFV vectors[8,9] but the role of sand flies should not be ignored as they co-occur in the same areas. There are only two experimental studies on African sand flies; RVFV developed and disseminated in *Phlebotomus sergenti* and *Sergentomyia schwetzi*[10] and was transmitted to hamsters by *Phlebotomus duboscqi*[11]. However, nothing is known about the susceptibility of European sand fly species to RVFV although this knowledge could be crucial for risk assessment of RVFV spread to Europe.

In addition to phleboviruses, sand flies also transmit *Leishmania* parasites which co-circulate in the same areas as TOSV and RVFV[5,8]. However, no information is available about phlebovirus-leishmania interaction, even though this tripartite interaction may have significant epidemiological significance. We are the only laboratory in Europe keeping all three organisms (sand flies, phleboviruses, leishmania) and able to do such co-infections.

Results obtained within this complex project will clarify various aspects of phlebovirus circulation in nature, reveal the risk of TOSV and RVFV spread in Europe, and help to understand the co-circulation of sand fly-borne pathogens.

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L-19 UNDERSTANDING THE MOLECULAR MECHANISMS OF VIRUSES AND HOST INTERACTIONS

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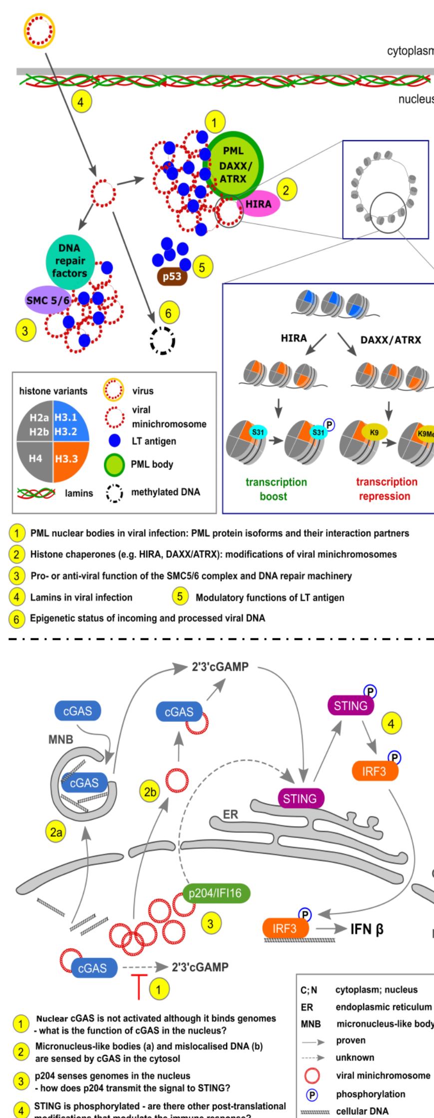
Our research is focused on two objectives, first, the study of interactions between nuclear proteins and viral components which could result in the restriction or establishment of the infection, and second, the elucidation of the mechanisms of innate immune response modulation in infected cells. For that, the model – Murine polyomaviruses (MPyV) and the human pathogen – BK polyomavirus (BKPyV) [1] are used in our lab.

In the first part, we are studying i) the role of PML protein isoforms in viral transcription; ii) the participation of PML-associated chaperons, the HIRA protein, and the DAXX/ATRXX in polyomavirus minichromosomes remodeling and regulation of expression iii) functions of the SMC5/6 protein complex in MPyV replication; iv) role of nuclear lamins in viral infection; v) possible new cell partners of the major viral regulatory protein, LT antigen (this part of the research is carried out in cooperation with Pichová's group from Institute or organic chemistry), and finally, vi) epigenetic modifications of the BKPyV genomes (isolated from patients) that could be connected with transcriptional repression (this work will be performed in cooperation with Stanton's group from Cardiff University, GB, – and Tachezy's group from Charles University). Furthermore, our group will support the studies of validation of the role of PML bodies and the dynamic of HBV DNA chromatinization in the induction of antiviral state in HBV-infected hepatocytes carried out by the group of Šašková, Charles University (our group will perform the standardization of protocols for in-situ DNA hybridization and confocal and super-resolution microscopy studies).

In the second part, we are studying i) the DNA sensors and adaptors involved in the immune sensing of BKPyV in the microvascular endothelial cells which are possible reservoir cells for the virus ii) the mechanisms of activation of the canonical and possibly non-canonical pathways leading to the production of interferon and pro-inflammatory cytokines in BKPyV and MPyV infected cells. For the studies of non-canonical pathways, we focused on understanding the possible activation of STING via TRAF62,3 iii) the mechanisms of modulation of the innate immune responses during MPyV and BKPyV infection by post-translational modification of cGAS DNA sensor and adaptor protein, STING and iv) cross-talk of cell innate immunity and viral components by exploring the interaction between virus early antigens and DNA sensors and adaptors). Furthermore, our group will cooperate with the group of Šašková- Charles University, on studies of the role of plasmacytoid dendritic cells (pDC) in the response to BKPyV. The proposed research is summarized in Scheme 1.



Scheme 1. **Summary of the research topics. The top figure-presents the topics of research for objective 1 and the bottom figure introduces the current model for MPyV immune sensing and the questions that are still unresolved.**

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L-20

CURRENT RESEARCH ON A-to-I EDITING IN THE LABORATORY OF RNA BIOCHEMISTRY, FACULTY OF SCIENCE, CHARLES UNIVERSITY

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Current research in the Laboratory of RNA Biochemistry at the Faculty of Science, Charles University is mainly focused on characterization of 5' mRNA end formation, mRNA modifications and translation initiation in eukaryotes and their viruses. Recently, a possible role of the adenosine deaminase acting on RNA 1 (ADAR1) in eukaryotic translation and mutual interactions between viruses and the human host came in the centre of our interest.

ADAR1 is one of two enzymatically active human RNA adenosine deaminases and is responsible for most of the adenosine to inosine (A-to-I) RNA editing events in human cells. ADAR1 is inducible by interferon and constitutes part of the cellular innate immunity and antiviral defence machinery. However, detailed function of ADAR1 in viral infection can differ from virus to virus, can be manifested both as antiviral and/or proviral, and is poorly understood. Dysregulation in ADAR1 protein level or ADAR1-dependent editing were observed in plethora of cancer types. Reduced ADAR1 activity causes Aicardi–Goutières Syndrome that is characterized by childhood severe encephalopathy and high mortality. For a recent review on ADAR1 refer e.g. to Song et al. 2022 (1).

It is assumed that some of the ADAR1 cellular activities are not related to its deaminase enzymatic activity. We prepared an array of human cell lines bearing disrupted genes in the ADAR1 signalling pathway including ADAR1 itself which we use for investigation into the role of ADAR1 A-to-I editing both in translation and during the virus infection. Methods used to achieve our goals comprise, but are not limited to, RNAseq combined with polysome profiling and translatome analysis to map and evaluate A-to-I edited sites in the viral and cellular RNAs and to decipher a role, which A-to-I editing plays in synthesis of celullar and viral RNAs and proteins. Investigation of the ADAR1 regulon has a potential to either establish ADAR1 as an emerging target for new broad-range antivirals or to find novel cellular and viral targets specific for the particular viruses.

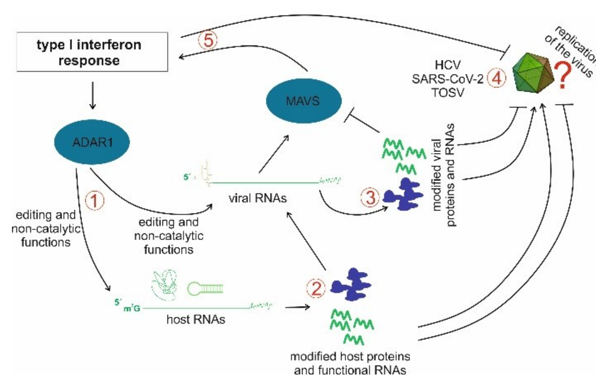

Fig. 1. Overview of the possible research tasks concerning function of ADAR1 regulon during virus infection. Production of ADAR1 is stimulated by IFN. ADAR1 edits both viral and host RNAs, including mRNAs, small RNAs, miRNAs, various ncRNAs etc. It can act both as adenosine deaminase and also by a non-catalytic manner (1). A-to-I edited cellular RNAs can manifest different protein coding, in case of mRNAs) and/or can change their association with other RNAs and proteins. These modified molecules can directly (2) or indirectly (4) influence replication of the virus. The same is valid also for the viral proteins and RNAs (3). The newly-emerged network of modified proteins and RNAs can finally act as either proviral or antiviral environment, depending of the virus and perhaps also the cell origin (4). A-to-I edited RNAs also inhibit MAVS signaling and thus attenuate type I IFN response (5).

Acknowledgement
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L-21
GROUP OF CANCER IMMUNOTHERAPY AND MOLECULAR EPIDEMIOLOGY OF VIRUS INFECTIONS

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The group is composed of the team of Laboratory of Molecular and Tumor Virology (headed by Ruth Tachezy) and Laboratory of Immunotherapy (headed by Michal Šmahel). While the research of the team led by Ruth Tachezy focuses mainly on the molecular epidemiology of anelloviruses, polyomaviruses, herspesviruses, and papillomaviruses with the aim of evaluating the involvement of these viruses in different benign and malignant disorders, the research of the group of Michal Šmahel is focused on the optimization of combined cancer immunotherapy. The research of both teams complements each other. Finding the association of viruses with particular disorders opens the possibility of searching for diagnostic, therapeutic, preventive, and prognostic markers of these diseases. Therapeutic markers are being further explored in both *in vitro* and *in vivo* model systems and combined immunotherapy is further evaluated.

