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Book of Abstracts

CEITEC MUNI

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CEITEC PhD Conference posters will be on display
at CEITEC MU, Building A35, Atrium
(10th – 15th February 2020)

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WELCOME ADDRESS

Welcome Word

Dear participants,

Five years ago, many of us moved to the new buildings of CEITEC, equipped with the best research infrastructure that money could buy. Nevertheless, it was clear that all of these investments would be for naught without talented people to use them to push the boundaries of human knowledge. Therefore, the initiative to create an international PhD program that is known beyond the borders of the Czech Republic and is attractive to talented students worldwide is, according to my opinion, even more important than investments into research infrastructure. What would all of these machines be good for without the curiosity, devotion, and talent of the people who can use them to solve exciting scientific problems? You are among the first generations of PhD students that joined CEITEC, and the success of your current research projects, as well as your future careers, will be one of the most important indicators that defines whether CEITEC indeed succeeds in its mission. So, let´s take this conference as an opportunity to take a look at science that has been done here during the past years, let´s learn about projects that are being performed next door, and let´s engage in discussions on topics that may go beyond our primary research interests. I am convinced that we will all benefit from new ideas, unorthodox questions, and perhaps new collaborations.

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Ethylene Signaling and its Paths: a Fascination Wrapped in Mystery

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Ethylene has been shown to be involved in controlling cytokinin-mediated root growth. Current research attempts to uncover several candidates that link between ethylene (C2H4) and cytokinin (CK) at the signaling level in the root. We have recently shown that ethylene contributes into the MSP pathway via histidine kinase activity of ETR1, controlling the activity of several response regulators (ARRs). To continue the research, we explored several components of C2H4 and CK signaling pathways as potential targets of the signaling crosstalk in the root. We found out that there is additional histidine kinase (AHK5) that is involved in CK-C2H4 crosstalk at the signaling level in the root. We also found out that the ethylene-mediated MSP activation is independent on its own canonical signaling pathway (ETR1-CTR1-EIN2) but not on its transcription factor EIN3. Thus, the canonical ethylene signaling components EIN2 and EIN3 show a differential role in the ethylene-mediated MSP activation. Our current findings propose two possible mechanisms for ethylene-dependent activation of MSP pathway in the root at the signaling level. The signal is perceived by C2H4 receptors (e.g. ETR1) and either directly or in cooperation with another histidine kinase phosphorylates AHPs, which transfer the signal to the nucleus and activate ARRs, controlling the transcription of the MSP target genes. The other possible CK-ethylene signaling hub is the transcription factor EIN3, which can control MSP e.g. via regulating the transcriptional activity of ARRs-A.

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Human Telomere Repeat Binding Factor TRF1 Replaces TRF2 Bound to Shelterin Core Hub TIN2 when TPP1 Is

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Telomeric repeat binding factor TRF1, TRF2 together with TIN2 protein create important roles as a shelterin-core subunits. Their dynamics is important for regulating the assembly of shelterin complex. We applied FCCS – Fluorescence Cross-Correlation Spectroscopy as an in vitro single molecule fluorescence microscopy approach to quantitatively describe the exchange of TRF1 and TRF2 in complex of TIN2. We found that TRF1 can effectively exchange TRF2 in TIN2-TRF2 complex which is essential regarding the function of shelterin during specific recognition of chromosome ends and telomerase activity regulation. We extended the FCCS study with addition of TPP1 which is TIN2 binding partner and tested whether TPP1 presence could change the TIN2-TRF2 interaction and enable TIN2 interact simultaneously with TRF1 and TRF2, hence allow to originate TRF1-TIN2-TRF2 shelterin-core complex. Our FCCS data shows that TPP1 indeed upon binding to TIN2 induce allosteric effect improving the binding capacity so the complex TPP1-TIN2 can accommodate both TRF1 and TRF2 and it is essential for the proper formation of TRF1-TIN2-TRF2 shelterin-core complex.

Mutational Landscape Analysis in Systemic Anaplastic Large Cell Lymphoma Identifies Novel Prognostic Markers

