Session 3. Gene Expression Regulations: Epigenetics, and Alternative Splicing

Lectures

L3.1

Epigenomics — the road to functional genomics

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Epigenetic concepts have greatly influenced our view on mechanisms of genetic regulation and modes of inheritance. Epigenetic control involves multiple layers of molecular information including DNA-methylation, histone modifications and non-coding RNAs. Each cell type of a multicellular organism contains characteristic patterns of such epigenetic modifications. During development the single genome of an organism is epigenetically "translated" into hundreds of cell type specific epigenomes. NGS based methods allow to precisely map and interpret cell specific epigenomes. These data will revolutionize our interpretation of genome function in the cellular contect. The development of novel mathematical concepts and computational approaches will be crucial to understand biological complexity of epigenetic control. DNA-methylation is an epigenetic key modification forming stable covalent long term modifications of certain DNA bases. The recent discovery of novel oxidized forms of modified DNA bases creating hydroxyl-, formyl- and carboxy-methylcytosine has revolutionized our view of DNA methylation and challenges „traditional" concepts of DNA-methylation as a simply "on-off signal". Using ultra deep sequencing and immunofluorescence techniques we have begun to decipher the *in vivo* control of DNA methylation during critical phases of mammalian development. In my presentation I will discus our recent findings pointing towards a fine tuned and context dependent in vivo control of enzymatic control of DNA-methylation inheritance and DNA-methylation reprogramming.

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L3.2

Global and specific roles of linker histones in chromatin

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Linker (H1) histones are ubiquitous structural elements of eukaryotic chromatin occurring on average at one molecule per nucleosome. In both plants and animals, numerous cell-type and stage-specific non-allelic isoforms of H1 have been described. This functional significance of this complexity is not well understood. Compared to animals, plants represent a simpler model in which to study the function of linker histones. *Arabidopsis* has three H1 variants, two closely related main variants (H1-1 and H1-2) and a stress-inducible variant (H1-3) [1]. Stress-inducible H1 variants are evolutionarily conserved in flowering plants and their occurrence has been shown to positively correlate with abiotic stresses [2]. Plant stress-inducible H1 variants differ both structurally and in their *in vivo* chromatin binding properties from the main H1 variants. We characterized in more detail the nature of these differences and showed that the may underlay a major mechanism responsible for plant adaptation to changing environmental conditions.

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L3.3

Posttranscriptional regulation of microRNA expression in plants

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Plant microRNA genes are usually long, and very often contain introns. In the majority of cases a miRNA/miRNA* hairpin is found in the first exon of pri-miRNAs, but there are also plant miRNA genes where a miRNA is encoded in the second or third exon. Biogenesis of miRNAs requires complex and multistep processing. These maturation steps include: constitutive and alternative splicing of pri-miRNAs, alternative polyadenylation of miRNA primary transcripts, miRNA-containing hairpins excision, miRNA/miRNA* duplex formation, and miRNAs incorporation into the RISC complex. Our studies on A. thaliana and barley miRNA genes have revealed that miRNA genes organization is similar in both species, and probably reflects general miRNA genes organization in higher plants. Our results show that alternative miRNA precursor isoforms are specific to the particular organ and/or developmental stage of *A. thaliana*. To answer the question whether splicing plays an essential role in the efficiency of mature miRNA production, we analyzed processing of five different intron-containing *A. thaliana* pri-miRNAs in several Arabidopsis SR protein mutants. The data show that in some of the SR mutants tested there are only small changes in the pri-miRNA level, when compared to wild type plants, but the level of mature miRNAs, originated from these intron-containing genes, is significantly decreased. Next, we asked the question whether the observed by us changes in the level of mature miRNAs are caused by direct or rather indirect effects. To test it we introduced three variants of the MIR163 gene: a native form containing one intron, a gene containing mutated 5' and 3'ss, and an intronless variant, into the *A. thaliana* mir163-2 mutant (SALK_034556), in which T-DNA insertion had disrupted the endogenous MIR163 gene. At least two independent lines for each transgenic construct were analyzed for pri-miRNA163 and mature miR163 levels. Introduction of the wild type form of the MIR163 gene showed the same level of pri-miRNA 163 and its mature form, as it was observed in wild type plants. In the case of the intronless MIR163 construct we observed the accumulation of pri-miRNA 163, while the level of mature miRNA 163 was decreased about three times. In the case of transgenic plants containing the MIR163 gene with mutated both splice sites, the level of mature miRNA 163 was again lower when compared to wild type *Arabidopsis* plants. Altogether, our results show that splicing stimulates biogenesis of plant miRNAs derived from intron-containing genes. In addition, we showed that two factors involved in miRNA biogenesis in plants, SERRATE (SE) and AtCBC (AtCBP20/AtCBP80) are key players in the crosstalk between miRNA biogenesis and splicing.

L3.4

Reading and writing of methylation marks

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DNA methylation is an essential epigenetic signal. In my presentation the mechanisms of reading and writing methyl marks by human and mouse DNA Methyltransferases will be discussed. In cells, Dnmt1 is mainly responsible for copying the methylation patterns after DNA replication. Hence its recognition of hemimethylated CpG sites is essential for epigenetic inheritance. I will report recent data on the mechanism and basis of specificity of Dnmt1. Dnmt1 is guided to replicating DNA by UHRF1. I will present new data on the interaction of UHRF1 with Dnmt1 and its regulation of Dnmt1 activity. In addition, I will discuss *de novo* methylation of DNA by Dnmt3a and focus on the chromatin reading, multimerisation and localization of Dnmt3a as well as the role of Dnmt3L.

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L3.5

Assembly, structural dynamics and function of the spliceosome

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The spliceosome is a multi-MDa RNP machine that consists of the small nuclear (sn)RNPs U1, U2, U4/U6 and U5, and numerous non-snRNP proteins. The stepwise interaction of the snRNPs with the pre-mRNA during spliceosome assembly culminates with the formation of the so-called B complex which still lacks an active site. During the subsequent catalytic activation step major RNA-RNA and RNP remodelling events occur, generating the activated B complex, which then catalyses the first step of splicing to yield the C complex. We recently established an in vitro splicing complementation system that allows us to reconstitute both steps of yeast splicing with purified components and have now extended it to the disassembly stage of the spliceosome. Using this system, we have investigated the factor requirements and kinetics of the various remodelling steps of the yeast using fluorescence cross-correlation spectroscopy. We are also employing electron cryomicroscopy for the investigation of the 3D structure of yeast spliceosomes at defined stages of assembly. Finally, I will report on the crystal structure of two proteins involved in the catalytic activation of the spliceosome.

L3.6

To replicate or not to replicate? Regulation of the initiation step of bacterial chromosome replication

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Faithful transmission of genetic material to daughter cells requires precise regulation of chromosome(s) replication and its coordination with the cell cycle. Chromosome replication in all three domains of life is mainly regulated at the initiation step — one of the important checkpoints in the cell cycle. In bacteria, chromosome replication is initiated by cooperative binding of the initiator protein, DnaA, to multiple sequences termed DnaA boxes — 9-mers within the oriC (origin of chromosomal replication) region. The activity and availability of both key elements of the replication initiation, DnaA and oriC are tightly regulated to ensure that replication initiates only once per cell cycle. Several factors that regulate replication initiation have been identified in both Gram-negative (*Escherichia coli, Caulobacter crescentus*) and Gram-positive (*Bacillus subtilis, Streptomyces coelicolor*) bacteria. Few regulatory systems, such as inactivation of DnaA-ATP by ATP hydrolysis, are presumably used universally across all of the bacteria, whereas others systems are supposed to be specific for a particular bacteria. In bacteria that undergo a complex life cycle, a transcription factors temporarily and spatially coordinate the initiation of replication with cell differentiation and cell cycle progression. Surprisingly, little is known about regulation of DNA replication in response to various environmental conditions and factors. Recent studies indicate the existence of global correlation between metabolic status and the key cellular processes of cell cycle (e.g. replication) leading to bacterial proliferation.
I will overview the regulation of chromosome replication in bacteria focusing specifically on how transcription factors coordinate the initiation of replication with cell differentiation and cell cycle progression. Furthermore, regulation of DNA replication in response to various environmental conditions and factors will be discussed.

L3.7

If there's an order in all of this disorder…: structural bioinformatics of the human spliceosomal proteome

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The spliceosome is one of the largest molecular machines known. It performs the excision of introns from eukaryotic pre-mRNAs. In human cells it comprises five RNAs, over one hundred "core" proteins and more than one hundred additional associated proteins. The details of the spliceosome mechanism of action are unclear, because only a small fraction of spliceosomal proteins have been characterized structurally in high resolution. To aid structural and functional analyses of the spliceosomal proteins and complexes, and to provide a starting point for multiscale modeling, we carried out a comprehensive structural bioinformatics analysis of the entire spliceosomal proteome.

First, we discovered that almost a half of the combined sequence of proteins abundant in the spliceosome is predicted to be intrinsically disordered, at least when the individual proteins are considered in isolation. The distribution of intrinsic order and disorder throughout the spliceosome is uneven, and is related to the various functions performed by the intrinsic disorder of the spliceosomal proteins in the complex. In particular, proteins involved in the secondary functions of the spliceosome, such as mRNA recognition, intron/exon definition and spliceosomal assembly and dynamics, are more disordered than proteins directly involved in assisting splicing catalysis. Conserved disordered regions in splicing proteins are evolutionarily younger and less widespread than ordered domains of essential splicing proteins at the core of the spliceosome, suggesting that disordered regions were added to a preexistent ordered functional core. The spliceosomal proteome contains a much higher amount of intrinsic disorder predicted to lack secondary structure than the proteome of the ribosome, another large RNP machine. This result agrees with the currently recognized different functions of proteins in these two complexes.

For the ordered part of the spliceosomal proteome, we have carried out protein structure prediction. We identified new domains in spliceosomal proteins and predicted 3D folds for many previously known domains. We also established a non-redundant set of experimental models of spliceosomal proteins, as well as constructed in silico models for regions without an experimental structure. Altogether, over 90% of the ordered regions of the spliceosomal proteome can be represented structurally with a high degree of confidence. The combined set of structural models for the entire spliceosomal proteome is available for download from the SpliProt3D database (http://iimcb.genesilico.pl/SpliProt3D).

Finally, we analyzed the reduced spliceosomal proteome of the intron-poor organism Giardia lamblia, and as a result, we proposed a candidate set of ordered structural regions necessary for a functional spliceosome.

The results of this work enable multiscale modeling of the structure and dynamics of the entire spliceosome and its subcomplexes and will guide further research toward understanding of the molecular mechanism of mRNA splicing.

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Oral Presentations

O3.1

Regulation of alternative mRNA splicing: factors, mechanisms, networks

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The complexity of gene expression in the human system is largely determined by posttranscriptional processes such as alternative splicing, which multiplies the diversity of the proteome. Most human protein-coding genes undergo alternative RNA processing and yield functionally diverse proteins, regulated in a tissue- and developmental-specific manner. Alternative splicing is determined by the combinatorial control of a relatively small set of splicing regulators. We focus on developing and applying genome-wide RNA-based deep-sequencing (RNA-Seq), with the aim to identify all functional targets of specific splicing regulator proteins. Initial approaches are to knock-down by RNAi or overexpress specific regulator proteins and to analyze the resulting global changes in alternative splicing patterns. In addition, we use genome-wide iCLIP-Seq techniques (individual-nucleotide crosslinking-immunoprecipitation combined with deep-sequencing) to identify all in vivo binding sites of splicing regulators.
Our goal is to integrate both datasets, that is splice isoform changes and *in vivo* RNA binding sites, to generate a functional RNA map. These approaches will be illustrated by our ongoing studies on two splicing factors, hnRNP L and U1C. First, the heterogeneous ribonucleoprotein L (hnRNP L), a multifunctional RNA-binding protein, specifically recognizes CA-repeat and CA-rich RNA elements and participates in diverse functions of mRNA metabolism. HnRNP L can regulate alternative splicing either as an activator or a repressor. Based on iCLIP-Seq and genomic mapping, we derived a comprehensive genome-wide map of hnRNP L RNA interaction sites in HeLa cells. Analysis of the crosslink sites revealed that hnRNP L RNA binding follows distinct patterns for exons activated or repressed by hnRNP L, suggesting a positional code and a predictive RNA map. Second, the U1C protein, a constituent of the U1 snRNP, is important in the first step of spliceosome assembly, the precise recognition of the 5' splice site by the U1 snRNP. Our RNA-Seq analyses in both the zebrafish and the mammalian systems demonstrated that U1C not only functions as a general splicing factor, but can also determine 5' splice site choice during alternative splicing.

O3.2

DNA methylation markers in cancer diagnostics

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Cancers are widely characterized by two epigenetically-mediated phenomena which directly affect gene expression namely, the aberrant silencing of tumor suppressor genes in parallel with the activation of protooncogenes and transposons. It has been repeatedly shown that different tumor types display specific profiles of DNA methylation changes. This knowledge can be applied in oncology for both clinical diagnostics and therapy.
Squamous cell carcinomas of the head and neck region show unsatisfactory treatment outcomes, where the major cause for treatment failure is the development of a local relapse or a second primary tumor. This phenomenon is partly explained by the presence of a large field of molecular changes in the affected mucosa in most of the patients. Since no definite genetic markers have been identified that could be used for prognostic purposes in this group of patients, attention was focused on possible epigenetic biomarkers. Similarly, therapy outcomes in patients with brain tumors are highly unsatisfactory and epigenetic markers could be applied for better prediction of sensitivity to therapy regimens.
Our studies investigating the characteristic profile of DNA methylation changes in patients with laryngeal or oral cavity cancers show a high prevalence of hypermethylation of the promoters of several well-characterized tumor suppressor genes, such as p16, RARbeta, RASSF1A, FHIT, MGMT, DAPK, CDH1. Importantly, this aberrant DNA methylation is a frequent event not only in tumor cells but also in clinically and histologically normal mucosa from distant points in relation to the centre of the tumor. This epigenetic field defect may be an early event in head and neck carcinogenesis and may reflect the long-term exposure to environmental epicarcinogens. However, the functional relevance of these epigenetic changes in driving carcinogenesis is yet to be elucidated.
On the other hand, the analysis of DNA methylation profiles in brain tumors shows a frequent correlation between tumor and plasma methylation patterns. This may possibly serve for the development of a predictive epigenetic biomarker panel suitable for the improvement of treatment choice.
The analysis of the DNA methylation pattern is a promising biomarker strategy in the diagnostics of head and neck and brain tumors. However the assessment of the clinical validity of the proposed markers requires further studies.
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O3.3

Regulation of small RNA-guided gene silencing

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Small regulatory RNAs such as short interfering RNAs (siRNAs), microRNAs (miRNAs) or Piwi interacting RNAs (piRNAs) are more and more emerging as small molecules that have key-regulatory functions. Small RNAs are found in all higher eukaryotes and play important roles in cellular processes as diverse as development, stress response or transposon silencing. Members of the Argonaute protein family are mediators of gene silencing and bind to small regulatory RNAs. Ago proteins are characterized by PAZ, MID and PIWI domains and facilitate target RNA cleavage in RNA interference (RNAi) or miRNA-guided repression of gene expression. For a detailed understanding of Ago protein function, it is important to identify their cellular binding partners. Here, we report the analysis of Ago protein interactions in miRNA-containing and miRNA-depleted cells. Using Stable Isotope Labeling in Cell Culture (SILAC) in conjunction with Dicer knock out mouse embryonic fibroblasts (MEFs), we identify proteins that interact with Ago2 in the presence or the absence of miRNAs. In contrast to our current view, we find that Ago-mRNA interactions can also take place in the absence of miRNAs. Our proteomics approach provides a rich resource for further functional studies on the cellular roles of Ago proteins.
In addition to the protein interactors, we have also characterized small RNAs that bind to the different human Ago proteins. In contrast to the published literature, we do not find evidence that miRNAs are selectively sorted into specific Ago proteins.

