2nd Danube Conference on Epigenetics

Research Center for Natural Sciences, Budapest, Hungary

5-8 October 2016

Book of Abstracts

2nd Danube Conference on Epigenetics

Following the success of the previous Epigenetic Conferences in Budapest (2012 and 2014) we are delighted to announce that the next **Danube Conference on Epigenetics** will be held **October 5-8th, 2016** again in Budapest. The main objectives are to bring scientists together from these fields of epigenetics and promote their intensive interdisciplinary interactions facilitated by the medium sized meeting.

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Contents

Sponsors…….....**3**
Venues……**4**
Overview timetable……**5**
Lecture abstracts……....……**6**
Poster abstracts……**52**
Sponsor advertisements……..**115**
Floorplan……...…**118**

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Venues

October 5, 2016
Hungarian Academy of Sciences
H-1051 Budapest, Széchenyi tér 9.

October 6-8, 2016
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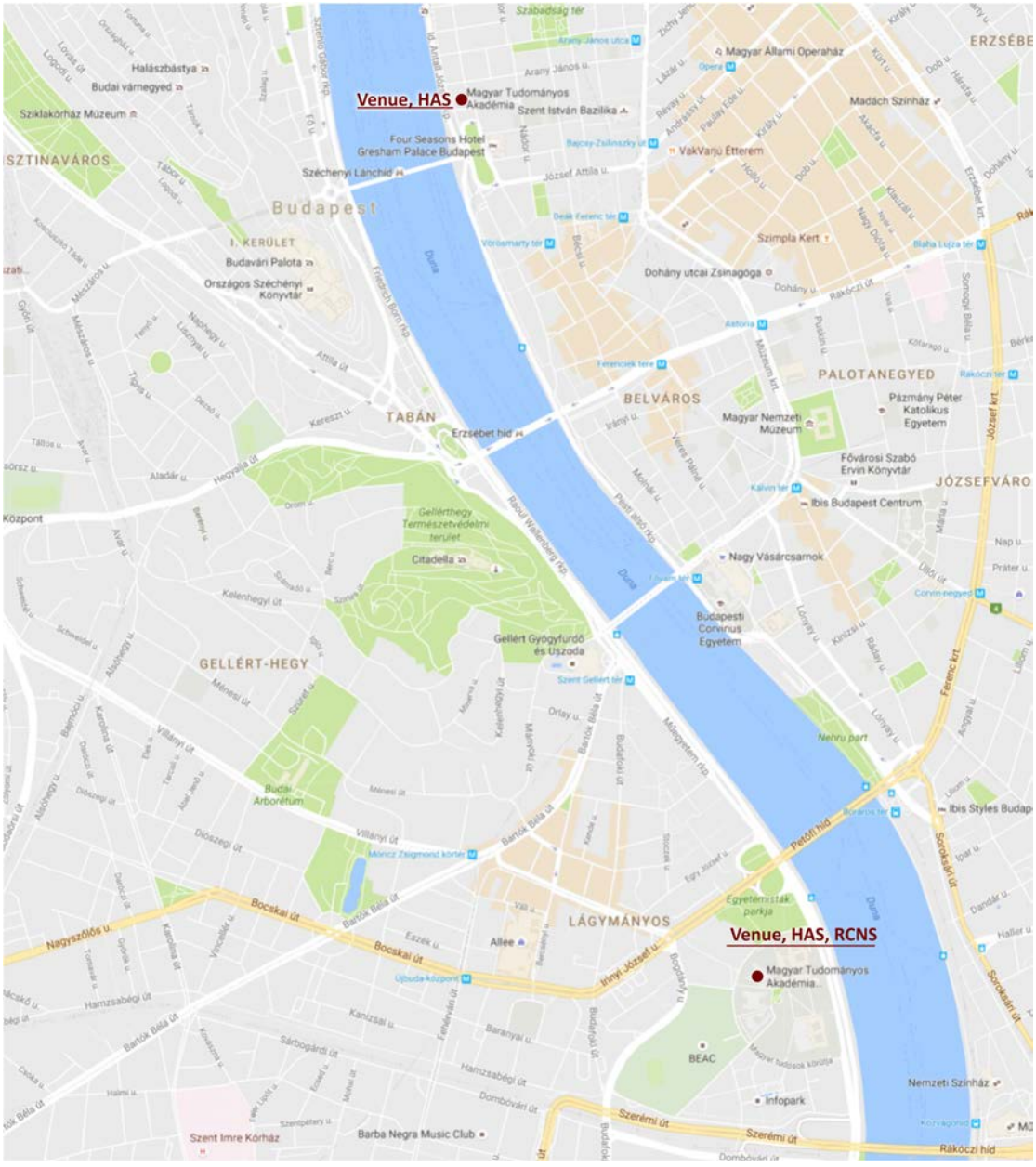

Overview timetable

5th October (Wednesday) Venue: HAS		6th October (Thursday) Venue: RCNS		7th October (Friday) Venue: RCNS		8th October (Saturday) Venue: RCNS	
14:00-	Registration	9:00-10:30	Metabolism and epigenetics	9:00-10:30	Chromatin architecture	9:00-10:30	Transcriptional regulation and epigenetics
		10:30-11:00	**Coffee break**	10:30-11:00	**Coffee break**	10:30-10:50	**Coffee break**
		11:00-12:00	Metabolism and epigenetics	11:00-11:45	Chromatin architecture	10:50-11:35	Transcriptional regulation and epigenetics
		12:00 - 13:00	**Lunch**	11:45-13:45	**Poster viewing**	11:35-13:00	**Lunch & poster viewing**
		13:00 - 14:30	Developmental epigenetics	13:45-	**Sandwiches, Free afternoon**	13:00-14:30	Transgenerational inheritance
		14:30-15:00	**Coffee break** & Live DEMO Show on the exhibition booth of BioMarker QIAGEN			14:30-	Poster prizes and concluding remarks
16:00-16:30	Introduction	15:00-15:45	Developmental epigenetics				
16:30-17:30	Keynote Lecture Eileen Furlong	15:45-16:15	**Coffee break**				
17:30-20:00	Single-cell epigenetics	16:15-18:00	**Poster viewing**				
19:00-21:00	**Welcome reception**			20:00-22:00	**Gala dinner & River cruise**		

Keynote Lecture

Functional insights into chromatin topology and gene expression during embryonic development

Yad Ghavi-Helm, Sascha Meiers, Aleksander Jankowski, Lucia Ciglar, Rebecca Rodriguez Viales, Jan O. Korbel, **Eileen E. M. Furlong**

Head of the Genome Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

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Embryonic development requires the coordinated expression of genes in both a space and time manner. This complex regulation is controlled through the binding of transcription factors (TFs) to enhancer elements, sometimes located at great distances from their target gene. Chromatin conformation studies have shown that gene activation by remote enhancers is associated with the establishment of a chromatin loop to the promoter element. Our previous work indicates that enhancer-promoter loops are often formed prior to transcription, suggesting that chromatin loops are necessary, but not sufficient, for gene activation. The precise link between chromatin organization and transcription regulation remains therefore unclear.

To understand the functional links between chromatin 'interactions' and gene expression, we have engineered both minor and major genome rearrangements and examined their effects on both transcription and chromatin conformation. First, to examine the effect of small and targeted mutations or deletions, we used CRISPR-Cas9 genome editing to produce targeted deletions of interacting regions in *Drosophila*. Second, to examine the effect of major rearrangements, we are taking advantage of *D. melanogaster* strains with highly scrambled chromosomes. These combined approaches are allowing us to decipher the link between chromatin organization and transcription regulation at multiple scales, for example, by examining the transcriptional effect of the precise deletion of an individual enhancer to a major modification disrupting a large chromatin domain. Although the analysis is still on going, the results are revealing surprising insights into the relationship between chromatin topology in *cis* and transcriptional regulation.

Single-cell epigenetics

Revealing novel cell types, cell-cell interactions, and cell lineages by single-cell sequencing

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In this talk I will report on our progress on detecting new cell types, cell-cell interactions, and cell lineages using single-cell sequencing. I will present three recent approaches, RaceID, StemID, and ProximID, that are used to detect rare cells, stem cells, and cell-cell interactions respectively. These algorithms are applied to several experimental model systems including the mammalian intestine, pancreas, and bone marrow. I will also present new data on detecting 5-hydroxycytosine methylation (5hmC) in single cells. This method does not only reveal extensive cell-to-cell heterogeneity of this epigenetic mark but also provide a powerful tool to perform endogenous lineage tracing at the single cell level.

Contribution of parental histone dynamics to epigenome stability after DNA damage

Juliette Dabin[1*], Salomé Adam[1,2,4], Odile Chevallier[1], Olivier Leroy[3], Céline Baldeyron[2,5], Armelle Corpet[6], Patrick Lomonte[6], Olivier Renaud[3], Geneviève Almouzni[2], Sophie Polo[1]

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Keywords: *DNA damage, histone variants, epigenome integrity*

DNA damage challenges not only genome stability but also the integrity of its organization into chromatin, which conveys critical epigenetic information governing gene expression and cell identity. How the chromatin landscape is modified in response to DNA damage while preserving the information that it carries is thus a key issue. We address this fundamental question by focusing on histone H3 variants, which we showed are deposited *de novo* in UVC-damaged chromatin regions in human cells. The deposition of newly synthesized histones raises questions about the fate of parental histones, present in chromatin before damage infliction and carrying the original epigenetic information.
To tackle this issue, we have developed an innovative approach based on the SNAP-tag technology and UVC laser micro-irradiation, allowing real-time tracking of parental and newly synthesized histone dynamics at sites of UVC damage. Thus, we have uncovered a redistribution of parental histones around damage sites and we have examined their contribution to repaired chromatin.
I will present the underlying molecular mechanisms and discuss the implications of our study for understanding how epigenetic information can be maintained during the chromatin rearrangements that accompany DNA repair.

Daxx and PRC1 control integrity and segregation of paternal chromosomes in mouse early embryos

Zichuan Liu[1], Mathieu Tardat[1,3], Mark Gill[1,3], Alexander Hanzl[1], Raphael Thierry[1], Antoine Peters[1,2]

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[3] *Equal contributions*

Keywords: *DAXX, H3.3, mouse zygotes, PRC1, PCH*

Constitutive heterochromatin surrounding centromeres is required for proper chromosome segregation in yeast, fly and mammals. The evolutionary conserved Suv39h histone H3 lysine 9 methyltransferases and Hp1 proteins serve crucial functions in this process. In mouse early pre-implantation embryos, maternal peri-centromeric heterochromatin (PCH) is constituted by these proteins, like in any somatic cell. Members of PRC2 and the canonical PRC1 complex and associated histone modifications define an alternative repressive state at PCH of paternal chromosomes. Paternal PCH is also enriched for the histone H3.3 variant. We identify the histone chaperone proteins Daxx and Atrx as two new components of paternal PCH that control local incorporation of H3.3. Targeting of Daxx and Atrx to paternal PCH is dependent on the core PRC1 components *Ring1* and *Rnf2*. Genetic deficiency of *Daxx* or *PRC1* components abrogates H3.3 incorporation and induces breakage at PCH of exclusively paternal chromosomes, leading to their mis-segregation. Defects in chromosome segregation in maternally deficient *Daxx* embryos are restored by exogenously provided Daxx protein indicating that maternally provided *Daxx* controls chromosome stability in early embryos. This novel targeting module enables incorporation of H3.3 at canonical endogenous PRC1 target genes in somatic cells as well. Together, we dissect a novel molecular pathway that ensures genome integrity of the paternal genome at the onset of life.

Hierarchical folding of chromosomes in neuronal differentiation and its link to epigenetics

Mario Nicodemi

Naples University, Napoli, Italy

Mammalian chromosomes fold into arrays of megabase-sized topologically associating domains (TADs). By combining Hi-C data and modeling techniques, we investigate TAD higher-order interactions through neuronal differentiation and find that they form a hierarchy of domains-within-domains (metaTADs) extending across genomic scales up to the range of entire chromosomes. TAD interactions are well captured by tree-like, hierarchical structures irrespective of cell type. The structure of metaTAD trees correlates with genetic, epigenomic and expression data, and tree rearrangements during differentiation are linked to transcriptional state changes. Using polymer modelling, we show that hierarchical folding promotes efficient chromatin packaging without the loss of contact specificity, highlighting a role well beyond the simple need for packing efficiency.

HIV and human chromatin

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To complete the infection cycle, HIV needs to be inserted in the genome of its host cell. This step is critical,as it allows the virus to become latent and to establish a reservoir of infected cells without any sign of infection. For this reason, patients infected with HIV cannot interrupt the antiretroviral therapy at any moment, otherwise the virus rebounds and all the symptoms of the infection quickly reappear. How and why HIV goes latent remains mostly speculative. Surprisingly little attention has been brought to the hypothesis that HIV may be silenced by the chromatin of the host. To test this idea, we have a developed a technology called BHIVE (Barcoded HIV Ensembles) in which we tag viral genomes with a barcode to follow their insertion and expression in the host cell. Using BHIVE, we have discovered that HIV latency depends on the insertion site. Insertions far from human enhancers are more likely to be latent. In addition, we have used BHIVE to test several latency reversal drugs and have discovered that their typical targets have distinct insertion sites. Our results thus suggest that the chromatin of the host plays a key role in the fate of the HIV infection and in the perspective of treatment.

Epigenetic biomarkers: Ready for clinical diagnostics

Christoph Bock[1,2,3*]

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The human epigenome is altered in numerous diseases, often correlating with clinically relevant information such as disease subtypes, prognosis, and drug response. With suitable assays and after validation in large cohorts, such associations can be exploited for clinical diagnostics and personalized treatment decisions. I will present recent research showing that epigenetic biomarkers are ready for widespread application in translational research and clinical diagnostics.

First, I will describe the results of a community-wide benchmarking study conducted in the context of BLUEPRINT, where we compared the performance of all widely used methods for DNA methylation analysis that are compatible with routine clinical use (*Bock et al. 2016, Nature Biotechnology, doi:10.1038/nbt.3605*). We shipped 32 reference samples to 18 laboratories in 7 different countries. These laboratories collectively contributed 21 locus-specific assays for an average of 27 predefined genomic regions, as well as 6 global assays. We evaluated assay sensitivity on low-input samples and assessed the assays' ability to discriminate between cell types. Good agreement was observed across all tested methods, with amplicon bisulfite sequencing and bisulfite pyrosequencing showing the best all-round performance. We provide detailed documentation of all contributed assays, such that this study can be used not only to guide assay selection, but also as a resource of validated protocols for epigenetic biomarker development.

Second, I will present a feasibility study focusing on chronic lymphocytic leukemia (CLL), which shows that chromatin can provide diagnostically relevant information in a clinical context (*Rendeiro, Schmidl et al. 2016 Nature Communications, doi:10.1038/ncomms11938*). Using the ATAC-seq assay from the Greenleaf lab and our lab's ChIPmentation assay (*Schmidl, Rendeiro et al. 2015 Nature Methods, doi:10.1038/nMeth.3542*), we established chromatin maps for 88 CLL samples from 55 patients using the ATAC-seq assay. Based on the resulting dataset, we devised and applied a bioinformatic method that links chromatin profiles to clinical annotations. Our analysis identified sample-specific variation on top of a shared core of CLL regulatory regions. IGHV mutation status – which distinguishes the two major subtypes of CLL – was accurately predicted by the chromatin profiles, and gene regulatory networks inferred for IGHV-mutated vs. IGHV-unmutated samples

identified characteristic differences between these two disease subtypes. Having optimized both ATAC-seq and ChIPmentation for a turn-around of 24 hours from sample to result, our study highlights the feasibility and relevance of chromatin biomarkers.

In summary, after extensive optimization of assays and bioinformatic methods, and a large multicenter validation study, the tools are now available for ambitious epigenetic biomarker development programs in academia and industry. It is expected that epigenetic biomarkers will become widely useful for early diagnostics and precision medicine, as companion diagnostics of targeted drugs, in forensic testing of tissue types, and in many other applications.

Funding

This work was performed in the context of the BLUEPRINT project (European Union's Seventh Framework Programme grant agreement No. 282510). Christoph Bock is also supported by a New Frontiers Group award of the Austrian Academy of Sciences and by an ERC Starting Grant.

Metabolism and epigenetics

H4K20 monomethylation regulates RNA Pol-II elongation and plays an important role in the transcriptional control of hepatic metabolic pathways

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[4] HHMI, Department of Biochemistry New York University School of Medicine, New York, USA

Monomethylation of H4 at Lysine 20 residue (H4K20Me1) is important for cell cycle progression and the maintenance of genome integrity. It is essential for proper mitotic chromatin condensation, DNA damage response and replication licensing. H4K20 monomethylation is catalyzed by PR-SET7 (Setd8/KMT5a).

Here we show that in non-dividing hepatic cells H4K20Me1 is specifically enriched in the gene bodies of the majority of active genes, but excluded from the promoter and promoter-proximal regions. H4K20Me1 levels positively correlate with gene activation. At early postnatal stages, liver specific inactivation of PR-SET7 (PR-SET7-LivKO) resulted in a partial but not complete loss of H4K20Me1 at most genes, suggesting that this modification is relatively stable. The extent of H4K20Me1 dynamics in the non-dividing hepatocytes correlated with the occupancy pattern of Phf8 (JHDM1F) demethylase.

Reduction of H4K20Me1-modification in PR-SET7-LivKO mice resulted in a decrease of RNA Pol-II occupancy mainly at the gene bodies and to a lesser extent in promoter regions, suggesting that this modification may function as a facilitator of RNA Pol-II release from the promoters. Interestingly, regulation of RNA Pol-II transition to the elongation stage is most prominent in genes regulating hepatic glucose and fatty acid metabolic pathways. Downregulation of glycolytic and de novo lipogenesis genes was accompanied by the activation of genes involved in fatty acid uptake, β-oxidation and ketogenesis. As a result, we detected extensive glycogen deposition in hepatocytes, impaired glucose tolerance, decreased serum cholesterol and triglyceride and increased serum β-hydroxybutyrate levels in PR-SET7-LivKO mice. PR-SET7 deficient hepatocytes display increased mitochondrial activity, which results in detectable reactive oxygen species (ROS) production.

These metabolic changes generate a sensitized state, which upon metabolic stress, quickly aggravate into an irreversible lethal phenotype characterized by severe hypoglycaemia, further enhancement of mitochondrial activity, the accumulation of excessive amounts of ROS, leading to oxidative DNA damage, nuclear swelling, cellular senescence and eventually to necrotic cell death.

The above results illustrate how defects in the process of transition from transcript initiation to elongation can lead to the development of metabolic disorders and associated genome instability.

Contribution of parental histone dynamics to epigenome stability after DNA damage

Florence Cammas

Institut de Recherche en Cancérologie de Montpellier, Montpellier, France

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Chromatin organization plays essential roles in cell identity and functions mainly through regulation of gene expression and is itself regulated by numerous protein complexes, including the family of chromatin associated proteins HP1. We demonstrated here that the absence of either HP1α or HP1γ predispose mice to develop tumours specifically within liver. By specific inactivation of HP1β and HP1γ within hepatocytes of WT or HP1αKO, we further showed that all HP1 are liver specific tumour suppressors. Transcriptomic analysis in young, 5 week old animals revealed that genes up-regulated in HP1αβliverKO and HP1αγliverKO livers were enriched in genes encoding several members of the family of KRAB-ZFPs. These repressors are known to act through the recruitment of the TRIM28 corepressor which has been demonstrated to require its interaction with HP1 for some of its functions. Disruption of the interaction between TRIM28 and HP1 within hepatocytes lead to spontaneous development of tumours and to over-expression of the same KRAB-ZFP as those deregulated in HP1 mutant. We further showed that these genes are direct targets of TRIM28 and HP1. Altogether, our data demonstrated that HP1 are liver-specific tumour suppressors and that although these proteins are known to interact with plethora of chromatin associated proteins, their main functions within liver seems to be the regulation of TRIM28 activity and thereby the expression and repression activity of KRAB-ZFP and ultimately liver homeostasis.

