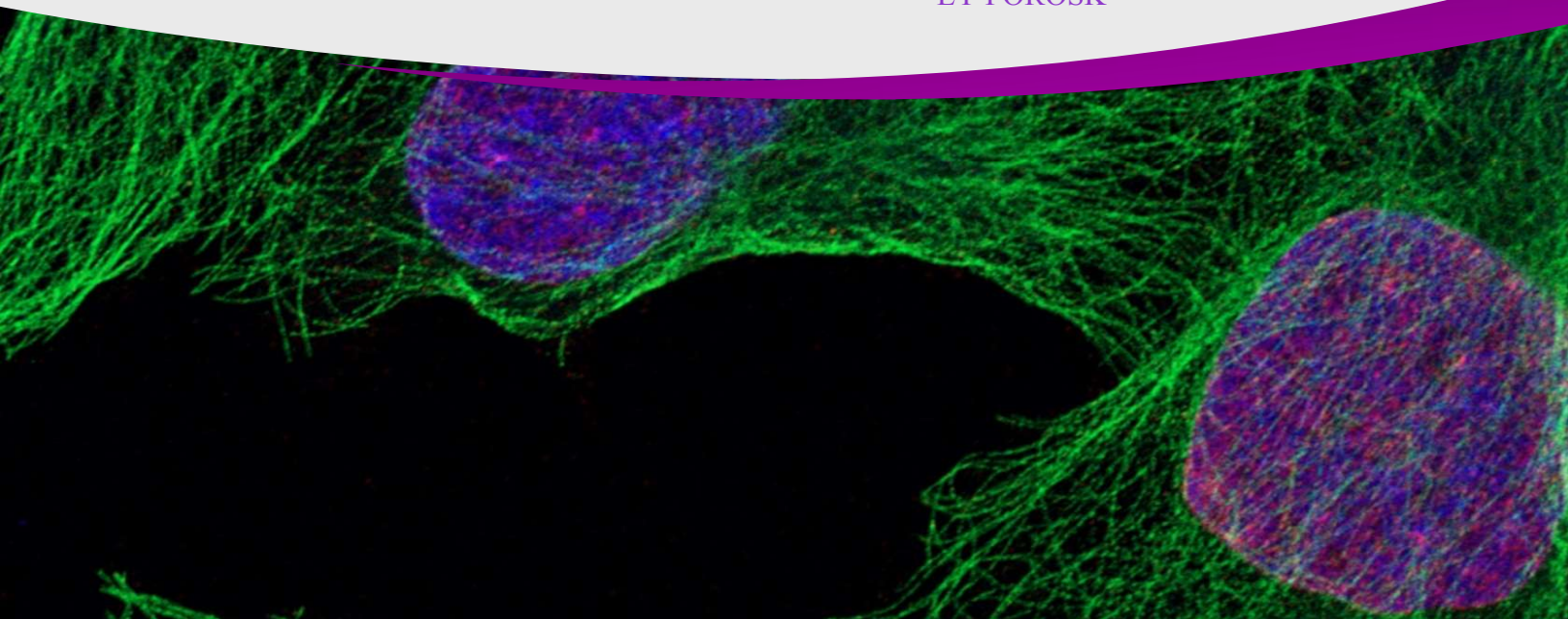
# 28th

# Wilhelm Bernhard Workshop

## on the Cell Nucleus

JUNE 16-20, 2025
TARTU, ESTONIA
LY POROSK



Organized by the University of Tartu, Institute of Technology

Local organizers: Prof. Reet Kurg, Margit Mutso, Kristiina Kurg, Merike Petuhov, Ly Porosk

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# Contents

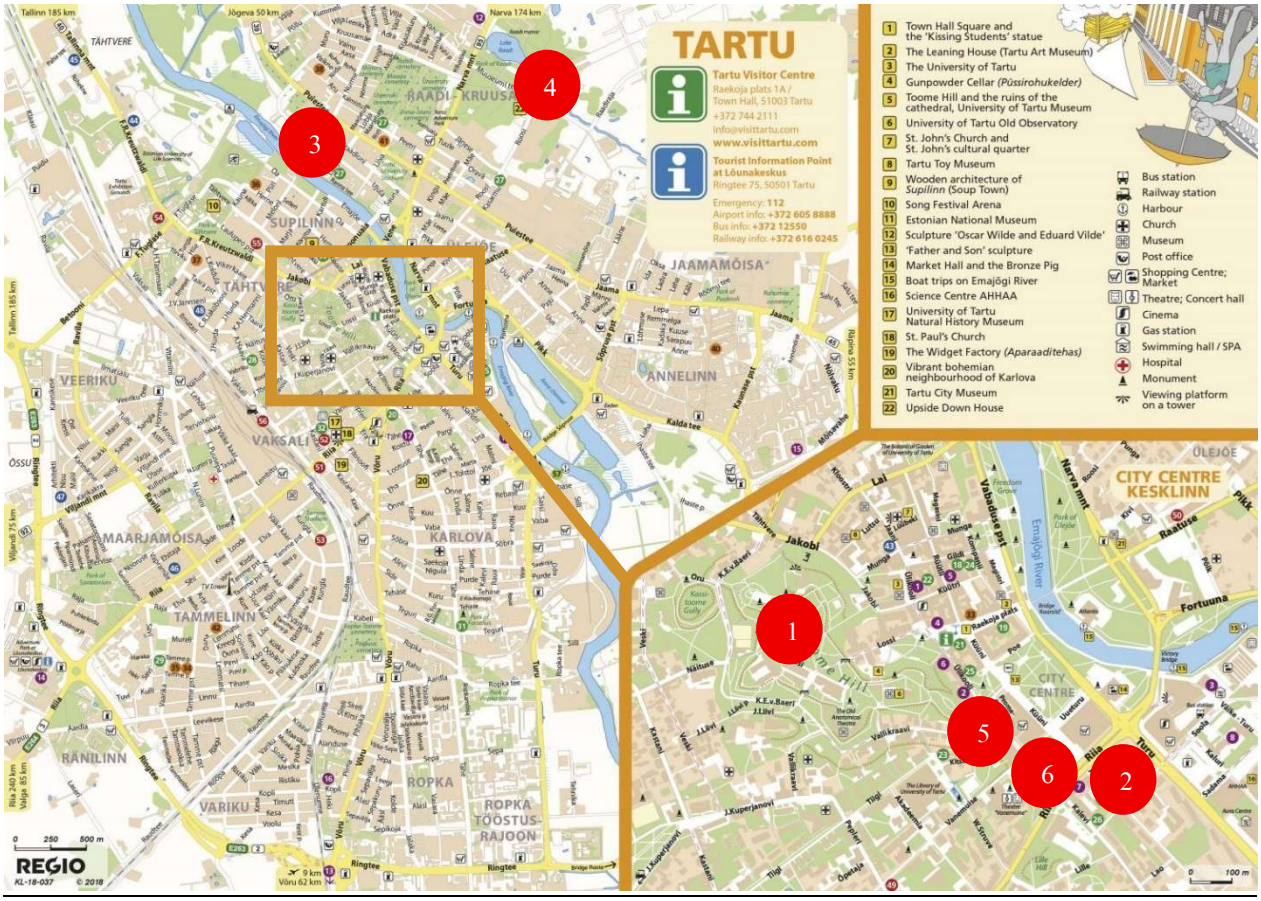
# Program and Guide

## Monday, 16th of June

**18:00** Registration at the University of Tartu History Museum (Marked with (1) on the included map). *The Museum will be open for the WBW28 participants from 18:00 to 21:00*

**Social program:**

**19:00 – 21:00** Welcome party at the same location, in the White Hall (Marked 1 on map)




(1) University of Tartu History Museum, and in the same building, the White Hall
(2) V Spa Conference Centre
(3) Lodjakoda
(4) Estonian National Museum
(5) Vilde ja Vine Restaurant
(6) Vanemuine Theatre parking lot

## Tuesday, 17th of June (V-spa and Conference Centre, marked with 2 on the map)

**9:00- 10:00** Scientific Board Meeting

Session 1 – Nuclear localization and epigenetic plasticity

**Chair: Yegor Vassetzky**

**10:00 – 11:00** Plenary – Chromatin landscape, histone variants and cell fate; WBW28 Medal Winner Geneviève Almouzni (France)

*Coffee break*

**11:30 – 12:00** Embryonal ovarian carcinoma PA1 treated by Etoposide: The journey through cellular senescence to the metastatic amoeboid phenotype (Jekaterina Erenpreisa, Latvia)

**12:00 – 12:30** Dynamics of the nano-scaled spatial organization of chromatin and repair foci during DNA repair and senescence after induction of double strand breaks (Michael Hausmann, Germany)

**12:30 – 13:00** Nuclear Reorganization During Hypoxia (Margherita Cavallo, Italy)

*Lunch*

Session 1 continues – Nuclear localization and epigenetic plasticity

**Chair: Reet Kurg**

**14:00 – 15:00** Plenary - Evolution of cellular and gene regulatory programs in mammalian cerebellum development; Young Researcher Awardee Mari Sepp (Estonia)

**15:00 – 15:30** Micromanagement of microexons in brain tumors (Andrei Thomas-Tikhonenko, USA)

**15:30 – 16:00** Chromatin mechanisms of neuronal maturation (Kärt Mätlik, Estonia)

*Coffee break*

**16:30 – 17:00** YEATS proteins – chromatin readers with additional functions (Arnold Kristjuhan, Estonia)

**17:00 – 17:30** Epigenetic Response to UV Irradiation in HEK293 Cells Expressing DDB2PCNA– (Claudio Casali, Italy)

**17:30 – 17:45** Essential roles of the histone variant H2A.Z in the development and function of reproductive organs of C.Elegans (Natsumi Horii)

Social program:

**19:00-21:00** - Boat trip with Suur Sume barge on river Emajõgi with snack. (Marked 3 on the Tartu city Map). The boat will leave at 19:00 sharp!

