# CEITEC PhD Conference

**30th May, 2022**

CEITEC MU, Brno, Czech Republic

# BOOK OF ABSTRACTS

CEITEC MUNI

# CEITEC PhD Conference

30th May, 2022
Brno, Czech Republic

CEITEC MU, Building B11, Auditorium 205

CEITEC PhD Conference posters will be on display
at CEITEC MU, Building E35, Atrium
(27/5/2022 – 3/6/2022)

## CONTENT

# WELCOME ADDRESS

## Welcome Word

Dear Students,

another year passed by and PhD conference is here again. Many things we used to take as granted changed over the past two years – including the way we discuss science. Due to COVID, discussions moved to virtual environment and we became masters of on line meeting platforms. This experience was priceless; COVID forced us to learn new venues of communication that seems to be in many aspects more efficient and convenient, and this will stay with us. However, we also realized that despite all the technology advances, person-to-person interactions are still irreplaceable. Many big discoveries start with a little talk with glass of wine. Therefore, let´s embrace the fact that we can have these little talks again and enjoy the conference!

**Karel Říha**
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# ABSTRACTS OF SPEAKERS

## Impact of Phosphorylation for Tau210-240 Peptide and Interaction of Small Molecules and 14-3-3ζ Protein Studies Using Computational Methods

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The conformational dynamics of intrinsically disordered proteins (IDPs) regulated by post-translational modifications such as phosphorylation is challenging to elucidate. A well-known IDP Tau is found hyper-phosphorylated in Alzheimer's disease (AD) in humans [1]. The proline-rich motif of Tau210-240 peptide directly interacts with proteins such as 14-3-3ζ. 14-3-3ζ is one of the crucial protein in the human brain and bind to a multiarray of proteins. It has a significant impact on forming and deforming neurofibrillary tangles, and it was shown that the 14-3-3ζ monomer has strong anti-aggregation properties [2]. It was shown that monomerization of 14-3-3z can be induced by phosphorylation of Ser58 at the dimeric interface [3, 4]

Designing small molecules aiming to modulate the strength of interactions of binding partners with 14-3-3ζ proteins and proper understanding of the conformational and dynamics changes upon phosphorylation of Tau can extend the therapeutic options for AD.

In the first objective, microsecond time scale, all atoms molecular dynamic (MD) simulation studies have been performed for apo and phosphorylated ($212^{P}$Thr, $217^{P}$Thr, $231^{P}$Thr, $235^{P}$Ser) Tau peptide210-240 using three different temperature variants (278K, 298K and 310K) and two different force field parameters (AMBER99SB-ILDN and CHARMM36m) with TIP4PD water model as these FF parameters combine with water model worked the best for IDPs from our group previous study [5]. These four-phosphorylations cause increase in compactness. The binding of associated proteins like 14-3-3 with Tau may alter by the strong salt bridges, forming nearby lysine and arginine due to the phosphorylation [6]. Phosphorylation induces a strong structural transition, with $Tau^{210–240}$ favouring a bent conformation. The MD simulation results were verified using NMR experimental parameters like chemical shift, $^{3}$J-coupling etc.

Secondly, molecular docking studies have been performed with selected FDA approved 1391 drugs and 14-3-3ζ dimer and monomer protein. I will narrow down the drugs based on interaction and docking scoring function based on binding energy. Further MM/PBSA based binding energy will be calculated from all-atom MD simulations of protein-drug complexes. The computational predictions will be validated experimentally by NMR, ITC and fluorescence titration experiments. I have also tested and explored relevant methods (molecular docking and MD simulation) for my planned studies by using different protein targets of our experimental partners (Dr Harekrushna Sahoo and Dr Monalisa Mishra) from India, and this resulted in five publications [7, 8, 9, 10, 11] and two manuscripts under peer-reviewed.

Project acknowledgement.

*Krishnendu Bera acknowledges funding from the Ministry of Education, Youth, and Sport of the Czech Republic (MEYS CR), grant number LTAUSA18168 (Inter-Excellence Inter-Action), and by the Czech Science Foundation (no. GF20-05789L). The computational resources were supplied by (Metacentrum and IT4Innovations National Supercomputing Center) the project "e-Infrastruktura CZ" (e-INFRA LM2018140 and ID:90140) provided within the program Projects of Large Research, Development and Innovations Infrastructures. Krishnendu Bera is also supported by Brno PhD Talent Scholarship – funded by the Brno City Municipality, Brno, Czech Republic. I am acknowledging Prof. Isabelle Landrieu and her group for the experimental part of tau$^{210-240}$.*

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# Application of Modern Microcolumn Separation Techniques Coupled to Mass Spectrometry for Analysis of Non-invasive Biological Samples

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Non-invasive biological samples such as saliva and exhaled breath condensate (EBC) are gaining attractivity in clinical research and disease diagnostics due to their simple collection and the rich analyte composition. Bile acids, a family of steroid compounds, were suggested to be a potential contributing factor for development of a gastroesophageal disease Barret´s esophagus (BE). Levels of bile acids in saliva or EBC could serve as possible biomarkers for the alternative non-invasive diagnostics. In this work, we focused on method development for sample collection, sample preparation, and analysis of bile acids by liquid chromatography-mass spectrometry.

Saliva was collected by simple spitting and the Salivette® saliva collection system that were compared, the latter was found unsuitable due to excessive retention of bile acids in the cotton swab. For EBC collection, we assembled a sampling device able to maintain a stable temperature between -7°C and +12°C during sample collection. Sample preparation included protein precipitation and bile acid enrichment in samples by solid-phase extraction.

Bile acids (unconjugated, glycine-conjugated) were quantified by ultra-high-performance liquid chromatography coupled to triple-quadrupole tandem mass spectrometry. Bile acid levels in EBC samples were generally below the detection limit, but it was possible to quantify bile acids in saliva. Therefore, bile acids were determined in saliva from healthy volunteers and BE patients. Saliva from BE patients contained higher levels of almost all bile acids and the tested groups could be distinguished by principal component analysis. Moreover, high resolution mass spectrometry was utilized for identification of new bile acids in saliva, i.e., taurine-conjugated bile acids and glycine-conjugated dihydroxy-bile acid sulfate. We propose that analysis of salivary bile acids including taurine conjugates could be applicable in diagnostics of BE, following a larger clinical study.

*This work was supported by the institutional support RVO: 68081715.*

# Prediction of Novel Treatment Options for CLL and AML Cells Based on Drug Repurposing

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Acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL) are the most frequently occurring leukemia types in adulthood. A significant barrier to their effective treatment is based on their molecular heterogeneity reflecting the variable clinical behaviour of the diseases. The presence of specific subclonal mutations often requires different therapeutic regimens on a patient-specific basis and thus, calls for the personalized medicine approach.

This kind of personalized approach can be obtained through drug repurposing. Compared to traditional drug development, drug repurposing enables the identification of novel therapeutic options among existing drugs and investigates new uses outside the scope of their original indication. This method is time-saving and cost-efficient and can vastly accelerate the lengthy approval process. Using this approach's advantages, we aim to identify novel treatment options through high-throughput screening of FDA approved drugs for AML and CLL cells.

CLL and AML patient samples were cultured in the presence of cytokines to mimic the protective tissue microenvironment and maintain viability during the screening. Drug screening is performed with a library of 859 clinically approved compounds covering the broad chemical and pharmacological diversity. It consists of drugs approved by the US FDA and European EMA agencies for clinical use in diverse indications but is enriched explicitly for drugs approved for oncology. The library's standard concentration (10 µM) of drugs is automatically added to cells on 384-well-plates with a programmed liquid handling system epMotion (Eppendorf). The incubation of cells lasts 72 hours, and then the cell viability in response to drugs is determined by a Cell titer Glo assay. The results are normalized to DMSO and analyzed for the most effective compounds. These top hits are further validated to assess the reproducibility and the magnitude of the effect.

Overall, we performed and analyzed the drug screening on 29 primary CLL samples representing the four major genetic subtypes and several AML samples. We identified several drugs that were recurrently found among the most efficient for the majority of samples. We also detected some specific drugs unique for individual patients. However, it does not seem that there would be any shared sensitivities specific to concrete CLL genetic subtypes.

In summary, our research project points out the importance of a personalized treatment approach based on the application of approved-drug screening.

*This project was supported by the grants number MUNI/A/1330/2021, MUNI/A/1419/2021 and by OPRDE (No.CZ.02.2.69/0.0/0.0/19_073/0016943).*

# Long-term Adaptation to High Temperatures in Arabidopsis Thaliana: from Flowering to Seed Development

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In the last decades, we have witnessed the worldwide effects of climate change. The average global temperature and frequency of extreme temperatures increased year after year. The yield production of temperate crops has decreased due to high temperatures and drought and is expected to reduce even more in the upcoming years. In plants, the reproductive phase is a developmental stage sensitive to high temperature with heat-sensitive processes such as pollen development. The response to high temperature involves transcription factors, such as phytochrome interacting factors or heat shock factors, chaperone proteins, and phytohormones production, creating a complex response with different levels of regulation. However, most of the available data focus on heat shock and pollen development, while information regarding long-term adaptation is scarce, especially related to ovule and embryo development.

Using the tools available at the Core Facilities of Plant Sciences and CELLIM, we have performed a complete phenotyping analysis of the reproductive phase of wild-type Arabidopsis thaliana plants and two temperature-sensitive mutants, *hsbp-II* and *hot1-3*, with different high-temperature scenarios. Also, we analyzed the expression profile of genes regulating the heat response in pistils and siliques of Arabidopsis thaliana. Furthermore, with the assistance of Labdeers s.r.o., we studied the yield and quality of seeds produced in high-temperature conditions.