In the National Institute of Virology and Bacteriology, the team will explore the role of microbiome/virome in the pathogenesis of autoimmune and malignant diseases linked to human papillomaviruses and polyomaviruses. In the laboratory, the pipeline for the preparation and analysis of viromes was implemented and proved to allow the reliable detection of a wide range of viruses with different characteristics[1]. The method will be further optimized and utilized for virome analyses in patients with autoimmune disorder – psoriasis – since our previous research has shown that these patients on long-term treatment with biologics might be at increased risk of acquisition or reactivation of numerous viral infections[2].

The second direction of research will extend our recent findings where we focused on the analyses of the tumor microenvironment (TME) of head and neck squamous cell carcinomas (HNSCC; Fig. 1) in relation to their etiology to explain the prognostic advantages of patients with HPV-associated tumors. In the laboratory, mass cytometry for solid tumors[3] and multiplex multispectral fluorescent immunohistochemistry were implemented for the complex analyses of TME. In previous studies with the help of new techniques, we have analyzed TME of HNSCC of viral and non-viral etiology and detected PD1+CD8+ cells as an independent positive prognostic marker for patients with HNSCC[4]. Furthermore, we also focused on tumor-associated macrophages (TAMs), which represent the main immune population in TME with a controversial influence on the prognosis. We have observed more pro-tumorigenic M2 TAMs and higher mRNA expression of M2 markers – cluster of differentiation 163 (CD163), ARG1, and prostaglandin endoperoxide synthase 2 (PTGS2) in HPV non-associated tumors and M1 marker nitric oxide synthase 2 (NOS2) in an HPV+ group. The expression of ARG1 mRNA was revealed to be a negative prognostic factor for the overall survival of HNSCC patients[5].

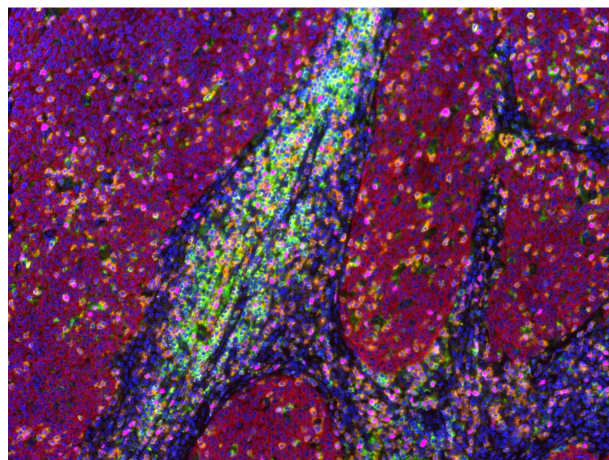

Fig. 1. **Analysis of the head and neck squamous cell carcinoma environment.** Multispectral fluorescent staining of formalin fixed paraffin embedded tumor samples (green – CD4, orange – CD8, yellow – CD3, magenta – FOXP3, red – panCytokeratin AE1/AE3, blue – DAPI).

In mouse models of HPV-induced tumors with reversible or irreversible MHC class I downregulation established in our laboratory, we studied combined immunotherapy aimed at repolarization of protumor M2 TAMs into anti-tumor M1 TAMs and found resistance to this treatment in tumors with irreversible MHC class I downregulation[6].

Within the project we will cooperate with the group of Sandra Huerfano from the Faculty of Science, Charles University on antiviral innate immune responses to human polyomaviruses; Pavel Dřevínek group from the 2nd Medical Faculty, Charles University on anelloviruses dynamics; Marián Hajdúch group at the Faculty of Medicine and Dentistry, Palacký University on the HPV-specific antibody prevalence; and Radin Nencka group from The Institute of Organic Chemistry and Biochemistry, Czech Academy of Science, on STING activation.

Acknowledgement
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L-22
EMERGING PATTERNS OF MICROBIOME AND RESISTOME IN THE HEALTHCARE ENVIRONMENT

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The project led by Prof. Dřevinek at the 2nd Faculty of Medicine has two main research objectives (see below). Active collaboration with the groups in the consortium is planned.

Research objective 1: Molecular epidemiology and antimicrobial resistance in healthcare-associated pathogens

Antimicrobial resistance (AMR) is one of the biggest public health challenges. A rapid spread of multi- and pan-resistant bacteria leads to difficult-to-treat infections and higher mortality in healthcare settings. To address this issue, Pavel Drevinek's research team initiated several national surveillance studies in the Czech Republic and Slovakia with the goal to map the epidemiology of healthcare-associated pathogens (1-3). In addition, large sets of colistin-resistant Enterobacterales and *Burkholderia cepacia* complex isolates were collected over the years during the grant projects (4).

The aim of the project is (i) to perform the prospective surveillance of the currently important nosocomial pathogens in the Czech Republic and Slovakia, i.e. *C. difficile*, *S. aureus* (including methicillin-resistant) and coagulase-negative Staphylococci, vancomycin-resistant Enterococci, and multidrug-resistant Gram-negative bacteria with plasmid-mediated resistance to the last resort antibiotics. The second aim is (ii) to gather deeper knowledge about major clones of antibiotic-resistant nosocomial pathogens and get insight into the forces driving their spread by studying isolates acquired during the prospective surveillance and from retrospective collections of the team. Comparative analysis of genomes and antimicrobial resistance of acquired isolates will allow to elucidate of the specific resistance determinants and their genetic context (plasmid or chromosome-associated genes, mutation) and also perform a phylogenetic analysis of isolates putting them in the local and global context of respective pathogen epidemiology. In addition, the biofilm formation and growth rates of resistant isolates in different conditions will be analysed as important factors influencing the clinical success of the pathogenic bacteria.

Research objective 2: The human virome in human disease and its interaction with other exposome components

The modern concept of the exposome encompasses all the multifaceted exposures acting upon the organism (the microbiota, diet, toxicants, climate), and the response of the organism induced by these exposures (e.g. changes in its transcriptome, proteome, metabolome). Microbes are very important exposome components, including bacteria, archaea, parasites, fungi or viruses. The technical advances in metagenomics over the last decade enabled researchers to define the microbial component of the exposome with reassuring accuracy, yet the knowledge is incomplete of the association of microbes with a great range of primarily non-infectious diseases, and gaps remain also in the definition of what can be regarded as a healthy microbiome.

The proposed project is a logical extension of the works on virome exploration that the laboratory under the leadership of prof. Cinek has been conducting for over 10 years (5–8). The main objective of this part of the project is to explore the virome in longitudinal sample collections from several diagnoses of medical and societal importance. The project will study the virome during the natural disease course, in reaction to standard treatment modalities, or to targeted interventions in ongoing clinical trials. This will be done in the context of other components of the microbial exposome (bacteriome, mycobiome, parasitome), with background metadata characterizing the available non-microbial exposome (diet, physical activity etc).

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-23
DEVELOPMENT OF ANTIMICROBIAL AGENTS WITH ACTIVITY AGAINST DRUG-RESISTANT BACTERIA

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Tuberculosis (TB) is a widespread infectious disease predominantly caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). The discovery and widespread use of antimicrobials effective against TB starting in the middle of the 20th century allowed a dramatic reduction in TB mortality. However, despite the success of chemotherapy, the disease became the leading infectious killer seven decades later. Recently, the COVID-19 pandemic has reversed years of progress in providing essential TB services and reducing the burden of TB disease. Reduced access to TB diagnosis and treatment has resulted in an increase in TB deaths. The World Health Organization (WHO) estimated 10 million new cases of TB and 1.3 million deaths from TB among HIV-negative people in 2020. Additionally, TB was a contributing factor of approximately 214,000 HIV-related deaths. COVID-19 and TB co-infection is another threat issue, even if some symptoms are identical for both diseases. By 2050, deaths due to antimicrobial resistance are expected to reach 10 million deaths/year if no serious measures are implemented. Among all, *Staphylococcus aureus* (SA) remains a challenging pathogen that causes common and life-threatening infections of different organ systems. SA is the leading cause of bacteremia, infectious endocarditis, osteomyelitis, as well as being the main causative organism of skin and soft tissue infections, device-related infections, and pleuropulmonary infections, including hospital-acquired pneumonia, ventilator-associated pneumonia, and health care-associated pneumonia SA possess the ability to form biofilms on host tissue or medical implants, which contributes to the persistence nature of chronic SA infections. The current spread of multi-drug resistance is often mentioned in connection with bacterial pathogens, including clinically important methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA strains have developed resistance to many commonly used antibacterial compounds from different groups, e.g., aminoglycosides, macrolides, fluoroquinolones etc. WHO has declared the antibacterial research against MRSA as one of the priorities. Therefore, there is an urgent need to develop antimicrobial agents with new mechanisms of action or with the ability to overcome resistance of (myco)bacterial strains.