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Systemic Anaplastic Large Cell Lymphoma comprises two different entities of T-cell non-Hodgkin lymphomas: ALK-positive ALCL characterized by the presence of ALK-translocation, and ALK-negative ALCL, lacking the latter. Both share pathologic features, such as lymphoid cells called 'hallmark cells' and the expression of CD30. ALK-positive ALCL shows a better prognosis compared with ALK-negative ALCL, with a 5-years overall survival of 70-80% and 15-45% respectively. Although more than 25% of ALK-positive ALCL patients show relapse. Systemic ALCL is a genetically heterogenous disease whose genomic characterisation has been improved through the implementation of high-throughput technologies. Despite this, the prognostic value of somatic mutations has been poorly described. Using targeted DNA sequencing, we investigated the whole coding region of 275 genes in a retrospective cohort of 82 systemic ALCL patients (47 ALK-positive and 35 ALK-negative), with the aim to improve risk stratification and predict disease outcome. We showed that STAT3 mutations associate with a shorter overall survival in ALK-negative ALCL patients. In addition, we identified TP53 as the most frequently mutated gene in systemic ALCL (5/47 ALK-positive and 8/35 ALK-negative). Moreover, we also showed association between TP53 mutations and shorter progression free survival and the comparison of samples at diagnosis and relapse revealed that clones harbouring aberrations in the TP53 may have a role in disease progression. Altogether, this study provides information about the genetic landscape in systemic ALCL across 275 genes. We identified novel diagnostic and prognostic biomarkers, showing the relationship

between mutated genes and outcome. For the first time we have shown a correlation between mutated STAT3 and TP53 with poor prognosis, highlighting the need to investigate the mutational status of those genes at the diagnosis.

This project has received funding from the European Union's Horizon 2020 Marie Skłodowska – Curie Innovative Training Networks (ITN - ETN) under grant agreement No.: 675712.

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Studying Tight Dimers Using Ordinary Fluorimeter

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The 14-3-3 proteins represent one part of the large group of dimeric proteins. Specifically, the 14-3-3 family consists of 7 isoforms, that can form many homo- and heterodimeric states, not even accounting for the possibility of changing the oligomerization properties by posttranslational modifications such as phosphorylation. In our study, we focused on the zeta isoform with most stable dimers and its phosphorylated form. Using standard biophysical methods we have only seen that the Kd is lower than 1μM. Therefore, we designed very sensitive fluorescence based methods to allow for study of such tighly bound dimers. Using these methods, we determined the dissociation constant to 5 nM, as well as kinetic parameters of the oligomerization process. Moreover, we studied the dependencies of the process on several buffer conditions. Also, we tested the proposed dimer disruption after phosphorylation at Ser58 located at the dimeric interface and measured the Kd and kinetic parameters for the mixed dimer (wildtype - phosphorylated form).

Tyrosine hydroxylase is one of many binding partners of 14-3-3 and an enzyme catalyzing the rate-limiting step in the synthesis of catecholamines (dopamine, noradrenaline, adrenaline). We study its regulatory domain that directly interacts with 14-3-3 and thus regulates the function of the whole enzyme. The domain is dimeric and each monomer consists of a structured and an unstructured part of similar size. This considerably restricts the possibilities how to study its structure. We use NMR as it can see with atomic resolution both parts and we can assess the dynamic properties of the domain. We studied the effects of phosphorylation on the structure and the resulting dynamic data for computational studies.

RecQ4 is a Hub in the Eukaryotic Replisome

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Efficient duplication of the genome requires the concerted action of helicases and DNA polymerases at replication forks to avoid stalling of the replication machinery and consequent genomic instability. RecQ4, a member of the RecQ helicase family, is required for the proper assembly of the Cdc45–MCM–GINS (CMG) DNA helicase, and consequently for the initiation of DNA replication in human cells. To better understand the mechanistic underpinnings of human RecQ4 in DNA replication we study the unique, mostly disordered, N-terminal region and its interactions with various DNA substrates, as well as components of the replisome.

Using mainly NMR and other biophysical methods, we have identified and characterized an unstructured RecQ4-specific motif (RSM) that a) binds diverse DNA structures, in a salt-dependent manner, forming fuzzy complexes (with the concept of disorder extending into the bound state), b) upon binding disrupts the non-canonical base pairing and unfolds the G-quadruplex DNA, c) interacts with the primary eukaryotic ssDNA binding protein, RPA, through an induced fit adoption of helical structure, d) interacts with a negatively charged part of RecQ4's own N-terminal region, implying the existence of self-regulation of function.

Our results identify RecQ4 as a key platform for multivalent interactions in the eukaryotic replisome. Rather than simply participating in the assembly of the DNA helicase, a RecQ4 homotetramer can couple the replication machinery to multiple factors that contribute to diverse aspects of chromosome duplication.

Highlights

- RecQ4 is a hub that links factors with diverse functions to the eukaryotic replisome
- RecQ4 maintains genomic integrity by resolving G4 DNA structures during replication

The Use of CRISPR/Cas9 Technology in the Study of Chronic Lymphocytic Leukemia

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Chronic lymphocytic leukemia (CLL) patients carry a variety of somatic mutations, whose exploration could shed light on the disease etiology, or even lead to the discovery of potential novel drug targets. To establish suitable models mimicking the genetic heterogeneity observed in patients, we generated isogenic cell lines harboring some of the most frequent CLL mutations. We aim to investigate unique vulnerabilities specific to these mutations.