O3.4

The Grainyhead-like 3 transcription factor and miR-21 micro RNA in skin cancer

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Squamous cell carcinoma (SCC) is a malignant tumor originating from epidermal cells. It bodes worse than basal cell carcinoma, as it can metastasize, mainly to the lymph nodes and has a faster growth. Despite its prevalence, the molecular basis of SCC remains poorly understood. We identified the Grainyhead-like 3 (GRHL3) transcription factor as a potent suppressor of SCC in humans and in the mouse model. We discovered that phosphatase and tensin homolog (PTEN) is the key target gene of GRHL3 in SCC and reduced GRHL3 expression in the skin leads to the reduced expression of PTEN. In this way we were able to explain why the decrease in the expression of PTEN is often observed in human SCC in the absence of genetic and epigenetic changes in the gene encoding PTEN. In more than half of the human SCC samples studied by us we found significantly lower levels of GRHL3 and PTEN expression. Decrease in the expression of PTEN leads to the amplification of PI3K/AKT/mTOR signaling and induction of SCC of both skin, and head and neck origins. Furthermore, we discovered that in human SCC GRHL3 is negatively regulated by proto-oncogenic microRNA miR-21, which underpins the development of SCC. In addition, miR-21 negatively regulates the expression of PTEN. To our knowledge, this is the first example of coordinated miRNA-mediated regulation of both transcription factor and its direct target gene for signal amplification.

O3.5

Prediction of hammerhead ribozyme intracellular activity with the catalytic core parameters fingerprint

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Catalytic RNAs are versatile tools for down-regulation of gene expression *in vivo*. Due to its small size and good activity in protein-free environment, hammerhead ribozyme is used as a model for RNA structure-function studies. We designed four hammerhead ribozymes targeting different mRNAs: minimal hammerhead ribozyme (HH-0) and three extended ribozymes with tertiary stabilizing motif (TSM) containing tetraloop receptor (TLR), differ from each other by the number of base pairs in helix I joining TSM with the catalytic core (HH-6, HH-5, HH-4). We describe a new universally efficient extended hammerhead ribozyme HH-5b. This ribozyme is very active in living cells but shows low activity *in vitro*.

To understand HH-5b specificity, we analyzed tertiary structure models of substrate:ribozyme complexes. We calculated six unique catalytic core geometry parameters — distances and angles between particular atoms that we call the ribozyme fingerprint. Flanking sequence and tertiary motif change a geometry of interactions between general base, acid, nucleophile and the leaving group, that affect the ribozyme catalytic properties. We found straight correlation between calculated parameters and the decrease of target gene expression in the cells. Efficient intracellular activity of HH-5b strongly correlates with bringing closer G8(2'O) to C1.1 (5'O) (D2) and withdrawing G12(N1) from C1.1(2'O) (D1). This relationship is described by the ratio D1/D2, that increase above 1 for more active ribozymes. There is also a straight correlation between stem I-II adjacent atoms distances and ribozymes intracellular activity. The closer are the stems I and II, the higher is D1/D2 ratio together with ribozymes efficiency.

Unique distribution of treats is specific and can describe the molecule, so can be called ribozyme fingerprint. Such ribozyme profile is a set of numbers that reflect ones catalytic core, which can be used as the identifier and be helpful in predicting its activity *in vivo*. Defined HH-5b ribozyme structure can be used not only as a therapeutic tool, but also in studying gene function in a way of reverse genetics. Proposed hypothesis of catalytic centre parameters compensation present an interesting and reliable alternative for traditional ribozyme design. Close correlation between structure modeling and intracellular experiments results shows, that improved RNA design supported by computational analysis could be a way to predict activity. Our approach could be widely adapt to characterize other RNAs.

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Acknowledgements

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O3.6

The role of free radicals in miRNA regulation of gene expression in K562 cancer cells

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Ionizing radiation (IR) is widely used in cancer radiotherapy. The main target for IR is DNA, which can suffer variable type of damage such as: single- and double-strand breaks, oxidative base damage, cell cycle disturbance, cytogenetic damage commonly leading to apoptosis, or other types of death. Increasing data however indicate that ionizing radiation can induce similar damage indirectly, e.g. by molecular signals like reactive oxygen species (ROS) and nitrogen species (RNS), also in RNA. Together with micro RNAs (miRNAs), 22-24 bp long RNA molecules containing sequences (seeds) that are fully or partially complementary to their target mRNA, the free radicals are important elements in regulating gene expression.

The goal of the present work was to study the influence of exposure cells to ionizing radiation on processes regulated by miRNAs. K562 cancer cells, originating from human leukemia line, were transfected with a vector (psiCHECK-2) containing a modified version of the renilla luciferase gene with eight recognition sites for let-7 miRNA in its 3'-UTR, and a reference firefly luciferase gene which is not influenced by let-7 miRNA. Transfected cells were exposed to 4 Gy of X-radiation, and after different times the levels of luciferase transcripts were assessed by RT-qPCR and the levels of luciferase protein by the Dual-Luciferase® Reporter Assay System (Promega). The levels of let-7 miRNA were followed by microarray assays and RT-qPCR. Additionally we performed studies on cyto- and genenotoxic effects of ionizing radiation in cells, comprised of: measurements of DNA strand breaks using comet assay, and phosphorylated histone H2AX level (gH2AX). We also performed analysis of 8-oxodG/8-oxoG using HPLC-EC; generation of ROS and nitric oxide in irradiated cells measured by flow cytometry with specific fluorescent markers (DCFH-DA and DAF-FM), and measurement of nitric oxide secretion to medium using Griess Reagent.

We observed a significant change in the levels of both renilla luciferase transcripts and protein in response to radiation. The results of comet assay and analysis of gH2AX foci show that directly irradiated K562 cells generate molecular signals, which induce DNA/RNA damage in cells. The level of damage in exposed cells is significantly higher than in control. Cytometric analysis indicates that irradiation of K562 cells causes an increase of ROS and NO, with the maximum level after 24 h. At the same time, the level of NO demonstrates also rapid increase. The level of oxidized bases 8-oxoG in RNA increased 1 hour after ionizing radiation. These all suggest that ROS and NO not only damage DNA or RNA in irradiated cells, but are among the signal mediators and together with miRNA influence the gene expression.

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Posters

P3.1

Computational prediction of novel microRNA candidates in *Pellia endiviifolia*

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Short 18-26nt RNA sequences (sRNA) are known as potent regulators of gene expression. Recently, next-generation sequencing (NGS) technology has provided new possibilities for identification of this class of sequences. Prediction methods based on NGS data, however, either focus on model organisms or at least are restricted to those for which reference genome sequences are available.
To analyze sRNA sequences from Pellia endiviifolia species B, a member of liverworts group, we have integrated four different strategies: 1) similarity search with 2) sRNA clustering without reference — for short sequence identification, and 3) window based sequence clustering on reference with 4) complementary sequence finding — for precursors prediction.
Similarity search approach was simply based on mapping of Pellia sequences to known plant microRNAs (miRNA). As a result, we have identified 960 known miRNAs from 472 families, 11 families were confirmed using northern hybridization. In sRNA clustering approach, short 18-26nt sequences from NGS data were grouped into clusters and ranked according to selected sequence features. Additionally, to distinguish between putative functional sequences and degradation products, NGS reads distribution and expression levels were analyzed. This method representing a new technique developed in our laboratory allowed us to select 74 candidates representing putative novel, liverwort-specific sequences. Expression of 51 was verified experimentally and 35 were confirmed as stable sRNA by northern hybridization and splinted ligation.
Methods for precursor prediction were focused on the level of reads accumulation on reference sequence and identification of putative miRNA* sequence. Using Pellia whole transcriptome data, we identified thousands of putative miRNA precursors. Among 20 sequences selected for experimental validation 13 were annotated as novel — several of them has been already confirmed by 5'RACE and genome walking.
Finally, functionality of known and novel miRNAs was assessed by target identification. At this moment, targets are validated by 5'RACE and splinted ligation.
In our work, we performed the first in-depth characterization of P. endiviifolia microtranscriptome. The results show that even without reference genomic sequence we are able to predict functional sRNA sequences using NGS data only. Since our functional sRNA identification method is based on the NGS reads analysis, it can be applied to any organism.

P3.2

Regulatory functions of small RNAs in stress adaptation and translation — lessons from novel mRNA-derived noncoding RNA

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In the past years, it became evident that small non-protein-coding RNAs play key roles in regulatory networks, shaping cellular life. While the list of validated ncRNAs that regulate crucial cellular processes grows steadily, not a single ncRNA has been identified, with a notable exception of the signal recognition particle that directly interacts and regulates the ribosome during protein biosynthesis. All of the recently discovered regulatory ncRNAs (e.g. microRNAs, siRNAs) target the mRNA rather than the ribosome. This is unexpected, concerning the central position the ribosome plays during the gene expression. The fundamental question that we are asking is: are there ncRNAs that directly bind and possibly regulate the ribosome during translation? To address this question, we started genomic screens for novel regulatory ncRNAs that associate with Saccharomyces cerevisiae ribosomes under specific environmental conditions.
We have constructed a specialized cDNA library and subjected it to high throughput deep sequencing analysis. The cDNA library included small RNAs (20–500 nt) that co-purified with S. cerevisiae ribosomes under 12 different stress conditions. We have also constructed a novel automated computational pipeline, named APART, providing a complete workflow for the reliable detection of RNA processing products from next-generation-sequencing data.
The cDNA library was not randomly fragmented like in usual mRNA profiling projects, therefore by employing the APART pipeline we were able to detect and confirm by independent experimental methods multiple novel stable RNA molecules differentially processed from well known ncRNAs, like rRNAs, tRNAs or snoRNAs, in a stress-dependent manner. Currently our interest is focused on revealing an unknown function of novel mRNA-derived small RNA in *Saccharomyces cerevisiae*, which is derived from the gene locus of TRM10 (systematic name: YOL093W). By combining traditional genetic, molecular biology and biochemistry approaches we are trying to investigate if and how the uncharacterized TRM10-derived small RNA is involved in regulating gene expression by interacting with the ribosomes. We have experimentally verified the presence of this novel small RNA under all investigated growth conditions and it's binding to yeast ribosomes in a stress-dependent manner. We have additionally verified the sequence specificity and its ability to inhibit protein biosynthesis in vitro and in vivo in yeast system as well as in various eukaryotic species. What is more, using the power of yeast genetics, we have implicated the TRM10-derived small RNA in stress adaptation process in *S. cerevisiae*.

P3.3

Interactions of Arabidopsis SWR1 complex proteins in stress

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Replacement of the canonical histone H2A with its highly conserved variant H2A.Z forms an important chromatin mark, affecting gene transcription. As loss of H2A.Z can result in both increase and decrease of transcriptional activity, depending on a locus, H2A.Z can be assigned neither transcriptional activator nor repressor function. However, H2A.Z tends to occur in genes that show regulated expression and avoids genes expressed at constant levels. Therefore, incorporation of H2A.Z can be viewed as an early step in adjusting expression levels of otherwise unresponsive genes in certain developmental or environmental situations. Deposition of H2A.Z into nucleosomes is catalyzed by a chromatin remodeling complex SWR1 (SWR1-C), which is well conserved among eukaryotes. Targeting of this complex and regulation of its activity in response to a signal seems to underlie directed H2A.Z deposition and subsequent transcriptional changes. Homologs of yeast SWR1 complex subunits exist in Arabidopsis thaliana which served as a model in some of the most important studies on H2A.Z involvement in the control of cell biology. However, to this date, no one reported purification of the complex from plants. Our main goal is to find out how the plant homolog of SWR1-C is activated and targeted during stress. We hypothesise that this can be achieved by association and dissociation of its particular subunits. To investigate these processes, we decided to use a combination of protein-protein interaction assays, mainly Bimolecular Fluorescence Complementation (BiFC) and Split Firefly Luciferase Complementation (SFLC). Here we present results of a comprehensive screen for interactions between plant homologs of SWR1-C subunits. We tested all possible configurations of EYFP fragment fusions for every pair of proteins, using Arabidopsis mesophyll protoplasts. We also present results of SFLC assay, which we used to confirm interactions detected by BiFC and also to search for modulation of the interactions under selected abiotic stresses. To meet the specific requirements of the project (121 potential protein-protein interactions to be experimentally verified) we developed sophisticated high-throughput strategies which enabled us to identify novel interactions within the Arabidopsis SWR1-C that provide a new insight into its structure and function.

P3.4

Highly conservative staphylococcal operon coding for a potential transcription factor

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Transcription regulation plays a crucial role in adaptation to constantly-changing environmental conditions, including pathogen-host interactions substantial for pathogenesis initiation and progression. Bacterial systems responsible for transcription regulation demonstrate a mostly high level of complexity, which allows for strict control of activation of a system and subsequent signal transduction. A wide search across GenBank database among available genomes of *Staphylococcus aureus* permitted us to find hypothetical proteins with putative DNA-binding motives: potential transcription factors. Further research was focused on one of the hypothetical proteins which contained two such motives. Utilising reverse transcription method we confirmed the predicted operon structure, which allowed for the generation of a hypothesis that it may be an operon coding for a more complex system involved in transcription regulation. Subsequent cross-genomes analysis revealed prevalence and high conservation of the operon across different species of the *Staphylococcus* genus. Cloning of the operon genes, overexpression under control of phage T7 promoter in *Escherichia coli* and partial purification demonstrated the ability of the investigated protein for DNA-binding. DNase I digestion of a partially purified and not denatured sample led to limited degradation of co-purified DNA as a small fragment remained intact when compared to the sample initially heat-denatured. Concurrently, the possibility of RNA co-purification was excluded by treatment the samples with RNase A. Isolation of the shielded fragment and cloning into a plasmid allowed for preliminary determination of a possible motif bound by the protein. Subsequently, whole genome analysis in search for the motif was carried out. The preliminary results indicate the possible physiological role of the newly described system and further research has the potential to expound the full meaning of the operon for *S. aureus* strains.

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P3.5

Interaction of polypyrimidine tract binding protein 1 with the 5′-terminal region of p53 mRNA

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The tumor suppressor p53 is one of the major transcription factors involved in cell cycle control, DNA repair and induction of apoptosis. p53 expression is regulated on several levels and one of them is translation initiation. In this process the 5'-terminal region of p53 mRNA plays a crucial role. This region is folded into a complex secondary structure through interaction of the 5' untranslated region and the proximal p53 coding sequence (Błaszczyk & Ciesiołka, 2011, *Biochemistry* **50**: **33**). It has been also suggested that this part of p53 mRNA expresses activity of an IRES element. Moreover, several protein factors have been identefied which interact with the 5'-terminal region of p53 mRNA to regulate not only translation of p53 but also its ΔNp53 isoform, which is synthesized from the downstream initiation codon. However, their exact role in translation regulation is largely unknown.

In the present study we have characterized ribonucleoprotein complexes that are formed between polypyrimidine tract binding protein 1 (PTB1) and the 5'-terminal region of p53 mRNA. Probing of RNA structure in the complex with this protein allowed us to identify possible regions of PTB1 interactions. PTB1 binds to the long polypyrimidine tracts located mainly in the stretch of coding sequence which is important for proper folding of the 5'-terminal region of p53 mRNA, as we have demonstrated recently. We also showed that PTB1 binding leads to the formation of higher-order complexes containing presumably multiple copies of the protein. Quantitative analysis of RNA-protein complexes formation by filter binding assay revealed that PTB1 has the greatest affinity for variants of the 5'-terminal region containing part of the coding sequence (K_d values were in the range of 27–175 nM). This indicates that proper structure of the 5'-terminal region of p53 mRNA is required for PTB1 binding. We also determined the influence of PTB1 protein on p53 and ΔNp53 *in vitro* translation. It turned out that PTB1 inhibited synthesis of both proteins. Moreover, the level of translation inhibition was correlated with the affinity of PTB1 to different fragments of p53 mRNA.

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P3.6

Influence of the SWI/SNF complex on alternative splicing in *A. thaliana*

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The SWI/SNF complex is an ATP-dependent chromatin remodeling factor which plays a key role in the regulation of eukaryotic chromatin structure. This dynamic multiprotein complex has the ability to remodel nucleosomes, preferentially at the promoter region of genes. The SWI/SNF complexes are recruited to their substrates by acetylated histone tails, and they use energy from ATP hydrolysis to modify DNA-protein contacts in the nucleosome. As a result, the processivity of RNA polymerase II can be changed, which, subsequently, influences processing of primary transcripts made by RNA pol II.