Our results provide new insight into long-term adaptation to high temperatures during the reproductive phase of plants. We observed that pollen, but especially ovule development, are negatively affected by high temperatures. Embryo development is accelerated by high temperatures, resulting in morphological abnormalities similar to defects in various auxin-related mutants. We showed that abnormal embryos have a different auxin signaling pattern when compared with WT-like embryos. The expression of some auxin biosynthetic genes is also altered. The reduction of fertility is translated to smaller seed production and a worsened quality of those seeds.

*We acknowledge the Core Facility CELLIM supported by the Czech-BioImaging large RI project (LM2018129 funded by MEYS CR), the Core Facility Plant Sciences of CEITEC Masaryk University, and the Brno Ph.D. Talent.*

# Study of the Genetic Pathways Controlling High-temperature Stress During Embryogenesis in *Arabidopsis Thaliana*

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The global climate system is warming up, and the increase in temperatures is one of the important factors affecting multiple plant developmental processes, like growth and flowering. The use of *Arabidopsis thaliana* as a model organism substantially increases our knowledge of the signaling and response mechanisms during plant exposure to elevated temperatures. A substantial amount of data has been gathered to elucidate the role of phytohormones and molecular chaperones, such as Heat Shock Proteins (HSPs), in this temperature response in vegetative tissues. My Ph.D. project investigates the molecular mechanisms controlling the temperature response during *Arabidopsis embryogenesis*. Based on reported data, the PHYB-PIF4 pathway is known for controlling high-temperature stress in the seedling. We assessed if the same pathway responds to the heat temperature during embryogenesis. Expression analysis of PIF4, using pPIF4: GUS reporter lines showed a PIF4 higher expression in the integuments of high-temperature-grown seeds than in the normal condition. We further confirmed this result using Fluorometric GUS quantitation Assay. Phenotyping analysis for some PIF4 pathway component mutant lines has been done by a light microscope (AxioScope). The results indicate a minor variation between mutant lines and wild-type background in the high-temperature condition. To assert this result, phenotyping is done using a laser-scanning confocal microscope (LSM700), which facilitates the detection of all the division and slight phenotypic changes in the embryo. Furthermore, phenotyping analysis is performed for ovules and anthers in normal and high-temperature conditions. We will later conduct RNA expression analysis for PIF4 pathway components to check their expression alteration in the high-temperature condition.

We also studied the MAPK (mitogen-activated protein kinase) kinase kinase gene *YDA* (YODA). Loss-of-function mutations in *YDA* affect zygote elongation and apical-basal embryonic polarity. We have observed similar phenotypes in the wild type, exposed to high-temperature stress. In addition, *YDA* was described for its role in regulating stomatal density, and heat stress suppresses stomatal development in an HSP90-dependent manner. Through their physical interaction with YDA kinase, HSP90s regulate the activation of signaling components downstream of YDA, specifically, MPK3 and MPK6 kinases. We hypothesized that YDA, its collaborating proteins, and the downstream activated transcription factors are good candidates to be examined in our study. The

phenotyping analysis of *cop1* (upstream of YDA) and *yda* mutant lines showed less embryo defect percentage than their wild-type backgrounds while exposed to high temperatures. These results imply that the YDA pathway may control high-temperature stress during embryogenesis. To assess the expression of YDA, some reporter lines have been constructed. Then, we will perform the expression analysis of the components involved in this pathway using generated reporter lines and RNA expression analysis. To study the function of HSP90s, we have generated a constitutively-active YDA (CA-YDA) line, which is insensitive to HSP90.

Altogether, investigating both pathways during embryo development in high temperatures will shed some light on the development response to this critical growth stressor.

*This work was supported by European Regional Development Fund-Project "SINGING PLANT" (No. CZ.02.1.01/0.0/0.0/16_026/0008446). And SE is financially supported by South Moravian Centre for International Mobility (JCMM).*

*We further thank Plant Sciences and the Cell Imaging Core Facilities of CEITEC Masaryk University for support with plant cultivation and access to the microscopy platform.*

# Mycobacterium Protein moaB2 Inhibits Sigma A against Proteolytic Cleavage

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Bacteria need to modulate their gene expression to adapt to changing external conditions for survival. Transcription initiation is the crucial focal point of gene expression in prokaryotes. The key players in this process, sigma factors (σs) associate with the catalytic core RNA polymerase to guide them through the essential steps of initiation: promoter recognition and opening, and synthesis of the first few nucleotides of the transcript. An immunoprecipitation study on M. smegmatis sigma factor A ($\sigma^A$) revealed an association with moaB2 which was later confirmed by size exclusion chromatographic studies and mass spectrometry analysis. The moaB family of proteins are known to be involved in catalyzing complex oxygen transfer reactions in molybdenum uptake pathways in all clades of life but, their bacterial homologs lack this function which is instead handled by another member of the family. To understand the biological role of moaB2 and $\sigma^A$, we prepared moaB2 and $\sigma^A$ depleted strains in M. smegmatis. The qPCR studies on the moaB2 depleted strains showed an increase in the level of expression of $\sigma^A$ and vice versa. But, In-vitro transcription assays did not show any significant changes in transcription activity upon introduction of moaB2 suggesting a lack of direct role in transcription. We hypothesize that the increased level of expression of the gene may suggest a role of moaB2 in conferring protection to $\sigma^A$ against proteolytic cleavage. To better understand structural role of moaB2- $\sigma^A$ interaction, we solved the crystal structure of moaB2 from M. smegmatis to a resolution of 2.6 $A^O$ by Molecular replacement. The data collection was performed at the DESY Beamline, Hamburg. The co-crystal structural characterization of $\sigma^A$ - moaB2 are under optimization trials. The significance of moaB2 may pave the path for a better understanding of the bacterial transcription pathway.

## Structural Characterization of Lectins from *Photorhabdus spp.*

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Lectins are proteins and glycoproteins able to reversibly bind saccharide moieties of glycoconjugates with high specificity. Lectins are involved in many physiological processes and play crucial roles in cell-cell communication or recognition of the host by a pathogen. The research is focused on the lectins produced by Gram-negative bacteria *Photorhabdus laumondii* and *Photorhabdus asymbiotica*. Bacteria of *Photorhabdus* genus live in symbiosis with *Heterorhabditis* nematodes. This symbiotic complex can be found in soil, where it searches for insect prey. Unusual dual behaviour makes *Photorhabdus* bacterium a compelling organism for further study of its biomolecules. Protein crystallography was used to obtain the 3D structure of lectins and to identify the key residues involved in the protein-saccharide interaction. It was revealed that *P. laumondii* produce five lectins with the seven-bladed β-propeller fold. Determined structures differ in the oligomerization and number of binding sites despite high sequential similarities. Lectin complexes with saccharides revealed two different binding sites per monomeric unit for the same ligand. Obtained results may reveal the importance of the lectins in the bacterial pathogenic or symbiotic stage of life.

*Funded under the FP7 project ,The ERA Chair Culture as a Catalyst to Maximize the Potential of CEITEC' (ID 621368) of the European Union.*

*This work was supported by the Czech Science Foundation (21-29622S) and CIISB research infrastructure project LM2018127 funded by MEYS CR.*

# Cytokinin-Induced Dirigent Gene AtDIR13 As Potential Player In Plant Stress Response

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Dirigent proteins mediate regio- and stereo-specific monolignol coupling during the lignans and lignins formation (1). As a complex phenolic polymer, lignin enhances plant cell wall rigidity, hydrophobic properties and promotes minerals transport through the vascular bundles in plant (2), whereas the ubiquitous but structurally diverse lignans are involved in plant defense (antioxidants, biocides, etc.) (3). Arabidopsis dirigent proteins (AtDIRs) represent a large and still unexplored gene family, consisting of 25 members. AtDIR5 and AtDIR6 are implicated in the (-)-pinoresinol biosynthesis (4), AtDIR10/ESB1 was shown to control Casparian strip formation in the root endodermis (5), and AtDIR12/DP1 is specifically expressed in seeds and involved in neolignans synthesis (6). According to the phylogenetic analysis *AtDIR13* and *AtDIR14* are paralogues of *AtDIR5* and *AtDIR6*, but they lack the conserved residues necessary for (-)-pinoresinol formation (7).

Hereby, we found *AtDIR13* to be root-specific, upregulated by cytokinins and active since early stage of postembryonic growth. AtDIR13-mCherry fusion protein is localised in the root endodermis of the differentiation zone with a strong expression in the overlying endodermal cells of developing lateral roots. We also tested different stresses on *35S::AtDIR13* lines. Thus, in the presence of NaCl salt, *35S::AtDIR13* lines showed better germination rate and primary root growth in comparison to Col-0. Under drought stress conditions *35S::AtDIR13* lines exhibited higher wilting resistance and better recovery after plant rewatering. Moreover, our preliminary results indicate that *35S::AtDIR13* leaves are hypersensitive to flg22-triggered ROS production in the apoplast. These findings suggest a potential role of AtDIR13 in abiotic and biotic plant stress responses.

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# Interrogating the Molecular Heterogeneity of Chronic Lymphocytic Leukemia through Computational Approaches

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Chronic lymphocytic leukemia (CLL) is an incurable lymphoproliferative disease of mature CD5+ B cells with very heterogeneous biological and clinical behavior. Understanding the molecular heterogeneity of CLL and linking it with the clinical outcome of patients requires evidence from multiple biological layers, which can be obtained using genomics and transcriptomics methods.

Firstly, we attempted to identify prognostic subtypes of CLL based on exome somatic mutation profiles. The main objective of this project was to build and test an analytical pipeline on a large patient cohort (n = 506). We mapped the somatic mutations to the molecular pathways and calculated a mutation score. Ensemble clustering of the pathway mutation score revealed four prognostic subtypes, which we subsequently characterized and associated with recurrently mutated pathways such as DNA-damage response, RNA processing, and calcium signaling. The developed pipeline is currently being applied in an ongoing project with an in-house dataset focused on the clonal development of *TP53* mutations during the CLL disease course.