## Wednesday, 18th of June

Session 2 – Nuclear pathologies and cancer

**Chair: Pavel Hozak**

**9:00 – 10:00** Plenary – Tumor biology- are we asking the right questions? (Toivo Maimets, Estonia)

**10:00 – 10:30** Trans-interactions in the cancer cell nucleus (Yegor Vassetzky, France)

**10:30 – 11:00** Nuclear Voyage of Extrinsic Cell Death Society Members (Oleg Demidov, France)

*Coffee break*

**11:30 – 12:00** Melanoma-derived exosomes induce transcriptional stress response in T cells, leading to intrinsic apoptosis of target cells (Piotr Widlak, Poland)

**12:00 – 12:30** Non-canonical targets for PARP-catalyzed ADP-ribosylation (Alexander Ishchenko, France)

**12:30 – 12:45** The role of phosphatidylinositol 4,5-bisphosphate in RNA polymerase II transcription (Ludovica Antiga, Czech Republic)

**12:45 – 13:00** Localization and molecular mechanisms of nuclear functions of phosphatidylinositol 4,5-bisphosphate (Ana Miladinovic, Czech Republic)

*Lunch*

**14:00 – 14:30** Poster session (Odd numbers)

Social program:

**15:00** – Bus from in front of the main venue VSpa to Estonian National Museum, guided tour starts from 15:30. (Rally point marked 2 on the map, ERM location marked with 4 on map)

## Thursday, 19th of June

Session 3 – Nuclear structure and function I

**Chair: Piotr Widlak**

**9:00 – 9:30** AIF/CHCHD4 complex: a key complex for nucleus-to-mitochondria communication and metabolism reprogramming (Catherine Brenner, France)

**9:30 – 10:00** Comparative Analysis of Redox Pathway Activation During Ferroptosis in Lung Cell Lines (Jakub Pawlikowski, Poland)

**10:00 – 10:30** Ferroptosis in IBD: ultrastructural and molecular correlates of cell death susceptibility (Małgorzata Adamiec-Organiściok, Poland)

**10:30 – 10:45** OneCell CUT&Tag maps chromatin modifications together with transcriptome and phenotype starting from a single cell (Anna Schwager, France)

**10:45 – 11:00** Methyltransferase Hemk2 impact on cell structures and cell cycle (Margit Mutso, Estonia)

*Coffee break*

**11:30 – 12:00** Nucleoli and ribosomal gene expression in the context of tissue regeneration, aging and nutrition (Christian Schöfer, Austria)

**12:00 – 12:30** Nucleocytoplasmic regulation of mRNA biogenesis by actin-associated protein Carmil 1 (Tomas Venit, UAE)

**12:30 – 13:00** Actin and actin-related proteins involved in nuclear structure and function (Masahiko Harata, Japan)

*Lunch*

**14:00 – 14:30** Poster session (even numbers)

Session 3 – Nuclear structure and function II

**Chair: Albert Jordan**

**14:30 – 15:00** Effect of induced degradation of HIRA on prostate cancer models (Alexander M. Ishov, USA)

**15:00 – 15:30** The Zta (ZEBRA) Epstein-Barr Virus transcription factor: Relevance in clinical pathology (Emmanuel Drouet, France)

**15:30 – 16:00** Human papillomaviruses: advances and challenges in targeting the infection (Alla Piirsoo, Estonia)

*Coffee break*

**16:30 - 17:45** Panel discussion: Future Frontiers in the Cell Nucleus Research

**16:45 - 18:00** Closing words

Social program:

**19:00** – Gala dinner in the restaurant Vilde ja Vine (Marked 5 on map)

## Friday, 20th of June

Social program:

**9:30** - Bus to Tallinn that leaves from the theatre Vanemuine parking lot (Marked 6 on map)

**12:00** - Bus stops at the airport for luggage storage, then continues on to Tallinn Old Town

**13:00-15:00** - Tallinn Old Town tour

Bus returns to Tartu after the tour

# Welcome message

Dear Colleagues and Friends,

On behalf of the University of Tartu and the Institute of Technology, we are delighted to welcome you to the 28th Wilhelm Bernhard Workshop here in beautiful Tartu, Estonia. This event brings together renowned scientists and young researchers from all over the world to explore the latest advances in the study of the cell nucleus and its complex roles in cell functions and structures.

Gathering in the spirit of collaboration and discovery, let us honour the legacy of Dr. Wilhelm Bernhard, whose pioneering work in electron microscopy transformed our understanding of cellular biology. This workshop serves as an essential platform for fostering interdisciplinary relationships and encouraging connections across borders and generations.

We are pleased to offer opportunities for young scientists to present their research, engage in meaningful dialogues, and learn from experienced colleagues in their field. Together, we will deepen our knowledge, share innovative ideas, and inspire each other to push the boundaries of science.

Thank you for joining us in shaping the future of cell nucleus research. We look forward to an enriching and fruitful workshop.

The local organizing committee

# AWARD WINNERS

# Wilhelm Bernhard Medal

**Geneviève Almouzni** (Institut Curie, CNRS, Paris, France) is a remarkable researcher whose groundbreaking studies on the epigenetic regulation of gene expression has opened new doors in our understanding of chromatin dynamics. She has played a significant role in deciphering the complexes of cellular processes. She is a world leader in understanding genome organization and function during development and disease in particular in cancer.

Her creative approach to experimental design and commitment to interdisciplinary collaboration embody the values that the Wilhelm Bernhard Medal celebrates. Almouzni's achievements highlight not only her exceptional talents but also her important role in advancing the scientific community, making her a worthy candidate for this prestigious award. Beyond her research, she is known for her collaborative spirit and dedication to mentoring young scientists, helping to nurture the next generation of innovators in the field.

# Young Researcher Award

The Wilhelm Bernhard Local Organizing Committee has granted the 2025 Young Researcher Award to Mari Sepp from Heidelberg University's Centre of Molecular Biology (ZMBH), Germany, in recognition of her contributions to developmental biology.

# ORAL COMMUNICATIONS

# Session 1 - Nuclear organization and epigenetic plasticity

Oral 1

# Embryonal Ovarian Carcinoma PA1 Treated by Etoposide: The Journey Through Cellular Senescence to the Metastatic Amoeboid Phenotype

Jekaterina Erenpreisa[1], Kristine Salmina[1], Inna Inashkina[1], Felikss Rumnieks[1], Dimitry Perminov[2], Jaroslava Zajakina[3], Juris Jansons[1], Baiba Brumele[4], Talivaldis Freivalds[5], Ross D Saliba[1,5], Marc Bayer[3], Michael Haussmann[3], Reet Kurg[4]

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*katrina@biomed.lu.lv*

¶The role of cellular senescence in reprogramming and resistance of cancer stem cells to treatments is under study. Here, the PA1 embryonal carcinoma cell line was treated with 8 μM etoposide (ETO) for 20 h. At the critical day 3, we revealed interruption of mitoses and the p53-induced dual, heterogenous activation of both stemness and senescence regulators, OCT4A and p21, in the same cells. Mechanistic experiments showed that OCT4A was moderating the p21 levels, while methylating own enhancers along with upregulation of OCT4 isoforms OCT4B and OCT4B1 and disjoining from the chromatin. Transient suppression of SOX2 and upregulation of G1/S checkpoint inhibiting counterpart p27 was coupled to inactivation of the embryonal stemness network.

Beside p21, the cells signalled senescence by the increase of p16, Sa-β-gal staining, and DNA strand-break marker γH2AX. This critical response was accompanied by dissolution of the large perinucleolar heterochromatin blocks, their DNA de-methylation, and spreading of the repressive pericentric H3K9me3 heterochromatin granules within the nuclei, intermingling with euchromatin marker H3K4me3, and by activation of ALU retrotransposons. On day 5, the ALU increased three-fold, FISH showed mobility and reposition of ALU elements, H3K9me3 revealed the heterochromatin coalescence, with low H3K4me3 level and loss of any chromatin compartmentation.

From day 5 to 7, there occurred both massive cell death by anoikis and reconstitution of stemness in the polyploidized surviving cells attached to chamber slides. The excess of p27 was seen shed out of cell nuclei by increased presence of SOX2, the accumulated p16 was shed out of nucleoli and cell nuclei by concurring Nanog, which appeared as ~1μm granules transited into cytoplasm and less, around cells. Molecular analysis of total RNA with different reverse transcription PCR (RT-PCR) chemistries and Sanger sequencing displayed the transcription of the NANOG and increasing portion of its pseudogen 8, likely in recombined forms. Mass-spectrometry of secretome revealed in String analysis DNA replication, repair and recombination.

From day 5-7 onwards, the mitotically dividing cells and among them the front-rear polarized multinuclear giant cells appeared. They exhibited a typical amoeboid actin-Cdc42-rich front cytoskeleton trailing by a tubulin bridge a rear set of subnuclei, free of γ-H2AX. We suggest that transient bi-potential senescence with persisting p21 signalling finally led to the PAK-Rho-induced amoeboidisation with formation of the multinuclear, epithelial-mesenchymal, metastatic giant cell clusters capable of circulating in blood.

**References:** [1] Salmina et al. 2010, DOI: 10.1016/j.yexcr.2010.04.030; [2] Jackson et al. 2013, doi: 10.4161/cc.23285; [3] Huna et al.2015, doi: 10.1080/15384101.2015.1056948; [4] Baryshev et al. 2018, doi: 10.1080/15384101.2018.1426412

Oral 2

# Dynamics of the nano-scaled spatial organization of chromatin and repair foci during DNA repair and senescence after induction of double strand breaks

Michael Hausmann[1], Myriam Schäfer[1,5], Bayer, Marc[1], Jaroslava Zajakina[1], Martin Weinreich[1], Timon Caltapanides[1], Jonathan Hering[1], Jacob Leuther[1], Marian Seidl[1], Kristine Salmina[2], Jekaterina Erenpreisa[2], Harry Scherthan[3], MartinFalk[4], Georg Hildenbrand[5]

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The cell nucleus is a complex, self-organized biological system in which simultaneous reactions and functions take place to keep the cell as a specialized system well running. The cell nucleus contains chromatin packed in various degrees of density and separated in volumes of chromosome territories and sub-chromosomal domains. Between the chromatin there is enough “free” space for floating RNAs, proteins, enzymes, ATPs, ions, water-molecules etc., which are trafficking mostly by super- or supra-diffusion to the interaction points being required. It seems that this trafficking works somehow self-propelled driving the system perfectly. Compact heterochromatin seems to control this trafficking to interactions points located in euchromatin. After exposure to ionizing radiation or etoposide causing chromatin double-strand breaks (DSBs), the whole system “cell nucleus” responds, and repair processes start to recover the fully functional, intact system. At the molecular level, many individual epigenetic pathways of DNA damage response or repair of single- and double-strand breaks are at work. How these responses are embedded into the response of the system as a whole is often neglected, although especially for damages in heterochromatin, chromatin reorganization is necessary for an improved accessibility of repair proteins. By means of single molecule localization microscopy (SMLM), we have measured the spatial organization of hetero- and euchromatin after DSB induction during repair or at several time points during senescence. We also analysed γH2AX, 53BP1, Mre11, pATM, Rad51, DNA-PKcs and L1 and ALU regions after exposure to X-rays, electrons, and particles or internally incorporated electron emitter. The application of Ripley statistics, persistent homology, persistent imaging and principal component analysis on pattern of the molecular coordinates showed defined geometric conditions of the labelled sites. Systematic changes in chromatin organization and clustering, topologically expressed by mesh sizes of knot formations of chromatin networks indicate chromatin (re)arrangements associated with repair activities or changes of the cell fate during senescence. During a successful repair of DSBs, the whole chromatin revealed a cyclic movement in the latent space of the two major principal components. The same occurred for γH2AX or 53P1. The application of novel mathematical approaches in SMLM indicate that during DSB repair not only the damage sites but also the whole chromatin changes the organization. In contrast, during senescence hetero- and euchromatin, usually separated, can intermingle and re-separate which may indicate that genetic activity may vary and fluctuate. Our results support the verification of the hypothesis of the impact of spatial chromatin organization on the control of epigenetic pathways and vice versa. The functionally determined networks of chromatin and proteins seem to drive and to control the repair process at damage sites.