Using the RT-PCR panel based on fluorescently labeled primers we have analyzed alternative splicing in Arabidopsis brm and swi3c mutants (BRM and SWI3c are two subunits of the A. thaliana SWI/SNF complex). The results showed a significant effect of both the proteins studied on selection of pre-mRNA splice sites. Among the 249 splicing events analyzed, significant changes were observed in about 20% of all analyzed cases. Most of the changes were observed in the brm mutant, or were common to both analyzed swi/snf mutants. Among different splicing events tested exon skipping and intron retention were the most frequently affected in the mutants. Additional experiments carried out on the brm point mutant (the mutation disrupts only the ATPase activity of BRM), and the double brm x swi3c mutant clearly show two different mechanisms of the SWI/SNF influence on alternative splicing in A. thaliana: one that depends on SWI3c and the BRM ATPase activity, and another one which seems to be independent from the BRM enzymatic activity. More detail analyses of the results obtained in the brm point mutant revealed a preferential usage of splicing sites which are synthesized as first in a sequence of pre-mRNA. Our data prove a role of BRM and SWI3c in regulation of alternative splicing, most likely through changing the elongation rate of RNA polymerase II.

P3.7

Chromosome segregation of *Streptomyces venezuelae*

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Streptomyces are famous as producers of diverse secondary metabolites including many antibiotics. These organisms are characterized by morphologically complex life cycle, during which they initially form a vegetative mycelium of branched hyphae. Upon nutrients depletion *Streptomyces* form sporogenic hyphae which differentiate into chain of exospores in the process called sporulation.
Streptomyces venezuelae is unique among *Streptomyces* genus, because of its ability to grow in diffuse and homogenous manner in liquid media, where it is able to sporulate to near completion. These features make *S. venezuelae* suitable for physiological analyses of sporulation.
ParA and ParB are crucial for efficient chromosome segregation during sporulation. ParA forms a sandwich dimer and can oligomerize to form extensive filaments stretched along the aerial hyphae. ParB is a DNA-binding protein, forming nucleoprotein complexes at partitioning sites (*parS*) clustered around origin of replication. These complexes are indispensable for proper positioning of chromosomes along growing aerial hyphae.
S. venezuelae parA, *parB* and *parAB* deletion strains exhibit disorders in septa location and chromosome segregation. We focus on localization and dynamics of the ParA and ParB during *Streptomyces* sporulation using fluorescence microcopy and time lapse experiments.
In order to localize proteins of interest within *S. venezuelae* hyphae, we constructed different strains, which produce chromosomally encoded EGFP or Cherry-tagged ParB and ParA instead of the wild type proteins.
Our studies allowed us to initially characterize chromosome segregation dynamics in *S. venezuelae*. We propose the model of sporulation, where active segregation directly follows the point when the aerial hyphae growth comes to an end.

P3.8

Unusual features of *Helicobacter pylori* origin of chromosomal replication

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Helicobacter pylori is a human pathogen responsible for such disorders as gastrisis and peptic cancer. It has been a subject of extensive studies for many years but still many aspects of its basic processes are poorly understood, including chromosome replication.
Replication of the bacterial chromosome is initiated by the interaction of the DnaA protein with oriC region (origin of chromosomal replication). Binding of the DnaA protein to specific DnaA boxes localized in the oriC leads to DNA melting within DNA unwinding element (DUE). Open complex is formed and subsequently further replication proteins are assembled.
H. pylori oriC containing a cluster of DnaA-boxes was previously identified upstream of a dnaA gene. However, despite many attempts, no DNA unwinding within the oriC region has been detected. Comprehensive in silico analysis allowed us to identify an additional region (oriC2), separated from the original one (oriC1) by the dnaA gene. By applying several in vitro and in vivo methods we proved that DnaA specifically binds both regions, but DnaA-dependent DNA unwinding occurs within oriC2. Surprisingly, oriC2 is bound exclusively as a supercoiled DNA, directly showing the importance of the DNA topology in DnaA-oriC interactions. Such feature highly resembles initiator-origin interactions in archea and eukaryota.
We conclude that *H. pylori* oriC exhibits bipartite structure being a first such origin discovered in a Gram-negative bacterium. *H. pylori* mode of initiator-oriC interactions with the loop formation between both parts of the discontinuous origin resembles those discovered in case of Bacillus subtilis chromosome and in many plasmids and might be involved in regulation of replication and/or dnaA gene expression.

P3.9

Ultrahigh-resolution crystal structures of Z-DNA in complex with $Mn^{2+}$and Zn^{2+} ions

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The X-ray crystal structures of the spermine form of the Z-DNA duplex $d(CG)_3$ in complexes with Mn^{2+} and Zn^{2+} cations have been determined at ultrahigh resolution of 0.75 and 0.85 Å, respectively. Stereochemical restraints were used only for the sperminium cation (in both structures) and for nucleotide with dual conformation in the Zn^{2+} complex. The Mn^{2+} and Zn^{2+} cations at the major site, designated as (1), bind at the N7 position of Gua12 by direct coordination. The coordination geometry of this site is octahedral, with complete hydration shells. An additional Zn^{2+}(2) cation is bis-coordinated in a tetrahedral fashion by the N7 atoms of Gua4 and Gua6 from symmetry-related molecules. The coordination distances of Zn^{2+}(1) and Zn^{2+}(2) to the O6 atom of the guanine residues are 3.617(6) and 3.254(5) Å, respectively. An additional chloride ionhas also been identified in the coordination sphere of Zn^{2+}(2). Alternate conformations are observed in the Z-DNA/Zn^{2+} structure not only at internucleotide linkages but also at the terminal C3'-OH group of Gua6. The conformation of the sperminium chain in the Z-DNA/Mn^{2+} complex is similar to the spermine conformation in other Z-DNA/Mg^{2+} structures. In the Z-DNA/Zn^{2+} complex, the sperminium cation is disordered and partially invisible in electron density maps. In the Z-DNA/Zn^{2+} complex the sperminium cation only interacts with the phosphate groups of the Z-DNA molecules, while in the Z-DNA/Mn^{2+} structure it forms hydrogen bonds with both the phosphate groups and DNA bases.

P3.10

Hybridization of complementary oligonucleotides in the 5'untranslated region of the coxsackievirus B3 genomic RNA and in the 3' terminal region of its replicative strand

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Oligonucleotide-based tools like siRNA, ribozymes or antisense DNA oligomers are considered as powerful approaches to specifically attack the RNA(+) viruses. The best regions to target in the dynamically changing viral genomes are highly conserved non-coding RNA stretches. Unfortunately, they are mostly involved in formation of highly structured RNA elements what makes them hardly accessible to oligonucleotide-based tools.

Against coxsackievirus B3 (CVB-3), a heart pathogen, no specific treatment exists to date. It's RNA(+) genome is flanked by untranslated regions (UTRs). The 5'UTR contains an IRES element that directs translation process and a cloverleaf structure that regulates viral replication. The complementary, 3'-terminal region of the replicative strand is believed to be crucial for the replication as well. Both regions are attractive targets for antiviral attack mainly because of their high sequence conservation.

Prior to applying oligonucleotide-based strategies to attack the viral RNA we determined sites accessible to hybridization in the 5'-terminal region of the viral genomic RNA strand and in the complementary 3'-terminal region of the viral replicative strand. Subsequently, we designed a set of 42 DNA 16-mers complementary to the mapped accessible regions and RNase H-assay was performed to estimate their ability to effectively hybridize to these targeted sequences. Seventeen oligomers that induced the most effective RNA cleavage by RNase H were chosen to analyse RNA target misfolding. To monitor changes in the secondary structure of viral RNA upon antisense oligomers hybridization the chemical modification with DMS followed by reverse transcription was applied.

We found that some of the tested antisense oligomers which were hybridized to the 5'UTR region induced RNA structure destabilization in domain II and III that potentially could interfere with biological functions of this region. However most of the studied oligomers did not change the RNA target structure significantly. In the 3' terminal region of the replicative viral strand we also observed changes in RNA folding not only in the close neighborhood of the hybridization sites but also in more distant regions. The obtained results should facilitate proper design of nucleic acid-based tools able to target highly structured non-coding viral regions.

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P3.11

Mechanism of BRCA1 mRNA down-regulation in BCR-ABL expressing cells

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The BCR-ABL oncoprotein plays a major role in the development and progression of chronic myeloid leukaemia (CML). To study the role of BCR-ABL, we employed mouse progenitor 32D cell line expressing high level of BCR-ABL corresponding to drug-resistant cells from blast crisis of the disease. We previously demonstrated that CML progression correlates with increased aneuploidy resulting from affected chromosome segregation. This was caused by BRCA1 down-regulation leading to decreased expression of protein members of spindle assemble checkpoint (like Bub1, Bub3, BubR1 and Mad2). In this study we investigated the mechanism responsible for BCR-ABL-mediated BRCA1 down-regulation. We analyzed distribution of mRNA in the polysome profile, formation of complexes with mRNA-binding proteins as well as compared 3'UTR activity in lucyferase-based assay. We found that the mRNA binding proteins could play central role in the regulation of BRCA1 mRNA translation in chronic myeloid leukemia cells.

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P3.12

Sequence variants of p21 gene of the MRL/MpJ mouse: a possible association with the deficit of p21 protein and regenerative phenotype

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The MRL/MpJ mouse, an inbred laboratory strain, is known for its enhanced regeneration abilities, observed in different tissues and manifested by scarless ear-hole closure.

The Cdkn1a gene encodes the p21 protein, a cyclin-dependent kinase inhibitor, which plays an important role in cell cycle regulation. By interacting with CDKs p21 may stop cell cycle progression at different stages. The p21 gene expression may be initiated from two independent promoters, the classical and the p53-induced one, what is important in case of DNA damage.

The p21 protein has not been detected using Western blotting in the MRL tissues, even after γ-irradiation, although p53, which up-regulates p21 expression after DNA damage, is constantly present. The deficit of p21 found in the MRL mouse is associated with the regenerative capacity as the p21 gene knockout in a mouse strain unrelated to the MRL mouse results in an ear-hole closure effect, similar to that observed in the MRL mouse.

In our research, we have attempted to investigate the genetic background of p21 deficiency in the MRL mouse. We sequenced the p21 gene of the MRL mouse and compared its nucleotide sequence with those already known of 17 other laboratory murine strains. We identified three in/del sequence variants that are unique for the MRL mouse in the first intron of the p53-dependent transcript and six single-nucleotide substitutions in the vicinity of the p53-dependent promoter. As we found no aberrant splicing variants, we quantified the levels of two p21 transcripts variants in various tissues of the MRL and the control B6 mouse. No dramatic decrease in the p21 transcript levels, both the classical one and the p53-dependent, in the MRL/MpJ mouse, which could explain the deficit of p21 protein in the MRL/MpJ, was revealed. On the contrary, the average levels of both p53-dependent and the classical p21 transcripts in spleen, blood and liver were a few times higher in the MRL/MpJ than in the C57BL/6J mouse.

The results indicate that the transcriptional regulation of p21 is not responsible for the reported deficit of p21 protein in the MRL mouse. The location of the MRL specific nucleotide sequence variants in the first intron of the p53-dependent p21 transcript and in the vicinity of the p53-dependent promoter may also be associated with the observation that the expression of p21 protein in the MRL mouse is not induced following γ-irradiation.

P3.13

The translational regulation of p53 protein and its ΔNp53 isoform by antisense oligonucleotide strategy

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The p53 protein is one of the major transcription factors involved in cell cycle control, DNA repair and induction of apoptosis. It has been revealed that p53 expression is regulated at many different levels, and that several p53 isoforms are synthetized in the cell. One of these isoforms, the ΔNp53 isoform, which is deprived of 39 N-terminal amino acids, can bind to the full-length p53 reducing its available pool. Interestingly, the level of ΔNp53 seems to be elevated during G1-S phase transition.
Recently, we have determined the secondary structure of the 5'-terminal region of p53 mRNA that includes two major translation initiation codons AUG1 and AUG2, responsible for the synthesis of p53 and ΔNp53 (Błaszczyk and Ciesiolka, Biochemistry 50, 7080, 2011). It turned out that the part of the p53 coding sequence is involved in the folding of 5'UTR p53. The most characteristic structural elements in the 5'-terminal region of p53 mRNA were two hairpin motifs. In the first hairpin, G56-C169, the initiation AUG1 codon was embedded while the other hairpin, U180-A218, has been earlier shown to bind to the Mdm2 protein.
In our report antisense oligonucleotide strategy was applied to modulate the p53 and ΔNp53 translation. We used the model mRNA construct, ΔNp53-Luc, which consisted of the 5'-terminal region of p53 mRNA with both the initiation codons AUG1 and AUG2, and a sequence encoding the reporter protein, luciferase. We confirmed that the most characteristic structural elements of the 5'-terminal region of mRNA p53 are preserved in the ΔNp53-Luc transcript in vitro and in rabbit reticulocyte lysate (RRL).
To design antisense oligonucleotides targeting the 5'-terminal region of ΔNp53-Luc sites accessible to hybridization in this region were determined using libraries of 6-mer DNA oligomers and RNase H digestion. Then, several antisense oligonucleotides (no. 1–7) were tested in translation system in vitro in RRL. Two out of seven oligonucleotides induced changes in translation level from both the initiation codons. The antisense oligonucleotide no. 1, which binds to the hairpin G56-C169, inhibited translation significantly, especially from AUG1. Interestingly, the opposite effect was observed in the presence of oligonucleotide no. 7, which hybridizes to the hairpin U180-A218. In this case, the level of translation from AUG2 was markedly increased. Currently, the antisense oligonucleotides are examined in human breast adenocarcinoma cell line (MCF-7), which has been shown to express the p53 and ΔNp53 isoform. Our results showed that the expression profile of p53 and ΔNp53 proteins might be modulated at translational level by antisense oligonucleotides. Such modulation would provide new information about the role of the 5'UTR region in p53 and ΔNp53 translation.

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P3.14

Alternative splicing events in two maize lines under herbicide stress conditions

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Until now, splicing and its role not only in diverse aspect of cell biology, but also pathology and stress response, has been poorly described for many plant species, including maize. It has been known for many years that primary transcripts (pre-mRNA) that have multiple exons and introns may undergo alternative splicing events. This process results in formation of many different mRNAs, can affect their stability and translational efficiency. It also gives a way of generating multiple proteins from single gene, influencing activity, cellular localization, regulation and stability of coding protein.
Plants as sessile organisms, to survive changing environmental conditions, biotic and abiotic stresses must adequately adapt their growth and development. Splicing is one of the mechanisms which play an important role in plant adaptation giving them a way to adjust quickly and effectively to adverse conditions regardless of the limited capacity of genome size.
For better characterization of alternative splicing role in plant herbicide stress response, we sequenced transcriptomes of two maize breed lines – that have differential sensitivity to herbicide RoundUp. We used Illumina next-generation sequencer Genome Analyzer IIx and conducted pair-end sequencing. As a result we obtained 35 to 76 mln 50nt reads per sample. We identified around 90 000 potential alternative splicing targets out of more than 150 000 mRNAs with more than one exon for both tested lines. Using bioinformatics tools such as BowTie, TopHat, Cufflinks, Cuffdiff and CummRbund we managed to identify 4914 transcripts with different splicing patterns, 5133 genes with different expression level and 55 genes, which differ in promotor sequence between the two maize lines.
In addition we confirmed expression for 6 401 novel transcripts in sensitive line and 3 696 in tolerant line compared to the sequenced genome of maize B73 line.

P3.15

KRAB domain triggers DNA methylation during reprogramming of human induced pluripotent stem cells

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Generation of induced pluripotent stem cells (iPSCs) from somatic cells landmarked a new field in stem cell research providing novel opportunities for basic research and regenerative medicine. During reprogramming cells undergo ordered epigenetic alterations including histone modifications and DNA methylation. The underlying molecular mechanisms remain largely unknown and are subject of intensive research. Our research interest focuses on a family of zinc finger proteins carrying KRAB domain. Binding of KRAB-ZFPs to DNA in the vicinity of gene promoters triggers transcriptional silencing through histone modifications that in some settings leads to DNA methylation. Although various KRAB-ZFPs have been associated with pluripotency and development, the physiological roles of these transcriptional repressors remain unclear.