Dynamic DNA methylation changes due to acute metabolic stress in mice

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Calorie intake abnormalities both in rhythm and amount are becoming nowadays epidemic. This is a well-known risk factor for diabetes, cardiovascular diseases and cancer. Our group focuses on the epigenetic changes occurring due to acute nutritional challenges. We use the C57BL/6 mouse model to study the effects of fasting (16 hours) and re-feeding (fasting 16h + 4 h re-feeding). This model is used as a model of gluconeogenesis when important gene expression changes occur. We have performed reduced representation bisulfite sequencing (RRBS) in order to unravel DNA methylation changes in the liver of these mice. RRBS investigates the methylation level of several hundreds of thousands of CpGs mostly located in gene promoters and CpG islands. We have observed pronounced DNA hypo- and hypermethylation both after the overnight fasting and the fasting followed by re-feeding suggesting general active DNA demethylation and de novo methylation. Our data indicate that almost 10% of CpGs loose DNA methylation during fasting while 5% gain of DNA methylation. We observed a partial regain of methylation after re-feeding. Surprisingly, most of the changes occurred in intergenic and CpG poor regions while promoters and CpG islands were less represented than expected. These data highlight the highly dynamic nature of DNA methylation and suggests an important regulatory role of the identified intergenic CpG poor regions.

Posttranscriptionally controlled ribosome assembly rhythms drive diurnal cycles in global liver mass and macromolecular content

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The liver plays a pivotal role in metabolism and xenobiotic detoxification, processes that must be particularly efficient during the activity phase when animals feed. We found that the size and macromolecular content of mouse hepatocytes follow a daily rhythm, whose amplitude depends on both feeding-fasting and light-dark cycles. In liver, ribosomes are rate-limiting for protein synthesis, and the daily oscillation of protein accumulation is indeed accompanied by a similar fluctuation in ribosome number. While the transcription of ribosomal RNA (rRNA) and ribosomal protein (RP) mRNAs remains nearly constant throughout the day, the translation efficiencies of many RP mRNAs follow high-amplitude diurnal cycles. Intriguingly, nuclear 28s and 18s rRNAs are polyadenylated at maximal rates when RP protein synthesis rates are minimal. Based on studies with cultured fibroblasts we propose that rRNAs not packaged into complete ribosomal subunits are polyadenylated by the poly(A) polymerase Papd5 and degraded by the exosome Exosc10.

Deciphering the transcriptional regulatory network of the human malaria parasite *Plasmodium falciparum*

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Keywords: *ATAC-seq, regulatory elements, malaria*

Despite global efforts, *Plasmodium falciparum* is still responsible for an estimated half a million deaths annually. Disease-related symptoms occur when the parasite develops and multiplies within the human red blood cell. Yet, regulatory processes responsible for orchestrating blood stage development remain to be unravelled. To identify and characterize active *cis*-regulatory DNA elements during this blood stage development we performed ATAC-seq and RNA-seq at eight consecutive time points 5h apart. This exercise revealed about 3000 sites of chromatin accessibility predominantly located 0-2000bp upstream of genes (>90%). In addition, accessibility appears highly dynamic during blood stage development, reminiscent of the dynamics of the transcriptional program. Accessible chromatin regions were subjected to a combination of *de novo* motif identification approaches and database searches to identify putative DNA elements responsible for transcription factor binding. Functional validation of these regulatory sequences is in progress including DNA pull-downs followed by quantitative proteomics. The latter have already been used on selected motifs to identify the interacting transcription factors. Together our analysis will unveil the complex interplay between *cis*-regulatory elements and transcription factors that underlies development and survival of the malaria parasite in the human bloodstream.

Transcriptional outcomes in response to DNA damage

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Various types of DNA damage interfere with key vital processes which use DNA as a template, like replication and transcription. Upon large amount of genotoxic impacts, transcription is over-activated and probably results in the activation of several DNA damage recognition processes. During transcription, numerous components of the transcription machinery may act as a platform to recruit repair proteins at break sites.
In contrast to that, when DNA damage occurs at a transcribing unit, it leads to transcriptional block. This multistep process involves several kinases and the ubiquitin ligases like NEDD4 and CUL3 leading to proteasome dependent degradation of RNA polymerase II (RNAPII) which happens at the site of the damage. Finally, at the break site ddRNA (a new class of noncoding RNA) production could be observed by controlling the DDR activation at sites of DNA damage.
Taken together these results support an uncharacterized function of RNAPII complexes which allow the recognition of DNA damages and like this enhance cell survival following DNA damage.

This work was supported by OTKA-PD [112118], and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences

β-cell PRC2 focuses transcription of select lineage genes and thus prevents de-differentiation and Diabetes in mouse and Man

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Type-2 diabetes affects ~10% of the global population. Recent data suggest that pancreatic β-cell 'de-differentiation' may underpin diabetes etiology, though mechanisms remain lacking. Here, we probed the role of Polycomb Repressive Complex 2 in maintaining β-cell function and identity. We find that β-cell specific Eed deletion in mice triggers fully penetrant and synchronized de-differentiation throughout the β-cell compartment. Knockout β-cells exhibit chromatin-state defined loss of transcriptional silencing of hundreds of transcripts enriched for immature an precursor-state genes. Intriguingly, de-differentiation appears to be driven by loss of expression of specific set of lineage defining genes characterized by extremely broad H3K27Ac 'super'-domains but that lack enhancer marking. We find strong evidence of comparable Polycomb-associated dysregulation in human type-2 diabetes including reduced β-cell H3K27me3 levels as well as antagonistic transcriptional regulation of H3K27me3 and H3K27Ac 'super'-domain marked genes in human type-2 diabetes transcriptome data. The results demonstrate in vivo roles for PRC2 maintaining differentiated cell identity, broad scale transcriptional silencing and positive transcriptional regulation of a novel set of selectively marked lineage genes. They identify Polycomb as one of the first epigenetic regulatory systems required for preventing β-cell de-differentiation and diabetes in mouse, and likely, in man.

Developmental epigenetics

Towards understanding of the molecular mechanisms of the developmental epigenetic reprogramming

Petra Hajkova

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During mouse embryonic development early postfertilisation zygotes and the developing primordial germ cells (PGCs, the precursors of gametes) undergo global epigenetic reprogramming. This process involves genome-wide erasure of DNA methylation as well as global changes in chromatin structure and histone modifications. Despite the efforts of numerous research teams the molecular mechanisms underlying these developmental reprogramming processes remain elusive.

I will present our recent results regarding the dynamics of DNA modifications and chromatin during the epigenetic reprogramming in mouse gonadal primordial germ cells (PGCs). I will also discuss our current understanding of mechanistic links between the global DNA demethylation and the execution of the germline developmental programme.

A histone mimic within DNA Ligase 1 recruits UHRF1 to sites of DNA replication: Implications for DNA remethylation

Pierre-Antoine Defossez[1], Alexandra Fournier[1], Laure Ferry[1], Takeshi Tsusaka[2], Tadahiro Shimazu[2], Kyohei Arita[3], Guillaume Adelmant[4], Yoichi Shinkai[2]

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DNA methylation is an essential epigenetic mark in mammals: it controls the expression of imprinted genes, germline genes, transposons, and is intimately linked to the local chromatin state. For all these reasons, the maintenance of correct patterns of DNA methylation is essential for the survival of mammalian cells. This pattern has to be re-established at each round of DNA replication. One of the key actors in this process is the protein UHRF1: it is essential for DNA remethylation after replication, but its mode of action is unclear. We have characterized the UHRF1 interactome by proteomics and found that DNA Ligase 1 (LIG1) is a highly abundant interactor of UHRF1. We have mapped the interaction domains and found that a Tudor domain of UHRF1 interacts with an H3-like histone mimic within LIG1. We show that the interaction requires the methylation of the LIG1 histone mimic by the lysine methyltransferases G9a or GLP. Finally, we find that the interaction with LIG1 promotes the recruitment of UHRF1 to sites of DNA replication and is required for normal DNA remethylation. These results prompt a reinterpretation of the function of UHRF1's Tudor domain, which we show can bind non-histone proteins. They also reveal a new level of complexity in DNA Ligase 1, identify a new non-histone target of G9a and GLP, and provide the first example of a histone mimic that coordinates DNA replication and DNA remethylation.

Small RNA-based regulation during temperature adaptation in *Arabidopsis*

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The sessile nature of plants necessitates that they grow and develop in response to environmental conditions. In the 12-27^{0}C ambient temperature range plants show large differences in growth and development. Flowering is the primary trait affected by ambient temperature changes. Although temperature influences plant's life in a large extent, the process, how temperature is sensed and integrated into development is largely unknown. Plant microRNAs (miRNAs) are small non-coding RNAs playing an important regulatory role both transcriptionally and post-transciptionally. To study the role of small RNAs in ambient temperature sensing and adaptation we used sRNA, mRNA and degradome libraries from *Arabidopsis thaliana* plants combined with genomic-scale deep-seguencing. We profile and grouped by annotations the ambient temperature regulated sRNS loci, miRNAs and their target genes. Our results suggets that during inductive photoperiod miR169 family could mediate ambient temperature signals to modify flowering time by regulating the expression of the subunit A of Nuclear Factor Y. The NF-Y transcription factor complex binds to regulatory elements of SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1) and demethylates H3K27me3 partly through RELATIVE OF EARLY FLOWERING 6 (REF6) demethylase. We propose a model which show that miR169 can signal information from the environmental into the flowering time pathway.

Single cell approaches to cellular memory

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We will describe approaches for single cell and single molecule analysis of genome regulation. These new methods provide us with high resolution maps of the transcriptional states of individual cells within tissues, and with data on the epigenetic and chromosomal mechanisms that regulate such states. In many cases, as we exemplified using data on mouse embryogenesis and hematopoiesis, the remarkable specificity of the transcriptional response cannot be explained by data on transcription factors activity, enhancer epigenetic states, or chromosome conformation. Potential methodologies for integrating these regulatory layers will be discussed. In particular we will discuss the possibility of synergistic enhancer hubs as drivers for specific regulatory decisions in repressive and active contexts.

MYC favors the onset of tumorigenesis by inducing epigenetic reprogramming of mammary epithelial cells towards a stem cell-like state

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Breast cancer consists of highly heterogeneous tumors whose cell of origin resulted difficult to be defined. Recent finding highlight the possibility that tumor-initiating cells (TICs) may arise from the dedifferentiation of lineage-committed cells by reactivation of multipotency in response to oncogenic insults. MYC is the most frequent amplified oncogene in breast cancer and the activation of MYC pathway has been associated with the basal-like subtype, which is characterized by poor survival lacking of a specific therapeutic strategy. Although MYC has been considered a driver oncogene of breast cancer, its mechanism of action in tumor initiation has been poorly addressed. Here we show that MYC acts as tumor reprogramming factor by inducing an alternative epigenetic program, which triggers loss of cell identity and activation of oncogenic enhancers. Overexpression of MYC induces transcriptional repression of lineage-specific transcription factors, causing decommissioning of luminal-specific enhancers. MYC-driven dedifferentiation supports the onset of a stem cell-like state by inducing the activation of *de novo* enhancers, which drive the transcriptional activations of oncogenic pathways. We will further illustrate the molecular mechanisms through which MYC participates in inducing epigenetic changes which support activation of oncogenic enhancers. Furthermore we will present data showing that the MYC-driven epigenetic reprogramming favors the formation and maintenance of basal-like TICs in vivo.

Developmental dynamics of epigenomic features of cis-regulatory elements in early embryo development

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Large-scale genomics programmes such as ENCODE and FANTOM resulted in prediction of previously unanticipated density of functional elements of the human genome. These predictions raise the need for validation models of predicted functional elements. We are assessing the degree to which zebrafish with the transparent, externally developing embryo can be used as a surrogate for validating cis-regulatory functions predicted by genome wide assays in mammals and in fish. While zebrafish provides a lucrative experimental system for functional validation, its utility is hindered by the lag in the annotation of its own genome. Improved functional annotation of the zebrafish genome is crucially required to assess the zebrafish model for its relevance in human genomics. Early development of zebrafish encompassing the maternal to zygotic transition and including zygotic genome activation provides an ideal experimental platform for elucidating the epigenomic features of gene regulation. It allows dissecting the temporal sequence and dynamics of establishing transcriptionally active chromatin state and helps in identifying the determinants of transcription activation of Polymerase II transcribed genes. The relatively large number of pluripotent cells generated by the fast cell divisions prior to zygotic transcription provides sufficient biomass for next generation sequencing technology approaches to establish the temporal dynamics of transcription regulatory events and suggest causative relationship between them. We have been annotating epigenetic features, transcriptional start site regions (core promoters) and distal cis-regulatory modules such as enhancers of the developing embryo by CAGE-seq, ChIP-seq ATAC-seq and RNA-seq during development. Analyses of these epigenomic datasets strongly support a model which suggest that epigenomic premarking mechanisms contribute to developmental gene regulation programme well ahead of commencement of the activation of gene activation.

Chromatin architecture

Genome Architecture Mapping: A spatial approach to map chromatin contacts

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The organization of the genome in the nucleus and the interactions of genes with their regulatory elements are key features of transcriptional control and their disruption causes disease. Technologies based on chromosome conformation capture (3C) have profoundly expanded our understanding of the role of genome architecture in gene regulation. We introduce Genome Architecture Mapping (GAM), a novel genome-wide method for measuring three-dimensional chromatin topology.

Exploration of the most prominent chromatin contacts detected in mouse ES cells using GAM identifies most specific chromatin contacts between active genes and enhancers across very large genomic distances. GAM also reveals abundant three-way contacts genome-wide, especially between the enhancers most highly occupied by pluripotency transcription factors and highly transcribed genomic regions.

Our results highlight a previously inaccessible complexity in genome architecture and a major role for contacts related with gene expression in the structural organisation of the genome in mammalian nuclei.

Heterozygous mutations in *DNMT3B* cause derepression of the subtelomeric D4Z4 macrosatellite array and contribute to the development of muscle disease

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Genetic and epigenetic mechanisms are involved in the spatio-temporal regulation of transcription, which is essential for all cellular processes. Proper chromatin state is required for coordinated regulation of gene expression. Chromatin accessibility is governed by epigenetic factors and deregulation caused by genetic variants in epigenetic modifiers might have deleterious consequences, leading to disease. Facioscapulohumeral muscular dystrophy (FSHD) is an exemplary disease of deregulation of chromatin accessibility. Chromatin relaxation leads to the expression of the germline transcription factor DUX4 in muscle cells resulting in cell death. A copy of the DUX4 coding sequence is present in every D4Z4 macrosatellite unit which are organized in tandem arrays in the human genome localized at 4q and 10q subtelomeric regions. D4Z4 arrays are transcriptionally repressed in somatic tissue, but contraction of the array below a critical length or mutations in the D4Z4 chromatin repressor SMCHD1 result in derepression of D4Z4 arrays. Since not all patients with FSHD phenotype could be explained by contraction of D4Z4 array or SMCHD1 mutations we performed whole exome sequencing in 8 families with DNA hypomethylation at D4Z4 and without any SMCHD1 exonic mutations. Potentially damaging variants in the de novo DNA methyltransferase DNMT3B were identified in 2 independent families.

Biallelic DNMT3B mutations are described in ICF1 syndrome characterized by a primary immunodeficiency phenotype but without any reported muscle weakness, and we could identify epigenetic commonalities between both disorders. Our work suggests that mutations in epigenetic regulators can lead to discordant phenotypes.

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A Set1C-entric view of meiotic recombination initiation

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During meiotic homologous recombination DNA double-strand breaks (DSBs) occur in the context of chromatin structure. The frequency and distribution of DSBs are significantly affected by various histone modifying enzymes and histone modifications. Herein I elaborate the role of the Set1 histone methylase complex (Set1C or COMPASS) in the process of meiotic DSB and crossover pattern formation. Based on various genome-scale approaches I show that the Set1C *i)* is highly-dynamic in nature upon chromatin binding, *ii)* governs the number and localization of DSBs, and *iii)* affects the outcome of DNA repair events with respect to crossover *vs.* non-crossover recombinant joint molecule formation.

Beyond memory: The secret life of Polycomb Response Elements

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Polycomb/Trithorax Response Elements (PREs) are epigenetic cis-regulatory DNA elements that work in concert with enhancers to ensure genome-wide transcriptional fidelity for hundreds of developmentally important target genes. For the *Drosophila* Hox genes, enhancers establish expression states early in development and PREs maintain these states epigenetically, long after the initial determining transcription factors have disappeared. However the Polycomb and Trithorax group proteins have several hundred additional target genes whose regulation shows variations on this basic theme of stable epigenetic memory of "On" and "Off" states. For example many target genes change their expression status dynamically upon developmental or environmental signals, implying that for these targets, PcG mediated memory readily allows switching. Other target genes are regulated by a robust network of transcription factors that are continuously present, thus these genes would seem to have no need for epigenetic memory. Understanding the full repertoire of PRE properties will be essential to understanding genome-wide Polycomb/Trithorax regulation. PREs respond to enhancer input and genomic and developmental context in a manner that depends on their DNA sequence, but how these parameters contribute to the quantitative readout of PRE activity is unknown. We have used stochastic mathematical modeling in combination with quantitative experimentation in *Drosophila* to dissect the contribution of each of these parameters to PRE output in terms of robustness vs. switchability of epigenetic memory. The results demonstrate that the interplay between PREs and enhancers can produce a rich repertoire of outputs beyond simple epigenetic memory, including noise suppression, thresholding, and stabilisation of intermediate transcriptional states. Fitting models to experimental data within the known time constraints of *Drosophila* development predicts that the relationship between stability and flexibility of epigenetic memory is critically dependent on the frequency of disruption by replication and mitosis. This work has broad implications for our understanding of the quantitative relationship between PRE sequence and developmental context in determining genome-wide transcriptional responses to PRE mediated regulation.

This work was performed by L. Ringrose while on sabbatical in the group of Martin Howard (Department of Computational and Systems Biology, John Innes Centre, Norwich, UK). For more information see Cell. 2015 Nov 5;163(4):788-9. doi: 10.1016/j.cell.2015.10.058. PMID: 26544929

Cell fusion-mediated reprogramming reveals a link between variability of human X chromosome inactivation in somatic cells and pluripotency-induced gene reactivation

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Reactivation of the inactive X-chromosome (Xi) has been used to model epigenetic reprogramming in the mouse but human studies are hampered by Xi epigenetic instability and difficulties in tracking partially reprogrammed iPSCs. Here, we use cell fusion to examine the earliest events in reprogramming-induced Xi reactivation of human female fibroblasts. We show that ahead of cell division a rapid loss of Xi-associated of H3K27me3 and *XIST* RNA occurs in fused cells and precedes bi-allelic expression of selected Xi genes. After cell division, RNA-FISH and allele-specific RNA-seq analyses confirm that Xi reactivation remains partial and that induction of human pluripotency-specific *XACT* transcripts is rare (1%). Interestingly, Xi-genes that are sensitive to pluripotency-induced reactivation also show heritable stochastic expression in different single-cell clones examined ahead of reprogramming. Treatment with 5-deoxy-aza-cytidine shows that DNA demethylation does not increase stochastic Xi expression ahead of reprogramming but predisposes a second cadre of Xi-genes to pluripotency-induced reactivation. Overall, these data separate pre- and post-mitotic events in Xi-reactivation and suggest that analysis of transcriptional stochasticity among human cells can be used to predict, and potentially engineer different epigenetic strategies for locus- or domain-specific human Xi reactivation.

