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Book of Abstracts

CEITEC PhD Retreat

MUNI

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Online Webinar

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WELCOME ADDRESS

Welcome Word

Dear Students,

this was not an easy year. The COVID pandemy substantially affected our everyday activities and we faced many limitations in our personal as well as professional life. While, in contrast to many institutions abroad, we managed to keep laboratories open to minimize disruptions in our experiments, the impact on our work has been substantial. What we all probably miss most are social contacts, being that with collaborators or competitors at international congresses, face to face discussions with colleagues at labmeetings, or small chats by coffee machine. Communication is the essence of science. Therefore, I am very thankful for the opportunity to learn about your research at the annual PhD conference. I am sure that despite all the hurdles and limitations, you kept pushing frontiers of knowledge and we will hear a lot of exciting stories.

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Baseplate Structure of Bacteriophage Phi812 Reveals Functions of Host Cell Recognition and Cell Wall

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Antibiotic-resistant strains of *Staphylococcus aureus* cause human infections that are difficult to treat and can lead to death. Bacteriophage (phage) phi812K1/420 from the family Myoviridae has been found to infect 95% of tested *S. aureus* strains and therefore is considered as a potential phage therapy agent. As the native phage particle approaches its host cell, primary phage receptors make a contact with the host cell wall. This interaction triggers cascade of structural changes in the baseplate, resulting in phage tail contraction and genome delivery. Mechanistic description of the baseplate re-organization, however, remains unknown.

Using cryo-electron microscopy (cryo-EM), we reconstructed the phage baseplate before and after the attachment to host cell. Moreover, we performed cryo-EM single-particle analysis of recombinant tail spike protein (TSP). Dislocation of the TSP from the center of native phage baseplate suggests that it may play a role in triggering the whole contraction mechanism.

The structures of the baseplate were reconstructed in resolution of 3.5-5 Å and we are in process of building individual protein structures. We have already solved the structure of the C-terminal domain of TSP with putative glycerol-diesterase activity, which sticks out from the phage baseplate. Such orientation suggests that the TSP can readily degrade host call wall upon phage attachment, making way for tail tube insertion. We assume that as the TSP C-terminal domain is drawn towards the cell wall, the N-terminal domain detaches from phage baseplate, causing instability and consequent baseplate re-organization.

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Structure Analysis of a Huge Algal Virus

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The *Emiliania huxleyi* virus 86 (EhV-86) infects the most abundant algae in marine ecosystems – the *Emiliania huxleyi*, which creates regular population explosions called blooms. The algal bloom collapses after infection by coccolithoviruses, in most cases by EhV 86. Among the other large double-stranded DNA viruses that infect green algae, EhV 86 differs in complex virions structure possessing inner membrane, capsid, outer membrane, and protein outer shell. The pleomorphism of viral particles and the layered capsid make EhV 86 virus challenging for 3-dimensional reconstruction.

To determine the high-resolution structure of the EhV 86 virion, we use cryo-electron microscopy (cryo-EM) imaging followed by a combination of so-called single particle analysis i.e. averaging over many particles, and tomography, i.e. few particles imaged at many angles. We also investigate the assembly process of viral capsids inside the alga cell in lamellas – thin cell sections produced by focused ion beam milling. The combination of our results obtained from various cryo-EM approaches will provide a comprehensive insight into the EhV 86 virion structure, life cycle, and interactions with the *Emiliania huxleyi* host cell.

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.

Multi-faces of Dishevelled Protein

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Dishevelled (DVL) is a scaffold protein known to play a central role in different branches of Wnt signaling pathway. Phosphorylation of DVL by Casein kinase 1ε/δ (CK1ε/δ) is crucial for activation of Wnt signal transduction. DVL has three isoforms in humans (DVL1, DVL2 and DVL3) that are functionally redundant. Each of the isoform has three modular domains, namely N-terminal DIX domain, central PDZ domain and C-terminal DEP domain, connected by unstructured linkers. Our biochemical and structural analysis coupled with cellular data shows that DVL function is governed by interactions between modular domains and unstructured regions of its own, CK1ε-mediated phosphorylation, or both. CK1ε phosphorylates DVL at multiple sites and at different levels affecting the DVL conformation. DVL conformational flexibility (multi-faces) is essential for the interactions with a wide range of substrates and these interactions can be modulated by a specific phospho-code.

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 (the heptads within the first half have highly conserved sequence while the heptads in the second half diverge in sequence). Several CTD's residues can be subjects of post-translational modifications. Tyrosine, threonine, and serine residues undergo dynamic de-/phosphorylation resulting in specific phosphorylation patterns throughout different stages of transcription cycle. These characteristic phosphorylation patterns are specifically 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 want to 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. pSer2 and pThr4 phosphomarks occur mainly during the late elongation and termination. RPRD2's preference for these two phosphomarks suggests possible involvement of RPRD2 in transcription termination. To provide mechanistic details of the interaction between the RPRD2 CID and pSer2,7/pThr4 CTD, solution NMR spectroscopy was used to collect structural data for these two complexes.

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Deletion of Trf4 Results in Accumulation of mRNA 3'End Extended Form in *Saccharomyces Cerevisiae*

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The yeast RNA polymerase II (Pol II) transcribes protein-coding genes and a wide spectrum of non-coding RNAs (ncRNA), such as small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs) and Cryptic Unstable Transcripts (CUTs). Eukaryotic cells contain several complexes that take part in RNA transcription termination and 3'end processing. In *Saccharomyces cerevisiae*, mRNAs are terminated and processed by the cleavage and polyadenylation complexes and the cleaved product is further degraded by Rat1, a 5′ to 3′ exonuclease from the Rat1-Rai1-Rtt103 complex (RRR complex). This is in contrast with ncRNAs, that are terminated and processed by the Nab3-Nrd1-Sen1 (NNS) complex that further draws the polyadenylation Trf4- Air1/2-Mtr4 (TRAMP) complex and the exosome to the ncRNAs leading to trimming or fully degradation of the nascent RNAs.

A recent study in our laboratory revealed that depletion of the noncanonical poly(A) polymerase Trf4p leads to accumulation of 3'end extended forms of several mRNAs (RT) as a result of Pol II termination defects or altered mRNA decay. To further investigate the phenomenon, we study the mechanism leading to the RT accumulation in the strain lacking Trf5p, homolog of Trf4p, and in different strain phenotype. We use small and high-throughput approaches such as ChIP-seq, ChIP-RT-qPCR and RNAseq to uncover the role of the TRAMP complex in gene expression regulation or transcriptional surveillance.

This work was supported from the Czech Science Foundation (17-20388S) and the Operational Programme Research, Development and Education – „Project Internal Grant Agency of Masaryk University" (No. CZ.02.2.69/0.0/0.0/19_073/0016943).

