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Book of Abstracts

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Wilhelm Bernhard Medal Lecture

Type of presentation: Oral

From nucleolar morphology to phase separation in the cell nucleus

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Processes such as gene expression or DNA repair are compartmentalized within eukaryotic nucleus, and nuclear environment contains dynamic membrane-less sub-compartments whose formation is prevalently driven by phase separation. It Apparently, formation of phase boundaries provides the surface for spatiotemporal control contributing to the high-rate kinetics of crucial processes such as transcription, ribosome maturation, splicing. I will briefly recapitulate the history of my research and devote a majority of my talk to recent findings from our Prague laboratory. findings We discovered the Nuclear Lipid Islets (NLIs) – globular ~100 nm structures containing PI(4,5)P2 (PIP2) at their periphery which associate with key transcription factors, and showed that NLIs are crucial for efficient Polymerase II transcription. To decipher whether the NLIs surface recruits a transcription regulatory proteins through PIP2 molecules in their surface, we employed a proteomic approach based on differential quantitative mass in combination with super-resolution microscopy. We identified more than 300 NLIs-associated proteins belonging to gene expression (53%) and pre-mRNA splicing (33%). Super-resolution microscopy confirmed that some candidate proteins form foci in nucleoplasm and associate with sub-population of NLIs. Further, our bioinformatical analysis of putative NLIs proteins revealed that majority of them contain Intrinsically Disordered Regions (IDRs). IDRs are known features of proteins undergoing phase separation under in vivo and in vitro conditions. Moreover, we found that the vast majority of these proteins contain K/R rich motifs, which were previously shown as recognition sites for phosphoinositide (PIPs) binding. We hypothesize that NLIs may serve as a structural platform integrating RNA Polymerase II transcription and pre-mRNA splicing by attracting proteins which are prone to form liquid-like particles.

Acknowledgement: This study was supported by the Czech Academy of Sciences (JSPS-20-06); Grant Agency of the Czech Republic (19-05608S, 18-19714S); IMG ASCR, v. v. i.(RVO:68378050); by the COST Action CA19105 and MEYS CR (COST Inter-excellence LTC19048), and Czech-BioImaging projects (LM2018129 and LM2023050, funded by MEYS CR).

Young Researcher Award Lecture

Type of presentation: Oral

ADP-ribose metabolism, DNA strand breaks and disease

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We focus on understanding how defects in DNA repair and/or ADP-ribosylation lead to the development of cancer and/or neurological disease, particularly neurodegeneration. We identified gene mutations in the DNA repair proteins XRCC1 and PNKP associated with a DNA single-strand break (SSB) repair-defective neurological disease and revealed a pathology associated with excessive activation of PARP1, an enzyme that detects SSBs and signals their presence by catalysing the rapid post-translational modification of proteins with ADP-ribose. We discovered a novel molecular defect in CONDSIAS (stress-induced childhood-onset neurodegeneration with ataxia and seizures), a neurodegenerative disease in which the ADP-ribose glycohydrolase ARH3, that removes ADP-ribose from chromatin, is mutated. We found that whilst the absence of ARH3 does not impede SSB repair, it instead results in the persistence of mono-ADPribose chromatin 'scars' at sites of SSBs that have long since been repaired. These most likely perturb the canonical histone code, might interfere with transcription and can trigger cellular dysfunction. Hence, our preliminary data suggest that alterations in SSB repair and chromatin ADP-ribosylation are major contributors to neuropathological events. We find that these mono-ADPribose chromatin 'scars' at sites of SSBs are HPF1-dependent. The DNA damage responsive protein histone PARylation factor-1 (HPF1) has been identified as an interaction partner of PARPs, specifically PARP1 and PARP2. Subsequent findings indicate that loss of HPF1 affects primarily serine ADP-ribosylation, which is the most abundant ADP-ribosylation in the cell after DNA damage. We explore the role of histone ADP-ribosylation at sites of DNA damage using HPF1- and/or ARH3-deficient cells and a mouse model. We aim to investigate the mechanism/s by which aberrant chromatin mono-ADP-ribosylation lead to neurological disease and pathology associated with ARH3 deficiency.

Nuclear Compartments and Gene Expression

Type of presentation: Oral

Exploring the Nuclear Functions and Nuclear Presence of Actin Via In Vivo Methods

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Actin, as an ancient and fundamental protein, participates in various cytoplasmic, as well as nuclear functions such as chromatin reorganization and transcription regulation of differentiating eukaryotic cells. Based on its manifold tasks in the nucleus, it is a reasonable assumption that the nuclear presence of actin is essential for the cell, and consequently, its nuclear localization is ensured by a robust system.
In our work, we generated a genetic system in Drosophila, that enabled us to inhibit actin functions specifically in the nucleus. In this in vivo system we rescued the lethal phenotype of the null mutation of the Actin5C gene with different derivatives of actin, including a Nuclear Export Signal (NES)-tagged isoform which ensures forced nuclear export of the protein. In order to further decrease nuclear actin levels, we eliminated the only known nuclear import factor of actin, Importin 9. The NES tagging of actin, and the simultaneous knock out of its importin, significantly reduced the amount of nuclear actin, and induced lethality. This confirmed that the presence of actin in the nucleus is essential, and thereby, over-secured. Supporting this, we identified novel nuclear importins specific to actin with the in vivo BiFC system, and thereby we shed light on the robustness of the nuclear import of actin.

Type of presentation: Oral

A various response to different modes of anti-cancer treatment

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Nowadays, chemo-, targeted, and immunotherapy are used extensively to treat oncological malignancies. All of them could induce various responses in cancer cells and non-tumor cells of the tumor microenvironment: multiple ways of cell death, permanent cell arrest - senescence, and autophagy. Here we present our findings on how different modes of treatment interact and potentiate each other. We will share our thoughts what are the crucial points of switching cell response to anti-cancer therapy and the benefits of modulation these responses to improve the efficiency of current-day anti-cancer therapy.

Acknowledgement: The study was supported by RSF19-75-20128

Type of presentation: Oral

Intrinsically Disordered RGG/RG Repeats in Drosophila Nucleolar Proteins Form Liquid Phase Condensates with Non-Coding Heat Shock Transcripts

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RGG/RG repeats constitute low sequence complexity domains within proteins that participate in liquid phase condensation of membrane-less organelles such as nucleoli, processing bodies, and stress granules (Lafontaine et al., 2021; DOI 10.1038/s41580-020-0272-6). Upon heat shock, *Drosophila* nucleolar proteins Fibrillarin and Nopp140-RGG containing prominent RGG/RG domains redistribute from nucleolar DFCs to form condensates directly on heat shock genetic loci 87C and 93D of salivary gland polytene chromosomes. These condensates appear dark by phase contrast microscopy and are reminiscent of nucleolar DFCs. *Drosophila*'s true Nopp140 orthologue (Nopp140-True) is well conserved with vertebrate Nopp140 in its carboxyl tail, but it fails to locate to 87C and 93D upon heat shock, indicating the RGG/RG domains in Fibrillarin and Nopp140-RGG are responsible for their localization to 87C and 93D. The 87C locus encodes *Hsp70* transcripts as well as *alpha* and *beta* ncRNAs; 93D encodes only the *omega* ncRNA. Conversely, the neighboring 87A heat shock locus encodes only *Hsp70* mRNAs, and it fails to form condensates with GFP::Nopp140-RGG or Fibrillarin upon heat shock, indicating that the ncRNAs expressed at 87C and 93D are responsible for condensate formation with the RGG/RG-containing proteins. Earlier we showed that GFP::Nopp140-RGG locates to multi-lobed nucleoli in egg chamber nurse cells and to embryonic nucleoli, but that it uniformly fills the transcriptionally silent oocyte nucleus without forming condensates. Based on these observations, we suggest that formation of RGG/RG condensates depends on the expression of select RNAs (pre-rRNAs, C/D box snRNAs, or ncRNAs) that share common yet undetermined structural features.

Acknowledgement: This work is funded by the NSF, award number MCB2141390.

Type of presentation: Oral

Quantitative super-resolution microscopy reveals the phospholipid identity of the gene expression compartments in human cells and tissues

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Accumulating evidence suggests the involvement of nPIPs in the regulation of gene expression, the precise sub-nuclear distribution of nPIPs and their relationships with the gene expression remains elusive. We used dual-color direct stochastic optical reconstruction microscopy and quantitative nearest neighbor distance analysis to show that in the nucleoplasm (NP) and at nuclear speckles (NS), phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) and phosphatidylinositol 3,4-bisphosphate (PI(3,4)P2) co-pattern in the close proximity with the subset of sub-diffraction limited RNA polymerase II (RNAPII) foci. Moreover, we analyzed the spatial relationship between the marker of RNAPII initiation or elongation phase with respect to PI(4,5)P2 or PI(3,4)P2 in the NP or at NS. We found the preferential spatial relationship of PI(4,5)P2 with the transcription initiation marker in the NP and PI(3,4)P2 with elongation marker at NS. We extended our analyses from cells to human clinical formalin-fixed paraffin-embedded (FFPE) tissue sections using stimulated emission depletion microscopy. We quantified the specific nanoscale co-patterning between PI(4,5)P2 and NS in two clinically relevant human samples, healthy skin and human papilloma virus (HPV) induced skin warts. We found a higher overlap and correlation of PI(4,5)P2 with NS in warts compared to skin, which is in line with previous findings that linked increased levels of nPI(4,5)P2 with HPV infection. Based on our data we propose a model in which nPIPs of the membrane-less nuclear sub-compartments accompany the molecular events of the progression of the RNAPII transcription. Moreover, our data document the feasibility of the nanoscale analysis of protein and lipid nuclear antigens in human clinical FFPE tissue sections, provide the detailed information about the nPI(4,5)P2 in human tissue and show differences between healthy and HPV infected skin.

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Type of presentation: Oral

HIRA-mediated loading of histone variant H3.3 regulates androgen-induced transcription in prostate cancer by retention of AR/BRD4 complex at enhancers

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Initiation and progression of prostate cancer (PC) is determined by, among other factors, transcription reprogramming that is controlled by epigenetic dysregulation. Incorporation of histone variant H3.3 comprises active territories of chromatin. Exploring the function of H3.3 in PC, we found that knockout (KO) of H3.3 chaperone HIRA obliterates PC growth in vitro and in xenograft settings, deregulates androgen-induced expression and reduces androgen receptor (AR) binding at androgen response elements (AREs) within enhancers of target genes. H3.3 affects transcription in multiple ways, including activation of p300 by phosphorylated H3.3S31, that results in acetylation of H3K27 (H3K2Ac) at enhancers. In turn, H3K27Ac recruits bromodomain protein BRD4 for enhancer-promoter interaction and transcription activation. We observed that HIRA KO reduces H3.3 incorporation, diminishes H3.3S31Ph, H3K27Ac, recruitment of BRD4, and, finally, affects androgen-induced enhancer/gene association. These data suggest that H3.3-enriched enhancer chromatin serves as a platform for H3K27Ac-mediated BRD4 recruitment, which interacts with and retains AR at enhancer AREs, resulting in transcription reprogramming. In addition, HIRA KO deregulates glucocorticoid-driven transcription, suggesting common H3.3/HIRA-dependent mechanism of nuclear receptors function. Expression of two members of HIRA complex (HIRA and UBN1) is increased in PC compared with normal prostate tissue, is elevated in high/very high-risk PC groups, and their accumulation is associated with negative prognosis. Collectively, our results demonstrate function of H3.3/ HIRA pathway in regulation of nuclear receptors activity and suggest an oncogenic function of HIRA-dependent H3.3 pathway in PC progression.

Type of presentation: Oral

Differential Genomic Distribution of Human Histone H1 Variants.

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Histone H1 binds to the linker DNA at the nucleosome, participating in the formation of higher-order chromatin structures. Human somatic cells may contain up to seven members of the histone H1 family contributing to the regulation of nuclear processes, apparently with certain subtype specificities. We have explored the functional role of histone H1 variants by shRNA-mediated knock-down of single or multiple H1s. In T47D breast cancer cells, the combined knock-down of H1.2 and H1.4 subtypes (multi-H1 KD) has a strong deleterious effect: coordinately deregulates many genes, promotes the appearance of accessibility sites genome-wide and triggers an interferon response via activation of heterochromatic repeats. Besides, multi-H1 KD translated into more de-compacted chromatin structures at the scale of topologically associating domains (TADs). Profiling of endogenous H1 variants in these cells revealed coexistence in the genome in two large groups depending on the local GC content: H1.2, H1.3, H1.5 and H1.0 were abundant at low GC regions while H1.4 and H1X preferentially co-localized at high GC regions. H1 abundance at different transposable element classes was also variant-specific. Interestingly, above-mentioned chromatin changes upon multi-H1 KD occurred with only slight H1 variant redistributions across the genome. Imaging experiments of H1 variants also support differential genomic patterns revealed by ChIP-Seq data and variant-specific association to particular chromatin environments, such as lamina-associated domains (LADs) or the nucleolus.

Type of presentation: Oral

The Essence of Existence: from the Finalization of the 3D Genome Organization to a Novel Generalized/Unified Evolution Theory

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Recently, we filled finally the debated centennial gaps in genome 3D architecture/dynamics: definately a chromatin quasi-fibre with 5±1 nucleosome per 11 nm, folding into stable(!) 40-100 kbp loops forming stable(!) aggregates/rosettes which are connected by a ~50 kbp chromatin linker exists in complete consistency with the entire field history of the last 170 years.
This leads instantly to a novel consistent, cross-proven systems statistical mechanics genomics framework elucidating genome intrinsic function and regulation. It balances stability/flexibility ensuring genome integrity, enabling expression/regulation of genetic information, as well as genome replication/spread. Furthermore, genotype and phenotype are multiplisticly entangled being evolutionarily the outcome of both Darwinian natural selection AND Lamarckian self-referenced manipulation - all embedded in broader unifying genome ecology (autopoietic) i(!)n- and environmental scopes. This allows formulating a new meta-level functional semantics of genomics, i.e. notions as communication of genes, genomes, and information networks, architectural and dynamic spaces for creativity and innovation, or genomes as central geno-/phenotype entanglements. Beyond and most fundamentally, the paradoxical-seeming hierarchical local equilibrium substance stability in its entity though far from a universal heat-death-like equilibrium is solved, and system irreversibility, time directionality, and thus the emergence of existence are clarified! This is nothing less than a - by the upper experiments and thus already partially proven - novel general and unified theory of evolution. Consequently, real deep understandings of genomes, life, and complex systems in general appear in evolutionary perspectives as well as from systems analyses, via system damage/disease (its repair/cure and manipulation) as far as the understanding of extraterrestrial life, the de novo creation and thus artificial life, and even the raison d'etre.

Acknowledgement: Knoch, T. A. How genomes emerge, function and evolve: living systems emergence, genotype-phenotype-multilism, genome/systems ecology. Res. Prob. Cell Diff. 70, Springer, ISBN 978-3-031-06572-9, DOI 10.1007/978-3-031-06573-6_4, 103-156, 2022.

Type of presentation: Oral

The Nuclear Import of the Drosophila Moesin Protein Is Regulated by Cytoplasmic Retention

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The actin-binding moesin protein is a member of the evolutionary conserved ERM (Ezrin-Radixin-Moesin) family and the only ERM representative in Drosophila melanogaster. The cytoplasmic function of moesin is the crosslinking of the actin cytoskeleton and plasma membrane proteins. However, moesin is also present in the nucleus, and under certain circumstances (e.g. heat shock, ecdysone treatment) it's amount there can increase significantly. Our laboratory has shown that the nuclear activity of moesin is pivotal in Drosophila development, but the exact mechanisms by which moesin enters and exits the nucleus are not known.
Our goal is to elucidate the dynamics and the underlying mechanisms of the nuclear transport of Drosophila moesin. For the investigation of the dynamics we use the fluorescence microscopy technique, called FRAP (Fluorescence Recovery After Photobleaching). Our FRAP experiments revealed that in contrast to its main binding partner, actin, moesin has a constant but weak nuclear import, and that upon induction of its import there is only a little increase in the dynamics of moesin's nuclear entry. This suggested the existence of a mechanism that retains the protein in the cytoplasm. With the help of data available in the literature we were able to predict a 25 amino acids long motif in moesin, which might be responsible for its cytoplasmic retention. By analyzing the nuclear import dynamics of wild type moesin under different conditions, and by comparing the behavior of various mutant forms we showed that the motif is indeed a functional Cytoplasmic Retention Signal (CRS). Further analysis of the CRS demonstrated that the motif is able to retain even the GFP protein in the cytoplasm. Currently, we are identifying the protein that binds the CRS thus, retains moesin in the cytoplasm.

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Type of presentation: Oral

The intrinsically disordered N-terminus of cancer-testis antigen MAGEA10 is responsible for its expression, nuclear localization and aberrant migration

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Melanoma-associated antigen A (MAGEA) subfamily proteins are normally expressed in testis and/or placenta, however, the aberrant expression is detected in tumor cells of multiple types of human cancer. MAGEA expression is observed mainly in cancers that have acquired malignant phenotypes, invasiveness and metastasis, and the expression of MAGEA family proteins has been linked to poor prognosis in cancer patients. Despite being discovered more than 25 years ago, biological functions of the MAGE family of proteins still remain poorly understood. All MAGE proteins share the common MAGE homology domain (MHD) which encompasses up to 70% of the protein, however the areas flanking the MHD region vary between family members and are poorly conserved. We performed the deletion and point-mutation analysis of MAGEA10 protein to investigate the molecular basis of MAGEA10 expression and anomalous mobility in gel. We demonstrate that the N-terminal intrinsically disordered domain and specifically the first seven amino acids are responsible for its expression, aberrant migration in SDS-PAGE and nuclear localization. The N-terminus of MAGEA10 must be freely accessible to be expressed in the cells. Our results suggest that the intrinsically disordered domains flanking MHD determine the unique properties of individual MAGEA proteins.

Type of presentation: Oral

Most of the human pol II promoters have small regions with a distinctive physical property

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The TATA box and initiator (Inr) sequences are well-known core promoter elements (CPEs), though both TATA-containing promoters and Inr-containing promoters are the minorities in the pol II promoters. These elements are only 2.4 nm in length, while, *e.g.*, the human genome is ~1 m long. A longstanding enigma is how these CPEs are recognized to initiate transcription. Using human pol II promoters, we previously found that TATA box and Inr comprise distinctively flexible (upstream half) and rigid (downstream half) sequences when compared with the other parts of the promoter region (Fukue et al., 2004). Interestingly, the same physical property (PP) lies in the transcription start site (TSS) in the case of “core-less” promoters that do not harbor any known promoter elements and form the majority in the human pol II promoters (Fukue et al., 2005). The distinctive PPs embedded in the promoters may function as built-in signals to show positions of the promoters. However, the findings described above were based on the averaged PPs of the respective groups of promoters. Thus, it remains unsolved whether each individual gene has and uses such a distinctive PP for transcription. The current study examined this issue and also performed clustering of genes based on PPs of their promoters.
Using the data set on “high-confidence TSSs” defined by the FANTOM5 consortium, we classified human pol II promoters. Those were largely divided into CPE-containing promoters and core-less promoters. The former was further sub-grouped into 67 groups according to the species of CPEs. Interestingly, core-less promoters accounted for more than 60% of the total. We confirmed that in most cases the TSS and position ~ –27 of each individual gene have a distinctive PP, irrespective of the promoter type. Furthermore, the clustering analysis unveiled an interesting aspect of the pol II genes. Here, we discuss genetic information carried in PPs of DNA.

Type of presentation: Oral

Nuclear actin regulates the 3D genome organization

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The eukaryotic genome is organized into a multilayered hierarchical structure with key roles in development and differentiation. 3D chromatin architecture refers to the organization of the genome into chromosomal territories, active and repressive compartments, topologically associating domains, and chromatin loops. We recently reported that nuclear β-actin regulates chromatin organization. Using a comprehensive genomic analysis utilizing HiC-Seq, ATAC-Seq, RNA-Seq and ChIP-Seq of various epigenetic marks in β-actin knockout cells, we showed that changes in β-actin levels influence chromatin structure by affecting the interplay between the chromain remodeler BAF (SWI/SNF) and polycomb repressive complexes. Changes in β-actin-dependent chromatin remodeling activities not only affect local chromatin accessibility but also induce reversable changes in 3D genome architecture at the level of A and B compartments. We next used β-actin knockout cells as a model to establish the relationship between changes in compartment-level 3D genome architecture and transcriptional regulation. Further, we studied the relevance of this relationship during development and differentiation using published genomic data generated in mouse embryonic fibroblasts (MEFs) reprogrammed into pluripotent stem cells (PSCs). We showed that transcriptional changes associated with compartment reorganization originate from changes in gene accessibility or accessibility of distal regulatory elements. β-actin loss triggers accumulation of enhancer-specific epigenetic mark H3K27ac and using the ABC model of enhancer annotation we demonstrate that this epigenetic change is associated with activation of enhancer elements. Using both β-actin knockout and MEF to PSC reprogramming data, we reveal that transcriptional changes in compartment switching regions are driven by changes in the epigenetic state of distal enhancers. Altogether this supports a key role for actin in the functional 3D genome organization.