Multi-contact 4C reveals multi-way three-dimensional chromatin conformation

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Spatial chromatin organization is increasingly recognized as a regulator of nuclear processes such as gene activity. Most methods developed to unravel three-dimensional (3D) chromatin conformation analyze pair-wise chromatin contacts, but are incapable of identifying simultaneous associations among multiple loci. Yet, exactly this type of 'beyond two-way' contact information will provide the insights required to start composing a more complete picture of 3D genome organization at the level of the individual allele.

We describe a novel chromatin conformation capture technology that allows direct identification of multi-way chromatin contacts. Multi-Contact 4C (MC-4C) applies the Pacific Biosciences third-generation long-read single molecule real time (SMRT) sequencing technology to an intermediate product of the conventional chromatin conformation capture (3C) protocol. For a given allele of interest, MC-4C can easily identify 4 to 8 spatial neighbors, based on proximity-ligation events. With many thousands of identified multi-way (>3) contacts per targeted locus, MC-4C presents a unique and promising new technology to distinguish co-operative from mutually exclusive 3D chromatin structures.

Transcriptional regulation and epigenetics

The ATAC and SAGA coactivator complexes are highly dynamic in the nuclear environment with fast and slow chromatin interacting populations

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Initiation of transcription by RNA polymerase II (Pol II) according to the textbook view is believed to be the outcome of a number of sequential events beginning with the binding of specific activators to their cognate binding sites. This initial step will trigger the recruitment of coactivator complexes and general transcription factors (such as for example TFIIB and TFIID) at promoters to allow the loading of Pol II into the preinitiation complex (PIC). In this process, coactivators play multiple crucial roles through enzymatic as well as non-enzymatic functions. SAGA (Spt-Ada-Gcn5-Acetyltransferase) and the ATAC (Ada-Two-A-Containing) complexes are two functionally distinct, but related, coactivator complexes with several enzymatic activities: histone acetyl transferase (HAT, for ATAC) or HAT and deubiquitinase (for SAGA). These complexes have been shown to regulate global gene expression and chromatin architecture through their enzymatic activities. With a combination of fluorescence recovery after photobleaching (FRAP), fluorescence loss in photobleaching (FLIP) and fluorescence correlation spectroscopy (FCS) we analyzed the dynamic behavior of TFIIB, TFIID, Pol II, SAGA and ATAC in living cells. Our FRAP and FLIP measurements indicate that TFIIB, TFIID, ATAC and SAGA subunits are highly dynamic and exhibit only transient interactions with the chromatin with no detectable immobile fractions. In contrast, we found that the recovery rates of the TATA binding protein (TBP, a subunit of Pol I, II and III transcription machineries), and RPB1 (Pol II subunit) were significantly slower than that of TFIIB, or TFIID, ATAC and SAGA subunits. Furthermore, our FCS measurements indicate that ATAC and SAGA have two distinct diffusing populations in the nucleus: a 'fast' population (having the approximate size of the studied complexes) and a 'slow' population (representing chromatin interacting, but still mobile complexes). The changes of the dynamic behavior of these complexes upon inhibition of transcription, or histone H3K4me3-ion, and their relevance in transcription regulation will be discussed.

Epromoters define a new class of regulatory elements with dual promoter and enhancer functions in mammals

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Keywords: *enhancer, promoter, high-throughput reporter assay*

Gene expression in mammals is precisely regulated by combination of promoters and gene-distal regulatory regions, known as enhancers. Recent studies have shown broad similarities between enhancers and promoters and suggested that some promoters might also play enhancer functions. However, the extent of this type of promoters and whether they actually function to regulate the expression of distal genes have remained elusive. Here, by exploiting CapStarr-seq, a high-throughput enhancer reporter assay, we unravel a substantial proportion of mammalian promoters displaying enhancer activity, named hereafter Epromoters. Compared to classical promoters and distal enhancers, Epromoters display distinct genomic and epigenomic features and are associated with stress response. By using comprehensive CRISPR/Cas9 genomic deletions we demonstrated that Epromoters are frequently involved in *cis*-regulation of distal gene expression in their endogenous context, therefore functioning as *bona fide* enhancers. Furthermore, human genetic variations within Epromoters were associated with a strong effect on distal gene expression. Our results highlight a new category of regulatory elements playing a dual role as transcriptional promoters and enhancers, thus ensuring rapid and coordinate regulation of gene expression. These finding have important implications for the understanding of complex gene regulation in normal development and disease.

Silencing transposable elements by PIWI-interacting RNAs

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Transposable elements (TEs) are targeted for transcriptional silencing through a mechanism mediated by small RNAs. In animal germ lines, the piRNA (PIWI-interacting RNAs) pathway has been identified as the major mechanism for mounting an effective defense against TEs. In *Drosophila*, both germ cells and their associated somatic cells possess a functional piRNA pathway, however the biogenesis of piRNA molecules differs. In both types of cells, a pool of primary piRNAs is presumably processed from long single stranded transcripts. These long transcripts are produced from discrete genomic loci (piRNA clusters) that mainly reside in pericentric heterochromatin enriched in TEs or their relics. One of these loci is the *flamenco* locus of *Drosophila* which has been identified as a master locus for three retrotransposon regulation: gypsy, ZAM and Idefix. Despite advances in understanding the processing events that generate piRNAs for silencing, little is known about piRNA clusters, their structural dynamics and expression. We will report our last data obtained from the *flamenco* analysis.

Epigenetic control of pervasive transcription and genomic stability

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The eukaryotic chromatin structure compacts and protects the genome but also limits the accessibility of the underlying DNA. Chromatin modifying activities can open the chromatin and provide regulated access to specific genomic loci. Nucleosomes are the basic building blocks of the chromatin, and the position, occupancy and turn-over rate of nucleosomes at a given genomic locus ultimately determine the accessibility of the underlying DNA sequence. Defects in nucleosome organization lead to increased levels of pervasive transcription outside of genetically defined transcription units. Despite the central role of nucleosomes in transcription regulation, our knowledge about the effects of different histone modifications and histone variants on nucleosome organization is very limited. We recently characterised the impact of various histone modifications on the position, occupancy and turn-over rate of nucleosomes and their effect on pervasive transcription. Increased pervasive transcription and the resulting nuclear accumulation of non-coding transcripts can lead to excessive RNA-DNA hybrid formation in the genome and subsequent genomic instability. Interestingly, we have recently identified an additional role for pervasive transcription in the DNA double-strand break repair pathway, thus revealing a surprising, positive role in genomic stability.

Super-enhancers and person-to-person genetic variability in the context of the 1000 Genomes Project

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Understanding the person-to-person variability in healthy and diseased states became a possibility by using the unprecedented amount of available genomic data. Super-enhancers seem to be key elements of transcription regulation both in cancer and healthy states.

We have investigated the mechanisms that drive transcription in the MCF-7 cancer cell line and in the LCL cell lines derived from a family of the 1000 Genomes project. Interestingly subsets of estrogen receptor (ER) dependent super-enhancers bind ER even without the estrogen signal (estradiol, E2). These binding sites will anchor the Mediator complex upon ligand activation and will serve as organizers of later events of the transcription.

In the cell lines of the 1000 Genomes project, investigating a specific family gives us the opportunity to better understand the genetic differences that are the basis of person-to-person differences in gene expression. The cell line specific master regulator transcription factor of LCL cells is PAX5. We have mapped the binding sites of PAX5 and the PAX5 super-enhancers in parents and a child of a well-characterized family. We have found that the inter-family variability of TF binding sites is significant; roughly 15% of binding sites might behave in a person specific manner. Interestingly on these differences can be explained in many cases by an allele specific binding of the TF to some genetic positions. The allelic differences might be explained only in a small subset by known SNP-s. Usually the known SNP-s localize between TF binding sites. Some allele specific differences in TF binding could be correlated with allele specific transcription.

Our results can bring a better understanding in how super enhancers work in the context of person-to –person genetic variability.

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RNA methylation in stem cells and cancer

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Whether protein synthesis and cellular stress response pathways interact to control stem cell functions was unknown. Our recent data show that skin stem cells synthesize less protein than their immediate progenitors in vivo, even when forced to proliferate in a tumor model. Our analyses reveal that activation of stress response pathways drives both a global reduction of protein synthesis and altered translation of specific mRNAs that together promote stem cell functions and tumorigenesis. Mechanistically we show that inhibition of post-transcriptional cytosine-5 methylation locks stem cells in this distinct translational inhibition program. Paradoxically, this stress-induced translation inhibition renders stem cells hypersensitive to cytotoxic stress, as tumor regeneration after treatment with 5-fluorouracil is blocked. Thus, stem cells must revoke translation inhibition pathways to regenerate a tissue or tutor.

Transgenerational inheritance

Transgenerational epigenetics: Lessons from Arabidopsis

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Until recently, a key assumption in biology has been that DNA mutations are the only source of heritable phenotypic diversification and therefore that adaptation is impossible in the absence of DNA sequence variants. However, this view is being increasingly challenged by the observation that changes in chromatin states, which are pivotal for the control of genome activity in eukaryotes, can sometimes become locked in and inherited across multiple sexual generations, independently of any change in the genome sequence. This system of inheritance, called epigenetic, is best documented in plants and often involves differential DNA methylation of repeat sequences, notably transposable elements. However, there is still a lack of systematic studies on the stability of epigenetic variants and their phenotypic consequences. Thus, the ecological or evolutionary impact of this type of variation, which could be different from that of DNA sequence variants by potentially providing a more rapid and reversible route to adaptation, remains unclear. I will present our efforts at addressing some of these issues experimentally using a population of near-isogenic, epigenetic Recombinant Inbred Lines (epiRILs) in Arabidospsis.

Drosophila gut microbiome is involved in transgenerational inheritance of acquired traits

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Keywords: *Transgenerational inheritance, drosophila gut microbiome, RNAseq*

Changes in the environment have influence on gene expression, which may lead to an adaptive phenotype. Some phenotypic alterations can be inherited to the next generation independent of changes in genotype. Studies of transgenerational inheritance of acquired traits has mainly focused on what is transferred through the germ line. Chromatin modifications (e.g. DNA methylation) and non-coding RNAs are the most studied mechanisms where there is also evidence of their involvement in such transgenerational inheritance. However, the microbiome is generally also transferred to the next generation. We hypothesized that the gut microbiome plays a role in transmitting environmental experiences to the next generation.
In this project, we study transgenerational inheritance of acquired traits using *Drosophila melanogaster* as a model organism. We have exposed flies to different temperatures and measured the effects on transcription by RNA-seq. We now have RNA-seq data from two generations of flies, from which only the first generation was exposed to the heat and cold condition and where the offspring received microbiomes from parents grown at different temperatures. The results show that some of the transcriptional response to the changes in temperature are transmitted to the next generation. Moreover, we show that the microbiome significantly contributes to the transgenerational inheritance of this transcriptional response. Our study has implications on the field of transgenerational inheritance of acquired traits. Clearly, the microbiome has to be taken into account when trying to understand the mechanisms behind this phenomenon.

The epigenetic modifier *Fam208a* is essential for mouse gastrulation

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The current study investigates the effect of Fam208a mutation on mouse gastrulation. Gastrulation initiates with the formation of the primitive streak and, during gastrulation, cells of the epiblast delaminate and ingress through the primitive streak to form the mesoderm and definitive endoderm. The gastrulation stage embryo is also the stage where the anterior-posterior (A-P) axis is established. At this stage, the pluripotent cell population of the epiblast undergoes very rapid cellular proliferation and extensive epigenetic programming. Previously we identified a novel gene, Fam208a, as a new epigenetic modifier essential for early postimplantation mouse development. Causative mutations in this gene were identified in two mouse strains obtained in an N-ethyl-N-nitrosourea (ENU) screen for modifiers of transgene variegation. These mutant strains, both suppressors of transgene variegation, were termed MommeD6 and MommeD20. Recently, the human orthologue FAM208A was highlighted as an essential member of a new epigenetic silencing complex (termed the HUSH complex), which also includes the histone H3K9me3 binding protein MPP8 and the H3K9 methyltransferase SETDB1. In our ongoing study, we conducted a detailed investigation of the signaling pathways that are disrupted by *Fam208a* mutation during early post-implantation stages, and which may further explain the failure of mouse mutants to gastrulate. We observed that homozygote mutants first appear growth retarded around 6.75 d.p.c., leading to their eventual resorption around 9.5 d.p.c. E6.5 mutant embryos exhibited significantly delayed epithelial-mesenchymal transition (EMT) as evidenced by diminished Snail expression. They also had delayed formation of the node, as shown by whole mount in-situ hybridization with Noto, Brachury & Shh. We further observed several genes, where mis- and or delayed-expression is suggestive of incorrect A-P patterning.

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How abnormal folate metabolism haunts our descendants

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Transgenerational epigenetic inheritance (TEI) involves the transmission of 'information' invoked by an environmental stressor via the germline over multiple generations and independent of the DNA base sequence. The epigenetic mechanisms behind this phenomenon remain unclear. Our goal is to use the *Mtrr*^{gt} mouse model of abnormal folate metabolism to explore mechanisms of TEI. Normally, folate metabolism is required for cellular methylation (e.g., of DNA, RNA, proteins) and the Mtrr enzyme progresses the cycle. The *Mtrr*^{gt} mutation in mice reduces *Mtrr* mRNA expression and sufficiently disrupts folate metabolism with similar effects to dietary folate deficiency. Remarkably, when a maternal grandparent is a carrier of the *Mtrr*^{gt} mutation, a wide spectrum of developmental phenotypes (e.g., growth defects, congenital malformations) associated with epigenetic instability results in their wildtype grandprogeny at midgestation. An embryo transfer experiment revealed that the congenital malformations were independent of uterine environment effects and rather were caused by epigenetic inheritance via the germline. Currently, we are investigating a number of avenues in this model to better understand the mechanism(s) behind TEI. This includes exploring the extent to which epigenetic instability affects genetic instability, searching for inherited epimutations, and defining the relationship between epigenetic instability and DNA methylation machinery as a means of perpetuating transgenerational phenotypes. There are numerous benefits to elucidating how our environment affects our health and that of our descendants: from a greater understanding of adaptation to disease prevention.

Poster abstracts

P-01
miR-548as could be a novel suppressor of TGFβR1 to inhibit epithelial-to-mesenchymal transition in NSCLC

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MicroRNAs (miRNAs) have been identified as important post-transcriptional regulators involved in various biological and pathological processes of cells, but their underlying mechanisms in epithelial-to-mesenchymal transition (EMT) and metastasis have not been fully elucidated. miR-548as is localized in 13q31.3, and is lowly expressed in NSCLC cells, suggesting that it might be a tumour repressor miRNA for NSCLC cells. However, it has not been reported that the functions of miR-548as in the carcinogenesis of NSCLC. Here, the objective of this study is to identify the role of miR-548as in EMT of NSCLC. To evaluate the effects of mir-548as on EMT, A549 cells were transfected with mimic-miR-548as or non-targeting control mimic. 24 h after transfection, we observed that overexpression of mir-548as could markedly inhibit TGFβR1 expression. Moreover, it was showed that overexpression of mir-548as induces expressions of E-cadherin and SATB2, downregulates also Zeb1 expression in A549 cells. This results strongly indicate that mir-548as could be a novel repressor for the induction of EMT by targeting TGFβR1 in NSCLC cells.

P-02
Applying environmental epigenomics to assess adaptation to global change in *Heliconius melpomene* butterfly populations

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Global change imposes a significant risk over biological systems, threatening their diversity and survival. Understanding how organisms can adapt to global change is important for the ecosystems, and also from an anthropocentric point of view. Epigenetic processes can produce phenotypic variation in short evolutionary time, promoting the apparition of diversity in populations. They represent a mechanistic link between the environment, the genome and the phenotype, hence playing a key role in stress responses and adaptation. Tropical ectotherms are particularly sensitive to environmental conditions, experiencing morphological and fenological changes, as well as modifications in their distribution, abundance and even extintions. Here we propose the butterfly *Heliconius melpomene* as a suitable model to study the role of epigenetic components in mediating adaptation to global change in an Amazon rainforest ecosystem. We explored the existing literature and genomic databases and found regions responsible for drastic phenotypic variation that are not linked to coding regions, where most probably epigenetic modifications are having a regulatory role. We present preliminary results to detect methylation patterns in these regions, as well as global chromatin structure analysis. We also use this research as an opportunity to increase environmental awareness in the local quichua communities, creating didactic materials and other butterfly-connected items that bring together the university and its social and cultural environment.

P-03
Developmental programming in response to maternal overnutrition

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Keywords: *developmental programming, epigenetics, metabolic syndrome*

Perinatal environmental exposure can permanently impact offspring health; a process called developmental programming. In particular, maternal high-fat-diet (mHFD) predisposes offspring to adult metabolic disorders including obesity and excess hepatic lipid storage (steatosis).

Molecular mechanisms leading to steatosis include enhanced fatty-acid uptake, increased triglyceride synthesis and decreased beta-oxidation. However, how mHFD exposure during prenatal or early postnatal development differentially impacts these pathways remains unclear. Evidence suggests that epigenetic modifications are likely to be crucial for developmental programming by modulating transcriptional network activity. In this context, our study aims to 1) discriminate the molecular changes induced by prenatal and postnatal mHFD exposure, and; 2) test whether epigenetic mechanisms underlie the relative contributions of these distinct time-windows to steatosis in later life.

Using a crossfostering strategy, C57Bl/6 mice were exposed to mHFD (45% fat), or corresponding control diet, during prenatal and/or postnatal development. At birth, weaning and adulthood, we performed metabolic tests and characterized histological and molecular changes in the liver.

At weaning, mice exposed to postnatal but not prenatal mHFD exhibit abnormal white adipose tissue accumulation and hepatic steatosis. This is associated with hepatic transcriptional changes including an increase in expression of the transcription factor Zac1.

Zac1 controls a coordinately expressed Imprinted Gene Network (IGN) shown to regulate lipid storage in vitro. However, the function of Zac1 and the IGN in the liver is unknown. We hypothesize that this network contributes to hepatic lipid storage during development, and that Zac1 constitutes an epigenetic mediator between mHFD and steatosis in later life.

P-04
Readers, writers and erasers of nuclear PIP3

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Keywords: *phopsholipids, PTEN, IPMK*

Structural analyses of the NR5A nuclear receptors (NR5A1 and NR5A2) have previously shown that PIP2 and PIP3 bind these trancription factors in a format where the acyl chains are buried deep in the core while the phospholipid headgroups are solvent exposed. The phosphoinositide headgroups of these complexes are accessible to nuclear lipid signaling enzymes, which directly modify these transcription factor-bound PIPs, altering the transcriptional output from NR5As. Here, we took a proteomics approach to ask if the solvent exposed PIP3 headgroup is available to bind nuclear proteins. We identified two PH-domain containing proteins that interact with NR5A1/PIP3, but not other phospholipid bound forms of NR5A1, and siRNA knockdown of these two genes decreases NR5A1 transcriptional activity. PH-domains are one of the most well represented domain classes in the human genome, and are widely thought to mediate membrane binding, however only 10% of the PH-domain proteins in yeast bind membranes. Our data suggest a fraction of PH-domain containing proteins act as readers of nuclear PIP3, while nuclear lipid signaling enzymes acts as writers and erasers of nuclear PIP3. These data provide a direct mechanism that explains how nuclear PIP3 mediates transcriptional activation in mammalian cells.