LYNX: an Interactive Bioinformatic Tool For Next-generation Sequencing Data Analysis in Lymphoid Malignancies

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Next-generation sequencing (NGS) is a modern expanding technology used in both biomedical research and clinical practice. A vast amount of data generated by NGS is nearly impossible to evaluate only with a naked human eye, therefore rather complex bioinformatic computational methods are required to proceed towards actual results. To perform an advanced bioinformatic analysis, a set of interconnected tools is required. This set of tools creates a so-called pipeline, which operates as one complex tool with multiple parameters. Most bioinformatic pipelines are operated via simple command lines and knowledge of computer science is expected. This fact often puts an obstacle between a molecular geneticist and data analysis. Thus, design of the pipeline should be only a part of modern bioinformatic software development. The other part is focused on user interface (UI) and user experience-driven design.

We aimed to develop LYNX (*Lymphoid NGS*) *analytical software* – a tailor-made bioinformatic tool for a new custom-designed capture-based NGS panel. With this tool, a user is able to analyze molecular markers (gene mutations, copy number variations, antigen receptor rearrangements and translocations) in the most common lymphoid malignancies. LYNX is developed with an idea of simplicity and modularity. In a web-based UI, a user is able to simply upload and select data, choose independent steps of the required analysis, start the analysis, and afterwards visualize results via interactive tables and charts. Thanks to the modular approach, LYNX is a future-proof platform opened for injection of new computational methods based on needs of a user. An important part of this project is cooperation of our team with a private company HPST, s.r.o., which is supported by a TACR ZETA funding scheme. This cooperation helps us to experience a private sector development approach and also to deliver our project to a wider professional community.

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

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In contrast to animals, plant meiosis is followed by several mitotic divisions to make functional gametes. The transition from meiosis to post meiotic development is poorly understood. We discovered that in *Arabidopsis* this transition governed by unknown mechanism that involves *SMG7* and *TDM1* genes. Mutations of these genes leads to third meiotic division and failure 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* has already been reported as 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 mechanism of CDM1 function in meiosis.

Genetic association studies and complementation experiments confirmed that mutations in *CDM1* restore fertility and microspore formation of *smg7. CDM1* encodes 308 amino acids protein containing two Tandem Zinc Finger (TZF) motifs separated by a linker at the C-terminus. *CDM1* construct lacking TZFs could not complemented the CDM1-null mutation demonstrating that TZFs are essential for molecular functions of CDM1. Reporter line expressing CDM1-GFP construct showed that CDM1 forms distinct cytoplasmic foci that are specifically present 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 indicate 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 in meiosis. Presence of TZF motifs suggests direct binding of CDM1 to RNA, and our further research is aimed at identification of transcripts that associate with CDM1 in meiosis.

I am also interested in addressing the question how evolutionarily conserved the mechanism governing meiotic exit. To this end, we identified homologs of *AtSMG7* and *AtTDM1* in monocots rice and barley and set up collaborations to generate null mutants in these crops using CRISPR/Cas9 tools. These mutants will be assessed for fertility and meiotic progression.

Topology of ER PINs: PIN5 versus PIN8 "the Heads and Tails" of Auxin Flux?

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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 have determined the plasma membrane topology of PIN5 and PIN8, in terms of the subcellular orientation of the central hydrophilic loop and the two terminals. We have used GFP tagged constructs which are ectopically localizing on the PM of epidermis and cortex of *Arabidopsis thaliana* root cells. The pH dependent GFP quenching, immuno-localization and protease protection assay techniques indicate that PIN5 and PIN8 exhibit opposite topology. We have also showed that these two proteins elicit distinct root phenotypes, which is likely caused by their antagonistic auxin transport activity.

Key words: Topology, Endoplasmic Reticulum PINs, *Arabidopsis thaliana*, Auxin.

Interphase Chromosome Organization in Crucifer Species

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During interphase, chromosomes assume a largely decondensed state. Each chromosome occupies a limited, exclusive nuclear subdomain, known as chromosome territory (CTs). In many plant species with relatively large genomes, chromosomes during interphase adopt Rabl configuration, with telomeres and centromeres located at opposite poles of the nucleus. In the small Arabidopsis genome, the non-Rabl interphase organization has been described as the chromocenter (CC)-loop model or rosette-like organization. This pattern is characterized by heterochromatic CCs located at the nuclear periphery with emanating euchromatic loops comprising the ten chromosome arms, and telomeres usually clustered around the nucleolus. Nevertheless, the small Arabidopsis genome is not necessarily representative of more than 3,600 crucifer species displaying a 50-fold genome size variation. We analyzed centromere/telomere position in somatic (floral, leaf, stem and root tip) and pre-meiotic nuclei of seven diploid Brassicaceae species with constrasting genome sizes and phylogenetic position. Our FISH data suggest that in somatic and pre-meiotic nuclei of species with small genome size, telomeres cluster around the nucleolus, whereas centromeres are dispersed within the areas of nuclear. In species with large genome size, telomeres and centromeres are mostly positioned within the nuclear interior and the nuclear periphery, respectively. In *Arabis cypria* with intermediate genome size and *Bunias orientalis* with large genome size, both patterns of telomere positioning were observed. We propose that contrasting patterns of telomere clustering in Brassicaceae are associated with genome size variation, but not with phylogenetic position.

Elucidating the Role of ADAR1 in the Innate Immunity Response

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The adenosine deaminase acting on RNA (ADAR) enzymes catalyse the hydrolytic deamination of adenosine (A) into inosine (I) in RNAs with double-stranded character, leading to the destabilization of RNA duplex structures and genetic recoding. Editing of cellular dsRNA by ADAR1 prevents aberrant activation of cytoplasmic antiviral dsRNA sensors which are induced by interferon (IFN).

Mutations in the *ADAR* gene encoding ADAR1, cause Aicardi-Goutières syndrome (AGS), an autoinflammatory disorder associated with spontaneous IFN production and neurological disorder. The importance of ADAR1 proteins is highlighted by the embryonic lethality in mice following genetic disruption of *Adar1* expression. Generation of mutant mice lacking both ADAR1p150 and ADAR1p110 isoforms or only p150 expression, display embryonic lethality around E12.51,2 with a type I IFN signature similar to that observed in the AGS patients. They display fatal liver disintegration, failed haematopoiesis, widespread apoptosis, overexpression of IFN and IFN-stimulated genes (ISGs) 3,4.

Our group generated double mutant mice that lack the mitochondrial antiviral signalling adaptor (MAVS), required for IFN induction signalling thus blocking the cellular response to cytoplasmic dsRNA. These *Adar,Mavs* double mutant mice die by P20, showing that the embryonic lethality is due to an aberrant innate immune response of RIG-I like receptors (RLRs) to unedited dsRNA.