The project aims to elucidate the structure-activity-toxicity relationships of the unique, in-house structural types of compounds with good anti(myco)bacterial activity and to elucidate their mechanism of action[1–4]. Based on the obtained results and *in silico* prediction[5], design and synthesis of new analogs with improved activity and ADMETox profile will be performed and their *in vitro* and *in vivo* pharmacokinetic and pharmacodynamic profile will be evaluated. The final goal of this project is to develop novel (pre)clinical antimicrobial candidate(s).

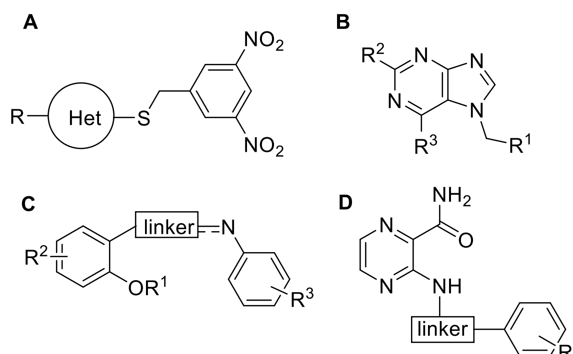

Fig. 1. **Structural types of compounds with significant anti(myco) bacterial activity developed by the authors that will serve as lead compounds in this project**

All prepared compounds will be subjected to basic anti (myco)bacterial activity screening *in vitro* in our established G+/G- bacterial and mycobacterial screening panels. Advanced testing will include static/cidal activity determination, evaluation of the anti(myco)bacterial activity against clinical isolates (including drug-resistant strains), time-kill assays, assays to evaluate the mechanism of action, and screening of antibiofilm activity. Checkerboard studies will be performed to evaluate the potential synergism between candidate compounds and standard anti(myco)bacterial compounds used in clinical practice. Candidate antibacterial compounds will be evaluated *in vivo* to assess their toxicity and antibacterial activity in the insect model of *Galleria mellonella*. In the case of potential antiTB agents, the ability of newly developed compound(s) to cure TB will be determined in pharmacokinetically-guided *in vivo* study in a murine model of TB.

Acknowledgement
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L-24

PREFERRED β-LACTONE SYNTHESIS CAN EXPLAIN HIGH RATE OF FALSE-NEGATIVE RESULTS IN THE DETECTION OF OXA-48-LIKE CARBAPENEMASES

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The resistance to carbapenems is usually mediated by enzymes hydrolyzing β-lactam ring. Recently, an alternative way of the modification of the antibiotic, a β-lactone formation by OXA-48-like enzymes, in some carbapenems was identified. We focused our study on a deep analysis of OXA-48-like-producing *Enterobacterales*, especially strains showing poor hydrolytic activity. In this study, well characterized 74 isolates of *Enterobacterales* resistant to carbapenems were used. Carbapenemase activity was determined by matrix-assisted laser desorption/Ionization time-of-flight mass spectrometry (MALDI-TOF MS), liquid chromatography/mass spectrometry (LC-MS), and Carba-NP test. As meropenem-derived β-lactone possesses the same molecular weight as native meropenem (MW 383.46 g/mol), β-lactonization cannot be directly detected by MALDI-TOF MS. In the spectra, however, the peaks of m/z=340.5 and 362.5 representing decarboxylated β-lactone and its sodium adduct were detected in 25 out of 40 OXA-48-like producers. In the rest 15 isolates, decarboxylated hydrolytic product (m/z=358.5) and its sodium adduct (m/z=380.5) have been detected. The peak of m/z=362.5 was detected in 3 strains co-producing OXA-48-like and NDM-1 carbapenemases. The respective signal was identified in no strain producing class A or class B carbapenemase alone showing its specificity for OXA-48-like carbapenemases. Using LC-MS, we were able to identify meropenem-derived β-lactone directly according to the different retention time. All strains with a predominant β-lactone production showed negative results of Carba NP test. In this study, we have demonstrated that the strains producing OXA-48-like carbapenemases showing false-negative results using Carba NP test and MALDI-TOF MS preferentially produced meropenem-derived β-lactone. We also identified β-lactone-specific peak in MALDI-TOF MS spectra and demonstrated the ability of LC-MS to detect meropenem-derived β-lactone.

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P-45
REVEALING NEW HEPATITIS B VIRUS-HOST INTERACTIONS BY PROXIMITY LABELLING

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HBV is a major health concern that affects the lives of more than 250 million people worldwide and causes more than 780,000 deaths annually. Current treatment helps reduce the risk of developing serious liver diseases, but does not completely eliminate the infection. Developing a complete cure for hepatitis B requires in-depth knowledge of the molecular and cellular mechanisms of HBV infection. A detailed analysis of virus-host interactions at the molecular level potentially reveals essential host factors as possible targets for new antiviral interventions.

In this study, we focused on exploring the interactome of HBV core protein (HBc) with respect to its pluripotent functions in the HBV life cycle. HBc provides building blocks for HBV capsid, and plays a role in cccDNA stability, transcription and epigenetic regulation, as well as in reverse transcription of HBV RNA and viral egress. We used a proteomic approach of proximity labelling (PL), called BioID2, which allows the detection of weak and transient protein-protein interactions directly in living cells. We mapped the flux of the assembly-incompetent HBV core protein fused with biotin ligase through the cytoplasm, nucleus, and nucleolus. Newly identified HBV core interacting partners are yet to be validated by co-immunoprecipitation and their molecular functions characterized in the context of HBV infection. To this end, viral parameters will be compared after knock-down of the respective genes in HBV infected cells versus control cells. The goal is to identify the key host proteins affecting viral replication and catalogize new target candidates for a possible therapeutic intervention.

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P-46
THE ROLES OF PML NUCLEAR BODIES IN THE EARLY STEP OF HBV INFECTION

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Promyelocytic leukaemia nuclear bodies (PML-NBs) orchestrate 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 depending on the cellular state and the role of PML-NBs needs to be further elucidated. Our main goal is to elucidate the interplay between PML-NBs and HBV in the early steps of HBV infection.

In contrast to herpesviruses, HBV infection does not induce IFN-signaling and our preliminary results show that HBV infection does not induce the formation of "antiviral" PML-NBs. Thus, we analyzed the effect of IFN-α-induced PML-NBs on the dynamics of the conversion of HBV rcDNA to cccDNA and HBV-DNA localization in the nucleus by FISH and qPCR. 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 and the secretion of HBV antigens. We also observed an incresing association of HBV-DNA with PML-NBs in the early steps of infection in IFN-α-pretreated HepG2-NTCP. These experiments have clinical relevance because of IFN-α therapy in chronically infected patients with HBV.

Acknowledgement
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P-47

ACTIVATION OF cGAS-STING PATHWAY BY STING AGONISTS INDUCES TYPE I INTERFERON SECRETION INSEPARABLE FROM CD14+ MONOCYTE CELL DEATH

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Stimulation of pattern recognition receptors of the innate immune pathways is a promising approach for the treatment of viral diseases and cancer. The cyclic-GMP-AMP synthase – stimulator of interferon genes (cGAS-STING) pathway is crucial for the detection of double-stranded DNA in the cytoplasm, which is a sign of cell damage or infection[1]. STING activation with natural or synthetic agonists induces in peripheral blood mononuclear cells (PBMC) type I interferon and proinflammatory cytokine secretion[1,2].

Although the overall effect of STING agonists on PBMC viability was minimal, it caused a complete loss of CD14+ monocytes[3]. Inhibition of the cGAS-STING pathway blocked both type I interferon secretion and depletion of the CD14+ monocyte. Furthermore, we identified that CD14+ monocytes underwent apoptosis. However, we could not exclude that other mechanisms were involved in CD14+ monocyte cell death, as the cGAS-STING pathway was also associated with pyroptosis and necroptosis[4]. Since STING agonists induced IL1β secretion[3], which requires processing by inflammasome[4], we speculate that pyroptosis is likely involved in the CD14+ monocyte death process. Finally, a phenomenon called PANoptosis, which combines pyroptosis, apoptosis, and necroptosis, has recently been described[5]. Since the cGAS-STING pathway represents a new target for the development of antiviral drugs, we will investigate the mechanisms of CD14+ monocyte cell death induced by the cGAS-STING agonists further.

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P-48

COMPARISON OF SUSCEPTIBILITY OF VARIOUS SAND FLY SPECIES TO TOSCANA VIRUS

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Toscana phlebovirus (TOSV) is an emerging but still neglected human pathogen circulating in the Mediterranean area, Middle East and North Africa. Its manifestation varies from non-symptomatic forms through febrile illness to CNS disease. Despite of its importance, the information about TOSV biology and epidemiology is very limited. Currently, only sand flies (Diptera: Phlebotominae), specifically *Phlebotomus perniciosus* and *Phlebotomus perfiliewi*, are considered as a proven vectors of TOSV[1,2]. However, the TOSV spread into the new geographical areas as well as the TOSV detection in several other sand fly species suggested that spectrum of TOSV vectors is much broader[2]. Here we aim to study in detail the vector competence of four sand fly species (*Phlebotomus tobbi*, *Phlebotomus sergenti*, *Phlebotomus papatasi* and *Sergentomyia schwetzi*) for TOSV A and TOSV B lineages.

Experimental infections showed that none of the sand fly species tested were susceptible to TOSV A infection. In TOSV B, the most susceptible vector was *P. tobbi,* with infection and dissemination rates of 59.5% and 46%, respectively. *Phlebotomus sergenti* seems to be less susceptible to TOSV B (dissemination rate 5.9%), however in all positive females virus disseminated to the head with salivary glands. *Phlebotomus papatasi* and *S. schwetzi* were refractory to TOSV B infection.