Secondly, we aimed to explore the cellular origin of CLL, which remains an open question until now. During the B cell development in bone marrow (BM), B cells transiently express CLL marker gene ROR1. However, we identified rare populations of non-malignant ROR1+ mature B cells in peripheral blood. We hypothesized that these cells evaded autoreactive checkpoints in BM and are predisposed to become malignant. Single-cell RNA-sequencing (scRNA-seq) data is valuable for exploring this phenomenon and allowed us to unravel the heterogeneity of ROR1+ B cells. To identify the most similar population of non-malignant B cells to CLL cells, we combined published datasets of B cells with in-house datasets of ROR1+ B cells and nine CLL samples. Then, we built a machine learning model and found that ROR1+ B cells are the most similar to CLL cells.

Altogether, we established and tested computational pipelines for mining insights from exome mutation and scRNA-seq datasets and for enriching in-house analyses with published findings. This provides us with a solid ground for exploring CLL using integrative approaches, which is necessary for a deep understanding of CLL biology and, therefore, the rational use of available treatment options and their development, leading towards personalized medicine.

*Supported by MH-CZ projects AZV NU20-08-00314 and RVO 65269705, and MEYS-CZ MUNI/A/1330/2021..*

# Bioinformatic Pipeline for Comprehensive Analysis of Small RNA-seq Data

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Next-generation sequencing (NGS) is a revolutionary method that allows massive parallel sequencing of millions of DNA or RNA fragments. Although NGS is considered a state-of-the-art method, there is still a need for more comprehensive bioinformatical approaches for NGS data evaluation, especially in the research of small RNAs. In sequencing of small non-coding RNAs, the critical problem is accurate identification and quantification of a full spectrum of small RNA pool. Most of the available pipelines are however targeted on microRNA only and ignore other RNA types such as snoRNA, snRNA, piRNA, or isomiRs.

We propose here a bioinformatic pipeline for accurate quantification of various small RNA classes. Our pipeline is divided into stand-alone modules, each focusing on one part of the sequencing data analysis (first quality control, pre-processing, RNA quantification and differential expression analysis). The most crucial is the RNA quantification module, where a successive number of mapping rounds ensure accurate quantification of all different small non-coding RNAs. For this purpose, we also utilize a set of reference sequences collected from several well-recognized resources. Custom Python tool was created to count reads assigned to different small RNAs that also address an issue of multi-loci RNAs (such as piRNA) and the problem of overlapping RNA annotations.

Each module ends with a PDF/HTML report summarizing results, including tables, plots and their explanation which guides user in further exploration of various small RNA expression levels. To facilitate utilization of report plots for publications, we also offer an interactive application implemented in Shiny for a real-time visualization of differential expression results where content and appearance of popular plots such as heatmap, PCA or volcano plot can be easily altered.

*Core Facility Bioinformatics of CEITEC Masaryk University is gratefully acknowledged for the obtaining of the scientific data presented here.*

# The Role of Long Non-coding RNAs in BCR-mediated CLL Activation

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The B cell receptor (BCR) provides a critical signal for the maturation and survival of B lymphocytes and its dysregulation is a fundamental feature observed in multiple B cell malignancies, particularly in chronic lymphocytic leukemia (CLL). Notably, CLL patients also show a universal clinical response to BCR inhibitor therapy and no recurrent mutations are found in the BCR-related genes of untreated patients.

We and others have shown that short non-coding RNAs namely microRNAs can (dys)regulate the BCR signaling propensity but it is still unclear if long non-coding RNAs (lncRNAs) play a role in BCR activation. Hence, we hypothesized that lncRNAs could be involved in BCR-mediated CLL activation.

To address our hypothesis, we performed differential lncRNAs expression analysis in CLL cells from patients treated with BCR inhibitors and cross-validated in CLL intraclonal subpopulations with high BCR activity ($CXCR4^{dim}$ $CD5^{bright}$) vs. low BCR activity ($CXCR4^{bright}$ $CD5^{dim}$). We found 12 lncRNAs related to the BCR pathway inhibition/activity. Out of these lncRNAs, we selected a lncRNA that belongs to a class of lncRNA called long intergenic non-coding RNA (lincRNAs) which often play a role in trans-activating signaling pathways.

The studied lncRNA was upregulated upon BCR activation (BCR crosslinking with anti-IgM) in CLL cells and had impaired upregulation when cells were treated *in vitro* with BCR inhibitors (ibrutinib/idelalisib). In line with this data, the lncRNA was also downregulated in CLL patients undergoing BCR inhibitor therapy ($p=0.003$, fold-change=3.84). Also, CLL patients with higher expression of the lncRNA have longer survival than those with relatively low levels ($n=100$, $p=0.04$, HR = 2.28; median survival of 9.7 years vs. 16.8 years).

To understand these observations mechanistically, we transcriptionally repressed the lncRNA using dCAS9-KRAB system in MEC1 cell line. To our surprise, the engineered cells showed higher BCR responsiveness as evidenced by the increased calcium flux (FLUO-4 assay) following BCR crosslinking. We further developed a novel functional assay for BCR activation using an engineered MEC1 cell line expressing a calmodulin fused with GFP protein containing an endoplasmatic reticulum (ER) retention sequence. The fused GFP is fluorescent only when calmodulin binds to calcium, and upon the BCR activation, the calcium is released from ER. Strikingly, the engineered cells with lower levels of the lncRNA released the calcium similarly to the control but had a delayed restoration of the calcium pool in the ER.

In summary, the studied lncRNA is directly regulated by the BCR activation and potentially acts in a negative feedback loop to limit BCR-mediated CLL activation.

*This work was supported by the Czech Science Foundation (20-02566S), by MH CZ - DRO (FNBr, 65269705) and by MUNI/A/1330/2021.*

# Genome Diploidization Associates with Cladogenesis, Trait Disparity and Plastid Gene Evolution in a Crucifer Tribe

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Angiosperm genome evolution was marked by many clade-specific whole-genome duplication (WGD) events. The Microlepidieae is one of the monophyletic clades in the mustard family (Brassicaceae) formed after an ancient allotetraploidization. Post-polyploid cladogenesis has resulted in the extant c. 17 genera and 60 species endemic to Australia and New Zealand (10 species). As post-polyploid genome diploidization is a trial-and-error process under natural selection, it may proceed with different intensity and be associated with speciation events. In Microlepidieae, different extent of homoeologous recombination between the two parental subgenomes generated clades marked by slow ("cold") vs. fast ("hot") genome diploidization. To gain a deeper understanding of post-polyploid genome evolution in Microlepidieae, we analyzed phylogenetic relationships in this tribe using complete chloroplast sequences, entire 35S rDNA units, and abundant repetitive sequences. The four recovered intra-tribal clades mirror the varied diploidization of Microlepidieae genomes, suggesting that the intrinsic genomic features underlying the extent of diploidization are shared among genera and species within one clade. Nevertheless, even congeneric species may exert considerable morphological disparity (e.g., in fruit shape), whereas some species within different clades experience extensive morphological convergence despite the different pace of their genome diploidization. We showed that faster genome diploidization is positively correlated with mean morphological disparity and evolution of chloroplast genes (plastid-nuclear genome coevolution). Higher speciation rates in perennials than in annual species were observed. Altogether, the newly acquired results confirm the potential of Microlepidieae as a promising subject for the analysis of post-polyploid genome diploidization in Brassicaceae.

*This work was financially supported by a research grant from the Czech Science Foundation (20-03419Y). Computational resources were supplied by the project "e-Infrastruktura CZ" (e-INFRA CZ LM2018140) supported by the Ministry of Education, Youth and Sports of the Czech Republic. Computational resources were also provided by the ELIXIR-CZ project (LM2018131), part of the international ELIXIR infrastructure.*

## PENGUINN-RNA: Prediction of RNA G-quadruplexes Using Interpretable Neural Networks

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G-quadruplexes (G4s) are non-canonical structures of nucleic acids that have gained increasing interest due to their involvement in a series of biological processes. While the first DNA G4 was identified more than 30 years ago, RNA G4s became known two decades ago. Since then there is accumulating evidence for their importance in cellular mechanisms, including translation regulation, telomere maintenance, and alternative splicing. Here we present PENGUINN-RNA, a machine learning method able to predict RNA G4s based on raw RNA sequence and highlight the regions of the sequence that contribute to the formation of the G4 structure. The trained model is available online and is also accessible through a user-friendly interface that can calculate the G4-forming propensity of user-submitted RNA sequences.

# Mechanism of Cell Wall Binding and Penetration by Bacteriophage phi812

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*Staphylococcus aureus* is a human pathogen contributing to the silent pandemic of antimicrobial resistance (Lin, 2017; Antimicrobial Resistance Collaborators, 2022). Phage phi812 infects 90 % of S. aureus isolates and, therefore, is a promising phage therapy agent (Nováček, 2016; Botka, 2019). To illuminate the initial stage of phage infection, we characterized structural changes in the phage baseplate that lead to the phage tail contraction and genome ejection.

Using a combination of cryo-electron microscopy and X-ray crystallography, we reconstructed the phage baseplate in the pre- and post-contraction states. The baseplate core proteins comprise specific structures that form a baseplate with the overall threefold symmetry. Phage phi812 lacks long baseplate fibers typical for other contractile systems. Instead, six rigid baseplate arms provide attachment sites for twelve 400kDa multidomain tripod complexes and six of each of two types of receptor-binding proteins. Upon the host cell engagement, the conformational change of the receptor-binding proteins and tripod complexes propagates through the core proteins to the tail sheath, resulting in the tail contraction. Sixfold symmetry of the post-contraction baseplate provides additional stability for the penetration of the tail tube through the host cell wall.