We made use of HG3 and MEC1 cells, two well-established CLL cell lines, and using CRISPR/Cas9 technology we generated monoclonal isogenic knockout cell lines in ATM and TP53 genes and a knock-in HG3 cell line in MYD88 gene. In addition to these monoclonal cell lines I also established a polyclonal CD20 knockout HG3 cell line.

The ATM knockout cell line was used for CRISPR/Cas9 functional screening to reveal genes synthetic lethal to ATM by comparing the abundance of gene knockouts in the beginning of the experiment vs. after 3-week incubation. Gene knockouts that were depleted in the ATM knockout cells but not in wildtype control should have a synthetic lethal relationship with the ATM gene. Altogether we obtained 11 candidate ATM-synthetic lethal genes, which are now being validated. After thorough validation, some of the most interesting genes might serve as potential therapeutic targets for patients with ATM mutations.

Apart from the functional screening, the established knockout and knock-in cell lines serve as suitable models for CLL and have a wide range of uses to develop novel personalized therapies. We have utilized these cells to generate CLL mouse models for CAR T-cell study, to investigate CD20 function and also for a drug screening with FDA-approved drug library.

This research is financially supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601).

Novel Disease-causing Mutations Discovered in Human ADAR2

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Double-stranded RNA-specific adenosine deaminases (ADARs) are a family of enzymes that catalyse the hydrolytic deamination of adenosine to inosine in dsRNA. The editing and RNA-binding activities of ADARs affect RNA processing, stability, and can even lead to RNA recoding [1]. The enzyme ADAR2 is essential for recoding of brain transcripts. Impaired ADAR2 editing causes early-onset epilepsy and premature death in mouse models [2].

ADAR2 variants were found in five unrelated individuals with microcephaly, intellectual disability, and epilepsy. One patient carried a homozygous mutation in one of the double-stranded RNA-binding domains, whereas, in the remaining four patients, homozygous or biallelic mutations located in or around the deaminase domain were identified. To evaluate the effects of these variants on ADAR2 activity, in vitro assays with recombinant proteins expressed in HEK293T cells and ex vivo assays with fibroblasts derived from one of the individuals were performed.

We demonstrate that these ADAR2 variants lead to reduced editing activity on a known ADAR2 substrate. We also detected changes in ADAR2 mRNA splicing induced by one of the mutations. Lastly, we observed altered ADAR2 mRNA and protein levels in patient fibroblasts. Together, these results provide evidence that the mutations have a small but significant effect on ADAR2 activity that may lead to the observed phenotype. Proper neuronal model is needed to characterise the effects of these mutations on brain development.

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MiR-29 Regulates the CD40 Signaling in the Microenvironment of Chronic Lymphocytic Leukemia

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The (de)regulation of microenvironmental interactions, primarily BCR signaling and T cell interactions play an essential role in chronic lymphocytic leukemia (CLL) pathogenesis. In the microenvironment, both BCR activation and T cell interactions are concurrently available to CLL cells. We and others have shown that CLL cells increase BCR signaling propensity in the lymph node microenvironment by modulating the levels of microRNAs (miRNAs) and their targets. However, it is unknown if miRNAs are involved in modulating T cell interactions and their synchronization with BCR signaling in the context of CLL (or normal) microenvironment.

To describe the changes in miRNA expression in the CLL microenvironment, we performed the first complex profiling of short non-coding RNAs by analyzing CXCR4/CD5 intraclonal cell subpopulations (CXCR4[dim]CD5[bright] vs. CXCR4[bright]CD5[dim] cells). This identified dozens of differentially expressed miRNAs including ones that have been previously reported to modulate BCR signaling but also other candidates with a potential role in microenvironmental interactions. Particularly, all 3 miR-29 family members (miR-29a, miR-29b, miR-29c) were down-modulated in the immune niches, and lower miR-29s levels associated with significantly shorter overall survival and presence of unfavorable prognostic markers such as unmutated immunoglobulin heavy chain variable (IGHV) genes and ZAP-70 expression in CLL. We further performed an unbiased search for miR-29 targets and identified a novel direct target of miR-29s and showed that its higher levels increase CLL propensity to CD40 activation and downstream NFkB signaling. In CLL, BCR-induced miR-29 repression via MYC stabilization allows simultaneously stronger CD40-NFkB signaling, and this regulatory loop is obstructed by "BCR inhibitors" (ibrutinib/idelalisib).