P-05
Exploring the role of histone modifications in Huntington's disease

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Huntington's disease (HD) is a devastating autosomal dominant neurodegenerative disorder that primarily affects striatal neurons. HD is caused by expansion of a polymorphic CAG repeat in the first exon of the huntingtin (*htt*) gene that translates to a pure polyglutamine repeat region. While in healthy individuals the polyglutamine repeat have less than 36 glutamine residues, mutant alleles with more than 39 glutamines cause HD with complete penetrance. Mutant Htt proteins with an expanded polyglutamine repeat form intracellular aggregates and participate in aberrant protein-protein interactions that lead to a multifaceted pathogenesis. We and others have shown that mutant Htt induces epigenetic changes and transcriptional dysregulation. In this study we aim to determine whether specific histone marks have a critical role in HD pathogenesis and/or progression using a *Drosophila* model of the disease. For this purpose we introduced point mutations mimicking post-translational modifications of specific lysine residues in the *His3.3A* and *His4r* variant histone genes *in vitro* and used these constructs to generate transgenic flies. Immunofluorescence microscopy revealed that FLAG-tagged His3.3A is localized to the nucleus and is incorporated in chromosomes. Preliminary data indicate that PTM mimic transgenes of variannt histones expressed in the nervous system can exert functional effects.

Funding source: NKFI grant 112294.

P-06
Canonical elements drive super-enhancer formation

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Keywords: *super-enhancer, nuclear receptor, estrogen receptor*

Super-enhancers (SEs) are well-studied regulatory units of the genome and were defined as clusters of outstandingly active enhancers, which regulate cell-type specific genes. In this study we examined the formation of estrogen receptor driven super-enhancers in MCF-7 cell line. We found that in the lack of stimulation, the future super-enhancers were represented by one or few ChIP-seq peak(s) (called mother enhancers), and upon estradiol treatment, the appearing further peaks (named daughter enhancers) were recruited nearby to form eventually SEs.
Mother enhancers always possessed a canonical binding element, while the subsequent binding sites, if they did, had poor ones. We also investigated the simultaneous presence of MED1, BRD4, P300 transcription factors (TFs), signal of DNase I hypersensitivity and the active histone mark H3K27ac. Interestingly, all of them are specific for mother enhancers. We were curious whether the mother and daughter enhancers are specific for other TFs, so we examined further TFs, such as FoxA1, AP2γ, AR, RAR, VDR and JUNB in other cell types. All these TFs showed similar patterns as obtained for estrogen receptor, which indicates that we found a general phenomenon.
Although numerous studies suggested that in the DNA binding of transcription factors, protein-protein interactions are more important than the presence of canonical elements, based on our results it seems that certain response elements themselves are able to guide transcription factors, and interestingly, not only to the given site, but also to neighboring regions and typically each super-enhancer is dominated by a single transcription factor, which recruits the further proteins primarily to the canonical elements.

P-07
Hypermethylation of the TERT promoter predicts biochemical relapse in prostate cancer

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In order to determine the role of a cancer specific epigenetic control of telomerase, THOR (TERT Hypermethylated Oncological Region) as a diagnostic and prognostic tool in prostate cancer (PCa) we performed Genome-wide 450k methylation arrays were used to analyse THOR in common adult cancers. Methylation status of the whole TERT gene in patient specific normal and malignant prostate samples was determined by MeDIP-Seq. The prognostic role of THOR was assessed by pyrosequencing on a discovery and validation cohorts from PCa patients submitted to radical prostatectomy with long-term follow-up data.
Most cancers (n=3056) including PCa (n=300) exhibited hypermethylation of THOR. THOR was the only region within the TERT gene which is differentially methylated between normal and malignant prostate tissue ($p<0.0001$). THOR was significantly hypermethylated in PCa when compared to paired benign tissues (n=164, $p<0.0001$). THOR correlated with Gleason scores and was associated with tumor invasiveness ($p=0.0147$). Five-year biochemical progression free survival (BPFS) for PCa patients in the discovery cohort was 87% (95%CI 73-100) and 65% (95%CI 52-78) for THOR non-hypermethylated and hypermethylated cancers respectively ($p=0.01$). Similar differences in BPFS were noted in the validation cohort ($p=0.03$). Importantly, THOR was able to predict outcome in low and intermediate-risk PCa (Gleason 6 and 7(3+4)) PCa ($p=0.007$). For this group, THOR was an independent risk factor for BPFS with a hazard-ratio of 3.685 ($p=0.0247$). Finally, THOR hypermethylation more than doubled the risk for recurrence across all PSA levels (OR 2.5, $p=0.02$).

P-08
The interplay between transcription factor GRHL2 and epigenetics in the regulation of EMT in ovarian cancer

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Grainyhead-like 2 (GRHL2) is a transcription factor that regulates a repertoire of epithelial genes during Epithelial-mesenchymal transition (EMT). Here, we describe the transcriptional and epigenetic landscapes regulated by GRHL2. Using Illumina HumanMethylation450K array, we identified differentially methylated CpG sites of ovarian cancer (OC) cells in correlation with EMT. These differentially methylated sites were found significantly enriched in the DNA binding motif of GRHL2, suggesting that its target genes are regulated by DNA methylation. The DNA methylation of GRHL2 target genes were then analysed in a GRHL2 knockdown model. Overall, there was a negative correlation between the changes in CpG methylation and the alterations of gene expression after GRHL2-knockdown. An OC cell line panel and the isogenic knockdown model were also used to explore the genome-wide histone modifications involving five marks (H3K4me1, H3K4me3, H3K27Ac, H3K27me3 and H3K9me3) by ChIP-sequencing. The loss of GRHL2 resulted in a general decrease in the active marks (H3K4me1, H3K4me3, H3K27Ac) and a gain in the repressive mark H3K27me3 at GRHL2 binding sites. These changes reflected the transcriptional states of GRHL2 target genes, which showed transitions from active to low activity (47%), active to PRC2-repressed (9.75%), active to poised (2.1%), and active to heterochromatin (1.52%). Finally, we demonstrated that the EZH2 inhibitor GSK126, which suppressed the H3K27me3 level, could enhance the GRHL2 activation of its target gene E-cadherin. Therefore, the function of GRHL2, which depends on the accessibility to its target genes, could be affected by the existing histone modifications at the promoters or the GRHL2-binding sites.

P-09
Role of the chromatin-remodelling complex NURF and NuRD in epigenetic regulation of melanoma gene expression

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Keywords *: Chromatin remodelling, NuRD, melanoma*

The transition between the proliferative and invasive states of melanoma cells is epigenetically and transcriptionally controlled by changes in the levels of functional MIcrophthalmia-associated Transcription Factor (MITF). Our previous work showed that in human melanoma cells MITF interacts physically and functionally with several chromatin remodelling complexes, PBAF, Nucleosome Remodelling factor (NURF) complex and the Nucleosome Remodelling and histone Deacetylation complex (NuRD).
NURF comprises BPTF (Bromodomain, PHD-finger Transcription Factor) which is the defining and only unique subunit of NURF. We showed that NURF acts downstream of MITF in proliferative melanoma cells co-regulating the expression of genes required for proliferation and oxidative metabolism. RNA-seq shows that BPTF silencing in invasive melanoma cells, that do not express MITF, leads also to reduced oxidative metabolism and to an arrest of the cell cycle in G2M, but that BPTF regulates distinct gene expression programs in invasive and proliferative cells.
NuRD is a multisubunit chromatin remodelling complex containing either the Chromodomain-Helicase-DNA-binding protein 3 (CHD3) or CHD4 with ATP-dependent chromatin remodelling activity. We found that NuRD complexes containing either CHD3 or CHD4 are expressed in proliferative melanoma cells, but expression of both of these factors is strongly downregulated in invasive melanoma cells. We showed that CHD4 has a critical and specific function that cannot be replaced by CHD3 and that the level of CHD4 is a major determinant of melanoma cell proliferation. RNA-seq following confirms the distinct role of NuRD complexes containing either CHD3 or CHD4 in melanoma cells.
Together our results demonstrate the critical, but highly specific and distinct functions of two different chromatin-remodelling complexes in epigenetic regulation of gene expression in melanocytes and in melanoma cells in vitro.

P-10
Genome-wide studies during adipocyte differentiation from mouse pluripotent stem cells

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Obesity is worldwide public health issue due to its prevalence and its morbimortality. Thus, Adipose tissue has been recognized as an endocrine organ since both the excess and the lack of this tissue are associated with altered metabolism and lead to major diseases. Consequently, several models have been used for studying fat cell differentiation. For this study, we set up an improved adipocyte differentiation protocol for mouse pluripotent stem cells: embryonic stem cells (ESCs) and induce pluripotent stem cells (iPS); that enable us to get insight in the early and late stages of adipogenesis due to its monolayer output and high efficiency. We performed genome-wide analysis during adipocyte differentiation (RNA-seq, ChIP-seq and ATAC-seq): samples for ChIP-seq were taken at day 0, 15 and 27 in ESCs (For RXR and histone modification markers), at day 0, 15, 18, 21 and 27 for PPAR antibody. RNA seq samples were taken at different time points along the differentiation (day 0, 3, 4, 7, 8, 15, 16, 18, 21 and 27). For PPARg KO and control iPS cells ChIP-seq and RNA-seq samples were taken at day 15, 16, 19 and 27 meanwhile ATAC-seq samples were taken at day 15 and day 19 of the differentiation; Hierarchical clustering of the RNA-seq data combined with H3K27ac, an active histone mark (also recognize super-enhancers), ChIP-seq results in a dynamic change in gene expression and enhancer map during adipocyte differentiation identifying clusters for general gene expression analysis and transcription factor expression. Dissection of this data will allow us to get insight in adipocyte differentiation and potential new targets for therapy in obesity.

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P-11
Epigenetic in the development: Detection of histones deacetylases in *Melipona scutellaris* (Apidae, Meliponini)

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Keywords: *histone deacetylases, development, Melipona*

Epigenetics studies in the development and females' polyphenisms in social insects reached a new dimension. Morphological and molecular markers added evidences to the hypothesis of a mechanism involving interaction between genes and environment (food) for the differentiation of castes in bees of the genus *Melipona*. We analyzed, by RT-qPCR, the stages of development and phenotypic divergences in *Melipona scutellaris* females. For the first time in this species, expression of genes encoding enzymes histone deacetylases HDAC1 and HDAC4 were identified and quantified. Results showed expression of transcripts in stages of larvae, pupae and newly emerged adult (queen and workers), with fluctuations in stages of development and castes. In queens, occurred peak of expression of *hdac4* in white pupae (Pw). In workers, this peak occurred later in brown pupae (Pb). In queens, expression of *hdac1* and *hdac4* showed decline throughout development. Expression of *hdac1* and *hdac4* in stages Pw and Pp (pink pupae) was greater in queen than in workers ($P<0.05$). Expression of *hdac4* in Pb was higher in workers than in queens ($P<0.05$). The expression pattern of *hdac1* and *hdac4*, throughout workers' development, is similar. The same is observed for queens. This suggests that these genes act synergistically in the establishment of epigenetic patterns necessary for production of the phenotypes of females. Fluctuations of *hdac1* and *hdac4* expression may promote a timely and refined control of caste-specific gene modulation throughout development. Environmental factors might be translated into epigenetic signals, which added to genetic and metabolic pathways, could control the females' polyphenism.

P-12
Genome-wide mapping of COUP-TFII and ERα co-occupancy in breast cancer cells

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Keywords: *nuclear receptors, breast cancer*

Chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) is an orphan nuclear receptor having no identified ligands. Its expression level has been shown to be associated with the prognosis and outcome of breast cancer, and correlates with lymph node and estrogen receptor (ERα) status. Gene regulation processes controlled by COUP-TFII are unknown, therefore our aims were to investigate the COUP-TFII binding events alone and together with ERα by genomic and bioinformatic approches in breast cancer cells.

COUP-TFII and ERα ChIP-seq data derived from MCF-7 cell line were collected from NCBI Sequence Read Archive and were reanalysed by our bioinformatic pipeline. Then, we separated the common and individual COUP-TFII and ERα binding sites. These sites were classified based on the binding of the pioneer factors of ERα such as FoxA1 and AP2γ, and we found that unique ERα binding sites contain only ERE motif in the absence of pioneer factor binding. Common COUP-TFII/ERα binding sites showed the presence of FoxA1 and AP2γ as well as DNase I hypersensitivity. In contrast, FoxA1 or AP2γ together with COUP-TFII did not show DNA accessibility. Some of the unique COUP-TFII binding sites showed opened chromatin without ERα binding. Finally, pathway analysis demonstrated that some of the resulted signaling pathways are regulated by COUP-TFII in MCF-7.

Taken together, these data suggest that COUP-TFII might have a key role in ERα enhancer activation, therefore, in our future studies, we would like to investigate the role of COUP-TFII in relation to the progression and metastasis of breast cancer by using genome-wide approaches.

The project is funded by Internal Research University Grant entitled „Dissecting the genetic and epigenetic components of gene expression regulation in the context of the 1000 Genomes Project".

P-13
In silico analysis of potential cernas in renal cell carcinoma

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Keywords: *Renal cell carcinoma, miRNA, ceRNA, in silico analysis*

Renal cell carcinoma (RCCa) constitutes 2% of all adult malignancies and is the most lethal among urologic cancers. Nowadays, new information on the biology of RCCa is uncovered. MicroRNAs (miRNAs) having epigenetic role are expressed differentially in different pathologies. Competing endogenous RNAs (ceRNAs) are RNA transcripts regulating other RNA transcripts by competing for shared microRNAs. Deletions in some loci on the short arm of chromosome 3 are observed in RCCa. Consequently, miRNAs targeting RNA transcripts of these deleted genes are expected to search for different targets. The expression levels of ceRNAs targeted by these miRNAs would decrease.

Some genes (VHL, ITPR1, PPAR, GPD1L, ABHD5, IMPDH2, CHDH, DRR1, PDHB and FHIT) on the short arm of chromosome 3 was observed to be deleted in renal cell carcinoma cases according to previous studies. MiRNAs targeting at least 5 of these genes were determined using miRWalk database. The genes targeted by all of these miRNAs and showing the most potential ceRNA activity were detected by ComiR database (Figure 1).

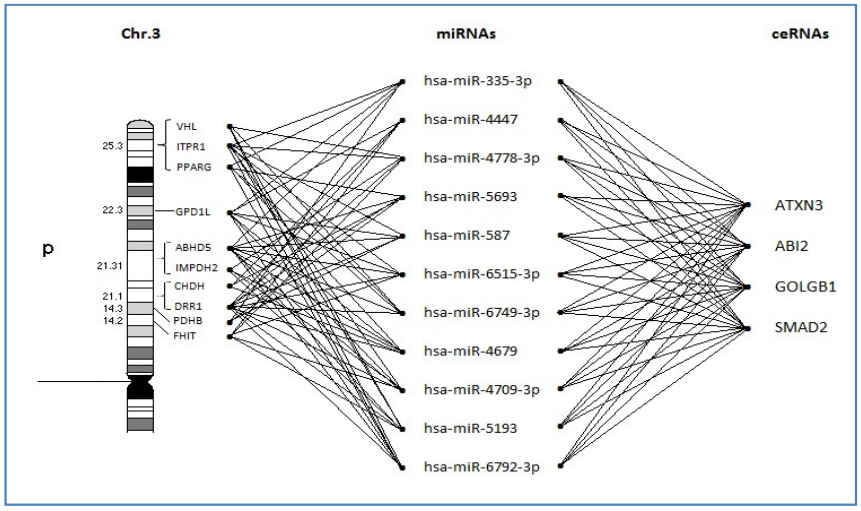

Figure 1: *In silico ceRNA analysis in renal cell carcinoma*

Since these potential ceRNAs' (ATXN3,ABI2, GOLGB1 ve SMAD2) expression levels are expected to decrease, we think that these genes may have tumor suppressor function. Moreover, these genes have previously not associated with kidney cancer pathology so they may be new tumor biomarkers for RCCa. In the future, it is intended to demonstrate the relationship between these genes and clinicopathological parameters of RCCa patients. By this way, these potential prognostic and predictive markers may enable RCCa to detect early from serum or urine.

P-14
Analysis of 3'UTR shortening of *ABCB1* gene in Imatinib-resistant CML cells in terms of potential ceRNAs by a computational study

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Keywords: *CML, Imatinib-resistance, miRNA, ceRNA*

Imatinib, a potent BCR-ABL tyrosine kinase inhibitor, has been widely used for chronic myeloid leukemia (CML). Imatinib is a substrate for *ABCB1* gene product. Accordingly, *ABCB1* contribute to resistance by extruding Imatinib from hematopoietic cells. According to a study, Imatinib-resistant CML cells expressed predominantly shorter 3'-UTRs (248 bp) instead of full length 3'UTR (384 bp) of *ABCB1* gene. MiRNAs targeting lost part of *ABCB1* search for new targets, which are potential competing endogenous RNAs (ceRNAs) regulating other RNA transcripts by competing for shared microRNAs, and regulate their expressions in relation with Imatinib resistance. This study purposes to find miRNAs targeting shortening part of 3'UTR of *ABCB1* gene in imatinib resistant CML cells and to search for new genes targeted by these freed miRNAs.

Firstly, the nucleotide sequence of *ABCB1* gene's shortened 3'UTR in Imatinib-resistant CML cells was specified by using NCBI database. By miRWalk database, miRNAs targeting this lost portion were determined. The genes targeted by all these miRNAs were found by ComiR database.

MiRNAs targeting shortening part of 3'UTR of *ABCB1* gene were found as hsa-miR-4708-3p, hsa-miR-451b, hsa-miR-3682-5p, hsa-miR-138-1-3p, hsa-miR-129-5p, hsa-miR-130a-5p, hsa-miR-3117-5p, hsa-miR-543, hsa-miR-6733-5p, hsa-miR-940 and hsa-miR-4672. Moreover, potential ceRNAs specific to Imatinib resistance in CML were detected as *DGKH, SLC35E3, ZNF26, NTRK3, ZC3H14, PEAK1, TNRC6B, FAM204A* and *GREM1*. It is thought that the expression levels of these genes showing potential ceRNA activity decrease with the effect of shortened 3'UTR of *ABCB1* gene in in Imatinib resistant CML cells indirectly. This fact supports these found genes to have potential tumor suppressor role in molecular mechanism of CML.