We present the first detailed structural characterization of a contractile phage infecting Gram-positive bacterium. Comparison of the two distinct baseplate states allows the description of the initial stage of phage infection on the molecular level. Finally, our results provide a framework for engineering phage particles to combat *S. aureus* infections in humans.

*We acknowledge the Cryo-electron microscopy and tomography core facility and the Biomolecular interaction and crystallization core facility of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127).*

# Structural Analysis of Transcription Termination Machinery in Yeast

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Gene expression is one of the most fundamental processes conserved from bacteria to humans. One of the key steps, transcription, is performed by RNA polymerase II (RNAPII). In *Saccharomyces cerevisiae*, the largest subunit of RNAPII, Rpb1, contains an unstructured and highly dynamic C-terminal domain (CTD) consisting of 26 repeats of $Y^1S^2P^3T^4S^5P^6S^7$ sequence. Five out of seven amino acids per repeat can be posttranslationally modified (e.g. phosphorylation) which allows CTD to serve as binding platform for various transcription factors.

During transcription termination, subunit of CPF-CF complex, recognizes Ser2 phosphorylated (pSer2) CTD, allowing CPF–CF to interact with the nascent transcript and cleave it within poly(A) site exposing free 5'-end of RNA. The exact mechanism, however, of how the RNAP II is then released from the remaining RNA transcript is unknown. Recent studies in yeasts showed that exposed and unprotected free 5'-end of RNA serves as an entry point for Rat1 yeast 5'-3' exonuclease stimulated by its cofactor, Rai1 forming Rai1/Rat1 (RR) complex. Findings also show that Rat1 mediated termination is coupled to recruitment of RR by Rtt1031 recognizing pSer2 phosphorylation. This allows us to hypothesize that Rtt103 recruits 5'-3' RNA processing machinery in order to trigger RNAPII release from the DNA template, so-called "torpedo model".

To determine the principles that govern association of RR to Rtt103 we performed a series of in vitro pull-down experiments, which allowed us to identify key part of each protein necessary to form the complex. Based on these results, we performed fluorescence anisotropy experiments.

Data shows that short peptides corresponding to interaction regions of Rat1 and Rai1 occupy the same binding site on Rtt103 as CTD pSer2 peptide. We show that the tested peptides compete with CTD pSer2 for binding to Rtt103 CTD-interacting domain (CID), so the recognition of RR complex by RNAPII could depend on Rtt103 and the competition-based mechanism between RNAPII CTD and RNA degradation machinery. In order to understand the architecture of Rtt103 mediated complexes, and their involvement in transcription termination we produced recombinant full-length variants of all three proteins in *E. coli*. Our results reveal that Rtt103 indeed binds to RR, based on size-exclusion chromatography profile of purified protein complex. We speculate two scenarios how interaction between RR and Rtt103 is realized. Based on sequence analysis

of RR and Rtt103 the interaction includes individual CTD-mimicking motifs of RR and two CIDs of dimeric Rtt103[2]. Different theory about formation of complex is based on our interaction prediction by AlphaFold[3], where the interaction between RR and Rtt103 relies on one region of monomeric Rtt103 and one region of RR. To decipher which scenario is correct, individual RR complexes and the RRR complexes are now investigated via cryo-electron microscopy.

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## Dissecting CIPK-Mo25 Regulatory Module in Plant

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Meiosis is a specialized cell division that produces haploid spores from diploid precursors. Meiosis is the main source of genetic diversity in eukaryotes and directly contributes to adaptation. In our lab, we study the mechanisms that regulate meiosis in *Arabidopsis*. We designed a forward genetic screen to identify genes involved in meiotic progression. One gene found in screen encodes MO25A, an evolutionarily conserved protein known to regulate the activity of LKB1 and MST4 kinases in animals. *Arabidopsis* encodes four paralogues of MO25, but their function is unknown. In yeast two-hybrid screen, we found that MO25A and its homologue MO25C interact with seven members

of the CIPK kinase family that are known for their role in calcium-mediated responses in plants together with Calcineurin B-like family (CBL). *Arabidopsis* has 26 genes for *cipks* and 10 for *cbls*, but only some of them have been characterized. MO25 interacts only with the subset of CIPKs without introns. MO25A (*At4g17270*) and MO25C (*At5g47540*), share 80% of the amino acid sequence, but the double mutant is lethal, suggesting that while these genes act redundantly, their function is essential.

To get cues on the function of the MO25-CIPK in plants, we performed systematic localization of CIPK and MO25 in the leaf's protoplasts and confirmed by BIFC (bimolecular fluorescence complementation) that MO25 and CIPK interact. We also defined the localization of MO25 in all plant tissues using reporter constructs stably expressed in Arabidopsis plants and found that the MO25 is highly expressed during the growth of the pollen tube. Different phylogenetic approaches confirmed that the CIPKs are highly conserved in all green lines of the Bikonta species, and the MO25-CIPK module appears to be present in all lineages of green plants. In the end, we described that the

expression and localization of CIPKs are affected by MO25. In conclusion, we discovered that MO25 represents a new regulator of CIPKs that may play a wide role in several plant physiological processes.

*Project acknowledgment:*
*We acknowledge the core facility CELLIM supported by MEYS CR (LM2018129 Czech-BioImaging) and the Plant Sciences Core Facility of CEITEC Masaryk University.*

# Exploring The Role of Cell-type Specific Expansion Overexpression in The Control of Cell Wall Biochemical Properties and Root Growth of *Arabidopsis*

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The cell wall (CW) is an important structure of plants as it determines the shape of cells, enables cell connections and acts as a protective barrier against pathogens and the environment. The primary CW consists of polysaccharides cellulose and hemicellulose that provide the basic mechanical strength, and pectins that can alter the viscoelastic properties of the matrix. Expansins (EXPAs) are cell wall-loosening proteins activated during CW acidification triggered by a number of stimuli through the plasma membrane H+-ATPase proton pump. EXPAs are known to disrupt non-covalent bonds between CW polysaccharides but without structural changes of the CW. EXPAs do not possess a hydrolytic activity but they facilitate the CW loosening by mediating slippage of carbohydrate polymers at load-bearing elements of the CW. However, the molecular mode of action of EXPAs that enables cell wall expansion remains elusive.

Our previous results suggested that spatial-specific distribution of expansins and disruption of fine-tuned pH and strain-stress optimum leads to growth arrest of *Arabidopsis* roots. Hence, we propose that tightly controlled spatiotemporal specificity of expansin expression and hormonal-mediated pH distribution within the root apoplast plays an important regulatory role controlling the root growth and development in *Arabidopsis*. To decipher the riddle, we induced ectopic expression of α-expansin 1 (*EXPA1*), in each individual layer of the root apical meristem (RAM) using cell type-specific activators of the chemically inducible transcription activation system pOp6/LhGR. We measured the size of root and RAM but interestingly found no significant differences. Next, we plan to follow more closely the growth of selected lines after applied hormonal treatment and pH changes using confocal laser scanning microscopy and 4D imaging. Also, we will try to lower the pH in specific cell type by overexpressing the H+-ATPase using the inducible system above and investigate the dynamics of root growth and finally associated changes in the cell wall biomechanical properties.

*This work was supported by Czech Science Foundation 22-17501S, MEYS (CZ.02.1.01/0.0/0.0/16_019/0000738) and LTAUSA18161.*

## Cdk11: New Player in Pre-mRNA Splicing

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Cdk11 is a poorly characterized cyclin-dependent kinase implied in control of mRNA transcription, alternative splicing, 3'end processing of HIV mRNA and expression of replication dependent histones. Recently discovered inhibitor of Cdk11 gave us powerful tool to assess its roles in cellular context following rapid inhibition. RNAseq in HCT116 cells showed widespread disruption of pre-mRNA splicing. Several proteins involved in splicing were identified in screen as candidate interacting partners of Cdk11 and tested in immunoprecipitations. Direct effect of Cdk11 activity on splicing was verified *in vitro* and also *in vivo*.

# Versatile Coculture System to Study Chronic Lymphocytic Leukemia Proliferation

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Malignant cells of chronic lymphocytic leukemia are characteristic by their dependence on signals from the lymphoid microenvironment. We aimed to prepare a coculture model for CLL cells inducing their proliferation by signals mimicking interactions in their microenvironment. Since it has been proved that CLL proliferation occurs mainly in sites with close contact with T cells, we engineered an immortalized supportive fibroblast-like cell line to express up to three factors normally produced by activated T cells. We have altogether prepared a panel of 6 such supporting cell lines expressing one factor, or various combinations of two out of the three factors.

To reveal the relevance of our coculture model with in vivo stimulation, we have performed transcriptome profiling (Illumina) of CLL cells cultured on a layer of mitotically inactivated wild-type supportive cells, or the engineered supportive cell line. We compared changes in gene expression induced by an engineered cell line with changes detected in the intraclonal CXCR4dimCD5bright CLL subpopulation, which is considered to consist of the activated CLL cells recently exited from lymphoid niche. We have detected 1743 genes (out of 2080), which overlap with differentially expressed genes in CXCR4dimCD5bright cells ($p<0.0001$), including those related to metabolism and antigen processing. We further validated increased expression of anti-apoptotic proteins Mcl-1 and Bcl-xL, and cell cycle drivers, namely cyclin D1 and MYC.

Using different variants of supportive cell lines, we triggered CLL cells into sporadic proliferation or induced their relatively massive cell division (indicated by CFSE dilution). The model enables long-term culture of proliferating CLL cells for >60 days, which is not possible in culture without supportive cells. The coculture model is highly reproducible and enables the studies of CLL cell proliferation, CLL-T cell interactions, and testing of drugs targeting pathways required for proliferation of malignant B cells.