Here, we aimed to probe if KRAB domain may trigger DNA methylation of exogenous PGK (phosphoglycerate kinase) promoter during the reprogramming of human primary fibroblasts to the iPS cells. We took advantage of inducible system that relies on conditional, doxycycline (Dox)-dependent binding of chimeric tTRKRAB transrepressor to tetO element from *E. coli* tetracycline operator. In order to engineer the reporter cell lines, the primary fibroblast cells were transduced with the lentiviral vectors carrying PGK-GFP expression cassette flanked by tetO sequences and cDNA encoding tTRKRAB. The engineered cells were induced to pluripotency by forced expression of Oct4, Klf4, Sox2 and Myc genes and cultured in the presence or absence of doxycycline. The obtained individual iPS colonies were isolated and propagated. The pluripotency of the obtained cells was confirmed by analysis of expression of embryonic markers by RT-PCR and immunofluorescence. The methylation of the PGK promoter in the iPS cells that were obtained in the absence of doxycycline was quantified by bisulfite sequencing FACS analysis of GFP fluorescence. The histone modifications of the integrated expression cassette will be further analysed by chromatin immunoprecipitation.

The obtained results using the lentivector-based conditional system will help us to further investigate potential role of KRAB-containing zing fingers in methylation of endogenous promoters during the reprogramming process.

P3.16

Expression of genes involved in iron metabolism in colorectal cancer

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Colorectal cancer is the third most common cancer in human population and the second most common cause of cancer-related death. The prognosis is related to the stage at which the disease is detected. Unfortunately, blood tests, such as carcinoembryonic antigen (CEA) have been disappointing due to their low sensitivity in patients with early stage of disease. Also other serum biomarkers such as MMP-9, complement C3a des-arg and α-defensins are not sufficient, however they are more sensitive and specific than CEA. Thus, specific diagnostic biomarker of early stage colorectal cancer is needed.

To significantly reduce the mortality, the faecal occult blood test is still used in screening programmes. Chronic occult blood loss may cause anaemia, which is a common presenting symptom of colorectal cancer. Recent study has shown that up to 6% of patients with iron deficiency anaemia (IDA) had colorectal cancer and the induction of hepcidin release stimulated by IL-6 is crucial to this process. Hepcidin prevents cellular iron export by internalization and degradation of ferroportin. At the level of the macrophage, which are responsible for iron recycling, this results in iron sequestration and interrupts iron delivery to erythroid precursor cells thus causing anaemia. Increased hepcidin levels can also cause an accumulation of iron in other ferroportin expressing cell lineages such as colonocytes. Raising colonocyte iron levels can result in increased Wnt signaling which has been shown to be crucial in colorectal carcinogenesis.

The aim of our study was to analyze the expression of several genes, which are involved in iron metabolism or regulation of hepcidin expression in colorectal cancers. We collected tissue samples from 70 colorectal cancer patients and found few miRNA genes, which expression is significantly different ($p<0.0001$) in specimens of colorectal carcinoma *vs.* normal colonic tissues from the same resection specimen in patients with early stage of disease. Now we are going to investigate whether this difference is also observed in serum.

P3.17

Phosphorylation of splicing factors: the curious case of topoisomerase I (acting as a kinase)

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Human topoisomerase I (topo I) is a bifunctional nuclear enzyme: it resolves supercoiled DNA and phosphorylates serine/arginine-rich splicing factors (SR proteins). The latter activity is much less studied and a key features of the enzyme, i.e. ATP binding site, catalytic centre or substrate specificity remain unclear. Topo I phosphorylates SRSF1 taking part in constitutive and alternative splicing, while it does not recognize similar SR protein, SRSF9, as a substrate. Interestingly, GST pull-down assay confirmed that SRSF9 interact in the same way as SRSF1 with topo I [1]. This observation arose the question: how topo I distinguishes SR proteins available as a substrate from others?
To elucidate most important features of topo I as a kinase, we constructed several recombinant substrates containing point mutations within RS domain of SRSF1 or RS domain originating from different SR proteins. Using them as a protein substrate during topo I kinase assay lead us to following conclusions: (1) properties of RS domain are solely responsible for the phosphorylation catalyzed by topo I, (2) proper interaction of SR proteins with topo I do not guarantee phosphorylation of RS domain, (3) putative motif, a target of topo I, within RS domain (RX_7RSRSX_4R) might be an initial contact site during phosphorylation, and (4) the Arg208 residue in RS domain of SRSF1 is critical for phosphorylation catalyzed by topo I, which suggest an engagement of topo I residues interacting with Arg208 in forming of topo I active site.
To date, the molecular structure of the topo I–SRSF1 complex remains unsolved as both protein partners contain intrinsically unfolded domains. Instead, our approach employes molecular modeling and wet verification of the topo I–SRSF1 complex. Our previous study confirmed the accuracy of the molecular model [1], and therefore, next step in our study should be the site-directed mutagenesis of topo I, which will clarify molecular mechanism of SR protein phosphorylation catalyzed by topo I — the hallmark of coordination between DNA replication and transcription [2].

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P3.18

Evaluation of ABCB1 expression in *Helicobacter pylori*-infected patients

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Helicobacter pylori is gram negative bacterium. Its characteristic feature is the production of urease which is used to identify infection by diagnostic tests. The *H. pylori* infection is one of the most common factor of inflammatory changes of stomach mucosa membrane, peptic ulcer of the stomach and duodenum disease, and gastric cancer. Antibacterial therapy is recommended for all *H. pylori*-infected patients. Effective removal of the infection can lower the risk of recurrent gastric and duodenal ulcers. Nowadays the big problem is effective eradication of *H. pylori* infection. Uneffective therapy can lead to chronic inflammation and even to cancer. Every year is noticeable increase bacterial resistance to antibacterial treatment One of the reason of this situation can be connected with the transporters encoded by genes from ABC family.
P-glycoprotein encoded by the ABCB1 gene is a conventional transporter. It works as ATP-dependent pump responsible for removing xenobiotics from the cell. It is known that drugs used in the eradication of H. pylori infection belong to P-gp substrates. It is proved that the increased expression of the ABCB1 is one of causes of multidrug resistance. This can lead to lack of efficacy of drug therapy. For this reason, it seems to be important to determine the level of expression of ABCB1 in patients infected with H. pylori.
Material: examined group: stomach mucosa samples collected during endoscopy, control group: blood samples from donors.
Methods: urease test — detecting the presence of *H. pylori* infection, assessment of ABCB1 gene expression: qualitative and quantitative PCR.
Results: Qualitative assessment of ABCB1 gene expression were performed in 78 samples. In 69 cases, expression of the gene was observed. These samples were subjected to quantitative analysis. So far, 43 samples have been subjected to quantitative assessment of ABCB1 gene expression.
We observed differences in the level of the expression in tested samples.
The difference in the level of the ABCB1 expression in the examined group compared with the control group can affect the level of P-gp. It can cause the diversified effectiveness of the eradication of *H. pylori* infection.
Low expression level of glycoprotein P can lead for increasing the risk of the development of the gastric ulcer.

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P3.19

Formation of nucleoprotein filaments by the mammalian DNA methyltrasferase Dnmt3a

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We have shown previously that the C-terminal domains of Dnmt3a and Dnmt3L form a heterotetrameric complex, which polymerizes on the DNA forming linear filaments [1, 2]. The structure of Dnmt3a/3L tetramer shows that Dnmt3a has two interfaces for protein/protein interaction. One is the RD interface that mediates the Dnmt3a-3a contact, the second is the FF interface, that is used in the structure to mediate the Dnmt3a-3L contact [1]. Amino acid similarity and modeling suggests that the FF interface could also support self interaction of Dnmt3a, suggesting that Dnmt3a may form linear polymers. Analytical centrifugation confirmed that Dnmt3a-C can form dimers, tetramers and higher aggregates in solution. Each RD interface of the Dnmt3a linear polymer creates a putative DNA binding site, suggesting that such polymer could bind several DNA molecules oriented in parallel. DNA binding and scanning force microscopy experiments show that in the absence of Dnmt3L Dnmt3a also polymerizes on the DNA forming nucleoprotein filaments, however in contrast to the Dnmt3a/3L complex, Dnmt3a alone tends to synapse bringing two (or more) molecules of DNA together. We show additionally that both interfaces are necessary for the heterochromatin localization of Dnmt3a in the cells. Overexpression of the wt Dnmt3L leads to the release of Dnmt3a from pericentromeric heterochromatin, Overexpression of Dnmt3L in cells leads to the release of Dnmt3a from heterochromatic regions, which may increase its activity for methylation of euchromatic targets like the differentially methylated regions involved in imprinting. We propose a novel function for Dnmt3L that would cap on the Dnmt3a linear polymers and therefore prevent vertical polymerization of Dnmt3a [3].

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P3.20

Correlation of changes in total DNA methylation level and maize resistance to herbicide stress

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The term "epigenetics" defines heritable states of gene activity level not encoded in the DNA sequence. In recent years, it has become clear that dynamic changes in chromatin properties like DNA methylation and histone modification also contribute to transcriptional and post-transcriptional regulation of gene expression, important for stress responses. Epigenetic regulation of gene expression is dynamically developing field of research, especially in plant biology.

The maize is one of the most important fodder crops cultivated in our climatic zone, with great economic consideration and wide industrial application. In cultivation of maize widely applied is Roundup, a foliar herbicide of the systemic activity. It is absorbed by green parts of plants, and then translocated through the whole plant causing their necrobiosis.

In research carried out in our laboratory we observed various reaction of different strains of maize to herbicide. For our experiments we selected two strains displaying the natural resistance or sensitivity to glyphosate. The aim of the work is examining the molecular mechanisms which determine this feature.

The use of the microarray technology with probes specific for *Zea mays*, allowed us to infer about changes in the genes expression level in maize under stress conditions caused by herbicide Roundup, depending on the time (days 0 and 7 after treatment). Bioinformatic analyses of results were carried out to select genes involved in the plant response to the abiotic stress.

To investigate the effect of stress conditions on m^5C level in plants that could reveal difference in stress resistance, we applied two-dimensional TLC separation method of radioactive labelled nucleotides from enzymatic hydrolysate of total DNA. The quantified signals of spots of m^5C, C and T were measured and the amount of m^5C in relation to pyrimidines C and T was evaluated with the Graph Pad Prism Software. Subsequently we examined level of m^5C in sequence of genes which indicated significant changes in expression level.

In our observations we imply that there is statistically significant difference in total DNA methylation level in plants sensitive and resistant to herbicide stress and its correlation with changes in gene expression level.

P3.21

Genetic and epigenetic analysis of GRHL genes in human skin cancers

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Grainyhead-like proteins (GRHL) belong to a highly conserved family of transcription factors that are critical for development and homeostasis of the surface ectoderm across a wide range of species [1]. Mammalian GRHLs are necessary for maintaining the structure and balance of proliferative epidermis. They control transcription of genes coding: E-cadherin, desmoglein 1, transglutaminase I, keratin VIII, RhoGEF19 and PTEN, FAS and DR5. Based on literature data and our preliminary results we hypothesize that there is a link between GRHL genes and skin cancers. According to recent studies, the expression levels of GRHL3 in human head and neck non-melanoma skin cancers are reduced by 90% in over half of the samples studied [2]. We predict that this phenomenon will extend to other non-melanoma skin cancer types, as well as to melanoma, and that the alterations in GRHL1 and GRHL2 geneswill also be found in these cancers. Our preliminary studies have shown that reduced level of Grhl1 expression in mice increases the incidence of chemically induced (DMBA/TPA) non-melanoma skin cancers. Links to theprocess of carcinogenesis have also been shown for GRHL2. This transcription factor is involved in telomerase activation and immortalization of keratinocytes during malignant transformation, leading to the formation of oral squamous cell carcinoma [3].
The aim of the proposed research is to investigate whether various types of human skin cancers are accompanied by changes in the expression levels as well as genetic and epigenetic changes of GRHL1-3 genes. We are going to specifically search for: upregulation or downregulation of the GRHL expression, specific pointmutations, loss of heterozygosity, copy number variation and changes of methylation profile in regulatory sequences. To detect and identify GRHL gene disruptions in skin cancers, we will use genetic and epigenetic approaches, such as New Generation Sequencing, DNA-methylation analysis and qRT-PCR.
Our findings will provide new molecular insights into the links between the GRHL genes and epidermal neoplasia, which will enhance our knowledge of the molecular basis of skin carcinogenesis in the human context.

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P3.22

Evaluation of progression of esophageal cancer patients in the Caucasian population based on analysis of expression of selected genes

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Esophagus cancer is classified in sixth place in terms of new cases and fifth in terms of mortality among men in the world. In Poland, there are only few scientific reports of research on esophageal cancer at the molecular level. Most of the publications regarding this type of cancer in the Polish population are review articles summarizing the knowledge of high risk areas. In countries considered high-risk research is being conducted at the level of transcription and translation of genes involved in the development of cancer in the esophagus. The few reports on global miRNA expression correlate with the genes and polymorphisms responsible for the etiopathogenesis of esophageal cancer. In our research we selected some of the genes responsible for etiopathogenesis, including miRNA genes and analyzed them using samples of esophageal cancer and compared them with negative control. We used real-time PCR. The aim of this study was to find a correlation between expression of studied genes related to pathogenesis and miRNA genes with the progression of esophageal cancer.

P3.23

Blood circulating miRNAs as potential predictive biomarkers in prostate cancer patients

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Introduction: Prostate cancer is the second diagnosed cancer and sixth leading death cancer in males worldwide. This cancer develops in men over fifty and is classified as an adenocarcinoma originates, in most cases, from prostate basal cells. This cancer can metastasize to the bones, lymph nodes, rectum, bladder and lower uretrus. Prostate tests screening are controversial and may lead to mistaken procedures in some cases. There is a need to create simple and sensitive diagnostic tool for therapy monitoring. Nowadays, diagnostic researches are focused on measurement of circulating miRNAs in blood. miRNAs are small, non-coding RNAs, that regulate gene expression. It's proven, they play crucial role in tumorigenesis and metastasis. miRNAs from cancer cells can enter to blood stream, be protected against RNase activity and as stable tumor-derived miRNAs used to detection of presence and development of cancer. In this study we compare levels of selected miRNAs: -21, -100, -141, -143 and -221 in prostate cancer patients and healthy control.
Purpose: Investigation of blood circulating miRNAs as predective biomarkers in prostate cancer patients.
Methods: Total RNA has been isolated from 44 cancer patients and 41 healthy controls using TRI reagent method. TaqMan MicroRNA Assay (Applied Biosystem) has been used to determine selected miRNA expression levels. The miRNA levels of cancer patiens have been compared to healthy controls using the $2^{-\Delta\Delta CT}$ method. Statistic analysis were performed with MedCalc Software 10.3.2 and Statistica 10.
Results: Four of analyzed miRNAs significantly differ between cancer patients and healty one, showing lower expression level for healty group. One of analyzed miRNAs – miR-100 did not show prognostic properties (AUC=0.551, p=0.41) The best single miRNA was miR-143 with the area under ROC curve (AUC) 80.3% (p 0.0001) and correct overall clasification of 71.8%.The best diagnostic model was observed for panel consisting of three miRNAs: miR-100, miR-141, miR-143 (AUC=83.9%, p<0.0001) resulting the highest overall correct classification 78.8% compared to all five miRNAs panel with overall correct classificaton 67.06%.
Conclusions: Analysis of expression miRNAs -100, -141 and -143 may be used as diagnostic tool for monitoring the course of treatment prostate cancer patients. Research leading to combine miRNA expression analysis with clinical applications are done currently.