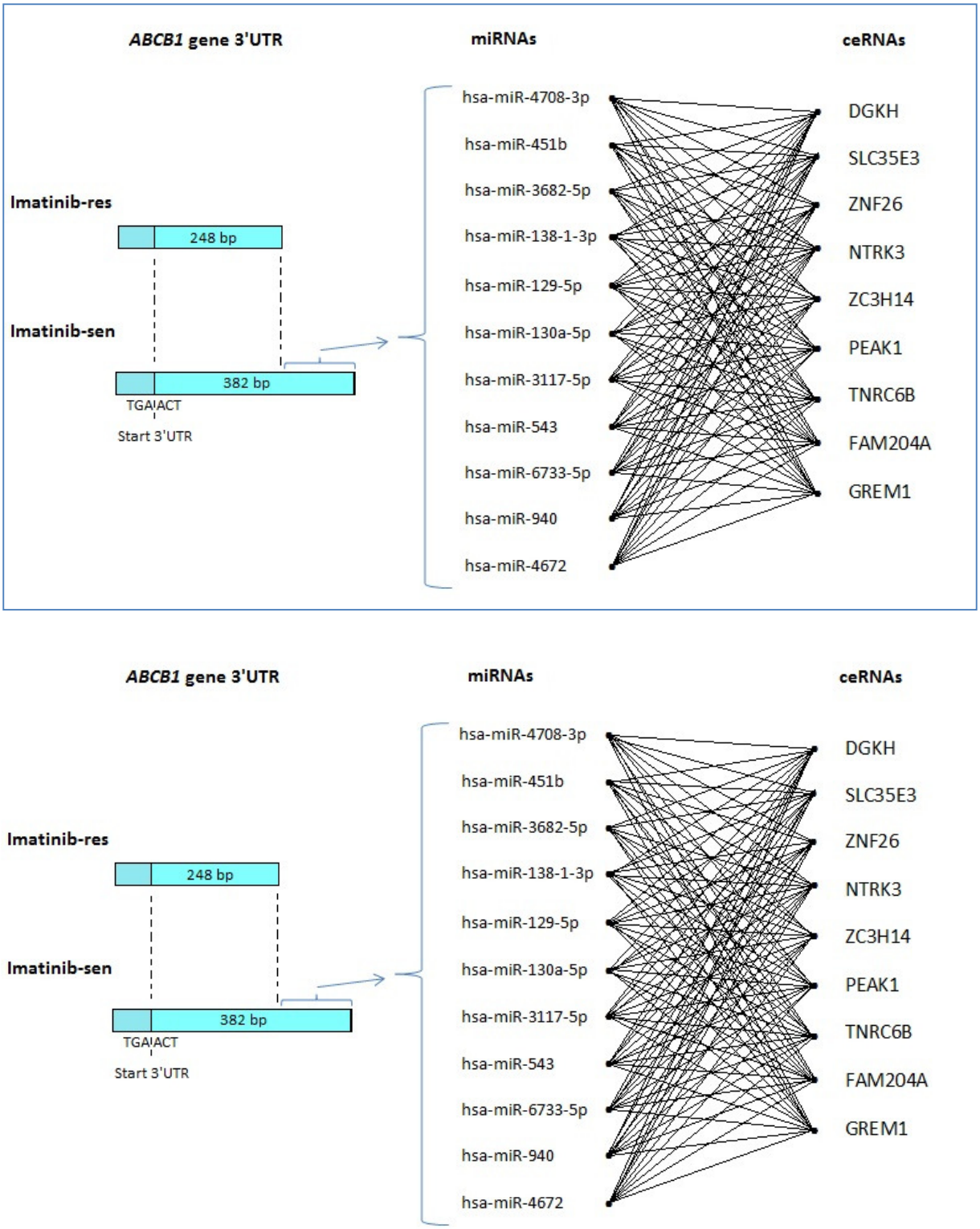

Figure 1: *Computational ceRNA analysis of ABCB1 gene in imatinib resistant CML*

P-15
Next generation epigenetics – Innovative research tools for the accurate analysis of DNA modifications

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Keywords: *WGBS, oxidative bisulfite sequencing, 5-methylcytosine, 5-hydroxymethylcytosine*

DNA methylation (5mC) is central to early development, stem cell dynamics, ageing and has been implicated in the control of a wide range of diseases including cancer and neurodegenerative disorders. Although several studies have shown that 5-hydroxymethylcytosine (5hmC), a TET-oxidized derivative of 5mC, is functionally distinct, traditional epigenetic research tools cannot accurately distinguish between these modifications, confounding analysis.

Cambridge Epigenetix (CEGX) has developed TrueMethyl® oxidative bisulfite (oxBS) technology to quantitatively measure 5mC and 5hmC at single base resolution. Compatible with conventional array and enrichment techniques such as the 450K and EPIC array, amplicon sequencing and pyrosequencing, TrueMethyl® can also be coupled with NGS for targeted sequencing or whole genome analysis.
To take Whole Genome Bisulfite Sequencing (WGBS) from niche to mainstream, CEGX has developed TrueMethyl® Whole Genome (TMWG). This innovative, all-in-one workflow brings together TrueMethyl® oxBS technology with a single-stranded, template independent, post-conversion library construction technology and a ready-to-use bioinformatics pipeline for data analysis, revolutionizing the quality and simplicity of conducting WGBS experiments.

TrueMethyl® Whole Genome libraries overcome existing limitations of traditional pre-BS and random-priming PBAT library construction methods, and offer high yield, high diversity and low duplicate libraries at low DNA input burden, for outstanding data quality and cost-efficient WGBS sequencing.

P-16
Histone type, modification and cell cycle phase specific characterization of nucleosome stability in *situ*

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Keywords: *histone PTMs, γH2A.X, nucleosome stability, laser scanning cytometry*

Measurement of nucleosome stability *in situ,* in a histone type, posttranslational modification (PTM) and cell-cycle specific manner is made possible by the laser scanning cytometric (LSC) method presented. The assay involves elution of histones using intercalators or salt, to assess DNA conformation dependent and intrinsic stability, respectively, and measuring of the fraction of histones remaining chromatin-bound in the individual nuclei using histone type- or PTM-specific monoclonal antibodies. The approach has been validated using different intercalators, by the assessment of the intercalator induced chromatin loop relaxation required for nucleosomal destabilization and via demonstrating the preferential eviction of the promoter proximal H3K4me3 nucleosomes in parallel chipseq and LSC experiments. The advantages of the method are demonstrated by comparing the binding strength of the histone variant H2A.X with that of its phosphorylated form γH2A.X generated in the course of DNA damage response, revealing a major role of the DNA relaxing breaks themselves in the relative destabilization of the latter.

P-17
Measurement of interstrand DNA crosslinks generated by anticancer agents through a modified alkaline comet assay

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Keywords: *Cisplatinum, interstrand crosslink (ICL), laser scanning cytometry (LSC)*

Agents that are able to crosslink the two strands of the DNA, e.g. Cisplatin, are widely used in cancer chemotherapy. Correlations between the expression profiles of enzymes involved in the regulation of the topological state of DNA and the relative sensitivity to DNA crosslinking agents across defined cell line panels, as well as genomic studies on the relationship between psoralen crosslinking and DNA superhelicity raise the possibility that modulation of crosslinking efficiency via changing superhelicity using intecalating agents could be exploited for therapeutic purposes. In this context, and also to better understand the role of interstrand crosslinking (ICL) relative to other forms of alkylation in the toxicity of Cisplatin and related agents, we have set out to develop a laser scanning cytometric method based on the alkaline nuclear comet assay. Our technique utilizes a frequent-cutter nickase enzyme to achieve optimal size single-strand (ss) fragments after denaturation, and ss-specific S1 nuclease to digest away all non-reannealed, i.e. non-crosslinked fragments. Ongoing experiments address the question if the load of ICLs achieved can be modulated by the simultaneous administration of intercalator agents, what was suggested by the apparent antagonism of their toxic effects observed in certain dose windows, as measured in the Alamar Blue assay.

P-18
Histone dynamics in response to DNA damage in heterochromatin domains

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Keywords: *histone dynamics, DNA damage, heterochromatin*

DNA damage challenges not only genome stability but also the integrity of its organization with histone proteins into chromatin, which governs gene expression and cell identity. How chromatin organization is altered after DNA damage while preserving its functions is thus a central issue. Previous studies by our group and others characterized new histone deposition pathways in UVC-damaged chromatin and their critical role in transcription recovery after repair. However, whether such histone dynamics operate similarly in poorly transcribed heterochromatin, and with what consequences on heterochromatin structure and function, is unknown. To address these questions, we have established a unique cellular model in mouse fibroblasts for studying histone dynamics in UVC-damaged heterochromatin domains, focusing on the histone variant H3.3. This variant is of particular interest because it is the most conserved histone H3 variant, it is associated with transcription regulation and it is deposited *de novo* into UVC-damaged chromatin regions in human cells. Using our cellular model, we show that UVC damage repair takes place efficiently in mouse pericentric heterochromatin domains and we demonstrate that H3.3 histones accumulate *de novo* in UVC-damaged heterochromatin. Our findings also provide insights into the underlying molecular mechanisms, with the characterization of H3.3-specific chaperone accumulation in damaged heterochromatin. We are currently exploring the consequences of UVC damage repair and associated histone dynamics on heterochromatin organization and transcriptional activity. Altogether, this study sheds new light on fundamental mechanisms involved in the maintenance of higher-order chromatin structures following DNA damage.

P-19
Inflammation differentially affects histone methylation state of promoters of inflammatory genes and structure proteins

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Pro-inflammatory conditions in periodontal disease result in the activation of NF-κB, which in turn regulates the transcription of pro-inflammatory cytokines and structure proteins, through changes in the histone methylation state at gene promoters. Changes in histone methylation state are accomplished by histone methyltransferases and histone demethylases. We have focused on the effect of inflammatory conditions on SetD1, a lysine-specific transferase that trimethylates the histone mark on lysine 4 as a major epigenetic mechanism involved in active gene transcription.

Objective: To determine the epigenetic mechanism by which inflammatory conditions affect gene expression.

Methods: Human periodontal ligament (PDL) cells were stimulated with LPS. Gene expression analysis was conducted using RT real-time PCR. Histone methylation state was examined using chromatin immunoprecipitation analysis and relative enrichment was determined.

Results: LPS stimulation increased inflammatory gene expression and occupancy of H3K4me3 while decreased bone marker gene expression and reduced occupancy of H3K4me. SetD1 knockdown decreased H3K4me3 occupancy on inflammatory gene promoters and increased occupancy on bone marker promoters under inflammatory conditions. NF-kB enrichment was increased on inflammatory gene promoters and reduced on bone markers and upon NF-kB knockdown, occupancy of both SetD1 and H3K4me3 was increased on bone marker promoters upon stimulation with LPS.

Conclusion: Inhibition of SetD1 altered H3K4me3 marks while inhibition of NF-kB affected the enrichment of both SetD1 and H3K4me3 on both inflammatory genes and bone marker promoters in LPS challenged PDL cells. Studies using NF-kB inhibitors and histone methylation modifiers may provide the basis for future Epi-therapeutics to improve the periodontal health.

P-20
Expression levels of Warburg-effect related microRNAs in hematological malignancies of the adults

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The role of epigenetics in cancer development has got into the center of interest in recent years, and have been proved to be involved in the multi-step process of carcinogenesis, during which both genetic and epigenetic alterations are widely accumulated. A metabolic shift in glucose metabolism from oxidative phosphorylation towards aerobic glycolysis, even in the presence of abundant oxygen, is also a unique characteristic of cancer cells, referred to as the Warburg-effect. MicroRNAs are small noncoding RNA molecules, regulating gene expression at posttranscriptional level. Many of them are involved in the regulation of hemopoesis, and several possess oncogenic or tumorsuppressor property. Multiple relationships have been described between the microRNAs and other epigenetic regulatory mechanisms, furthermore, the role of microRNAs in the Warburg-effect is also being increasingly emphasized.

In our study we investigated the expression levels of microRNAs via RT-qPCR in bone marrow specimens of adult patients suffering from hematological malignancies, especially acute myeloid leukemia.

The levels of the examined Warburg-effect related microRNAs positively correlated with each other and with an oncogenic microRNA as well, while negatively with the level of a well-known tumorsuppressor microRNA. Our results suggest that the altered expression levels of Warburg-effect related microRNAs may have pathogenetic role in the development of leukemia.

It can be suspected, that besides the most widely examined characteristics of the Warburg-effect (such as elevated glycolytic rate and lactate production), unique features of the disruption of metabolic regulation in distinct leukemia types may also exist, controlled by epigenetic factors in a considerable manner.

P-21
Molecular mechanisms of elicitor-induced epigenetic changes in Apple and *Arabidopsis thaliana*

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Keywords: *epigenetic memory, stress perception, Arabidopsis, apple*

Epigenetics is an important and growing field in plant science. However, not much is known about epigenetic mechanisms in crop plants like apple. With this work we want to uncover possible epigenetic mechanisms involved in the long term epigenetic memory of plant-pathogen interactions. Defence promoting compounds are widely used in commercial apple breeding since many years. The aim of this study is to uncover genes with the potential to memorize treatments with defence promoting compounds and to uncover the underlying epigenetic mechanisms. We work at two levels: (i) At the basic level where we aim to understand how treatments are memorized in Arabidopsis (ii) At the applied level by testing our findings in apple. Preliminary results show that different combinations of treatments with elicitors can be memorized over long periods in apple and Arabidopsis plants. Transcriptome analysis of elicitor-treated plants show that genes are differently expressed even weeks after the last elicitor treatment. Moreover we could show, that the expression profile changes depending on the type of elicitor being applied. Here we show that plants can memorize stresses over long periods of time and show how subsequent stresses perceived by the plant can affect this memory.

P-22
The cyclin-dependent kinase CDK6 as key regulator of the cancer epigenome

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The G1 kinase CDK6 is considered a redundant homologue of CDK4 for cell cycle progression. Both proteins become activated when binding to D-type cyclins and phosphorylate the retinoblastoma (Rb) protein, thus releasing the Rb-mediated inhibition of E2F transcription factors, and promoting the G1 to S progression. Recently, functions of CDK6 not shared with CDK4 have been identified and raised great interest as CDK4/6 kinase inhibitors entered clinical trials and have been approved for the treatment of breast cancer. CDK6 is frequently upregulated in hematopoietic tumors, and is essential in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). We discovered a key role for CDK6 in transcription, both in lymphoid leukemia and in AML, as well as in hematopoietic stem cells. CDK6 is found associated with promoters and influencing the expression levels of key genes involved in leukemia development and stem cell activation. Transcriptional targets of CDK6 include *Vegf-A*, *p16INK4A, and Egr1 that are regulated* in a kinase independent manner, and *Flt3* and *Pim1* for whose activation the kinase activity of CDK6 is required. Members of the STAT, AP-1, and NF-kB families have been shown to interact with CDK6 at promoters. We have conducted genome-wide CDK6 Chromatin Immunoprecipitation experiments, alongside RNA-Seq, ATAC-Seq (Assay for Transposase-Accessible Chromatin), and RRBS (Reduced Representation Bisulfite Sequencing) to understand the transcriptional functions of CDK6, the extent to which they depend on the kinase activity, and the changes in chromatin architecture that accompany CDK6 binding at promoters. An overview of our current understanding will be the topic of my talk/poster.

P-23
Characterization and modeling of lineage-specific enhancer states and transitions in macrophages

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The emerging concept of tissue specific gene expression posits that cell type specific transcription factors termed lineage-determining transcription factors largely determine the cell type-specific enhancer repertoire. However, it has not been systematically evaluated how the binding of lineage-determining and other collaborative transcription factors to chromatin is related to nucleosome-free regions and enhancer activity. We sought to address this issue by determining the interrelationship among the chromatin states, transcription factor binding and enhancer activity and modeling the identified distinct states and transitions between them using murine bone marrow-derived macrophages as the model system. By using highly integrated genome-wide technologies (ChIP-seq, ATAC-seq and GRO-seq) we show that only one third of the cistrome of the lineage-determining transcription factor Pu.1 is associated with highly accessible chromatin. We found that several collaborating transcription factors (Irf8, Junb, Cebpa and Runx1) contribute to enhancer openness and activation organized in a hierarchical manner. In addition, the chromatin openness can be predicted from TFs' occupancies using a Support Vector Machine classifier. Finally, we have identified four distinct states of the regulated enhancers including a novel class (labeled enhancers) characterized by Pu.1 binding to low accessible regions in the unstimulated state. These analyses coupled to modeling allowed the construction of a Nondeterministic Finite Automaton to formally describe these states and the transitions between them.

P-24
Adipocyte gene expression and DNA methylation patterns differ significantly between lean and obese pigs

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Adipose tissue plays a central role in metabolic diseases, and, in particular, abdominal obesity is considered unfavorable, due to its tight relationship with development of diabetes and cardiovascular diseases. As both genetic and environmental factors contribute to the development of obesity and its co-morbidities, we aimed to explore how the DNA methylome contribute to an altered transcriptome profile of adipocytes in obesity.
The gene expression profile of mature adipocytes isolated from the retroperitoneal fat pad was compared between a group of lean and a group of obese pigs. Simultaneously the epigenetic signature was profiled by analyzing the global methylation of the DNA isolated from the same adipocytes. The pigs used in this study were housed in the same farm under the same environmental conditions with free access to food and water. This very stringent and controlled environment suggests that the differences in epigenetic profile detected in the study primarily reflect variations due to obesity.
We found a total of 1155 coding genes and 66 non-coding RNAs that were differentially expressed between the lean and obese animals. The methylation analysis revealed more than 6000 differentially methylated regions. Combining the transcriptome and the methylation analysis disclosed almost 100 genes, which have expression profiles that seems directly influenced by methylation. Gene ontology analyses of these genes show an overrepresentation of genes involved in lipid and fatty acid metabolism, indicating that the altered methylation profile of the adipocytes is implicated in development of obesity in pigs. We are currently validating the most relevant results.

P-25
Erythroid activator NF-E2, TAL1 and KLF1 play roles in forming the LCR HSs in the human adult β-globin locus

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The β-like globin genes are developmental stage specifically transcribed in erythroid cells. The transcription of the β-like globin genes requires erythroid specific activators such as GATA-1, NF-E2, TAL1 and KLF1. However, the roles of these activators have not fully elucidated in transcription of the human adult β-globin gene. Here we employed hybrid MEL cells (MEL/ch11) where a human chromosome containing the β-globin locus is present and the adult β-globin gene is highly transcribed by induction. The roles of erythroid specific activators were analyzed by inhibiting the expression of NF-E2, TAL1 or KLF1 in MEL/ch11 cells. The loss of each activator decreased the transcription of human β-globin gene, locus wide histone hyperacetylation and the binding of other erythroid specific activators including GATA-1, even though not affecting the expression of other activators. Notably, sensitivity to DNase I was reduced in the locus control region (LCR) hypersensitive sites (HSs) with the depletion of activators. These results indicate that NF-E2, TAL1 and KLF1, all activators play a primary role in HSs formation in the LCR. It might contribute to the transcription of human adult β-globin gene by allowing the access of activators and cofactors. The roles of activators in the adult β-globin locus appear to be different from the roles in the early fetal locus.

P-26
Rybp plays essential role in neural differentiation of mouse embryonic stem cells

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Polycomb Group (PcG) proteins are epigenetic regulators. Ring1 and Yy1 Binding Protein (Rybp) is a recently discovered member of the PcG protein family. We have previously reported that lack of Rybp causes early embryonic lethality in mice and that a portion of heterozygotes exhibit neurological disorders (e.g. lack of cerebellum, exencephaly, disorganized neurocortex), which showed the essential role of Rybp in the development of the central nervous system. In current work, we used wild type *(rybp$^{+/+}$)* and *rybp null* mutant *(rybp$^{-/-}$)* mouse embryonic stem (ES) cells and differentiated them towards neural lineages *in vitro* with the purpose to uncover underlying molecular events that are responsible for the *in vivo* phenotypic changes. Our results revealed that *rybp$^{-/-}$* ES cells are able to differentiate to neural stem cells and neural precursors, but they cannot form matured neurons and glias. Furthermore, lack of *rybp* coincided with altered gene expression of key neural markers, retinoic acid pathway members and transcription factors including Pax6 and Zac1 pinpointing a possible transcriptional regulation circuit among Rybp and these genes. Together, these findings support critical roles for Rybp in neural lineage commitment and that epigenetic machinery may play important role in this process. To clarify the genetic underpinnings of differentiation has important implications not only for understanding neurological diseases but also for the possibility of neural repair through genetic reprogramming of non-neural cells to a neurogenic fate.