*Supported by: Czech Science Foundation (project No. 20-02566S), European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 802644), Ministry of Health of the Czech Rep. grant nr. NU20-03-00292 and NU22-03-00117, MH CZ - DRO (FNBr, 65269705), and MUNI/A/1330/2021.*

# Mechanism of Virion Formation of the *Emiliania Huxleyi* Virus 201 Enveloped by Two Membranes

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*Emiliania huxleyi* is a worldwide distributed unicellular marine alga whose cells are covered by calcite disks called coccoliths. By reflecting light, the coccoliths influence retention of heat in oceans, which impacts planetary climate [1]. *Emiliania huxleyi* virus 201 (EhV-201) and related nucleocytoplasmic large DNA viruses limit the population growth of the *E. huxleyi* alga [2].

We show that virions of EhV-201 are pleiomorphic in shape with a complex ultrastructure, comprising an inner membrane, capsid, outer membrane, and surface protein envelope. Furthermore, we used focused ion beam milling and cryo-electron tomography to characterize the formation of EhV-201 virions in *E. huxleyi* cells. The particle assembly is initiated on membrane fragments, which separate from the endoplasmic reticulum. Assembly of the capsid proteins at the outer surface of the membrane fragment induces its bending and gradual formation of capsids containing a membrane sack. Virus DNA is packaged into the pre-formed particles through an opening in the capsid and inner membrane. The genome-filled intermediates bud into intracellular vesicles, and in this process, acquire the outer membrane and protein envelope. Virions are released from the cell by exocytosis or lysis of the infected alga. Our results give structural insight into the formation of EhV-201 – a pathogen that influences the Earth's climate.

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# Skin-Wash: A 3D Printed Sweat Sampling Device for Diagnosis of Cystic Fibrosis

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Cystic fibrosis (CF) is a rare incurable inherited disease caused by a mutation in cystic fibrosis transmembrane conductance regulator gene. The disease affects mostly lungs and leads to various symptoms such as lung infections, difficulty breathing or defective chloride transport channels. The elevated chloride concentration in sweat is one of the important parameters in CF diagnosis. Therefore, appropriate method for sweat collection and for its further analysis is needed.

Macroduct is commonly used method for sweat collection carried out right after a sweat induction based on pilocarpine iontophoresis. However, sufficient amount of sweat is not always obtainable, or it might be overly time consuming. Also, this sampling method may be considered as semi-invasive and expensive.

Recently, new sweat sampling method called Skin-Wipe was developed. This method is based on wiping the spontaneously formed sweat from skin by a moistened cotton swab followed by its extraction. However, the necessary material preparation turned out to be time consuming because of the sample contamination risk. Moreover, sale of cotton swabs with a plastic stick became prohibited by the European Union.

On the basis of the above, in this work we focused on developing a new sweat sampling device and method, which we call Skin-Wash. This new method allows effective and quick collection of sweat specimens completely noninvasively. The main part of the Skin-Wash device is produced by AnyCubic resin 3D printer. During sampling, a flow-through channel of the 3D-printed part is placed on the patient's forearm skin. 500 µl of deionised water is flushed through the device from a syringe so that the spontaneously formed sweat from the skin is washed into a plastic vial.

The sampling method using the Skin-Wash device was tested in a study comparing it with Skin Wipe and Macroduct sweat samples and involved a cohort of 22 CF patients, 22 CF disease carriers and 21 healthy controls. The subsequent analysis of the obtained Skin-Wash sweat samples was carried out by capillary electrophoresis with contactless conductive detection. The ion ratio method (developed by us earlier) was used for evaluation of the measured data. The results show an excellent correlation for both selected ion ratios (Cl-/K+) and ((Cl-+Na+)/K+) between the Skin-Wipe and the Skin-Wash samples. Moreover, using the ion ratios listed above as a cut-off value, the Skin-Wash method enables diagnosis of CF patients with comparable sensitivity and selectivity as the common diagnosis by Macroduct sample with coulometric chloride analysis. Therefore, it might offer a completely non-invasive, simple, and faster alternative that has the potential to become a new diagnostic or screening approach for CF.

*Project acknowledgement: This research was funded by the Ministry of Health of the Czech Republic, grant number NV18-08-00189 and by the Czech Academy of Sciences, the institutional support RVO: 68081715. Participating researchers and data contributors participate in the ERN LUNG.*

## Structural Characterization of the Interaction Between BRCA1-BARD1 and RNA Polymerase II

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Transcription is considered one of the major threats for genome stability; as the conflicts of the transcription machinery with the replication fork, or another barrier, can lead to double-stranded DNA breaks. Although maintaining genome integrity is crucial for the cell viability, the mechanisms responsible for avoiding these conflicts are poorly characterized. Therefore, my research project focuses on structural characterization of the interaction between RNA polymerase II (RNAPII) and BRCA1-BARD1 complex, one of possible players involved in maintaining the genome stability. Recently, we have confirmed the interaction between the C-terminal domain of RNAPII and the BRCT domains of BRCA1 and BARD1, respectively, and we are reconstituting the full-length complex for cryo-electron microscopy studies. Structural characterization of the complex, as well as description of the conditions under which it is formed will help us to analyse its function in preventing transcription-borne DNA damage. This, in turn, will help us to understand how cells coordinate transcription and other competing processes on DNA, such as replication or DNA repair.

# Characterization of Lectin PluLec from *Photorhabdus Luminescens*

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Lectins are ubiquitous proteins of non-immune origin that can reversibly and specifically interact with carbohydrates. They are involved in recognition events in various physiological and pathological processes like intercellular communication, adhesion, migration and host-pathogen interactions. Unlike antibodies, they are not product of immune response and do not possess any enzymatic activity. Lectins are commonly used for characterization of carbohydrate structures, for purification of glycoproteins and to specifically label the cell surface structures

*Photorhabdus luminescens* is a naturally bioluminescent Gram-negative bacterium and an insect pathogen, which symbiotically lives in Heterorhabditidae nematodes. PluLec is a putative lectin from *Photorhabdus luminescens* and a homologue of PA-IL lectin, which is D-galactose specific, Ca2+ dependent, cytotoxic lectin from opportunistic pathogen *Pseudomonas aeruginosa*, involved in facilitating infection in patients with compromised immunity.

This research is focused on structural-functional characterization of recombinant protein PluLec using various methods like isothermal titration calorimetry, hemagglutination, glycan array, analytical ultracentrifugation, protein X-ray crystallography and toxicity tests made on insect models.

The study revealed that lectin crystallizes as a homotetramer with four binding sites for D-galactose (one per monomer). Shows specificity towards beta anomers of D-galactose. Preliminary results of toxicity test made on insect models shows clear negative effect on survival of insect. Obtained results of the structure and function of PluLec may reveal importance in the pathogenic or symbiotic stage of life.

*This work was supported by the Czech Science Foundation (21-29622S) and we acknowledge the Biomolecular Interaction and Crystallization Core Facility and the Proteomics Core Facility, CEITEC, Masaryk University supported by the CIISB research infrastructure (project LM2018127 funded by MEYS CR) for their support with obtaining scientific data presented in this paper.*

## Dual Binding Mode of Dishevelled PDZ Domain

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PDZ is a widespread modular domain known to function as scaffold in protein complexes. This is achieved by the specific recognition of a free carboxyl group in the interacting partners, present, either at the end of the linear sequence (terminal mode) or the sidechain of aspartate residues anywhere in the linear sequence (internal mode). Here we studied the binding mode of Dishevelled (DVL) PDZ domain which is known to interact with its own C-terminus (DVL_C). Interestingly, the crystal structure of PDZ domain with DVL_C revealed that both binding modes are plausible, because the native ligand comprises both terminal and internal carboxyl groups. The dual binding mode was confirmed by mutational analysis using NMR spectroscopy. A thorough analysis of other PDZ structures with peptide ligands reported on the presence of one or another binding mode, but not on the ability to switch between them. Our work expands the ligand-recognition space explored by PDZ domains.

## Recognition of RNA Polymerase II C-terminal Domain by RPRD2

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The largest subunit of human RNA Polymerase II contains highly flexible C-terminal domain (CTD) that is composed of 52 heptapeptide repeats (first half of repeats with consensus sequence YSPTSPS and second half largely degenerated in sequence). Several CTDs canonical and non-canonical residues can be subjects of post-translational modifications. Tyrosine, threonine, and serine residues undergo dynamic phosphorylation/dephosphorylation resulting in specific phosphorylation patterns throughout different stages of transcription cycle. These phosphorylation patterns are recognized by various transcription and processing factors during the transcription cycle. Therefore, CTD plays an important role in the regulation of transcription and coupling of transcription to post-transcriptional processes such as mRNA processing.

In this study, we show that human transcription factor, RPRD2, recognizes specifically pSer2 or pThr4 phosphorylated forms of CTD via its CTD-interacting domain (CID) in a similar way to its yeast homologue, Rtt103. The interaction of RPRD2 CID with pSer2 phosphorylated CTD is further enhanced by additional phosphorylation on pSer7. To provide mechanistic details of the interaction between RPRD2 CID and pSer2,7 CTD, the solution structure was obtained using NMR spectroscopy. pSer 2 and pTh4 phosphomarks occur mainly during the late elongation and termination. RPRD2s preference for these two phosphomarks suggests possible involvement of RPRD2 in transcription termination.

## How Phosphorylation Impacts 14-3-3ζ Dimerization

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The 14-3-3 proteins represent a large group of dimeric proteins. Specifically, the 14-3-3 family consists of 7 isoforms, that can create many homo- and heterodimeric forms, not even accounting for the possibility of changing the oligomerization properties by posttranslational modifications such as phosphorylation. The functions of 14-3-3 are very often dependent on its oligomeric state. Therefore, the parameters of oligomerization are very interesting in order to correctly understand the regulation and behavior of 14-3-3 itself.