Oral 3

# Nuclear Reorganization During Hypoxia

Margherita Cavallo[1], Claudio Casali[1], Lucia Giulini[1], Adel Diaf[1], Davide Tunesi[1]; Gloria Milanesi[1], Giuliano Mazzini[2], Marco Biggiogera[1]

*[1]Department of Biology and Biotechnology, Laboratory of Cell Biology and Neurobiology, University of Pavia, Pavia, Italy, [2]Institute of Molecular Genetics, National Research Council (IGM-CNR), Italy*

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The spatial organization of the cell nucleus bears functional relevance in both maintaining cellular homeostasis and facilitating pathological transformations. In response to external cues, it undergoes dynamic reorganization of chromatin architecture, causing the spatial relocalization of nucleic acids and specific proteins into defined nuclear clusters, whose stimulus-driven compartmentalization remains largely elusive. Among the various environmental stimuli capable of triggering such nuclear reorganization, oxygen availability plays a pivotal role. Scarce oxygen levels lead to hypoxia, which is characterized by the activation of the hypoxia-inducible factor (HIF), a heterodimeric transcription factor composed of a stable β-subunit and an oxygen-sensitive α-subunit. While transient HIF activation contributes to cellular adaptation and tissue homeostasis, its chronic stabilization promotes the expression of genes associated with key cancer hallmarks. Recent studies have highlighted the role of hypoxia in reshaping the epigenetic landscape, suggesting that HIF-1α upregulation may drive chromatin remodelling events governing the expression of genes essential for cellular adaptation. Nevertheless, the intricate dynamics of nuclear reorganization elicited by hypoxic stress remain poorly understood. Therefore, this work is aimed at investigating chromatin reorganization in mouse hepatocytes exposed to hypoxia-mimicking conditions and during a subsequent reoxygenation phase. Our analyses suggest that cells adapt to hypoxic stress by reshaping the epigenetic landscape, resulting in a more accessible chromatin state that may support enhanced transcriptional activity, as indicated by the increased density of perichromatin granules (PGs). However, the highly decondensed and transcriptionally active environment may render the genome more susceptible to DNA damage, potentially activating cell cycle checkpoints, as evidenced by the accumulation of hypoxic cells in G2/M phase. Notably, this arrest coincides with the clustering of subnuclear components, including PGs and RNPs assemblies, which may serve as reservoirs for mature RNA and RNA-processing factors, respectively. The pronounced enrichment of these compartments suggests a regulatory mechanism aimed at either concentrating specific transcriptional regulators or transiently sequestering HIF-1α-responsive transcripts. In this context, we explored the role of the lncRNA MALAT1, which may contribute to the spatial organization of these functional nuclear assemblies. Interestingly, reoxygenation restored the epigenetic landscape and stabilized cell cycle progression, as evidenced by the resolution of G2/M arrest and accelerated S-phase progression. Further investigation could offer valuable insights into the intricate mechanisms of cellular adaptation to hypoxia, with significant implications for understanding tumour progression within hypoxic microenvironments.

Plenary 2

# Evolution of Cellular and Gene Regulatory Programs in Mammalian Cerebellum Development

Mari Sepp

*Heidelberg University, Centre of Molecular Biology (ZMBH)*

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The cerebellum expanded in parallel to the neocortex during human evolution and is increasingly recognised to play important roles in the evolution of cognition. Such phenotypic innovations are thought to be largely driven by evolutionary shifts in time- and cell-type-specific gene regulation. I will present studies where we used single-nucleus measurements of gene expression and chromatin accessibility to characterise mammalian cerebellum development from early neurogenesis to adulthood. Our data from six species (human, bonobo, macaque, marmoset, mouse, opossum) include approximately 780,000 single-nucleus profiles. We found largely conserved developmental dynamics of cell-type generation, except for Purkinje cells, which showed an expansion of early-born subtypes in humans. Gene expression analyses revealed that cerebellar cell type-defining programs have been preserved for at least 160 million years of mammalian evolution. However, we also observed widespread gene repurposing at the cell-type level, identifying numerous genes that gained or lost expression during evolution. To investigate cis-regulatory elements (CREs) driving interspecies expression differences, we trained a sequence-based deep learning model on chromatin accessibility data, revealing conserved regulatory codes. By predicting chromatin accessibility across 240 mammalian species, we reconstructed the evolutionary histories of human CREs, identifying sets associated with positive selection and gene expression changes. We performed enhancer reporter assays for a selection of CREs in ex vivo cultures of cerebellar granule cells, confirming that our model's predictions align with the evolution of enhancer activity. Altogether, our work unveils shared and lineage-specific programs governing cerebellum development, and expands our understanding of mammalian brain evolution.

Oral 4

# Micromanagement of microexons in brain tumors

Andrei Thomas-Tikhonenko

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Oral 5

# Chromatin Mechanisms of Neuronal Maturation

Kärt Mätlik[1,2], Matthew R. Paul[3], Eve-Ellen Govek[2], Natarajan Bhanu[4], Lijuan Feng[5], Benjamin A. Garcia[4], Thomas S. Carroll[3], C. David Allis[5], Mary E. Hatten[2]

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Neuronal development is controlled by epigenetic mechanisms, including histone and DNA modifications, which regulate gene expression patterns during lineage decisions, differentiation, and maturation. Histone methylation patterns are tightly regulated during neuronal maturation and influence chromatin accessibility and interactions with transcription factors, ensuring that appropriate genes are expressed at each stage of development. Using mouse cerebellar granule neurons as a model, we characterised changes in histone modifications during key stages of neuronal development: proliferation, glial-guided migration, and maturation. We show that the repressive histone modification H3K27me3 undergoes dynamic changes during development, including a local remodelling of H3K4me3/H3K27me3 bivalent domains at gene promoters, and a global increase in H3K27me3 at broad intergenic regions. Here, we will discuss the underlying molecular mechanisms, as well as the functional importance of H3K27me3 regulation on neuronal maturation.

Oral 6

# YEATS proteins - chromatin readers with additional functions

Arnold Kristjuhan[1], Signe Värv[1], Henel Jürgens[1], Kadri Peil[1]

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The YEATS domain is a highly conserved protein structure that interacts with acetylated and crotonylated lysine residues in the N-terminal tails of histones. YEATS proteins are found in all eukaryotes, including yeast and humans, where they assemble into large protein complexes involved in chromatin modification and transcription regulation. The genome of the budding yeast Saccharomyces cerevisiae encodes three YEATS domain proteins (Taf14, Yaf9, and Sas5), which are subunits of complexes involved in histone acetylation, gene transcription and chromatin remodelling. As yeast strains deficient in all three of these genes are inviable, it has been proposed that the YEATS domain is essential for yeast survival. We have investigated the functions of YEATS proteins in yeast and discovered that Taf14's primary role is to stabilise the transcription pre-initiation complex (PIC). Additionally, Taf14's linker region provides the PIC with extra DNA-binding capacity, which becomes essential for cell survival when chromatin accessibility is impaired.

Oral 7

# Epigenetic Response to UV Irradiation in HEK293 Cells Expressing DDB2PCNA–

Claudio Casali[1], Margherita Cavallo[1], Adel Diaf[1], Lucia Giulini[1], Davide Tunesi[1], Martina Furfaro[2], Anna Tricarico[2], Gloria Milanesi[1], Paola Perucca[2], Ornella Cazzalini[2], Marco Biggiogera[1]

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Nuclear and chromatin organization respond to the general needs of the cell through precise local arrangements. This allows for regulation of gene expression in response to various stimuli, promoting pathways related to adaptation, survival, or cell death. Depending on chromatin organization, DNA accessibility to transcription or repair factors may be favored or hindered. This occurs following several regulatory aspects, such as protein-protein and protein-DNA interactions, and epigenetic mechanisms involving DNA and histone tail post-translational modifications or ncRNAs activity. Such orchestrated system also functions in response to exogenous factors, since maintaining genome integrity is essential not only for cell survival, but also for the correct transmission of genetic information. To investigate how the nuclear environment responds to DNA damage, this study focused on DNA Damage Binding Protein 2 (DDB2), a protein involved in the early steps of DNA damage recognition in the nucleotide excision repair process, which specializes in removing UV-induced DNA photoproducts. Using HEK293 cells stably overexpressing either wild-type DDB2 (DDB2Wt) or its mutated form DDB2PCNA-, which is unable to interact with PCNA, we analyzed the effect of UV radiation from the epigenetic perspective. UV irradiation induces a time-dependent increase in acetylated H3K9 levels in DDB2PCNA- cells. Likewise, histone methylation displays an analogous dynamic course with an increment in trimethylated H3K9 compared to DDB2Wt. Similar trends are also verified for 5-methylcytosine and p300 histone acetyltransferase, key actors in genome organization and maintenance, respectively. Interestingly, both cell lines show a reduced presence of phosphorylated histone H2A.X compared to baseline expressing DDB2 cells. Notably, ultrastructural visualization demonstrated its preferential localization in euchromatin regions in DDB2PCNA- cells, further suggesting the always more widespread idea about a strict relationship between a local chromatin condensation state and DNA damage susceptibility.