P3.24

A GntR-like protein SCO6294 from *Streptomyces coelicolor* A3(2) is involved in the regulation of secondary metabolism and differentiation

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Majority of medically important natural compounds are secondary metabolites of bacteria from the genus Streptomyces. Genomic studies revealed that single strains have dozens of gene clusters for potentially active compounds which have not been detected under laboratory conditions. Secondary metabolites production is correlated with the process of mycelium differentiation and strongly depends on the environmental conditions. A broad range of regulatory proteins are involved in the control of both differentiation and secondary metabolizm. We investigated the role of SCO6294 protein, a putative transcriptional regulator belonging to HTH GntR family.
A SCO6294 deletion mutant of *Streptomyces coelicolor* A3(2) and SCO6294 overproducing strain were constructed. Both of them retained the ability to sporulate and to produce actinorhodin, prodiginines and coelimycin. The effect of SCO6294 level on the secondary metabolites production depended on the medium composition. Overproduction of the regulatory protein inhibited actinorhodin synthesis in the absence of glucose. Deletion of SCO6294 gene resulted in a delay in aerial mycelium formation and sporulation. A consensus sequence recognized by SCO6294 protein was proposed using a bacterial one hybrid system. Promoter of SCO6294 was found active in the wild type S. coelicolor strain carrying a luciferase reporter plasmid as opposed to the overproducing strain where no luciferase activity was detected.
We conclude, that the protein SCO6294 is not necessary for the production of three colored secondary metabolites. It is involved in the control of actinorhodin production. Its activity depends on the medium composition. The regulator also affects the process of differentiation. SCO6294 is likely an autorepressor.

P3.25

Quantitative polymorphism of linker histone subtype H1.d

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Qualitative polymorphism of avian histone H1 subtypes is usually identified by the presence of two or three allelic electromorphs differently located in a polyacrylamide gel due to alterations in their net charges and/or molecular weights (Kowalski & Pałyga, 2012). On rare occasions, however, a differential abundance of allelic variants may reflect a quantitative polymorphism of a histone H1 subtype. Differential quantities of erythrocyte histone subtype H1.d detected during quail population screening may represent a quantitative polymorphism associated with a differential level of the allelic variant expression.

Histone H1 preparations isolated from Japanese quail erythrocytes have been found to contain histone H1.d bands and spots of high, intermediate and low intensities in one-dimension acetic acid-urea and two-dimension SDS-polyacrylamide gels, respectively, These phenotypes were designated as d^h, d^hd^l and d^l. The quantitative variation in histone H1.d was recorded in 10 (phenotype d^h), 36 (phenotype d^l) and 30 (phenotype d^hd^l) individuals in the tested quail population. To find differences in the staining intensities of histone H1.d bands, we measured the area under the peaks in the densitometric tracings of histone H1.d bands resolved in acetic acid-urea polyacrylamide gel and the results were expressed as a percentage of H1.d peak area of respective phenotype in relation to the total peak area of all histone H1 subtypes in a given preparation. The mean values of protein band intensities for phenotypes $H1.d^h$, $H1.d^hd^l$ and $H1.d^l$ amounted to 7.23, 3.2 and 1.31, respectively. The differences in the staining intensities of histone H1.d spots separated in the two-dimension SDS-polaycrylamide gel were calculated based on an integrated density that represented the sum of pixel values in a given protein spot. The mean value of integrated density was 6.77 for the spot $H1.d^h$, 3.03 for the spot $H1.d^hd^l$ and 1.35 for the spot $H1.d^l$. A coefficient of variation was calculated as a ratio of standard deviation and the mean. The estimated values of the variation coefficients for histone H1.d bands ($d^h = 0.08$, $d^l = 0.17$ and $d^hd^l = 0.1$) and H1.d spots ($d^h = 0.1$, $d^l = 0.14$ and $d^hd^l = 0.14$) were below a threshold value of 0.25, indicating a low relative variability within the protein bands as well as protein spots of the H1.d allelic variants.

These data together with a co-dominant inheritance of the alleles at a locus H1.d in progeny from various quail crossings support the occurrence of quantitative polymorphism of the quail histone H1.d.

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P3.26

Application of epigenetic switch for generation of human iPS cells

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Human induced pluripotent stem cells (iPS) are generated by reprogramming of somatic cells through enforced expression of embryonic transcription factors. However, clinical applications require that expression of introduced transgenes must be permanently switched off in the iPS cells and obtained differentiated progenies.

Here, we took advantage of epigenetic switch that relies on doxycycline (dox)-controllable binding of tTRKRAB transrepressor to tetO element. In the presence of dox, tTRKRAB is sequestrated from tetO allowing for transgenes expression. In contrary, dox removal allows for tTRKRAB binding to tetO what results in tight transcriptional repression of proximal promoter through heterochromatin formation. In order to apply this system for reprogramming, the tetO element was inserted into pSTEMCCA lentiviral vector carrying OCT4, SOX2, KLF4 and cMYC under control of EF-1alpha promoter. Co-transduction of human skin fibroblasts with obtained pSTEMCCA-tet and pLV-HK carrying tTRKRAB cDNA followed by culture in presence of doxycycline allowed for expression of reprogramming factors and thus efficient generation of human iPS clones. Obtained clones were picked and further cultured in the absence of dox. Tight repression of introduced transgenes in all human iPS clones was analysed by RT-PCR and confirmed full functionality of our system. Pluripotent phenotype of iPS cells was revealed by analysis of endogenous embryonic genes expression using RT-PCR and cell surface protein markers by immunofluorescence. tTRKRAB-mediated epigenetic repression persisted through prolonged culture of obtained iPS cell lines. Importantly, expression of introduced transgenes remained undetectable after differentiation into embryonic bodies.

Our results confirm that our epigenetic switch effectively prohibits re-expression of embryonic transgenes in human iPS cells and their differentiated progenies paving the way for their applications in various fields of regenerative medicine, disease modelling and drug discovery.

P3.27

The effect of histone deacetylase inhibitor on expression levels of histone H3/f

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There are a lof of different modifications of histone tails like acetylation, metylation or phosphorylation. Acetylation is a reversible process and leads to loosening the histone — DNA interactions, it allows *trans* factors to join on the DNA sequences. This junction initiates the transcription. The reverse process, deacetylation, leads to repression of the transcription. In many human diseases, it is necessary to use a histone deacetylase inhibitor (HDACi) like sodium butyrate. Sodium butyrate selectively affects the activation of genes encoding proteins which block cell cycle such as eg. $p21^{WAF1}$ protein and promote apoptosis of cells. Most of HDAC inhibitors block the expression of D, E and A cyclins so they prevent cells mostly in G1 phase of cell cycle. In our work, we want to estimate the influence of sodium butyrate treatment on measurable changes in mRNA levels of histone H3/f subtype. In this case, expression of H3/f is used as a proliferative marker of cells because it is tightly correlated with S phase of cell cycle. Primers for replication –dependent H3/f were designed using Primer Express™ 2.0. The study material consisted of RNA isolated from fibroblast cell cultures. Evaluation of gene expression was performed using quantitative Real time™ PCR (with reverse transcription at the beginning) which enabled quantification of mRNA levels for each reaction cycle. Sodium butyrate was used in a two different concentrations: 3 mM and 10 mM. The inhibitor was added and left for 2 days. After that time it was removed with medium and RNA was isolated in two-hours periods of time. Applying sodium butyrate allows to answer the question whether the inhibition and unlock of cell proliferation results in measurable changes in mRNA levels of H3/f in analyzed periods of time compared to the control (without addition of inhibitor) and permanently blocked cells.

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P3.28

Methylated circulating tumour-derived DNA as a serum biomarker of brain cancer patients

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Tumor-derived DNA has been detected in the serum of patients with various cancers. This fact triggered numerous studies to explore the diagnostic and prognostic potential of circulating DNA, including the detection of aberrant methylation in the promoter region of specific genes. However, this DNA is present in serum in only minute concentration and is often highly fragmented, what makes its isolation difficult.

In this study we applied sodium iodide/glycogen method for DNA extraction from serum of patients diagnosed with brain tumour of glial origin (17 samples) and other CNS tumours (10 metastatic and 6 meningiomas) to evaluate the methylation status of promoter region of *MGMT*, *RASSF1A*, *p15INK4B* and *p14ARF* genes. Apart from serum sample, also brain cancer tissue was available for 16 cases.

The recovery of DNA was sufficient to detect methylation status of selected genes using MSP technique. Among 33 serum samples examined, 13 showed *RASSF1A* promoter methylation (39.39%), 11 *p14ARF* methylation (33.33%), 6 *p15INK4B* methylation (18.18%) and 4 *MGMT* methylation (12.12%). Moreover the correlation between methylation profile in tumour tissue and serum DNA was found in 50% of patients.

We report frequent methylation of *RASSF1A* in DNA found in the serum of brain cancer patients, both diagnosed with primary as well as metastatic brain tumours. Although *MGMT* was found to be methylated in only 3 of 17 glial tumours, for these particular patients it can be an important information, since they can possibly benefit from the alkylator-based therapy. *p14ARF* and *p15INK4B* methylation was also found in both cohorts, which indicates the role of epigenetic inactivation of these tumour suppressors in brain cancers.

This study confirms the presence and acceptable prevalence of circulating tumour-derived DNA in the serum of patients with brain cancers, when isolated with sodium iodide/glycogen method, and suggests great potential for its future use in methylation biomarker detection in the clinics. Circulating tumor-derived DNA in serum of cancer patients shares similar methylation status to those found in the corresponding brain tumour tissue, however not all methylation markers detected in the tumour could be found in the corresponding serum sample. Therefore more research should be done to allow the widespread implementation of methylated genes found in serum into the routine clinical setting.

P3.29

Role of KAP1 protein in reprogramming and self-renewal of mouse induced pluripotent stem cells (miPS)

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Induced pluripotent stem cells (iPS) are obtained by epigenetic reprogramming of adultsomatic cells by forced expression of few embryonic transcription factors. The iPS cells may have a significant impact on basic research and regenerative medicine due to their self-renewal capability and pluripotnent potential. A growing body of evidence suggests that epigenetic mechanisms are involved in the process in which somatic cells acquire embryonic phenotype. Here we probe potential role of KAP1 in the reprogramming of mouse fibroblasts and self-renewal of the obtained miPS cells. KAP1 protein together with repressor transcriptional repressors carrying KRAB domain regulate expression of specific genes through histone modifications and DNA methylation.

In the first series of experiments we have achieved an efficient knockdown of KAP1mRNA in mouse fibroblasts (MF) using lentiviral vectors carrying KAP1-specific shRNA.Next, obtained KAP1-KD and WT fibroblasts have been de-differentiated to miPS cells by lentivector-mediated delivery of Oct4, Klf4, Sox2 and cMyc cDNAs. Phenotype of the miPS colonies was confirmed by expression analysis of selected embryonic markers using RT-PCR and immunofluorescence with specific Abs. Specific effect of KAP1 knockdown was revealed by different morphology and growth kinetic of the emerging miPS colonies.The obtained results suggests that epigenetic mechanisms controlled by KAP1 protein are involved in the reprogramming process that leads to the appearance of the miPS cells.

P3.30

Quantitative assessment of DNA methylation level of repetitive sequences in childhood acute lymphoblastic leukemia

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Aberrant DNA methylation in cancer cells typically comprises local, gene-specific hypermethylation and global hypomethylation. Genome-wide hypomethylation predominantly occurs at DNA interspersed repetitive sequences and is thought to promote tumorigenesis by bringing about genomic instability. The retrotransposable element LINE-1 (Long interspersed nuclear element 1) and element Alu which are largely used as determinants of genome-wide DNA methylation level are found to be highly methylated in normal cells whereas in cancer cells significant hypomethylation has been frequently observed. In some cancer types their methylation was shown to have prognostic value. Among hematological malignancies significant LINE-1 and Alu hypomethylation was found in chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML), whereas in acute myeloid leukemia (AML) only significant hypomethylation of Alu sequences was observed. LINE-1 and Alu methylation status was not reported in childhood acute lymphoblastic leukemia (ALL).

The aim of the present study was to assess the methylation level of LINE-1 and Alu elements in 37 pediatric ALL patients. DNA from whole peripheral blood or peripheral blood mononuclear cells was bisulfite converted and LINE-1 and Alu CpGs methylation level was quantified using a pyrosequencing methylation assay.

The mean methylation level of analyzed CpGs in LINE-1 repeats ranged between 71% and 81%, whereas in Alu elements between 24% and 30%. In peripheral blood and bone marrow samples from healthy donors LINE-1 and Alu methylation statuses were observed at comparable levels. Thus we did not notice clear evidence of global hypomethylation measured by the methylation level of LINE-1 and Alu repetitive elements in childhood ALL.

P3.31

Cloning and the initial characterization of a new murine 3110001I22Rik gene induced during the heat shock

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A mouse 3110001I22Rik gene is located on chromosome 16 in the first intron of the BFAR (bifunctional apoptosis regulator) gene. Data from the BioGPS database (http://biogps.org/#goto=genereport&;id=66598) shows a high expression of the 3110001I22Rik gene mainly in cells with a low level of differentiation (oocytes, fertilized egg, stem cells-like cells), and the immune response cells (T-cells, B-cells, NK-cells). In this study, the expression of 3110001I22Rik was analyzed by RT-PCR in mouse liver, testes, isolated spermatocytes, kidneys, gastrointestinal tract, and several cell lines. The analysis revealed a generally slight expression of the gene at physiological temperature: the strongest expression was observed in stomach, small intestine and in HECa10 cell line (endothelial cells of peripheral lymph nodes). However, the gene was strongly activated following heat shock at 43ºC. This activation is not dependent on HSF1 (heat shock transcription factor 1), which is the main regulator of the heat shock response. By chromatin immunoprecipitation (ChIP), HSF1 binding to the 3110001I22Rik promoter was not stated.
The 3110001I22Rik gene encodes a hypothetical protein LOC66598 containing serine-rich domain. The protein consists of 312 amino acids, its calculated molecular weight is 35.22 kDa. In order to identify the intracellular localization of the protein, 3110001I22Rik cDNA was amplified by PCR and cloned in a frame with the EGFP coding sequence (under the control of the CMV promoter). After the transient transfection of the constuct to the NIH3T3 cells, the LOC66598-EGFP fusion protein was observed mainly in the nucleus and, to a lesser extend, in the cytoplasm. To determine the LOC66598 protein molecular weight by Western blot, the 3110001I22Rik cDNA was cloned in a frame with the HA coding sequence and anti-HA antibody was used. Molecular weight of the LOC66598 protein was experimentally assessed on approximately 45 kDa. To silence the 3110001I22Rik gene expression, three different shRNA sequences were designed and cloned. The effect of silencing on the phenotype and behavior during thermal stress will be studied in cell line HECA10.
Observed pattern of the 3110001I22Rik gene expression indicates that the gene is repressed after zygotic genome activation. Its expression would be dependent on a high level of calcium ions, since it is a common feature of all 3110001I22Rik expressing cells.

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P3.32

7-methyl guanosine 5'-diphosphates modified in bridging and non-bridging position as inhibitors of Decapping Scavenger enzyme (DcpS)

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DcpS (Decapping Scavenger) enzyme affects regulation of gene expression by hydrolysis of short cap structures (m^7GpppN_n) remaining after mRNA 3'-5' decay. The natural cap is an interesting structure occurring at the 5' mRNA chain which protects mRNA from degradation by cellular 5' exonucleases and influences many processes via interactions with cap binding proteins, such as translation initiation factor 4E or nuclear cap binding complex CBC. DcpS acts against the accumulation of free cap structures remaining after the mRNA decay, thereby preventing the inhibition of proteins crucial for mRNA splicing and translation and consequently potentially toxic effects.
The interplay between DcpS and cap binding proteins can be exploited for the therapeutic purpose. DcpS-resistant inhibitors of translation initiation factor 4E may reduce level of translation process in cancer cells and prevent production of overexpressed proteins. DcpS is also a target protein in spinal muscular atrophy which is connected with deficient SMN (Survival Motor Neuron) protein production. Inhibiting DcpS by means of quinazoline derivatives, was shown to augment expression of SMN but the mechanism of this process is not exactly understood.
In this work we have examined mononucleotide cap analogs bearing either a bridging or non-bridging modification within the diphosphate bridge as inhibitors of human DcpS enzyme. The analogs bearing the non-bridging phosphorothioate or boranophosphate modification: m^7Gp_Sp and $m^7Gp_{BH3}p$ exist in a form of two diastereomers, which were resolved by means of RP HPLC and studied separately. The rationale for introducing the selected modifications was to enhance compounds enzymatic stability and evaluate influence of the atomic mutations (O to S, O to BH_3, O to CH_2, O to NH substitutions) on interaction with DcpS. We developed an RP-HPLC based assay to study the kinetics of hydrolysis of a dinucleotide analog m^7GpppG in the presence of the inhibitors. The determined IC_{50} values reveal that the potency to inhibit DcpS strongly depends on the type of modification, as well as on phosphorus center stereochemistry.