Acknowledgement: We thank the Bioinformatics Core Facility, NYU Abu Dhabi. This work is supported by core funding from NYU Abu Dhabi and by Tamkeen under the NYU Abu Dhabi Research Institute Award to the Center for Genomics and Systems Biology (ADHPG-CGSB) to PP

Type of presentation: Oral

Topological arrangement of poised rRNA genes and the nucleolar response to anti-cancer drug CX-5461

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The nucleolus serves as an integral hub to translate environmental cues to the biosynthetic activity of cells. Here, we studied (1) the effects of a drug to reduce specifically nucleolar activity, CX-5461, and (2) the hitherto unknown localization of poised rRNA genes.
CX-5461 is a recently introduced inhibitor of ribosome synthesis. We used time-lapse imaging, immunofluorescence and ultrastructural analysis and detected a profound impact on nucleolar morphology and function upon exposure of cancer cells to CX-5461: nucleoli became compact, and spherical in shape and display enlarged, ring-like masses of perinucleolar condensed chromatin. In addition, intranucleolar tunnels that consist of chromatin developed as transient structures running through nucleoli. The nucleolar components involved in rRNA transcription, fibrillar centres and dense fibrillar component with their major constituents, ribosomal DNA, RNA polymerase I and fibrillarin maintain their topological arrangement but become reduced in number and move towards the nucleolar periphery. We demonstrate that incubation with CX-5461 leads to specific morphological alterations of the nucleolar structure and show that CX-5461 is well suited as nucleolar inhibitor for use in basic research applications.
Poised rRNA genes carry bivalent histone modification marks (i.e. both activating and repressing marks) to allow quick recruitment for transcription if cells encounter favourable environmental conditions. We applied a combined IF-FISH approach and consistently localized poised genes exclusively at the nucleolar periphery in the perinucleolar chromatin. We hypothesize that the location of poised rDNA genes in the perinucleolar environment enables access of histone modifying enzymes for maintaining the poised state as well as to those factors responsible for activation of poised rRNA genes.
Both studies shed light on the topology and dynamic interplay between ribosomal DNA chromatin structure and nucleolar morphology.

Type of presentation: Oral

Variability of Human rDNA and Transcription Activity of Ribosomal Genes

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In humans, ribosomal DNA (rDNA) comprises multiple repeats, each containing a 13 kb segment with genes encoding 18S, 5.8S, and 28S ribosomal RNAs, and a 30 kb intergenic spacer (IGS). However, not all repeats are transcribed, and the active ones are characterized by specific epigenetic marks associated with active chromatin, including hypomethylation of the promoter and adjacent IGS regions. It is still not clear what determines the differentiation of rDNA genes into active and silent. Our study investigates whether the nucleotide sequence of the IGS influences this differentiation. rDNA was isolated from nucleoli of human fibrosarcoma cells (HT1080), and methylated and non-methylated DNA were separated using chromatin immunoprecipitation. A 2 kb long region upstream of the transcription start site was amplified using PCR, and the resulting product was analysed by Next Generation Sequencing. The analysis revealed significant sequence variability, including single nucleotide variations (SNVs), short insertions, and deletions. Six SNVs showed a correlation with DNA methylation status, and there was a strong association between the methylation status and the number of simple (tetrameric) repeats, which are favourable for the formation of G-quadruplexes. Similar variants were observed in the normal human fibroblasts and in the samples of brain tumours. These findings suggest that the IGS structure is involved in the differentiation of ribosomal genes into active and silent states. Notably, one of the identified SNVs belongs to a known micro-RNA (miR) gene that participates in cancer-related pathways. Additionally, two other SNVs are situated in regions that are well-matched with miRs produced in other parts of the genome. Therefore, our data suggest several pathways that may connect variations in rDNA sequence with its transcriptional activity.

Acknowledgement: This study was funded by the Grant Agency of Czech Republic (19-21715S) and the Charles University (Cooperation - Oncology and Hematology program).

Type of presentation: Oral

Molecular principles of Cajal body formation

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The cell nucleus is a highly heterogeneous environment crowded with numerous macro-molecules. Part of the nuclear complexity rises from the presence of a number of different bodies, non-membrane bound structures, which accumulate various proteins and, often RNAs. One of the “classical” examples of a nuclear body is the Cajal body (CB). CBs are involved in biogenesis, quality control and recycling of spliceosomal snRNPs. Coilin, the essential scaffolding protein of CBs, self-oligomerize and interacts with numerous proteins including snRNPs, and these interactions are important for CB formation. However, the basic information regarding coilin dynamics, structure and function are lacking. To uncover molecular principles of CB formation we analyzed snRNP role in coilin self-oligomerization and CB formation. We prepared several different mutated versions of coilin that prevent self-association, interaction with snRNPs or both, express these mutants in coilin KO cells and apply different fluorescence microscopy techniques (FRAP, single-point and imaging FCS and 3D-SIM SPT) to determine coilin dynamics in the nucleoplasm and the CB. Our data show that abolishing coilin interaction with snRNPs does not inhibit coilin self-association but prevent CB formation. We propose a two-component model where a coilin-snRNP complex is necessary to form CBs.

Type of presentation: Oral

Hyperinsulinemia in Brown Adipocytes: Both Insulin and Phosphorylated Insulin Receptor Enter the Nucleus and Reside in Heterochromatin Region

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Insulin (INS) is considered a major modulator of thermogenic function of brown adipocytes (BA). INS activate various signalling pathways through the phosphorylation of insulin receptor (IR), leading to the upregulation of genes involved in thermogenesis and energy expenditure. INS can also enter the nucleus of various cell types and exert the similar effects. Hence, we induced hyperinsulinemia to study the effects of INS on BA nucleus.
Adult male rats were treated with INS (0.4IU/kg or 4IU/kg body mass, i.p.), acutely (one day) or chronically (three days). Three hours after the last injection the animals were sacrificed and the interscapular portion of BAT was isolated. Nuclear ultrastructural examination was convoyed with INS and phosphorylated IR (IRp) immunohistochemistry (IHC) and immunogold labeling (IG).
Microscopy analysis revealed that both acute doses and a low chronic dose of INS induced similar changes: an increase in volume and a change in the shape of the nucleus to a spherical one, followed by appearance of euchromatin and nucleoli. Chronic high dose of INS led to the recurrence of polygonal nuclei characteristic for the control. Using IHC, we discovered the presence of INS in the nuclei of BA, heterochromatic nuclei being more receptive to INS than euchromatic ones. IG confirmed dominant INS presence in heterochromatin region of the nucleus irrespective of dose applied and treatment duration. We also examined the nuclear presence and distribution of IRp and show that INS and IRp simultaneously enter BA nucleus.
These results reveal that INS plays a key role in the regulation of nuclear events in BAT leading to decrease in chromatin compactness and changes in distribution of heterochromatin and euchromatin, thus affecting the expression of genes involved in thermogenesis and energy homeostasis. Understanding these mechanisms could have important implications for the development of new therapies for metabolic disorders such as obesity and diabetes.

Acknowledgement: This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia [grant numbers 451-03-68/2022-14/200178].

Type of presentation: Oral

Epigenetic regulation and its modulation via the C-terminal tail of H2A.Z.

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Since the nucleosomal structure is, in general, repressive for transcription, replication and repair, the strategy of eukaryotic cells to regulate these activities involves de-repression by destabilizing or mobilizing particular nucleosomes; therefore, the stability of nucleosomes is of utmost regulatory importance and it is expected to be modulated by posttranslational modifications, the reader proteins binding to them as well as histone variant composition. In this context, destabilizing effects are expected in the case of activating functions, while nucleosome stabilization would suit repressive roles. In a puzzling manner, there are observations suggesting that the presence of the histone variant H2A.Z in the nucleosomes can increase, while others indicating that it may rather decrease nucleosome stability,
Using an in situ assay of nucleosome stability in nuclei of DT40 cells expressing engineered forms of the variant we show that native H2A.Z is released from nucleosomes of peripheral heterochromatin at unusually high salt concentrations, compared to cells expressing C-terminally truncated H2A.Z. Upon treatment of HeLa nuclei with the tail-peptide (C9), the H2A.Z-nucleosomes were destabilized, peripheral heterochromatin became dispersed and overall nuclease sensitivity increased, recapitulating tail-dependent differences in DT40. Binding of C9 in reconstituted nucleosomes and to DNA, as well as to the nuclear lamina were demonstrated. Thus, H2A.Z has a large-scale impact on chromatin structure suggesting that epigenetic modulation can be achieved by targeting molecular interactions involving its C-terminal tail.

Acknowledgement: This work has been supported by OTKA 128770 and 138524, COST CA15214 and CA18127.

Type of presentation: Oral

Mercury Chloride reshapes heterochromatin domains and increases nucleolar activity in mouse hepatocytes

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Introduction
Mercury is a highly toxic element, widespread in the environment, that induces a broad range of adverse effects on health. It is metabolized primarily in the liver. We analysed the effects of $HgCl_2$ on the nuclear architecture in mouse hepatocytes, focusing specifically on the organization and some epigenetic features of the heterochromatin and on the nucleolar morphology and activity.
Methods
Mouse hepatocytes were exposed to $HgCl_2$ for 1h. To investigate in detail the heterochromatin degree of condensation after $HgCl_2$ treatment, we stained the hepatocytes with osmium ammine. Immunogold staining at TEM was used to image and quantify the distribution of some epigenetic markers of heterochromatin. Finally, nucleolar size and morphology were evaluated by TEM and agNOR staining, while ribogenesis was estimated by RT-qPCR quantification of the rRNAs.
Results
We observed an evident heterochromatin decrease that assumes a loose conformation after $HgCl_2$ exposure as revealed by osmium ammine staining: on average heterochromatin is less contrasted and less dense in treated cells compared to control. Analyzing the distribution of epigenetic markers of heterochromatin: H3K9me3, H4K20me3 and H3K27me3, we found a strong decrease of all these three markers both in nucleolus associated domains (NADs) and lamina associated domains (LADs) in cells exposed to $HgCl_2$. Finally, hepatocytes treated with $HgCl_2$ showed a clear extension of nucleolus and NORs size and an increase in rRNA transcription.
Conclusions
Our data suggest a general cell activation after HgCl2 treatment, which is realized by an extensive heterochromatin decondensation, clearly visible by our microscopy approaches and reflected by a decrease of some epigenetic markers of heterochromatin. This heterochromatin derepression seems to be accompanied by nucleolar activation, maybe to rapidly translate the enzymes necessary for detoxification.

Acknowledgement: This research was supported by the Italian Ministry of Education, University and Research (MIUR): Dipartimenti di Eccellenza Program (2018–2022)—Dept. of Biology and Biotechnology, University of Pavia.

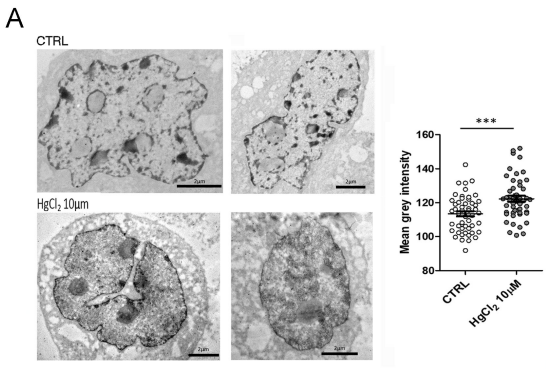

Fig. 1: Osmium ammine staining revealed different heterochromatin morphology and organization in control (top) and after HgCl2 treatment (bottom) in cell culture hepatocytes, bars: 2µm (a) and in liver tissue, bars: 1µm (b). The graphs show the heterochromatin mean grey intensity ± SEM. Statistical significance was evaluated using unpaired Student's t test

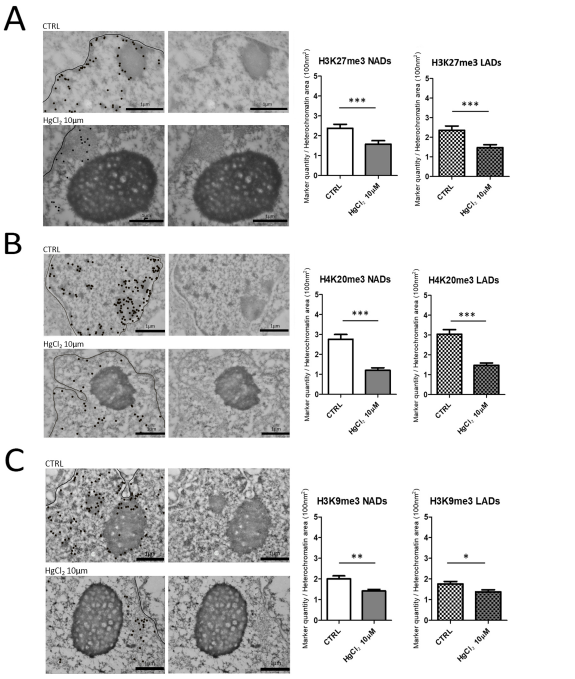

Fig. 2: The micrographs show the localizations of H3K27me3 (a), H4K20me3 (b) and H3K9me3 (c) in mouse hepatocytes, highlighted by black dots, the line indicate nuclear envelope. The corresponding original images are shown on the right. Bars: 1µm. Histograms show the mean ± SEM quantity of the HPTMs density per heterochromatin area in NADs and LADs for control and HgCl2 treated cells. Statistical significance was evaluated using unpaired Student's t test.

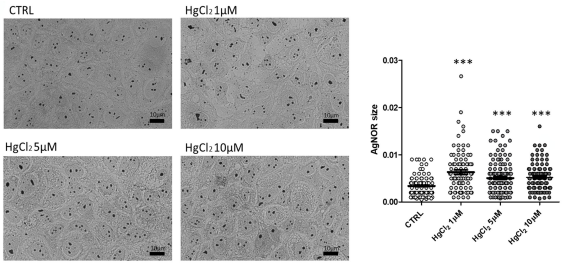

Fig. 3: Silver staining of the NORs. AgNOR staining for control and hepatocytes treated with increasing doses of HgCl2. Bars: 10µm. Histograms show the size of the NORs. Bars indicate mean ± SEM. Statistical significance was evaluated using One Way ANOVA test

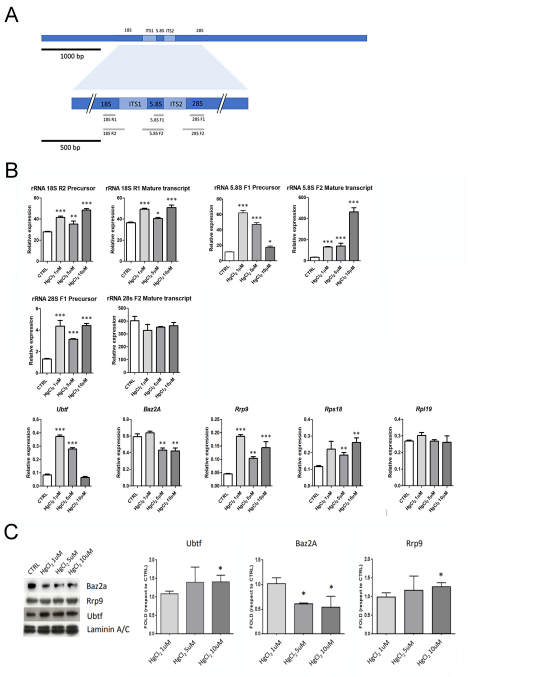

Fig. 4: (a) Schematic representation of primers design for rRNAs quantification. (b) RT-qPCR to quantify the expression of rRNAs and some proteins involved in the ribogenesis. Histograms: mean ± SD of the relative gene expression (One Way ANOVA test). (c) Western blot analysis to quantify the expression of proteins involved in the ribogenesis process. Histograms: mean ± SD of the fold change protein quantity relative to the control (One Way ANOVA test)

Nuclear Lipids and Phase Separation in Health and Disease (COST Action 19105, EpiLipidNET)

Type of presentation: Oral

The role of phosphatidylinositol 4,5-bisphosphate in transcription

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The RNA Polymerase II (RNA Pol II) transcription is tightly regulated process in eukaryotic cells. The assembly of RNA Pol II initiation complex is associated with the capacity of its components (eg. RNA Pol II and transcriptional factors) to form condensates by phase separation. Our laboratory discovered that Nuclear Lipid Islets (NLI) are phosphatidylinositol 4,5-bisphosphate-rich structures whose periphery is associated with Pol II transcription initiation (Sobol et al., 2018). Our recent study identified over 500 protein interactors of phosphatidylinositol 4,5-bisphosphate involved in different biological processes such as gene expression, chromatin organization and cell cycle regulation (Sztacho et al., 2021). Nevertheless, the precise role of phosphoinositides in the nucleus is still largely unknown. The aim of this project is to elucidate in more detail the role of phosphatidylinositol 4,5-bisphosphate in the regulation of RNA Pol II gene expression.

References:

Sobol M. et al., Nuclear phosphatidylinositol 4,5-bisphosphate islets contribute to efficient RNA polymerase II-dependent transcription, J Cell Sci (2018)

Sztacho M. et al., Limited Proteolysis-Coupled Mass Spectrometry Identifies Phosphatidylinositol 4,5-Bisphosphate Effectors in Human Nuclear Proteome, Cells (2021)

Acknowledgement: GACR 19-05608S,18-19714S;CAS JSPS-20-06; IMG RVO: 68378050;MEYSCR LTC19048,LTC20024,LM2018129 Czech-BioImaging;COS EpiLipidNET CA19105;European Regional Development Funds CZ.02.1.01/0.0/0.0/16_013/0001775, CZ.1.05/1.1.00/02.0109;TACR(TN02000122)

Type of presentation: Oral

DNA methylation, methyl readers and heterochromatin organization

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Heterochromatin is the highly compacted form of chromatin and hallmarked by high DNA methylation. MeCP2 is a DNA methylation reader, which is mutated in a human neurological disorder called Rett syndrome. MeCP2 and other members of the methyl-CpG binding domain protein family have also been reported as heterochromatin organizers. We combined liquid-liquid phase separation (LLPS) analysis and single-molecule tracking with quantification of local MeCP2 concentrations in vitro and in vivo to explore the mechanism of MeCP2-driven heterochromatin organization and dynamics. We show that MeCP2 alone forms liquid-like spherical droplets via multivalent electrostatic interactions and with isotropic mobility. Crowded environments and DNA promote MeCP2 LLPS and slow down MeCP2 mobility. DNA methylation, however, restricts the growth of heterochromatin compartments correlating with immobilization of MeCP2. Furthermore, MeCP2 self-interaction is required for LLPS and is disrupted by Rett syndrome mutations. In summary, we are able to model the in vivo heterochromatin compartmentalization as well as MeCP2 concentration and heterogeneous motion in the minimal in vitro system.

Acknowledgement: Funded by DFG.

Type of presentation: Oral

Nuclear Inositide Signalling: Role of PLCs in Health and Disease

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Since 1987 (Cocco & Irvine, B.J., 248, 765-7015) evidence from several laboratories has highlighted the presence of autonomous nuclear inositol lipid metabolism. The evidence suggests that lipid signalling molecules are important components of signalling pathways operating within the nucleus. The findings are important given the fact that nuclear signalling activity controls cell growth and differentiation.
Among the nuclear enzymes involved in this system, inositide-specific phospholipase C (PI-PLC) β1 has been one of the most extensively studied enzymes (Martelli & Cocco, Nature, 358, 242-45). Besides the studies on its signalling activity in physiological conditions, clinically oriented ones have shown that PI-PLCβ1 gene is associated with several pathological conditions. Nuclear PI-PLCβ1 is involved in the early stages of hemopoiesis and, namely, in the control of cell-cycle progression in progenitor hemopoietic cells. In addition nuclear PI-PLCβ1 plays a crucial role in the initiation of the genetic program responsible for muscle differentiation in that the enzyme activates the cyclin D3 promoter during the differentiation of myoblasts to myotubes. Down regulation of this enzyme is associated with progression of myelodysplastic syndromes (MDS) into acute myeloid leukaemia as well as with myotonic dystrophy or DM, both type 1 and type 2. Here we briefly highlight the most important evidence of the role of nuclear PI-PLCβ1 in these pathologies as well as the significance of subcellular localization of PI-PLCs. In addition it is quite clear the potential role of PLCβ1 as biomarker in high-grade gliomas.

Type of presentation: Oral

Mechanism of phase separation at PML nuclear bodies

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PML nuclear bodies (PML NBs) play an important role as hubs of biochemical pathways in the nucleus of mammalian cells. Despite three decades of intense research, the precise biochemical function of PML NBs is still elusive. We are mainly interested in the assembly mechanisms of these multi-component structures. The assembly of PML NBs is mainly driven by poly-SUMOylated PML molecules. Here we established an *in silico* model of the assembly of a complete PML NB employing molecular dynamics simulation. Modeling-derived 'Virtual Microscopy' allowed us to assess the spatial relationship between PML NB components at molecule resolution. Strikingly, in an iterative process we have identified a novel SUMO self-interaction mechanism. We show that short SUMO3 peptide chains are able to self-assemble into dynamic biomolecular condensates in live-cell nuclei as well as *in vitro*. Sophistcated fluorescence fluctuation and super-resolution microscopy assays (FCS, RICS, FRAP, STED) were employed in living cells to assess the biophysical parameters of condensate formation. Our findings reveal a novel polySUMO chain-driven mechanism of liquid-liquid phase separation at PML nuclear bodies.

