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International Symposium:

"Spatial-temporal genome regulation in stress-response and cell-fate change“

Lecture Hall and Virtual
BIOMEDICAL RESEARCH AND STUDY CENTRE (BMC), RIGA
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PROGRAMME & BOOK OF ABSTRACTS

Organization:
Jekaterina Erenpreisa, Latvian Biomedical Research and Study Centre, Riga
Michael Hausmann, Kirchhoff-Institute for Physics, Heidelberg University

Virtual Participation via: https://meet.jit.si/Spatial-temporal_genome_reguation

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Programme

09:00	Ieva Pranka, Baltisch-Deutsches Hochschulkontor: ***Opening and welcome***
09:05	J. Erenpreisa, Riga, and M.Hausmann, Heidelberg: ***Welcome***
09:10	J. Erenpreisa, Riga: ***Genome regulation by positional information: in space and time***
09:50	M. Falk, Brno and Heidelberg: ***How nanoscale chromatin architecture and chromatin topology within the cell nucleus participates in cancer development – an example of pathogenesis of three different leukemia types***
10:30	Coffee Break
10:50	Felikss Rūmnieks, Riga: ***Scale-free organisation of pericentric chromatin domains in MCF-7 breast cancer nuclei***
11:10	Michael Hausmann, Heidelberg: ***From Schrödinger´s cat to his chromosomal aperiodic crystal and what an irradiated cell nucleus "thinks" about it***
11:50	K. Salmina, Riga, Poster Presentation (Flashtalk): ***Spatial relationship between ribosomal and mRNA transcription/splicing conveyer, nuclear lamin rigidity, and actin cytoskeleton tension***
12:00	Lunch Break and walk (Restaurant „Lido", Imanta)
14:00	A. Guiliani, Rome: ***The guardians of stability are the same that initiate revolutions: the peculiar character of gene expression dynamics***
14:40	G.Hildenbrand, Heidelberg: ***Sequence Composition and 3D-Genome Structure***
15:20	Coffee Break
15:40	N.M. Vainshelbaum, Riga: ***Circadian clock and cancer***
16:00	K. Yoshikawa, Kyoto: ***Change of the higher-order structure in DNA causes significant effect on genetic activity: a physical view.***
16:25	Final Discussion and closing remarks
17:00	End

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Abstracts

Genome regulation by positional information: in space and time

Jekaterina Erenpreisa

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All cells of the multicellular organism contain the same linear sequence of genes in a ~2 m of DNA. Clearly, additional information should be produced for the diversity of tissue cell types. The chromosome territories generally occupy in cell nucleus the radial position and also display the radial gradient of gene activity, reduced at the nuclear envelope by tight chromatin packaging. Heterochromatin with its silencing/compacting cis-effect creates the positional information and likely establishes the tissue-specific „address“ and epigenetic formula by replication timing. It is paced by circadian and sub-verged cell-cycle molecular oscillators [3]. This epigenetic memory is stable in cell generations. For the ribogenesis-transcription-splicing conveyor, we deduced from our work and literature [1, 2] the radial-concentric organisation of cell nuclei. It represents a dynamic network clipped by clusters of scale-free pericentromeric domains (PADs) and nucleolus-associating domains (NADs) enclosing transcription hubs with spliceosomes. Its activity is functionally and physically linked to the elastic radial-concentric actomyosin cytoskeleton. This network mediates transcriptional pulsing capable of feedback sensing the environment, thus adjusting actual transcription „to the tune of life“ (Denys Noble). The profile in transcription burst frequencies is tissue-specific. However, a crucial change in cell fate (e.g. in early embryo, re-differentiation of cancer cells) needs genome re-patterning by critical self-organisation [4]. The positional information is erased „in a jump“ by splitting of PADs under the silencing threshold [1], circadian clock dispensed, and at the „edge of chaos“, the tide energy of bi-phasic stress-response (AP-1, FOS-L1, c-MYC) opening the chromatin is used to synchronise the transcription avalanche, starting the whole genome change [4]. The re-differentiation is then determined at the recurrent phase of this giant wave.

[1] Krigerts et al., Differentiating Cancer Cells Reveal Early Large-Scale Genome Regulation by Pericentric Domains. Biophys. J 2021 doi: 10.1016/j.bpj.2021.01.002

[2] Erenpreisa et al., Heterochromatin networks: topology, dynamics and function. Cells 2021 https://doi.org/10.3390/cells10071582

[3] Vainshelbaum et al., Role of the Circadian Clock “Death-Loop” in the DNA Damage Response... Cells 2022 https://www.mdpi.com/2073-4409/11/5/880

[4] Tsuchiya et al., A Unified Genomic Mechanism of Cell-Fate Change. Chapter in a Springer Nature book series: Results and Problems in Cell Differentiation. Eds. M. Kloc and J. Kubiak, (in print).