Overall, our study showed for the first time that a miRNA-dependent mechanism acts to modulate CD40 signaling/T-cell interactions in a CLL microenvironment and described a novel miR-29-TRAF4-CD40 signaling axis.

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T6A modification of A37 Position in tRNA and Its Role in Plant Development

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RNA modifications are involved in many essential biological processes, representing a rapidly developing research field. Our project focuses on the role of tRNA modification threonylcarbamoyladenosine (t6A), which is present at postition A37 of nearly all tRNA molecules decoding ANN codons. t6A is conserved virtually in all organisms and known to play a role in the translation process. However, despite high effort, the deeper understanding of its function, in particular in multicellular organisms, is still incomplete. We are characterizing Arabidopsis thaliana homologs of t6A forming enzymes. We isolated candidate mutants with the abolished t6A formation and we show that t6A biosynthesis genes are essential for the earliest steps of plant morphogenesis, including gametophyte development. We also examined subcellular localization of t6A biosynthesis proteins and reveal that different steps of t6A biosynthesis take place in different compartments of plant cell, such as nucleus, plastids and mitochondria.

Architecture of the Human SMC5/6 Complex

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So far, three eukaryotic (cohesin, condensin, and SMC5/6) and three prokaryotic (SMC/ScpAB, MukBEF, MksBEF) SMC complexes have been described. They share common SMC-kleisin circular architecture allowing them to embrace and hold DNA. In addition, prokaryotic SMCs and the eukaryotic SMC5/6 complex share even more structural similarities. Their NSE subunits interact with the kleisin subunit and form stable homo- or hetero-dimers through winged-helix (WH) domains, thus they were re-classified into a new protein family called KITE (Kleisin Interacting Tandem winged-helix Elements of SMC complexes).

Here, we analysed the human KITE NSE1-NSE3 interaction within the complex by crosslinking coupled with MS/MS. In addition, we mapped the interaction between human NSE2, NSE5, NSE6 subunits and the SMC5/6 core complex using yeast two-hybrid system. All together, we gained insight into the complex architecture by specifying positions of the NSE subunits within the complex, and between the NSE subunits themselves.

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Breaching the wall: Structural Studies of Phage Infection

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Infection caused by antibiotic-resistant S. aureus strains are difficult to treat and can induce life-threatening symptoms (1). Bacteriophage (phage) phi812K1/420 from the family Myoviridae can infect 95 % of S. aureus strains, including those resistant to antibiotics, which makes the phage a promising agent for phage therapy (2,3).

Tail spike protein (TSP) is located at the centre of the phage phi812K1/420 baseplate (4). Putative hydrolase domain of TSP probably degrades S. aureus cell wall by creating a hole for tail tube insertion. Moreover, detachment of TSP from the baseplate is suggested to trigger structural changes in virion leading to genome release.

We aim to obtain high-resolution structures of TSP with combination of cryo-electron microscopy and X-ray crystallography. Structures will be evaluated both individually and upon fitting into the map of the phage baseplate. Analysis of the TSP active site architecture will be complemented with zymography to define key determinants of the phage degradative machinery.

Trimeric organisation of the TSP resulting in near 300kDa protein assembly allowed to collect cryo-EM data for single-particle reconstruction. Preliminary results correspond with the overall TSP arrangement as observed in whole phage baseplate reconstruction.

Achieving TSP characterisation from the structural and functional point of view will help to complete our understanding of the phage behaviour and infection. Comprehensive description of the phage phi812K1/420 is necessary for its approval as

pharmaceutical agent, which will be used with, or instead of, antibiotics for treatment of S. aureus infections. TSP hydrolase domain can be also exploited to supress infection and/or degrade bacterial biofilms.

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Cdk11 is a Novel Player in Non-coding RNA Expression

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Cdk11 is a poorly-characterized cyclin-dependent kinase with described functions in transcription of mRNA, alternative splicing and 3'end processing of HIV mRNA. Our aim was to better define its cellular roles via identification of novel interacting partners and candidate substrates combined with data from microarray experiment after knock-down of Cdk11. We utilized BioID method to probe for relevant proteins connected to Cdk11. Microarray data showed differential expression of a subset of non-coding genes. Proteins implicated in control of their expression and processing were identified in BioID screen as candidate interacting partners of Cdk11. We utilized also Cdk11 analog-sensitive mutant (designed using Shokat's lab approach) to test the candidate interacting partners as substrates of Cdk11 in vitro. Altogether, our data uncover novel role of Cdk11 in the expression of subset of non-coding RNAs

Toward Structure Analysis of a Huge Algal Virus

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The Emiliania huxleyi virus 86 (EhV-86) is an important agent in terminating regular population explosions of Emiliania huxleyi – the most abundant alga in marine ecosystems. We are attempting to solve the structure of EhV-86 using several cryo-electron microscopy techniques.