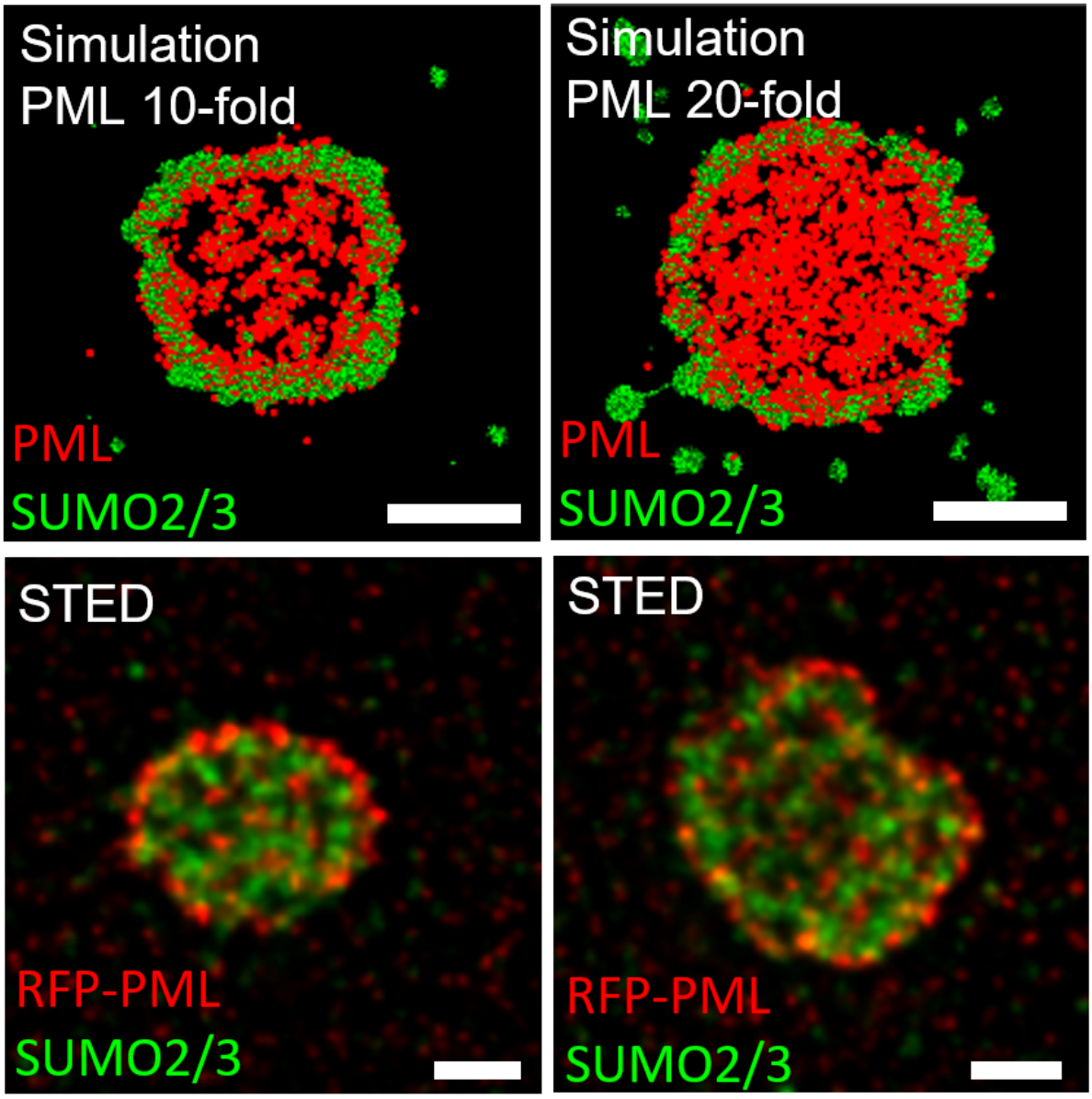

Fig. 1: PML nuclear bodies at nanometer resolution

Type of presentation: Oral

HP1 mediated phase separation regulates gene expression in development and disease

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Liquid-liquid phase separation has drawn great attention as a physical process that mediates the formation of membraneless organelles or microcompartments in cells to coordinate biological reactions in space and time. Initially, it was proposed that the formation of phase-separated droplets is a unique property of HP1α but we could show that also HP1β forms phase separated droplets which, however, is dependent of nucleosomes and H3K9me3 and thus provides a direct link to repressive epigenetic pathways. As mutations in the HP1β locus were linked with autism spectrum disorder (ASD), a neurological and developmental disorder, we investigated functional consequences of HP1β phase separation and found that it is required for neural progenitor cell (NPC) differentiation. RNA-seq analyses show that depletion of HP1b impairs the expression of bivalent developmental genes. We found that HP1β condensates regulate bivalent chromatin states during differentiation by promoting H3K27me3 removal and protecting them from hyper DNA methylation. We will discuss how HP1β condensates selectively recruit and exclude epigenetic regulators and thus control the expression of developmental genes. We found that ASD-causing mutations prevent the formation of these microcompartments and cause NPC differentiation defect. These results show how HP1 mediated phase separation may contribute to epigenetic regulation during development.

Type of presentation: Oral

Phosphoinositide 3-Kinase Signalling in the Nucleolus

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Many cellular processes are regulated by the phosphoinositide 3-kinase (PI3K) signalling pathway. The mode of action of this pathway has mostly been elucidated in the cytoplasm and can be orchestrated by two ubiquitously expressed PI3K catalytic isoforms, p110α and p110β. They both have the same enzymatic activity, which produces the polyphosphoinositide phosphatidylinositol (3,4,5)-trisphosphate (PIP3). Although both isoforms are localised in the cytoplasm and share common properties, recent evidence points to the existence of p110β and PIP3 in the nucleolus, where it can drive complementary functions. The nucleolus is the site of ribosome biogenesis through the synthesis of 47S pre-rRNA and processing into 18S, 5.8S and 28S, and assembly with ribosomal proteins. Ribogenesis is a rate limiting process for cell proliferation and requires tight control. The signals regulating nucleolar processes are however largely unknown, but we have now shown that the selective inhibition of p110β induced a decrease in 47S rRNA levels and cell proliferation. In addition, we mapped the nuclear interactome of its product, PIP3, by combining quantitative mass spectrometry and lipid pull down and identified a substantial number of PIP3-interacting proteins, about half of which were common to nucleolar protein datasets. We have so far focused our efforts on two of these proteins, proliferation associated protein 2G4 and poly(ADP-ribose) polymerase 1 and validated their direct interaction with PIP3. We identified the sites of interaction which consists of polybasic regions (PBR) and showed that these PBR also mediate the nucleolar localisation of these proteins. We are currently focusing on the functional characterization of the effect of PIP3 interaction of these proteins in the context of nucleolar processes.
In sum, the PI3K pathway can regulate processes in the nucleolus, upstream from its well-recognised role in protein translation in the cytoplasm.

Type of presentation: Oral

Biomolecular condensates as a storage site for immature transcripts in plant cells

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Membraneless biomolecular condensates play key roles in many cellular functions. Most often they are formed by phase separation, which is crated as a result of biomolecules accumulation within scaffold proteins and nucleic acids. This compartmentization of specific proteins and nucleic acids in subcellular environments is crucial for the proper regulation of many cellular functions. One of these condensates that we observe in European larch microsporocytes are known as extremely dynamic structures called Cajal bodies (CBs). They play a role in many processes related to the i.a. metabolism of small nuclear (sn)RNPs and nucleolar snoRNPs. However, this is the first time that their participation in the global retention of polyadenylated transcripts has been observed in plant a cells. We discovered that CBs are the side of the poly(A)RNA accumulation, but mainly nonfully spliced transcripts with retained intron(s) (Fig.1.). Among them were: transcripts coding for the Sm proteins: SmD1, SmD2, and SmG as well as mRNAs encoding the RNA polymerase II subunit RPB10. The fully mature transcripts were observed in the nucleoplasm and weren't accumulated in CBs (Fig.1.). We hypothesize that splicing of the retained intron(s) takes place immediately after the release of these transcripts from CBs and before they are exported to the cytoplasm.
Our research indicate the crucial role of these biomolecular condensates in the process responsible for post-transcriptional regulation of gene expression. This regulation allows the synthesis of specific proteins in a strictly controlled time points, which can be important for the proper differentiation and development of cells. Further investigation of condensates functions in normal and aberrant cellular states, can provide new insights into plant disease and the possibilities of increasing their resistance to stress conditions.

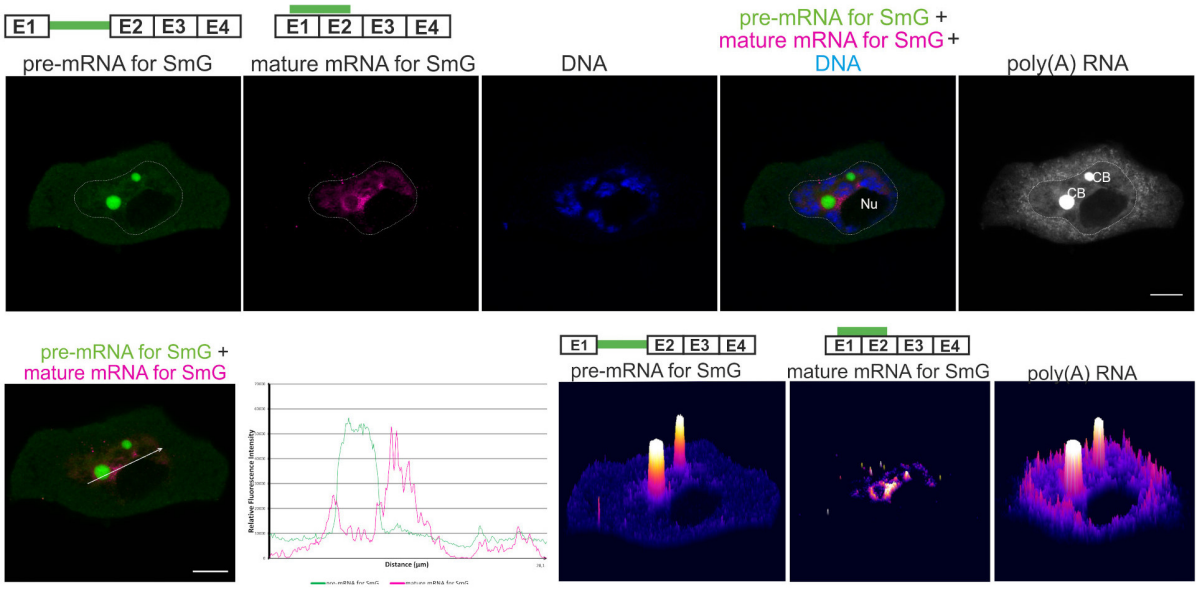

Fig. 1: Figure.1.Comparison of the distribution of immature and mature SmG mRNAs. Multi-labeling of the immature and mature form of SmG mRNA (upper). C: The fluorescence intensity of the SmG pre-mRNA and SmG mature mRNA along the white line is shown (lower). Right, 3D models of the general signal intensity of the analyzed cell.

Type of presentation: Oral

Localization and molecular mechanisms of nuclear functions of phosphoinositide-3,4-bisphosphate

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Phosphoinositides belong to the group of amphipathic phospholipid molecules. Enzymatically exchangeable phosphorylation pattern of 3'-, 4'-, and 5'-hydroxyl groups in the inositol head group yields seven isoforms of the phosphoinositide family. Consequently, each phosphorylation pattern results in diversified physical and chemical properties of the isoforms. Concerning their role in the cytoplasm and membrane transport, phosphoinositides are well-studied signalling molecules. However, recent findings suggest their active roles in various nuclear processes.
Although nuclei contain only membraneless structures, it is established that phosphoinositides play a role in transcriptional regulation, cell cycle progression, and chromatin organization. However, the function of phosphoinositide-3, 4-bisphosphate [PI(3,4)P2] in the nucleus is largely unexplored.
Our preliminary results indicate that phosphoinositide-3, 4-bisphosphate interacts with many RNA-binding proteins, namely ones involved in mRNA export. Therefore, we aim to explore the impact of modulated phosphoinositide levels on mRNA export.
The main objective of our study is to functionally characterize phosphoinositide-3, 4-bisphosphate in the regulation of nuclear export processes by defining the localization and interactome of PI(3.4)P2, as well as determining the conditions of interaction with its binding partners. We hypothesize that nuclear phosphoinositide pools might promote the nuclear compartmentalization of export machinery. As a result, our research will contribute to the understanding of the nuclear functions of phosphoinositides.

Acknowledgement: GACR (19-05608S and 18-19714S); IMG CAS (RVO: 68378050); TACR (TN02000122); CAS (JSPS-20-06); COS EpiLipidNET CA19105; MEYS CR (LTC19048, LTC20024, LM2018129); CzechBioImaging (CZ.02.1.01/0.0/0.0/16_013/0001775, CZ.1.05/1.1.00/02.0109)

Type of presentation: Oral

Molecular basis for interaction of intrinsically disordered proteins with nuclear PIP2 compartments.

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Phosphatidylinositol 4,5 bisphosphate (PIP2) is the most abundant phosphoinositide in eukaryotic cell nucleus. The majority of nuclear PIP2 is located in the nuclear speckles and nucleoli a two archetypical membrane-less compartments formed by phase separation. Moreover, the nucleoplasmic PIP2 pool is represented by Nuclear lipid islets (NLIs) that participate in the RNA polymerase II (Pol2) mediated transcription. The formation of nuclear membraneless sub-compartments depends on RNA. Similarly, the appropriate PIP2 localization within the nucleus is vastly affected by removal of intact RNA molecules. We speculate that PIP2-RNA association cooperates the establishment of nuclear architecture. Therefore, we sought for the proteins, which associated with nuclear PIP2 in RNA dependent manner. In order to identify the RNA-dependent PIP2-associated nuclear proteome (RDPA) in human cells we developed comparative mass spectrometry approach. The follow up bioinformatic analysis revealed that RDPA proteome participates in all steps of gene expression and typical RDPA protein possesses RNA binding and phase separation capacity. We show that RDPA proteins associate with PIP2 by electrostatic interactions, which depend on intact RNA and phase separation capacity of the participating proteins. Moreover, we identified that intrinsically disordered regions (IDRs) with polybasic PIP2-binding K/R-motif are common structural features of RDPA proteins. Further, we revealed the physicochemical properties of these IDRs and revealed that manipulation of PIP2 level results in changes of nuclear localization patterns of RDPA proteins in human cells. We identified that PIP2 spatiotemporally orchestrates nuclear processes through association with RNA and RDPA proteins and their capacity to phase separate. Thus, we described first evidence of PIP2 being crucial factor participating in still-to-be fully clarified establishing mechanism of nuclear architecture competent for gene expression.

Acknowledgement: GACR 19-05608S, 18-19714S; CAS JSPS-20-06; IMG RVO: 68378050; MEYSCR LTC19048,LTC20024, LM2018129 Czech-BioImaging; COS EpiLipidNET CA19105; European Regional Development Funds CZ.02.1.01/0.0/0.0/16_013/0001775, CZ.1.05/1.1.00/02.0109

DNA Replication, Repair, Disease

Type of presentation: Oral

Repair and misrepair of telomeric DNA in dynamic interactions with PML bodies and nuclear lamina in doxorubicin-treated cancer cell lines

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Previously, we described the alternative telomere lengthening (ALT) in TRF2-RAD51-positive PML bodies (APB) occurring after mitotic slippage in senescent breast cancer MDA MB 231 cells treated by doxorubicin (DOX). Transient ALT was accompanied by cutting off the DNA-damaged telomere ends with telomerase, while returning to telomerase mechanism in the recoved mitotic survivors. A number of meiotic genes (DMC1, SPO11, Mos, Vasa, Fragilis) were found activated in this model, and we suggested that the meiotic recombination nuclease SPO11 and inverted meiosis may be involved (Salmina, doi:10.3390/ijms 21082779). Here, the experiments with various concentrations of DOX were set on this and melanoma cell line SkMel28. We could confirm the association of SPO11 with APB after DOX and also RAD51-positivity in both cell lines indicating telomere DNA repair by HR. At high or sub-lethal concentrations of DOX the formation of the thready PML structures (PML isoform II) was occurring. These threads were composed of dimeric PML rods tandemly joined by the γH2AX -TRF2-marked chromatin and RAD51 absence. The PML threads were radially extending and circumventing the nuclear periphery, also intergrating into the dashed segments of nuclear lamin B1. The accumulated unrepaired DNA formed near nucleoli large γH2AX-positive clusters interknotted and fused to PML fibrils to result in a few mega-PML-bodies. The collapsed lamin B1 intruded and convoluted inside these structures. Both PML threads and defective nuclear lamina are known from progeria disease; somatic telomere movement and clustering driven by Hop2-Mnd1 heterodimers, which are essential for homologous chromosome synapsis during meiosis, were observed in ALT cells (Cho, doi: 10.1016/j.cell.2014.08.030). The dynamic PML-telomere-lamina interactions in the DOX-induced reversible and irreversible senescence of cancer cells will be discussed. Funding: Project No: lzp-2022/1-0114.

Type of presentation: Oral

In-vivo approaches to study DNA repair in plant roots and protoplasts: rebooting laser microirradiation for plant samples

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Plants are sessile organisms faced with constant threats to the genome integrity due to various exogenous and endogenous factors that introduce DNA lesions. While the spectrum of DNA lesions that plants face varies (e.g. base mismatches, photo-products, strand gaps, double-stranded breaks), the repair pathways with the exception of photo-reactivation are generally conserved among living organisms (Nisa et al., 2019). One crucial problem with studying the plant response is the lack of tools for in-vivo mapping of protein recruitment and dynamics in sites of DNA lesions, since the plant equivalent of mammalian cell culture systems (e.g. cell suspension cultures) is not well suited for microscopy. We have recently implemented a versatile approach to study DNA repair in-vivo in plant roots and protoplasts, which is based on laser microirradiation after sensitization with the DNA binding dye Hoechst (Nespor Dadejova et al, 2022). We have shown that the nature of DNA lesions induced by this system is complex, with pronounced and rapid recruitment of DNA repair factors such as PCNA or MRE11 (a double-strand break repair factor) to sites of DNA lesions. Besides monitoring the involvement of a protein of interest in DNA repair processes, the dynamics of recruitment can be estimated in a quantitative manner. As an extension of the system, the irradiation can be performed in protoplasts transformed with vectors designed for bimolecular fluorescence complementation assay, which can be used to assess conditional interactions of proteins tied to the DNA damage response. Using this system we were able to observe the assembly of the CAF-1 histone chaperone to the sites of DNA lesions (Nespor Dadejova et al., 2022). Coupled with related approaches such as UV-irradiation of stably transformed lines (e.g. PCNA-GFP; Yokoyama et al., 2016) and exposure to chemical genotoxic agents (bleomycin, hydroxyurea), it is possible to map the response of plants to DNA lesions by microscopy.

Acknowledgement: „We acknowledge the CEITEC core facility CELLIM supported by MEYS CR (LM2023050)"

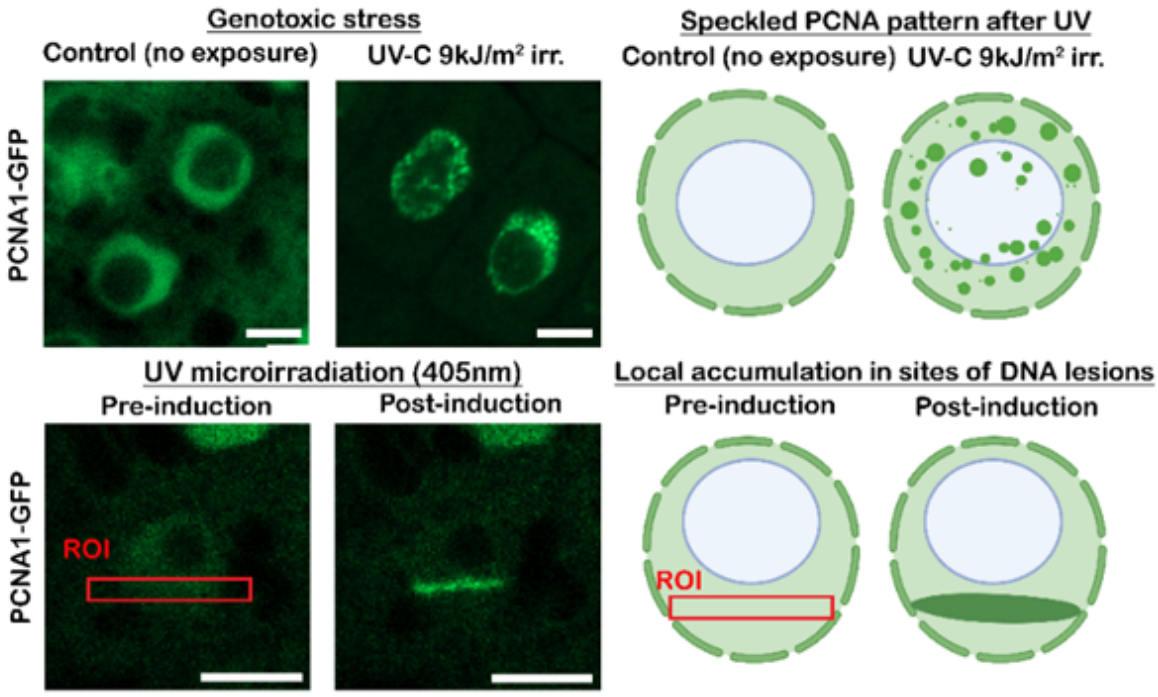

Fig. 1: Figure 1. Microscopic monitoring of the plant DNA damage response using stably-transformed linesTop left – Changes in the distribution of PCNA-GFP after genotoxic stress (UV-irradiation), schematically depicted in the top right. Bottom left – Application of the laser microirradiation system to monitor the recruitment of PCNA-GFP to sites of DNA damage, drawn schematically drawn in the bottom right. Scale bar – 5 μm.