How nanoscale chromatin architecture and chromatin topology within the cell nucleus participates in cancer development – an example of pathogenesis of three different leukemia types

Martin Falk,[1,2)] Michael Hausmann,[2)] Wisam Mohammed Hikmat,[2)] Georg Hildenbrand,[2)], Ivan Dellino,[3)] Mario Faretta,[3)] Iva Falková,[1)] Olga Kopečná,[1)] Eva Pagáčová,[1)] Emilie Lukasova,[1)] Alena Bačíková,[2)] Pier Giuseppe Pellicci,[3)] Götz Pilarczyk,[2)] Ema Huščavová,[1,4)] Myriam Schäfer,[2)] Ruth Winter,[2)] Karel Štěpka,[5)] Kyra Michalová,[6)] Zuzana Zemanová,[6)] Elham Parsimehr,[1,4)] Jiří Toufar,[1,4)] Lucie Dobešová,[1,4)]

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Chromatin architecture, starting with the nucleotide motifs of DNA and ending with the chromatin topology in the nucleus, has been recognized as a critical factor determining both the expression of individual genes and the functionality of the nucleus as a whole. Alterations of chromatin architecture at various levels therefore play a crucial role in the pathogenesis of countless diseases, including cancer. In our paper, we reveal how specific characteristics of chromatin architecture or alterations of this architecture contribute to acute/chronic myeloid leukemia (AML/CML), myelodysplastic syndrome (MDS) and acute promyelocytic leukemia (APL). Leukemias typically result from a single translocation leading to expression of an oncogenic protein, with DNA breaks occurring at sharply defined chromosomal loci of characteristic chromosomes. However, in the case of AML/CML, we observed, to varying degrees in individual patients, incomplete maturation of chromatin architecture in granulocytes. This type of chromatin defect persisted in granulocyte nuclei even after molecular remission of the oncogenic translocation and could be partly responsible for leukemia-related immunodeficiency. In contrast to AML/CML, MDS is not caused by a dominant oncogenic translocation between sharply defined chromosomal loci. Instead, it is associated with various deletions and translocations that occur at preferred but not sharply defined loci. We will discuss how chromatin nanoarchitecture conditions the formation of double-strand breaks in these more broadly defined MDS loci and how chromatin topology subsequently determines the preferential types of chromosomal aberrations and the loci that will be affected by these aberrations. APL is another unique example of leukemia which can be ascribed to altered chromatin architecture. While there is a dominant oncogenic translocation leading to APL, like in AML/CML, the pathogenic effect of the resulting fusion oncoprotein is mostly exerted through the alteration of chromatin architecture.

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Scale-free organisation of pericentric chromatin domains in MCF-7 breast cancer nuclei

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Our working hypothesis was that constitutive silencing pericentric chromatin domains (PADs) may change for the genome re-patterning in differentiation commitment. We reproduced the model system of MCF-7 breast cancer cells treated with the ErbB3 ligand heregulin (HRG), (which is used in the therapy of cancer), with known dynamically traced transcriptome data. PAD-repressive heterochromatin (H3K9me3), centromere-associated-protein-specific, and active euchromatin (H3K4me3) antibodies, acridine orange DNA structural test, qPRC for stress response proteins, and microscopic image analysis were applied (Krigerts et al., 2021). In starving control, the average number of PADs per cell correlated with their average size by power-law and the number of individual PADs with their size, by exponential decay. The centromere clustering in turn correlated with PADs' size, both indicating that PADs may create and modulate a suprachromosomal network by fusing and splitting a constant proportion of the constitutive heterochromatin. Between 15-20 min of HRG treatment, the splitting of PADs mostly to ~ 1μm^2 units occurred with bursting from the nucleolar boundary to the nuclear border. It coincided with opening the chromatin registered by Acridine orange structural test and homogeneity of H3K4me3 in nuclear staining, upregulation of stress response gene FOS, and the earlier reported splash of accelerated genome transcription involving thousands of genes (Tsuchiya et al., 2016). This suggests that splitting the PADs under threshold of silencing effect induces critical phase transition of the active genome compartment as a prerequisite for re-patterning, to start cancer cells differentiation.

References: Krigerts et al., Differentiating Cancer Cells Reveal Early Large-Scale Genome Regulation by Pericentric Domains. Biophys. J 2021 doi: 10.1016/j.bpj.2021.01.002; Tchuchiya et al. PLoS One. 2016 Dec 20;11(12):e0167912. doi: 10.1371/journal.pone.0167912. eCollection 2016.