P3.33

Small RNA competition for binding to the bacterial chaperone protein Hfq

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Small regulatory RNAs affect bacterial cell's adaptation to changes in their extracellular environment. The bacterial chaperone protein Hfq facilitates the binding of these regulatory RNAs to their target mRNAs, thus allowing for the control of translation of selected genes. This homohexameric ring-shaped protein is homologous to eukaryotic Sm proteins and was shown to bind to RNAs using two distinct binding sites. Recent data showed that the concentration of the Hfq protein in cells is limiting for the regulation of translation by trans-encoded small RNAs (sRNAs). This could possibly lead to competition among sRNAs for access to Hfq.
Here, the thermodynamic and kinetic properties of different sRNA binding to Hfq were compared using high-throughput filter binding assays to determine their properties in the competition for binding to this protein. The results showed that Hfq bound different sRNAs with similar affinities and kinetics. However, the sRNAs widely differed in their ability to outcompete other sRNAs from the complex with Hfq. The differences in the competition performance of the individual sRNAs, which have been observed here, could serve to modulate their access to Hfq, thus allowing for flexible bacterial cell adaptation to changing environmental conditions.

P3.34

Influence of divalent cations on G-quadruplex DNA structures

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G-quadruplexes are four stranded DNA structures formed from guanosine residues. Most information on G-quadruplexes comes from chemical and structural biology studies. Monovalent cations (sodium and potassium) nucleate the G-quadruplex structure and are found trapped between the base planes. There are still questions about the physiological formation and role of G-quadruplexes although potential repeat G-tracts are found with higher than expected frequency in DNA sequences. Little is known about the influence of divalent cations, such as calcium and zinc, whose concentrations are known to either vary, or be modulated, inside cells. We are currently working on a telomeric G-quadruplex sequence for which zinc-binding data has been reported by titrations followed by UV and Circular Dichroism (CD). Our first aim is to measure accurate binding data for zinc (1 to 50 nanomolar can be considered the intracellular zinc signalling range). We will extend the studies with PAGE and NMR studies of the zinc complex to further characterize the zinc-telomere quadruplex and compare it with the sodium and potassium forms. We will also measure competition between the cations. Our aim is to extend the studies to potential G-quadruplexes associated with calcium and zinc modulated proteins. In conclusion, divalent cations associated with signalling pathways may influence genetic events by binding directly to DNA elements and the formation of G-quadruplex structures.

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P3.35

An GntR like protein SCO3932 is a potential regulator of the coelimycin producing gene cluster from *Streptomyces coelicolor* A3(2)

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Introduction: *Streptomyces* are bacteria with complex life cycles involving mycelium differentiation and production of a broad range of secondary metabolites with a number of biological activities including the majority of antibiotics of natural origin. Consequently the genus *Streptomyce*s is of major interest in the search for new biologically active compounds. *Streptomyces coelicolor* A3(2) is a well known producer of coloured polyketide antibiotics: red undecylprodigiosin, blue actinorhodin and recently reported coelimycin.
We have observed a that production of coelimycin is dependend on medium composition, (glucose presence) [1] similarly Gottelt *et al.* [2] showed influence of glutamate as nitrogen source on coelimycin production.
Materials & methods: Protein was caught by affinity chromatography and analyzed in electrospray mass spectrometry. Interactions were confirmed by electromobility shift assays with non-labelled and radiolabeled probes.
Results: Here we present the search results for direct regulators of coelimycin polyketide synthase gene cluster (*cpk*). Using the DNA region between genes *cpkA* i *cpkD* we caught protein SCO3932. Find protein is homological to GntR protein family containing wHTH motif and UTRA effector domain. We have found protein SCO3932 binds putative promoter region of structural genes cpkABC, and genes cpkO and cpkN coding for SARP-like activators. Also its own promoter region is bound by protein SCO3932.
In conclusion: We suspect that the protein SCO 3932 is responsible for the regulation of expression gene cluster *cpk*.

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P3.36

Comparative analysis of laminopathies-associated mutations: L263P, E358K, D446V, H222P, D50 and its effect on proliferation, nuclear envelope morphology and cell cycle

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Laminopathies are group of degenerative disorders with not fully understand pathogenesis. They are caused by mutations in genes of lamins and nuclear lamina associated proteins. Lamins are nuclear envelope main structural proteins. They are responsible for mechanical functions, organization of chromatin, DNA replication, regulation of transcription factors, epigenetics, DNA repair, transcription, cell cycle regulation, cell development and differentiation, nuclear migration and apoptosis.
To investigate mechanism of laminopathies, we prepared series of constructs of lamin A mutants associated with most common lamin disorders: Emery-Dreifuss Muscular Dystrophy type 2 (EMD2) and Hutchinson-Gilford Progeria Syndrome (HGPS). Mutants were prepared as fusion proteins GFP-lamin A in plasmid pEGFP-C1 with neomycin resistant marker. We have chosen novel, not analysed yet, dominant, point mutations: L263P, E358K, D446V and well characterized H222P as models of EMD2. As model for HGPS we used well characterized construct D50 that reflects most common dominant mutation causing alternative splicing that result in producing protein without 50 amino acids.
Overexpression of those constructs in cell lines Hek 293 and NHDF show that mutations results in broad spectrum of lamin A nuclear phenotype. Mutants L263P and D446V form different deposits in nuclear envelope and nucleoplasm, while E358K have even smaller tendency do create deposits than overexpressed wild-type lamin A. Each mutation can disrupt nuclear envelope and nuclear lamina, influence level and localization of endogenous lamin A and lamin C.
We also developed cell lines Hek 293 with permanent overexpression of lamin A and its mutants D446V and D50. Cell lines D446V and D50 show diminished proliferation, alternated morphology similar to starved cells and disrupted endogenous lamin C localization. FACS analysis also showed changes in cell cycle.

P3.37

Comparative analysis of laminopathies-associated mutations: L263P, E358K, D446V, H222P, D50 and its effect on proliferation, nuclear envelope morphology and cell cycle

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Laminopathies are group of degenerative disorders with not fully understand pathogenesis. They are caused by mutations in genes of lamins and nuclear lamina associated proteins. Lamins are main structural proteins of nuclear envelope. They are responsible for mechanical functions, organization of chromatin, DNA replication, regulation of transcription factors, epigenetics, DNA repair, transcription, cell cycle regulation, cell development and differentiation, nuclear migration and apoptosis.

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Overexpression of those constructs in cell lines Hek 293 and NHDF show that analysed mutations results in broad spectrum of lamin A nuclear phenotype. Mutants L263P and D446V form different deposits in nuclear envelope and nucleoplasm, while E358K have even smaller tendency do create deposits than overexpressed wild-type lamin A. Each mutation can disrupt nuclear envelope and nuclear lamina, influence the level and localization of endogenous lamin A and lamin C.

We also developed cell line Hek 293 with permanent overexpression of lamin A and its mutants D446V and D50. Cell lines D446V and D50 show diminished proliferation, alternated morphology similar to starved cells and disrupted endogenous lamin C expression. FACS analysis also showed lower level of chromatin amount in D446V cell line.

P3.38

αB-Crystallin as a novel target in malignant glioma therapy

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Glioblastoma multiforme (GBM) is a the most common and deadliest malignant brain tumor in adults, which is characterized by highly invasive growth, a rampant genetic instability and intense resistance to apoptosis. Even with aggressive surgical resections, along with recent advances in radiotherapy and chemotherapy, the prognosis for GBM patients remains poor: median survival after diagnosis is about 8–12 months. Brain tumors preferentially express a number of specific proteins and RNA markers, that may be exploited as potential therapeutic targets for design of new treatment modalities based on nucleic acids.

Recently, we have identified a strong overexpression of αB-crystallin (CRYAB) in malignant glioma tissues. αB-Crystallin is a member of the small heat shock protein family (sHsps) and was identified as the anti-apoptotic agent that is connected with many human disorders. It was detected in brains of patients with neurological diseases, including Alzheimer, Parkinson, Alexander and Huntington diseases as well as in human carcinomas of lungs, head and neck, breast, kidney or liver.

We designed and implemented the experimental therapy of patients suffering from malignant brain tumors based on application of long (~155 pz) double-stranded RNA (dsRNA) specific for CRYAB mRNA. That therapeutic agent, called ACRY-RNA, induces RNA interference (RNAi) pathway to inhibit the synthesis of CRYAB and, thus, enables the activation of apoptosis in cancer cells. We showed that ACRY-RNA specifically and efficiently inhibited CRYAB synthesis in human cell culture including glioblastoma cell line (U118-MG). Moreover, we showed a higher level of apoptosis in cancer cells transfected with ACRY-RNA. Further implementation of ACRY-RNA to the experimental treatment of GBM patients in clinic is under evaluation.

P3.39

Epigenetic regulation and regeneration: the search for differentially methylated genes in the MRL mouse

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The MRL mouse is an inbred laboratory strain, which was developed in the 60's of the 20th century and has been extensively used as a model of lupus erythematosus. The regenerative abilities of the strain were discovered in the 90' when the MRL mouse was shown to close 2 mm hole punches made in the ear pinnae four weeks after injury without scarring. The phenomenon has not been observed in other mouse strains, where the holes remain for life. The process is accompanied by cartilage regrowth, normal vascularisation, the restoration of hair follicles and peripheral nerves. Enhanced regenerative response in the MRL mouse has been observed in heart after cryo-injury of the right ventricle, spinal cord following hemi-section, digits after amputation, and articular cartilage. A remarkable feature of the strain is that the MRL mice retain several foetal biochemical characteristics as adults, including increased expression of stem cell markers. The epigenetic regulation, though known to play a key role in the organ development, has not been examined with respect to regeneration.
The idea of our project is to examine the epigenetic basis of regeneration by comparing genome-wide DNA methylation and expression profiles in two murine strains: the MRL mouse which displays enhanced regeneration abilities and the control C57BL/6J mouse. In our experiments we applied methylated DNA immunoprecipitation followed by the analysis using NimbleGen microarray platform (Roche) which covers all promoter regions in the murine genome. We examined the promoter methylation profiles in heart, liver and spleen samples. The preliminary results allowed us to extract a number of genes that show the most spectacular differences in the DNA methylation of promoter regions between the MRL and the control strain (i.e. 100% methylated and 100% unmethylated). Our particular interest is focused on these among the differentially methylated genes which display similar methylation pattern in the MRL mouse and murine embryonic stem cells. The microarray data are being verified by quantitative Real-Time PCR.
We believe that the elucidation of the epigenetic basis of regeneration phenomenon in the MRL mouse will contribute to a better understanding of regeneration processes, as well as it may provide clues on how cross the limitations of regeneration in mammals.

P3.40

FUS protein as a new factor interacting with U7 snRNA/snRNP

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The U7 snRNP is an essential factor for replication-dependent histone RNA 3'end formation. However, it has also been suggested to be involved in other RNA processing events and in the transcriptional regulation of certain genes. To further elucidate the mechanism of histone mRNA 3' end processing and to detect unknown proteins associated with the U7 snRNP we used affinity purification strategy based on tagged U7 snRNA.
For this purpose U7 snRNA was extended at its 5' end by short hairpins recognized by the bacteriophage MS2 coat protein. Protein extracts from cells stably expressing tagged U7 snRNAs were incubated with recombinant MS2 protein fused to maltose binding protein (MBP), then bound to amylose resins and eluted with an excess of maltose. Western blots confirmed the presence of U7 snRNP-specific proteins and other components of the histone RNA 3' end processing complex in the purified fractions. Mass spectrometric analysis of such affinity-purified fractions then revealed the presence of new, unknown proteins interacting with U7 snRNA. One of the most interesting is multifunctional, RNA/DNA-binding protein FUS/TLS. Preliminary experiments confirmed the interaction between FUS and the U7 snRNA/snRNP. Interestingly, this FUS:U7 snRNA interaction seems to be activated in S phase where the core histone genes are expressed. Moreover, we found that FUS coimmunoprecipitates with NPAT and ZFP100, although it does not directly influence histone pre-mRNA 3' end processing.

P3.41

DNA methylation analysis of hypoxia-inducible factor-1α (HIF-1α), prolyl hydroxylases (PHDs) and asparaginase hydroxylase (FIH) in colorectal cancer

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Colorectal cancer (CRC) is one of the leading causes of death from malignant disease among adults [1]. The development of solid tumors is usually associated with hypoxic conditions characterized by overexpression of hypoxia-inducible factor-1α (HIF-1α), which encodes an essential component of the HIF-1 transcription factor [2]. HIF-1 plays a pivotal role in cellular adaptation in an oxygen depleted milieu [2]. HIF-1 is post-translationally regulated by prolyl hydroxylases (PHD1, PHD2, PHD3) and factor inhibiting HIF-1 (FIH) that target HIF-1α subunit for proteasomal degradation in normoxic conditions [3]. All PHDs, FIH and HIF-1α genes posses CpG island within their promoter region. It has been shown that DNA hyper or hypomethylation of gene regulatory sequences may change the expression of cancer related genes in CRC [4].
Using high-resolution melting analysis we detected higher level of DNA methylation within promoter region of PHD3 gene in primary colonic adenocarcinoma compare to histopathologically unchanged tissue isolated from the same 90 patients. Moreover, the high level of DNA methylation correlated with decreased PHD3 transcript and protein level. There was no evidence of methylation in PHD1, PHD2, FIH and HIF-1α genes and impact on the altered mRNA and protein levels of PHD1, PHD2, FIH and HIF-1α in primary colonic adenocarcinoma compare to histopathologically unchanged tissue.
Observed methylation-induced epigenetic silencing of PHD3 may help to elucidate the mechanisms controlling of HIF-1α gene expression in colorectal cancer.

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P3.42

The effect of PMCA2/PMCA3 suppression on PC12 cells sensitivity to retinoic acid.

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The plasma membrane calcium ATPase (PMCA) is a transmembrane protein which maintains low cytosolic calcium concentration. There are four PMCA isoforms, which composition varies in membranes of different kinds of cells. This phenomenon can be explained by functional specificity of PMCAs and cell-specific requirements for calcium homeostasis. In this study examined whether PMCA composition is crucial for the response of cells to administration of retinoic acid (RA). We used stably transfected pseudoneuronal PC12 cells with suppressed expression of PMCA2 or PMCA3 (isoforms typical for neurons) and incubated them with various concentrations of RA for 24, 48 and 72 hours. Microscopic observation of morphology indicate that the differentiation of cells into neuron-like phenotype was most efficient under the influence of 100 μM RA. Moreover, it occurred that PMCA2 and PMCA3 suppression inhibits the formation of neurites. The western blotting analysis of the expression of GAP-43 (neurodifferentiation marker) showed significant increase of this protein in all types of cells during differentiation. However, the increase of GAP-43 expression was significantly higher in PMCA2-supressed cells. Another examined concentration of RA (250 μM) turned out to greatly reduce the viability of control and PMCA2-supressed cells. Interestingly, PMCA3-supressed cells showed high resistance for toxic concentration of RA. These results indicate that the changes in the expression of particular PMCA isoforms specifically affect regulation of RA-dependent neurodifferentiation and susceptibility to RA cytotoxicity.