Type of presentation: Oral

Identification of potential readers of ADP-ribosylated DNA adducts in cell-free extracts.

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Poly- and mono-ADP-ribosylation catalyzed by poly(ADP-ribose) polymerases (PARPs) is a highly conserved post-translational modification of proteins involved in DNA damage recognition, signaling and repair, regulation of cell division, transcriptional regulation and regulation of protein degradation. During DNA damage response, ADP-ribose (ADPr) modifications onto target nuclear proteins, including histones, are recognized by chromatin remodeling and other factors, allowing chromatin to be opened and repair to be orchestrated. It has been demonstrated that mammalian DNA-dependent PARP1-3 have the ability to modify not only nuclear proteins but also terminal phosphates at DNA strand breaks. This modification is reversible and can by removed by PARG and other cellular glycohydrolases, such as TARG1, MACROD1, MACROD2 and ARH3. Currently, a number of critical questions remain unclear regarding the molecular mechanisms and role of PARP-mediated ADP-ribosylation of DNA strand break termini. We hypothesized that ADPr adducts at DNA ends may recruit some specific DNA damage signaling, processing and other factors.
Here we used proteomics-based approach to purify and identify proteins in cell-free extracts of HeLa PARGKD cells that specifically bind to mono-ADP-ribosylated (MARylated) ss/dsDNA oligonucleotides bound to streptavidin-coated magnetic beads. We observed an enrichment of about 70 factors and a similar loss of other proteins on MARylated versus non-MARylated dsDNA. Notably, most of the proteins lost their affinity for ssDNA after its MARylation. The depletion of several enzymes with DNA exonuclease activity on MARylated compared to non-MARylated DNA oligonucleotides suggests a protective role for the ADPr moiety at the DNA ends. The data obtained provide new insights into the protein-DNA interaction network modulated by ADP-ribosylation of DNA termini.

Acknowledgement: This work was supported by Fondation ARC (PJA-2021060003796).

Type of presentation: Oral

Protein crowding in DNA repair foci probed by fluorescence lifetime imaging microscopy

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Induction of DNA damage leads to the recruitment of repair factors and formation of a DNA repair focus. Since even an individual DNA break results in the accumulation of a large number of molecules, is it reasonable to expect that they transiently create a distinct local micro-environment surrounding a DNA lesion. We exploited the fact that there exists an inverse quadratic relationship between the fluorescence lifetime of a labelled protein and the local refractive index (RI, n), which, in turn, depends on a local protein concentration. Using Fluorescence Lifetime Imaging Microscopy (FLIM) we demonstrate that the local concentration of all proteins residing in a double-strand repair focus is 30 – 120 mg/mL higher than their concentration in the nucleoplasm, an increase of up to 2.2 times. We hypothesize that a microenvironment characterized by such a high protein concentration may stabilize the DNA repair complex and facilitate a liquid-liquid phase transition.

Type of presentation: Oral

Why chromosmal translocations are cell linage-specific?

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Most cancer-related chromosomal translocations appear to be cell type-specific. It is currently unknown why different chromosomal translocations occur in different cells. This can be either due to the occurrence of particular translocations in specific cell types or adaptive survival advantage conferred by translocations only in specific cells. We experimentally addressed this question by double-strand break (DSB) induction at *MYC, IGH, AML, ETO* loci in the same cell to generate chromosomal translocations in different cell lineages. Our results show that any translocation can potentially arise in any cell type. We have analyzed different factors that could affect the frequency of the translocations and only the spatial proximity between gene loci after the DSB induction correlated with the resulting translocation frequency, supporting the “breakage-first” model. Furthermore, upon long term culture of cells with the generated chromosomal translocations, only oncogenic *MYC-IGH* and *AML-ETO* translocations persisted over a 60-day period. Overall, the results suggest that chromosomal translocation can be generated after DSB induction in any type of cell, but as to whether the cell with the translocation would persist in a cell population depends on the cell type-specific selective survival advantage that the chromosomal translocation confers to the cell.

Acknowledgement: This study was supported by grants from the TA2019 and TA2021, Université Paris Saclay, SFCE (France) and the IDB RAS government basic research program 0088-2021-0007.

Type of presentation: Oral

PML compartment and nucleolus: when both structures meet together

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Promyelocytic leukemia protein (PML) functions as a scaffold for the accumulation of proteins in specific nuclear sites. This membraneless compartment is essential for regulating various nuclear processes such as DNA repair, telomere maintenance, or chromatin modification. Under specific genotoxic stress, PML interacts with the nucleolus, forming the PML-nucleolar compartment, which undergoes dynamic structural changes due to the inactivation and reactivation of RNA polymerase I (RNAPI)-mediated transcription. Generation of PML nucleolar associations depends on the PML SUMO-interacting motif (SIM) and adjacent serine-rich region, which phosphorylation by casein kinase II regulates the interaction of SIM with SUMOylated proteins. PML nucleolar interaction is also positively regulated by p14ARF/p53 tumor suppressors. Besides the canonical PML interactors (DAXX, SP100, SUMO1, and SUMO2), this compartment accumulates damaged rDNA sorted away from the reactivated nucleolus. Using the I-PpoI endonuclease, we demonstrated that this interaction is triggered by unsolved rDNA damage suggesting that the PML compartment plays a role in maintaining the rDNA locus.
Altogether, these findings highlight the role of the PML-nucleolar compartment in rDNA maintenance and extend the knowledge about this multitasking protein.

Acknowledgement: Grant Agency of the Czech Republic (Project 19-21325S and 23-07273S).

Type of presentation: Oral

Radiation-Induced Bystander Effect Mediated by Exosomes Involves the Replication Stress in Recipient Cells

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Exosomes released by irradiated cells mediate the radiation-induced bystander effect, which is manifested by DNA breaks detected in recipient cells; yet, the specific mechanism responsible for the generation of chromosome lesions remains unclear. In this study, naive FaDu head and neck cancer cells were stimulated with exosomes released by irradiated (a single 2 Gy dose) or mock-irradiated cells. Maximum accumulation of gamma H2A.X foci, a marker of DNA breaks, was detected after one hour of stimulation with exosomes from irradiated donors, the level of which was comparable to the one observed in directly irradiated cells (a weaker wave of the gamma H2A.X foci accumulation was also noted after 23 h of stimulation). Exosomes from irradiated cells, but not from control ones, activated two stress-induced protein kinases: ATM and ATR. Noteworthy is that while direct irradiation activated only ATM, both ATM and ATR were activated by two factors known to induce the replication stress: hydroxyurea and camptothecin (with subsequent phosphorylation of gamma H2A.X). One hour of stimulation with exosomes from irradiated cells suppressed DNA synthesis in recipient cells and resulted in the subsequent nuclear accumulation of RNA:DNA hybrids, which is an indicator of impaired replication. Interestingly, the abovementioned effects were observed before a substantial internalization of exosomes, which may suggest a receptor-mediated mechanism. It was observed that after one hour of stimulation with exosomes from irradiated donors, phosphorylation of several nuclear proteins, including replication factors and regulators of heterochromatin remodeling as well as components of multiple intracellular signaling pathways increased. Hence, we concluded that the bystander effect mediated by exosomes released from irradiated cells involves the replication stress in recipient cells.

Acknowledgement: This research was financed by the National Science Centre (Poland) as a part of a Preludium-Bis grant (no. 2020/39/O/NZ4/02838).

Type of presentation: Oral

CHD5 promotes homologous recombination-specific chromatin changes to govern DNA double-strand break repair pathway choice

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Chromatin remodelers play a key role in facilitating precise DNA damage response (DDR) pathways. Altered expression of these proteins results in chemoresistance and survival of cancer cells by fostering genomic instability. Of all the known CHD proteins, the tumor suppressor protein chromodomain-helicase-DNA binding 5 (CHD5) is the least characterized and its functional role in the DDR remains unclear. Here, we show that CHD5 promotes homologous recombination (HR)-specific chromatin changes to govern DNA double-strand break (DSB) repair pathway choice. CHD5 deficiency in cells causes impaired HR and an increase in classical non-homologous end joining (c-NHEJ), thereby promoting genomic instability. Consistent with its role in HR, CHD5 depleted cells are hypersensitive to ionizing radiation (IR) and PARP inhibitors. Importantly, CHD5 mutations that impair its helicase activity are unable to promote chromatin remodeling dependent-DNA repair pathway choice. Mechanistically, we demonstrate that loss of CHD5 regulates HR via impaired deposition of H3K36me3, which in turn results in impaired CtIP recruitment at DSB site. Consistent with decreased CtIP recruitment, CHD5 depleted cells also show impaired IR-induced BrdU exposure, which can be rescued by expression of wildtype-CHD5 but not with the catalytic dead CHD5 mutant, suggesting that CHD5 helicase activity promotes DNA end resection. Interestingly, the eviction of H3K36me3 at HR prone DSB sites, is accompanied with increased deposition of H4K20me2 and H3K4me3 deposition at the NHEJ-prone repair site. Enhanced H4K20me2 and H3K4me3 accumulation facilitates 53bp1 and RIF1 recruitment, counteracting DNA end resection and engaging c-NHEJ DNA repair pathway. Collectively, our findings define a new and previously unidentified role for CHD5 in altering the chromatin landscape to facilitate HR over c-NHEJ to dictate DSB repair pathway choice.

Novel Methods in Nuclear Research and Diagnostics

Type of presentation: Oral

Detecting DNA Breaks, DNA Fragments and Cytosolic DNA in Cells *In Vitro* and Tissue Cryosections Using STRIDE Techniques

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STRIDE technique was originally developed as a very sensitive method of detecting DNA single- and double-strand breaks (sSTRIDE and dSTRIDE) in cells cultured in vitro [1]. It was also adapted for detecting DNA damage in sperm cells. STRIDE has now been optimized further and adapted to quantitative studies of DNA breaks in cryosections of various mouse tissues (Fig. 1). Current research and development of this technology is focused on increasing the detection yield, developing an ability to quantitate DNA ends of various types, distinguishing genomic DNA breaks from short DNA fragments residing in the cell nucleus, and detecting individual cytosolic DNA fragments derived from nuclear and mitochondrial DNA.

[1]. Kordon MM, Zarębski M, Solarczyk K, Ma H, Pederson T, Dobrucki JW. STRIDE-a fluorescence method for direct, specific in situ detection of individual single- or double-strand DNA breaks in fixed cells. Nucleic Acids Res. 2020 Feb 20;48(3):e14

Acknowledgement: We gratefully acknowledge financial support of Polish National Science Center grant OPUS-LAP 2020/39/I/NZ3/02545 (JD).

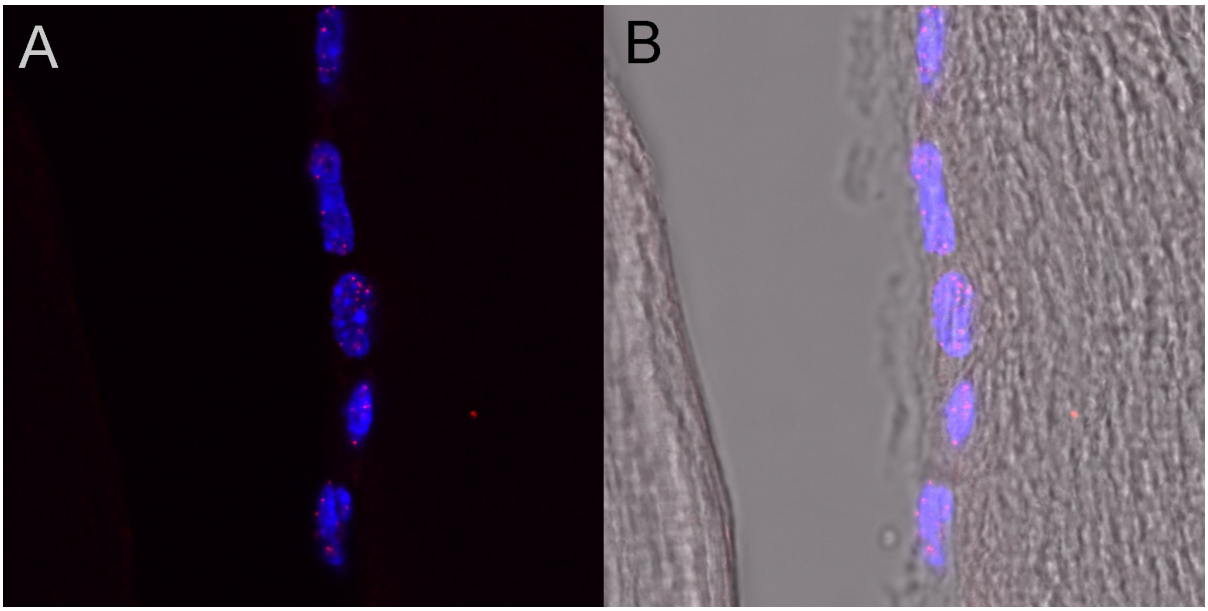

Fig. 1: Single-strand DNA breaks detected by sSTRIDE in nuclei of a cryosection of skeletal muscle cells of a mouse. A - sSTRIDe (red) and nuclei (DNA stained with DAPI, blue). B - fluorescence image overlayed onto transmitted light image (greay scale). Field of view 76 x 76 µm.

Type of presentation: Oral

Advanced correlative workflows for cryo-electron tomography on frozen-hydrated lamella for the study of multicellular organisms

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Cryo-electron tomography of frozen-hydrated samples provides unparalleled level of details from native biological structures. Together with cryo-FIB lamella preparation, it allows us to peek deep inside a cell or multicellular organism without introducing any artefacts and deformations caused by more traditional sample preparation techniques such as chemical fixation or (cryo)ultramicrotomy. While the results provided by the method are exceptional, the workflow is technically challenging, requiring expensive equipment and consumables and trained operators. Extensive optimizations of all steps is needed to prevent heat, beam and mechanical damage as well as ice contamination during the whole process of sample preparation, lamella fabrication and transfer.
We demonstrate optimization of the semi-automated cryo TEM lamella preparation workflow on a variety of samples, including C.elegans model, using TESCAN AMBER Cryo FIB-SEM system equipped with the Leica VCT500 cryo transfer stage for operation in cryogenic conditions. To reduce the time invested in processing of a single sample, it is reasonable to be as precise as possible with selection and targeting of what to actually process. For that purpose, we utilize cryo-fluorescence microscope (Leica CryoCLEM) that allows us to quickly inspect the grids without going into the vacuum of SEM chamber, and pinpoint a specific structure of interest.
We have optimized the workflows for side entry TEM holders to make the method accessible to wider microscopic community compared to autoloader-cartridge focused work.

Acknowledgement: MEYS CR (LM2018129, LM2023050), ERDF (No. CZ.02.1.01/0.0/0.0/18_046/0016045)., and IMG grant (RVO: 68378050)

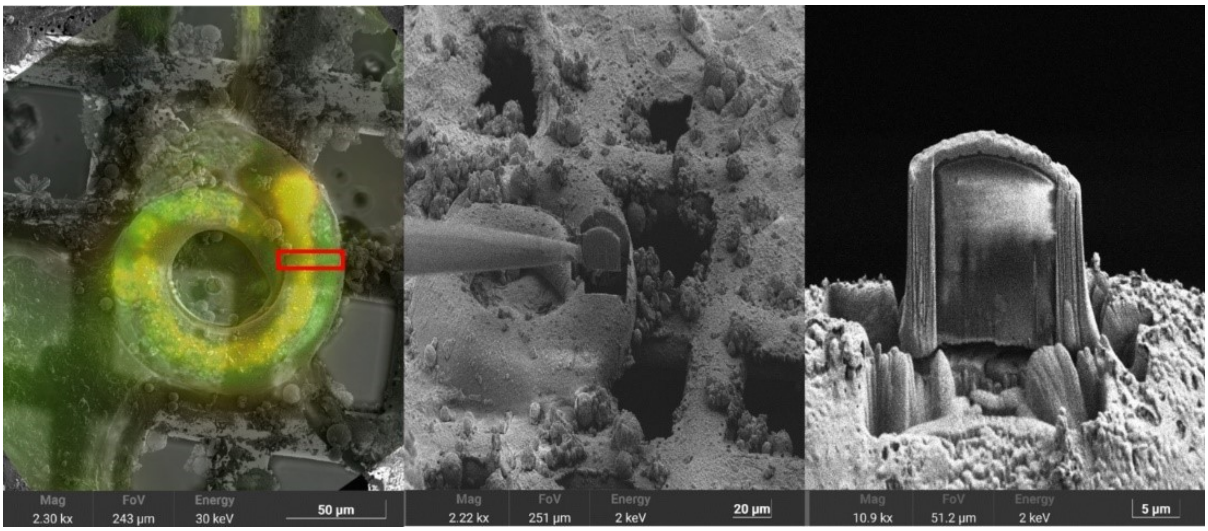

Fig. 1: Overview of the FIB-SEM lift-out lamella preparation procedure. Left – overlay of LM and EM images with selected feature of interest; middle – lamella extraction from the body; right – pre-polished lamella

Type of presentation: Oral

Pollutants – single worm proteomics bring the environment to the lab

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The current pollution crisis manifests by a global distribution of xenobiotics to remote locations such as the arctic and deep sea. Anthropogenic neurotoxicants poison species from polar foxes to arthropods. Despite this knowledge chemicals like nanomaterials are industrially produced and released to the environment on a scale of hundreds of thousand tons annually.
We previously showed in cell culture and the animal model *C. elegans* that silica nanoparticles efficiently transferred to the cell nucleus and induced amyloid protein aggregation specifically in the nucleolus. This amyloid aggregation inhibited nuclear functions including replication, transcription and protein homeostasis. Mass spectrometry-based analyses of nano silica-exposed *C. elegans* (N2) identified an aggregome that among other contained 9 super aggregators comprising the rRNA 2'-O-methyltransferase fibrillarin (fib-1) and ribosomal proteins rpl-2, rpl-7 and rpl-22. In addition, nano silica promoted dipeptide condensates in intestinal cells, interfered with peptide metabolism and reduced translation resulting in worms with a petite phenotype (Piechulek at al., 2019).
The petite phenotype goes with neurodegeneration, axonopathy and neuropathy in single serotonergic and dopaminergic neurons that in turn reduce locomotion fitness and the health span of nano silica-exposed reporter *C. elegans*. Gene expression studies in these worms identified significant changes in the gene ontology (GO) groups serotonin, dopamine and neuropeptide signaling. Consistently, respective nlp-reporter worms showed redistribution of neuropeptides which suggests their role in cross talk between the portal of entry of silica nanoparticles into intestinal cells and neuronal cells, e.g. neurodegeneration.
Currently, we apply single worm proteomics in order to improve characterization of (i) individual resilience pathways against pollutants and (ii) *C. elegans* sampled from the environment, specifically contaminated sites.

Acknowledgement: von Mikecz and Scharf, 2022 Piechulek et al., 2019

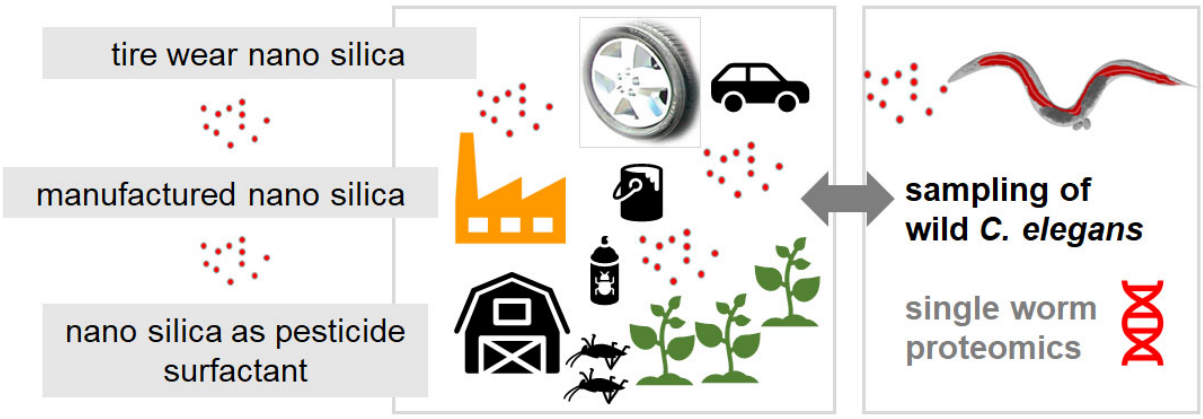

Fig. 1: The schematic depicts the production, use and distribution of silica nanoparticles (red dots) into the environment. Major sources of nano silica are agriculture (pesticide surfactants) and traffic (tire wear). Wild C. elegans are sampled form environmental and analyzed with respect to gene expression, e.g. resilience pathways to pollutants.