This information is crucial from an epidemiological point of view as due to climate change the sand flies are expanding in Europe, together with pathogens they transmit[3].

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P-49

UNDERSTANDING THE MOLECULAR BASES OF INNATE IMMUNE RESPONSES TO FOREIGN OR MISLOCALIZED SELF-DNA

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We previously described that Murine polyomavirus MPyV induces type I interferon (IFN) responses via the sensors, p204 (human IFI16 homolog) and cGAS which sense viral genomes and micronucleus-like bodies generated during infection[1]. Sensing by both DNA sensors leads to canonical STING activation. In the canonical pathway, STING recruits and activates TBK1, which phosphorylates STING and IRF3 transcription factor to induce IFN and other cytokines. During MPyV infection, there are additional cell responses activated, i) DNA damage response and ii) low levels of apoptosis – the so-called sub-lethal apoptosis. Importantly, in the last years, DNA damage induced by different stimuli, among them sub-lethal apoptosis (in which caspase-activated Dnase (CAD) generates DNA breaks) has been shown to induce IFN responses[2,3]. Moreover, a novel, noncanonical pathway for IFN production was proposed in the responses to DNA damage. In this pathway, DNA sensing is mediated by IFI16 and DNA damage response factors. After sensing, STING signaling complex that includes the p53 and TRAF6 is assembled. TRAF6 ubiquitinylates STING, leading to the activation of the transcription factor, NF-κB[3]. Here, we investigated a possible contribution of the STING non-canonical pathway and the role of sublethal apoptosis in the IFN and other cytokine responses to the infection by MPyV. For the study, we followed innate immune responses in the mouse embryo fibroblasts (MEF) with knockout (KO) of *TRAF6* or *CAD* gene.

We found that TRAF6 is essential for immune responses to MPyV, since the absence of TRAF6 impairs the translocation of NF-Kβ to the nucleus and leads to the downregulation of IFN and pro-inflammatory cytokines, IL-6 and CXCL10. Furthermore, our results suggest that sublethal apoptosis is responsible at least in part for the immune responses to MPyV since CAD-KO displayed lower levels of IL-6 and CXCL10.

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P-50

ROLE OF PML NUCLEAR BODIES DURING MOUSE POLYOMAVIRUS INFECTION: PML PROTEIN ISOFORMS AND THE NON-CANONICAL HISTONE H3.3

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The role of promyelotic nuclear bodies (PML NBs) in virus infection have been intensively studied. However, their possible restriction function and the role of proteins associated with PML NBs is not well understood yet. The first aim of this study was to characterize the possible restriction function of the main structural component of PML NBs, PML protein, and its individual isoforms during Murine polyomavirus (MPyV) infection. The mouse *PML* gene is composed of nine exons that are alternatively spliced to produce isoforms. Only 3 isoforms (mPML1-3) have been described in the mouse model. At early times post MPyV infection, we observed mPML NBs to localize in close proximity to MPyV transcriptional and replication centres. In *PML* KO cells, transcription from the early gene promoter was more efficient in comparison with that in wt cells, suggesting trascription restriction by PML protein(s). During transient expression, the largest isoform, mPML2, became incorporated into endogenous PML NBs and also in *Pml* KO cells, it formed speckles. Nevertheless, the overexpression of mPML2 isoform did not significantly affect MPyV infection in both *Pml* KO and wt cells. Further, we detected cellular expression of two predicted mPML isoforms, mPMLX4 and mPMLX6, and one novel isoform, named by us mPMLXK. Further studies have been carried out to evaluate their functions. The second aim of this study was to reveal the possible participation of proteins transiently interacting with PML NBs, chaperones HIRA and DAXX/ATRX, in deposition of non-cannonical histone H3.3 into viral minichromosomes and the functional consequences of such incorporation. We found H3.3 incorporated not only in condensed minichromosomes in virions but also accumulated at the sites of MPyV replication. It suggests that incorporation of H3.3 to the viral chromatin is important either for the regulation of viral expression or for genome packaging. Further, the massive recruitment of DAXX to the sites of MPyV replication was observed. Hovewer, the absence of PML or DAXX did not prevent the H3.3 incorporation into viral minichromosomes. This indicates the contribution of other chaperones, e.g. HIRA, to the deposition of H3.3 into the viral minichromosomes.

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TRANSCRIPTION APPARATUS OF THE YEAST VIRUS-LIKE ELEMENTS IS CLOSELY RELATED TO THE POXVIRAL TRANSCRIPTION APPARATUS

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We employed virus-like elements (VLEs) pGKL1,2 from *Kluyveromyces lactis* as a model to investigate the previously neglected transcriptome of the broader group of yeast cytoplasmic linear dsDNA VLEs. We found that RNA polymerase encoded by the yeast cytoplasmic linear dsDNA plasmids, and also promoters recognized by this polymerase, shows high similarity to the poxvirus RNA polymerase and to the promoters of poxvirus genes. We show that the two RNAP subunits encoded by pGKL2 element interact *in vivo*, and this complex interacts with another two VLE-encoded proteins, namely the mRNA capping enzyme and a putative helicase. RNAP, mRNA capping enzyme and the helicase also interact with VLE-specific DNA *in vivo*. We performed 5' and 3' RACE analyses of all pGKL1,2 mRNAs and found them not 3' polyadenylated and containing frequently uncapped 5' poly(A) leaders that are not complementary to VLE genomic DNA. Moreover, we found the expression of pGKL1,2 transcripts is independent of eIF4E and Pab1 and is enhanced in *lsm1Δ* and *pab1Δ* strains. We believe that yeast linear plasmids has most likely an origin close to poxviruses and therefore are ideal non-infectious model for study of transcription mechanisms of viruses belonging to the family *Poxviridae.*

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SKIN AND ORAL VIROME IN PSORIASIS PATIENTS ON BIOLOGIC THERAPY

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The viral communities represent the most abundant microbiota population in humans. Viruses, together with other microbiota such as bacteria and fungi, form a dynamic ecosystem that influences the health of the host. The human microbiota is involved in the pathogenesis of psoriasis, a chronic skin inflammatory disorder that affect 2–4% population. Various not yet well-known environmental triggers, including infections, can initiate psoriasis; however, the molecular mechanisms of host-microbe interaction are still unknown (1). Psoriasis is now treated with newly discovered biologic drugs that have shown excellent efficacy. However, patients on biologics are at increased risk of infections (2). Patients using interleukin (IL)-17 blockers are at increased risk of mycotic and respiratory infections, patients treated with TNF-α inhibitors are at risk of reactivation of latent tuberculosis. However, information about the influence of other viruses and viral communities is limited or completely lacking. In our preliminary studies, we found higher prevalence of human papillomaviruses (HPV) in the oral cavity of patients treated with biologic therapy (anti-TNF-alpha, anti-IL-12/23, and anti-IL-17), compared to patients on topical treatment suggesting that the long-term immunosuppression increases the risk of oral HPV acquisition (3). The prevalence of Merkel cell polyomavirus was statistically significantly higher in the genital area of these patients (4). The following study will focus on a) virome analysis of lesional and non-lesional skin of psoriasis patients, b) oral virome analysis, c) influence of long-term treatment with biologics on virome changes. To conclude, our preliminary data show that even when traditional methods, that can detect only known sequences of a small number of targets, are used some alterations in the viral communities of patients on biologic therapy can be detected. The next-generation sequencing methods recently optimized in our laboratory will expand our knowledge, helping to identify patients at increased risk of viral reactivation and improve patients' management and therapeutic approaches.

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P-53

BIOINFORMATICS ANALYSIS OF IMMUNE CHARACTERISTICS IN TUMORS WITH ALTERNATIVE TUMORIGENESIS PATHWAYS ASSOCIATED WITH HUMAN PAPILLOMAVIRUSES

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Human papillomaviruses induce a subset of head and neck squamous cell carcinomas (HNSCC) and anogenital cancers, particularly cervical cancer (CC). The main viral proteins that contribute to tumorigenesis are the E6 and E7 oncoproteins, the expression of which is usually enhanced after the integration of viral DNA into the host genome. Recently, an alternative tumorigenesis pathway has been suggested in about half of HNSCC and CC cases associated with HPV infection (1). This pathway is characterized by extrachromosomal HPV persistence and increased expression of the viral E2, E4, and E5 genes. The E6, E7, E5, and E2 proteins were shown to modify the expression of numerous cellular immune-related genes. The antitumor immune response is a crucial factor in the prognosis of HPV-driven cancers and its characterization can contribute to the prediction and personalization of increasingly used cancer immunotherapy. Therefore, we analyzed immune characteristics of HPV-dependent tumors and their association with types of tumorigenesis. For this analysis, transcriptomic HNSCC and CC datasets from The Cancer Genome Atlas were used. Although patients with tumors with high E2/E4/E5 expression showed better survival, only small differences were found in the composition of tumor-infiltrating immune cells and the expression profiles of immune-related genes. In patients with high E2/E4/E5 expression, a higher age at the time of tumor diagnosis was found for CC and a lower Winter hypoxia score for HNSCC. Unsupervised clustering of both HNSCC and CC samples identified a group of patients with better overall survival, a lower Winter hypoxia score, and lower keratinization of tumors. These characteristics were associated with enhanced infiltration of immune cells and markedly different expression profiles of immune-related genes. Our analysis suggests that while detection of immune reactions against HPV-driven tumors may be a marker of a better prognosis and an important factor in therapy selection, the type of tumorigenesis seems not to play a decisive role in the induction of antitumor immunity.