From Schrödinger´s cat to his chromosomal aperiodic crystal and what an irradiated cell nucleus "thinks" about it

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There are a lot of open questions concerning the understanding of radiation DNA damaging mechanisms and repair processes within the light of radio-sensitivity or radio-resistance. The three-dimensional architecture of genomes on the micro-, meso- and nano-scale acts in combination with epigenetic modifications as an important player of gene regulation and, consequently, fundamental biological processes such as DNA damage response and repair. Based on early ideas of Erwin Schrödinger to build up a cell nucleus ab initio from quantum mechanics to a solid state of chromosomal aperiodic crystals, we will ask, how does **a cell nucleus as system as a whole**, process DSBs and re-organize the chromatin towards functionally intact repair units? We present investigations of spatial and topological parameters of chromatin and DNA repair foci during a time period of repair to glimpse key aspects related to this question. Nano-probing in combination with super-resolution Single Molecule Localization Microscopy (SMLM) are powerful methods for geometric and topological analyses of nano-structures in single cells and at single DSB sites and, thus, to study mechanisms of their formation and repair pathway regulation. We used variable tools for such investigations based on image-free high-precision SMLM, nano-scaled molecule distribution analyses, appropriate metrics following Ripley´s distance frequencies and cluster formation analyses, as well as topological quantifications employing persistence homology. Comparing the topology of repair foci by persistence homology suggests general similarities in repair cluster formation, indicating a well-defined non-random, molecule topology at given time points during repair. Characteristics of chromatin architecture around complex damage sites, repair focus nano-architecture or (similar) spatial arrangements of repair proteins may contribute to control repair process. Our studies contribute to the understanding of whole system cellular radiation response in cancer and non-cancer cells.

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Spatial relationship between ribosomal and mRNA transcription/splicing conveyor, nuclear lamin rigidity, and actin cytoskeleton tension

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The topological relationship between the nucleolar fibrillar centres, perinucleolar heterochromatin, nuclear speckles and lamin-associated heterochromatin after RNA transcription inhibition was studied. MCF7 breast cancer cells were treated in chamber slides by increasing concentration/time of Actinomycin D (AcD) and evaluated by immunofluorescent confocal image analysis. In control cells with active rRNA and mRNA synthesis, the perinucleolar repressive heterochromatin labelled by H3K9Me3/cen forms extended structures bent around nucleoli, speckles located more externally are also extended, lamin B1 ideally outlines the nuclear envelope (NE), while actin filaments form fibrils both circular around NE and perpendicular or at angles to it, attached to the cellular membrane. When the nucleolar synthesis is initially suppressed by low AcD, the remnant Pol I cofactor RPA194 forms a few large granules at the nucleolar margin, H3K9Me3 heterochromatin condenses in round clumps between them, while speckles also condense as regular circular structures around the latter – all together revealing a radial-concentric nuclear network. The lamin B1-positive nuclear contour becomes irregular and lamin B1 forms intranuclear channels reaching the nucleoli. The perinuclear actin ring thickens, while its radial cytoplasmic fibrils become less tense. Full suppression of both syntheses by high dosage/prolonged AcD or a-amanitin brings to disorganization of the radial-concentric nuclear order. We conclude that the links of the perinucleolar and lamin-associated heterochromatin with speckle intranuclear compartments are involved in topological coordination between the ribogenesis and mRNA maturation, where the radial tension of the actin cytoskeleton exerted via concentric elasticity of the nuclear lamina forwards this conveyor towards translation sites.

References: Erenpreisa et al., Heterochromatin networks: topology, dynamics and function. Cells 2021 https://doi.org/10.3390/cells10071582; Scholarly Encyclopedia MDPI_ https://encyclopedia.pub/12670

The guardians of stability are the same that initiate revolutions: the peculiar character of gene expression dynamics.

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The analysis of the time course of entire genome expression when in presence of a transition highlights a typical behaviour of complex multidimensional systems embedded into a continuously slightly changing environment. This behaviour is named 'Self Organized Criticality' (SOC) and encompasses a relentless variability of the elements at the periphery of the system that buffer the environmental perturbations so allowing to keep the core of the system largely invariant. This particular form of homeostasis (shared by very different biological systems like heartbeat dynamics, ecological communities, protein molecules, gene expression..) stems from the contemporary presence of a crystallized (core) and a fluid (periphery) phase in the same system. In the case of gene expression, these two phases are very evident in terms of genes with a negligible temporal variance (crystallized core) and genes endowed with an high temporal variability (fluid periphery). When, following an external input or for a purely chance effect, the motion of the fluid phase exceeds a certain threshold, the motion is transmitted to the core invading the entire system, a transition (e.g cell fate change, allosteric effect) starts. This mechanism will be thoroughly described to the case of cell differentiation compared to allosteric signal transmission in proteins.