In our study, we focused on the zeta isoform (most abundant isoform in human brain, also forming most stable dimers) and its phosphorylated form. Using standard biophysical methods we have only seen that the Kd is lower than 1 μM. Therefore, we designed very sensitive fluorescence based methods to allow for study of such tightly bound dimers. Using these methods, we determined the dissociation constant, as well as kinetic parameters of the oligomerization process.

In order to determine the effect of phosphorylation on Ser58, which the literature is ambiguous about, we used our fluorescence assays. The serine S58 is located at the dimeric interface and therefore its phosphorylation affects the dimerization process. We succeeded in determination of both kinetic and equilibrium parameters of the interaction between non-phosphorylated and phosphorylated 14-3-3ζ protein.

The results of this work were published recently.

*ACKNOWLEDGEMENT*
*This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic within programme INTER-ACTION (project No. LTAUSA18168) and by the research grant from the Czech Science Foundation, grant no. GA15-34684L and GF20-05789L. Research infrastructure was supported by CEITEC 2020 (LQ1601) and the CIISB research infrastructure project supported by MEYS CR (LM2018127).*

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# The Role of Long Non-coding RNAs in the Microenvironmental Interactions of Malignant B Cells

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Chronic lymphocytic leukemia (CLL) is a disease largely dependent on the interactions of malignant B lymphocytes with the components of the tissue microenvironment (TME). This complex crosstalk between the neoplastic B cells and the different immune cells in the microenvironment provides stimuli that favor clonal expansion and drug resistance in CLL cells, and thus contributes to the aggressiveness of the disease. It has been shown by us and others that microRNAs act as regulators of B cell receptor (BCR) signaling propensity in the lymph node microenvironment by directly or indirectly targeting downstream effectors of the BCR pathway. However, the role of long non-coding RNAs (lncRNAs) in regulating the BCR activation and coordinating microenvironmental crosstalk remains poorly understood.

To address this issue, we carried out RNA-seq profiling of lncRNAs differentially expressed pre- and post-idelalisib treatment *in vivo* and lncRNAs differentially expressed in intraclonal subpopulations of CLL cells, which identified dozens of lncRNAs. We focused on the subset of lncRNA candidates likely to be involved in TME crosstalk and selected one of these candidates for further investigation.

The candidate lncRNA was found to be enriched in leukemic cells in comparison to healthy B cells and our data shows a difference in the lncRNA levels in CLL patients undergoing treatment with BCR inhibitors, which was also confirmed on primary CLL cells *in vitro*. To understand the role of the candidate lncRNA in microenvironmental interactions, we stimulated primary CLL cells with stimuli mimicking the pro-survival and pro-proliferation signals CLL cells receive in the tissue microenvironment. Our preliminary data suggests that microenvironmental stimuli strongly affect lncRNA levels in a time-dependent manner. Specifically, we observed a significant decrease of lncRNA levels in response to T cell-derived signals, such as CD40L, IL-10, and activated T cell conditioned media, as well as CpG stimulation. On the contrary, stimulation with T cell-derived IL-4 resulted in increased levels of the candidate lncRNA. Additionally, BCR ligation by bead-bound anti-IgM revealed a pattern of tight regulation of lncRNA expression in response to the different stages of BCR-mediated activation of leukemic cells. Furthermore, the

candidate lncRNA was found to be localized in the cytoplasmic compartment of CLL cells, suggesting that it might interact with some of the key effectors regulating BCR-transduced signaling.

We identified a novel microenvironment-dependent lncRNA involved in facilitating the crosstalk of CLL cells. However, further investigation is needed to understand its precise functional role in the context of microenvironment-dependent regulatory networks.

*This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 802644). Supported by the Ministry of Health of the Czech Republic, grant nr. NU20-03-00292. All rights reserved. This work was supported by the Czech Science Foundation (20-02566S). Supported by MH CZ - DRO (FNBr, 65269705) and MUNI/A/1330/2021.*

# Natural Genetic Variability in Multistep Phosphorelay as a Tool for Elucidating Drought Adaptation in *Arabidopsis Thaliana*

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Improving plant productivity by developing climate-adaptable varieties using advanced molecular breeding strategies and high-throughput phenotyping technologies is one of the promising avenues for overcoming the challenges on food production posed by severe weather unpredictability, diminishing arable lands, and resources, and a growing global population. To adapt to changing climatic conditions, plants evolved molecular networks governed by endogenous elements such as plant hormones and external environmental stimuli such as temperature, light, and humidity.

The goal of this research is to find natural genetic variants of critical regulators that increase the integration of environmental and hormonal (cytokinin) signaling pathways in plants, as well as to assess their relevance as prospective targets for generating climate-adaptable crops. Arabidopsis histidine kinases (AHKs) are a critical component of such a route. Natural variation in three cytokinin-responsive histidine kinases, AHK2, AHK3, and AHK4/CRE1, was identified using publicly accessible resources such as 1001 Genomes. Accessions with SNPs discovered near a known protein function location were chosen for further investigation. Using a root-elongation experiment and transcriptomic analysis of CK signaling reporter genes, the role of the revealed genetic diversity to the responsiveness of MSP signaling to CKs was investigated. Accessions with either lower or higher CK sensitivity were identified. Further analysis of the root morphology upon exogenous CK treatment showed a stronger reduction of root apical meristem size in the more responsive AHK variants. The size of the root apical meristem, previously shown to be CK-controlled, correlated well with the reduced root length of particular accession, supporting the observed altered CK sensitivity in selected accessions. A ligand-binding assay was also conducted and found no significant changes in the CK affinity to AHK4 variants harboring the SNP of ecotypes with increased CK sensitivity. This suggests that the discovered SNPs may affect AHK4's ability to transduce the signal down the pathway rather than the sensor's CK binding-mediated activation. A detailed phenotypic analysis of the accessions was performed to correlate particular SNP in AHK to the decreased or enhanced cytokinin signaling and drought stress response.

The information generated from this study can be used for the targeted improvement of the Brassica sp. genome resulting in higher yield and better tolerance to different abiotic stress.

*This work was supported from European Regional Development Fund-Project „SINGING PLANT“ (No. CZ.02.1.01/0.0/0.0/16_026/0008446).*

## Multifunctional Disorder in RECQ4 Helicase with the Inherent Ability to Trap G-quadruplexes and Form Phase-separated Condensates

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Biomolecular polyelectrolyte complexes can be formed between oppositely charged intrinsically disordered regions (IDRs) of proteins or between IDRs and nucleic acids. Here, we show that a positively charged IDR within the human ATP-dependent DNA helicase Q4 (RECQ4) efficiently binds G-quadruplexes (G4s). Phase-separated droplets form, containing high-order assemblies of RECQ4/G4 complexes. We describe a two-step mechanism featuring electrostatics to form an encounter complex, that eventually partitions to the coacervate phase. The IDR traps G4s and presents a physical barrier for other G4 unwinding helicases, suggesting a novel signaling function for polyelectrolyte complexes. We also discover a physical interaction with RPA, the most abundant single-stranded DNA binding protein, and demonstrate that the IDR is able to switch between the two extremes of the structural continuum of complexes. The structural, kinetic, and thermodynamic profile of its interactions revealed a dynamic disordered complex with nucleic acids and a static ordered complex with RPA, both of them dominated by electrostatic interactions. The two binding modes are mutually exclusive suggesting a regulatory role for the IDR in RECQ4 function by enabling molecular handoffs. Our study extends the functional repertoire of IDRs and demonstrates a novel role of polyelectrolyte complexes involved in G4 trapping.

# Unravelling the Process of Thermoregulation During the Seed Development in *Brassica Napus*

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Global warming and its effect on crop yield are among the most significant problems faced in the 21st century. Over the past decades, the gradual rise in global temperatures has reached above 1℃, affecting the yield of important crops like wheat, rice, and maize. *Brassica napus* (rapeseed or canola) comes from the agronomically important Brassicaceae plant family and is the second most widely produced oilseed worldwide. Studies on the development of plants with rising temperature conditions provide knowledge about the temperature influence on crop yield. This project studies the development of three *B. napus* cultivars, Westar, Topas, and DH12075, in three temperature regimes, 21℃, 28℃, and 34℃ in long-day conditions. Characterizing the thermo-morphogenesis of B. napus grown in long-term heat stress conditions identified accelerated plant growth, reduced fertilization rate and increased seed abortion rate. The accelerated embryo development, defective embryo and seedling development and pre-harvest sprouting phenotypes, which might result from reduced seed dormancy, reduce the viable seed set. From an RNA-Seq analysis and hormonal profiling data followed by RT-qPCR studies, we identified that the ABA and auxin hormonal pathways are misregulated in plants grown at high temperatures. Interestingly, some of the genes involved in the pre-mRNA splicing are upregulated in heat-stressed plants. Also, seed oil content measurements show the reduced quality of seeds developed at higher temperature conditions. Further studies in this research area will pave the way towards producing thermotolerant varieties of *B. napus* with better crop yield, thus contributing to improved global food production.