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THE HUMAN VIROME IN HUMAN DISEASE AND ITS INTERACTION WITH OTHER EXPOSOME COMPONENTS

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The virome has been an understudied component of the human microbiome. While most studies focus on bacteriome profiles sequenced by simple amplicon profiling of the 16S rRNA marker gene, the viral fraction is often overlooked due to technical difficulties inherent to the work with minute amounts of virus-derived nucleic acids on the background of human, bacterial and food-derived background.

The present project will focus on the viromes and their interaction with other components of the exposome (bacteriome, parasitome, and possibly also mycobiome) in three conditions whose prognosis has just recently dramatically changed with novel therapies. Firstly, this is the application of novel cystic fibrosis transmembrane conductance regulator (CFTR) modulators. We will focus on the restoration of the gut microbiome following to application of the elexacaftor/tezacaftor/ivacaftor modulator. The analytic goal of the study in Crohn's disease upon anti-TNF-alpha therapy is to find such microbial taxa that associate with the restoration of gut health (therefore the parallel investigation of juvenile idiopathic arthritis that has no gut involvement). In faecal microbiota transfer therapy for CDI, the observation will focus on the restoration of the trajectories of the evolving microbiome towards the donor or other stable healthy state. The cohorts are being actively followed up and sample collections are growing.

The methods comprise mechanical virus enrichment, enzymatic treatment, nucleic acid extraction, sequence-independent single-primer amplification, construction of metagenomic libraries, and massively parallel sequencing. The ensuing reads are then filtered and mapped to panels of virus-derived sequences and large panels of common motifs of presumed viral origin. The statistical analysis focuses on dimensionality reduction ordination methods, on building models explaining the development of the virome in time and by diagnosis, and the identification of novel virus motifs.

Our team has so far produced viral metagenomes from over 2000 stool samples of various origins. Experience in laboratory procedures, as well as in bioinformatic and statistical analyses will be instrumental also in the present project. The poster presents results obtained in previous projects, exemplifying the use of the above methods.

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P-55

MOLECULAR EPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE IN HEALTHCARE-ASSOCIATED PATHOGENS

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A rapid spread of multidrug-resistant bacteria leads to difficult-to-treat infections and higher mortality. Thus, monitoring the emergence of new epidemic lineages of nosocomial pathogens and or novel determinants of antimicrobial resistance in healthcare settings is of utmost importance.

Applicability of the Fourier transformation infrared spectroscopy in healthcare pathogen typing

Molecular typing is one of the important tools for infection prevention control. The Fourier transformation infrared spectroscopy (FT-IR), represents a promising new technology for strain typing in real-time. Ongoing projects are focused on FT-IR-based typing of multiresistant pathogens, i.e. carbapenemase-producing *Enterobacteriaceae,* bacteria infecting patients with cystic fibrosis (*Burkholderia cenocepacia* complex, *Pseudomonas aeruginosa*) and others.

Accumulation of antimicrobial resistance in epidemic and non-epidemic *Clostridioides difficile* strains

The accumulation of antimicrobial resistance in *C. difficile* is one of the main forces for *C. difficile* infection development.

A large set of *C. difficile* isolates currently circulating in patients hospitalized in the Czech Republic and Slovakia, as well as *C. difficile* isolates from samples of pets and their owners, were sequenced and bioinformatics analysis is currently underway.

Study on the population structure of vancomycin-resistant Enterococci

Due to the observed increase in the prevalence of vancomycin-resistant *Enterococcus faecalis/faecium* isolates, a national collection of these isolates was launched in September 2022 in 20 Czech hospitals, including susceptible strains. Until now almost 200 isolates were collected. The strains will be subjected to whole genome sequencing, and their relatedness and localization of resistance mechanisms will be studied.

Characterisation of Methicillin-resistant *S. aureus* epidemic clones

To increase our understanding of the driving forces behind the success of epidemic clones in the Czech Republic, we start to analyse the growth kinetics of selected isolates of epidemic MRSA clones under various conditions with a focus on antimicrobial and other drugs that are frequently used in human medicine.

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EPIDEMIC SPREAD OF KPC-PRODUCING BACTERIA IN THE CZECH REPUBLIC

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Carbapenems are currently last-resort antibiotics for the therapy of infections caused by multidrug-resistant Gram-negative bacteria. Therefore, the resistence to those drugs represents a significant threat of current medicine. Genes encoding for carbapenemases are mainly spread on mobile-genetic elements, especially plasmids. As demonstrated by several studies, they can be efficiently spread in bacterial populations. For surveillance purpose, it is crucial to understand evolution and spread of those resistence determinants on molecular-genetic level. In the Czech Republic, carbapenemase-producing bacteria are monitored in a routine level by diagnostic clinical laboratories and confirmed at National Reference Laboratory for Antibiotics of National Institute of Public Health and at Biomedical Center of Faculty of Medicine in Pilsen, Charles University. Whole-genome-sequencing-based molecular surveillance of those bacteria has been established since 2014. Among three main molecular groups of carbapenemases, KPC-type enzymes are spread globally, causing high-level of resistence to carbapenems. In this study we present the ongoing spread of the KPC-producing strains, which is evolving to an epidemic in Czech hospitals. During the period of 2018–2019, a total of 108 KPC -producing Enterobacterales were recovered from 20 hospitals. Analysis of long-read sequencing data revealed the presence of several types of bla_{KPC}-carrying plasmids; 19 out of 25 bla_{KPC}-carrying plasmids could be assigned to R (n = 12), N (n = 5), C (n = 1) and P6 (n = 1) incompatibility (Inc) groups. Five of the remaining bla_{KPC}-carrying plasmids were multireplicon, while one plasmid couldn't be typed. Additionally, phylogenetic analysis confirmed the spread of bla_{KPC}-carrying plasmids among different clones of diverse Enterobacterales species. Our findings demonstrated that the increased prevalence of KPC-producing isolates was due to plasmids spreading among different species. In some districts, the local dissemination of IncR and IncN plasmids was observed. Additionally, the ongoing evolution of bla_{KPC}-carrying plasmids, through genetic rearrangements, favours the preservation and further dissemination of these mobile genetic elements. Therefore, the situation should be monitored, and immediate infection control should be implemented in hospitals reporting KPC-producing strains.

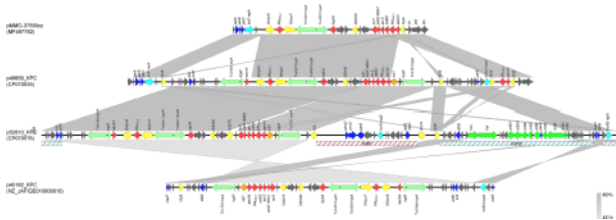

Fig. 1. **Linear comparisons of the KPC-encoding plasmids p48659_KPC and p45182_KPC.** Arrows show the direction of transcription of open reading frames (ORFs). Resistance genes are shown in red. IS elements and transposases are shown in yellow and light green, respectively. intI1 genes are shaded purple. Genes encoding replication, stability and transfer systems are shown in aqua, blue and green colors, respectively. The remaining genes are shown in gray. Homologous segments (representing ≥ 85% sequence identity) are indicated by gray shading.

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GUT MICROBIOME DIVERSITY OF PORCINE PERITONITIS MODEL OF SEPSIS

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Animal models are essential in understanding of the mechanisms of sepsis moreover the development and the assessment of emerging therapies. In clinically relevant porcine model, however, a significant variability in the host response has been observed among animals. Thus, there is a strong demand to better understand the potential sources of this heterogeneity. In this study, we compared faecal microbiome composition of 12 animals. Three samples were collected at different time points from each animal. Bacteriome was subjected to 16S rDNA profiling. A significant difference in bacterial composition was associated with the season ($p<0.001$) but not with the sex of the pig ($p = 0.28$), the timing of sample collection ($p = 0.59$), or interactions thereof (all $p > 0.3$). The season batch explained 55% of the total variance in the bacteriome diversity. The season term was highly significant from the high-resolution level of the bacterial amplicon sequencing

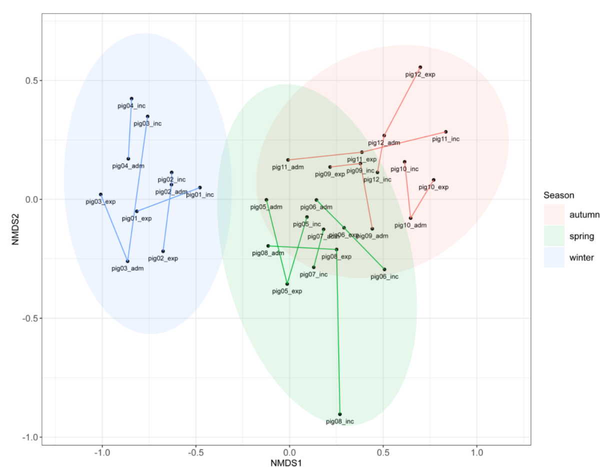

Fig. 2. **Non-metric multidimensional scaling on the quantitative Bray-Curtis dissimilarity (beta diversity – dissimilarity between communities).** Testing was performed by PERMANOVA at the genus level. Pigs are identified according to their numbers and the time of their admission into the animal facility ("pig*Nr*_adm"), immediately before the experiment ("pig*Nr*_exp"), and after incubation in isotonic saline before peritonitis induction ("pig*Nr*_inc").

variants up to the level of phylum. The diversity of the microbiome composition could significantly influence experimental model of sepsis, and studies are warranted to demonstrate the effects of gut microbiome diversity on the host-response. If confirmed, control of the gut microbiome should become a standard part of the pre-clinical sepsis experiments.