Type of presentation: Oral

The nucleus is the command center for populations and ecosystems

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The current crisis of biodiversity loss through reduced habitats, pollution, and climate change, forces us to develop new strategies to protect species. To develop innovative new strategies, we need to gain better knowledge of the underlying principles that drive population survival. Countless studies in the last decades have focused on how the genome and the structure of the nucleus shape the building blocks of individual organism from cells to tissues and how to assemble these into complex organism. However, the genome in the nucleus also encodes the roadmap for populations and ecosystems by controlling how individuals interact with each other; but this aspect is far less studied since it is neither covered by cell- or developmental biology nor by ecology. To bridge these disciplines, we developed a laboratory ecosystem with the genetic model organisms *Caenorhabditis elegans* and *Escherichia coli* including a complementary computational simulation (1). With this system, we can now study how changes in single genes will affect the higher-level emergent property population dynamics of ecosystems under controlled laboratory conditions (2). An important aspect is to analyze how different alleles and epigenetic changes that affect the architecture of the nucleus will have consequences not only on the cellular and organism level but also on the population and ecosystem level. Here, we will decode how the nucleus functions as command center for populations and ecosystems.

1 Scharf, A., Mitteldorf, J., Armstead, B., Schneider, D., Jin, H., Kocsisova, Z., Tan, C.H., Sanchez, F., Brady, B., Ram, N. and DiAntonio, G.B., 2022. A laboratory and simulation platform to integrate individual life history traits and population dynamics. Nature Computational Science, 2(2), pp.90-101.

2 von Mikecz, A. and Scharf, A., 2022. Pollution-bring the field into the lab. Nature, 602(7897), pp.386-386.

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Pathogenesis and Cancer (Symposium of the Society for Histochemistry)

Type of presentation: Oral

The role of nuclear PI(4,5)P2 in papillomavirus associated cancer development.

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Human papillomaviruses (HPV) are small DNA viruses, which infect either mucosal or cutaneous epithelia. Oncogenic HPV types can cause cancer at these anatomic sites. However, the mechanism of cancer progression in patients with persistent HPV infections is not well understood. Recently, we found that nuclear phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) levels are high in keratinocytes expressing HPV oncoproteins and in HPV-infected epithelia, resulting in the phospholipidation of nuclear proteins in HPV positive skin cancer as well as HPV16 positive cervical intraepithelial neoplasia and cervical cancer. Elevated PI(4,5)P2 levels seem to be a common phenomenon in oncogenic-HPV infected keratinocytic lesions, since skin tumors which are not triggered by HPV, were found to be PI(4,5)P2 negative. These findings raise important fundamental questions regarding the oncogenic role of PI(4,5)P2 in HPV-induced epidermal cancer development.
We are currently using cellular models as well as human tumor tissues / tumor biopsies to investigate the levels of PI(4)P, PI(3,5)P2, PI(3,4)P2, PI(4,5)P2 and PI(3,4,5)P3 in HPV-associated as well as non-HPV tumors. We expect the acquired data to be of pivotal importance in paving the way for the identification of novel molecular pathways driving tumor development.

Type of presentation: Oral

Targeting mitochondria as a novel therapeutic approach to overcome TKI resistance of NSCLC cells: the role of paraptosis

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Non-small cell lung carcinoma (NSCLC) is the most common type of lung cancer, accounting for about 80-85% of cases. One of the main causes of tumor progression in NSCLC is aberrant regulation of the epidermal growth factor receptor (EGFR) gene. In addition to overexpression, the appearance of activating deletions/point mutations (e.g. exon 19del, L858R) in the EGFR protein are responsible for its ligand-independent hyperactivation and augmented proliferative signaling and tumor growth. However, patients with such tumors are efficiently treated with tyrosine kinase inhibitors (TKI), e.g. gefitinib and erlotinib. Unfortunately, most of the patients eventually develop resistance to these drugs. Among those TKI-resistant tumors, more than half of them carry a point mutation (T790M) in the EGFR gene.

To find novel therapeutic approaches to tackle TKI-resistant NSCLC we screened a library of 2300 FDA approved drugs for their ability to kill the T790M-resistant cells in combination with gefitinib. We found several drugs that synergize with gefitinib/afatinib in selective killing these cells both in vitro (cell culture) and in vivo (xenografts in immune-deficient mice). Importantly, on the molecular level most of these drugs mediated their cytotoxic effects via destabilization of mitochondria. The RNA-seq data coupled with the SeaHorse energy profiling revealed that these drugs increased the production of ROS, resulting in DNA damage and subsequent cell death. Furthermore, the morphological analysis of mitochondria by electron microscopy together with the results of fluorescent reporter assay suggested that these cells were dying because of paraptosis, a form of cell death induced by mitochondrial influx of Ca2+. In accordance with the obtained results, we propose a novel therapeutic scheme to overcome the rapidly developing resistance of NSCLC cells to TKI monotherapy.

Type of presentation: Oral

Effects of Heat and Cold Shock on Epigenetics: Insights on Methylation and Chromatin Structure

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During their existence, cells must cope with several alterations in their environment, including exposure to toxic substances or radiations, variations in oxygen levels and osmolarity. Stress response is based on profound modifications of the gene expression pattern, which is achieved acting at different levels, such as structural and architectural nuclear rearrangement and epigenetic modifications, hence resulting in transcriptional regulation.
Whereas various stress responses have largely been investigated, major features of certain insults remain obscure and elusive. For example, temperature alterations are among the most common stresses cell must deal with. Hyperthermia is widely studied as the response is similarly activated by a range of cellular stressors and by pathophysiological conditions. On the contrary, hypothermia has been far less studied, but it is currently gaining major relevance for its clinical, therapeutical, and industrial outcomes. Since the direct effect of exposure to non-optimal temperature on epigenetic modification is still unclear, we aimed at investigating this putative relationship by focusing our attention on DNA methylation and demethylation, histone modifications and the influence of epigenetics on chromatin organization. Our data highlight distinct epigenetic fingerprints in response to the two types of insult. Hyperthermia is strictly associated with increments in DNA and histone methylation and induces a more compact chromatin organization. Conversely, hypothermia does not determine a univocal epigenetic response: indeed, different epigenetic markers are contemporarily exhibited, confirming the delicate gene expression regulation pathways required when dealing with severe condition alterations. In the light of its involvement in stress adaptation, deepening the knowledge about the impact of environmental temperature on human epigenome may underline potential outcomes for medical, pathological, and commercial applications.

Acknowledgement: Authors sincerely thank Gloria Milanesi and Paola Veneroni (DBB, University of Pavia); Massimo Boiocchi and Amanda Oldani (CGS, University of Pavia).

Type of presentation: Oral

Fat induced Nuclear Rupture

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Excessive fat is pathological for many reasons and perhaps even carcinogenic, but direct physical interactions with the nucleus might contribute. Although the nucleus in many cell types is a stiff organelle, fat-filled lipid droplets (FD's) in the cytoplasm can be seen to indent and displace the nucleus. FD's are phase-separated liquid 'condensates' with a poorly understood interfacial tension that determines how FD's interact with other organelles. Here, micron-sized FD's remain spherical as they indent both the nucleus and peri-nuclear actomyosin, dilute Lamin-B1 locally independent of Lamin-A,C, and trigger rupture with locally persistent accumulation in the nucleus of cGAS, a cytosolic DNA sensor. DNA density increases and lamin-A,C recovers at rupture sites. However, FD-nucleus interactions initiate rapid mis-localization of the essential DNA repair factor KU80, and nuclear rupture associates with DNA damage and delayed cell cycle. Misrepair of damage is conceivable and is being studied. Spherical shapes of small FD's are consistent with a high interfacial tension that we measure for FD's that are mechanically isolated from fresh adipose tissue as ~40_mN/m. The value is far higher than other liquid condensates, but typical of oils in water and sufficiently rigid to disrupt cell structures including the nucleus.

Acknowledgement: NCI and NSF grants.

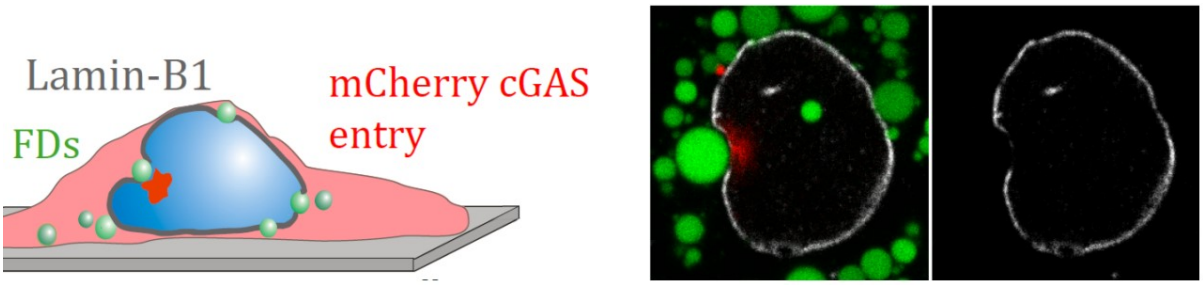

Fig. 1: Fat droplet indents and ruptures nucleus, with gap in Lamin-B.

Type of presentation: Oral

Targeting the 3D genomic and epigenetic changes in Mantle Cell Lymphoma

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Recurrent chromosomal translocations found in most lymphomas frequently lead to overexpression of a certain oncogene, but in many cases, the expression of the oncogene alone does not suffice to produce a malignant phenotype. This is the case in mantle cell lymphoma (MCL), an aggressive B-cell non-Hodgkin lymphoma associated with the t(11;14)(q13;q32) translocation that results in the overexpression of cyclin D1 (CCND1), a potent cell-cycle regulator. Nevertheless, not all MCLs overexpress *CCND1*, and the *CCND1* overexpression alone does not lead to malignancies in animal models. Thus, the development of MCL should be triggered by additional factors, which may guide the development of new therapies once discovered.

A chromosomal translocation can trigger large-scale changes in the 3D genome organization, as well as the transcriptional and epigenetic changes in the translocated loci. Here we demonstrated that the regions in the vicinity of the translocation breakpoint on derivative chromosomes 11 and 14 are relocated closer to the nuclear center in MCL cells. This was accompanied by the upregulation of gene expression in these regions, as well as the global perturbation of the enhancer landscape of MCL cells. Several novel enhancers and superanhancers predicted to regulate the genes overexpressed in MCL were discovered, suggesting the potential utility of the enhancer-modifying substances for MCL treatment. We tested two substances with such properties, Abemaciclib and Minnelide, in MCL cell lines and the B cells from the venous blood of MCL patients. Both substances effectively reduced the viability of the malignant cells. These results provide valuable preclinical data and novel insights into the MCL pathogenesis.

Type of presentation: Oral

Gene expression profiles of GABAergic neuronal loss by nanoparticle exposure

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In aging societies the increasing cases of neurodegenerative protein deposit diseases urge the identification of the underlying mechanisms. Formation of amyloid-like aggregates unifies superficially unrelated human conditions in neurodegenerative deposition diseases such as Alzheimer's with amyloid β peptides (Aβs) or Huntington's disease with polymeric glutamines (polyQ). The cell nucleus is a primary target of amyloid-like protein fibrillation in various disorders that are characterized by widespread aggregation of proteins with unstable homopolymeric amino acid repeats, ubiquitin, and other proteinaceous components. We are investigating the influence of pollutants on neurodegenerative diseases by studying the effects of nano-silica on neural signaling and protein homeostasis in the nematode *Caenorhabditis elegans* from young to middle-aged cohorts. We found that loss of GABAergic neurons occurred in the nano silica-exposed cohort and this also correlated with premature neuromuscular behavior defects in young worms. Fibrillarin (*fib-1*) has been identified as a super aggregator in the constitution of a nano silica-induced aggregome which resulted in formation of nucleolar amyloid. Nano silica also induced nucleolar amyloid formation in intestinal cells. Age-groups vulnerable to nanosilica are being studied in respect to protein homeostasis by mass spec proteomics analysis with different Gene Ontology categories. We show that nanosilica disturbs the resilience pathways that normally preserve cellular homeostasis and thereby causes the toxic effects on neuronal cells and induces corresponding neuromuscular defects.

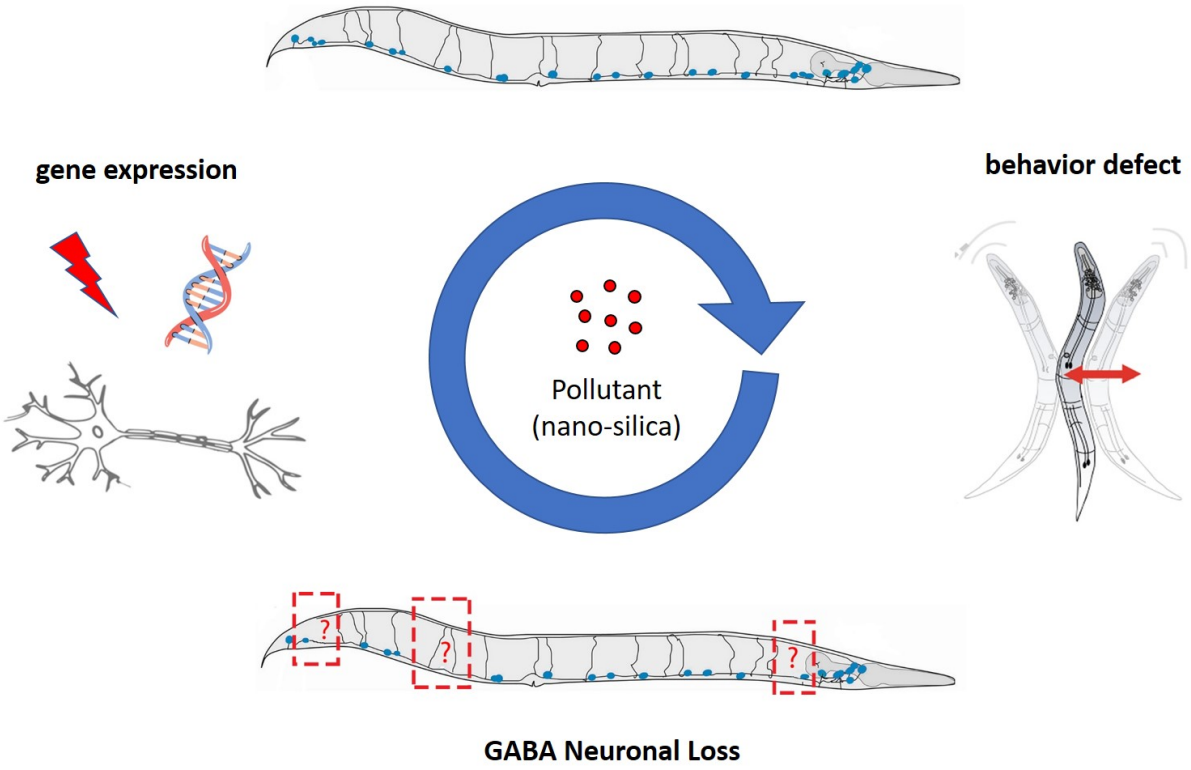

Fig. 1: From gene expression to behavior defect through GABA neuronal loss induced by pollutant in C. elegans

Type of presentation: Oral

Negative prognostic signature in ovarian cancer

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In our previous microarray study we identified the 96-gene signature significantly related to the worse survival of patients with high-grade serous ovarian cancer [1]. Top differentially expressed genes were e.g. POSTN, COL11A1, SFRP2, MFAP5, ITGBL1, LOX, FN1. Similar mesenchymal signature with negative impact on survival, has been observed also by others. However, it has been regarded rather as a specific feature of cancer associated fibroblasts, while not epithelial cells.

We postulate that these genes can be also expressed by cancer cells themselves and affect their phenotype. Here, we will present results of our studies on the role of selected genes from the negative prognostic signature. We analyzed both, biological role of these genes in ovarian cancer cells, and their significance as potential prognostic biomarkers [2-4].

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Type of presentation: Oral

The interaction of two viral nuclear transcription factors HIV-1 Tat and EBV Zta induces immune evasion of EBV-infected B cells.

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Compared to general population, people living with HIV are more likely to be seropositive for EBV, more often present EBV reactivation and are at a higher risk of developing aggressive EBV-associated B-cell lymphomas. Tat protein of HIV-1 and Zta protein of EBV possess similar features (both are nuclear transcription factors) and can be found simultaneously in blood or cells of people living with HIV. This study aimed to investigate the physical and functional interaction between Tat and Zta. Co-immunoprecipitation, in vitro binding, FRET and YFP reconstitution assays were used to study protein-protein interaction. The effects on gene expression were analysed via RNA-sequencing in primary human B-cells treated with Tat and Zta. Tat and Zta directly and specifically bind each other in human B and T-cells and blood serum through C-terminal domains of both proteins. YFP reconstitution and FRET demonstrated that the interaction occurred predominantly in the nucleus. Analysis of primary human B-cells treated with Tat and Zta showed that the subset of genes that trigger an immune response and antigen presentation in B-cells remained unchanged when two proteins were combined as opposed to their single action. Tat and Zta interaction induced downregulation of HLA-ABC surface and total expression in B-cells; this effect was blocked by inhibiting proteasome or E2-ubiquitin-conjugating enzyme. Pre-treatment of EBV-infected B-cells with Tat and Zta resulted in decreased cytotoxic lysis of these cells by EBV-specific CD8+ T-cells. This study provides evidence that the direct interaction between HIV-1 Tat and EBV Zta can lead to immune evasion in B-cells through E2-dependent HLA-ABC proteasomal degradation. Our findings suggest that the interplay between these two viruses may have important implications for the pathogenesis of HIV-EBV co-infection and associated malignancies.

Acknowledgement: This study was supported by ANRS and Cancéropole IdF.

Type of presentation: Oral

Transcriptional regulation of oxidative phosphorylation by nuclear myosin 1 protects cells from metabolic reprogramming and tumorigenesis in mice

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Metabolic reprogramming is one of the hallmarks of tumorigenesis. By using a combination of multi-omics approaches, here we show that nuclear myosin 1 (NM1) serves as a key regulator of cellular metabolism. As part of the PI3K/Akt/mTOR pathway, NM1 forms a positive feedback loop with mTOR and directly affects mitochondrial oxidative phosphorylation (OXPHOS) via transcriptional regulation of mitochondrial transcription factors TFAM and PGC1α. NM1 depletion leads to suppression of the PI3K/Akt/mTOR pathway, underdevelopment of mitochondria inner cristae, and redistribution of mitochondria within the cell, which is associated with reduced expression of OXPHOS genes, decreased mitochondrial DNA copy number, and deregulated mitochondrial dynamics. This leads to metabolic reprogramming of NM1 KO cells from OXPHOS to aerobic glycolysis associated with a metabolomic profile typical for cancer cells, namely increased amino acid-, fatty acid-, and sugar metabolism, and increased glucose uptake, lactate production, and intracellular acidity. We show that NM1 KO cells form solid tumors in a mouse model, despite the suppressed PI3K/Akt/mTOR signaling pathway, suggesting that the metabolic switch towards aerobic glycolysis provides a sufficient signal for carcinogenesis. We suggest that NM1 plays a key role as a tumor suppressor and that NM1 depletion may contribute to the Warburg effect at the early onset of tumorigenesis.

Posters

Type of presentation: Poster

P-1 Mouse PML Protein Isoforms and their Role in Mouse Polyomavirus Infection

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Promyelocytic leukaemia nuclear bodies (PML NBs) are dynamic, spherical, membrane-less structures composed of the main scaffold PML protein and a variety of stable or transient partner proteins. Apart from many endogenous functions, PML NBs play an important role in antiviral defence, both as direct restriction factors and regulators of the interferon responses. Hence, many viruses developed effective mechanisms to counteract this restriction. This project uses Mouse polyomavirus (MPyV) as a model for studying interactions of PML and viral components. The mouse PML (mPML) protein occurs in three confirmed (mPML1-3) and six predicted (mPMLX1-X6) isoforms. Individual isoforms may affect the composition and functions of PML NBs and mediate antiviral effects.
Our data showed, that during MPyV infection, mPML NBs appeared in close proximity to viral replication centres. In *Pml* KO cells, the transcription of MPyV regulatory genes was significantly increased and the amount of viral progeny was approximately two times higher. These results indicate a potential restriction function of mPML NBs and/or mPML protein in MPyV infection. Therefore, we focused on the role of individual mPML isoforms. Their expression has been examined in different mouse tissues. The expression of all, confirmed and predicted isoforms was proved and, in addition, a novel isoform (named by us mPMLXK) was detected in all tested samples. Subsequently, the analysis of PML NBs formation and restriction functions was initiated. The longest mPML2 isoform formed speckles when expressed in *Pml* KO cells and in WT cells, it incorporated into endogenous mPML NBs. However, its overexpression did not significantly affect MPyV infection neither in *Pml* KO nor in WT cells. The proposed antiviral role of other mPML isoforms is now under investigation.

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

Type of presentation: Poster

P-2 A novel role for linker histones in human centromeres

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Linker histone H1 consists of a family of 11 positively charged lysine-rich proteins that compact chromatin. Recent studies implicate histone H1 mis-regulation and epigenetic alterations can lead to diseased states, including cancer. In this study, we investigate a role for linker histone H1.5, which is upregulated in glioblastomas. We find this this variant H1.5 is localized to centromeres and partners with centromeric histone H3 variant, CENP-A, both in vitro and in vivo. Additionally, both Atomic Force Microscopy (AFM) and MNase digestion of in vitro assembled H1.5 containing nucleosomes reveal it binds H3 and CENP-A nucleosomes differently -increasing the diameter of H3 but not CENP-A nucleosomes. Finally, H1.5 repression leads to significant increase in mitotic defects such as misaligned metaphase plate and multipolar spindles, which can be attributed to loss of key centromere proteins, and of noncoding centromeric transcription.