ADAR2 is most highly expressed in brain and it is primarily required for site-specific editing of glutamate receptor transcripts. Mutations in ADAR2 contribute to excitability syndromes such as epilepsy. Mice with a homozygous *ADAR2* null mutation die of seizures shortly after birth because of transcripts encoding *Gria2* RNA are unedited at the Q/R site and there is a consequent excessive Ca2+ influx into neurons 5.

The principal aim of my PhD project is to investigate at molecular level the reason why Mavs mutant does not fully rescue the *Adar1* deletion. During my project I tested if the deletion of specific genes; (*Tp53, Eif2ak2, Casp4*), involved in the apoptotic pathway or in the innate immune signalling pathway could rescue the mutant lethality.

I demonstrated that the intestine of mice *Adar1,Mavs* have massive apoptosis localized at Lieberkühn's crypts specifically along the small intestine, spleen morphology is destroyed and the mice have a mild inflammation in the brain. Moreover, embryos at 12 days of development used for mouse embryonic fibroblasts derivation, show a high expression of ISGs which decrease during the culturing time.

The second aim of my project is to investigate, by using LC-MS/MS, if the lack of inosine in the mutant murine models is affecting the equilibrium of the others main mRNA modifications. mRNA analyses by LC-MS/MS revealed that mice *Adar2* null show an evident increase in N6-Methyladenosine (m6A) level and mice with no editing activity (*Adar1* and *Adar2* knockout) show a slightly increase. Moreover, we confirmed that *Adar2* is the main actor in brain RNA editing by measuring I level in our *Adar* mutant mice.

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Patient-derived Xenograft Model of Chronic Lymphocytic Leukaemia Based on Mimicking Its Microenvironment

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Chronic lymphocytic leukemia (CLL) is an incurable B-cell malignancy with characteristic dependence on human immune microenvironment. Interactions in the microenvironment are provided by various types of cells and factors, but of them only CD4-positive T-cells can promote proliferation of leukemic cells. This makes T-cells also indispensable for current CLL-derived xenograft (PDX) mouse models. However, limited availability of autologous T-cells and their rapid spontaneous growth in immunodeficient mice, led us to an idea of replacing them in in vitro and in vivo models with a novel, genetically engineered cell line.

We prepared an adherent supportive cell line, expressing T-cell factors, which induces long term survival and major proliferation of primary CLL cells in vitro. Using this cell line, we developed a coculture system for testing drugs with antiproliferative effect and identified a novel inhibitor with such activity in CLL.

We introduced the cell line into immunodeficient mice (NSG) to mimic human lymphoid microenvironment. We seeded collagen scaffolds, implanted them subcutaneously and injected mice with 30–350×106 purified CLL cells (containing undetectable number of T-cells). By this, we achieved engraftment of B-cells, but these were positive for Epstein-Barr virus (EBV). As CLL malignancy in vivo is not based on EBV activation and CLL cells are virtually free of EBV, subsequently we attempted to implant mice with small number of CLL cells containing statistically no healthy B-cells (healthy B cells from CLL patients can carry EBV). The first strategy to achieve that is to pre-expand small number of CLL cells (103 – 104) cocultured on supportive cells in vitro. The second strategy is based on direct implantation of mice with supportive cells and 105 CLL cells into spleen. Currently we are waiting for the first results of both strategies, as none of them was ever applied in CLL research so far. The established model will be used for testing of novel therapeutic combinations and studies of CLL biology.

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Transposable Elements Activity in Hematological Malignances

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Transposable elements, or transposons, are sequence units present in eukaryotic genomes in a broad diversity of their structures. They have played an important role in evolution of many genomes. In the human genome, the vast majority of transposons is represented by retroelements (REs) that can generate new copies and integrate them in genomic DNA through RNA-mediated mechanisms. The long interspersed nucleic elements (LINE-1 or L1) utilize a "copy-and-paste" mechanism to retrotranspose copies into new genomic loci. Active L1 retrotransposons also drive retrotransposition of other mobile DNAs, such as short interspersed elements (SINEs), a group including Alu elements, and SVA transposons. RE activity is mostly silenced by various control mechanisms, however, genomic instability, especially in cancer cells, can be associated with RE reactivation. To our best knowledge, no systematic analysis has been performed to date to study RE activity in hematological malignancies.

We aim to explore RE activity in different types of hematological malignancies. To identify tumor-specific RE insertions, we adopted a protocol based on high-throughput sequencing 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. In total, 118 samples (73 tumor and 45 normal DNA) from 49 patients and 4 samples from 2 control cell lines were analyzed. We found 26 candidate insertions in 13 tumor samples. These insertions will be validated using PCR and Sanger sequencing.

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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 these meristems defines both the final shape of body and longevity of plant; when meristems proliferation ceases, plant stops its indeterminate 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 activity (SAM) and, hence, plant longevity are coupled with reproduction. Once plant produces a predeterminate number of seeds, it inhibits activity of all apical meristems and stops forming new flowers. This phenomenon is called 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, auxin 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.

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Study of Conformational and Dynamic Changes upon Phosphorylation of Proline Rich Region of $Tau_{210-240}$ Peptide Using Molecular Dynamic Simulation

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The conformational and dynamic changes of protein interaction regulated by post-translational modifications such as phosphorylation of intrinsically disordered proteins (IDPs), remains challenging to elucidate. Tau, which is a well-known IDP and its phosphorylation is of particular interest because Tau is found hyper-phosphorylated in Alzheimer's disease (AD). Therefore, it's pertinent to understand the conformational and dynamic changes upon phosphorylation of Tau. The proline-rich motif recognized within Tau210-240 peptide directly interact with AD progression protein such as 14-3-3.

Microsecond time scale molecular dynamic simulation studies performed for apo and phosphorylated residues (212^{P}Thr, 217^{P}Thr, 231^{P}Thr, 235^{P}Ser) Tau peptide210-240 using three different temperature variants (278° K, 298° K and 310° K) and two different force field parameters (AMBER99SB-ILDN and CHARMM36m) with TIP4PD water model. These four-phosphorylation causing increase in compactness. The strong salt bridges are forming with nearby lysine and arginine due to the phosphorylation, which may alter the binding of associated protein like 14-3-3 with Tau. 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, 3J-coupling etc. The experimental part has been carried out by our collaborator Prof. Isabelle Landrieu.