Short talk 1

# Essential roles of the histone variant H2A.Z in the development and function of reproductive organs of C. elegans

Natsumi Horii[1], Saho Kitagawa, Yukako Oma[1], Nami Haruta[2], Masato T Kanemaki[3], Masahiko Harata[1]

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Epigenetic regulation of genes governs the development and function of eukaryotic organisms. Recent molecular epigenetic studies have revealed that the histone variant H2A.Z is highly conserved among eukaryotic organisms, suggesting that it plays essential roles in cell differentiation. However, the functions of H2A.Z in multicellular organisms remain largely unknown because the knockout of the H2A.Z function causes lethality in the organisms. In this study, we established a method to inducibly knock down the function of H2A.Z in Caenorhabditis elegans in a whole-body manner using the auxin-induced degron (AID) system (Ref.). Using this method, induced whole-body knockdown of H2A.Z resulted in abnormalities in the nuclear morphology of the gonad, leading to the Endomitotic oocyte (Emo) and the Masculinization of the germline (Mog) phenotypes. Moreover, these observations suggest that the essentiality of the function of H2A.Z differs depending on developmental stages and that H2A.Z cooperates with various transcription factors and chromatin-associated factors. Based on these results, we propose novel and essential roles of H2A.Z in the development and function of reproductive organs. This study with the AID system could provide a better understanding of the H2A.Z function in multicellular organisms. Acknowledgment: This work was supported by the Pioneering Research Support Project of Tohoku University.

**Reference**: Negishi, T. et al. 2022, DOI: 10.1093/genetics/iyab218.

# Session 2 – Nuclear pathologies and cancer

Plenary 3

## Tumor biology- are we asking the right questions?

Toivo Maimets

*Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia*

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Cancer is a serious medical, social and economic problem. Over the past half century, there have been several waves of optimism about finding solutions, and the effectiveness of some forms of cancer treatment has indeed improved significantly, mostly thanks to new biological drugs. However, we are still quite far from solving the problem, and the incidence and mortality of some forms of cancer are increasing. This has forced us to ask whether our fundamental understanding of cancer biology is relevant.

In this presentation, I will talk about my personal experiences gained over four decades in understanding cancer. At the beginning of this period, the general understanding of cancer as a genetic problem prevailed. However, new accumulating data indicate that it is at least as important to understand cancer as a developmental biological problem. Together with my colleagues, I have thoroughly studied two cancer-related genes: p53 (the classic "tumor suppressor gene") and AHR ("oncogene"), and we have published dozens of articles on them. I have come to the conclusion that it is really not possible to speak of 'oncogenes' or 'tumor suppressor genes'. Different genes act as members of Gene Regulatory Networks (GRNs) and their effect on tumorigenesis (or its prevention) depends to a large extent on the context of other genes and their products. Just as the function of any gene in the production of a biological trait depends on the context. The context is determined by the developmental status and history of a particular cell/tissue. In this way, a single gene can participate in very different biological activities, which can even lead to opposite final outcomes. Thus, we have come to understand that cancer is not (only) a genetic disease but is largely determined by the developmental biological context.

Oral 8

# Trans-interactions in the cancer cell nucleus

Yegor Vassetzky

*Institut Gustave Roussy, Université Paris Saclay, France*

*vassetzky@gmail.com*

Oral 9

# Nuclear Voyage of Extrinsic Cell Death Society Members

Oleg Demidov

*University of Burgundy, France*

*oleg.demidov@u-bourgogne.fr*

Oral 10

# Melanoma-derived Exosomes Induce Transcriptional Stress Response in T cells, Leading to Intrinsic Apoptosis of Target Cells

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Extracellular vesicles (including exosomes) are important mediators of interactions between cancer and immune cells (or other components of the tumor microenvironment). Tumor-induced immune suppression, involving so-called exhaustion of T cells, is an important factor affecting the efficacy of anticancer treatment. Extracellular vesicles released by cancer cells (Tumor-derived exosomes, TEX) can induce apoptosis of T cells by poorly characterized mechanisms. We aimed to study these mechanisms using an in vitro model involving exosomes released by melanoma Mel526 cells (MTEX) and CD8+ Jurkat cells as a target. Co-incubation with MTEX resulted in the induction of apoptosis, suppression of proliferation, and inhibition of cytokine production in target Jurkat cells. MTEX-induced changes in the transcriptional profiles of Jurkat cells were analyzed by RNAseq after 2h and 4h of treatment, identifying 909 differentially expressed genes (FDRs<0.05). The analysis revealed deregulation of transcripts for genes involved in stress responses, major metabolic pathways, mitochondrial functions, apoptosis, and autophagy. Furthermore, transcriptional reprogramming was associated with changes in the abundance of proteins involved in response to stress, cellular signaling, apoptosis, and autophagy. A map of molecular changes occurring during MTEX-induced T cell reprogramming was generated, indicating that upon entry, MTEX induce activation of genes associated with cellular stress in T cells, which could be followed by intrinsic apoptosis in part of the target cells.

Oral 11

# DNA Substrate Specificity and Mechanisms of DNA ADP-Ribosylation

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Poly(ADP-ribose) polymerases (PARPs) use nicotine adenine dinucleotide (NAD) as substrate to synthetize monomer or a branched polymer of ADP-ribose (MAR or PAR, respectively) covalently attached to the acceptor residue of target proteins. PARPs It is generally accepted that PARP1, PARP2 and PARP3 act as DNA break sensors, which at lower levels of cellular DNA damage regulate the DNA repair pathways by recruiting chromatin remodeling and DNA repair factors to double- and single-strand breaks (DSB and SSB) and at a higher level of DNA damage, promote cell death via necrosis, apoptosis or both. It is shown that PARPs differently influence the relative contribution of canonical and alternative non-homologous end joining (cNHEJ and aNHEJ, respectively) and homologous recombination pathways in DSB repair. Recently, it was showed that PARP1-3 proteins can directly attach mono- or poly(ADP-ribose) moieties to the DNA termini at the sites of DNA strand breaks. This new type of DNA modification provides a heuristic insight into molecular mechanisms involved in DNA repair and cancer drug resistance, transcription and chromatin dynamics. Each PARP recognizes distinct sets of DNA structures with breaks, suggesting that PARP1, 2 and 3 have non-overlapping functions in DNA repair. At present, the detailed molecular mechanisms of PARP-dependent DNA breaks ADP-ribosylation and repair remain unknown. Here, we examined DNA substrate specificity and mechanisms of DNA PARylation by PARP1 in vitro and ex-vivo and we examined the stabilization of MAR/PAR adducts in the cell using different NAD+ analogs. Preference for a particular configuration of protruding DSB terminus for PARP-dependent ADP-ribosylation was revealed. Also we addressed the role of DNA ADP-ribosylation in repair and processing of DSBs via NHEJ assays by using different cell extracts, purified proteins and DNA substrates prone to ADP-ribosylation. We demonstrated that ADP-ribosylation of DSB termini can lead to inhibition of their repair if not removed by PARG glycohydrolase. MARylated oligonucleotides bound to beads were used to purify proteins from HeLa PARGKD cell extracts, which are able to specifically bind MAR-DNA adducts. The effect of DSB MARylation on affinity of multiple DNA binding proteins was estimated and potential MAR-DNA “readers” were identified. Finally, our results suggest that the biological effects of ADP-ribosylation may strongly depend on the configuration of complex DNA strand breaks. The new knowledge about the role and mechanisms of PARPs actions in DSB repair will identify novel therapeutic or diagnostic targets in cancer and other age-related diseases.

Short talk 2

# The Role of Phosphatidylinositol 4,5-bisphosphate in RNA Polymerase II Transcription

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The RNA Polymerase II (RNA Pol II) transcription is a 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) is phosphatidylinositol 4,5-bisphosphaterich structures whose periphery is associated with Pol II transcription initiation (Sobol et al..,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).

Our recent findings highlight that the manipulation of PI(4,5)P2 levels lead to a changes in the number of aggregates of protein involved in different cell processes in vivo.

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.

This work was supported by 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)

Short talk 3

# 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 membrane-less 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.

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

# Session 3 – Nuclear structure and function

Oral 12

# AIF/CHCHD4 complex: a key complex for nucleus-to-mitochondria communication and metabolism reprogramming

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Oral 13

# Comparative Analysis of Redox Pathway Activation During Ferroptosis in Lung Cell Lines

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Ferroptosis, a non-apoptotic form of programmed cell death driven by iron-dependent lipid peroxidation, is tightly regulated by endogenous antioxidant systems. Among them, the transcription factor NRF2 and the thioredoxin pathway (TRX/TXNRD1) play essential roles in cellular defense against oxidative damage. However, their contribution may vary across cell types and genetic contexts, influencing the susceptibility to ferroptosis. Aim: This study aimed to dissect and compare the oxidative stress responses between cancerous and non-cancerous lung epithelial cells by analyzing NRF2, TRX, and TXNRD1 expression under ferroptotic conditions.

Our study involved three human lung epithelial cell lines: A549 (cancer-derived), BEAS-2B wild-type (WT), and BEAS-2B GPX4-knockout (KO) cells generated using CRISPR/Cas9. All cell lines were treated with erastin (5 and 10 µM), a ferroptosis inducer that blocks the system $Xc^-$ cystine/glutamate antiporter. mRNA expression of NRF2, TRX, and TXNRD1 was assessed by RT-qPCR.

A549 cells exhibited a dose-dependent upregulation of NRF2 and TXNRD1, indicating an active antioxidant response. In contrast, BEAS-2B WT cells showed a significant downregulation of NRF2 at 5 µM, with partial restoration at 10 µM; TXNRD1 was upregulated only at the higher dose. Strikingly, GPX4-KO cells revealed a pronounced suppression of NRF2 and TRX expression at 5 µM, and only partial recovery at 10 µM, while TXNRD1 remained reduced. These findings point to a compromised oxidative defense system in GPX4-deficient cells, highlighting the interplay between GPX4 and NRF2/TRX signaling.

Our results reveal fundamental differences in redox homeostasis and stress response activation among lung epithelial cell types. A549 cells retain a strong antioxidant defense under ferroptotic stress, while BEAS-2B WT cells respond in a more limited fashion. The BEAS-2B GPX4-KO model, lacking a key ferroptosis regulator, displays severely impaired NRF2 and thioredoxin system activation. These insights shed light on the complexity of ferroptosis regulation and suggest that the redox landscape is highly dependent on both the genetic and cellular context—offering potential avenues for selective therapeutic targeting in diseases associated with oxidative stress.

Oral 14

# Ferroptosis in IBD: Ultrastructural and Molecular Correlates of Cell Death Susceptibility

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Ferroptosis, an iron-dependent form of programmed cell death associated with lipid peroxidation, plays an increasingly recognized role in the pathophysiology of inflammatory bowel disease (IBD). In this study, the sensitivity of various IBD-related cell lines to ferroptosis induction was assessed, along with a characterization of the ultrastructural and molecular changes accompanying this process. Using transmission electron microscopy (TEM), characteristic ferroptosis-associated mitochondrial changes were observed, including shrinkage, membrane densification, and loss of mitochondrial cristae. Concurrently, nuclear chromatin condensation and microscale structural damage were noted. Immunohistochemical analysis of γH2AX expression, a marker of DNA damage, revealed significant differences between cell lines with high and low susceptibility to ferroptosis. The results suggest that the extent of ferroptosisrelated response may determine genome integrity and organelle functionality in intestinal cells, which could be crucial for future targeted therapies in IBD.