Type of presentation: Poster

P-3 Does heme oxygenase 1 play a role in the cell cycle?

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Heme oxygenase 1 (Hmox1) is an anti-inflammatory enzyme that degrades free heme and reduces both hemolytic and oxidative stress. Our previous studies have shown that Hmox1 protecs cells against elevated levels of G-quadruplex formation and the accumulation of replication stress-induced dsDNA breaks. Consequently, such multi-processes impact of Hmox1 deficiency may lead to the activation of cell cycle checkpoints and the inhibition of one of the G1/S/G2 phases. Our aim was to verify, whether Hmox1 may play a protective role in the regulation of the cell cycle.

To conduct the experiments, we used iPS and HEK293T cells i) devoid of endogenous heme oxygenases, with or without constitutively active nuclear or cytoplasmic Hmox1 transgene ii) wild-type or Hmox1 deficient cells. Analysis of S phase by EdU stainig and percentage distibution of the cell cycle by using propidium iodide do not showed any alerts in Hmox1 deficient cells, despite the presence of increased level of inhibited replication forks. However, time-lapse analysis showed that heme oxygenase-1-deficient cells have significantly longer duration of the cell cycle, resulting in slower proliferation. Moreover, RNA-seq analyses of iPSC with nuclear or cytoplasmic form of Hmox1 identified differences in cell cycle-related genes comparing to Hmox1-deficient cells. Furthermore, in Hmox1-deficient cells, we observed lower nuclear translocation of crucial proteins involved in the cell cycle, such as CDC25C, cyclin B1 and p53. Such disfunction of nuclear translocation may results from a disturbed lamins distribution. Surprisingly, our RNA-seq results and immunocytochemical staining show a strong downregulation of lamin A/C, B1 and B2 level in heme oxygenase-1 deficient cells.

To summarize, our results suggest that Hmox1 regulates all phases of the cell cycle by targeting p53 and lamins.

Acknowledgement: The study was supported by NCN grants: MAESTRO10 (2018/30/A/NZ3/00495:A.J.) PRELUDIUM20 (2021/41/N/NZ3/03709:P.C.)

Type of presentation: Poster

P-4 Hypoxia sensitive polymersomes loaded with doxorubicin in anti-cancer therapy

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Solid tumors are one of most common form of cancer; in 90% of cases they exhibit hypoxia (regions of underoxygenation, below 2% O2). Specificity of such microenvironment allows for development of functionalized carriers. Cancer nanomedicine is revolutionary field which employs nanoparticles to target and destroy cancer cells. Nanoscale drug delivery systems improve anticancer efficiency with simultaneous reduction of off-target toxicity. Polymersomes (synthetic amphiphilic block copolymers) have attracted a lot of attention thanks to their low toxicity, high stability, and drug loading capacity. Hypoxia sensitive polymersomes exhibit longer circulation and increased chemical versatility with their specific degradation in tumor tissue. Encapsulation of doxorubicin inside of functionalized hypoxia sensitive polymersmomes allows for more effective treatment while decreasing cardiotoxicity.

Hypoxia-responsive polymersomes (poly(lactic acid)−azobenzene−poly(ethylene glycol) diblock copolymer) were loaded with doxorubicin. The experiments were conducted on murine cancer cells (4T1 – breast cancer and B16-F10 –melanoma). We assessed cytotoxicity of obtained polymersomes in different environments (normoxia and hypoxia) using MTS assay. Internalization and degradation of synthesized polymersomes in different time points and different concentrations were observed using confocal microscope.

Synthesized hypoxia-sensitive polymersomes were characterized (size and morphology) by electron microscopy. We confirmed that synthesized hypoxia-responsive polymersomes degrade faster in underoxygenated conditions. Tested cancer cells accumulate polymersomes in cytoplasm, and release doxorubicin in hypoxia. Higher tumor cells toxicity of doxorubicin-loaded polymersomes was observed in hypoxia condition.

In further studies we plan to combine doxorubicin-loaded polymersomes with STING agonistoaded polymersomes to activate the immune system for the eradication of tumor in murine models.

Acknowledgement: The work is a result of the research projects no. UMO-2020/39/B/NZ5/00745 financed by National Science Center.

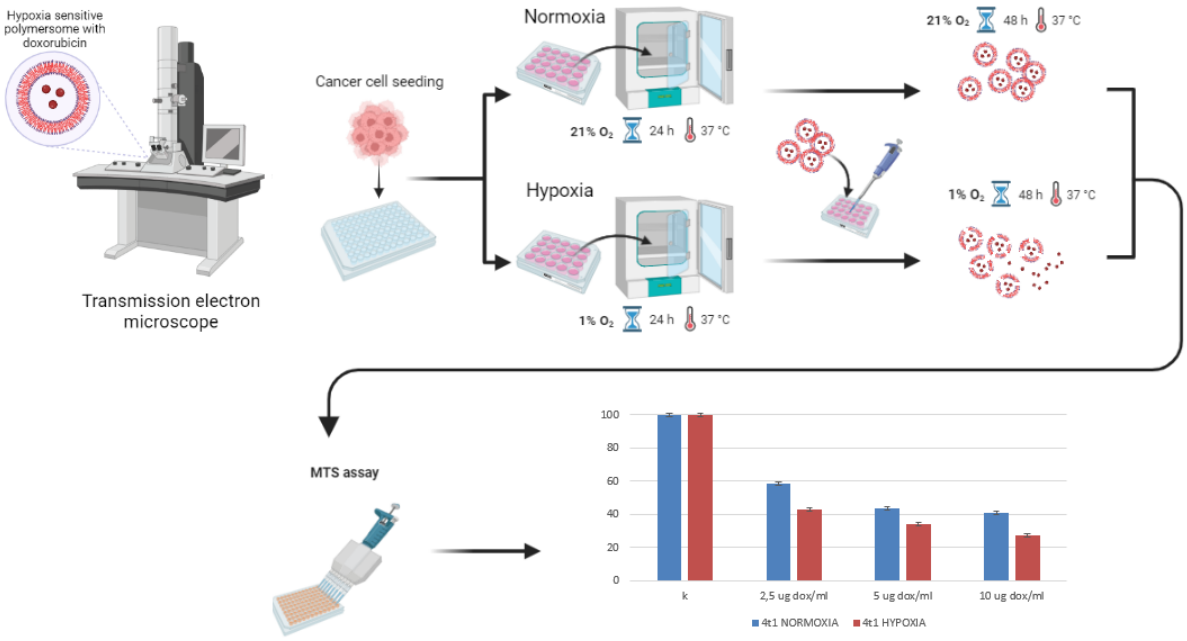

Fig. 1: Size and morphology of synthesized hypoxia sensitive polymersomes loaded with doxorubicin were characterized using transmission electron microscopy. Breast cancer cells 4T1 were treated with different concentrations of polymersomes and incubated in normoxic and hypoxic conditions for 48h, following with MTS assay.

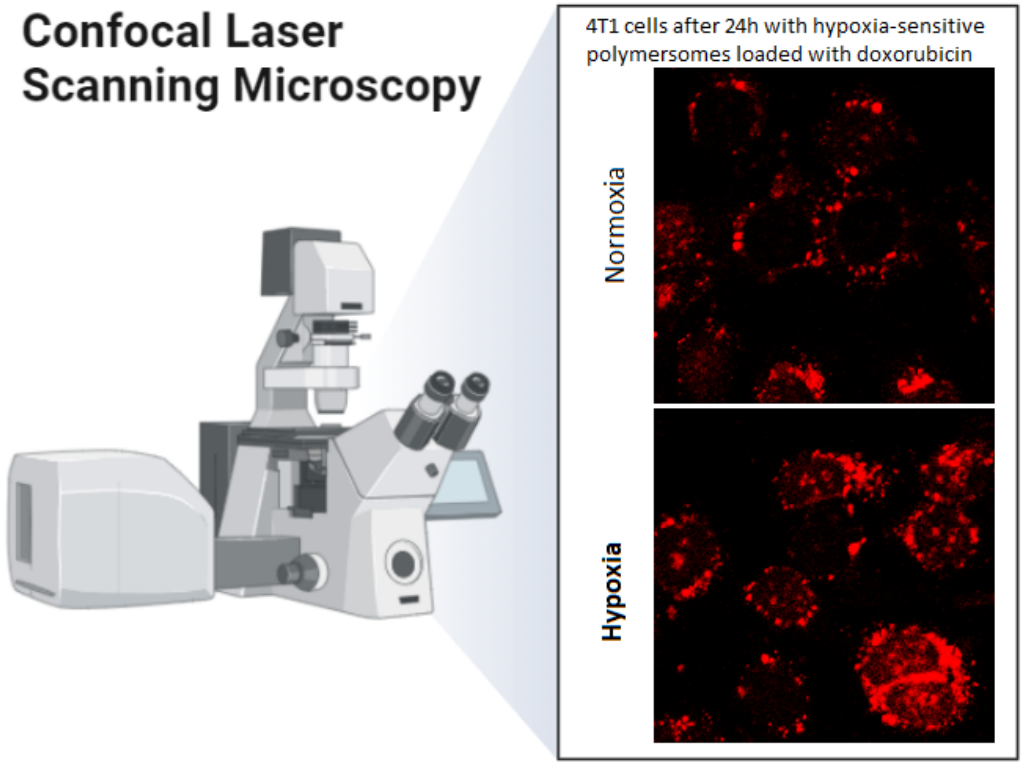

Fig. 2: Breast cancer cells 4T1 incubated with hypoxia sensitive polymersomes in normoxic and hypoxic conditions for 24h were observed using confocal microscope. Internalized polymersomes accumulated in cytoplasm. Doxorubicin, released in hypoxic condition from polymersomes entered cells nuclei and intercalated into DNAs.

Type of presentation: Poster

P-5 Effect of Hypoxia on Sensing and Repair of DNA Double-Strand Breaks

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The most deleterious DNA lesions are double-strand breaks (DSBs). The earliest factors recruited to DSBs are PARP1 and MRN complex [1, 2]. These sensors result in activation of downstream signalling cascades, and play important role in the recruitment and activation of ATM kinase. Activated ATM phosphorylates histone H2AX, which is critical for early DNA damage signal transduction [3].
Hypoxia contributes to genomic instability by hampering cellular responses to DNA damage [4]. The aim of this study was to investigate the influence of low oxygen and nutrient concentration on recruitment of factors involved in response to DNA double-strand breaks. DSBs were induced by a low power focused beam as in [5], in nuclei of cells in in vitro cultures equilibrated with air or under hypoxia, in a custom made sample holder on a microscope stage [6]. Inhibition of DNA damage response was quantified in terms of the proportion of cells responding to DNA damage.
Reduction of poly(ADP-ribosyl)ation and recruitment of MRN complex at the site of local DNA damage, as well as partial inhibition of activation of ATM kinase was observed under hypoxia. Also lower numbers of γH2AX foci in cells in response to DNA damage were observed. Proximity Ligation Assay was used to detect protein-protein interactions at the sites of DNA damage and revealed that MDC1 and γH2AX interactions are disrupted under hypoxic conditions.
We conclude that low oxygen concentration and nutrient depravation result in impaired response to DNA damage already at the stage of its recognition.
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Type of presentation: Poster

P-6 Anticancer activity of the novel 4-thiazolidinone derivatives tested against breast cancer (MDA-MB-231, MCF-7) and neuroblastoma cell lines (SH-SY5Y).

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Cancer remains to be one of the most alarming diseases with 19,3 million new cases identified in 2020. Nearly 10 million patients died due to cancer in the mentioned year. With the current tendency, it is expected that there will be about 28,4 million new cases in 2040. Another major problem is the steadily increasing amount of cancer cases with progressive drug resistance. For this reason, there is a demand for new anticancer medicines. Here we present 6 novel 4-thiazolidinone compounds with anticancer properties. 4-Thiazolidinone moiety has a confirmed anticancer activity against various types of cancer such as leukaemia, CNS cancer, non-small cell lung cancer, renal cancer, colon cancer and breast cancer. Therefore, the cytotoxicity of the new structures has been researched on the breast cancer cell lines: MDA-MB-231 and MCF-7 and neuroblastoma cell line SH-SY5Y in concentrations ranging from 0,1μM to 50μM using the MTT assay method. The results showed that the synthesized compounds are much more active against SH-SY5Y and MCF-7 than MDA-MB-231 cell line. The most cytotoxic against MCF-7 were Les-6416 and Les-5357 with IC-50 values of 3.181μM and 4.267μM respectively. The tested compounds were the most active against SH-SY5Y. The IC-50 value of all tested compounds did not exceed 30μM. However, the most prominent results have displayed Les-5357 and Les-6381 with IC-50 values of 1.999μM and 2.692μM correspondingly. Regarding MDA-MB-231 cell line, only two compounds demonstrated potent cytotoxic activity with IC-50 value less than 50μM. Those are Les-6418 and Les-5357 with IC50 values of 34.681μM and 47.126μM accordingly. In addition, *in silico* methods have been applied to investigate their ADME properties and possible sites of metabolism. In conclusion, we determined that those structures have promising anticancer properties and tests regarding their potential cytotoxic activity against other cell lines have to be conducted.

Type of presentation: Poster

P-7 The potential role of phosphatidylglycerol in epigenetics

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In the pathophysiology of ARDS (Acute respiratory distress syndrome), the gene expression of inflammatory mediators increases, while the expression of anti-inflammatory mediators decreases. Such changes in gene expression can be achieved through epigenetic regulation. Histone deacetylases (HDACs) are enzymes that deacetylate lysine residues and play an important role in epigenetics. Valproic acid (VPA) has been shown to modulate DNA transcription through HDAC inhibition, thereby limiting nitric oxide and nuclear factor-κB production. As an HDAC inhibitor, VPA has been reported to reduce inflammation through cytokine expression in lipopolysaccharide-stimulated human cells. However, a similar anti-inflammatory effect was also observed when phosphatidylglycerol (PG) was applied. Moreover, PG alone can specifically reverse the inhibitory effects of histones in vitro and is an important physiological regulator of nuclear protein kinase C βII. It was also proven in yeast that PG regulates the activity of the enzyme Isc1, which is a homolog of human sphingomyelinase 2. Sphingomyelinase 2 regulates the metabolism of sphingomyelin, the most abundant nuclear sphingolipid, which is part of the nuclear matrix and plays both structural and regulatory roles in chromatin assembly and dynamics. The biosynthesis of PG is regulated by phosphatidylglycerol phosphate synthase, an enzyme whose activity, like that of HDCA, is affected by the presence of VPA. Therefore, the question remains whether during inflammation VPA directly affects the expression of cytokines or whether this anti-inflammatory effect is mediated by increased synthesis and the presence of PG. To answer this question, we would like to take a closer look at the changes in the lipid composition of the nuclear membrane before and after inducing inflammation.

Acknowledgement: This work was supported by an ITC Conference Grant from the COST Action CA19105 Stratagem, VEGA grant number 2/0030/22 and grant number APVV-20-0129.

Type of presentation: Poster

P-8 Specific downregulation of progerin level in fibroblasts from patients with Hutchinson-Gilford progeria syndrome

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder with severe symptoms. The disease is caused by de novo point mutation in LMNA gene, which codes for lamin A/C – structural proteins of nuclear lamina. The mutation leads to a deletion of lamin A sequence crucial for posttranslational maturation. A shorter version of lamin A – progerin can not undergo the final cutting of the C-terminus, which anchors protein in the nuclear membrane. Progerin accumulation leads to various alterations, such as nuclear lamina disruption, abnormal nuclear morphology, changes in signalling pathways activity and chromatin organization, increased DNA damage (Fig. 1).

We assumed that progerin downregulation, possibly with an additional delivery of LMNA coding sequence, is sufficient for progeroid phenotype reversion. We established that efficient siRNA should decrease progerin level at least by 50% without lamin A level decrease. First, a set of siRNA was selected in HGPS cellular model based on HeLa cells. The most effective siRNAs decreased progerin level by up to 30%. We demonstrated that a combination of siRNA and the clinical drug lonafarnib has an additive effect on progerin downregulation. We also designed siRNA with modified nucleotides in order to increase siRNA stability. Sequential treatment of patients' fibroblasts confirmed the efficiency of selected siRNA, progerin level was decreased by up to 30% (Fig. 2).

We believe that preservation of lamin A level is important in Hutchinson-Gilford progeria syndrome therapy as it was shown previously the importance of lamin A and progerin ratio for progeroid phenotype removal. We have shown for the first time specific downregulation of progerin level with the siRNA approach along with preserved lamin A level in treated cells. For further application, siRNA sequences could be coded in plasmid as shRNA or miRNA with polymerase II-dependent promotors and delivered with viral vectors, or used as small oligonucleotide drugs.

Acknowledgement: The study has been funded by the grant from The National Centre for Research and Development Grant E-Rare-3 Treat HGPS (ERA-NET-E-RARE-3/III/TREARHGPS).

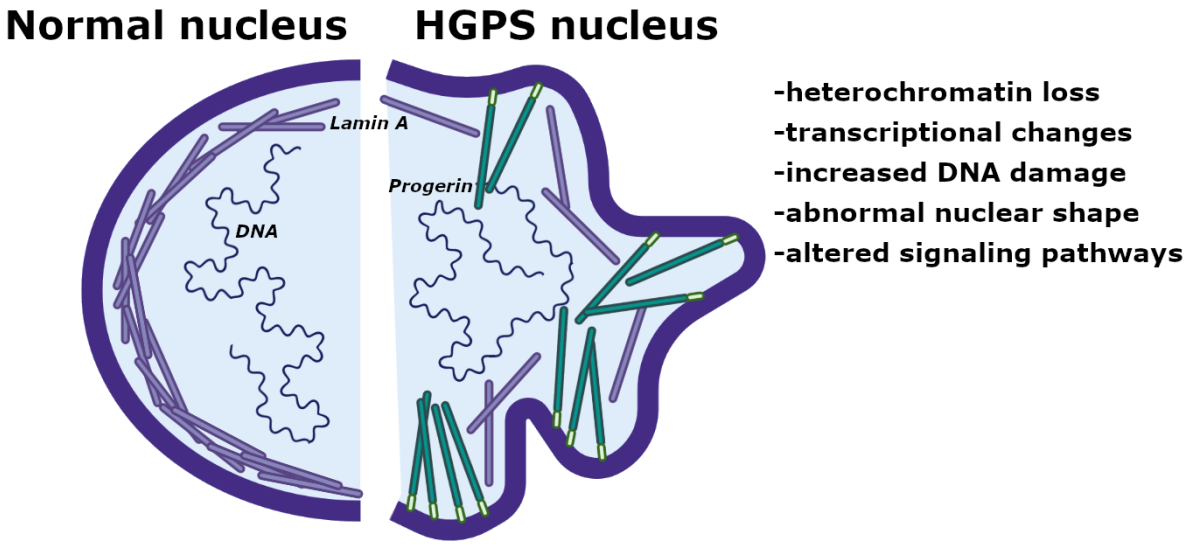

Fig. 1: The comparison of a normal nucleus with lamin A expression and nucleus of cells with HGPS with an expression of lamin A and progerin. Accumulation of progerin in the nucleus leads to various changes in cell physiology.

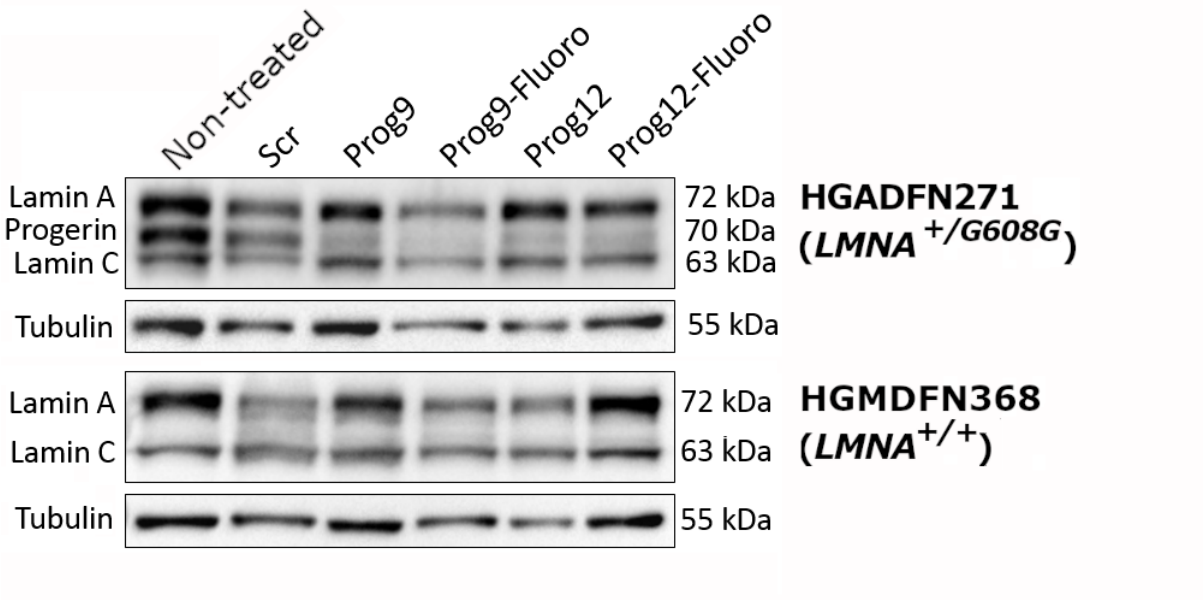

Fig. 2: Downregulation of progerin expression in patients' fibroblast (HGADFN271) after siRNA treatment. The level of lamin A and C was analyzed in patients' fibroblasts and fibroblasts from a healthy donor (HGMDFN368).