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Ethylene Regulates MSP Pathway signaling via ETR1 and EIN3 in the Root

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Ethylene is a gaseous molecule shown to function as a hormone that is synthesized by plants to control plant growth, development, and stress responses via a well-studied signaling pathway. We have shown that apart from its own canonical signaling (ETR1-CTR1-EIN2-EIN3), ethylene contributes into the multistep phosphorelay (MSP) pathway where ethylene via histidine kinase activity of ETR1, directly or in cooperation with another histidine kinase (AHK5), controls the transphosphorylation cascade mediated by AHPs and their nuclear targets ARRs. In line with that, we demonstrated that ethylene activates the MSP reporter *pTCSn:GFP* in columella and lateral root cap in AHK5-dependent manner. We found out that the constitutive ethylene response of *ctr1-1* mutant, defective in the Ser/Thr kinase acting downstream of ETR1 and inactivating the ethylene signaling in the absence of ethylene, associates with upregulation the *pTCSn::GFP* in the root transition zone, columella and lateral root cap. Furthermore, the *pTCSn::GFP/ctr1-1* line showed stronger response to the 1-aminocyclopropane-1-carboxylate (ACC), an ethylene rate-limiting precursor, thus further substantiating the ETR1-mediated MSP regulation independent on the ethylene canonical signaling. Interestingly, our data also show that ethylene activation of *pTCSn::GFP* in the root transition zone is dependent not only on the ethylene sensor ETR1, but also its downstream target, the ethylene-stabilized transcription factor EIN3. EIN3 was previously implied to be a negative regulator of ARRs-A, the cytokinin primary response genes. In contrary to that, using both RT qPCR and promoter transcriptional fusions, we observed positive role of EIN3 in the ethylene-mediated upregulation of *ARR3* and *ARR5*. In conclusion, our work reveals novel aspects of how plants perceive ethylene, particularly highlighting the unexpected complexity of the crosstalk between the MSP and canonical ethylene signaling pathways.

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Application of Separation Techniques in Diagnostics of Gastroesophageal Reflux Disease

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Gastroesophageal reflux (GER) is a physiological process characterized as a spontaneous movement of gastric contents from the stomach into the esophagus. Similarly, bile reflux is a retrograde movement of duodenal contents from the duodenum of the small intestine. Prolonged and more frequent episodes of GER and bile reflux can cause a variety of troublesome symptoms such as heartburn and regurgitation that were defined as Gastroesophageal Reflux Disease (GERD). GERD is primarily diagnosed by 24-hour multichannel intraluminal impedance and pH monitoring that is invasive, unpleasant, and time-consuming. In contrast, saliva collection is simple, non-invasive, inexpensive, and repeatable. Saliva protects the esophagus against chemical damage, caused for instance by hydrochloric acid or bile acids during the reflux episodes. Bile acids are a group of steroid compounds essential for lipid digestion and absorption. Bile acids compose bile and are secreted postprandially into the duodenum. The detection of bile acids is not performed routinely due to the lack of sensitive diagnostic methods.

The first part of this work investigates the hypothesis that bicarbonate and phosphate ions, being the major salivary buffer components, might be elevated in saliva from patients suffering from GERD, because they compensate for the acidic damage caused by recurrent reflux episodes. The second part of this work focuses on method development for bile acid analysis in biological samples. Since bile acids are present in saliva in trace concentrations, they might serve as a possible biomarker of GERD.

Seven inorganic ions (including bicarbonate and phosphate) and four organic acids were determined in saliva by capillary electrophoresis with capacitively coupled contactless conductivity detector (CE-C4D). The background electrolyte was composed of 20 mM Arginine, 10 mM 2-(N-morpholino) ethanesulfonic acid (Mes) and 30 μM cetyltrimethylammonium bromide (CTAB). Metabolite levels were compared in saliva from 13 patients suffering from GERD and saliva from 12 healthy subjects. Bicarbonate levels were significantly different between the two groups that suggest possible applicability of bicarbonate in saliva as a GERD diagnostic biomarker. Secondly, the method was developer for bile acid analysis by ultra-performance liquid chromatography coupled to mass

spectrometry (UPLC-MS/MS). The 11 bile acids (glycine conjugated and unconjugated) were separated in 10 minutes using a C18 column in the gradient elution of methanol and 0.1% formic acid. Ions [M-H]- and [M+HCOO]- were identified as the most abundant in the full scan MS. Bile acids were detected and quantified in the multiple reaction monitoring mode. The method for sample purification by solid-phase extraction will be developed and subsequently bile acids in saliva and exhaled breath condensate of healthy volunteers and GERD patients will be analyzed by the developed UPLC-MS/MS method.

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Study of the Involvement of COP1-YODA Pathway in High-temperature Response During Embryogenesis of 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 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 hormones and molecular chaperones, such as Heat shock proteins (HSPs), in this temperature response in vegetative tissues. In my Ph.D. project, our ultimate goal is to uncover the molecular mechanisms controlling the temperature response during *Arabidopsis embryogenesis*. Based on reported data, some candidate signaling pathways could play a role during this stress response. We have started our study by analyzing of PHYB-PIF4 pathway, which is known for controlling high-temperature stress in the seedling. However, we could not assertively confirm this pathway's involvement in the embryonic response to warm temperatures.

We further focused our analysis on the MAPK (mitogen-activated protein kinase) kinase kinase gene YDA (YODA). Loss-of-function mutations in YDA affect zygote elongation and apical-basal polarity of the embryo. We have observed similar phenotypes in the wild type, exposed to the 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. Phenotyping analysis of some mutant lines for the genes in this pathway is in progress. And I am generating some reporter lines to analyze their expression to test our hypothesis about this signaling pathway. If we get positive results from the first part of the assessment, we will perform further experiments like hormonal profiling and RNA expression analysis to confirm the results.

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The Role of Long Non-coding RNAs in the BCR-mediated Activation of Malignant B Cells

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The B cell receptor (BCR) is responsible for triggering the canonical signalling to mediate B cell activation and its transduction is tuned by a network of kinases and phosphatases capable to dictate B cell fate. Remarkably, B cell malignancies frequently exhibit a gene expression profile of constitutive BCR activation. The essential role of BCR signalling in B cell malignancies is highlighted by the major success of single-agent BCR inhibitor treatment in chronic lymphocytic leukaemia (CLL) patients.

Dysregulation of BCR signalling in CLL cells is the major drive force for proliferation and tumour maintenance, partially mediated by non-coding RNAs. MicroRNAs (miRNAs), such as miR-155, were described to be directly involved in the regulation of the BCR pathway targeting kinases and phosphatases, or even the downstream effectors of the BCR pathway. Although microRNAs play an essential role in regulating BCR pathway, it is unclear if long non-coding RNAs (lncRNAs) are involved in BCR signalling. LncRNAs can serve as a sponge for miRNAs, as a scaffold for proteins, as regulators for transcription factors, or even participate in signal transduction. LncRNAs were shown to be involved in several lymphocyte pathways and based on this rationale, we questioned whether any lncRNAs could be involved in the BCR pathway.