Short Talk 4

## OneCell CUT&Tag maps chromatin modifications together with transcriptome and phenotype starting from a single cell

Anna Schwager

Short Talk 5

# Methyltransfrease Hemk2 impact on cell structures and cell cycle

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Oral 15

# Nucleoli and ribosomal gene expression in the context of tissue regeneration, aging and nutrition

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Oral 16

# Nucleocytoplasmic Regulation of mRNA Biogenesis by Actin-associated Protein Carmil 1

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Beta-actin was shown to be responsible for genome organization, maintenance of genome integrity, and DNA transcription in the cell nucleus. In these processes, actin's roles are orchestrated by a plethora of actin-binding proteins, which have been previously described to regulate actin polymerization state in the cytoplasm. Some studies suggested that actin's function in the nucleus is not limited to chromatin regulation, and actin could accompany newly made mRNA during splicing and nuclear export. However, no direct evidence, mechanism, or single canonical actin-binding protein has been shown to be a part of the mRNA splicing machinery to date. We discovered that Carmil 1, which regulates actin polymerization in the cytoplasm, is present in the nucleus. Upon blocking transcription, Carmil 1 accumulates in nuclear speckles and its deletion leads to global misregulation of mRNA gene expression/splicing. We also discovered a shorter Carmil 1 variant localized into nuclear speckles but also to centromeres, suggesting that Carmil 1 could chaperone spliced mRNA into the cytoplasm. Carmil 1, as the first cytoplasmic actin-binding protein directly involved in mRNA splicing, could therefore be a missing link between actin-regulated transcription and mRNA processing

Oral 17

# Actin and Actin-related Proteins Involved in Nuclear Structure and Function

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The actin family consists of conventional actin and actin-related proteins (Arps). Monomeric G-actin and the nuclear Arps are known to contribute to genome functions, including transcription and DNA damage repair, as components of chromatin remodeling complexes. Nuclear actin filament (F-actin) is also involved in genome functions and nuclear organization. Although a sufficient amount of actin is found in the nucleus, information regarding factors involved in regulating the formation of nuclear F-actin is still limited. We showed that Arp4, one of the nuclear Arps, is a suppressor of nuclear F-actin formation, suggesting that crosstalks among actin family proteins in the nucleus perform important roles in chromatin functions and nuclear organization.

We also investigated the involvement of nuclear F-actin in Hutchinson-Gilford progeria syndrome (HGPS). HGPS is a premature ageing disorder caused by a lamin A mutant named progerin. The expression of progerin leads to structural disruption of nuclear organization and misregulation of gene expression. Since progerin lacks a part of the actin-binding site of lamin A, we hypothesized that nuclear actin dynamics are altered in HGPS cells. We found that progerin expression decreases nuclear F-actin formation and impairs F-actin-regulated transcription in HGPS cells. Importantly, when the nuclear F-actin level is artificially increased by overexpressing nuclear-targeted actin, the irregularity of nuclear shape and defects in gene expression can be reversed. Our observations suggest that crosstalks among nuclear actin family proteins perform essential roles in chromatin functions and nuclear organization.

Oral 18

# Effect of Induced Degradation of HIRA on Prostate Cancer Models

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Prostate cancer (PC) is the second leading cause of cancer mortality in men. Initiation and progression of PC are determined by androgen receptor (AR)-driven transcription. Recently diagnosed PC is treated with androgen deprivation therapies (ADT). Although effective in most patients, ADT offer only temporary relief, and the disease recurs as castration-resistant PC (CRPC), that is the cause of almost all PC-related deaths and is often presented with metastasis (mCRPC). To date, treatment options for CRPC are scarce and include taxanes. However, clinical use of taxanes is limited due to the acquired therapeutic resistance, thus necessitating discovery of new rationally identified druggable targets. Multiple evidences point to an essential function of histone variant H3.3 and its chaperone HIRA in the initiation and progression of cancer. Exploring the function of HIRA/H3.3 pathway in PC, we found that [1] expression of HIRA complex proteins HIRA and UBN1 is increased in tumor compared with normal prostate tissue, is elevated in high/very high-risk PC (including CRPC), and is associated with negative prognosis. [2] Knockout (KO) of HIRA suppresses PC cell growth in vitro and in xenograft settings and attenuates androgen-induced transcription by altering AR binding at enhancers of target genes. [3] CRPC cells expressing gain-of-function AR variants (AR-Vs, drivers of ADT resistance and metastatic CRPC), develop addiction to HIRA. Lethality of HIRA-KO in AR-V cells prevents functional analysis of HIRA/H3.3 pathway in CRPC. To bypass this problem, we employed the auxin-inducible degron (AID) technology. Degron method allows rapid, extensive, and reversible degradation of target proteins fused with AID in cells expressing E3 Tir1 only in the presence of auxin. We established isogenic AR-WT and AR-V cells expressing Tir1 and micro-AID-mNeonGreen-tagged endogenous HIRA. We observed rapid dynamics of HIRA degradation by auxin treatment, and restoration of HIRA levels after auxin withdrawal, that is accompanied by H3.3 dynamics. Importantly, HIRA degradation reduced proliferation of AR-V cells much stronger compared to AR-WT cells, recapitulating HIRA-KO and -KD results. Altogether, our data indicate that augmented HIRA complex elevates deposition of H3.3 that activates the AR-driven transcription, thereby driving CRPC progression. We will present data characterizing mechanism of HIRA-dependent proliferation in CRPC model. Our data strongly suggest HIRA/H3.3 pathway as a new treatment target in CRPC

Oral 19

# The Zta (ZEBRA) Epstein-Barr Virus Transcription Factor: Relevance in Clinical Pathology

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EBV is a member of the herpesvirus family infecting primates only, with tropism for B cells and epithelial cells that establishes a life-long persistent infection in more than 95% of the world's population. After the episode of primary infection has resolved, EBV enters a latent phase. Following primary infection, the virus establishes lifelong persistence within the host memory B cell compartment utilizing restricted latent gene expression programs. Like all herpesviruses, The Epstein-Barr virus (EBV) can choose between two alternative lifestyles: latent or lytic replication. EBV Lytic replication, which is required for horizontal spread of the virus from cell to cell, and from host to host, occurs both in epithelial cells and B cells. The switch from latent to lytic infection is mediated by the immediate-early (IE) proteins ZEBRA (BZLF1 Orf) and R (BRLF1 Orf). ZEBRA and R proteins are transcription factors which activate lytic EBV promoters. In combination, ZEBRA and R induce expression of all early (E) lytic viral proteins, allowing the virus to replicate. This protein belongs to the bZIP family of transcription factors and is homologous to c-jun and c-fos that binds to the consensus AP1 motif, as well as atypical AP1-like motifs known as Z-responsive elements (ZREs). ZEBRA expression in the nucleus of EBV-infected cells is critical for viral activation, disease pathogenesis, and, from an immunologic standpoint, may represent a prime target of adaptive immune responses (T cell and humoral responses). ZEBRA is highly immunogenic and elicits robust T-cell responses. As ZEBRA was able to activate host cellular genes - i.e. the immunomodulatory genes IL-10 & IL-13, it was also shown that the reactivation of EBV may contribute to the growth of latently infected cells by promoting the release B-cell growth factors. ZEBRA is a transcription factor involved in tumor progression: We have shown that this protein does not remain in the cell nucleus, a situation that is quite similar to that observed in the case of the HIV virus (Tat protein). Tat and ZEBRA are viral transcription factors with the capacity to be excreted into the circulating blood, where they act as a toxin (toxoid proteins). By crossing cell membranes without being degraded, ZEBRA can penetrate the cytoplasm of B lymphocytes or epithelial cells, before crossing the nuclear membrane to activate strategic genes. Described as a very early protein in the lytic cycle, it would be interesting to be able to use ZEBRA either as a therapeutic target to block the progression of the virus, or as a predictive biomarker in assessing the risk of complications arising in patients at risk. Finally, cell penetrating peptides (CPPs in the basic region) from the ZEBRA protein have been depicted, and these are promising candidates to exploit in therapeutic cancer vaccines, since they can transport antigenic cargos into dendritic cells and induce tumor-specific T cells.

Oral 20

# Human papillomaviruses: advances and challenges in targeting the infection

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Human papillomaviruses (HPVs) are associated with the development of various epithelial cancers, typically resulting from persistent infections that can last for years. Currently, there are no HPV-specific antiviral therapies, and while prophylactic HPV vaccines are highly effective, they do not eliminate existing infections. The viral proteins E1 and E2, as key regulators of HPV genome replication, represent promising targets for antiviral drug development. However, their roles vary throughout the infectious cycle, with at least two replication modes observed: an E1/E2-dependent mode, and a potentially E1/E2-independent one that may operate during latent infection, when viral genome copy number remains stable. Recently, through high-throughput screening of a library of small-molecule compounds, we identified a potent inhibitor of E1/E2-dependent replication of cutaneous pro-oncogenic HPV types. This finding is particularly important, as no vaccines currently target skin-infecting HPV types, which are associated with several cancer types in immunocompromised individuals. Further understanding of HPV latency and replication control will be essential for the development of effective antiviral strategies.