P-27
Direct decarboxylation of 5-carboxylcytosine by DNA C5-methyltransferases

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Keywords: *5-carboxylcytosine, DNA cytosine-5 methyltransferases, decarboxylation*

S-Adenosylmethionine-dependent DNA methyltransferases (MTases) perform direct methylation of cytosine to yield 5-methylcytosine (5mC), which serves as part of the epigenetic regulation mechanism in vertebrates. Active demethylation of 5mC by TET oxygenases produces 5-formylcytosine (fC) and 5-carboxylcytosine (caC), which were shown to be enzymatically excised and then replaced with an unmodified nucleotide. Here we find that both bacterial and mammalian C5-MTases can catalyze the direct decarboxylation of caC yielding unmodified cytosine in DNA in vitro but are inert toward fC. The observed atypical enzymatic C–C bond cleavage reaction provides a plausible precedent for a direct reversal of caC to the unmodified state in DNA and offers a unique approach for sequence-specific analysis of genomic caC.

P-28

Free hemoglobin change gene expression involving in cell-cell signaling through different DNA methylation in THP-1-derived macrophages

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Background: Free hemoglobin formed from hemolysis of red blood cell, was known a predicator of many disease such as cancer, sclerosis and delirium. That also related with cytokine production in leukocytes. In this study, we elucidated change of gene expression through DNA methylation difference by free hemoglobin in THP-1 derived macrophage.

Methods: PMA induced THP-1 derived macrophage were treated 50ug/ml of free hemoglobin (purchased from Sigma). DNA methylation were analyzed through whole genome bisulfate sequencing (WGBS) using TruSeq® DNA Methylation Kit (illumina) and 을 Illumina HiSeq. platform (illumina). Sequence quality were checked by FastQC and Trimmomatic and data analyzed by BSMAP. Difference of DNA methylation were determined in delta-mean>[0.75] and p <0.05). Expression of genes were determined by reverse transcriptional PCR (RT-QCR) and q-PCR.

Results: DNA methylation of 29,706 CpG sites in total genome and 553 CpG sites in promoter region were changed by hemoglobin. Among 553 CpG sites in promoters, 267 CpG sites were hypomethylated and 286 sites were hypermethylated. GO annotation showed genes involving in neurological system process, ion transport, cell-cell signaling and skeletal system development were significantly affected by hemoglobin. Genes involved in p53 cardiovascular system, cell adhesion molecules were significantly affected. Among of them, we focused 23 genes, which were included in cell-cell signaling, and checked gene expression of five genes. IL-7 and PCDN1 showed significant change of gene expression.

Discussion: Our results showed that increase of free hemoglobin affects gene expression through change of DNA methylation and these might suggest that free hemoglobin level in vessel should be affect cell-cell signaling and free hemoglobin might predictor of some diseases.

P-29
The non-coding gene *Ftx* promotes *Xist* upregulation at the onset of X-chromosome inactivation

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***Keywords**: WGBS, DNA methylation, haemoglobin, IL7, PCDN1*

The transcriptional inactivation of one X-chromosome is strictly required for successful female development, and multiple factors have co-emerged and co-evolved to ensure its regulation. Many of these factors, including the X-inactivation master regulator *Xist*, are long non-coding RNAs (lncRNAs) produced by the X-inactivation center (*Xic*), a region of the X-chromosome that is particularly rich in *cis*-acting non-coding genes. Our laboratory has identified *Ftx,* a *Xic*-linked non-coding gene, as a putative regulator of *Xist*. *Ftx* is located 5' to *Xist* and produces several polyadenylated isoforms. During the differentiation of female mouse embryonic stem cells (mESCs), *Ftx* expression increases concomitantly with *Xist* lncRNA accumulation on the inactive X and *Ftx* escapes from X-linked silencing.
Here, we reveal that X-inactivation is strongly perturbed in female mESCs with impaired *Ftx* expression. Differentiation of *Ftx* mutant mESCs is associated with high mortality rates as well as with defective *Xist* upregulation and accumulation, preferentially from the *Ftx*-deleted chromosome. These results indicate that *Ftx* is required, in *cis,* for proper X-inactivation establishment. Ongoing analyses are aimed at investigating the mechanisms of the *Ftx*-mediated *Xist* regulation.

P-30

The epigenetic regulator complex polycomb/H3K27me3 plays a role in the epigenetic memory induced by morphine upon EpiLC differentiation

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Keywords: *Epigenetic modifications, Cellular epigenetic memory, H3K27me3*

A presence of external stimuli, in contact with the blastocyst before or during epiblast cells differentiation, might affect the developmental process through the cellular memory. Due to the fact that morphine is the most active component of opium and its therapeutic value for pain relief, our aim is to elucidate the effect of morphine on differentiation and to identify the existence of a cellular memory produced by morphine upon EpiLCs differentiation.

The transcriptome analysis by the microarray technique showed 87 significantly modified genes in mESC, related to the function of the cell membrane. On the other hand, there was a 1462 genes deregulation in EpiLCs, showing a down-regulation on genes associated to male reproductive processes, while those related to neural development functions were up-regulated. The massive deregulation of gene expression induced by morphine in mEpiLCs, when the stimulus is absent, suggests the existence of cellular memory generated by the morphine exposure.

The transcriptome and qRT-PCR analysis in mESC showed a down regulation on EZh2 gene, which belongs to the epigenetic regulator Polycomb Complex, associated with inactive genes through the H3K27me3. Therefore, we verified the down regulation of H3K27me3 in morphine treated mESC, by immunoblotting, and also analyzed the distribution of H3K27me3 in paternally and maternally imprinted gene promoters after morphine exposure in mESC and mEpiLCs by Chip-PCR assay,suggesting that the complex Polycomb/H3K27me3 can be involved in the epigenetic memory induced by morphine during EpiLCs differentiation. This together with the altered reproductive proceses, also provides possible evidences for transgenerational epigenetic inheritance.

P-31
IL-4 reshapes the RXR cistrome in mouse bone marrow-derived macrophages

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Keywords: *RXR, PPARg, IL-4, STAT6, macrophage*

Bone marrow-derived macrophages (BMDMs) differentiated in the presence of macrophage colony-stimulating factor (M-CSF) have a quasi-static retinoid X receptor (RXR) cistrome collaborating mainly with peroxisome proliferator-activated receptor gamma (PPARγ) and liver X receptor (LXR) heterodimerizing partners. Alternative activation with interleukin-4 (IL-4), however, initiates a transcription factor cascade by the activation of signal transducer and activator of transcription 6 (STAT6) including PPARγ, which extend the RXR cistrome. Although we found more than 5000 IL-4/STAT6-dependent RXR-bound sites, only a small fraction of *de novo* sites was bound by STAT6 upon IL-4 treatment. Importantly, these sites became bound and activated much faster than those without STAT6 binding. The later occupied population of regulatory sites probably needed other transcription factors induced by STAT6. Based on PPARγ knock-out experiments, most of the *de novo* regulatory sites needed PPARγ to recruit RXR, then the coactivator P300 and e.g. RAD21. RAD21 is a component of the Cohesin ring, which holds DNA loops together, thus it appears that the activated regulatory sites communicate with other regions e.g. the promoter of regulated genes. Interestingly, PPARγ agonist and antagonist could not largely affect PPARγ/RXR binding, enhancer transcription and gene expression, so the regulation rather depends on the presence of the proteins than their ligand binding, which is an unusual property for this kind of nuclear receptor heterodimers.

P-32
The effect of histone modifications and DNA superhelicity on nucleosome stability

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The effect of various posttranslational histone tail modifications (PTMs) on nucleosome stability was compared by exposing agarose embedded nuclei of human HeLa, Jurkat, peripheral blood mononuclear cells and mouse embryonic stem cells to treatments with salt or intercalator dyes, determining the remaining fraction of histones using PTM specific antibodies and laser scanning cytometry. Elution profiles reflecting relative destabilization could be measured in the case of the H3K4me3 and H3K27ac active marks in nuclei of all phases of the cell cycle by both salt and intercalator treatment, while the nucleosomes carrying any of a number of different other PTMs were significantly more resistant, just like bulk histone-GFP. Destabilization of the H3K4me3 marked TSS proximal nucleosomes could be seen in the case of all active genes as revealed by chip sequencing, when doxorubicin was used as the intercalator. Nicking treatments did not affect the stability of nucleosomes carrying the active marks, while those of the second group were all destabilized, as seen exclusively by intercalator elution. These data support the notion that superhelicity has got a gene regulatory role. The H3K4me3 and H3K27ac active marks appear to specify dynamic nucleosomes accomodating already relaxed DNA sequences, while most other nucleosomes hold the DNA in constrained superhelices. This conclusion is supported by the results of mapping of DNA breaks via DNA immunoprecipitation – NGS. The relationship between H3K4me3 and DNA breaks has been further investigated by targeting a histone methylase enzyme to an artificial chromatin domain.

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P-33
Nucleosome stability through the spectacles of quantitative imaging

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Nucleosome stability is an essential determinant of most DNA directed cellular activities in the genome of eukaryotes. Available approaches have limitations in providing insight into the functional properties of particular nucleosomes in their native molecular environment, thus there is urgent need for improved techniques. Here we describe a salt/intercalator elution based assay, Quantitative Imaging of the Nucleus after Elution (QINE), for evaluating nucleosome stability in situ in a histone type, posttranslational modification (PTM) and cell cycle-specific manner, by laser scanning cytometry (LSC). The method involves elution of histones using intercalators, and in the other format of the protocol just salt, to assess stability features dependent on DNA superhelicity or relying mainly on electrostatic interactions, respectively, and measurement of the fraction of histones remaining chromatin-bound in the individual nuclei using histone type- or PTM-specific antibodies. QINE has been validated using three different intercalators, by the quantitative assessment of intercalator induced chromatin loop relaxation required for nucleosomal destabilization and via demonstration of the preferential eviction of promoter proximal H3K4me3 nucleosomes in parallel ChIP-seq and LSC experiments. Its utility in the analysis of different histones and histone PTMs is demonstrated by verifying the difference in nucleosomal binding strength between histone variant H2A.X and its phosphorylated form (γH2A.X) generated in the wake of DNA damage.

The benefits of the in situ experimental scenario are illustrated by revealing a marked effect of nickase treatment on H2A, H2B, H2AX and H3 histone eviction highlighting the powerful potential of topological relaxation in remodelling. The assay system described here will provide a powerful, convenient and versatile tool to analyze nucleosome stability in conditions approximating the native.

P-34
Epigenetic role of Vitamin C in the pathomechanism of Arterial Tortuosity Syndrome

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***Keywords**: ascorbate, Arterial Tortuosity Syndrome, 5-hydroxymethylcytosine*

Vitamin C (Ascorbate; AA) is a cofactor for iron/2-oxoglutarate dependent dioxygenases. Such dioxygenases are located in the nucleoplasm and have been shown to participate in the epigenetic regulation of gene expression *via* histone and DNA demethylation. Thus, transport of AA or its oxidized form dehydroascorbic acid (DHA) into this compartment is required for the functioning of nuclear AA/DHA dependent enzymes, for the maintenance of the redox/antioxidant homeostasis of the organelle and for the epigenetic regulation. The proposed project is based on the assumptions that nuclear AA concentration has a regulatory role in epigenetics. Deficiency of GLUT10 (mutation in the SLC2A10 gene) results in arterial tortuosity syndrome (ATS), a rare heritable disease of the Extracellular Matrix (ECM). Considering that GLUT10 is a DAA transporter in the endomembranes, ATS can be regarded as a vitamin C compartmentation disease. We hypothesized that nuclear AA concentrations – modulated by nuclear AA or DAA transporter(s) – are regulatory with respect to the nuclear Fe2+/2-oxoglutarate dependent dioxygenases. Global DNA modification (5mC and 5hmC) levels were assed by ELISA Kit (MethylFlash; Epigentek) and Mass Spectrometry. Global 5hmC levels increased after AA treatment, especially in the control group (control $p<0.05$; patient $p=0.12$). Measurement of locus specific DNA modification (MeDIP, hMeDIP) are planned for ECM hydroxylase genes.

P-35
Controlling for cell composition of peripheral biological samples in candidate gene DNA methylation analyses

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Keywords: *candidate genes, DNA methylation, cell heterogeneity*

An increasing number of studies have been using surrogate tissues (blood or saliva) for large-scale DNA methylation analyses. The most commonly used samples are derived from blood although another easily accessible surrogate sample, buccal epithelial cells, could serve as more relevant peripheral tissue (Lowe et al., 2013). It is now standard for epigenome-wide analyses to control for cell heterogeneity using cell count estimates derived from DNA methylation profiles of purified cell types. However, cell composition is rarely included as a technical variable in candidate gene studies.

Our pilot study aimed to evaluate the effect of cell heterogeneity on DNA methylation levels of candidate gene regions in frequently used peripheral tissues. Samples were collected from 16 adult males participating in a Montreal-based longitudinal cohort. Whole blood, peripheral blood mononuclear cell (PBMC), T and B lymphocytes were compared to non-invasively collected buccal, mouthwash and saliva samples. Psychiatric candidate genes (FKBP5, SLC6A4, TPH2) and tissue-specific CpG-sites were assessed by pyrosequencing.

DNA methylation levels of the SLC6A4 promoter CpG-island region varied little, but mouth-related samples had lower methylation level compared to blood samples. The low CpG density regions of FKBP5 intron 7 and TPH2 promoter were highly variable across samples, especially in saliva. The effect of cell composition was substantial at FKBP5 intron (epithelial cell ratio had R^2=0.5-0.7 in mouth-related samples, lymphocyte ratio had R^2=0.3-0.4 in whole blood and PBMC samples) and at SLC6A4 promoter (epithelial cell: R^2=0.4-0.6). Therefore, cell composition should be taken into account at both low and high CpG density regions.

P-36
Association of global DNA hydroxymethylation with cadmium in gastropod hepatopancreas

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Keywords: *5-hydroxymethylcytosine, cadmium, land snails*

Background: DNA methylation at the *5 position* of cytosine (5-methylcytosine or 5mC) is pivotal to gene expression and DNA metabolism in most eukaryotes. A recently discovered oxidation product of 5mC, 5-hydroxymethylcytosine (5hmC) is also an important, yet poorly understood epigenetic mechanism. Cadmium is ubiquitous in the environment and is associated with aberrant genome-wide 5hmC levels in humans. The interplay between Cd and 5hmC in sentinel organisms for environmental cadmium, including gastropods has not been investiaged before.

Objective: We determined the changes induced in global 5mC levels in DNA of the hepatopancreas of land snail *Cantareus aspersus* following dietary Cd exposure at different concentrations.

Methods: We measured the percentage of 5hmC in hepatopancreas samples using capture and detection antibodies followed by colorimetric quantification. We assessed cadmium concentrations in this organ using Flame Atomic Absorption Spectrometry.

Results and Discussions: For the first time, we show the presence of 5hmC in *C. aspersus* and reveal the potential effects of Cd exposure on global DNA hydroxymethylation in gastropod hepatopancreas. Low, but detectable levels of 5hmC were found in hepatopancreas samples. Subtle changes in global hydroxymethylation levels (analysis in progress) were observed in response to dietary cadmium exposure.

Conclusions: Our findings provide evidence that global 5hmC levels in hepatopancreas of land snail *Cantareus aspersus* may be affected by low-level cadmium exposure.

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P-37
Functional and molecular characterization of an epigenetically controlled PUMILIO-regulatory protein in Arabidopsis

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Keywords: *APUM9, transposon, plant development, heat stress*

Although great progress has been made recently to understand how transposable element (TE)-mediated epigenetic regulatory pathways have been co-opted to serve essential functions in the host, many questions remain unanswered. For example, how can developmental and environmental signals affect the TE-mediated epigenetic control?

Our research is *focused* on a conserved plant RNA binding protein, APUM9, regulated by TE-mediated epigenetic control. APUM9 belongs to the extremely conserved family of PUMILIO RNA-binding proteins. They are well characterized in all examined eukaryotes, playing essential roles in developmental regulation and in stem cell control. However the functional and molecular details of plant PUMILIO protein mediated gene regulations are largely unknown.

Our main objectives are to unravel the biological function and molecular mechanism of APUM9 and define the biological relevance of TE-mediated epigenetic control of this protein.

Here we show that APUM9 binding to mRNAs can trigger the rapid decay of these targets. It is likely that the TE located in the promoter is responsible for the highly tissue-specific expression of *APUM9*, restricted to the siliques. The release of APUM9 suppression results in phenotypical consequences suggesting that *TE*-mediated repression of *APUM9* plays an important role in normal growth of *Arabidopsis*. Indeed APUM9 repression is partially released during heat-stress and APUM9 overexpressing plants show enhanced heat tolerance.

These data suggest that TE-mediated epigenetic control of APUM9 might have important role not only in plant development, but also in plant adaptation to heat-stress conditions.

P-38
Differential DNA methylation associated with Autism Spectrum Disorder in a South African cohort

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Keywords: *DNA methylation, Autism Spectrum Disorder, EWAS*

Autism Spectrum Disorder (ASD) is defined by deficits in social communication and repetitive behaviours and restricted interests. ASD is a complex, quantitative and highly heritable trait that encompasses a number of known genetic disorders (e.g. Fraxile X, Rett Syndrome); however, for the majority of cases the genetic causes are unknown. This is partly due to the wide variance in ASD phenotypes, which range from individuals who remain pre-verbal with severe behavioural challenges, to individuals who have fluent language and impaired social abilities. This highlights the need for a phenotyping tool that partitions the autism spectrum into discrete endophenotypes. Recent work on monozygotic twin pairs, discordant for ASD phenotype, implicated DNA methylation as a contributor to the aetiology of ASD.

We report differential methylation using a whole-epigenome methylation assay on a South African cohort of children with ASD compared to children with a typical developmental profile. Given the complex quantitative nature of the autism spectrum, all participants were phenotyped using a standardised assessment tool, the Autism Diagnostic Observation Schedule-2 (ADOS-2). The ADOS also allowed the identification of ASD endophenotypes. The data was analysed using Minfi and MissMethyl in R. This resulted in the identification of differentially methylated sites in both our case-control comparison, and across endo-phenotypes. These sites mapped to both previously identified ASD candidate loci, as well as novel loci. Our study is the first to analyse a South African cohort and highlights the role of DNA methylation in specific ASD endophenotypes.

P-39
The serotonin transporter gene (SLC6A4) shows differential regulation between children with ASD and typically developing children in a South African population

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Keywords: *DNA methylation, Autism Spectrum Disorder, serotonin transporter gene, SLC6A4*

Autism spectrum disorder (ASD) is defined by defcits in social interaction, communication, repetitive and compulsive behaviours. Its highly heterogeneous phenotpye is underpinned by complex genetic networks. The serotonergic system has been implicated in the aetiology of ASD, with differential expression of the serotonin transporter gene (SLC6A4) linked to ASD. DNA methylation at the promoter of SLC6A4 is known to affect SLC6A4 expression. We tested the hypothesis that there is differential regulation of SLC6A4, mediated by DNA methylation, between an ASD and control cohort. We also predicted that methylation will differ between ASD endophenotypes based on severity levels categorised by ADOS-2 (Autism Diagnostic Observation Schedule-2).