To address this question, we investigated differentially expressed lncRNAs from CLL patients treated with BCR inhibitors (Ibrutinib and Idelalisib) and we cross-validated these lncRNAs between high BCR activity ($CXCR4^{dim}CD5^{bright}$) vs low BCR activity ($CXCR4^{bright}CD5^{dim}$) in intraclonal CLL cell subpopulations. We found 12 lncRNAs (named lncRNA-BCR1 to lncRNA-BCR12) related to the BCR pathway, including one, which is the host gene for miR-155, and we selected lncRNA-BCR1 for further studies. We hypothesized that lncRNA-BCR1 is regulated in a BCR-dependent fashion.

We confirmed that lncRNA-BCR1 is upregulated upon BCR activation and impaired when treated with BCR inhibitors. In line with this, lncRNA-BCR1 was also downregulated in CLL patients undergoing Ibrutinib therapy. However, a contra-intuitive observation was made when we analyzed the expression of this lncRNA in a CLL cohort. CLL patients with high expression of lncRNA-BCR1 have longer survival compared to those with relatively low levels. In order to understand these observations in more detail, we transcriptionally repressed lncRNA-BCR1 using a dCAS9-KRAB system in CLL cell lines. These engineered cell lines will be used for further functional assays to explain the molecular mechanism underlying the BCR activation and its effect on the survival of CLL patients.

In summary, we identified lncRNAs potentially participating in the BCR-mediated activation of malignant B cells. Our data suggest that lncRNA-BCR1 is involved in the BCR pathway and elucidating this interplay could improve the understanding of the pathogenesis of B cell malignancies.

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Chemical Inhibition of Cdk11 Leads to Defect in Splicing

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Cdk11 belongs to the group of cyclin-dependent kinases responsible for control of transcription of mRNA. Its additional roles are control of alternative splicing, 3'end processing of HIV mRNA and expression of replication dependent histones. Despite several decades of research, only siRNA knock-down experiments were available for probing of Cdk11's role in the cell. Finally, in late 2019, long-sought inhibitor of Cdk11 was characterized and published. Using said inhibitor, we show by RNAseq that general splicing in HCT116 cells is disrupted after 4 hours of treatment. We utilized BioID method to probe for relevant proteins connected to Cdk11. Multiple proteins involved in splicing were identified and one of them was verified as Cdk11 interacting partner using immunoprecipitation experiments.

Assembly of Human Guardians of Telomeres: TRF1 Exchanges TRF2 Bound to TIN2 but Tolerates TRF2 in TPP1

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Shelterin - protein complex formed by six telomere-specific proteins, is essential for protection of chromosome ends against unwanted DNA damage response machinery. Shelterin plays a major role in shaping the architecture of telomeric DNA. The formation of shelterin core complex and the dynamics of core subunits are critical for the assembly of completely functional complex.

We quantitatively described the order and binding preference of telomere repeat binding factors TRF1 and TRF2 in complex with TIN2 at physiological conditions at single-molecule level. We used fluorescence cross-correlation spectroscopy technique to monitor the coincident fluctuations of differently labeled shelterin proteins as they move through the confocal volume of a microscope.

We observed that TRF1 effectively exchanges TRF2 on TIN2. Furthermore, we extended the study to another shelterin unit TPP1 that directly recruits telomerase to telomeres. We found that the presence of TPP1 expands TIN2 binding capacity and enables the interaction with TRF1 and TRF2 simultaneously, hence allowing formation of TRF1-TIN2-TRF2 core complex. We present a molecular model explaining a key role of TPP1 for the stable formation of shelterin core complex.

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A Structural Basis for the Crosstalk Between Histones and RNA Polymerase II

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Transcription of eukaryotic protein-coding genes requires transfer of RNA polymerase II (Pol II) through nucleosomes. Nucleosomes are inherent barriers of transcription, and Pol II stalls at multiple locations within a nucleosome. Nucleosome core particle (NCP) consists of 145–147 base pairs of DNA wrapped around a histone protein octamer. Transcription elongation factors accompany Pol II to facilitate efficient transcription. They enable polymerase progression through NCPs and ensure re-establishment of chromatin after polymerase passage. The mechanisms underlying these processes, however, remain puzzling and poorly understood.

Our aim is to present molecular details underlying Spt6 (histone chaperone and transcription factor) binding events. In our study we are revealing this long-standing open question by identifying elements of Spt6 that mediates interactions between Pol II and nucleosome. Cryo-electron microscopy, X-ray and Small Angle X-ray Scattering (SAXS) are used to study the macromolecular complex. Our findings provide a fundamental mechanistic insight into the functional specialization of Spt6 and have implications for the understanding of crosstalk between RNAP II and chromosomes.

Identification of Novel Therapies for Genetically Defined CLL Cases Through Approved Drugs Repurposing

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Chronic lymphocytic leukemia (CLL) is a heterogeneous malignancy of slowly proliferating B lymphocytes known for the limited therapeutic options, mainly due to increasing resistance rates. Thus, new strategies incorporating personalized medicine approach are being explored and pioneered to target the disease based on the mutational background of the patient. Drug repurposing provides a tool in the search for new indications of already approved drugs outside their original indications. While the traditional drug discovery process must submit to several lengthy stages, drug repurposing offers an accelerated bench-to-bedside transition and provides faster, cheaper and safer ways to treat patients.

In this research project, we applied a library of 859 FDA approved drugs at a single concentration with diverse chemical, structural and pharmacological features to both patient primary cells and genome-edited cell lines. Data from these screens were normalized to DMSO and hierarchically clustered in a heatmap by using the R software. The primary drug screen revealed the most effective compounds, which were further validated on a 10-point dose-response curve with 2-fold drug dilutions to assess the reproducibility, the magnitude of the effect and to define other potentialities. These top hits showed higher efficacy compared to the standard targeted therapies represented by ibrutinib, venetoclax or idelalisib.

Up to date, we performed drug screens on 24 primary patient samples covering major CLL genetic subtypes with different mutational status of IGHV, ATM and TP53. We demonstrated that all patient samples share the common sensitivity to 16 drugs, pointing out the group of proteasome and histone-deacetylase inhibitors. These compounds were efficient independently on the chosen mutational status. On contrary, CLL patient cells bearing an ATM mutation were found to be exquisitely sensitive to dasatinib.

While these results demonstrate drug repurposing as a useful tool for potential personalized therapeutic applications, further examinations and drug screens need to be performed with validations of the top performing compounds.

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Dimerization of 14-3-3 Proteins and the Influence of Phosphorylation

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14-3-3 proteins are universal regulatory proteins and their function depends on their oligomeric form which may alter between the monomeric, homodimeric and heterodimeric states. The populations of individual oligomeric forms are controlled by Kd values of the dimer-monomer equilibria between the involved isoforms. This complex picture is extended by post-translational modifications, e.g. phosphorylation. In this work, we describe the equilibria between monomers, homo- and heterodimers of the 14-3-3ζ isoform in the unmodified and phosphorylated form. To cover a wide range of relevant dimerization affinities, we combined solution NMR, microscale thermophoresis, native PAGE, and a set of novel fluorescence assays. Using a FRET based assay, we also determined the kinetic parameters of dimerization.