The unicellular alga Emiliania huxleyi is widely distributed photosynthesizing marine eukaryote, creating regular population explosions called blooms. These algal blooms, visible from space, influence the global climate by absorption of carbon dioxide as E. huxleyi uses this molecule for produc-tion of calcareous plates, covering its surface. The algal bloom collapses usually one week 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 possessing a lipid membrane outside of the capsid shell, which it acquires while exiting the algal cell via budding. The membrane and large dimensions of the EhV-86 virus particle make it quite challenging for purification and subsequent 3-dimensional reconstruction.

To determine the near-atomic resolution structure of the EhV-86 virion, we use cryo-electron microscopy (cryo-EM). The observed heterogeneity of viral particle morphology pushes reconstruction by so-called single particle analysis, i.e. averaging over many particles – toward tomography, i.e. using only few particles, each imaged at many angles. We also investigate the rep-lication machinery of EhV-86 inside the algal host cell using lamellas – thin cell sections produced by focused ion beam milling. The combination of our results obtained from various cryo-EM approaches will provide a compre-hensive insight into the EhV-86 life cycle and interactions with the Emiliania huxleyi host cell.

A Structural Basis for the Cross-Talk 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.

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

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Transcrption 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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Wnt signaling pathway plays an important role during embryogenesis and adult homeostasis. Dishevelled (Dvl), a scaffold protein, is crucial to relay the signal to different branches of this pathway. Upon Wnt stimulation, Dvl gets heavily phosphorylated by CK1ε/δ and the rearrangements induced by these modifications are of paramount importance in understanding the mechanisms of Wnt signaling. 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. The different conformations (multi-faces) allow Dvl to interact with a wide range of substrates and these interactions can be modulated by a specific phospho-code which eventually can determine the output of the Wnt pathway.

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.

Deciphering the Role of Strigolactone Hormone in Plant Developmental Processes

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Plant hormones are endogenous signalling molecules that modulates the physio-morphological and molecular responses in plants. Individual and joint action of multiple hormones regulate diverse array of metabolic pathways which in turn triggers the phenotypic response. A recently identified plant hormone Strigolactones, (SLs) is known to control several above and below ground growth and developmental events in plants. In plant root, SLs promote primary root growth, root hair elongation and repress lateral root development. Whereas, in plant shoot, SLs regulate shoot branching, leaf senescence, stem secondary thickening, photo morphogenesis and stem elongation. In my present thesis, we explored different aspects of Strigolactone signaling in plant. Genetic studies on SMXL7 and SMAX1 demonstrated distinct developmental roles for each, but very little is known about these repressors in terms of their sequence features. In this study, we performed an extensive comparative analysis of SMXLs and determined their phylogenetic and evolutionary history in the plant lineage. In the first chapter, our results show that SMXL family members can be sub-divided into four distinct phylogenetic clades/classes, with an ancient SMAX1 and further, we identified the clade-specific motifs that have evolved and that might act as determinants of SL-KAR signalling specificity. In the second chapter, we focused on role of strigolactone in photosynthesis and high light stress tolerance through transcriptome-based study. And their chapter is focused on the cross talk of SL with the miRNA pathway controlling root and shoot development.

Deletion of TRF4 Results in Accumulation of mRNAs 3'end Extended Forms in Saccharomyces Cerevisiae

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Gene expression in eukaryotic cells is regulated at multiple levels. The most critical is the processing and stability of mRNA production followed by translational regulation. Pre-mRNA processing involves a large set of proteins as well as other so-called non-coding RNAs (ncRNAs). In our lab we investigate how yeast and mammalian cells control RNA stability and quality.

In my project I focus on the role of the yeast non-canonical poly(A) polymerase complex, TRAMP4, in the formation of functional 3' termini of mRNAs. To date, TRAMP4 has been known to play a role in RNA processing and surveillance of ncRNAs, however our findings are broadening its role also to mRNAs.

Eukaryotic cells contain several complexes that take part in RNA transcription termination and 3'end processing. In Saccharomyces cerevisiae, cleavage and polyadenylation complexes mediate transcription termination of mRNAs and Rat1p, 5′ to 3′ exonuclease from the Rat1p-Rai1p-Rtt103p (RRR) complex, further degrades the cleaved product. On the other hand, ncRNAs are terminated and processed by the Nab3p-Nrd1p-Sen1p (NNS) complex that further draws the polyadenylation Trf4p- Air1/2p-Mtr4p (TRAMP) complex and the exosome to the ncRNAs leading to trimming or fully degradation of the nascent RNAs. A recent study of our lab on the role of ncRNAs transcription termination in S. cerevisiae, revealed that the strain lacking TRF4 (Δtrf4) displays RNA Polymerase II termination defects on TUB1 gene (so called read through, RT, phenotype). My task is to uncover whether Trf4p and thus the whole TRAMP4 complex participate in 3' end processing of mRNAs. For that I combine several different approaches, such as RNA analyses and RNA polymerase II ChIP analyses together with cell growth phenotypes.