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P3.43

Regulation of expression of the MboII restriction-modification system

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Type IIS restriction endonucleases recognize asymmetric DNA sequences and cleave outside both DNA strands at fixed positions. The host DNA is resistant to cleavage as all recognition sites are modified by cognate methyltransferase. The opposing functions and activities of proteins that build up restriction-modification (R-M) system are balanced by proper regulation of their expression. The genetic organization of MboII R-M system from *Moraxella bovis*, the object of our study, has no exact counterpart in the other discovered to date systems. It consists of three genes of known function, *mboII*R-encoding a restriction endonuclease, and *mboIIM2* and *mboIIM1*, genes encoding M2.MboII M1.MboII methyltransferases, and moreover the gene of unknown function — *orf654*. The Orf654 proteins is predicted to be 25 kDa in size. The location of this gene between genes of methyltransferases suggests that this protein may be involved in the regulation of gene expression MboII R-M system. Our goal is to examine the degree of orf654 gene polymorphism by screening *M. bovis* strains from different continents, and to investigate the properties of this protein. Full gene orf654 is found always in a genetic linkage of genes MboII R-M system. Cloning and expression of the gene orf654 allowed to overproduce of proteins in the T7 system. The proved sequence of N-terminus of purified protein is consistent with the nucleotide sequence of orf654. We found that the protein has the property of binding to double-stranded DNA. Computational analysis of the orf654 gene promoter region revealed that there are at least two promoters consisting of consensus-like sequences for RNA polymerase subunit σ^{70}. Both promoters are located in the distal part of the gene mboIIM2. Analysis of the efficiency of transcription from $P2_{orf654}$ promoter in fusion with lacZ reporter gene demonstrated its very low level (230 Miller units of activity compared to 60 Mu of background), and the relative strength of $P1_{orf654}$ was 10-fold higher (2000 Mu). In addition, we observed rare phenomenon of transcriptional frameshifting of ribosomes into -1 direction in case of the defective mboIIM2 gene, which suppressed the nonsense mutation located inside of the gene.

P3.44

Expression patterns and diagnostic significance of HOXA5 mRNA in bladder cancer

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The homeobox gene HOXA5 is a transcription factor controlling the embryonic morphogenesis and pathways connected with remodeling and pathogenesis of an adult tissue. Impaired function of the HOXA5 gene has been implicated in the downregulation of the TP53 gene transcription. The purpose of the present study is to investigate the gene expression level of HOXA5, and to correlate it with the HOXA5 promoter methylation, the p53 status, clinicopathological variables, and outcome. The expression level of HOXA5 mRNA in normal and tumor tissue was evaluated by real-time PCR, and the methyltion status was analyzed by COBRA method. The decreased level of HOXA5 mRNA was observed in 44% (22/51) of tumor samples, while remaining showed higher or no difference in HOXA5 expression. High-stage tumors with low expression of HOXA5 mRNA displayed aberrant methylation (>50%) of the promoter region in 28% of cases. HOXA5 mRNA level was significantly lower in invasive tumors ($P < 0.05$; Mann-Whitney U-test) compared to superficial tumors. ROC curve analysis showed that the differential HOXA5 gene expression may distinguish superficial from invasive bladder cancer. There was no significant association between the HOXA5 expression and both the TP53 gene status and overall survival. In conclusion, low expression of HOXA5 mRNA was associated with promoter hypermethylation and higher stage of bladder cancer at the same time higher expression of HOXA5 mRNA was more specific for superficial tumors; therefore, the level of HOXA5 mRNA expression may indicate that HOXA5 could function as a tumor marker for bladder cancer, however, further investigation is needed for validation.

P3.45

MiRNA biogenesis proteins shape differently precursor processing depending on the structure of miRNA gene

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MicroRNAs (miRNAs) are small non-coding RNAs of about 21 nt in length, which take part in a wide variety of physiological and cellular processes. miRNAs act by regulating gene expression in a sequence-specific manner. The target mRNAs are first recognized by miRNAs and subsequently cleaved or blocked inhibiting translation. In plants, miRNAs are encoded mostly by independent transcriptional units and are transcribed into long non-coding primary transcripts (pri-miRNAs), containing miRNA sequences. They can be found mostly in exons, but there are examples where miRNA are located within introns.
We have already identified the structure of 32 Arabidopsis MIR genes. It has been also reported that 11 plant miRNAs are encoded within introns of protein-coding genes. Our further bioinformatic analyses revealed there the existence of additional 17 microRNAs within introns of functional genes. Expression of these miRNAs is probably regulated by their host-gene promoters.
Primary transcripts of miRNA genes are synthesized almost exclusively by RNA polymerase II and contain characteristic hairpin-like secondary structures, in which sequences of mature miRNAs are embedded. Pri-miRNAs are processed by DCL1 (DICER-LIKE 1) accompanied by CBC (a nuclear cap-binding protein complex, composed of two subunits, CBP20 and CBP80), HYL1 (HYPONASTIC LEAVES 1) and SE (SERRATE, zinc-finger protein) proteins into miRNA precursors (pre-miRNAs), which are further cleaved into mature miRNA/miRNA* duplexes. Interactions between these five proteins are necessary to improve the efficiency and precision of miRNA formation, however, apart from DCL1, the function of the rest of plant miRNA processing proteins is still unclear.
To outline their role in miRNA biogenesis we performed studies using our comprehensive platform, called mirEX, based on the high-throughput real-time PCR technique. It allows us to check the level of all known A. thaliana pri-miRNAs (289) at the same time. In our experiments we tested 14-day old se-1, cbc, hyl1-2 mutants and wild type plants. The expression level of over 50% precursors was changed in se-1 and cbc mutants and about 40% in hyl1-2 plants. We divided 60 miRNA genes whose structure is known into three categories: miRNA encoded by the independent transcriptional units without introns or containing introns and miRNA located within introns of a given host-gene. Next, we compared the influence of a given mutant on miRNA biogenesis. Among pri-miRNAs which are encoded by the independent transcription units (intron-less and intron-containing precursors), almost all were HYL1-, SERRATE- and CBC-dependent. In the case of miRNAs embedded in the introns of protein-coding genes, we observed disturbances in pri-miRNA expression level in all analyzed mutants in less than 50% cases. It clearly shows there are other players, apart from known miRNA biogenesis machinery proteins that influence the maturation process of miRNAs located in host-gene introns.

P3.46

KAP1 protein controls sensitivity of breast cancer stem cells to chemotherapy

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Cancer stem cells (CSCs) play an essential role in the development of various types of cancer and are largely responsible for resistance to chemotherapy and radiotherapy, metastases and relapse. Thus, various novel therapeutic approaches specifically targeting CSCs are currently being investigated by academic community and pharmaceutical companies. It has been documented that self-renewal of CSCs is controlled by epigenetic mechanisms that regulate the expression of CSCs-specific genes. Modulation of specific histone modifications and DNA methylation may be exploited to induce differentiation of CSCs and sensitize them to standard chemotherapeutics.
Here, we investigated a role of KAP1 protein in the hemostasis of breast cancer stem cells. KAP1 protein regulates expression of large group of genes through recruitment of histone methylases and deacetylases to specific genomic regions that leads to local DNA heterochromatization. In a first series of experiements, we have silenced KAP1 gene expression in a panel of five breast cancer cell lines using lentiviral vectors carrying specific shRNAs. The knockdown efficiency was confirmed using RT-qPCR, Western blot and immunofluorescence using specific antibodies. Using FACS analysis have evaluated the percentage and morphology of $CD44^{+}/CD24^{-/low}$ CSCs population in the $KAP1^{KD}$ and WT breast cancer cells. We have also tested the effect of KAP1 knockdown on tumor formation ability of the breast cancer cells using the soft agar assay. Next, the modified and control cells were subjected to treatment with serial dilutions of doxorubicin, DNA intercalating drug that is a standard treatment for many types of cancer including breast tumors. We have observed that silencing of KAP1 protein leads to decrease of ED50 of doxorubicin in some of the tested breast cancer cell lines. Our current work is focused on investigating molecular mechanisms that mediates KAP1-dependent sensitivity of breast cancer cells to chemotherapeutics and radiation. Ultimately, our findings may pave the way to novel and more effective therapies for breast tumors.

P3.47

Non-coding RNAs as a molecular markers in malignancy of gliomas

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Tumors and complex tissues consist of mixtures of communicating cells that differ significantly in their gene expression status. In order to understand how different cell types influence on another's gene expression, it will be necessary to monitor the RNA profiles of cells to dissect the mechanisms that regulate their gene expression outcomes.
In recent years, the discovery of non-coding RNA (ncRNAs) has unveiled a slew of powerful regulators of gene expression. In fact, an increasing number of studies have implicated ncRNAs in human health and disease ranging from metabolic disorders to diseases of various organ systems as well as various forms of cancer.
Glioblastoma multiforme (GBM) is the most common type of malignant gliomas, characterized by genetic instability, intratumoral histopathological variability and unpredictable clinical behaviour. GBM is a leading cause of death among children and adults diagnosed with a neoplasia of the brain. The molecular mechanisms that contribute to gliomagenesis have become increasingly clear in recent years, yet much remains to be learned. It is now evident that ncRNAs can regulate a wide variety of tumorigenic processes such as cellular proliferation, differentaiation, angiogenesis, invasion or apoptosis.
a. BC 200 RNA
BC200 RNA (brain cytoplasmatic ncRNA), a small functional RNA (200 nt) has been implicated in the regulation of local synaptodendritic protein synthesis in neurons. It is a small untranslated RNA polymerase III transcript that contains no open reading frames to encode protein amino acids sequences. That RNA molecule operate as a modulator of translation in the activity-dependent synthesis of new protein at the synapse. Subject to activity-dependent regulation, BC200 RNA is specifically transported to dendrites where they are located in synaptodendritic microdomains to regulate on-site translation of local mRNAs.
Translational control, a key element in the modulation of gene expression, is frequently deregulated in cancer cells. We have found that BC200 RNA is atypically expressed in brain tumor tissue.
The expression profile with the Northern blot and real-time PCR proved that BC200 RNA is present at low levels in invasive gliomas (grade WHO IV). In normal brain tissue or in not highly invasive tumors (grade WHO II, III), in contrast, we found RNA detected at higher levels.
b. HAR 1
We have analyzed also an expression level of HAR1 RNA (human accelerated region 1). Many of the human accelerated regions are supposed to be associated with genes known to be involved in transcriptional regulation and neurodevelopment. HAR1, the most dramatically changed element, is part of a novel RNA gene expressed during human cortical development.
In this cease we have also noticed the strong correlation between the malignancy of the tumor and the HAR1 RNA expression level– the most invasive tumor the smaller expression is observed.
These results bring to the hypothesis, that these RNAs can act as a molecular markers for malignancies and as a molecular indicators of diagnostic and prognostic value in the glioblastoma.

P3.48

Splicing stimulates biogenesis of plant miRNA derived from intron-containing genes

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Plant microRNA genes are usually long, and very often contain introns. In the majority of cases a miRNA/miRNA* hairpin is found in the first exon of pri-miRNAs, but there are also miRNA genes where a miRNA is encoded in the second or third exon. Biogenesis of miRNAs is a multistep and complex process. miRNA maturation steps include: constitutive and alternative splicing of pri-miRNAs, alternative polyadenylation of miRNA primary transcripts, miRNA-containing hairpins excision, miRNA/miRNA* duplex formation, and miRNAs incorporation into the RISC complex. Our studies on A. thaliana and barley miRNA genes have revealed that miRNA genes organization is similar in both plant species, and probably reflects general miRNA genes organization in higher plants. Our results show that alternative miRNA precursor isoforms are specific to the particular organ and/or developmental stage of A. thaliana. To answer the question whether splicing plays an essential role in the efficiency of mature miRNA production, we analyzed processing of five different intron-containing A. thaliana pri-miRNAs in several Arabidopsis SR protein mutants. The data show that in some of the SR mutants tested the level of mature miRNAs originated from intron-containing genes is significantly decreased. Next, we asked the question whether the observed changes in the level of mature miRNAs were caused by direct or rather indirect effects. To test it, we introduced three variants of the Arabidopsis MIR163 gene: a native form containing one intron, a gene containing mutated 5` and 3`ss, and an intronless variant, into the A. thaliana mir163-2 mutant (SALK_034556), in which T-DNA insertion had disrupted the endogenous MIR163 gene. Introduction of the wild type form of MIR163 showed the same level of pri-miRNA 163 and its mature form, as it was observed in wild type plants. In the case of the intronless MIR163 construct we observed the accumulation of pri-miRNA 163, while the level of mature miRNA 163 was decreased about three times. In the case of transgenic plants containing the MIR163 gene with mutated both splice sites, the level of mature miRNA 163 was again lower when compared to wild type plants. Altogether, our results show that splicing stimulates biogenesis of plant miRNAs derived from intron-containing genes. In addition, we showed that two factors involved in miRNA biogenesis in plants, SERRATE (SE) and AtCBC (AtCBP20/AtCBP80) are key players in the crosstalk between miRNA biogenesis and splicing.

P3.49

Histone variant H2A.Z changes transcription of stress-specific genes in drought, but not by direct remodelling of chromatin structure at their promoters

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H2A.Z is a universal and highly evolutionarily conserved histone H2A variant, essential for viability in *Tetrahymena thermophila*, *Drosophila melanogaster*, *Xenopus laevis* and *Mus musculus*. It is involved in a great variety of biological processes, including gene expression. However, the mechanism by which H2A.Z influences transcription is still not clear. Models, which have been proposed so far, focus mainly on its presence in gene promoters. In *Arabidopsis thaliana* it has been suggested that H2A.Z poises genes for transcription, and its removal from nucleosomes flanking the transcriptional start site (TSS) is necessary for full gene activation. Transcription-specific loss of H2A.Z from promoter region may directly or indirectly (by affecting nucleosome repositioning) facilitate recruiting of the transcription machinery, and/or binding of activators/repressors to allow high-level expression under the appropriate conditions. As most of *A. thaliana* genes contain H2A.Z at their promoters, many genes expressed at specific environmental and developmental conditions may be regulated by H2A.Z in an indirect way, by H2A.Z-driven transcriptional modulation of specific transcription factors (TF). Therefore, impact of the histone variant H2A.Z on global gene expression is very complex, and requires further investigations to be fully understood.

Here, by comparing transcription of selected genes in H2A.Z-depleted mutant lines, we show that H2A.Z regulates expression of several genes engaged in response to drought stress in *A. thaliana*, and may promote both their activation or repression. Using our *in vivo* biotinylation system we tagged endogenous H2A.Z and used it for chromatin precipitation. The DNA obtained was further used to test for the presence of H2A.Z along *COR15A* gene promoter by Q-PCR with tilling primer sets. In parallel, we investigated changes in nucleosome positioning within the *COR15A* promoter by micrococcal nuclease mapping. Our data shows that the increased expression of *COR15A* in drought stress is related to H2A.Z loss from promoter, although not related to nucleosome eviction or repositioning by H2A.Z. Therefore, our results suggest that in case of *COR15A*, H2A.Z affects its enhanced transcription in drought either by direct regulation of TF expression, or their specific binding to the promoter region.

P3.50

The impact of water deficiency on micro RNA production in barley (*Hordeum vulgare*)

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Micro RNAs (miRNAs) are a class of small RNAs, mostly of 21 nucleotides in length acting as posttranscriptional gene expression regulators. They decrease protein level mostly through cleavage of the corresponding mRNA but they also may act as translation inhibitors. In plants the miRNAs modulate plant development, signal transduction, protein degradation, pathogen invasion and response to environmental stresses. However, the role of miRNAs in barley response for drought is not known.

In this study we carried out the Illumina deep sequencing of small RNAs obtained from the barley plants grown in a water deficiency conditions for 24h and in the control ones. From the Illumina microRNAs sequences data sets, we extracted eight conservative miRNAs - miRNA156, miRNA159, miRNA166, miRNA168, miRNA171, miRNA172 and miRNA530-5p, which show the differences in the read numbers in dehydrated compared to control plants. The sequencing data were validated using northern hybridization to test the alterations of the microRNAs expression levels in plants grown in the water deficiency stress for 24h and 5 days. The expression of miRNA156 and miRNA172 slightly increased in drought treated plants. In the case of barley miRNA166 and miRNA168 the expression levels were stable, while the remaining four miRNAs showed a slight to major decrease in the expression. Moreover, the northern experiments revealed that the level of miRNA530-5pdepends not only on growth conditions, but is also regulated throughout the ontogeny. Altogether northern hybridization results are in agreement with these obtained from Illumina sequencing.

We provide new data describing changes in the expression profiles of the miRNAs in the control and drought conditions. Our next step is to look for targets of these micro RNAs and to study their expression variation in barley plants treated with severe drought and after rehydratation.