We found that phosphorylation of 14-3-3ζ at Ser58 increases its homodimeric Kd value by 6 orders of magnitude.

The developed assays allow to efficiently monitor 14-3-3ζ dimerization as a function of external factors, such as temperature, salt concentration, and client protein binding. For instance, we obtained values of both transient and equilibrium thermodynamic constants for the dimerization. In summary, our work provides a conceptual framework to characterise the isoform exchanges of homo- and heterodimers which can significantly deepen our knowledge about the regulatory function of 14-3-3 proteins.

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Multivalency of the Photorhabdus asymbiotica Lectin PHL2

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Lectins are ubiquitous proteins and glycoproteins with the ability to specifically, non-covalently and reversibly bind to the mono-, oligo- and polysaccharides. These sugar-binding proteins can be found in most organisms, ranging from viruses and bacteria to plants and animals. They play an important role in many processes occurring in nature, such as cell-cell interaction or recognition of the host by a pathogen. Lectins represent a heterogeneous group of proteins that vary in size, oligomeric state, structure as well as in exhibit specificity.

Research is conducted on the lectins from gram-negative entomopathogenic bacteria *Photorhabdus asymbiotica*, which live in symbiosis with *Heterorhabditis* nematodes. This symbiotic complex can be found in soil, where it searches for the insect prey. Unusual dual behaviour makes bacteria *Photorhabdus* compelling organisms for further study of its biomolecules.

Beside functional characterization, structural information is essential for the discovery of the key residues involved in interaction which may be modified to confirm or enlarge the specificity and/or affinity with the ligand. For this purpose, the protein crystallography was used to determine the 3D structure of lectins and their complexes with binding partners in atomic resolution.

The study revealed that lectin PHL2 has a seven-bladed β-propeller fold. PHL2 crystallizes as a homodimer with fourteen binding sites for D-galactose (seven per monomer). Conducted research shows key residues involved in the interaction with saccharide. Obtained results may reveal importance of the PHL2 in the pathogenic or symbiotic stage of life.

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Cytokinin-Induced Dirigent Genes *AtDIR13* and *AtDIR14* As Potential Players in Stress Response

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Cytokinins are N^6-substituted adenine derivatives that play diverse roles in plant growth and development, including meristem function, leaf senescence, sink/source distribution, vascular development and (a)biotic stress responses (1). Dirigent proteins (DIRs) have been found to mediate regio- and stereo-specific monolignol coupling during formation of lignans that might serve as (a)biotic stress-delimiting plant 'biological weapons' (2). The presence of DIR protein epitopes in lignifying tissues and lignin initiation sites provided the basis for the hypothesis that DIRs guide regio- and stereo-specificity not only of lignans, but of the lignin, as well (3). Root endodermis differentiation involves formation of the Casparian Strip (CS), an apoplastic barrier analogous to animal endodermal tight-junctions, consisting of a ring-like impregnation of the primary cell wall with lignin (3). EBS1/DIR10 was shown to mediate CS formation via precise targeting of the lignification-mediating complex (4). Members of the DIR family were suggested to be direct targets of cytokinin signaling (5). We propose that cytokinin can control the cell wall composition and/or properties possibly in a response to (a)biotic stresses or as a part of developmental regulations via regulation of dirigent genes *AtDIR13/14*.

By using transcriptional fusion lines *pAtDIR13::NLS:3XGFP* and *pAtDIR14:GUS*, we found *AtDIR13* and *AtDIR14* to be root-specific and active since early stage of postembryonic growth. We observed *pAtDIR13* being strongly activated by cytokinins, particularly in the root tip. The detailed root histology employing thin polyacrylamide sections of *pAtDIR14:GUS* roots revealed that *pAtDIR14* expression shifts from procambium to meta- and protoxylem cells after cytokinin induction. The

cytokinin-mediated upregulation of endogenous *AtDIR13* and *AtDIR14* was confirmed by RT-qPCR analysis. Moreover, it was shown that *AtDIR13/14* expression is affected by type-B ARRs, the members of cytokinin signaling cascade. Localization of AtDIR13 was assayed in transgenic lines homozygous for *pAtDIR13:AtDIR13::mCherry* translational fusion. We observed a low level of expression in the endodermis of the root differentiation zone, where *pAtDIR13:AtDIR13::mCherry* seems to locate to the cell wall and might be a part of CS. The effects of CKs on AtDIR13 localization was also tested by application of 5 uM BAP, the aromatic cytokinin, for 24h. In line with the effect seen with the promoter fusions, we observed upregulation of *pAtDIR13:AtDIR13::mCherry* signal in the early differentiation zone of the root. In comparison to WT plants, the *AtDIR13* OE lines seem to produce more biomass and reveal better wilting resistance and faster recovery after rewatering.

In our further work, we will focus on the identification of potential role of *AtDIR13/14* in Casparian strip formation by investigating CRISPR-Cas9 knock-out single *AtDIR13* and *double AtDIR13/14* mutants and available overexpression lines. Abiotic stress application and additional examination of drought stress tolerance of *AtDIR13/14* OE (iOE) and mutant lines will be performed and potentially implemented other stress conditions, such as salt, cold, and biotic stresses.

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Atomistic Characterization of Microtubule Associated Protein 2c

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Microtubule associated protein 2c (MAP2c) is a juvenile splicing variant of the MAP2 gene product, assumed to regulate growth of dendrite in neurons of developing brain. MAP2c is an intrinsically disordered protein containing several sequence regions associated with known or predicted functions. The functional regions correlated with transient structural motifs identified by NMR. The most important functional region is the microtubule binding domain (MTBD) , regulating dynamic instability of microtubules. MTBD of MAP2c exhibits a high sequence homology with Tau, but MAP2c does not form aggregates like those found in brains of patients suffering from the Alzheimer's disease. MAP2c, as a regulatory protein, is itself regulated by numerous post-translational modifications, including phosphorylations, and by interactions with upstream regulatory proteins such as 14-3-3 and Neurosteroids. We have recently described that the 14-3-3ζ isoform interacts with MTBD of unphosphorylated MAP2c, competing with the microtubules and that phosphorylation by the PKA kinase creates additional binding sites(Melkova K et al., 2018; doi: 10.1074/jbc.RA118.001769). This interaction may represent a branching point in the MAP2c and Tau regulatory cascades because PKA phosphorylates different residues in MAP2c and in Tau. As such, we have co-crystallized MAP2c peptides designed based on our NMR studies with 14-3-3 to gain insight on the local conformations propensities. Another important binding site of MAP2c (not present in Tau) is a sequence binding the regulatory subunit RII of PKA. The regulatory subunit II of cAMP-dependent protein kinase (RII-PKA) is an important control factor, interacting with so-called A-kinase anchoring proteins (AKAP). PKA regulatory domain II binds in a region of MAP2c which also exhibits contacts with Fyn kinase and neurosteroids which hints at a comlex signalling cascade. Our aim is to determine structure of a complex of the full-size RII-PKA and/or of the dimerization domain of RII-PKA with a peptide derived from microtubule associated protein 2 and sharing sequence similarity with the RII-binding motif of AKAP. ITC revealed nanomolar affinity of the peptide to RII-PKA and the interaction has been verified by NMR. We also plan to address the binding of MAP2c with microtubules with cryo-EM trials and in-situ cryo-ET to study the interactions of the cytoseketal components.