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NOVEL 4-AMINOSALICYLIC ACID ANALOGUES ACTIVE AGAINST MULTIDRUG-RESISTANT TUBERCULOSIS

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Spread of drug-resistant *Mycobacterium tuberculosis* (*Mtb.*), together with latent tuberculosis (TB), COVID-19 co-infection and increasing prevalence of non-tuberculous mycobacteria (NTM), is a serious threat for public health justifying a strong need for new antimycobacterial agents. Modification of established drugs to obtain derivatives with improved properties represents a viable approach[1].

p-Aminosalicylic acid (PAS) is a prodrug targeting folate biosynthesis used for treatment of TB. Recently, we have published three promising PAS derivatives and their peptide conjugates as antitubercular agents[1].

Therefore, we have designed a series of novel imines and ureas based on PAS scaffold (free acid, esters, amides). Ureas were prepared from aliphatic, alicyclic, and phenylalkyl isocyanates, imines from halogenated salicylaldehydes in good yields. Some compounds were prepared to be conjugable with oligotuftsin-based peptides to improve especially their cellular uptake. Peptides were prepared by solid phase synthesis and coupled with small molecules on resin or in solution[1].

The compounds were evaluated against a panel of mycobacteria ($H_{37}Rv$ and drug-resistant *Mtb.*, NTM) and other microbes, for their cytotoxic/cytostatic action, cellular uptake and intracellular antimycobacterial activity.

Our PAS derivatives inhibited all mycobacterial strains with MIC ranging from 1 μM including multidrug- and extensively resistant TB strains (MIC ≥2 μM). In general, they showed higher potency than the parent PAS. Ureas were more active than imines, favouring *n*-alkyls from C_8 to C_{13}, cycloheptyl and 1-adamantyl. Most derivatives lacked cytotoxic or cytostatic effect on eukaryotic cell lines (e.g., HepG2, MonoMac6). Their coupling with oligotuftsin peptides improved physicochemical properties, cellular uptake, and intracellular activity against mycobacteria. Their mechanism of action is under investigation.

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PALACKÝ UNIVERSITY OLOMOUC

L-25
INNOVATIVE SIDEROPHORE-BASED RADIOPHARMACEUTICALS FOR SPECIFIC IMAGING OF INFECTIONS

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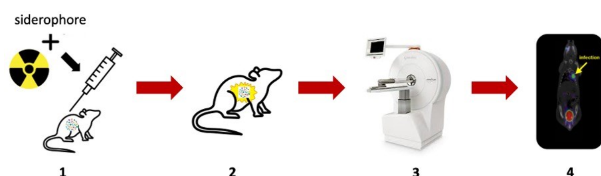
Microbial infections remain one of the leading cause of death globally[1]. Currently, hospital-acquired infections are among the most important public health issues, associated with high mortality and dramatic impact on healthcare costs[2]. Early and accurate detection and localization of infection is essential for effective treatment of patients and prevention of pathological complications. A number of diagnostic tests and methods are currently used in clinical practice[3]. However, most of these methods lack sufficient specificity and/or sensitivity. The availability of a rapid and reliable tool for the diagnosis of infectious diseases represents a major unmet need in the treatment of critically ill patients.

Molecular imaging, in particular positron emission tomography (PET), has the potential for specific and sensitive detection of microbial infections[4]. The siderophore-based iron acquisition system could be an interesting target for molecular imaging. Siderophores are specific iron chelators produced by many microorganisms that are recognized by specific microbial transporters[5]. Radiolabelled siderophores could be a highly specific tool for infection imaging, considering the essential role of the siderophore system for iron acquisition and virulence of microorganisms together with its upregulation during infection, whereas they are not utilized by mammals.



Scheme 1. **Schematic illustration of molecular imaging of an infected animal using a radiolabelled siderophorrer - preparation of the radiotracer and injection into the animal (1), distribution of the radiotracer in the infected animal (2), imaging of the animal using a dedicated imaging system (3) and an example of a PET image of an animal with lung infection (4).**

We have recently demonstrated that several siderophores can be radiolabelled, replacing iron without loss of bioactivity and allowing molecular imaging of microbial infections by PET[6–8]. We want to further investigate in detail the role and specificity of radiolabelled siderophores for imaging mainly bacterial infections, as we believe that targeting siderophore transporters with radiolabelled siderophores could open new diagnostic imaging strategies for microbial infections.

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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.

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L-26
OCCURRENCE AND DETECTION OF BETA-LACTAMASES IN BACTERIA

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Milan Kolář's group is part of the Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc. Milan Kolář's research group is active in analysing characteristics of bacterial pathogens, their resistance to antimicrobials, and developing new antimicrobial compounds.

The research team is represented by one excellent researcher (Prof. MUDr. Milan Kolář, PhD), one key researcher, two senior researchers, five junior researchers and three PhD students.

Milan Kolář's research group focuses on the following topics: 1) antimicrobial resistance, 2) testing the antimicrobial activity of new substances, and 3) determining bacterial pathogens in clinically significant infections, especially nosocomial and antibiotic treatment options.

As for the section on the development of antimicrobial resistance in bacteria, we focused on the search for the occurrence and detection of beta-lactamases in bacterial genera. In addition, in connection with the increasing resistance of bacteria to antibiotics, we also investigated the relationship between the proportion of regulatory genes to the presence of beta-lactamases and the size of the genome.

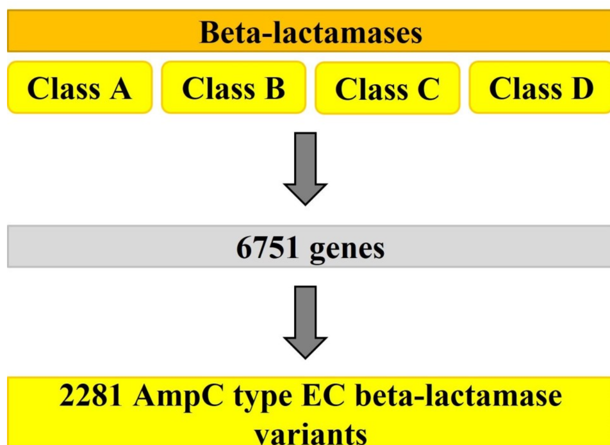

Figure. **PCR detection of the cephalosporinase-encoding genes *bla*$_{EC}$ by three primer pairs**

Up to now, 320 different types of genes encoding beta-lactamases, including class A (135 types; 1393 variants), class B (109 types; 757 variants), class C (55 types; 3551 variants) and class D (21 types; 1050 variants), have been described in bacteria using the Beta-Lactamase DataBase (BLDB, http://bldb.eu), representing more than 6751 individual genes with different properties in terms of their hydrolysis activity.

Among the beta-lactamases described so far, oxacillinases and AmpC beta-lactamases of the *bla*$_{EC}$ type account for more than 49% (3309 variants). Therefore, our previous study focused on this particular type of *ampC* gene. A total of three primers were designed to detect 2281 variants of *bla*$_{EC}$ genes (Figure) in *Escherichia* and *Shigella* strains (manuscript in preparation).

Search the BLDB and BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) databases showed that a wide range of beta-lactamases had been described in different bacterial genera, with the highest numbers of these enzymes being found in the genera *Pseudomonas* (46 types of beta-lactamases), *Enterobacter* (44), *Klebsiella* (39), *Escherichia* (35), *Serratia* (34), *Aeromonas* (29), *Citrobacter* (29), *Acinetobacter* (21), *Proteus* (19) and *Salmonella* (19).

Computational analysis of regulatory motifs in five genomes did not confirm that the proportion of regulatory proteins increases with increasing bacterial genome size. For example, regulatory motifs are found in 7.93% of *Pseudomonas aeruginosa* genes, 7.77% of *Klebsiella oxytoca* genes, 7.1% of *Serratia* sp. genes, 6.78% of *Escherichia coli* genes, and 7.66% of *Enterobacter hormaechei* genes (sorted by genome length). Furthermore, considering the highest proportion of regulatory genes described so far within the selected species and the total number of detected beta-lactamases in individual bacterial genera (as described above), we did not see any correlation. However, it should be noted that bacterial genomes still have many hypothetical proteins that, if determined, could more clearly point to a possible correlation. For example, in the bacterial genomes of *K. oxytoca* and *P. aeruginosa*, more than 1100 hypothetical proteins may have regulatory potential or impact on antibiotic resistance and therefore need to be annotated to determine their biological significance.

A database search showed that a wide range of beta-lactamases had been described in different bacterial genera. Based on this, more comprehensive information about the occurrence of these beta-lactamases may be helpful for the general public in terms of their detection. Furthermore, our results have not yet confirmed that a higher proportion of regulatory genes in the bacterial genome may be related to the overall distribution of beta-lactamases and genome size.