*Funding: This work was supported by European Regional Development Fund-Projects "SINGING PLANT" (CZ.02.1.01/0.0/0.0/16_026/0008446), "Centre for Experimental Plant Biology" (No. CZ.02.1.01/0.0/0.0/16_019/0000738), and by the Czech Science Foundation (project 19-05200S). We acknowledge the CEITEC Cellular Imaging core facility supported by the MEYS CR (LM2018129 Czech-BioImaging) and the CEITEC Plant Sciences core facility.*

## An Unbiased Analysis of Interplay between Adenosine Methylation and Editing

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The precise and unambiguous detection and quantification of internal RNA modifications represents a critical step for understanding their physiological function. Only handful of marks can be detected by reverse transcription and sequencing, some of those thanks to the additional chemical conversions of isolated RNAs. Detection and quantification of $m^6A$, $m^6Am$ and $m^1A$ modifications still remains one of the biggest challenges in the field. Second intriguing and timely question that remains to be addressed is the extent to which individual marks are co-regulated or potentially can affect each other. Here we present a study where we detect and quantify several key mRNA marks in human total RNA and mRNA. We show that the adenosine demethylases FTO and ALKBH5 primarily targets different modifications, thus specifying their targets and potential RNA substrates in HEK293T cells. Interestingly, upregulation of both FTO and ALKBH5 is accompanied by an increase in inosine level in overall mRNA, bringing up the role of innate immune response during protein overexpression in cells.

# CLLue: An Interactive Bioinformatic Tool for Data Exploration, Segregation, and Important Variables Selection

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**Introduction and aims**

In the era of personalized medicine, it is essential to grasp and understand all the available patient data. With a deeper understanding of the relevance, importance and relations of various clinico-biologic parameters, it is possible to give clinicians a clue about which variables in the dataset could be used for patients' segregation into groups with e.g. different clinical outcomes. On top of that, we are able to develop computational methods using those variables to segregate the data automatically and possibly predict disease evolution and the most efficient therapy.

**Methods**

The CLLue is a web-based application written in the R language. The user interface (UI) and interactive visualizations are built with Shiny and plotly R packages. For the data analysis, the following approaches are used: basic R functions for data manipulation and basic clustering, factoextra, tsne, and umap packages for dimensionality reduction, and XGBoost and e1071 for machine learning techniques. The web application is deployed online on a server with computational resources provided by MetaCentrum.

**Results**

We have developed a web-based interactive tool CLLue with a UI that enables users to inspect their data and give them a clue about the importance of single variables and connections between the variables either within given groups or newly created ones. To attain this, we have implemented methods for data cleansing and filtering, visual exploratory data analysis, statistical testing, data clustering, machine learning and interactive visualizations for most of these methods.

**Discussion and conclusion**

The CLLue has been initially created to investigate a preselected dataset of biological and clinical metadata of CLL patients and to identify significant variables defining given groups of patients. The original groups were used to segregate the patient cohort into new groups. Subsequently, the new groups were compared with the original ones, and the observed differences were used for more precise patient distribution. The CLLue has

been developed with regard to transferability, therefore, it can be used for analyzing any dataset containing both numerical and categorical data. Currently, we apply the tool to a new dataset from hematooncological patients generated by our separate bioinformatic platform LYNX, which provides information about single nucleotide variants, copy number variations, antigen receptor rearrangements, and translocations. We have annotated the data with routine diagnostic conclusions, which will be used as the original patient grouping information. The heterogeneity and complexity of this new dataset bring new questions and challenges, leading to further improvements of the CLLue.

*Supported by the Ministry of Health (CZE) projects RVO 65269705, AZV NU21-08-00237, NV19-03-00091, NU22-08-00227. Computational resources supplied by e-INFRA LM2018140.*

## Unravelling the Molecular Function of CDM1 Zinc-finger Protein in Meiotic Progression in *Arabidopsis Thaliana*

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

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

*Acknowledgments This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic, European Regional Development Fund-Project „REMAP“(No. CZ.02.1.01/0.0/0.0/15_003/0000479).We further thank to Plant Sciences Core Facility of CEITEC Masaryk University for support with plant cultivation.*

# ER Localized PIN5 and PIN8 Possess Opposite Topological Orientation of the Central Hydrophilic Loop and the C-terminal End

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PIN (PIN-FORMED) proteins are plant specific auxin transporters, which contain variable length of hydrophilic loop linking two alpha-helical regions. The long loop PINs localizing at the plasma membrane regulate polar auxin transport (PAT) and establish auxin gradients that subsequently maintain plant growth and development. However, the shorter loop PINs (PIN5 and PIN8) which localize at the endoplasmic reticulum (ER) are not directly involved in PAT, instead they maintain intracellular auxin homeostasis. Although the developmental role of the ER localized PINs has been studied, the structure and function relationship of these proteins remain less understood. In this study, we investigated membrane topology of PIN5 and PIN8, in terms of the subcellular orientation of the HL, the N- and C-termini ends. Our data revealed that PIN5 and PIN8 have opposite topology, despite their similarities in the topological arrangement of their N-termini.

Key words: *Arabidopsis thaliana*, Topology, ER, PIN auxin efflux carriers, Hydrophilic loop

# Characterization of Staufen1 Ribosome Interactions

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Human Staufen1 (hStau1) is a double-stranded RNA binding protein involved in mRNA transport and localization, Staufen mediated mRNA decay (SMD) as well as regulation of mRNA stability and translational efficiency. Staufen1 contains dsRNA binding domains (dsRBD2, dsRBD3, dsRBD4, dsRBD5), a Staufen Swapping Motif (SSM) and a Tubulin Binding Domain (TBD). In cells Staufen1 is found at the rough endoplasmic reticulum where it is associated with translating ribosomes. This interaction requires RNA binding activity of dsRBD3 and protein binding activity of dsRBD4 and TBD. Protein-protein interactions are proposed to occur at the P1 stalk while rRNA-Staufen1 interactions occur mainly with expansion segments (ES) of the 18S and 28S rRNA. The structural basis of these interactions is still unknown. In order to understand translational control and SMD, we focused our experiments on hStau1-ribosome association. We used techniques such as cell fractionation and pelleting assay. Further Staufen-ribosome binding was confirmed by electrophoretic mobility shift assay (EMSA) and western blot assay. Structural analysis of hStau1-ribosome was performed using cryo-electron microscopy (cryo-EM).

During cell fractionation and pelleting assay, Stau1 co-fractionated with ribosomes and associated with all 40 S, 60 S, 80 S ribosomal subunits as well as polysomes. Furthermore, we confirmed by EMSA and western blot assay that Staufen is more strongly bound with 60 S subunits and 80S ribosomes. These findings suggest that Staufen1 binds to ribosomes in 1:8 ratio. On the other hand, cryo-EM reconstruction of the 60S ribosome-Stau1 complex and 80S ribosome-Stau1 complex did not reveal any additional densities on ribosomes which could be identified as a Staufen protein. The possible explanation could be that bound protein can be more flexible comparing to the rigid structure of the ribosome and therefore we cannot visualize it with cryo-EM. More studies with different techniques such as XL-MS should be done to find the exact binding site of Staufen to ribosomes.

## Elucidating the Biological Role of ADAR1 in the Innate Immunity Response

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ADAR enzymes are responsible for the deamination of adenosine into inosine in cellular dsRNA allowing the cell to distinguish endogenous RNA from exogenous RNA and preventing an aberrant immune response. Indeed, accumulation of endogenous unedited ADAR1 RNA substrates within cells triggers type I IFN production through the anti-viral MDA5/MAVS pathway. Three members of the ADAR gene family (ADAR 1-3) have been identified in vertebrates. In addition, two isoforms of ADAR 1 are synthesized by translation initiation at alternative start codons, an interferon-inducible, cytoplasmic 150-kDa protein (p150) and a constitutive, nuclear 110-kDa protein (p110). Loss of function mutations in the human *ADAR1* gene cause Aicardi-Goutières Syndrome (AGS), a rare human congenital encephalopathy that resembles congenitally acquired viral infection.

*Adar1* deficiency in murine models lead to embryonic lethality by E12.5 with aberrant IFN induction, widespread apoptosis and failed hematopoiesis. Concurrent deletion of *Mavs* or *Mda5* rescues the embryonic lethality, allowing the pups to survive few days after birth. *Adar1*, *Mavs* mice show an increased apoptosis in the intestine, a mild inflammation, and a disrupted spleen morphology.

We have shown that the apoptosis detected in the small intestine of *Adar1*, *Mavs* mice at P14 it's absent at embryonic stage and it is spreading from the proximal part along the whole small intestine few days after birth. *Adar1* deletion causes activation of dsRNA-dependent protein kinase *Pkr*, encoded by *Eif2ak2* gene, an IFN stimulated gene responsible of translation inhibition by phosphorylating eIF2a and of cell death pathways activation. We have shown that the mortality, the intestinal apoptosis and the minute phenotype present in *Adar1*, *Mavs* mutant mice are rescued after *Eif2ak2* deletion. Moreover, the heterozygous gene loss of Pkr is enough to rescue the intestinal apoptotic phenotype and the spleen phenotype is not the cause of death in the *Adar1*, *Mavs* mutants.

Our goal at the moment is to investigate at molecular level why *Pkr* knockout is able to rescue the *Adar1*, *Mavs* lethality. Preliminary data show that Pkr activation is prevented in the enzymatically inactive Adar1 embryos suggesting that Adar1 and Pkr could compete for the same dsRNA substrate and that the presence of the inactive Adar1 is sufficient to avoid Pkr pathway activation.

# Tail Proteins of *Podoviridae* Phage SU10 Reorganize into the Nozzle for Genome Delivery

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*Escherichia coli* phage SU10 belongs to the genus *Kuravirus* from the family Podoviridae of phages with short tails. However, in contrast to other podophages, the tails of Kuraviruses were shown to elongate upon cell attachment. We used cryo-electron microscopy of SU10 particles before and after genome ejection and cryo-electron tomography of infected *E. coli* cells to describe the structural changes of the phage tail that are required for its genome ejection and delivery. The virion of SU10 has a prolate head, containing genome and ejection proteins, and a tail, which is formed of portal, adaptor, nozzle, and tail needle proteins and decorated with long and short fibers. The binding of the long tail fibers to the receptors in the outer bacterial membrane is followed by the straightening of nozzle proteins and rotation of short tail fibers. In the new arrangement, the nozzle proteins and short tail fibers alternate to form a nozzle that extends the tail by 28 nm. To open the tail channel, the tail needle detaches from the nozzle proteins. Five types of ejection proteins , one of which has the predicted peptidoglycan-degradation activity, are released from the SU10 head before or together with the genome. The nozzle with the putative extension formed by the ejection proteins enables the delivery of the SU10 genome into the bacterial cytoplasm. It is likely that this mechanism of genome delivery, involving the formation of the tail nozzle, is employed by all Kuraviruses.