Structural Basis for DNA Recognition by Human RECQ4 DNA Helicase

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RECQ4, a member of the RecQ helicase family, plays a role at the intersection of telomere maintenance, DNA damage response and replication, although the exact mechanism of function for this enzyme is not yet known. Using NMR and other biophysical methods, we study the mostly disordered N-terminal region of RECQ4. We identify a RECQ4 specific motif (RSM) that binds with high affinity various DNA structures (such as such as single-stranded, double-stranded, Y-form, bubble, or G-quadruplex), forming fuzzy complexes that critically depend on electrostatics. Additionally, the RSM interacts with an abundant component of the replisome via an induced fit helix. Our study demonstrates a disordered peptide that has the inherent ability to switch between DNA and protein transactions by using distinct and competitive mechanisms. Functionally, such a plasticity could enable accurate control and progression of sequential events in DNA replication or other genome maintenance processes.

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Effect of High Temperatures on Flowering *Arabidopsis Thaliana*

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In the last decades, the average annual temperatures have increased. According to the European Environment Agency (EEA, 2017), a further increase is anticipated in the frequency and intensity of extreme climate conditions, such as heatwaves, intense precipitations, and drought. High ambient temperatures impair crop production, shortening the life cycle of crops, and reducing the grain yield.

The reproductive phase has been suggested to be the most sensitive to high-temperature conditions due to heat-induced sterility and defective female and male gametophyte development. This results in reduced pollen tube growth in the stigma and style, leading to a lower number of fertilized ovules. The auxin and cytokinin plant hormones regulate plant development, including one of seedpods, ovules, seeds, and embryos. Heat stress has shown to alter hormone metabolism. Our project aims to study how high temperatures affect the development of flowering Arabidopsis thaliana, focusing on the development of seeds.

Our results show that high temperatures accelerate the inflorescence stem growth, increasing the number of flowers in the primary inflorescence. Nevertheless, the length of siliques and the pollination rate are reduced when temperatures are high. Embryo development is also accelerated, and, in many cases, embryos display developmental defects, similar to what is observed in various auxin mutants. These results suggest that high temperatures alter the proper development of the inflorescence and embryos in a hormone-dependent way.

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miR-29 Modulates CD40 Signaling in Chronic Lymphocytic Leukemia by Targeting TRAF4: an Axis Affected by BCR

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B cell receptor (BCR) signaling, and T cell interactions play a pivotal role in chronic lymphocytic leukemia (CLL) pathogenesis and disease aggressiveness. CLL cells can utilize microRNAs (miRNAs) and their targets to regulate microenvironmental interactions in the lymph node niches. To identify miRNA expression changes in the CLL microenvironment, we performed complex profiling of short non-coding RNAs in this context by comparing CXCR4/CD5 intraclonal cell subpopulations ($CXCR4^{dim}CD5^{bright}$ vs. $CXCR4^{bright}CD5^{dim}$ cells). This identified dozens of differentially expressed miRNAs including several that have previously been shown to modulate BCR signaling (*miR-155, miR-150, and miR-22*), but also other candidates for a role in microenvironmental interactions. Notably, all three *miR-29* family members (*miR-29a, miR-29b, miR-29c*) were consistently down-modulated in the immune niches, and lower *miR-29(a/b/c)* levels associated with an increased relative responsiveness of CLL cells to BCR ligation, and significantly shorter overall survival of CLL patients. We identified Tumor-Necrosis Factor Receptor-Associated Factor 4 (TRAF4) as a novel direct target of *miR-29s* and revealed that higher TRAF4 levels increase CLL responsiveness to CD40 activation and downstream NF-kB signaling. In CLL, BCR-represses *miR-29* expression via MYC, allowing for concurrent TRAF4 upregulation and stronger CD40-NFkB signaling. This regulatory loop is disrupted by "BCR inhibitors" (BTK inhibitor ibrutinib). In summary, we showed for the first time that a miRNA-dependent mechanism acts to activate CD40 signaling/T-cell interactions in a CLL microenvironment and described a novel miR-29-TRAF4-CD40 signaling axis modulated by the BCR activity.

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Virion Structure and Mechanism of Genome Delivery of Bacteriophage SU10

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Bacteriophages are molecular machines evolved to infect cells. Historically bacteriophages from the family *Podoviridae* were characterised by short non-contractile tails. Here we present the structure of native and genome releasing virion of phage SU10.

SU10 has a prolate capsid with a dodecameric portal complex that features a prolonged crown-barrel. The tail of SU10 is decorated by long and short tail fibers, both present in six copies. Tail needle protrudes out from the center of the base plate.

Our observations by CryoEM allows us to propose mechanism of the early stages of the phage infection. SU10 binds to the cell surface by primary and secondary receptor binding proteins. Primary receptor binding proteins, which bind reversibly, are located at distal ends of the long tail fibres. After primary receptor recognition, short tail fibres flip towards the cell surface. C-terminal receptor binding domains of short tail fibres serve as secondary irreversible receptor binding proteins. Extended nozzle is formed by a remodeled tail complex together with flipped short tail fibres. During attachment of secondary receptor binding proteins the tail needle mechanically disrupts the outer cell membrane and dissociates from the virion. Phage core proteins are ejected from the capsid and form a translocation channel. Transglycosylase domains, which are located on the outer surface of the translocation channel, hydrolytically degrade the cell wall. Translocation channel together with extended nozzle serve as a tunnel for phage DNA delivery into the bacterial cytoplasm.

Our study reveals major structural changes of podovirus tail upon phage attachment on the cell surface were not observed up to date.