High Throughput Sequencing Data Analysis of IG/TR Rearranged Genes in Leukemia Clinical Research

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Background:

High throughput sequencing/next-generation sequencing (NGS) is a rapidly evolving technology that is widely used in almost every branch of biology and medicine. Data generated by NGS are usually hard to process by a simple human interaction, and rather complex computational bioinformatics algorithms are necessary for further analyses and data exploitation. Designing analytical algorithms is only one part of a bioinformatics work; the other part is to develop a user interface simple enough to give an opportunity to end-users (e.g. biologists, clinicians) to apply computational pipelines seamlessly on their data straight after finishing wet lab experiments.

History:

With my education background in computational biology and bioinformatics I have become involved in a team mainly focused on research and development of tools for antigen receptor (AR; i.e. immunoglobulin (IG) and T cell receptor (TR)) sequence analysis in clinical research. We closely collaborate with the University Hospital Brno (FN Brno) and with hospitals within EuroClonality-NGS Working Group, a division of ESLHO (European Scientific foundation for Laboratory HematoOncology). The tools and pipelines developed by our team are mainly gathered as components of our 'Antigen Receptors Research Tool' / ARResT bioinformatics platform (bat.infspire.org/arrest), but thanks to TACR ZETA support programme, I am also involved in the development of a separate bioinformatics tool LYNX in collaboration with a private company HPST, s.r.o.

Results:

During my PhD studies I have been directly involved in the development of multiple bioinformatics tools: 1) ARResT/Interrogate, a web-based, interactive application for immunoprofiling IG/TR sequence data from both NGS and Sanger sequencing; 2) ARResT/Subsets (aka Encyclopedia of CLL Subsets), a unique knowledgebase on the homogeneous subgroups of CLL patients sharing virtually identical ARs on their leukemic cells; 3) genomePD/GLASS, an assisted and

standardized assessment of gene aberrations from Sanger sequence trace data; 4) ARResT/CLLpedia, a standalone version of ARResT/Interrogate modified for an everyday use in FN Brno extended by internal database and reporting functionalities for clinicians; 5) LYNX, a tool for analysis of gene mutations, copy number variants, and AR rearrangements from capture-based NGS data. Although some of the tools are already published, we keep working on improvements and update the tools regularly based on our collaborators' needs.

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Genetic Transformation of Fast Flowering Mini Maize (FFMM)

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Our lab aims at understanding molecular processes that underpin plant sexual reproduction. We are particularly interested in processes that regulate meiosis, a specialized cell division that defines differentiation of germline and formation of haploid gametes. We have developed a gene discovery pipeline for new meiotic regulators in Arabidopsis thaliana, the most popular model plant species. To assess whether the knowledge we acquire in Arabidopsis generally applies to other plants as well, we want to functionally validate some of our discoveries in a monocot plant. Maize is an important staple food, feed and fuel crop, and also an established good model systems for monocot research. However, the existing varieties of maize are relatively large plants with a long generation time, features not well suited for cultivation in indoor plant growth facilities. Recently, a novel cultivar of maize, Fast Flowering Mini Maize (FFMM), was created that is perfectly fit for indoor cultivation facilities. It grows less than one meter in height and has generation time of 60 days allowing fast seed production and rapid breeding. We are currently establishing FFMM maize as model to study plant sexual reproduction and developing regeneration and transformation protocols that would allow efficient induction of mutations in genes of interests by using CRISPR/Cas9 gene editing technology. Our ultimate goal is to modify selected genes we have discovered in Arabidopsis and study their function in maize.

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Interphase Chromosome Organization in the Brassicaceae

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During interphase, chromosomes assume a largely decondensed state. However, chromatin is still nonrandomly arranged within the nuclear space. 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. However, this model is not universal as showed for grass species with small genome sizes. 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. Apart the Arabidopsis genus, surprisingly little is known about the side-by-side arrangement of hetero- and homologous interphase CTs in other crucifer or eudicot species.