DNA methylation was quantified using the MALDI-TOF MS assay (Epityper Assay) in South Afdrican children with ADS and age-matched children without ASD; all participants were phenotyped using a standardised assessment tool, ADOS-2. Significant differences were found in SLC6A4 regulation between the ASD and control group. Overall DNA methylation at the SLC6A4 promoter region was significantly decreased in the ASD cohort, as well as in specific endophenotypes (e.g. the moderate ASD endophenotype, the language impaired endophenotype and the groups with more severe repetitive and restricted behaviours). Reduced levels of DNA methylation was observed at specific CpG sites and some of these sites were associated with higher levels of repetitive behaviour. The data from this study implicates the involvement of the serotonin transporter in ASD aetiology, specifically within the language impaired and repetitive and restricted behaviour endophenotypes.

P-40
Genomic determinants of molecular phenotype differences between B-lymphobastoid cells of a CEU trio

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In our research we are focusing on the extent of variability in different molecular phenotypes including genome-wide active histone signal, Pax-5 master regulator transcription factor binding and steady-state mRNA levels in connection with personal genomic variability. Our model cells are immortalized B-cells from an apparently healthy Utah trio of European origin. Making use of our in-house ChIP-seq and RNA-seq data and publicly available personal genome sequences we found that differentially occupied cis regulatory sites (i.e. enhancers) are rarely affected by motif-disrupting SNPs either in Pax-5, or in collaborating transcription factor motifs (0.4% of Pax-5 peaks). However, we found that the loss or gain of both Pax-5 and active histone mark peaks correlate at larger regions (average size is 206 kb) with clear boundaries. These regions are associated with topologically associated domains and partially overlap with super-enhancers. Also, differential Pax-5 binding results in differential histone acetylation and a corresponding change in the expression of one or more proximal genes. Allele-specific binding analysis using heterozygous single nucleotide variants showed that in case of binding loss, most signal comes from one parental allele. By applying genome-wide technologies we have the necessary tools to extend our understanding of molecular phenotype differences between individuals, thus paving the way for precision medicine. The project is funded by an Internal Research University Grant. Research head Balint L. Balint is a Szodoray fellow at the Medical Faculty of the University of Debrecen.

P-41
MicroRNA regulation in Tourette Syndrome candidate genes

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Background: Tourette Syndrome (TS) is a neurodevelopmental disorder marked by involuntary motor and vocal tics. Despite a strong genetic contribution, the molecular mechanisms behind TS are still unclear, although research suggests involvement of specific candidate genes and epigenetic mechanisms via miRNA regulators.

One of these candidates is the nicotinic acetylcholine receptor alpha 7 subunit (CHRNA7) known to regulate several developmental and secretory functions. Another candidate is Netrin 4 (NTN4) involved in axon outgrowth and guidance. Our goal was to investigate selected candidate genes in a case-control setup along with functional validation involving miRNA regulation.

Methods: An OpenArray platform was used for case-control analysis of TS patients (N=564) and healthy controls. We screened SNPs in 3'UTR regulatory regions by mirSNP and polymiRTS databases and identified 32 SNPs predicted to change miRNA binding. For functional validation SKNF1 and HEK human cell lines were co-transfected with luciferase reporter-3' UTR constructs of CHRNA7 and NTN4 and corresponding putative miRNAs (miRNA-106b and miRNA-198b) with non-targeting controls.

Results: Case-control analysis identified significant differences among the selected candidates (GDNF, LHX6, DRD2, CNTNAP2). Luciferase assays characterized the regulatory effect of predicted miRNAs on the expression in a concentration-dependent manner showing a dramatic change in gene expression of CHRNA7 and NTN4.

Discussion: To increase our understanding of the underlying genetic and epigenetic mechanisms of TS, we aimed to study the possible miRNA regulation processes to better understand the genetic architecture of TS and to determine how miRNAs contribute to the complexity of gene regulation in the development of disease.

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P-42

Changes in DNA methylation profiles followed by sub-chronic treatment with psychiatric drugs aripiprazole and riluzole in the rat striatum and prefrontal cortex

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Introduction: Studies in neuropsychiatric disorders have shown that epigenetics may play an important role in pathogenesis and therapy. The aim of this study is to investigate the effect of drugs used in Tourette Syndrome (TS) and other psychiatric disorders on DNA methylation in the CNS to find epigenetic targets that may influence efficacy of treatment.

Methods: Male Wistar Kyoto rats were treated from post-natal day 35 to 50 by intraperitoneal injection of either aripiprazole (neuroleptic), or riluzole (glutamatergic) or vehicle. Then rats were sacrificed and DNA was isolated from brain regions for methylation studies.

Reduced Representation Bisulfite Sequencing (RRBS) is a high-throughput technique used to analyze genome-wide methylation profiles at the single nucleotide level. We analyzed striatum and prefrontal cortex of 2 rats per drug group in order to identify DNA methylation changes due to these medications.

Results: We observed differences in overall methylation levels between the two drugs and controls. We identified hundreds of differentially methylated CpG sites and characterized their distribution across the genome (eg. promoter, intron, exon, UTR, etc.). The recognized CpG sites were annotated, a candidate gene list was created and pathway analysis was also performed on these genes.

Conclusion: The known functions of implicated genes suggest that some of the observed epigenetic changes might underlie the amelioration of symptoms by these drugs and account for certain adverse effects. The results give insights into the mechanism of action of aripiprazole and riluzole, as well as the side effects regarding these psychiatric medications.

This work is supported by EU funding under FP7-PEOPLE-2012-ITN, TS-EUROTRAIN, GA 316978

P-43

Dynamic changes of epigenetic biomarkers (5mC and 5hmC) in biofluids of prostate cancer patients undergoing hormonal treatment

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***Keywords**: methylation, prostate cancer, biomarkers*

Development of prostate cancer (PC) biomarkers to guide therapeutic decision at the time of diagnosis is difficult, but essential due to high rates of PC overtreatment and clinical relapse. Although there is a growing evidence for prognostic values of the newly emerging biomarkers, none of the available clinical protocols are taking molecular events into consideration. Epigenetic markers hold a significant prognostic potential in the carcinogenesis of PC since epigenetic alterations appear earlier and more frequently then genetic sequence changes.

This preliminary study involved 50 newly diagnosed PC patients, 80 patients with benign prostatic hyperplasia (BPH) and 10 healthy patients. The patients were reevaluated 3 moths and 6 moths after initial diagnosis and treatment. All patients we examined clinically and confirmed by PSA and Gleason score. Blood, serum/plasma and urine samples were collected at the time of evaluation. The Ethic Commission in Research approved the study and all patients signed the informed consent for their participation. DNA was extracted from blood (Qiagen) and urine (Norgen Biotek). The global 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) levels were assessed by Quest DNA ELISA Kits (ZymoResearch) and MethylFlash (Epigentek). Global methylation was also determined by direct digestion of DNA using EpiJET DNA Methylation Kit (ThermoFisher). The data were statistically analyzed to reveal their clinical significance either alone or in association with clinicopathological variables.

Detectable level of 5mC and 5hmC were observed in all samples. Variable methylation patterns were obtained depending on severity of PC and age. Our preliminary data show that the genomic 5hmC level is markedly changed and can be detected from body fluids in PC patients.

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P-44
Plasma DNA methylation profiles of genes associated with metastasis in breast cancer patients

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Keywords: *methylation, breast cancer, metastasize*

Breast cancer is driven by progressive genetic changes and epigenetic abnormalities. It includes changed patterns of histone modification, which result in remodeling of chromatin structure and hypo- and hypermethylation of DNA. These changes cause deregulation of the transcription activity of many genes.

The aim of this study was to compare the relationship between DNA methylation levels of 5 genes participated on metastasis formation, namely ADAM23, CXCL12, CDH1, TIMP3, BRMS1 containing CpG islands in promoter region, in invasive breast cancer. The goal of the study was improvement of an early detection of breast cancer and prediction of therapy effectiveness and prognostic response.

Total of 185 paraffin-embedded tumor tissue samples from non-familiar Slovak patients with primary breast cancer and 55 healthy controls as well as plasma and peripheral blood cells were analyzed.

We observed higher methylation status in 2 genes - CXCL12 and ADAM23. Average DNA methylation levels in tumor tissue were 9.36% ± 10.58 for the gene ADAM23 and 11.83% ± 12.01 for the gene CXCL12.

We can conclude that the quantitative analyses of tumor DNA methylation in ADAM23 and CXCL12 genes could have prognostic potential.

The research was supported by the Slovak Research and Development Agency (APVV) contract No. APVV-0076-10; the Research and Development Operational Programme (ERDF), contract No.26240220058; grants KEGA 023UK-4/2016 and UK/97/2016.

P-45
Methylation of genes associated with invasiveness of breast cancer

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Keywords: *breast cancer, methylation, invasiveness*

DNA methylation as an early marker in breast carcinogenesis has been frequently studied in tumor samples. The aim of our study was to compare the relationship between DNA methylation levels of six genes, namely *APC*, *ESR1*, *PGR B*, *RASSF1A*, *SYK* and *SOCS1*, associated with invasiveness and aberrant protein expression.

Bisulfite pyrosequencing was used to investigate DNA methylation profiles in formalin fixed paraffin-embedded tissues, blood cells and plasma samples in 185 patients with primary breast cancer.

The highest promoter methylation level was 88%, detected in APC and RASSF1A genes. Their average methylation levels were 38.57% ± 25.76 for *RASSF1A* gene and 23.73% ± 22.38 for *APC* gene.

The quantitative analyses of tumor DNA methylation in *RASSF1A* and *APC* genes could have prognostic potential. Surprisingly, the determination of methylation in genes *ESR1* and *PGR B* encoded hormonal receptors have not a relevant for prognosis or development of the disease.

The research was supported by the Slovak Research and Development Agency (APVV) contract No. APVV-0076-10; the Research and Development Operational Programme (ERDF), contract No.26240220058 and grant KEGA 023UK-4/2016.

P-46
The histone H3 lysine 36 demethylase KDM2A/FBXL11 is essential for Polycomb-mediated gene repression during germ cell development

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Keywords: *epigenetics; histone demethylation; transcriptional regulation; meiosis; Polycomb repression; spermatogenesis*

KDM2A/FBXL11 is a Jumonji-domain containing lysine demethylase catalyzing removal of mono and di-methylation of histone H3 lysine 36. While *Kdm2a* is required for mouse embryogenesis, its role in adult physiology is unknown. Using an inducible deletion approach, we demonstrate that *Kdm2a* deficiency causes male infertility, due to apoptosis of spermatogonia and early meiotic cells. Using RNA-sequencing, we could show that over 700 genes undergo repression during normal spermatogonial differentiation, and that this process is controlled by KDM2A. CpG-rich promoter genes upregulated in *Kdm2a* deficient cells are generally marked by Polycomb Repressive Complexes (PRC) and associated modifications in wildtype male germ cells suggesting that KDM2A is required for PRC-mediated repression. Our study thus identifies critical roles for KDM2A in coordinating gene expression programs during spermatogonial differentiation and meiosis which are essential for male germ cell development.

P-47

Epigenetic regulation of *P21*, *RASSF1*, *PTEN*, and *P53* in human breast cancer cells using different chemotherapeutic drugs

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Breast cancer is considered one of the most heterogeneous diseases that might be difficult to characterize and then to treat. Several research groups worldwide have explored different genetic and epigenetic approaches that might help. In the present study, different kinds of chemotherapeutic drugs have been applied to breast cancer cells (MCF-7) to assess their role in changing the methylome of these malignant cells. Global methylation was quantified in treated and untreated cells, and the results obtained indicated that the drugs/drug combinations applied (temozolomide, carboplatin, sodium phenylbutyrate, cyclophosphamide, erlotinib, procaine, vorinostat, and combinations) have a tremendous effect on the methylation landscape of the cells. Real time PCR was employed to assess the level of expression of different tumor suppressor genes; *RASSF1*, *PTEN*, *P21*, and *P53*. The results indicated that applying different drugs/drug combinations has affected the level of gene expression of the above mentioned genes. All drugs/drug combinations were incubated for 4 and 8 day with the cells. The four-day and the eight-day incubation have resulted in upregulation of *P53*, *PTEN*, and *P12* while a downregulation of *RASSF1* gene was obtained. This might indicate that the time of incubation has no effect on the regulation of the expression of these genes. However, this study needs more conformational investigation to elucidate the mode of action of the used drugs in epigenetically regulating these genes.

P-48
Procaine induces epigenetic changes in *HCT 116* colon cancer cells

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Keywords: *Epigenetics, Procaine, HCT 116*

Colon cancer is the third most commonly diagnosed cancer in the world, and it is the major cause of morbidity and mortality throughout the world. The present study aimed at treat colon cancer cell line (HCT116) with different chemotherapeutic drugs/drug combinations (procaine, vorinostat "SAHA", sodium phenylbutyrate, erlotinib, and carboplatin). Two different final concentrations were applied; 3µM and 5µM. Several assays were employed to study the effect of these combinations on the viability of malignant cells. Trypan blue test was performed to assess the viability of the cell before and after being treated with the drugs. The data obtained showed that there was a significant decrease in the viability of cells after applying the chemotherapeutic drug combinations. Also, DNA fragmentation assay was carried out to study the effect of these drugs on the activation of apoptosis-mediated DNA degradation. The results indicated that all the drugs/drug combinations had a severe effect on inducing DNA fragmentation. Global DNA methylation quantification was performed to identify the role of these drugs individually or in combinations in hypo- or hyper-methylating the CpG dinucleotide all over the genome of the HCT116 colon cancer cell line. Data obtained indicated that different combinations had different effects in reducing or increasing the level of methylation, which might indicate the effectiveness of combining drugs in treating colon cancer cells.

P-49
Retrotransposition caused and autophagy-aided heterochromatin release in etoposide treated senescent teratocarcinoma PA1 cells

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Genotoxic stress may induce epigenetic instability by altered DNA methylation pattern. Here we studied fragmentation and release of heterochromatin in PA1 embryonal carcinoma cells after etoposide treatment. Cells enter long G2 arrest, display DNA repair marks, however retain or obtain secondary DNA damage and markers of senescence (p21CIP21 and p16INKA4a). ETO treatment caused demethylation of heterochromatin as tested by 5'Me-cytosine IF staining. qRT-PCR analysis revealled a 3-fold increase of ALU transcription and FISH label clustering along the chromatin strands, indicating to retrotransposition as a source of secondary DNA damage and increased genome instability. We also found release of the nucleolar organisers and associated satellites (acro-p-arm FISH probe) into the nuclear buds, as well as their enrichment with multiple pericentric fragments (CREST and HP1 antibodies). The perinucleolar heterochromatin formed and released circular structures. As well, the TTAGGGn labeling displayed the enrichment of the budded heterochromatin with telomeric sequences. Altogether our study shows that accelerated senescence causes epigenetic and genetic instability and persistent DNA damage due to mobilization of normally silent transposable elements, which favour heterochromatin release and ultimate nuclear destruction. The latter was found much enhanced by inhibition of autophagic flux by Bafilomycin A1. This study was supported by the Latvian Scientific Council, grant No2012/341.

P-50
What happens to the Epigenome in 2D/3D cell culture?

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Keywords: *model system, epigenetics, methylation, acetylation, 2D/3D cell culture*

The field of epigenetics has rapidly evolved into one of the most influential areas of scientific research and has been shown to regulate essential biological processes such as aging, development, memory formation and carcinogenesis. This broad impact primes its implicat ions in basic as well as applied research, diagnostics, drug development and many more. Numerous studies are being performed in classical cell culture model systems, even though there is only little understanding of the epigenetic alterations in cultured cells versus human tissue. In this study we compare epigenetic features of non-small cell lung cancer (NSCLC) formalin-fixed paraffin embedded tissue samples to immortalized NSCLC cells growing in 2D or more complex 3D cell culture. Among the epigenetic traits of interest are primarily modificat ions that alter accessibility of DNA. The analyzed parameters include DNA methylation and hydroxymethlyation (5-mC and 5-hmC), the status of histone acetylation, as well as a global representation of chromatin organization in terms of heterochromaticity.
The aim of this study is to assess the epigenetic characteristics of human tissue samples, to compare these findings with the characteristics of 2D and 3D cell culture models to fina lly identify the most appropriate model system for epigenetic investigations in vitro. We hope that the obtained research insights enable an optimization of research settings for drug discovery, molecular pathology, epigenetic biomarker investigation and many other applications.

P-51
qRT-PCR evaluation of selected microRNAs' expression in amniotic fluid and chorionic villus samples from Down syndrome pregnancies

Anda Cornelia Vizitiu[1], Anda Alexa[2], Cristina Bejinar[2], Andrei Anghel[2], Danae Stambouli[3], Cezara Muresan[4], Doru Anastasiu[4], Ioan-Ovidiu Sirbu[2]

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Keywords: *microRNA, microRNA, trisomy 21*

Trisomy 21 (responsible for 95% of Down syndrome cases) is the most common viable aneuploidy, the prenatal diagnosis of which is based on genetic tests run on samples obtained by invasive procedures. Of note, the integrated screening (first and second trimester quad screen) can identify up to 94% of cases with Down syndrome.

Here we show preliminary data on the expression of hsa-miR-371-3 placenta specific cluster and of 5 microRNAs mapped on chromosome 21 (hsa-miR-99a, hsa-let-7c, hsa-miR-125b-2, hsa-miR-155, hsa-miR-802).

The biological samples obtained by amniocentesis (AF) and chorionic villous sampling (CVS) as part of the diagnostic procedure, were preserved in RNAlater at -80ºC until further use. microRNA quantification was performed using dedicated TaqMan assays on cDNAs synthesized using the a two-step Cells-to-Ct kit procedure; the changes in microRNA expression were calculated using the ΔΔCt method.

We found important, statistically significant changes in the expression of three microRNAs (one belonging to the miR-371-3 cluster, the other two mapped on chromosome 21) in Down syndrome samples compared to euploid samples. Of note, there are marked differences between microRNAs' expression in AF vs. CVS samples and furthermore, between microRNAs' expression in samples obtained from male fetus vs. female fetus pregnancies. Data regarding the transcriptional profiles the respective primary microRNAs are provided and a putative mechanism explaining the results is advanced. The biological and clinical significances of our data are discussed.

P-52
Plasma microRNA expression profiles in Parkinson's disease

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Keywords: *microRNA, Parkinson's disease, Levodopa*

The second most common neurodegenerative disease of the elderly, Parkinson's Disease (PD) is a progressive, incurable disease which clinically manifests when most of the patients' dopaminergic neurons are already affected. The diagnostic and prognosis of PD are entirely clinic, based on specific signs and the positive response to therapy; currently there are no specific diagnostic or prognostic markers for PD. The lack of appropriate, specific and sensitive biomarkers hinders the diagnostic, prognosis and therapeutic management of PD, and impinges on the development of effective prevention studies and clinical trials.
Here we describe a two-step biomarker analysis procedure based on qRT-PCR array (discovery step) and individual TaqMan qRT-PCR assays (validation step) and show preliminary data arguing for its usefulness in the analysis of plasma microRNA associated to PD. We propose a set of 5 plasma microRNAs to be associated to Parkinson's Disease, and show preliminary data for their validation as diagnostic and/or therapy-monitoring biomarkers in a cohort of patients diagnosed with sporadic PD.