# POSTER SESSION

Poster 1

# Explorative adaptation of somatic tumors to treatments via reprogramming to ancientreproductive attractors linked to polyploidy and circadian clock deregulation

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Metastatic malignant tumors are difficult to cure due to high adaptive plasticity and epigenetic reprogramming to multiresistant attractor states of the gene regulatory network (GRN) [1]. Recent studies directly show that resistance and metastasis of somatic cancer originate from poly-aneuploid cells (PACCs) [2], earlier proven to be associated with embryonal stemness and ectopic expression of meiotic genes [1, 3].
To investigate the hypothesis that cancer cells can assume evolutionary attractors of reproduction through polyploidy and epigenetic reprogramming, we have performed a systems-bioinformatic meta-analysis of -omics data(13509 samples in total) from tumor sample databases, diploid and polyploid normal tissue transcriptome datasets and in-house time-series RNA-seq of MDA-MB-231 breast cancer cells in the process of treatment resistance development after doxorubicin treatment [4-8].
Our results have shown that gametogenesis-related (GG) genes (mostly of unicellular and early multicellular origin) are not only ectopically expressed in malignant tumors, but also incorporated into highly interconnected networks (e.g. in the proteomics data of late-stage melanoma, 30.7% of the whole GG gene set (n=1474) was non-randomly ($p<0.05$) interlinked)and polyploidy-upregulated in several cancer types, suggesting the involvement of these genesin a cooperative process of reprogramming to a reproductive attractor [4].
MDA-MB-231 doxorubicin-treated PACCs also demonstrated a transcriptome rich in cell communication gene modules reliant on the early stress response (FOS/JUN), which notably include genes involved in placentation, suggesting importance of PACC-specific interactions with the tumor microenvironment in the process of cancer evolution [5]. Late-evolved reproductive CTA genes are linked to the latter GRNs through the stress-response and also upregulated in some metastatic cancers [6].
Additional evidence linking polyploidy with cancer via developmental bivalent gene activation and circadian clock dysfunction, which is also characteristic of embryonic stem cells, indirectly suggests genome regulation in both by explorative adaption (“order out of chaos”) [4, 7-9].
The results show that cancers use and combine phylogenetically diverse reproductive attractors as part of stress-induced explorative chaotic adaptations, which explain their extraordinary treatment resistance.
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Funding: Research grant lzp-2022/1-0114

Poster 2

# Nuclear vitamin D receptor gene polymorphisms are associated with multiple sclerosis therapy efficiency

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Background: The treatment for multiple sclerosis (MS) generally focuses on expediting recovery from attacks, diminishing relapses, decelerating disease development, and alleviating MS symptoms. Numerous studies have indicated an association between the risk of developing multiple sclerosis and serum vitamin D levels. Vitamin D is a potent immunomodulator that can suppress inflammatory signals and influence the immune system by binding to the nuclear vitamin D receptor (VDR). Genetic variations in the VDR gene are potential molecular markers of MS in different populations.

Aim: To identify the possible association of four significant genetic variations in the VDR gene with the outcome of multiple sclerosis therapy in a Latvian disease cohort.

Methods. In the Latvian MS cohort, 230 of 296 patients received 342 distinct pharmacological treatments. Data were collected regarding alterations in EDSS (Expanded Disability Status Scale) scores and NEDA (No evidence of disease activity) within two years following the initiation of therapy. Four SNPs of the VDR gene were genotyped by the restriction enzyme site polymorphism method: rs2228570, rs1544410, rs7975232 and rs731236.

Results. Statistical analysis revealed a significant association with EDSS change after starting therapy in the first and second year with VDR gene polymorphism genotypes. It was revealed that homozygous genotypes of common alleles CC (rs2228570) and TT (rs731236), as well as the heterozygous form of polymorphism rs1544410 in GA are a risk form in the case of Glatiramer acetate therapy, or an association with a higher increase in EDSS than in other forms of therapy. At the same time, patients with the heterozygous form of polymorphism rs1544410 in GA have a worse response to IFN therapy in the second year of therapy after EDSS change.

Conclusions. We present evidence that the VDR gene polymorphisms may contribute to the therapy of multiple sclerosis in the Latvian disease cohort.

Acknowledgments. The study was funded by UL project No.1.1.1.2/VIAA/4/20/718 “The role of vitamin D and its receptor gene polymorphisms in the modulation of intestinal inflammation in patients with relapsing and progressive forms of multiple sclerosis”, and European Regional Development Fund project 1.1.1.1/16/A/016.

Poster 3

# Single strand DNA breaks at active promoters facilitate +1 nucleosome eviction

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Stability of nucleosomes harboring various posttranslational histone tail modifications (PTMs) was compared in an in situ assay involving agarose-embedded nuclei. The promoter proximal H3K4me3, H3K27ac and H4K8ac positive nucleosomes exhibited relative sensitivity to intercalators as compared to bulk H3-GFP or nucleosomes carrying any of the following marks: H3K27me1, H3K27me2, H3K27me3, H3K9me1, H3K9me2, H3K9me3, H3K36me3, H3K4me0,H3K4me1, H3K4me2, H3K9ac, and H3K14ac. The posttranslational modifications of the histones were labeled using a panel of monoclonal antibodies (Kimura et al. Cell Struct Funct., 2008).Nickase or DNase I treatment of the nuclei, or bleomycin treatment of live cells, did not affect the stability of nucleosomes carrying H3K4me3 or H3K27ac, while those of the second group were destabilized upon treatment with intercalators. These observations support the possibility that the promoter proximal marks specify dynamic nucleosomes accomodating relaxed DNA sequences due to ss DNA breaks, nicks, generated in vivo. In line with this interpretation,endogeneous, 3'OH nicks were mapped within the nucleosome free region of promoters controlling genes active in human mononuclear cells co-localized with RNA polymerase, a conclusion supported by superresolution studies. Interestingly, the stability of the H3K4me3-nucleosomes proved to be antibody-dependent. Using a polyclonal H3K4me3-specific antibody (Abcam) the nucleosomes detected exhibited a much higher stability. When the binding sites of the monoclonal and polyclonal anti-H3K4me3 antibodies were mapped along the genome by CUT&RUN, peak coverages of both antibodies showed similar genomic distribution in the sense that 85-95% of peaks were detected in the 3 kb range of promoters. In the case of peaks flanking the transcription start sites (TSS), the monoclonal antibody detected a subpopulation of all those detected by the polyclonal. On the other hand, the H3.3 coverage immediately downstream of the TSS (i.e. the number of promoters harboring H3.3 there) is higher among the promoters detected exclusively by the polyclonal anti-H3K4me3 antibody, relative to those detected by both antibodies.
Thus, H3K4me3 nucleosomes showed a marked heterogeneity based on their different stability,juxtaposition with endogenous DNA discontinuities and arrangement of H3.3 containing nucleosomes around the TSS.

Poster 4

# The Role of Phosphatidylinositol 4,5-bisphosphate in RNA Polymerase II Transcription

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The RNA Polymerase II (RNA Pol II) transcription is a 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) is phosphatidylinositol 4,5-bisphosphaterich structures whose periphery is associated with Pol II transcription initiation (Sobol et al..,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).

Our recent findings highlight that the manipulation of PI(4,5)P2 levels lead to a changes in the number of aggregates of protein involved in different cell processes in vivo.

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.

This work was supported by 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)

Poster 5

# Tissue-Specific Epigenetic Remodelling During Prostate Aging: A Divergent Chromatin Landscape

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Epigenetic mechanisms play a pivotal role in regulating gene expression across a wide range of biological processes. Aging, for instance, is typically associated with a global reduction in heterochromatin and widespread DNA hypomethylation, as demonstrated in aged murine fibroblasts. However, the functional specificity of each organ suggests the existence of more heterogeneous, tissue-specific epigenetic patterns. The prostate, in particular, displays distinctive aging features that contrast with the atrophic trends observed in other tissues. Rather than undergoing mass reduction, the aging prostate is often associated with tissue expansion and growth-related pathologies, implying that chromatin remodeling and epigenetic alterations in this organ may substantially diverge from the canonical aging-associated patterns observed elsewhere. This study explores age-related changes in the epigenetic landscape of prostate epithelial luminal cells from young (5-month-old) and old (20-month-old) C57BL/6J mice. In contrast to the global heterochromatin loss typically observed in fibroblast-based aging models, the prostate follows an inverse epigenetic trend. Old murine prostate cells exhibited an expansion of chromatin domains, occurring as dispersed chromatin clumps, and an enrichment of H3K27me3, a hallmark of facultative heterochromatin, alongside a concomitant loss of transcription-associated marks, such as H3K4me3 and H3K9Ac. On the contrary, a decline in H3K9me3, a modification linked to constitutive heterochromatin and deposited at repetitive elements, was also reported, suggesting a potential association with telomere attrition. This shift towards a more compact chromatin state and the overall decline in histone modifications associated with transcription elongation is consistent with perichromatin granules (PGs) density reduction, reflecting a broader decline in transcriptional output. Furthermore, we investigated the distribution of TEX10, a key pluripotency factor coordinating histone acetylation and DNA demethylation, whose role and distribution in the prostate remain unexplored. Spatial analysis of TEX10 showed a preferential localization within nuclear regions associated with active transcription, RNA processing, and, generally, within the interchromatin compartment. Notably, this spatial enrichment was observed predominantly in the young condition, suggesting a functional role closely tied to its nuclear distribution as confirmed by colocalization of TEX10 signals with nascent mRNA fibrils. Although its global expression diminishes in old prostate cells, its presence in heterochromatin regions is relatively preserved, suggesting that this redistribution may reflect a shift in TEX10 function as cells age. A deeper understanding of the role and distribution of key factors involved in chromatin remodeling during aging in the prostate is essential, given its implications for age-related diseases, such as prostate cancer.

Poster 6

# Nuclear Reorganization During Mammal Erythropoiesis at EM

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The compartmentalization of the genome within the nucleus is a complex and highly dynamic phenomenon, and it responds to a plethora of endogenous and exogenous stimuli. Acting on cells, these signals remodel the three-dimensional organization of chromatin, hence altering the transcriptional activity. Mammalian erythropoiesis represents an excellent model for the study of nuclear reorganization and its impact on gene expression in response to endogenous signals, as the maturation of erythrocytes unfolds through a sequence of events including progressive gene silencing, chromatin remodeling, organelle clearance, and culminating in nuclear extrusion. This series of events constitute a highly regulated and coordinated mechanism of nucleoplasm remodelling leading to terminally differentiated cells. This work faces this point by the application of imaging techniques, which are particularly suitable for investigating nuclear organization. In particular, transmission electron microscopy is an ideal method due to its excellent resolving power and the availability of cytochemical and immunocytochemical methods for the selective or preferential visualization of the key players involved in such reorganization. Supported by other studies, the morphological analyses showed major cellular alterations, highlighting a gradual clearance of cytoplasmic organelles concurrently with the spread of heterochromatic regions. The progressive compaction of DNA was assessed through osmium ammine and by immunogold labeling of H3K9 trimethylation, an epigenetic marker of chromatin condensation. The osmium ammine staining allows precise DNA visualization and renders thresholding efficient and easily standardized. As chromatin condenses, profound variations occur in the genome organization, correlating with gene expression silencing. This trend is reflected in the immunogold analysis detecting an increase in H3K9me3 labeling from the first stages of chromatin condensation and its relocalization in the perichromatin region. Changes in nuclear complexity were also assessed by localization of RNA and ribonucleoproteins. RNA was visualized both via terbium citrate vapor staining, allowing the detection of individual RNA fibrils at the ultrastructural resolution, and through an electronmicroscopy in situ hybridization, which selectively targeted pre-mRNA fibrils. The EDTA regressive staining, by effectively bleaching and de-staining condensed chromatin, enabled the preferential visualization of ribonucleoprotein particles, whose distribution was shown to vary depending on the maturation stage and chromatin condensation level. Altogether, this combination of methodologies allows for deep insights into the investigation of the intricate nuclear features.