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P-59
BURKHOLDERIA CEPACIA COMPLEX INFECTION IMAGING USING RADIOLABELLED SIDEROPHORES

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Although generally considered non-pathogenic to healthy population, bacteria from *Burkholderia cepacia* complex (BCC) may cause severe hospital acquired pneumonia in immunocompromised patients. In these patients, the infection behaves unpredictably and is often resistant to medication. Therefore, it is necessary to accurately diagnose this causative agent, so that an appropriate treatment could be initiated. For this reason, we present here the use of radiolabelled ornibactin (ORNB), a chelator produced by BCC for iron scavenging, for positron emission tomography imaging of BCC infection.

Ornibactin was labelled with gallium-68 and radiochemical purity of the resulting complex was evaluated on RP-HPLC. *In vitro* characteristics of ^{68}Ga-ORNB complex were tested and its *in vitro* uptake in various microbial cultures was evaluated. *Ex vivo* biodistribution studies were performed on non-infected mice and on mice in muscle infection model. PET/CT imaging was performed on rats in lung infection model and on mice in muscle infection model.

^{68}Ga-ORNB complex has high radiochemical purity, it has hydrophilic properties and low protein binding values. It is stable in human plasma, although its stability is decreased in solutions with high concentrations of iron or competitive chelator. Its uptake is highest in members of the BCC compared to other respiratory pathogens. Both *in vivo* imaging and *ex vivo* results showed rapid biodistribution of the complex with no excessive accumulation in organs, renal excretion and accumulation of the complex in the site of infection.

68Ga-Ornibactin shows promising in vitro characteristics, high specificity for BCC, optimal pharmacokinetics and can be used for in vivo imaging of Burkholderia infection.

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P-60
DETECTION OF BETA-LACTAMASES IN BACTERIA OF ANIMAL ORIGIN

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Milan Kolář's research group focuses on the following topics: 1) antimicrobial resistance, 2) testing the antimicrobial activity of new substances, and 3) determining bacterial pathogens in clinically significant infections, especially nosocomial and antibiotic treatment options.

Regarding the research section on the development of antimicrobial resistance in bacteria, we focused on the search for the occurrence of beta-lactamases in bacteria of animal origin.

This study analyses 123 isolates of *Enterobacterales* and gram-negative non-fermenting bacteria of animal origin resistant to cefotaxime, ceftazidime, or meropenem. There were identified 22 bacterial species among the samples. Nominally, three strains of *Brevdundimonas diminuta*, three strains of *Citrobacter freundii*, five *Enterobacter* spp, 50 *Escherichia coli*, one *Myroides odoratimimus*, two *Ochrobactrum intermedium*, 43 *Pseudomonas* spp., one *Serratia fonticola* and 15 strains of *Stenotrophomonas* spp. They contained various combinations of AmpC variants (e.g. bla_{ACT}, bla_{CMY}, bla_{EC}, bla_{OCH}, bla_{PDC}, bla_{SFDC}; the total number of identified genes was 80, not including putative beta-lactamase genes), class A beta-lactamase genes (e.g. $bla_{\mathrm{CTX\text{-}M}}$, bla_{FONA}, bla_{L2}, bla_{SHV}, bla_{TEM}; the total number of identified genes was 92), class B beta-lactamase genes (e.g. bla_{L1}, bla_{MUS}, bla_{POM}, bla_{PST}; the total number of identified genes was 16) and oxacillinases (bla_{OXA}; the total number of identified genes was 25). New beta-lactamase variants (not yet described in the Beta-Lactamase DataBase) were found in 37 cases. For example, 26 times, these were new variants of the bla_{L1} and bla_{L2} genes in *Stenotrophomonas maltophilia.* In addition, 33 putative genes encoding new unknown beta-lactamase genes were found. For example, ten times, these genes were detected in *Pseudomonas* spp. Our genome analyses approved the co-occurrence of these genes with other resistance genes, such as *tet*, *sul*, *mph*, and *mdf*, confirming a multidrug resistance profile to beta-lactams, aminoglycosides, macrolides, sulfonamides and tetracyclines.

From a clinical point of view, the biggest problem is the constant growth of new beta-lactamases, especially carbapenemases, and thus the continuing evolution of beta-lactamases towards a broader spectrum of activity. This fact is

all the more worrying when considering bacterial species such as *Serratia marcescens*, which are naturally resistant to polymyxins. These facts highlight the importance of implementing molecular assays and phenotypic methods in recognizing new beta-lactamases.

This study represents only part of the studied issue of gene resistance in animal isolates. Antibiotic resistance in bacteria is dynamic and constantly growing. That means the current epidemiology of beta-lactamase-producing bacteria in veterinary medicine cannot be fully understood yet, so continuous monitoring is essential.

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L-27
SUMMARY OF RESEARCH DIRECTIONS – LABORATORY OF VIRAL AND CELLULAR GENETICS, INSTITUTE OF MOLECULAR GENETICS

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Avian leukosis virus (ALV) is a virus complex diversified through the virus-host coevolution (virus-host arms race) into several subgroups, each of which recognizes different cell surface receptor. In our laboratory, we employ this unique example of coevolution to study processes of virus adaptation, broadening the host range, and heterotransmission (in general, all zoonotic viruses arose in a process of heterotransmission). This knowledge could help in artificial creation of resistant animals using the CRISPR/Cas9 technology of gene editing in chicken, the natural host of ALV. Receptor alleles bearing simple substitutions of critical amino-acids at the virus binding domains (e.g., the recently prepared resistance to ALV-J subgroup[1]) are a good material for studying the escape mutations of the virus adapted to the new versions of receptors.

Antiviral restriction factors and virus dependence factors are other subjects of virus-host coevolution and bidirectional evolutionary approach is necessary to characterize viral adaptations to new host species during the process of heterotransmission and zoonotic events as well as the changes in relevant host genes. Many host cell-encoded anti-HIV-1 activities already have been characterized in human cells. In our laboratory, we focus on the effects of chicken tetherin against avian viruses[2]. Chicken tetherin will be the primary target to genetic knock out or gene editing in vivo using the CRISPR/Cas9 with the final goal to demonstrate its role in a resilience to chicken diseases.

The evolutionary approach enables the characterization of viral adaptations to new host species as well as adaptations necessary for long-term coevolution with hosts. These might include the appearance of novel antiviral activities of existing genes, the emergence of novel genes, or in some cases evolutionary losses of genes. In this respect, we focus on innate immunity factors in chicken, which exert antiviral effects, particularly against avian leukosis viruses, avian influenza, avian coronaviruses, etc. Chicken RIG-I and MDA-5[3] are studied with the ultimate goal to edit their genes in vivo using the CRISPR/Cas9.

All three aforementioned research directions will profit of our original technique of transgenesis and gene editing in chicken (Fig. 1), which consist of primordial germ cell (PGC) manipulation by CRISPR/Cas9 and orthotopic transplantation of PGCs into sterilized roosters[4].

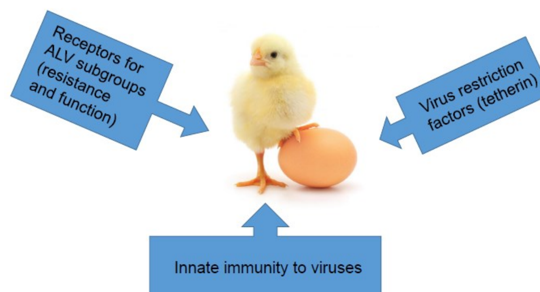

Figure 1. **Targets for gene knock-out and gene editing in chicken**

In our laboratory, we also stdy cell-to-cell fusion during placenta development, which is critical for the proper formation of its outer layer called syncytiotrophoblast. Fusion of mononuclear cytotrophoblast and formation of the multinuclear syncytium is largely dependent on the human proteins called Syncytins. Syncytin-1 and Syncytin-2, the retroviral envelope glycoproteins of two distinct human endogenous retroviruses. Using our original heterologous ectopic system of retrovirus-receptor interaction and dual luciferase cell fusion technique[5], we will analyze contribution of syncytin-1/2 and receptors hASCT1/2 for trophoblast fusion and identify amino-acids critical for syncytin-receptor binding. CryoEM structural analysis will be performed to support the functional analyses. In parallel, epigenetic repression of syncytins in non-placental tissues will be studied. This projet might result in identification of mutations behind the human idiopathic infertility.

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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.

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L-28
MOLECULAR MECHANISMS OF ADAPTIVE IMMUNITY AGAINST INFECTIONS

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T cells are the major orchestrators of the adaptive immune responses against pathogenic bacteria and virus by inducing direct cytotoxicity ($CD8^+$ T cells) and by instructing the antibody production by B cells ($CD4^+$ T cells). On the other hand, the overt activation of the adaptive immunity leads to collateral damage to tissues and to the development of autoimmune diseases. Thus, the understanding of the molecular mechanisms of T-cell activation and regulation is crucial for desigining the therapeutic modulators of the immune response.

LCK is a major protein tyrosine-kinase associated with the T-cell antigen receptor (TCR) triggering. It is bound to CD4 and CD8 co-receptors, which facilitate the TCR activation. We have generated novel mouse models to elucidate the role of CD8-LCK and CD4-LCK interaction. Surprisingly, we observed much greater importance of the CD4-LCK than CD8-LCK interaction for proper T-cell responses. Subsequently, the development and immune response to viral and bacterial infections was almost normal in cytotoxic T cells in mice with disrupted LCK-coreceptor interactions. In contrast, the development and the immune reponse was largely impaired in helper T cells in the same mouse. We observed that the CD4-bound LCK has an important kinase-independent function by stabilizing the CD4 on the cell surface. The kinase-dependent role of CD8- and CD4-bound LCK is largely limited to the response to suboptimal antigens.