We aimed to verify the hypothesis that crucifers exhibit at least two different patterns of interphase organization and identify the determining (phylo)genomic features (e.g. genome size, phylogenetic position). We used the method of fluorescence in situ hybridization to analyze telomere/centromere positioning in interphase nuclei in selected Brassicaceae species with constrasting genome sizes and evolutionary history. Our preliminary data suggest that Brassicaceae species with (very) small genome sizes have the rosette-like interphase organization in somatic cells isolated from different tissues (flower, leaf, stem and root), and that telomeres do not cluster around the nucleolus in interphase nuclei in crucifers with large genomes. Interestingly, interphase organization seems to be tissue-specific in the species with intermediate genome size, both rosette-like organization and random telomere/centromere positioning was observed.

Structure of Bacteriophage SU10

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Bacteriophage SU10 was isolated from sewage water from Käppala waste water treatment plant in Stockholm. It belongs to the family Podoviridae and infects wide range of E. coli strains. The phage has 77 kbp dsDNA genome, prolate capsid and short tail with base plate.

We determined the molecular structure of capsid, portal and tail-base plate complex of SU10. Capsid is formed by 11 pentons and 110 hexons of major capsid protein, which has HK97 fold. The dodecameric portal complex features a prolonged crown-barrel on the top, similar to that of phage P22. Phage particle is decorated by two types of fibers, both present in six copies. Collar fibers are connected to the neck protein complex. Long tail fibers, possible host recognition device, are connected to the tail protein. Hydrolytic tail needle is pointing out from the center of the base plate.

Our high-resolution reconstruction of the phage SU10 is the first reported detailed insight into the structure and build up of Gram-negative bacteria attacking phages.

Elucidating the Biological Role of ADAR1 in the Innate immunity Response

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One of the most common and best studied type of RNA editing in higher eukaryotes is the hydrolytic deamination of adenosine to inosine within double-stranded RNAs (dsRNAs), by the enzyme family adenosine deaminases acting on RNA (ADAR). As inosine base-pairs with cytidine (C), it is translated and reverse-transcribed as a guanosine (G), changing the sequence of an RNA.

ADAR1 edits cellular dsRNA to prevent aberrant activation of cytoplasmic antiviral dsRNA sensors and missense mutations, that change ADAR1 residues and reduce RNA editing activity, cause Aicardi-Goutières Syndrome, a childhood encephalitis and interferonopathy.

ADAR2 is most highly expressed in brain and it is primarily required for site-specific editing of glutamate receptor transcripts. Mutations in ADAR2 could contribute to excitability syndromes such as epilepsy, to seizures and to diseases involving neuronal plasticity defects.

Mice deficient in Adar1 show embryonic lethality by embryonic day E12.5 1,2 with a type I interferon (IFN) signature similar to that observed in the AGS patients. The most prominent cellular phenotypes of these embryos are: fetal liver disintegration, failed hematopoiesis and widespread apoptosis, overexpression of IFN and IFN-stimulated genes (ISGs) 3,4. This immune response is initiated by the MDA5/RIG-I/MAVS pathway which indicates that endogenous unedited transcripts are recognized as foreign by the cells.

We generated Adar1;Mavs double mutant mice that lack the essential adapter protein required for interferon induction signaling from both RIG-I and Mda5. These Adar1;Mavs double mutant mice survived till 20 days after birth, showing that the embryonic lethality is due to an aberrant innate immune response of RLRs to unedited dsRNA.

The principal aim of my project is to characterize the immune signaling pathway aberrantly activated in the absence of Adar1. The cause why the Adar1;Mavs mice are dying is still unknown and during my project I will test if the knockout of specific proteins involved in the apoptotic pathway or in the immune signaling pathway could rescue the mutant lethality. I found that the mutant mice show a mild inflammation in the brain and an increase in the

apoptotic level in the intestine, brain and kidneys.

The second aim of my project is to investigate, by LC-MS/MS, if the lack of inosine in the mutant murine models is affecting the equilibrium of other main RNA modifications. A first analysis revealed that mice with no editing activity (Adar1 and Adar2 null mice) show a slightly increase in m6A level and confirmed that ADAR2 is the main enzyme responsible for editing in the brain.

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The RNA-editing enzyme ADAR1 controls innate immune responses to RNA. Cell Rep., 9 (2014), pp. 1482-1494

Development of Novel Patient-derived Xenograft Model of Chronic Lymphocytic Leukemia

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Patient-derived xenografts (PDX) enable therapy testing and study of cancer evolution on a genetic background of individual patients. We aim to develop a novel murine PDX model of chronic lymphocytic leukemia (CLL), an incurable B-cell malignancy with characteristic dependence on human immune microenvironment. In currently available PDXs proliferation of CLL lymphocytes is maintained by presence of autologous T-cells, however, in few weeks these spontaneously overgrow and eliminate engrafted CLL cells. Our intention is to replace T-cells and supply pro-proliferative and pro-survival signals by their introduction into supportive cell line co-implanted with purified CLL cells.