Sequence Composition and 3D Genome Structure

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Several strongly conserved DNA sequence patterns in and between introns and intergenic regions (IIRs) consisting of super short tandem repeats (SSTRs) with repeat lengths <3 bp have been described in many domains of Eukaryotes. We find that the high correlations within introns, intergenic regions and between the two are a result of conserved abundancies of SSTRs with repeat units ≤2 bp (e.g., (AT)n). The highly contributing SSTRs do have specific features as length and word composition. Regions of strong deviations from the average distribution of k-spectra or SSTRs do occur on all chromosomes and may show correlations to known chromosomal features.

Circadian clock and cancer

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The "attractor state" of developed malignancy is associated with polyploidy giant cancer cells (PGCCs) possessing embryonal features. Their proportion highly increases in response to the stress of anti-cancer treatments and later depolyploidizing and spawning resistant progeny that repopulates the tumour and leads to disease relapse. The expression of the stress-induced accelerated senescence, stemness and germ markers, alongside a shift of the transcriptome age index towards evolutionarily ancient unicellular and early multicellular genes, points toward a radical rewiring of the gene regulatory network in aggressive cancers. The molecular circadian clock (CC) is linked to differentiation and to regulation of the cell cycle checkpoints, however, it is down-regulated in mammalian polyploidy cells [1] and in the ESCs induced by Yamanaka factors. We addressed the relationship of CC with polyploidy in 11 cancer types from the TCGA (The Cancer Genome Atlas) database [2]. There was observed a high (Spearman's rho=0.87, $p<0.01$) positive association between polyploidy and the coefficient of circadian deregulation DeltaCCD (a value calculated from the divergences in the co-expression network of key circadian clock genes). The results suggest that circadian clock deregulation is linked to an adaptation to stress-induced DNA damage through bypassing cell cycle checkpoints, which enables polyploidization. We propose that the measure of circadian deregulation within a tumour can potentially have diagnostic and/or prognostic significance.

[1] Anatskaya et al. Phylostratic Shift of Whole-Genome Duplications in Normal Mammalian Tissue towards Unicellularity Is Driven by Developmental Bivalent Genes and Reveals a Link to Cancer. *Int. J. Mol. Sci.* 2020, *21*, 8759. https://doi.org/10.3390/ijms21228759

[2] Vainshelbaum et al., Role of the Circadian Clock "Death-Loop" in the DNA Damage Response Underpinning Cancer Treatment Resistance Cells 2022, 11, 880. https://www.mdpi.com/2073-4409/11/5/880

Change of the higher-order structure in DNA causes significant effect on genetic activity: a physical view

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During the past triple of decades, it has been getting clearer that long genomic DNA undergoes discrete folding transition, exhibiting all-or-none transition or intrachain segregation for individual DNA chain. In the present talk, we will discuss the biological significance of the discrete nature of the conformational transition in relation to the robust on/off switching of large number of genes in living cells. The main topics are as follows.

1.Adapting cell-free gene expression systems, it is found that longer DNA molecules exhibit significantly greater potency in gene expression; for example, the expression level for DNA with 25.7 kbp is 1000‐times higher than that for DNA of 1.7 kbp. We propose an underlying mechanism for the favorable effect of longer DNA on gene expression in terms of the enhancement of access of RNA polymerase to the shrunken conformation. It is expected that the enhancement of gene expression efficiency with a shrunken DNA conformation would also be a rather general mechanism in living cellular environment.

2.It is found that Na^+ and K^+ exhibit markedly different effects through competitive binding with a cationic polyamine, SPD, to DNA, which causes a large difference in the higher-order structure of genomic DNA. It is concluded that the larger favorable effect of Na^+ than K^+ on in vitro gene expression observed in this study is well attributable to the significant difference between Na^+ and K^+ on the competitive binding inducing conformational transition of DNA.

3. It was found that polyamines exert opposite effect, enhancement and inhibition, on gene expression depending on their concentrations. Such an opposite effect is argued in relation to the conformational change of DNA: enhancement is due to the parallel ordering of DNA segments that is accompanied by a decrease in the negative charge of double-stranded DNA, and inhibition is caused by the compaction of DNA into a tightly packed state with almost perfect charge-neutralization.

References:

1)T. Nishio, et al., Longer DNA exhibits greater potential for cell-free gene expression. Sci. Rep., 11, 11739(2021).

2)T. Nishio, et al., K^+ promotes the favorable effect of polyamine on gene expression better than Na^+. Plos One, 15, e0238447(2020).

3)A. Kanemura, et al., Opposite effect of polyamines on In vitro gene expression: Enhancement at low concentrations but inhibition at high concentrations. Plos One, 13, e0193595(2018).