Poster 7

# Functional and proteomic profiles of CD3(+) plasma-derived small extracellular vesicles differentiate melanoma patients from healthy donors

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Small extracellular vesicles (sEV) released by T cells play a key role in immune regulation. Immune capture with anti-CD3 antibodies was used to isolate and study T cell-derived CD3(+)sEV from the plasma of patients with melanoma (MPs) and healthy donors (HDs). CD3(+)sEV of MPs coincubated with CD8+ Jurkat T cells or melanoma Mel526 cells serving as targets differed functionally from CD3(+)sEV of HDs: they promoted pro-tumor activities approximating functions mediated by melanoma cell-derived sEV (MTEX).

Proteomics profiling confirmed functional differences between CD3(+)sEV of MPs and HDs. Of 294 sEV-specific proteins identified in CD3(+)sEV, 226 were detected in the parent T cell proteome, confirming that the CD3(+)sEV proteome mimics that of the parent T lymphocytes. Among them were 66 differentially expressed proteins (DEPs) that differentiated vesicles from MPs and HDs. These DEPs were associated with the processes linked to cancer-related functions. DEPs upregulated in MPs' CD3(+)sEV were associated with Rho GTPase, cytokine, and MAPK-related signaling pathways. Moreover, the abundance of ITGB3 and YWHAB in MPs, two sEV proteins linked with BRAF-related pathways, correlated with the BRAF mutation status.

We concluded that T cells of MPs were reprogrammed to produce CD3(+)sEV that differed from CD3(+)sEV produced by HD's T cells, partly recapitulated features of the tumor proteome and functionally resembled MTEX. Thus, in melanoma, CD3(+)sEV might potentially serve as a liquid biopsy of tumor-reprogrammed T cells.

Poster 8

# Analysis of the effect of F-actin on the protein phase separation in the cell nucleus

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Purpose

In the cell nucleus, liquid-liquid phase separation (LLPS) of disordered proteins plays a central role in forming various membraneless domains, including the nucleolus. However, the details of the mechanism regulating LLPS in the nucleus haven't been cleared yet. Nuclear filamentous actin (F-actin) is supposed to contribute to forming the nuclear membraneless domains by regulating LLPS, but the details of the mechanisms haven't been fully analyzed yet. We used FUS(Fused in Sarcoma) protein as a marker for visualization of LLPS in the nucleus and analyzed how nuclear F-actin affects LLPS.

Methods

EGFP-FUS was over-expressed in NIH3T3 cells, and the foci of FUS accumulation were observed under a fluorescent microscope. The cells over-expressing EGFP-FUS were treated with 1,6-hexanediol to confirm that the FUS accumulation foci were formed by LLPS. The appearances of FUS foci and nuclear F-actin were analyzed, and the nuclei were classified according to their appearances. Co-localization of FUS foci and nuclear F-actin was analyzed by line profiling. The effect of the depolymerization of F-actin on the formation of FUS foci was also investigated. A cell line stably expressing FUS was established and analyzed for the change of FUS foci by overexpressing actin.

Results and discussion

We revealed that FUS foci were formed by LLPS, because the treatment with 1,6-hexanedioldecreased the ratio of nuclei with the foci. When the nuclei with F-actin were compared to those without nuclear F-actin, it was shown that the former contains more FUS foci. On the other hand, it was observed that F-actin and FUS foci were not colocalized in the nucleus. We also found that the depolymerization of nuclear F-actin decreases the ratio of nuclei with FUS foci and that FUS foci size in the nucleus tended to increase by the overexpression of actin. These results suggest that nuclear F-actin formation promotes LLPS of FUS in an indirect manner and supports the possibility that nuclear F-actin regulates the formation of nuclear domains, including the nucleolus, through enhancing LLPS. We are now proceeding with the analysis of LLPS in cells of Hutchinson-Gilford Progeria Syndrome, suggesting the formation of nuclear F-actin is decreased and the nuclear domain formation is misregulated (Ref.).

Reference: Takahashi, Y. et al. (2020) 'Impairment of nuclear F-actin formation and its relevance to cellular phenotypes in Hutchinson-Gilford progeria syndrome', Nucleus, 11(1), pp. 250–263.doi: 10.1080/19491034.2020.1815395.

Poster 9

# Cross-Reactivity of N6AMT1 Antibodies with Aurora Kinase A: An Example of Antibody-SpecificNon-Specificity

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Primary antibodies are one of the main tools used in molecular biology research. However, the often-occurring cross-reactivity of primary antibodies complicates accurate data analysis. Our results show that three commercial polyclonal antibodies raised against N-6 adenine-specific DNA methyltransferase 1 (N6AMT1) strongly cross-react with endogenous and recombinant mitosis-associated protein Aurora kinase A (AURKA). The cross-reactivity was verified through immunofluorescence, immunoblot, and immunoprecipitation assays combined with mass spectrometry. N6AMT1 and AURKA are evolutionarily conserved proteins that are vital for cellular processes. Both proteins share the motif ENNPEE, which is unique to only these two proteins. We suggest that N6AMT1 antibodies recognise this motif in N6AMT1 and AURKA proteins and exhibit an example of "specific" non-specificity. This serves as an example of the importance of controls and critical data interpretation in molecular biology research.

Poster 10

# Essential roles of the histone variant H2A.Z in the development and function of reproductiveorgans of C. Elegans

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Epigenetic regulation of genes governs the development and function of eukaryotic organisms. Recent molecular epigenetic studies have revealed that the histone variant H2A.Z is highly conserved among eukaryotic organisms, suggesting that it plays essential roles in cell differentiation. However, the functions of H2A.Z in multicellular organisms remain largely unknown because the knockout of the H2A.Z function causes lethality in the organisms. In this study, we established a method to inducibly knock down the function of H2A.Z in Caenorhabditis elegans in a whole-body manner using the auxin-induced degron (AID) system (Ref.). Using this method, induced whole-body knockdown of H2A.Z resulted in abnormalities in the nuclear morphology of the gonad, leading to the Endomitotic oocyte (Emo) and the Masculinization of the germline (Mog) phenotypes. Moreover, these observations suggest that the essentiality of the function of H2A.Z differs depending on developmental stages and that H2A.Z cooperates with various transcription factors and chromatin-associated factors. Based on these results, we propose novel and essential roles of H2A.Z in the development and function of reproductive organs. This study with the AID system could provide a better understanding of the H2A.Z function in multicellular organisms.

Acknowledgment: This work was supported by the Pioneering Research Support Project of Tohoku University.

**Reference:**

Negishi, T. et al., Genetics 220, iyab218 (2022)

Poster 11

# Rhabdomyosarcoma cell line RD contains a subpopulation of polyploid giant cancer cells (PGCCs) capable of proliferation

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Despite decades of intensive research, cancer remains one of the leading causes of death worldwide, with major clinical challenges being therapy resistance and cancer recurrence. Emerging evidence suggests that polyploid giant cancer cells (PGCCs), a rare subpopulation of tumour cells, could be key drivers of therapy resistance and cancer recurrence. PGCCs are characterized by their atypical nuclear morphology, in which the nucleus is multinucleated or multilobular. Cancer-testis antigens (CTAs) are a group of proteins that have restricted expression to the testis, ovaries and placenta, and are aberrantly re-expressed in cancer, where they have the potential to be immunogenic. The Melanoma Antigen Gene (MAGE) protein family is a large, highly conserved group of proteins that share a common MAGE homology domain. A subset of these >40 human proteins are classified as cancer-testis antigens. MAGE family proteins function during embryonic development after which, these genes are subsequently deactivated. During neoplastic transformation, these genes are re-activated, expressed, and maybe invoked in cell transformation.

In this study, we profiled the MAGEA expression via real time PCR in 20 cell lines, including seven melanoma and skin cancer, three cervical, three bone and muscle cell cancer, three miscellaneous, two trophoblast and two non cancerous cell lines. Using immunofluorescence microscopy, MAGEA10 was mainly expressed as a strong nuclear signal. Strong MAGEA10 signals were found in all cancer line categories and were specific to the cancer cell line not the cancer type. The bone and muscle cancer cell lines U2OS, RD and RH30 had a positive MAGEA10 nuclear signal of 6.8%, 95.5% and 0%, respectively. In this subgroup, further investigation of PGCCs was carried out – RD had an average of 17.5% PGCCs from all cells, while RH30 had 1.2%and U2OS 0.2%. In RD, a distinct PGCC subpopulation, which uses alternative proliferative mechanisms for cell division, was identified. These mechanisms, including endoreplication, defective mitosis, and cell fusion, contribute to the generation of cells with genomic instability.

Our findings confirm that PGCCs in the RD cell line are not senescent but contribute actively to tumour biology. These results highlight the need for further research on PGCCs and the identification of specific markers for their detection and therapeutic targeting. A deeper understanding of PGCCs could reveal novel therapeutic targets, improving treatment outcomes and reducing cancer recurrence.

Poster 12

# Methyltransferase HEMK2 is involved in the regulation of tubulin isotypes and microtubuledynamics

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HemK methyltransferase 2 (HEMK2) is an evolutionary conserved methyltransferase which has been associated with different pathological conditions, such as diabetes, various types of cancers and neurological malfunctions. While many of these diseases have been attributed to HEMK2 role in regulating gene expression, the exact molecular pathways and cellular processes which HEMK2 is associated with have not yet been fully described. Our analysis of HEMK2-depleted cells suggests that HEMK2 is involved in the regulation of the cytoskeleton and in particular microtubule dynamics in cancer cells. In cells depleted of HEMK2, the levels of different tubulin isotypes were altered with a notable downregulation of a tumour prognostic marker β-tubulin 3 (TUBB3). Furthermore, live-cell imaging combined with automatic particle tracking analysis (PTA) revealed that HEMK2 depletion might cause changes in microtubule dynamics by increasing their growth velocity and decreasing their growth duration. This was further validated by using functional and methylation-deficient HEMK2 compensation cell lines, which showed that the methylation activity of HEMK2 is also necessary for it to induce changes in microtubule dynamics. Finally, we found that HEMK2 depletion decreased microtubule regrowth after its complete depolymerization independent of its methylation activity suggesting that HEMK2 might also be important for microtubule nucleation. Taken together this points to HEMK2 role in the regulation of microtubule dynamics, but further studies are needed to determine whether these changes 1) result from alterations in tubulin isotype composition or 2)from the disruption of other processes governing microtubule dynamics. Understanding the mechanisms through which HEMK2 regulates microtubule dynamics, and potentially the entire cytoskeleton, could be used to better discern diseases associated with HEMK2.