P-53
miR-183 and miR-21 expression as biomarkers of progression and survival in tongue carcinoma patients

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Keywords: *Oral Cancer, Tongue Carcinoma, microRNA, miR-183, miR-21, Overall Survival, Alcohol*

Introduction: Tongue carcinoma is known for its high rate of nodal metastasis and mortality. Micro RNAs (miRNAs) act as negative regulators of gene expression and have a key role in epigenetic regulation in cancer, including oral carcinoma. The aim of the current study was to examine miR-183 and miR-21 expression in tongue carcinomas and to correlate their expression with the clinical status, prognosis and survival.

Material and method: For qPCR of miR-183 and miR-21 expression, total RNA isolated from 60 fresh-frozen tissue of tongue carcinomas was converted into cDNA by TaqMan MicroRNA Reverse Transcription Kit, and quantified by TaqMan Micro RNAs Expression Assays. Fold changes in the miRNAs expression, normalized to RNU6B, were determined using $2^{-\Delta\Delta Ct}$ method. The fold changes in miRNAs expression were dichotomized into high and low according to cut-off values derived from ROC curve analysis.

Results: miR-183 emerged as more promising discriminatory biomarker of poor outcome (AUC of 0.700, *p=0.009*). Tissue over-expression of miR-183, observed in 41 (68.3%) of tongue carcinomas, was associated with clinical stage (*p=0.037*), tumor size (*p=0.036*), and high alcohol intake (*p=0.034*), and showed a tendency of association with lymph node metastases (*p=0.066).* Moreover, miR-183 over-expression predicted the worse overall survival in tongue cancer patients (*p=0.006*), but not as an independent prognostic factor. miR-21 over-expression carried a tendency towards poorer overall survival, *p=0.073.*

Conclusion: Our results indicate the potential clinical utility of miR-183 expression as a biomarker of progression and a poor survival in tongue carcinoma patients.

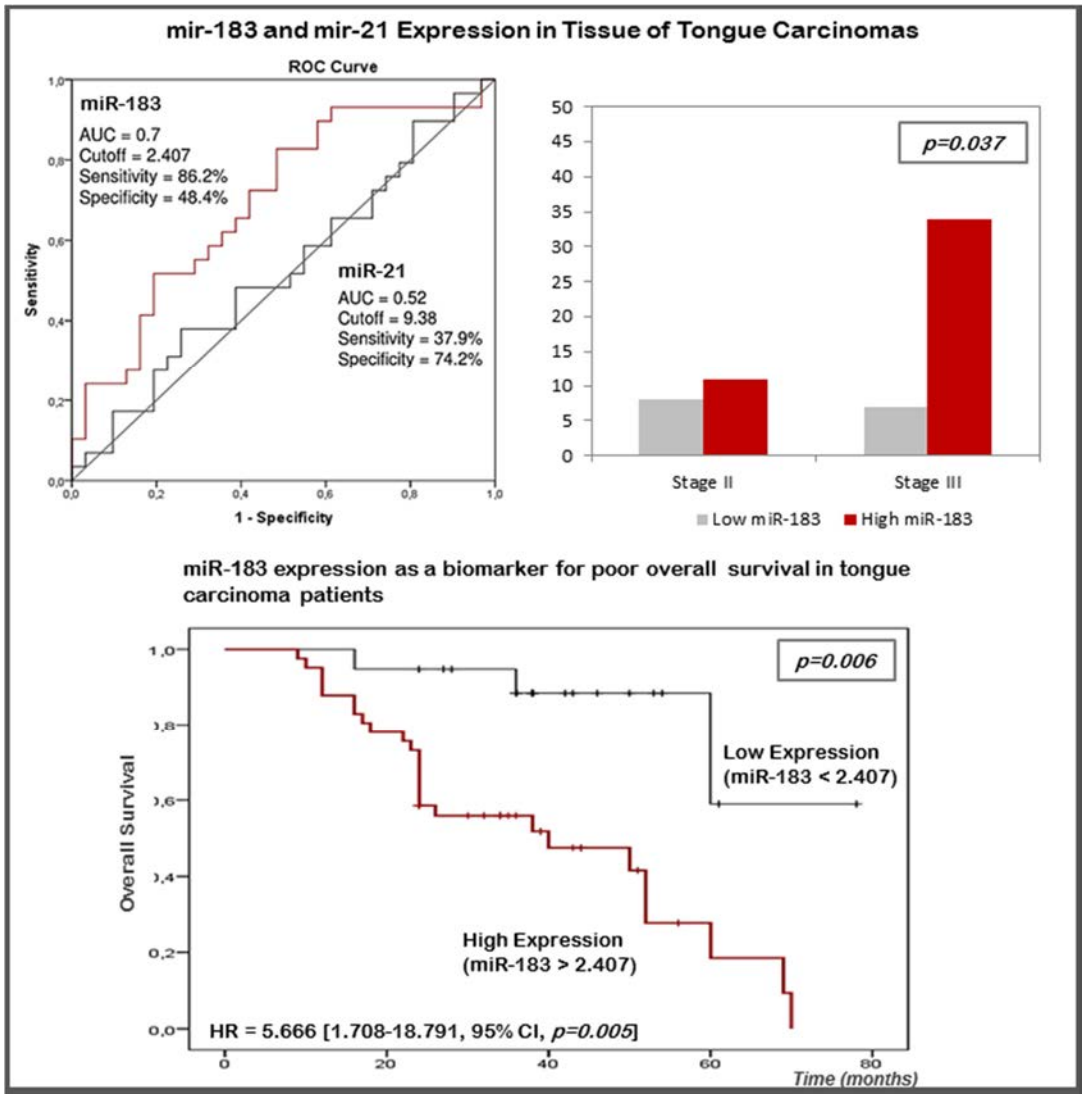

Figure 1 - Graphical Abstract: miR-183 and miR-21 Expression as Prognostic Marker in Tongue Carcinomas

P-54
The effect of histone modifications and DNA superhelicity on nucleosome stability

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Keywords: *nucleosome stability, endogeneous DNA breaks, H3K4me3, H3K27ac, laser scanning cytometry*

The effect of various posttranslational histone tail modifications (PTMs) on nucleosome stability was compared by exposing agarose embedded nuclei of human peripheral blood mononuclear cells, mouse embryonic stem cells and cell lines harboring the distinctly perceptible chromatin domain of a genome integrated LacO array, to treatments with salt or intercalator dyes, determining the remaining fraction of histones using PTM specific antibodies and laser scanning cytometry. Elution profiles reflecting relative destabilization could be measured in the case of the H3K4me3 and H3K27ac active marks in nuclei of all phases of the cell cycle by both salt and intercalator treatment, while the nucleosomes carrying any of a number of different other PTMs were significantly more resistant, just like bulk histone-GFP. Destabilization of the H3K4me3 marked TSS proximal nucleosomes could be seen in the case of all active genes as revealed by chip sequencing, when doxorubicin was used as the intercalator. Nicking treatments did not affect the stability of nucleosomes carrying the active marks, while those of the second group were all destabilized, as seen exclusively by intercalator elution. These data support the notion that superhelicity has got a gene regulatory role. The H3K4me3 and H3K27ac active marks appear to specify dynamic nucleosomes accomodating already relaxed DNA sequences, while most other nucleosomes hold the DNA in constrained superhelices. This conclusion is supported by the results of mapping of DNA breaks via DNA immunoprecipitation – NGS.

Support: OTKA 72762, 101337, Fulbright fellowship (G.Sz), TÁMOP 4.2.2.A-11/1/KONV-2012-0023, TÁMOP 4.2.4. A/2-11-1-2012-0001

P-55
Intrinsic protein disorder in histone lysine methylation

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Histone lysine methyltransferases (HKMTs) catalyze mono-, di- and trimethylation of lysine residues, resulting in a regulatory pattern that controls gene expression. Their involvement in many different cellular processes and diseases makes HKMTs an intensively studied protein group, but scientific interest so far has been concentrated mostly on their catalytic domains. We set out to analyze the structural heterogeneity of human HKMTs and found that many contain long intrinsically disordered regions (IDRs) that are conserved through vertebrate species. Our predictions show that these IDRs contain several linear motifs and conserved putative binding sites that harbor cancer-related SNPs. Although there are only limited data available in the literature, some of the predicted binding regions overlap with interacting segments identified experimentally. The importance of a disordered binding site is illustrated through the example of the ternary complex between MLL1, menin and LEDGF/p75. Our suggestion is that intrinsic protein disorder plays an as yet unrecognized role in epigenetic regulation, which needs to be further elucidated through structural and functional studies aimed specifically at the disordered regions of HKMTs.

Funding: Odysseus grant G.0029.12 from Research Foundation Flanders, Korean-Hungarian Joint Laboratory grant from Korea Research Council of Fundamental Science and Technology, OTKA grant PD-OTKA 108772, the Bolyai János Research Scholarship of the Hungarian Academy of Sciences and MedInProt Protein Science Research Synergy Program.

P-56
Apolipoprotein E upregulation by dexamethasone in macrophages

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Keywords: *apolipoprotein E, macrophages, dexamethasone*

Introduction: Macrophage-derived apolipoprotein E (apoE) plays an atheroprotective role, while its overexpression in the liver causes hypertriglyceridemia. Here we investigated the mechanism of glucocorticoid-induced macrophage-specific apoE gene regulation.

Materials and Methods: ApoE expression was determined by Real-Time PCR using TaqMan probes. Dexamethasone capacity to modulate the activity of apoE regulatory elements was assessed by transient transfections using plasmids containing apoE proximal promoter. Glucocorticoid receptor (GR) binding to apoE promoter was tested by DNA pull-down assays and chromatin immunoprecipitation.

Results: Dexamethasone induced a 5-6 fold increase in apoE mRNA levels in mouse peritoneal macrophages, but not in hepatocytes. Mifepristone (GR antagonist) significantly decreased apoE expression in macrophages. One-week dexamethasone administration to C57BL/6J mice increased apoE expression in macrophages, but not in liver. Dexamethasone-activated GRs specifically increased the apoE promoter activity in macrophages. DNA pull-down assays and chromatin immunoprecipitation showed that dexamethasone induced the recruitment of GRs to the -115/-65 region of the apoE promoter.

Conclusions: Glucocorticoids have a differential effect on apoE expression in macrophages and hepatocytes. GR binding site is functional in both cell types, but dexamethasone upregulates apoE expression only in macrophages. This data may contribute to the identification of specific drugs that, increasing apoE level in macrophages, stimulate the cholesterol efflux from the atherosclerotic plaque.

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P-57
Inhibition of DNA methyltransferase leads to increased genomic 5-hydroxymethylcytosine levels in lymphoid cells

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5-hydroxymethylcytosine (5hmC), a recently discovered epigenetic modification, is produced from 5-methylcytosine (5mC) by TET dioxygenases. Therefore, 5hmC is an active player in demethylation processes. The role of 5hmC is multi-faceted: it has crucial roles in cellular development and differentiation. In addition, impaired regulation of 5hmC can lead to carcinogenesis. 5hmC levels are low in the genome (~10% of 5mC and ~0.4% of all cytosines). We have developed a mass spectrometry method which can detect low amount of 5hmC in the DNA, as well as distinguish between 5mC and 5hmC compared to adequate standards. The epigenome reflects the influence of dynamically changing environment. Ascorbate was reported to regulate DNA methylation as a substantial cofactor for the catalytic activity of TET dioxygenases. We showed that vitamin C elevates the levels of both 5mC and 5hmC. Furthermore, different types of cells were treated with methyltransferase inhibitor 5-deoxy-azacytidine, which lead to the drastic decrease of 5mC together with great increase of 5hmC levels. Interestingly, we found that it is a characteristic of lymphoid cells.

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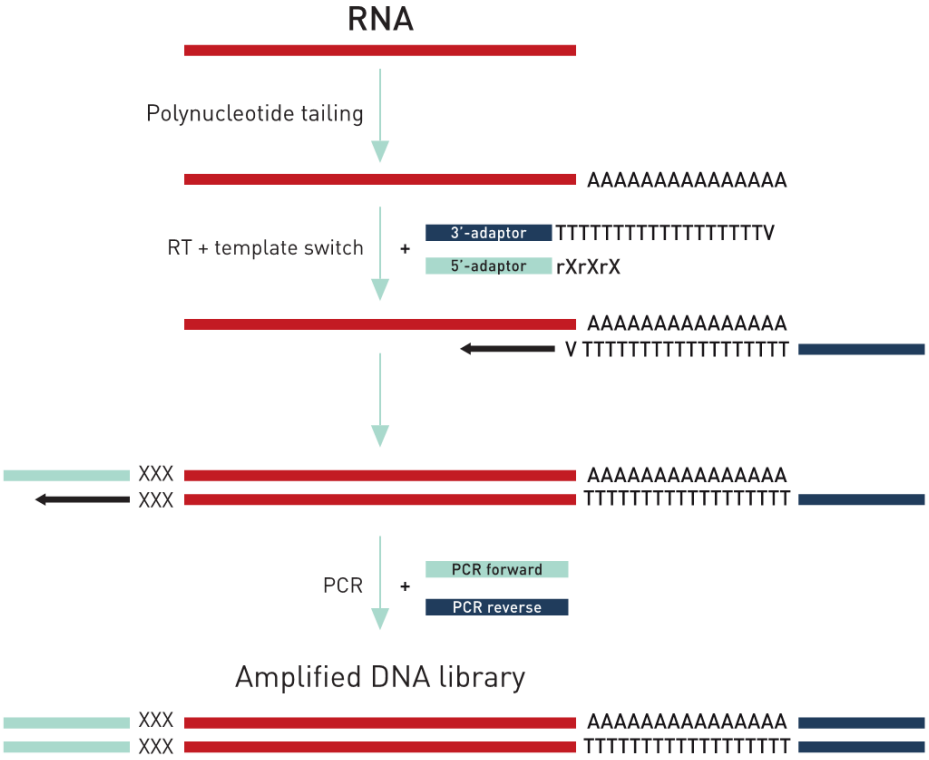

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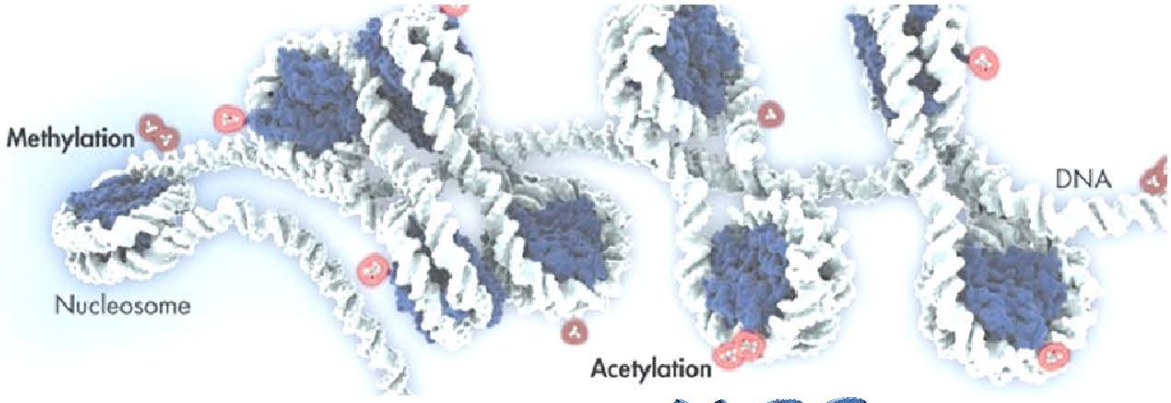

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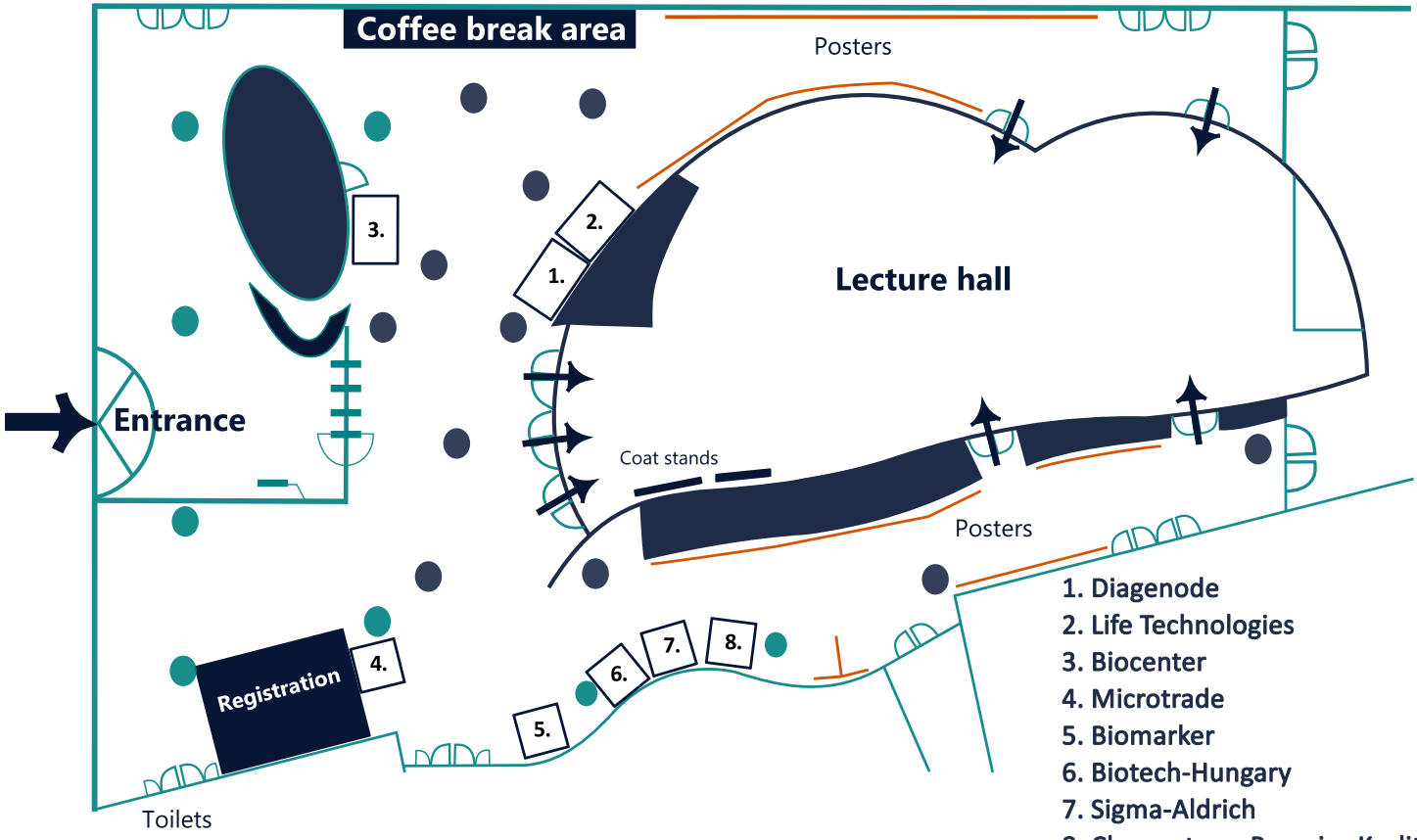

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