Type of presentation: Poster

P-9 Novel pyrrolidinedione-thiazolidinone compound binds with DNA molecule and induces its damage

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The 4-thiazolidinone derivatives were reported as a prospective scaffolds for the development of new anticancer agents. DNA damage leading to apoptosis and/or inhibition of DNA replication are important indicators of chemotherapy efficacy. The aim of our study was to evaluate both the anticancer activity and DNA-targeting properties of novel 4-thiazolidinone derivative - 3-{5-[(Z,2Z)-2-chloro-3-(4-nitrophenyl)-2-propenylidene]-4-oxo-2-thioxothiazolidin-3-yl}-1-(4-hydroxyphenyl)-pyrrolidine-2,5-dione.

This derivative demonstrated toxicity towards human T-leukemia cells of Jurkat line, human breast carcinoma MCF-7 and MDA-MB-231 cells, human cervical carcinoma HeLa cells, and human colon carcinoma HCT-116 cells. It induced DNA damage (single-strand brakes and inter-nucleosomal fragmentation) in Jurkat cells. Taking into account the results of conducted cellular and acellular types of comet assay, one may assume that the compound under study could cause an indirect effect on DNA molecule, while stronger effect of this compound was detected in the Cellular type of DNA comet assay. Molecular docking studies showed that compound binds to DNA strand 1DNE via the minor groove binding with the Gibbs free energy (ΔG) of −10.38 kcal/mol. The compound slightly replaced methyl green dye from its complex with the DNA. The absorption spectrum of studied derivative was found to change significantly in the presence of increasing concentrations of salmon DNA that decreased the absorbance of the compound at 300 and 400 nm. Besides, a shift in red zone of spectrum was detected. Thus, studied compound slightly intercalated into DNA.

Thus, the novel 4-thiazolidinone derivative has the ability to bind with DNA molecule. The DNA damaging effect of novel 4-thiazolidinone derivative in treated cancer cells might take place as a secondary event induced by apoptosis processes switched on by the studied compound.

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Type of presentation: Poster

P-10 The sensitivity of cholin inclusive phospholipids of rat brain cells nuclear to progesterone in vivo action.

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The lipid composition of the nucleus has been the subject of study for 40 years. Nuclear lipids are vital for cell life. They are components of the nuclear membrane, nuclear matrix, nucleolus and chromatin and are involved in numerous biological processes, including signal transduction and a variety of metabolic pathways. The metabolism of nuclear lipids is regulated independently of that of the cytoplasm.
Progesterone is known to stimulate metabolic processes in target cells. For example, progesterone suppresses the production of pro-inflammatory cytokines and chemokines in monocytes and the receptors of this steroid plays a major anti-inflammatory role in human myometrial cells.
Taking into consideration the dose depended active part of nuclear lipids in regulating of many essential cellular processes, investigation of action of progesterone on phospholipid content of nuclear fraction from rat brain cells is also of great interest.
The results show that the progesterone injection leads to increase in total amount of phospholipids in studied preparations nearly by 20%. Progesterone action caused significantly alterations of absolute quantity of all phospholipid fractions in compare to baseline. It is worthy to mention the increase in quantity of sphingomyelin by 120%. Phosphatidylcholine is the least sensitive to progesterone among the phospholipid fractions: its amount has been increased by only 11%. This is probably due to a 120% increase in sphingomyelin content and indicates a shift in sphingomyelin and phosphatidylcholin synthesis towards sphingomyelin.

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Type of presentation: Poster

P-11 Functional analysis of the histone variant H2A.Z of C. elegans in tissue- and stage-specific manners

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Recent molecular epigenetic research has revealed that histone variants have important roles in the epigenetic regulation of gene expression and cell development and differentiation. The histone variant H2A.Z is evolutionarily conserved in eukaryotic organisms and has been suggested to play important roles in epigenetic phenomena, including cell development and differentiation. However, since conventional knockout of H2A.Z induces cell lethality, the functions of H2A.Z in multicellular organisms are largely unknown. In this study, we established a method to knockdown the H2A.Z function in Caenorhabditis elegans in a whole-body or a gonad-specific manner using the auxin-induced degron system (AID). Using this method, the knockdown of H2A.Z in the whole body resulted in abnormalities in the gonads and exhibited the Endomitotic oocyte (Emo) and the Masculinization of the germline (Mog) phenotypes. In contrast, the knockdown of H2A.Z in a gonad-specific manner didn't cause these abnormalities, suggesting an important role of H2A.Z in inter-tissue functional communication. In this study, we propose new insights into the reproduction of H2A.Z in multicellular organisms.

Type of presentation: Poster

P-12 Mouse Polyomavirus Affects Nuclear Lamina in the Late Phase of Infection

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One of the important components of nuclear cytoskeleton is nuclear lamina. It is composed of intermediate filaments V, A type lamins (lamin A/C) and B type lamins (lamin B1 and B2). Although nuclear lamina maintains the structural integrity of the nucleus, it plays an important role in fine-tuning of DNA related processes, e. g. replication or transcription and is also a natural barrier for viruses replicating in the nucleus. Hence, viruses evolved different mechanisms ensuring exploitation of the lamina for their own purposes.
In this study, we followed the changes of lamina in cells infected with mouse polyomavirus (MPyV). At late times post infection, MPyV major capsid protein, VP1, accumulates in a close proximity of nuclear lamina. We were interested if replication of MPyV in cells affects nuclear lamina and if the lamina plays a role in virus replication. Despite that the defects in lamin A/C and B1 staining were observed, the nuclear lamina breakdown was not proven. After in situ fractionation, VP1 and the viral non-structural protein large T antigen (responsible for viral genome replication) were found together with lamin A/C and lamin B1 in the last insoluble fraction, indicating possible complex formation. In addition, VP1 protein together with lamin B1, was found in the last insoluble fraction after in situ fractionation of cells with lamin A knockdown (LMNA KO), suggesting that lamin B1 serves as a scaffold for virus replication centres formation. Further, during the course of the infection, the level of lamin A/C was decreasing and level of lamin B1 was increasing. Moreover, the higher solubility of lamin A/C was observed and changes in lamin A/C phosphorylation were detected in the infected cells. Also, in LMNA KO cells, the transcription of viral genes was decreased. These data suggest that MPyV infection affects lamin A/C network which becomes solubilized and that the solubilization of lamin A/C is required for efficient viral gene transcription.

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Type of presentation: Poster

P-13 Fluorocarbon solvents enhance growth and reduce oxidative stress in liquid culture of bacteria/yeast

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E. coli and budding yeast are widely used to produce recombinant DNA, proteins, and industrial products. Therefore, the efficiency of their culture affects the yield of the products. Chemical inert fluorocarbon solvents, called FC, were researched in liquid breathing and blood substitute because of their oxygen solubility and low toxicity and were reported to improve the growth of bacteria. However, whether oxygen dissolved in FC is responsible for improving cell growth has not been still unclear. So, we aimed to disclose the mechanisms of the effect of FC in bacteria/yeast liquid culture. Equal amounts of FC were added to the bacteria culture medium, sealed to prevent evaporation, and incubated in a 96-well microplate. The growth of bacteria was evaluated using the turbidity method (O.D 600nm) by spectrophotometer. We observed that hypoxic FC improved the growth of E. coli (Dh5α) similarly to untreated FC. This result suggests that the dissolved oxygen in FC is not solely responsible for the enhancement of bacterial growth. We also revealed that by using a mitochondrial gene-deficient yeast strain, rho0, the respiration process is required for growth enhancement in the budding yeast culture by FC. Moreover, under a microscope, we observed that E. coli (BW25113) growth is promoted away from the interface between FC and the bacterial culture solution. In addition, some oxidative stress response genes in E. coli were repressed by the addition of FC. These results suggest a possibility that some oxidative stress inducer is adsorbed at the interface between FC and culture medium and that FC promotes the growth of bacteria/yeast through this mechanism. We expect that FC can be utilized to improve liquid cell cultures for bacteria, yeast, and cultured animal/plant cells and also to establish novel methods for VBNC (viral but nonculturable) bacteria.

Type of presentation: Poster

P-14 Novel hybrid pyridine-thiazole derivative Les-6485 as potential Poly(ADP-ribose) polymerase inhibitor

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Alterations in DNA repair pathways are one of the main whys of cancer development. However, cancer cells are more susceptible to DNA damage than normal cells and they depend on specific functional repair pathways for survival . The benefit of DNA repair inhibition therapy is that it selectively amplifies unrepaired endogenous DNA damage in tumor cells and thus appears to have fewer side effects on non-cancerous cells. Our work aimed to study the mechanism of cytotoxic action of 4-(2-{1-(2-fluorophenyl)-3-[4-methyl-2-(pyridin-2-ylamino)-thiazol-5-yl]-3oxopropylsulfanyl}-acetylamino)-benzoic acid ethyl ester (Les-6485), as a potential PARP inhibitor, on normal and tumor mammalian cell lines.
Derivative Les-6485 was applied toward 15 cancer cell lines. The IC50 of Les-6485 ranged from 2.79 μM to 8.05 μM for tumor cells and it was not harmful to the lymphocytes of the peripheral blood of healthy human. Preincubation of tumor cells with Fluzaparib (inhibitor of PARP1) reduced in 3 times the activity of the Les-6485. In addition, it affected the nativity of DNA and showed synergistic activity with the MGMT inhibitor. This compound demonstrated the red-yellow fluorescence in a region closer to the nucleus of MCF-7 cells in a timedependent manner.
Since the preincubation of the tumor cells with PARP1 inhibitor reduced the sensitivity of these cells to the Les-6485, it can be assumed that its mechanism of action is connected with PAPR inhibition. The action of Les-6485 could be interesting in combination with different DNA repair inhibitors for study of the effects of synthetic lethality or in a combination with DNA damage agents.

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Type of presentation: Poster

P-15 Class III PI3-kinase Vps34 is involved in the cellular stress response in human leukemic cell lines

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Vacuolar protein sorting 34 (Vps34) is a class III phosphatidylinositol 3-kinase responsible for the generation of phosphatidylinositol 3-phosphate (PI3P), a lipid signaling molecule engaged in regulating cell proliferation, autophagy, and endocytic trafficking. Besides being present in the cytoplasm, Vps34 can localize to the nucleus where it colocalizes with upstream binding factor (UBF) in the nucleolus (Bertović *et al., unpublished*). The nucleolus is a non-membrane-bound organelle with a key role in ribosomal biogenesis and is a central hub for cellular stress response. Therefore, we aimed to investigate how cellular stress affects Vps34 and nucleoli in human megakaryoblastic (DAMI, HEL) and myeloid (K562) cell lines. Western blot analysis showed that nucleolar markers (UBF, fibrillarin, and B23) are upregulated with no change in Vps34 expression during cell proliferation. Nucleoli are visible in the early stages of cell proliferation with Vps34 visible in the nucleolus. During early PMA-induced differentiation, immunofluorescence showed an increased number of nucleoli and nucleolar Vps34 localization, although the expression of nucleolar markers drops with time. Pharmacological inhibition of Vps34 (Vps34-IN1) time and dose-dependently upregulated a key marker of DNA damage, γ-H2A.X. Interestingly, this was coupled with downregulation of UBF, but only in DAMI and HEL which are known to carry an important hematological mutation JAK2V617F. However, this effect was not observed in K562 or primary murine megakaryocytes. When exposed to a DNA-damaging agent (UV radiation), DAMI and HEL cells that were pre-treated with Vps34-IN1 exhibited lower γ-H2A.X levels after UV exposure when compared to controls. We also observed Vps34 and PI3P localizing in the nucleus of cells with positive γ-H2A.X foci. Taken together, our preliminary results indicate the potential involvement of Vps34 and its lipid product PI3P in the cellular stress response.

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Type of presentation: Poster

P-16 Newly synthesized juglone-based thiopyranothiazole Les-6400 has a pronounced cytotoxic effect towards cancer cells in vitro and interacts with DNA in two different ways

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Cancer remains one of the main causes of mortality in the world population. Therefore, developing and bio-evaluating novel highly effective, selective antitumor agents is a crucial aim in modern pharmacology and biomedicine. Thiazole-containing derivatives are promising candidates for this role. Our previous studies proved that thiazole-based compounds exhibit high effectiveness, potent anticancer activity, and less general toxicity in vivo.
The aim of the work was to study the cytotoxic activity of new 1,4-naphthoquinone-based thiopyranothiazole derivative Les-6400 towards cells of different origin and investigate its ability to interact with DNA.

It was found that the Les-6400 (11-(furan-2-yl)-9-hydroxy-3,11-dihydro-2H-benzo[6,7]thiochromeno[2,3-d]thiazole-2,5,10-trione) is characterized by selective action, so its cytotoxic effect on pseudonormal cells is weaker than on neoplastic cells. The most sensitive to Les-6400 action were human colon cancer cell HCT-116 (IC50=0.6±0.24 μM) while the most resistant were pseudonormal human keratinocytes HaCat (IC50=37.16±0.56 μM). IC50 of Les-6400 for cancer cells ranged from 0.6 μM to 5.98 μM during pseudonormal cell lines it was from 2.59 μM to 37.16 μM. In addition, the anti-leukemic activity of the studied compound was investigated ex vivo towards lymphocytes of healthy donor as well as chronic lymphocytic leukemia cells (CLLs) of non-treated / bendamustine-treated donors and patients in relapse. Les-6400 may be a good addition to standard anti-CLL therapy because CLLs of the treated donor were sensitive to this compound (IC50=8.24±0.32 μM). At the same time IC50 for lymphocytes of healthy donor was >50 μM. Hoechst-33342 fluorescence assay, DNA laddering assay, FACS analysis (Annexin V / PI) indicate apoptosis-inducing properties of Les-6400. While Methyl Green test and KMnO4 DNA oxidation reaction confirmed that Les-6400 is able to interact with DNA in two ways namely electrostatic interaction and intercalation.

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Type of presentation: Poster

P-17 C-terminal tail-dependent and tail-independent molecular associations of histone H2A.Z

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The variant histone H2A.Z is involved in the regulation of diverse and basic cell functions, with activating as well as repressive effects that have proved difficult to reconcile in a unified model. We have determined that H2A.Z is present in euchromatic as well as heterochromatic locations, reflected also by their disparate stability features (see also other posters of our group). In the wake of marked effects of a peptide representing the C-terminal tail (C9) of the histone variant on nucleosome stability measured in situ, nuclear architecture and accessibility features of the chromatin, fluorescence correlation spectroscopy (FCS) measurements were performed to characterize its binding to reconstituted nucleosomes. We have demonstrated the binding of carboxyfluorescein-labeled C9 to reconstituted H2A and H2A.Z-containing nucleosomes and to naked DNA, using a labeled scrambled peptide as a control. In immunofluorescence studies performed on nuclear halo samples prepared by high salt treatment of the agarose embedded nuclei, we also detected H2A.Z in tight association with the nuclear lamina, what was confirmed by mass spectrometry. We propose that molecular interactions involving its unstructured C-terminal tail that may be bound alternatively to the internucleosomal DNA, increasing the stability of these nucleosomes, or to reader proteins targeting the tail, are to a great part responsible for the disparate roles of this variant histone. The high salt-resistant fraction of H2A.Z detected in the nuclear lamina may play a role in the peripheral tethering of constitutive heterochromatin.

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Type of presentation: Poster

P-18 Detecting cytosolic DNA using SYBR green and sSTRIDE technique

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DNA can be found in cytosol as a consequence of damage and fragmentation of nuclear or mitochondrial DNA, or internalization of microorganisms. Cytosolic DNA can activate cellular responses that have evolved to sense and destroy foreign DNA [1].
The goal of this work was to develop methods to detect and quantify cytosolic DNA.
Fluorescent probe SYBR Green exhibits affinity to dsDNA and stains mitochondrial nucleoids in live cells (nuclear DNA in live cells stains only weakly), however it stains DNA in the cell nucleus and DNA fragments in the cytoplasm of fixed cells. This particular feature of SYBR Green DNA staining makes it possible to assess the position of DNA nucleoids in a live cell and subsequently identify the positions and numbers of damaged DNA fragments in the same cell after fixation. We have used SYBR Green to detect small fragments of cytosolic DNA in cells grown under standard conditions, and micronuclei and numerous fragments of cytosolic DNA in cells following exposure to UV.
We also used STRIDE, a new very sensitive technique capable of detecting DNA ends in fixed cells [2], to detect cytosolic DNA fragments in cells exposed to UV. The advantages and shortcoming of both techniques in detecting cytosolic DNA in fixed cells will be discussed.

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Type of presentation: Poster

P-19 DNA damage and repair in wild wheat *Triticum dicoccoides*.

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Wild wheat *Triticum dicoccoides* Körn. (*Poaceae*, Triticeae) has evolved in the Near East Fertile Crescent across multiple geographic ranges and ecological habitats, resulting in broad physical and biotic heterogeneity of natural populations and high adaptive genetic diversity. We investigated the influence of environmental factors on DNA damage and repair in native plants from two adjacent geologically and edaphically contrasting microsites, with humid basalt and dry terra rossa soil types, in the Tabigha population (Upper Galilee, Israel). Our data show that, in plants of the water-deficient Terra Rossa microsite, the amount of DNA double-strand breaks (DSBs) in interphase nuclei increases significantly, and the expression of genes that play a key role in the DNA Damage Response (DDR), Non-Homologous End Joining (NHEJ), and Mismatch Repair (MMR) pathways is lower compared with that in plants of the humid Basalt microsite. The mean contribution of Homologous Recombination (HR) to DSB repair in both microsites seems to be similar; however, the average values mask genotype-dependent differences in the level of gene expression. Immunodetection of γH2AX, RAD51, and LigIV on condensed chromosomes indicates that both DSB repair pathways, HR and NHEJ, operate during the M-phase; nevertheless, a significant proportion of DSBs, which are marked by γH2AX foci, remain unresolved in late mitosis. Furthermore, γH2AX-associated RNA polymerase II (RNAPII) and S9.6 foci are revealed on condensed chromosomes, suggesting post-interphase R-loop formation that is a potential source of chromosome aberrations, posing a threat to genome stability. Together, these results show the influence of edaphic factors on DNA damage and repair processes in the wheat genome in contrasting environments.
Reference: Raskina et al. 2023. The Influence of Edaphic Factors on DNA Damage and Repair in Wild Wheat Triticum dicoccoides Körn. (Poaceae, Triticeae) https://doi.org/10.3390/ijms24076847

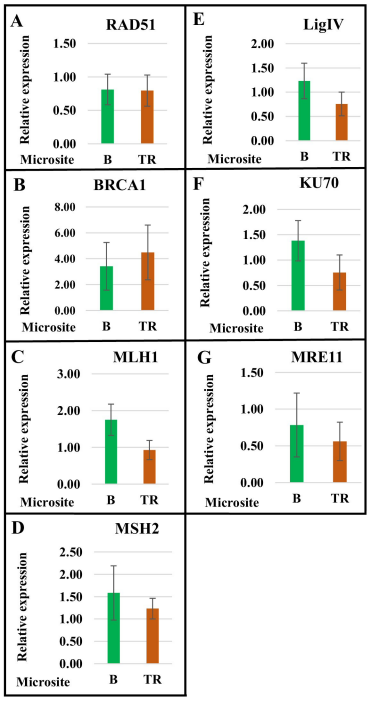

Fig. 1: Real-time qPCR analysis of the mean expression of the genes involved in DNA repair pathways in plants from the Basalt (B) and Terra Rossa (TR) microsites of the Tabigha population.

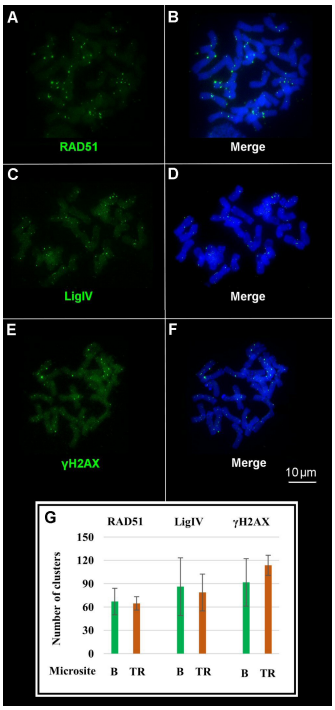

Fig. 2: Immunofluorescent localization of (A-B) anti-RAD51, (C-D) anti-LigIV, and (E-F) anti-γH2AX antibodies on metaphase chromosomes. Counterstaining with DAPI. (G) The mean number of clusters of RAD51, LigIV, and γH2AX on metaphase chromosomes in plants of the Basalt (B) and Terra Rossa (TR) microsites.