Deciphering the Abnormally Mutated Molecular Processes in Chronic Lymphocytic Leukemia: Identification of Clinically Relevant Mutation Subtypes

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Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in the Western world with a highly variable clinical course. Although the CLL genetic landscape has been well-described, patient stratification based on mutation profiles remains elusive mainly due to the heterogeneity of data. Mutations in some driver genes (e.g. TP53 and ATM mutations) are associated with worse clinical outcome whereas, in other instances, reports of prognostic relevance are inconsistent (e.g. NOTCH1 and SF3B1 mutations). Many driver genes cluster in specific signaling pathways, however, in a significant proportion of patients, no known recurrent mutation is found. Here, we aimed to identify clinically relevant patients' subtypes based on their abnormally mutated molecular processes.

Mutation data are inherently sparse which makes clustering challenging. Firstly, we attempted to decrease the heterogeneity of somatic mutation data limited to protein-coding regions by mapping mutated genes to the respective biological processes. We tested our approach on the sequencing data gathered by the International Cancer Genome Consortium for 506 CLL patients. We applied ensemble clustering on the pathway mutation score and extracted abnormal molecular pathways with a machine learning approach. We identified four clusters differing in pathway mutational profiles and time to first treatment. Among the most important signatures for the identified groups, biological processes previously described as recurrently mutated in CLL appeared (DNA-damage response, RNA processing, and inflammatory pathways). Additionally, processes known to play a vital role in CLL biology but without previously described mutated components in CLL, such as calcium signaling, were identified. Interestingly, common CLL drivers such as ATM or TP53 were associated with particular subtypes, while others like NOTCH1 or SF3B1 were not.

Since many mutations in non-coding regions can have a detrimental effect on the proteins, we decided to employ the CADD score that combines multiple diverse genomic features such as evolutionary constraint, epigenetic measurements, and functional predictions into one metric. Subsequently, we calculated pathway mutation score based on CADD. Moreover, this approach enabled us to combine somatic and germinal mutation profiles. Using the merged somatic and germline data from both coding and non-coding regions, we discovered novel disease subtypes. Currently, we are evaluating their clinical and biological relevance.

Finally, we leveraged the knowledge and computational approaches developed while exploring publicly available datasets on a local patient cohort from the University Hospital Brno. We collected samples before and after particular therapies from 52 CLL patients with known clinical courses and different scenarios of TP53 gene mutation expansion. We identified distinct mutated pathways (chromatin SWI/SNF complex, genome integrity, and RTK signaling) characteristic for defined patient groups, and co-occurring and mutually exclusive mutations. Then, we clustered patients based on their pathway mutation score and found clusters enriched with predefined particular groups.

Our results aid the understanding of mutational patterns in CLL, which is necessary for the accurate use of available treatment regimens and for the design of suitable diagnostic panels.

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Bioinformatic Approaches in Non-coding RNA Studies in Solid Cancer

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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 to analyze sequencing data. Especially in small non-coding RNAs research, the pivotal problem is accurate identification and quantification of a full spectrum of small RNA pool. Most of the available pipelines for analysis of small RNA-seq data are targeted mostly on microRNA and ignore other RNA types such as snoRNA, snRNA, piRNA, or isomiRs. This is especially problematic when studying small RNAs that are part of the circulatory system, which are known potential disease biomarkers and currently being heavily investigated due to biofluids being easier to collect than classical tissue samples.

To address the issue of accurate quantification of various small RNA, we have designed a bioinformatic pipeline for the detailed analysis of small RNA-seq data. Our pipeline is platform-independent, implemented in the Snakemake workflow management system, and freely available. All software tools are installed via Conda package manager, and the whole pipeline is versioned, which ensures reproducibility. We focused on producing transparent code as opposed to some currently used approaches for analysis of small RNA-seq data, which are set up as an online web service without the possibility of seeing what is actually happening with users' data.

All necessary steps for the analysis of small RNA-seq data are divided into multiple stand-alone modules. The main focus is centered on pre-processing and RNA quantification. Pre-processing step is capable of working with data prepared by various library prep kits, including these that incorporate UMIs into their reads. RNA quantification step is then carrying a subsequent number of mapping rounds, starting with removal of potential contamination by rRNA and continuing with mapping against the miRBase database for obtaining miRNA and isomiRs counts. The last mapping against a merged set of transcript sequences obtained from various databases ensures quantification of different small RNA classes. The final module for differential expression analysis reports significantly differently expressed RNAs for simple experimental designs. Parameters for different steps are fully adjustable by the user, but default parameters are set up based on the selected library prep kit used for data generation.

For particularly enquiring users, we have also created a Shiny application for real-time visualization of differential expression results where content and appearance of popular plots such as heatmap or volcano plot can be easily altered.

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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. This 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, in particular 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 t6A biosynthesis proteins and demonstrate that different steps of t^6A biosynthesis occur in different compartments of plant cells, such as nucleus, plastids, and mitochondria.

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Phylogeny of Microlepidieae (Brassicaceae) Based on Plastid and Nuclear Sequence Data

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The Microlepidieae (MICR) is a crucifer tribe of c. 17 genera and c. 56 species endemic to Australia (16 genera) and New Zealand (*Pachycladon*). To elucidate phylogenetic relationships within the MICR, we embarked on a comprehensive phylogenetic analysis to gain new insights into relationships and patterns of genome evolution within the tribe. In this study, sequence data from the plastid genomes, 45S nuclear ribosomal DNA and repeatomes of MICR taxa were used to infer the inter- and intra-generic relationships. The tribe MICR was retrieved as a monophyletic clade based on the analysis of plastid and rDNA data sets. However, *Arabidella* (F.Muell.) O.E.Schulz and *Menkea* Lehm. were found to be polyphyletic. The well-resolved and strongly supported plastome phylogeny revealed that the two *Arabidella* clades should be divided into two genera: clade A includes A. *filifolia*, A. *glaucescens*, A. *nasturtium* and A. *trisecta* (genus *Arabidella*), whereas Clade B included A. *eremigena*, A. *procumbens* and A. *eremigena* (genus *Micromystria* O.E.Schulz). A. *chrysodema* clustered with two Menkea species. Based on the three-codon-partitioned protein-coding gene (PCG) dataset, we estimated that the divergence of MICR started from the late Miocene, around 9.08 million years ago (Mya; 95% HPD: 7.36–11.24 Mya). The deepest split of Clade A (*Arabidella* s. str.) was dated to 7.50 Mya (6.04–9.33 Mya), and the subsequent diversification occurred c. 1.17 Mya (0.83–1.62 Mya) during the mid-Pleistocene. Clade B diverged from its sister lineage approximately 5.87 Mya (4.69–7.36 Mya) and the divergence between A. *eremigena* and *Cuphorulus andreanus* occurred roughly 2.60 Mya (1.89–3.48 Mya). In addition, we infered phylogenetic relationships among *Arabidella* genus using repeat sequence similarity matrices. The consensus tree, based on 37 out of top 100 abundant clusters retrieved from RepeatExplorer2 comparative clustering analysis, separated repeatomes of clade A and B species, whereas A. *chrysodema* formed a third clade.

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