It has been previously shown that the role of self-reactivity of naïve T cells is an important factor predicting their behavior during infections (Fulton et al.). Using a unique set of similar monoclonal T cells with a different level of self-reactivity, we addressed the role of self-reactivity in the development and immune response of mature peripheral T cells. We observed that highly self-reactive T cells form antigen-inexperienced memory-like T cells, but no a novel T cell subset with high expression of interferon type I-induced genes (Paprckova et al. 2022). Moreover, we did not observe any role of T-cell self-reactivity in the response of the T-cell clones during a bacterial infection.

IL-17 is a crucial cytokine for the response to extracellular bacterial and fungal infections. However, it can also trigger autoimmune disorders, such as psoriasis. We have identified a novel component of the receptor for IL-17A and IL-17F (Knizkova et al.), a tetra-transmembrane protein CMMT4. Using the CRISPR-Cas9 approach, we generated CMTM4-deficient cell lines and mice. We have observed that the CMTM4 is required for the cellular and systemic response to IL-17 in terms of the activation of down-stream signaling pathways, cytokine production, neutrophile recruitment, and generation of autoimmune pathology in the model of experimental psoriasis.

Regulatory T cells (Tregs) are crucial for maintaining the homeostasis of conventional T cells and for suppressing their over-responsiveness. We have focused on the mechanisms how Tregs suppress cytotoxic $CD8^+$ T cells. We have identified that the major mechanism is limiting the availability of IL-2, a key cytokine for the proliferation and survival of effector T cells (Tsyklauri et al. 2022). Surprisingly, the absence of Tregs and/or excess of IL-2 induces a formation of a previously unappreciated population of effector T cells expressing KLRK1, IL-7R, and lacking an established marker of effector T cells, CD49d (Figure 1). These KILR T cells show superior cytotoxic properties.

Overall, we have uncovered some molecular mechanisms behind the homeostasis and immune response of T cells.

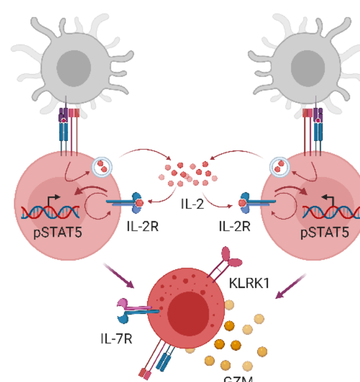

Fig. 1. **A schematic depiction of how high IL-2 levels in the absence of regulatory T cells contibute to the generation of an unusual population of effector CD8+ T cells with superior cytotoxicity.**

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DYNAMIC EVOLUTION OF AVIAN RNA VIRUS SENSORS: REPEATED LOSS OF RIG-I AND MDA5

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Two key cytosolic receptors belonging to the retinoic acid-inducible gene I (RIG-I)-like receptor (RLR) family detect the viral RNA-derived danger signals: RIG-I and melanoma differentiation-associated protein 5 (MDA5)[1]. Their activation establishes an antiviral state by downstream signaling that ultimately activates interferon-stimulated genes (ISGs). While in rare cases *RIG-I* gene loss has been detected in mammalian and avian species, most notably in the chicken, *MDA5* pseudogenization has only been detected once in mammals[2]. We have screened over a hundred publicly available avian genome sequences and described an independent disruption of *MDA5* in two unrelated avian lineages, the storks (Ciconiiformes) and the rallids (Gruiformes)[3]. Further, we detected 14 independent losses oft he *RIG-I* sensort in various bird species. In almost all cases, the *RIG-I* loss was coupled with loss of ist regulatory ubiquitin ligase *RIPLET/RNF135*.

We also used several approaches to assess the compensatory evolution of the remaining RLR sensor in cases of gene loss. Although the *MDA5* loss does not appear to have resulted in any compensatory evolution in the *RIG-I* gene, in the reciprocial situation we detect a possible pattern of compensatory evolution in *MDA5*. These findings provide further support for the dynamic evolution of RLR sensots and propose new questions about the redundancy and flexibility of the RNA virus-sensing apparatus in birds.

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QUANTITATIVE ANALYSIS OF SYNCYTIN-1 BINDING TO ITS RECEPTOR

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Syncytin-1 is a fusogenic glycoprotein of retroviral origin that is specifically expressed in the human placenta. Syncytin-1 induces the fusion of cellular membranes of cytotrophoblasts after interaction with its cellular receptor, ASCT2. This process results in the formation of a multi-nuclear syncytiotrophoblast layer, which facilitates the transport of nutrients and metabolites between the mother and the fetus and is required for a successful pregnancy. Mutations in Syncytin-1 or ASCT2 can impair their interaction which may lead to disorders like eclampsia, recurrent pregnancy loss or idiopathic infertility. Up to now, a proper description of the Syncytin-1-ASCT2 binding is missing.

We developed a multi-level approach to functionally analyze the efficiency of the Syncytin-1-ASCT2 interaction in a cell system. Ectopically expressed variants of ASCT2 are combined with fluorescent and luminescent proteins and can be instantly detected by microscopy, flow cytometry and common plate readers. We can routinely check for the receptor expression on mRNA and protein levels and validate its localization. We also prepared tools employing Syncytin-1: specific immunoadhesin, infectious virus and reporter cells detecting cellular fusion. With these tools, we can assess ASCT2 ability to bind and prime Syncytin-1.

Our system can facilitate precise characterization of the Syncytin-1 binding site on the receptor and lead to detailed molecular understanding of one of the critical steps in human placenta morphogenesis.

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KNOCK-ING ON HETEROCHROMATIN: STABILITY OF GAMMARETROVIRAL EXPRESSION AFTER INTEGRATION RETARGETING

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Retroviruses integrate their genome into the genome of the infected cell. Integrated proviruses form an inseparable part of the host genome and express viral genes effectively. Epigenetic silencing may however disrupt the proviral expression. We have previously shown that retroviruses differ in sensitivity to epigenetic silencing and that the features at the integration site can affect the stability of proviral expression. Vectors derived from the murine leukemia virus (MLV) – widely used representative of the gammaretrovirus genus – performed as the most efficient retrovirus-derived vector in human cells. Here, we further investigate the gammaretroviral expression in human cells.

We constructed retroviral vectors from diverse gammaretroviruses and transduced the human K562 cell line. We further tested if disruption of natural promoter/enhancer targeting affects the gammaretroviral expression. Our results indicate that the vectors express transduced genes stably in time irrespective of integration preference. We further corroborated the gammaretroviral expression stability in heterochromatin by CRISPR-directed knock-in into predicted lamin-associated domains.

Our results demonstrate the general ability of gammaretroviruses to stably express transduced genes in human cells. Moreover, we show that other-than-MLV gammaretroviruses may be used for the construction of heterochromatin-resistant expression vectors for transgenesis.

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CHICKEN DDNTFR23 AS A CANDIDATE RECEPTOR FOR AN ALV-F SUBGROUP

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Avian leukosis virus subgroups (ALV) A, B, C, D, E, J, and K are grouped according to their antigenic reactivity, superinfection interference, and cell receptor usage. However, these characteristics remain to be determined for the endogenous F subgroup. Therefore, we characterized the cell receptor usage for the F subgroup. We constructed a recombinant avian leukosis virus with the F subgroup *env* gene that replicates only in chicken cells. The F subgroup did not share the cell receptor usage with any other described ALV subgroup by the superinfection interference. We identified DDTNFR23 protein as the receptor in chicken and pheasant cells for the F subgroup by an infection of chicken/hamster radiation hybrids. Taken together, we provide a molecular characterization of the F subgroup avian leukosis virus and enhance our knowledge of receptor usage diversification within a closely related virus.

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PROTECTIVE AND PATHOLOGICAL TISSUE-INFILTRATING CELLS

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Tissue-infiltrating T cells 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 some infections. However, by killing pathogen-infected cells, cytotoxic $CD8^+$ T cells can also contribute to the disease pathology by damaging host tissues. Additionally, some $CD8^+$ T cells can be involved in autoimmunity. The diversity and function of these cells in tissues during infection and autoimmunity are not completely understood. For instance: It has been proposed that $CD8^+$ T cells infiltrating the central nervous system mediate immunopathology of tick-borne encephalitis[1] and play a role in the progression of experimental autoimmune encephalomyelitis (EAE) in mice as well as multiple sclerosis in humans[2]. However, other studies using the EAE model discovered a unique population of protective $CD8^+$ T cells, which suppress pathological $CD4^+$ T cells[3]. Recently it has been proposed that these regulatory cells have direct counterparts in human capable of suppressing pathological T cells in both autoimmunity and infection[4]. The aim of this project is to identify different infiltrating $CD8^+$ T cell subsets and uncover their functional differences in protection and pathology. Isolated T cells will be analyzed by current state-of-the-art methods. Gene expression profiles will be compared by single-cell RNA sequencing and subset diversity will be further confirmed by flow cytometry. The following steps are to describe the biological roles of different T cell subsets using model systems for generating monoclonal T cells.

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