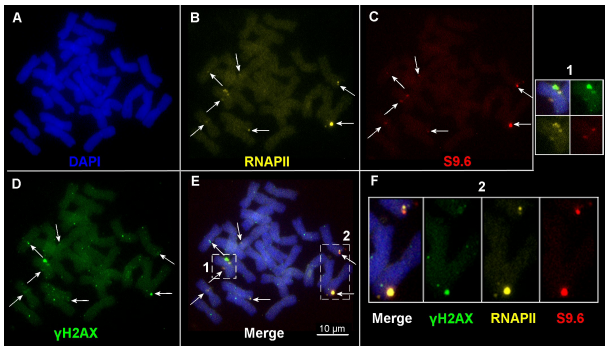

Fig. 3: Immunofluorescent localization of anti-RNAPII, anti-DNA-RNA hybrid [S9.6], and anti-γH2AX antibodies on metaphase chromosomes. (F) The fragments in dashed boxes 1 and 2 in E are shown as enlargements in small boxes. The seven co-localized clusters of RNAPII, S9.6, and γH2AX are indicated by arrows.

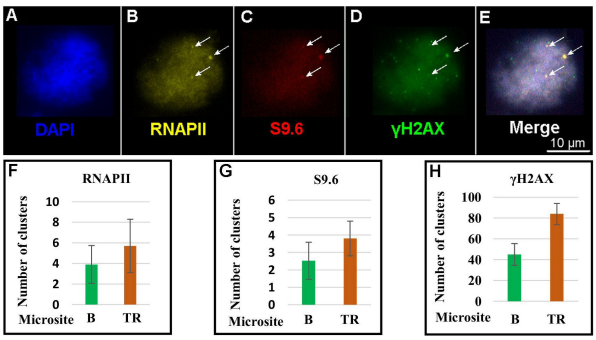

Fig. 4: Immunofluorescent localization of anti-RNAPII, anti-DNA-RNA hybrid [S9.6], and anti-γH2AX antibodies (A-E) and average number of clusters (F-H) in interphase nuclei in plants of the Basalt (B) and Terra Rossa (TR) microsites. The co-localization of RNAPII, S9.6, and γH2AX large clusters is indicated with arrows (A-E).

Type of presentation: Poster

P-20 Cytotoxicity of novel thiazolidinone derivatives on breast (MDA-MB-231, MCF-7), neuroblastoma (SH-SY5Y), and leukemia (RPMI-8866) cell lines.

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Cancer remains a significant healthcare problem worldwide. World Health Organization estimates that it represents the first or second cause of death for people under 70 years of age in 2019. This poses significant challenges to research focused on the development of novel and more effective pharmaceuticals without the disadvantages/adverse effects of classic/conventional anticancer chemotherapeutics. The molecular hybridization strategy is a popular trend and an attractive research direction, providing an endless source of significant opportunities in the design of new 4-thiazolidinone-bearing hybrid molecules with potential anticancer activity. Due to their biological activity, using a hybrid-pharmacophore approach, 4-thiazolidinones are promising compounds for modern anticancer therapy.

The aim of this study was to synthesize and evaluate the biological activity of the group of 11 novel

4-thiazolidinone derivatives in MDA-MB-231 and MCF-7 breast cancer cells and SH-SY5Y human neuroblastoma cells, RPMI8866 myelogenous leukemia and RAJI lymphoblast-like cells isolated of a Burkitt's lymphoma. MTT assay was used to assess cell viability. Cancer cells were incubated with the tested compounds and doxorubicin as a reference compound for 48 hours.

Among the 11 novel thiazolidinone derivatives, five had a significantly high cytotoxic activity with IC50 values range from 1 μM to 15 μM on the cell lines tested. The most active compound against the tested cell lines was compound A1 with IC50 values of 6.90 μM, 2.70 μM, 2.55 μM, 0.23 μM and 9.07 μM for MDA-MB-231, MCF-7, SH-SY5Y, RPMI-8866, and, RAJI respectively.

Type of presentation: Poster

P-21 The H3.3 histone variant and its chaperones in polyomaviral infection.

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Polyomaviruses (PyVs) are small non-enveloped viruses replicating in the host cell nucleus. Viral replication centers (VRCs) share features with cellular euchromatic or heterochromatic regions, depending on the stage of infection. In virion, the circular dsDNA genome and cellular histones are present in the form of a condensed minichromosome arranged into 24 nucleosomes. In this study, we demonstrate that, in addition to canonical histones, PyV minichromosome contains the non-canonical histone H3.3. Accumulation of H3.3 in VRCs occurs as early as 24 hours post infection (hpi) when viral genomes undergo massive replication. The DAXX (death domain-associated) and ATRX (6-alpha-thalassemia, mental retardation X-linked syndrome) proteins, which form a histone chaperone complex that resides in PML NBs (promyelocytic leukemia nuclear bodies), appear in the VRCs 24 hpi, but, in the late phase of infection (when heterochromatinization of viral genomes and assembly of virions take place), only DAXX without ATRX is located in the VRCs. PML NBs surround VRCs and increase in number and size as the infection progresses. The proximity of PML NBs to VRCs is retained even after inhibition of replication which indicates that PML NBs could directly recognize viral chromatin. Knockout of *Pml* or *Daxx* gene leads to increased accumulation of H3.3 in viral minichromosomes. Moreover, knockout of *Pml* gene is beneficial for PyV transcription. We suggest that PML NBs limit the accumulation of H3.3 into viral minichromosomes, and that also other histone chaperones than DAXX-ATRX (e.g., HIRA) are involved in PyV minichromosome modification. The ATRX-independent localization of DAXX in the VRCs during the late stage of infection suggests a yet unidentified function of the protein. The level of transcription of H3.3-deficient PyV, the distribution of H3.3 within the PyV minichromosomes and its post-translational modifications will be the subject of future research.

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

Type of presentation: Poster

P-22 DNA damage in the nucleated blood cells and production of free radicals in multiple sclerosis and type 1 diabetes mellitus patients

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The main objective of this is study was to evaluate level of DNA lesions and production of the free radicals in blood of the multiples sclerosis (MS) and Type 1 diabetes mellitus patients (T1DM). Whole blood of MS and T1DM patients was collected, lymphocytes were isolated, level of DNA breaks was determined using the alkaline comet assay, level of oxidized bases – by means of enzyme modified comet assay. Nitric oxide (NO) was measured by electron paramagnetic spectroscopy, metabolites of NO (NOx) we measure by luminescence. Significant increase in DNA damage was observed for T1D and MS patients. The level of oxidized bases was equal in MS patients and healthy subjects. Increase of NO level was observed in both pathologies, however in MS patients the NOx levels were also elevated, but they dropped down in the T1DM patients. MDA level evaluation showed a significant decrease in the MS study group, which also showed association with NOx level, but no associations with disease modifying therapies.

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Acknowledgement: The study was funded from the ERDF projects No. 1.1.1.1/16/A/016 and No. 1.1.1.2/VIAA/4/20/671

Type of presentation: Poster

P-23 Nanoscopy of complex promoter-enhancer topologies

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The 3D organization of the genome has increasingly been shown to play an important role in gene regulation. At a DNA level, distal regulatory elements such as enhancers, come into close spatial proximity to the promoters of their target genes, modulating their transcription. While most genes possess multiple enhancers, how multiple enhancers interact to regulate gene expression is still unclear. We hypothesize two extreme scenarios: either, the interaction could be competitive, meaning enhancers contact their target gene one by one, or, consistent with phase separation models, elements could converge simultaneously in an interaction hub. We employ a combination of fluorescence in situ hybridization (FISH) and superresolution microscopy to study these interactions in single cells. Using microfluidics, we have set up a sequential imaging system, which will allow us to visualize the 3D spatial positions of several individual genomic loci of interest, together with RNA and proteins. Focusing on a subset of 8 developmental genes, we are going to examine several promoter – enhancer interactions. Subsequently, we are going to use the transition from naive to primed mouse embryonic stem cells, a system in which major reorganization of enhancer patterns takes place, in order to observe if and how the proximity between these regulatory elements changes when they no longer interact. Correlating the enhancer interaction patterns with transcription will give us further insight into how different topologies influence gene expression.

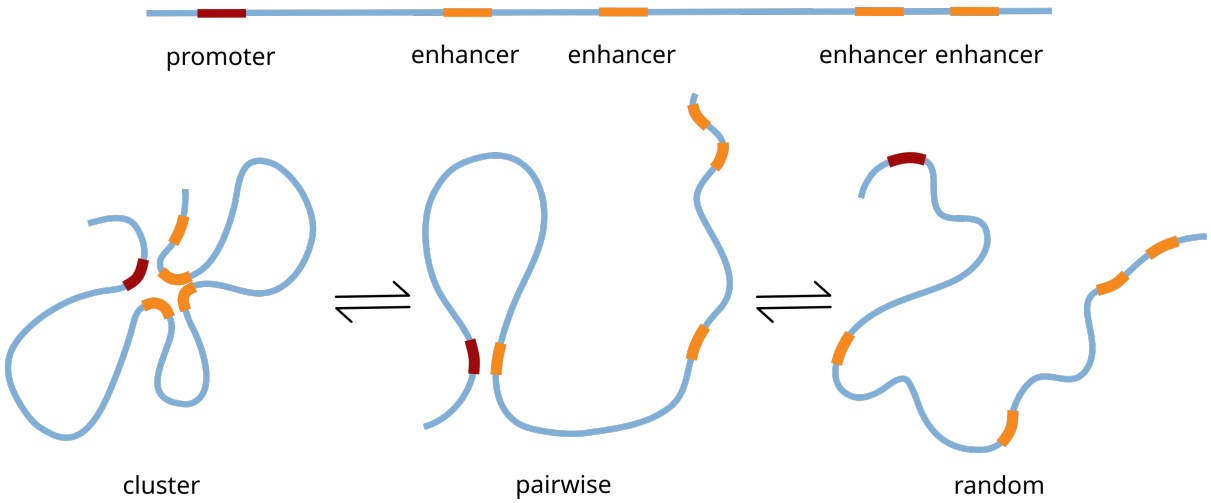

Fig. 1: Schematic representation of possible topological structures of a promoter (red) and enhancer sites (orange): representation of genomic distances (top) and examples of possible structures with different 3D distances (bottom).

Type of presentation: Poster

P-24 Lysyl oxidase propeptide (LOX-PP) inhibits ovarian cancer cells proliferation and sensitizes cells to chemotherapeutic agents.

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Introduction: Lysyl oxidase (LOX) is an enzyme engaged in the cross-linking of the extracellular matrix proteins. LOX proenzyme is secreted to extracellular space, then proteolitically cleaved resulting in the LOX enzyme and LOX propeptide (LOX-PP). Various studies showed contradictory results, indicating that LOX may act either as an oncogene or a tumor suppressor. More detailed analyses suggest that LOX enzyme may be responsible for oncogenic effects, while LOX-PP may act as tumor suppressor. Our aim was to investigate LOX-PP role in ovarian cancer cells.
Materials and methods: Ovarian cancer cell lines (SKOV3, A2780, ES2) with stable LOX-PP overexpression were established using lentiviral transfer system. Cell lines were validated by semi-quantitive RT-PCR and immunoblotting. Cells proliferation were assessed by MTS assay. In vitro cytotoxicity assay was performed with two major drugs used in ovarian cancer treatment: cisplatin (0,1-20μM range) and paclitaxel (1,56 nM - 30μM range).
Results: RT-PCR showed that the LOX-PP-containing cells had higher LOX-PP expression compared to control cells with an empty vector. Immunodetection was successful both in cells lysates and in culture medium suggesting that the protein is efficiently secreted. Cell proliferation assay showed lower proliferation rate of A2780_LOX-PP as compared to controls. No differences in proliferation rate was observed among cell lines SKOV3 and ES2. In vitro cytotoxicity assay demonstrated that LOX-PP overexpressing SKOV3 and A2780 cells were more sensitive to both cisplatin and paclitaxel. The A2780_ LOX-PP cells showed a 8,5% and 16% higher sensitivity to paclitaxel and cisplatin, respectively, while SKOV3¬_ LOX-PP cells showed 15% and 24% higher sensitivity to those drugs.
Conclusion: LOX-PP seems to exert an anti-cancer function in ovarian cancer, either by decreasing cells proliferation rate and/or by sensitizing of cells to chemotherapeutic agents.

Type of presentation: Poster

P-25 ERM protein Moesin shows nucleus-specific interaction with TE silencing factor panoramix

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ERM proteins are one of the main components of organizing the cell cortex by crosslinking membrane proteins to the underlying F-actin network. Through these interactions, ERM proteins mediate several cellular processes, such as cell adhesion, membrane trafficking and signalling pathways. In Drosophila melanogaster, Moesin (Moe) is the sole protein responsible for ERM function, and while several studies detail its role in cytoplasmic processes, not much is known about Moesin's nuclear purpose. In this work, we have shown that Moesin interacts with panoramix (panx), a Diptera exclusive transposable element (TE) silencing factor in a nucleus-specific manner. Pulldown experiments pinpoint the interaction surface to the nuclear localization signal (NLS) containing region of panoramix. Using a Drosophila model with decreased nuclear Moesin, we have detected an increase in the transcript level of TEs that are known to be influenced by panoramix, providing a functional link between the two proteins.

Type of presentation: Poster

P-26 Cytotoxicity of novel darbufelone derivatives on breast (MDA-MB-231, MCF-7), neuroblastoma (SH-SY5Y) and leukemia (RPMI-8866) cancer cell lines

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One of the novel pathways in the search for new drugs is the synthesis of thiazolidinone derivatives. These compounds have a wide range of biological functions and present a major opportunity for further chemical alterations [1].
Darbufelone finds application in the treatment of rheumatoid arthritis and osteoarthritis. Darbufelone is an inhibitor of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) [2]. Novel darbufelone derivatives with thiazolidinone core were synthesized (C1-C12) and their activity was assessed in MDA-MB-231 and MCF-7 breast cancer cells as well as SH-SY5Y human neuroblastoma cells, RPMI-8866 myelogenous leukemia and Raji lymphoblast-like cells isolated of a Burkitt's lymphoma. Cell viability was evaluated using the MTT test. For 48 hours, cancer cells were cultured with the tested substances plus doxorubicin as a control substance. The new thiazolidinedione compounds showed significant cytotoxic activity with the highest activity of compound C2, with IC50 values of 6.459 μM, 5.218 μM, 1.125 μM, 0.1 μM, and 6.979 μM for MDA-MB-231, MCF-7, SH-SY5Y, RPMI-8866, and, Raji respectively. The reference compound had similar IC50 values as compound C2.

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Type of presentation: Poster

P-27 Regulation of gene expression by retention of mRNAs encoding splicing factors in larch microsporocytes during diplotene

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Most synthesized pre-mRNAs transcripts undergo co-transcriptional splicing. This allows for the immediate transport of mRNAs to the cytoplasm, where translation and protein synthesis occurs. There are also transcripts in cells that do not undergo co-transcriptional splicing. Such transcripts are temporarily or permanently retained in the cell nucleus. Nuclear mRNAs retention allows for the degradation of defective transcripts, regulation of gene expression, and allows mRNA to be stored in case of stress.
In larch microsporocytes, in which transcription occurs in a pulsed manner, a significant part of mRNAs, formed as a result of one transcription pulse, is not transported to the cytoplasm immediately after synthesis. Our research shows that not-fully spliced mRNAs can be stored in the nucleoplasm and within sub-nuclear domain called Cajal bodies (CBs). We observe retention of mRNAs related to gene expression and RNA processing, translation, processes and organelle organization, metabolic, cell cycle. A significant part of retained in CBs mRNAs are these encoding splicing factors. Among them there are transcripts encoding proteins associated with the U snRNAs maturation process and those associated with U snRNAs molecules during splicing assembly. These transcripts are not fully mature and after the post-transcriptional splicing, they are exported to the cytoplasm. The mRNA retention phenomenon most likely serves to regulate the time of translation. Additionally, the retention of transcripts encoding splicing factors may regulate the retention process itself through feedback. The retention of mRNAs encoding splicing factors delays their export to the cytoplasm and the proteins biosynthesis, and the deficiency of splicing factors, in turn, affects the splicing of other pre-mRNAs.

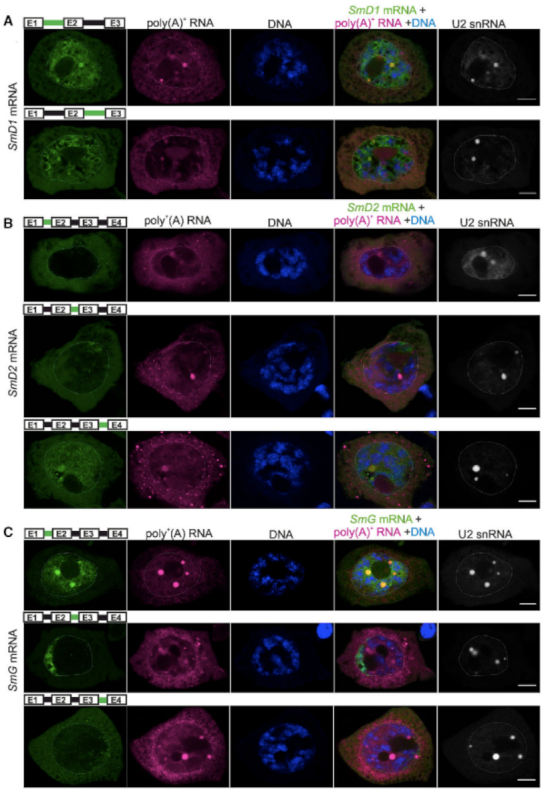

Fig. 1: SmRNAs are retained in the nucleoplasm and CBs as pre-mRNAs containing defined intron(s). pre-mRNAs for SmD1 has two retained introns. pre-mRNAs for SmD2 and SmG has only one retained intron. All these transcripts are accumulated in the nucleoplasm and CBs. (Rudzka et al. https://doi.org/10.1093/plcell/koac091)

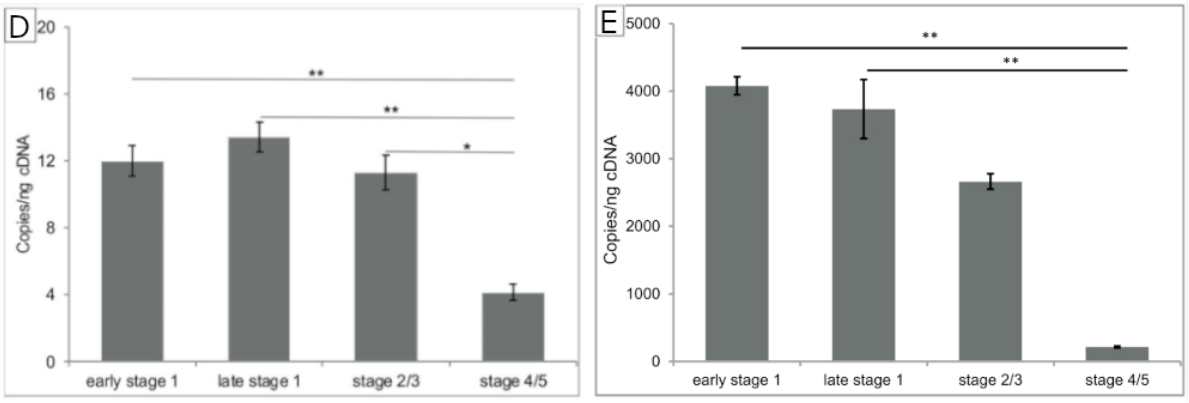

Fig. 2: Amount of the immature (D) and mature (E) forms of SmG pre-mRNA in successive stages of the cycle, as determined by RT-qPCR analysis. **P 5 0.01; *P 5 0.05 from ANOVA with Tukey's HSD (Rudzka et al. https://doi.org/10.1093/plcell/koac091)

Type of presentation: Poster

P-28 Lamins in fruit fly model - new protein complexes, new functions and strong muscle phenotype after lamins knockdown.

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A major part of the nuclear lamina are lamins, which are proteins responsible for maintaining mechanical stability, chromatin positioning, gene expression and many regulatory functions. In humans mutations in genes encoding for lamins and lamin interacting proteins, result in a set of rare, often neuromuscular, disorders. A great model organism to carry out the research of lamins is Drosophila melanogaster, which has two lamin types (A- and B-type). Our approach was based on the GAL4/UAS method which we have used for early embryo- and muscle- specific silencing of two lamin's genes. Downregulation of lamin C results in strong larval (on the 3rd instar) and muscle nuclei phenotype, also it is lethal at the imago stage. Immunofluorescence of larval muscles showed the presence of polymerized actin inside of the cell nucleus and disturbed cytoplasmatic actin neighbouring the nucleus. Also, nuclei were abnormally shaped. Depletion of lamin Dm gives a milder phenotype which was mainly irregular M and Z line distribution in muscle tissue and lower mobility of adult flies.
Also, a large effect was observed after the induction of heat shock on in vitro model (Kc and S2 cells) corresponding to lamin Dm. An experiment based on co-IP of lamin Dm combined with LC-MS/MS analysis showed that after stress induction the number of proteins interacting with lamin Dm increases drastically (for both tested cell lines). Detailed analysis showed that a large pool of the identified proteins is associated with stress granules (structures responsible for inhibition of protein biosynthesis, protection of mRNA and regulation of gene expression in response to heat shock). The obtained data indicate the involvement of lamin Dm in the mechanism of gene expression control (repression) after induction of heat shock. Interestingly, this effect was not observed for lamin C.

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