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P3.51

RNA interference in silencing the expression of BACE1 gene of Alzhaimer's disease in cellular model

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Alzheimer's disease (AD) is one of the most common forms of dementia. Existing therapeutics in AD are devoted to symptomatic relief. However, after the emergence of amyloid hypothesis, disease modifying therapeutics are being explored. A key step in the pathogenesis of AD is proteolysis of APP that results in the formation of the amyloid-b protein (Ab), the principle protein component of the characteristic cerebral plaques of the disease. Ab is produced from APP first by the action of β-secretase, a membrane-tethered enzyme that resembles pepsin and other water-soluble aspartyl proteases. This proteolysis leads to membrane shedding of the large luminal/extracellular APP domain. γ-secretase cleavage is performed by a high molecular weight protein complex containing presenilins (PSs), nicastrin, Aph-1 and Pen-2. Previous studies have demonstrated that the presenilins (PS1 and PS2) are critical components of a large enzyme complex that performs γ-secretase cleavage In this study we used RNA interference (RNAi) technology to examine the effects of small-interfering RNA (siRNA) against PS1 on expression levels of PS1 and Ab42 in IMR-32 Cells using RTPCR, western blotting and immunofluorescence techniques.

The results of the present study showed down regulation of PS1 and Ab42 in IMR32 cells transfected with siRNA against PS1.

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P3.52

Effects of food restriction on metallothionein gene expression in rat tissues

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Metallothionein (MT) is a cysteine-rich, low-molecular-weight protein that plays: 1) a regulatory role in the homeostasis of zinc and copper, 2) an antioxidant role, 3) role as a preventive agent against heavy metal toxicity by cadmium and mercury. Overexpression of MT is associated with resistance to both cisplatin chemotherapy and radiation. For these reason it is essential to know, how different factors affect MT gene expression. Four classes of MTs have been characterized in mammals. The MT1 and MT2 genes are expressed in many tissues particularly at high level in liver and kidney. Expression of MT3 is restricted to the brain and to male reproductive organs, while that of MT4 is specific to stratified squamous epithelia. Metallothionein gene expression is induced by metal exposure, oxidative stress, glucocorticoids, cytotoxic agents, as well as certain types of stress. Little is known about regulation of metallothionein gene expression in adipose. The purpose of present experiments was to determine whether metallothionein (each of its isoforms) is expressed in rats white adipose tissue (subcutaneous, epididymal, retroperitoneal). Diet restriction is one of the most popular way to lose weight thus it is relevant to know how food restriction (50% of the *ad libitum* energy intake for 30 days) influences the expression of metallothionein gene in WAT and liver. RNA was isolated from white adipose tissues, liver using Chomczynski-Sacchi method and reversely transcribed using hexamer primers and transcriptase. The gene expression levels were measured by real-time PCR. The results indicate that MT1, MT2, MT3 are expressed in subcutaneous, epididymal, retroperitoneal adipose tissue. MT1 mRNA was detected in the highest amount in each depot. MT4 expression in WAT and liver was undetectable. Caloric restriction induces a substantial increase of MT mRNA level in white adipose tissue and liver. The expression levels of MT1, MT2 had increased over fourfold in epididymal and perirenal adipose tissues. Induction of each isoform of MT expression was higher in WAT than in the liver (twofold). These results suggest that food restriction may significantly affect on the MT expression.

P3.53

Effect of C protein on transcription activity from C-box operator regulating Csp231I restriction-modification system

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Many bacteria produce restriction-modification (R-M) systems, in part to protect against invading DNA such as phage genomes. Most type II R-M systems include independently-active restriction endonuclease (REase) and protective DNA methyltransferase (MTase) proteins. After R-M genes enter a new cell, MTase activity must appear before REase or the chromosome will be cleaved. These R-M systems thus represent temporally-controlled genes, where the genes have a long-term relationship with their new hosts, and where the consequences of misregulation can be lethal. Some R-M systems achieve delayed REase expression by co-transcribing the REase gene with the gene for an autogenous transcription regulator (the controlling or "C" protein). R-M systems associated with C proteins fall into several groups based on their sequences and target DNA sequence specificities. All C proteins share a helix-turn-helix DNA binding motif resembling the N-terminal DNA binding domains of the well studied lcI repressor. The archetypes of three currently-recognized groups are the best studied for C.PvuII and C.EcoRV (names after first example discovered). The third group - C.EcoO109I comprising two members: C.EcoO109I and C.Csp231I is unexplored. The purpose of our research is to investigate C.Csp231I protein and its R-M system isolated from Citrobacter sp. RFL231I. Though, the crystal structure of C protein has been solved, there is no information on biological activity of C protein in vivo. C proteins bind to conserved operator sequences called "C-boxes" located upstream of their genes overlapping DNA promoter region. We tested the transcription activity in DNA regions carrying predicted C-boxes in series of experiments with lacZ reporter gene. Indeed, they show elevated expression unlike other fragments without C-box sequence. Next, we tested *in vivo* effect of C protein on the same DNA fragments when C gene is delivered in trans. The C gene was cloned under arabinose promoter to obtain a gradient of C expression after arabinose induction. We observed the transcription repression driven from C-box only in higher level of C protein.

P3.54

Is PIN3 involved in the regulation of *TP53* mRNA expression?

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Discrepancies between *TP53* cDNA and DNA sequencing results were noticed. We detected only mutated cDNA, and pattern of heterozygous mutations after DNA analysis. We hypothesized that different polymorphic *TP53* alleles can present different gene expression levels. *TP53* is polymorphic, and the best known polymorphisms are Arg72Pro and PIN3, where in the latter one, one of the alleles carries a duplication of 16 bp in intron 3 and is responsible for higher cancer risk. This insertion causes that alleles have different influence on *TP53* mRNA expression. 16 bp insertion/deletion in intron 3 may lead to alternative splicing of mRNA. Exon 3 (21bp) and intron 3 (112 bp) in *TP53* gene are relatively short, so elongation of intron 3 by 15% (16 bp insertion PIN3) can have significant influence on protein function. Gemignani *et al.* detected that the shorter allele (A1) provides higher expression rate of these gene, than the longer allele, A2. In this situation our hypothesis can be more detailed for selected group of cases (heterozygotes A1/A2). In reference to A1/A2 PIN3 heterozygous cases, mutation of allele allowing for higher *TP53* gene expression, prevents TP53 activity (it causes a definite predominance of mutated mRNA) despite the presence of wild type allele (haploinsufficiency). This assumption determined the main aim of our research – to answer the question in which allele, A1 or A2, heterozygous mutation occurs more often. For this purpose, subcloning of DNA fragments was performed. In all analyzed cases, which showed PIN3 heterozygosity, *TP53* heterozygous mutation and the lack or trace of normal cDNA, A2 (longer allele) was mutated. Moreover, ST486 cell line and analyses in reporter assay system can suggest higher efficiency of A2 transcrption. It suggests that mutation of allele with 16 bp insertion (if PIN3 heterozygosity occurs) can be partially responsible for the presence of *TP53* heterozygous mutations. We suggest that some of heterozygous mutations of *TP53* do not occur because of dominant-negative effect but for haploinsufficiency caused by *TP53* polymorphisms.

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P3.55

Potential regulatory role of PemIK$_{Sa}$ toxin-antitoxin system on gene expression in *Staphylococcus aureus*

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The toxin-antitoxin (TA) systems were initially characterized as components of low-copy-number plasmids which ensured their stable maintenance. However, the latter discovery of TA systems located in the bacterial chromosomes opened a still largely unsettled debate concerning the broader biological role of TA systems (Bukowski *et al.*, 2011). One of the largest classes of TA systems, class II, includes toxins characterized by ribonucleolytic activity. Unspecific ribonucleolytic toxins degrade total cellular RNA inhibiting cell growth and leading to cell death upon prolonged activation. However, many class II toxins are sequence-specific, that is, recognize and cleave only RNA molecules containing particular target sequence.

PemIK$_{Sa}$ is a TA system encoded on pCH91 plasmid carried by *Staphylococcus aureus* strain CH91. The toxin (PemK$_{Sa}$) is sequence-specific ribonuclease toward tetrad sequence UAUU, as determined using primer extension method. Such specificity of PemK$_{Sa}$ would constitute ideal means for global regulation of gene expression provided the recognized sequences are organized in the transcriptome in a nonrandom fashion. To evaluate if this was the case we calculated the expected distribution of UAUU sequence in open reading frames (ORFs) extracted from *S. aureus* genome and compared the expected values to those truly observed in the transcriptome. Among 2685 ORFs encoded in the genome of *S. aureus* we identified 56 ORFs in which the PemK$_{Sa}$ target sequence was absent and another 62 ORFs where the sequence was present but, statistically ($P \leq 0.01$) underrepresented. Most significantly, the identified groups were composed of nonrandom transcripts. Rather, they contained a substantial number of transcripts encoding proteins implicated in staphylococcal virulence. Among others, these included a transcription regulator Fur, delta-hemolysin, extracellular enzymes including metalloprotease, lipase and coagulase as well as host-protein-binding proteins such as clumping factor A, von Willebrant factor-binding protein, fibronectin binding protein, and serine-aspartate repeat proteins, all implicated in staphylococcal virulence. We also identified 202 ORFs in which the PemK$_{Sa}$ target sequence is statistically ($P \leq 0.01$) highly overrepresented. The vast majority of transcripts in this group encode proteins involved in membrane transport and signal transduction. Next, we experimentally verified the anticipated differential vulnerability of transcripts belonging to identified groups to PemK$_{Sa}$ degradation. The representative mRNA molecules from each group of transcripts were exposed to PemK$_{Sa}$ and their stability was monitored by electrophoresis. As expected, the more recognition sites were present in particular mRNA, the more efficiently was it degraded by PemK$_{Sa}$. This indicates that PemK$_{Sa}$ targets transcripts in the cellular mRNA pool selectively. As such, PemK$_{Sa}$ may possibly play a role of a global regulator of *S. aureus* gene expression, enhancing the transcription of virulence related genes and concurrently downregulating the housekeeping metabolism.

Reference
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P3.56

Molecular mechanisms involved in loss of betaglycan expression in endometrial cancer

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Recent studies has indicated that alterations of TGFβ signalling cascade play a vital role in development and progression of different cancer types. Canonical signal transduction in TGFβ pathway occurs *via* two types of signalling receptors, i.e., TGFβRI and TGFβRII, which posses serine/threonine kinase activity. Signal induced by TGFβ ligands can be also modulated by accessory receptors TGFβRIII. The main function of TGFβRIII is ligand presentation to its dedicated receptors. One of the members of TGFβRIII subfamily is betaglycan, which is a transmembrane proteoglycan characterized by the highest affinity to TGFβ2 isoform. We have previously reported that betaglycan mRNA expression has been significantly downregulated with concomitant protein upregulation, in endometrial cancer comparing to normal endometrial mucosa (Zakrzewski *et al*., 2011). Obtained results has become the basis for investigation of molecular alterations responsible for observed imbalance between transcriptomic and proteomic levels. The aim of the current study was methylation profiling of betaglycan promoter region, analysis of loss of heterozigosity in betaglycan gene and evaluation of GATA3 mRNA expression level and methylation status in endometrial cancer. GATA3 has been identified as the transcription regulator of betaglycan expression in renal cell carcinoma (Cooper *et al*., 2010).
MSP-PCR was applied for methylation profiling of studied genes. Loss of heterozigosity was evaluated with microsatellite PCR method using 5'fluorescently labelled primers. Gene expression level analysis was performed with qPCR technique using TaqMan® probes.
Betaglycan mRNA downregulation was found to be not associated with methylation silencing in its gene promoter region in endometrial cancer. Genetic imbalance analysis demonstrated LOH in 29% (n=7), 25% (n=6) and 21% (n=5) of analyzed cancer specimens, respectively for D1S188, D1S435 and D1S1588 microsatellite markers. mRNA expression analysis showed that GATA3 mRNA level was significantly (p<0.01) decreased in 33.3% (n=14), whereas significantly (p<0.01) increased in 66.7% (n=28) of endometrial cancer sales comparing to the normal tissue. GATA3 methylation profile also demonstrated similar diversity. GATA3 methylation was observed to be significantly (p<0.01) lowered in 66% (n=37) and significantly (p<0.001) elevated in 34% (n=19) of endometrial cacer specimens compared t nomaltissue.
Loss of betaglycan mRNA expression in endometrial cancer seems to be determined by different molecular processes, such as LOH of betaglycan gene locus and divers methylation status of GATA3 gene, but is not a result of methylation of betaglycan promoter region.

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Implication of single nucleotide polymorphism in alternative splicing of the CRHR1 receptor, a new link?

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Single nucleotide polymorphisms (SNPs) in the non-coding region of corticotropin-releasing factor receptor type 1 (*CRHR1*) gene was associated with several human pathologies including hypertension, abusive behavior and depression. Such as correlations were explained by epsitatasis or inverse effects, but presence of SNPs may play also regulatory role and may influence the expression and alternative splicing of CRHR1. Moreover, most of SNPs were mapped to intronic regions of the gene thus they do not have direct influence on CRHR1 protein sequence.
Corticotropin-releasing factor and its receptors play important role in regulation of hypothalamic-pituitary-adrenal axis, thus central response to the stress and their activity strongly influence not only human behavior but also immune and endocrine systems.
Here we are hypotheses that presence of SNP within the *CRHR1* gene influence its expression and alternative splicing and by this receptor properties. Interestingly, majority of SNPs related to human pathological conditions were located in sequence of introns surrounding exon 3, which is often involved in alternative splicing. At least 8 alternative variants of CRHR1 mRNA has been detected in human. The main isoform of CRHR1α is responsible for proper ligand binding and downstream signaling and other isoforms modulate its function. Recent studies have revealed that diverse physiological and pathological conditions including age of cell culture pathological conditions modulate alternative splicing of *CRHR1*, but the regulation mechanism and its significances is largely unknown. We are postulating here that presence of unique SNPs in CRHR1 gene might influence its expression and alternative splicing leading to modulation of CRH signaling. This hypothesis might explain observed correlation of several SNPs in CRHR1 with human pathologies and represent potential target for diagnostics and therapies.

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Revealing stable processing products from ribosome-associated small RNAs by deep-sequencing data analysis

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After the results of the pilot ENCODE project suggesting that up to 90% of the human genome is actively transcribed [1], the importance of RNA molecules which does not encode proteins (noncoding RNAs — ncRNAs) became unquestionable. The main difficulty in identification and characterization of novel ncRNAs is caused by their complex biogenesis, which often include multiple processing steps [2]. Thus, current methods are focused on identification of novel transcription units rather then on ncRNA species *per se*. We have addressed this challenge by developing a computational pipeline named APART (Automated Pipeline for Analysis of RNA Transcripts), which in combination with adequate preparation of cDNA library is able to detect a processing events within the longer RNA precursors. The analysis can be fulfilled in a fully automated fashion, starting with raw sequence reads and include genome mapping, contig assembly and annotation. One of the main advantages of APART is a unique way of handling of repeat-derived sequence reads. It enables the identification of expression of distinct loci of the same gene on one hand and removal of the redundancy caused by spurious matching of the reads to non-relevant short repeated sequence fragments dispersed across the genome on the other.
We have applied the APART analysis on the sequencing data obtained from a cDNA library of short RNAs associated with yeast ribosomes under different stress conditions. Within the 174 contigs assembled, we have identified 131 putative processing products of distinct origin, including intergenic regions, mRNAs, tRNAs rRNAs and snoRNAs. The most surprising was to observe the snoRNA-derived small RNAs to be associated with the ribosoms in the cytoplasm. Those small RNAs are believed to be localized speciffically within the nucleolus and to be involved in ribosomal RNA maturation. In order to verify the results we have performed a series of experiments, including northern blot analysis and others, which confirmed the cytoplasmic localization of APART-predicted snoRNA fragments and their association with translating ribosomes. Similar experiments have confirmed the differential stress-dependent processing of tRNAs and rRNAs which has been predicted by APART. The result suggest the existance of a new layer of modulation of gene expression, which is fulfilled by alternative processing of the transcripts.

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