## Automated Image Analysis of Grain Spike in Greenhouse and Field

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In greenhouse and field environment, an automated detection and segmentation of grain spikes in cereal crops is a highly demanded task for extracting phenotypes, such as yield biomass, time stamping of plant life cycle from images and other associated agronomics traits. The predictive power of trained deep neural networks changes for a spike depends on its spatial location in plant canopy. Our results show that the trained model on wheat show comparable performance (>80%) on unseen barley and rye images from the photo chamber with a similar optical setup. The image augmentation improves the average precision of detection DNNS by 3%. In the field environment having low illumination condition, a cascaded of ensemble DNNs with prior Lenet classifier on light and dark frame outperforms single trained DNNs. Our implementation is also available as a GUI-based tool SpikeApp which shows the application of detection and segmentation that extract 70 other spike phenotyping of greenhouse grown wheat plants.

## A Hunting Strategy and Virion structure of *P. Aeruginosa* Bacteriophage JBD30 Revealed by Cryo-electron Microscopy

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Pseudomonas aeruginosa is a human pathogen, whose treatment is complicated by its frequent antibiotic-resistance. *Siphoviridae* bacteriophage JBD30 infects and kills bacterium *P. aeruginosa*, which makes it a potential agent for phage therapy. Here we present the structure of bacteriophage JBD30 virion and its replication strategy, revealed by the combination of cryo-electron microscopy analysis techniques and cryo-electron tomography.

The virion of bacteriophage JBD30 is composed of non-enveloped icosahedral capsid, long flexible non-contractile tail and baseplate decorated with tail fibers. The capsid with a diameter of 60 nm is built from major capsid protein organised in T = 7 icosahedral lattice and decorated on three-fold and pseudo-threefold axis with trimers of minor capsid protein. In one vertex of the capsid, the penton of major capsid protein is replaced by dodecameric portal. The portal complex forms an interface between the capsid and 180 nm long tail. The tail is built from 44 hexameric discs of major tail protein. Distal tail protein trimer follows-up the last tail disc and forms an attachment site for the long tail fibers. The baseplate is terminated with a tripod complex of receptor binding protein trimers.

Using cryo-electron tomography we followed the infection process of *P. aeruginosa* by JBD30 phage from attachment to bacterial cell, to the production of new phage progeny and host cell lysis. Bacteriophage JBD30 uses its long tail fibres for binding to P. aeruginosa pili type IV. After attachment to pili, the virion either diffuses or is pulled towards the cellular surface, where it irreversibly binds by its receptor binding proteins. Afterwards, the phage punctures the outer cellular membrane, degrades the peptidoglycan layer and injects its DNA into host cell. New phage progeny is released approximately after 80 minutes post infection.

The combination of cryo-electron microscopy methods allowed us, to propose the mechanism of key stages of phage infection and describe it at molecular level.

*Core Facility Cryo-electron Microscopy and Tomography of CEITEC Masaryk University is gratefully acknowledged for the obtaining of the scientific data presented in this paper.*

# Retroelement Activity in Hematological Malignances

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Retroelements (RE) represent the vast majority of transposons in the human genome. Ongoing retrotransposition of RE copies into new genomic loci through RNA-mediated mechanisms in humans is attributed to long interspersed element-1 (LINE-1; L1) REs. A subgroup of the short interspersed elements called Alu is nonautonomous and relies upon L1-encoded proteins (ORF1 and ORF2) for their mobilization. Retroelement expression in normal human cells is suppressed by a variety of mechanisms, because of the potential harmful impact of these elements on genome stability. It is known, that REs are associated with carcinogenesis. Different cancer types are characterized by a different level of tumor-specific RE insertions.

We aimed to explore RE activity in chronic lymphocytic and acute lymphoblastic leukemias, and myelodysplastic syndrome. To identify tumor-specific RE insertions, we adopted an NGS protocol of amplicons containing a part of RE from Alu-Ya5, Alu-Yb8, or L1-HS families (the most active in humans), and its adjacent genomic region. First, the method sensitivity was evaluated revealing the 1% detection threshold for the proportion of cells with specific RE insertion. Following this result, we did not identify new tumor-specific RE insertions in the tested cohort of 14 adult ALL paired samples at the set level of sensitivity. Same result was also obtained using bioinformatics analysis (TraFiC-mem pipeline, MELT tool) to identify tumor-specific RE insertions in WGS data from 6 CLL patients. Our results indicate that RE transposition is not activated in adult leukemia. These results are in good agreement with previously published studies. One of possible explanations is that the studied leukemia types have a low tolerance to RE insertional activity in contrast to other cancer types.

*Supported by GACR 19-11299S and MH-CZ RVO 65269705.*

## Deciphering the Global Proliferative Arrest: an Elusive Link between Plant Reproduction and Longevity

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Plant growth is driven by cell proliferation in small clusters of stem cells called meristems. Proliferation of meristems defines both the final shape of body and longevity of plant; when meristems proliferation ceases, plant stops its growth and eventually dies. In many plant species, including annual crops such as rapeseed, rice, maize, as well as in model Arabidopsis thaliana, shoot apical meristem (SAM) activity and, hence, plant longevity are coupled with reproduction. Once plant produces a predetermined number of seeds, it inhibits activity of all apical meristems and stops forming new flowers. This phenomenon is termed global proliferative arrest (GPA) and indicates that there must be a systemic signaling mechanism that measures number of produced seeds and communicates it to meristems. The global proliferative arrest has important implications for crop yield, but little is known about its molecular underpinning.

We aim at providing cellular and molecular framework of global proliferative arrest by combining state-of-art imaging of shoot apex with classical forward genetic screen to identify genes involved in this process. We use unique technology developed in Riha lab that enables 3D reconstruction of meristems from light sheet microscopy data. We study how GPA affects proliferation of meristematic cells, hormonal signaling and size of stem cell niches using dedicated cellular markers.

In parallel, we have conducted a forward genetic screen to search for mutants that yield more fruits in comparison to wild type with no effect on fertility, which is indicative of delayed GPA. One promising line was mapped by genome sequencing, yielding a high confidence candidate gene from a gene family previously implicated in SAM maintenance and flower differentiation.

*This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic, European Regional Development Fund-Project „REMAP“(No. CZ.02.1.01/0.0/0.0/15_003/0000479).*

*This work was supported from Operational Program Research, Development and Education – "Project Internal Grant Agency of Masaryk University" (No.CZ.02.2.69 / 0.0 / 0.0 / 19_073 / 0016943).*

*We further thank to Plant Sciences Core Facility of CEITEC Masaryk University for support with plant cultivation.*

# $t^6A$ Modification of the A37 Position in tRNA and Its Role in Plant Development

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RNA modifications participate in many essential biological processes and represent a rapidly developing research field. Our project focuses on the role of a deeply conserved tRNA modification, threonylcarbamoyladenosine ($t^6A$). $t^6A$ is present at the A37 position of nearly all tRNA molecules decoding the ANN codons and is linked with the regulation of translation. However, despite a high effort, a deeper understanding of its function, particularly in multicellular organisms, is incomplete. Here, we characterize Arabidopsis thaliana enzymes involved in the $t^6A$ biosynthesis. We isolate mutants with the abolished $t^6A$ formation and reveal that the $t^6A$ biosynthesis genes are essential for the earliest steps of plant morphogenesis, including gametophyte development. We also examine the subcellular localization of the $t^6A$ biosynthesis proteins and demonstrate that different stages of $t^6A$ biosynthesis occur in separate compartments of plant cells, such as the nucleus, plastids, and mitochondria.

*Supported by the Ministry of Education, Youth and Sports of the Czech Republic (LTC18073) and Czech Science Foundation (19-23773S).*

# Atomistic Picture of Opening-Closing Dynamics of DNA Holliday Junction Obtained by Molecular Simulations

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Holliday Junction (HJ), or four-way DNA junction, is a non-canonical but common DNA motif prominent in gene repair and recombination. It is consisted of four DNA duplexes connected by two crossing strands. The four arms of HJ fluctuate between open and closed states in solution with the two states significantly differing in terms of interhelical orientations and playing a role in recognition by enzymes. Understanding of the open/closed transitions is therefore an important step towards understanding the sophisticated biological processes involving HJs. Although many experimental studies have unveiled some structural and dynamic aspects of HJ, the atomistic level details of HJ behaviours are still fully understood.

The molecular dynamics (MD) simulations appear to be ideal method to observe the open/closed transitions of HJs, however, we show in this study that this goal is not achievable with the standard molecular mechanistic force field which grossly overstabilizes the closed conformation of HJ to a point where the spontaneous opening of HJ cannot be observed in MD simulations on practical timescales. To resolve this problem, we propose applying a targeted modification of the Lennard-Jones interactions of the branch point nucleotides of the HJ, along with earlier proposed modifications of the ion-phosphate nonbonded interaction, allowing spontaneous opening/closing transitions in standard MD simulations. With these methods, we for the first time report spontaneous (unbiased) closing and opening of the HJ in standard MD simulations and provide structural basis for this transitions, including a previously unknown “half-closed” transition state of HJ.

*This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 765266.*

# NOTES

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