We genetically engineered an adherent supportive cell line, which in coculture induces long term survival and major proliferation of primary CLL cells in vitro. We seeded the cell line on collagen scaffolds, implanted subcutaneously into immunodeficient mice (NSG) and injected highly purified CLL cells ($65–350\times10^6$ cells per animal). By this we have achieved growth of B-cells in 2 out of 5 transplanted CLL samples; in both cases positive for Epstein– Barr virus (EBNA1 gene). CLL clonal relationship to the patient's sample was confirmed in one of the grafts by IGHV rearrangement analysis. In this case cells formed a solid tumor positive for CD20/CD45/CD79a cell-surface markers (detected by immunohistochemistry), and cells were found both in peripheral blood and murine spleen, which is a typical site of CLL infiltration in humans.

We plan to further assess different ways of transplantation and modify the supportive cells. The established model will be used for testing of novel therapeutic combinations and studies of CLL biology.

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The impact of Transposable Elements in Hematological Malignances

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Transposable elements, or transposons, are mobile genetic units that exhibit a broad diversity in their structure and transposition mechanisms. In the human genome, the vast majority of transposons is represented by retroelements (REs); they have a wide spectrum of functions, including gene expression deregulation via inserting their own copies within genes or gene proximity. The Long Interspersed Nucleic Elements (LINE-1 or L1) are the only active autonomous REs. They mobilize their own RNA to new genomic locations via a "copy-and-paste" mechanism and are able to retrotranspose other RE RNAs including Alu and SVA, and occasionally protein-coding RNAs. RE activity is mostly silenced by various control mechanisms, however, transposon reactivation has been implied in several medical conditions, including many cancer types (e.g. colorectal cancer, hepatocarcinoma, epithelial cancers). To our best knowledge, no systematic analysis employing sensitive high-throughput techniques has been performed to date to study RE activity in hematological malignancies.

Thus, we decided to explore RE activity in different types of hematological malignancies through detection of new RE insertions in primary patient samples. To identify tumor-specific RE insertions, we adopted a protocol based on high-throughput sequencing of amplicons containing a part of RE (i.e. belonging to Alu-Ya5, Alu-Yb8 or L1-HS families), and its adjacent genomic region. Using the unique molecular identifiers enabled calculation of a number of cells bearing each particular RE insertion. We performed a pilot experiment for a set of 36 samples from hematooncological patients, containing pretreatment tumor-normal pairs and follow-up tumor samples after anti-cancer therapy. The libraries were sequenced and analysed using the in-house bioinformatics pipeline for the identification of somatic RE insertions. A result of the analysis for the L1-HS family is 2 candidate insertions (one in a MDS and another in a CLL sample). We are going to further study these insertions in detail.

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Forward Genetic Screen as a Tool to Uncover Genes Governing Plant Meiosis

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Forward genetic screen is a powerful experimental method allowing to identify genes causing phenotype of interest. This method is becoming very popular again thanks to the progress in whole genome sequencing technologies that facilitate identification of causative mutations. In contrast to T-DNA insertional mutagenesis that causes gene disruptions, chemical mutagenesis used in forward genetic screens produces much wider range of mutations and, hence, higher diversity of phenotypes. This bring a new dimension in gene characterization efforts.

We use this technique to identify new genes governing cell fate transitions during germ-line differentiation. We are particularly interested in understanding mechanism by which non-sense mediated RNA decay factor, SMG7, regulates meiotic exit in Arabidopsis thaliana. We set up a suppressor screen in which we look for mutations that rescue infertility of SMG7 deficient plants. We have obtained at least 10 suppressor lines and we currently perform mapping in combination with whole genome sequencing to identify causative mutations. We expect that identified mutations will reveal new genes involved in regulation of meiotic progression.

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).

Initial Insights into the Topology of Endoplasmic Reticulum localized PINs in Arabidopsis Thaliana

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Auxin regulates plant growth and development through differential accumulation within tissues depending on the activity of PIN proteins that facilitate auxin efflux between cells. The plasma membrane PINs are involved in polar auxin transport (PAT), while the ER localized ones regulate intracellular auxin levels. The topology of plasma membrane (PM) localized ("long") PINs has recently been assessed. However, for the ER PINs this has not been done yet and we try to bridge this gap with our recent efforts. For the topology studies we have utilized GFP tagged PIN5 and PIN8 constructs, ectopically localizing on the PM of epidermis and cortex root cells. Here, we present experimental data showing the localization of PIN5 and PIN8 hydrophilic loops (HLs), using approaches based on pH-dependent quenching of fluorescent reporters, immuno-localization and protease protection techniques. The experiments were also guided by topology prediction software.

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

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