Poster 13

# Functional screening of cell-penetrating peptides to enhance the delivery of RNAi-loadednucleic acid nanostructures

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RNA-based therapeutics, particularly RNA interfering (RNAi) small RNAs such as microRNAs(miRs) and small interfering RNAs (siRNAs), have emerged as promising therapies for multiple pathologies, including cardiac repair after ischemia. These small non-coding RNAs can modulate key regenerative pathways, offering potential for functional recovery post injury. However, their clinical application is hindered by the lack of safe and efficient delivery systems.

In this context, DNA nanotechnology offers a unique platform to address these limitations. Nucleic acid nanostructures (NANs) are highly programmable, biocompatible and exhibit remarkable structural versatility, allowing precise control over size, shape and functionalization. Their ability to protect and transport unstable nucleic acid cargos while minimizing toxicity and immunogenicity makes them ideal candidates for miR delivery in cardiac regenerative medicine.

To enhance the cellular uptake of these NANs, cell-penetrating peptides (CPPs) are being explored as functional moieties. CPPs are short peptides with positively charged residues that facilitate translocation across negatively charged cellular membranes. Their integration into NANs is driven by electrostatic interactions between the cationic peptide's residues and the anionic phosphate backbone of DNA. A critical parameter in this assembly is the nitrogen to phosphate (N/P) ratio, which marks the stability of the peptide-DNA complex and its physicochemical properties. By fine-tuning the N/P ratio, it is possible to optimize the formation of stable, compact hybrids that maximize cellular internalization while preserving the cargo integrity.

Peptide-functionalized NANs were developed as advanced delivery vehicles for cardio-therapeutic miRs. DNA origami NANs were assembled via a stepwise self-assembly process and characterized by gel electrophoresis and dynamic light scattering (DLS), confirming consistent dimensions (~50 ± 5 nm) and high structural stability. Hybrid nanocarriers were subsequently engineered by incorporating CPPs into the different NANs through controlled N/P ratios, resulting in significantly enhanced cellular uptake in U87-luc2 cells.

Ongoing efforts focus on optimizing this strategy by evaluating alternative CPPs and expanding their application to other DNA nanostructures, including tetrahedron and DNA nanohydrogels. These advancements aim to maximize the intracellular availability of therapeutic miRs, with the final goal of enabling efficient and non-viral gene therapies in human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) for cardiac regeneration.

Poster 14

# 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 membrane-less 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.

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

Poster 15

# Dynamic interactions of PML nuclear bodies, nuclear lamina and repair of telomeric DNA indoxorubicin-treated cancer cells

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Accelerated cell senescence (ACS) induced by drug treatments in cancer cells is characterised by interruption of proliferation, telomere attrition and gradual degradation of lamin B1. Telomeres in epithelial tumours are maintained by telomerase, however, the breast cancer MDA-MB-231cells after doxorubicine (DOX) treatment, were found undergoing mitotic slippage, polyploidisation, telomere clustering, and shift to recombinative alternative telomere lengthening (ALT) in PML bodies (APBs) marked by the telomere repeat binding factor 2 (TRF2).ALT was transient and accompanied by cutting off the DNA-damaged telomere ends with telomerase, while in the recovered mitotic survivors telomerase mechanism was restored. A number of meiotic genes (DMC1, SPO11, Mos, Vasa, Fragilis) were found activated, suggesting that the meiotic type recombination may be involved in ALT. The association of meiotic recombination nuclease SPO11 with APB and DMC1 and RAD51 recombination foci resulting inmature APBs, were obseved after DOX treatment in 11-12% cells. Similarly this was found also in DOX-treated SK-MEL-28 melanoma cells, indicating telomere DNA repair of the meiotic type. At higher DOX concentration the formation of the thready PML structures (PML isoform II, known by affinity to the nuclear envelope) was occurring in a small proportion of cells. These threads were composed of dimeric PML rods tandemly joined by the γH2AX -TRF2-marked chromatin and mostly absent RAD51. The PML threads were radially extending and circumventing the nuclear periphery, also intermitting the dashed segments of the degrading nuclear lamin B1. Interaction of PML with the nuclear envelope and MOS-kinase-linked microtubule-driven rotation of DOX-treated cell nuclei was also observed. We conclude that mitotic slippage and ALT with the participation of the meiotic prophase proteins can potentially recombine telomeres, while ALT failure shifting to the fibrillar PML isoform mediates non-homologous telomere end-joining, the possible chance of survival, at the brink of nuclear degradation.

Funding: Project FLPP No: lzp-2022/1-0114.

Poster 16

# Early events, biomarkers, and therapeutic targets in lung cancer associated with 3p21 deletion

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Poster 17

# Specificity of human histone H1 variants in the organization and control of the genome

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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 (TEs) classes was also variant-specific. Interestingly, H1X was enriched at recently incorporated TEs. 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 universal enrichment of H1.2, H1.3, and H1.5 at the nuclear periphery and nucleolar H1X presence. Knock-down of particular H1 variants promotes transcription of non-coding RNAs genome wide and intragenic cryptic transcription. Overall, H1 variants show a differential and non-random genome-wide distribution, supporting their functional specificity.

Poster 18

# DNA substrate specificity and mechanisms of DNA ADP-ribosylation

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Poly(ADP-ribose) polymerases (PARPs) use nicotine adenine dinucleotide (NAD) as substrate to synthetize monomer or a branched polymer of ADP-ribose (MAR or PAR, respectively) covalently attached to the acceptor residue of target proteins. PARPs It is generally accepted that PARP1,PARP2 and PARP3 act as DNA break sensors, which at lower levels of cellular DNA damage regulate the DNA repair pathways by recruiting chromatin remodeling and DNA repair factors todouble- and single-strand breaks (DSB and SSB) and at a higher level of DNA damage, promote cell death via necrosis, apoptosis or both. It is shown that PARPs differently influence the relative contribution of canonical and alternative non-homologous end joining (cNHEJ and aNHEJ, respectively) and homologous recombination pathways in DSB repair. Recently, it was showed that PARP1-3 proteins can directly attach mono- or poly(ADP-ribose) moieties to the DNA termini at the sites of DNA strand breaks. This new type of DNA modification provides a heuristic insight into molecular mechanisms involved in DNA repair and cancer drug resistance, transcription and chromatin dynamics. Each PARP recognizes distinct sets of DNA structures with breaks, suggesting that PARP1, 2 and 3 have non-overlapping functions in DNA repair. At present, the detailed molecular mechanisms of PARP-dependent DNA breaks ADP-ribosylation and repair remain unknown. Here, we examined DNA substrate specificity and mechanisms of DNA PARylation by PARP1 in vitro and ex-vivo and we examined the stabilization of MAR/PAR adducts in the cell using different NAD+ analogs. Preference for a particular configuration of protruding DSB terminus for PARP-dependent ADP-ribosylation was revealed. Also we addressed the role of DNA ADP-ribosylation in repair and processing of DSBs via NHEJ assays by using different cell extracts, purified proteins and DNA substrates prone to ADP-ribosylation. We demonstrated that ADP-ribosylation of DSB termini can lead to inhibition of their repair if not removed by PARG glycohydrolase. MARylated oligonucleotides bound to beads were used to purify proteins from HeLa PARGKD cell extracts, which are able to specifically bind MAR-DNA adducts. The effect of DSB MARylation on affinity of multiple DNA binding proteins was estimated and potential MAR-DNA “readers” were identified. Finally, our results suggest that the biological effects of ADP-ribosylation may strongly depend on the configuration of complex DNA strand breaks. The new knowledge about the role and mechanisms of PARPs actions in DSB repair will identify novel therapeutic or diagnostic targets in cancer and other age-related diseases.

Poster 19

# PF14 analogs with induced alpha helix for siRNA functional delivery to the cells: Cell-penetrating peptides as a gene therapy delivery strategy

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Gene therapy has emerged as a promising approach in the treatment of various diseases by focusing on the root causes rather than merely alleviating symptoms, as is typical with conventional pharmaceuticals. Central to this innovation are nucleic acid therapeutics, which include both DNA and RNA-based techniques that promise targeted and efficient therapeutic strategies. The primary mechanisms deployed by these therapeutics involve the alteration of splicing processes with splice-correcting oligonucleotides (SCOs), gene silencing through the degradation of mRNA using short interfering RNAs (siRNAs), and the delivery of genetic instructions for protein synthesis via messenger RNA (mRNA).

However, despite their vast potential, nucleic acid therapeutics face significant challenges, notably their limited ability to penetrate cell membranes due to their large molecular structure and inherent negative charge. Added to this are difficulties in crossing the Blood-Brain Barrier and the risk of immune responses or degradation by nucleases when siRNAs are administered systemically. To overcome these obstacles, the development of effective delivery systems is crucial. Among the potential solutions are cell-penetrating peptides (CPPs), with PF14 (PepFect14), a modified analog of transportan10, standing out as highly efficient in vitro and in vivo. PF14-type CPPS have the unique capability to form noncovalent complexes with nucleic acids utilizing both electrostatic and hydrophobic interactions. Nonetheless, its delivery efficacy has driven the creation of PF14 analogs to improve their delivery efficay.

In this study, we present a series of novel PF14 analogs aimed at enhancing the delivery efficiency of nucleic acid molecules. Building on previous findings where positively charged ornithine residues were substituted with lysine, resulting in the PF14-Lys [1], we employed three distinct modification strategies: adjusting the ratio of cationic to hydrophobic faces, varying the lengths of fatty acid residues at the peptide's N-terminus, and altering the hydrophobic regions of the peptides.

The characterization of these new PF14 analogs focused on their ability to form nanoparticles with siRNA at different molar ratios, their efficiency in delivering reporter nucleic acids, the stability of the formed nanoparticles against proteases and polyanions, and their safety to cells. Predictive modeling confirmed that all analogs displayed an α-helical secondary structure, with several analogs effectively forming nanoparticles with siRNA. Furthermore, three analogs exhibited enhanced stability in the presence of polyanions and demonstrated a lower susceptibility to enzymatic degradation. Importantly, all CPP/siRNA nanoparticles tested were deemed safe for use in the selected cell lines for transfection assays. Our study emphasizes that three distinct analogs achieved a high functional delivery efficiency of siRNA to the U87-MGLuc2 cell line, while additional two analogs were also effective in delivering mRNA to the HaCaT cell line at tested charge ratios. Overall, our findings underscore the significance of optimizing CPP design in enhancing targeted therapeutic strategies within the field of gene therapy applications.

**References:** [1] Biswas A, et al. Engineered PepFect14 analog for efficient cellular delivery of oligonucleotides. Biomed Pharmacother. 2025 Mar;184:117872. doi: 10.1016/j.biopha.2025.117872.