Abstracts of papers presented at the
Conference RNA Club
16th September 2016

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GENERAL INFORMATION

Internet Facilities
Wi-Fi connection is available at the EDUROAM. The password will be given at the registration in the badge.

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The organizers of the conference do not accept liability for any injury, loss or damage, arising from accidents or other situations during the conference. Participants are therefore advised to arrange insurance for health and accident prior to travelling to the conference.

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Registration desk is located in the corridor of the building A11 (On the left side from the main University campus entrance). All participants must be registered before attending the lectures.

Registered and confirmed participant is entitled to:
• Admission to all scientific sessions
• Admission to the poster session
• All refreshment
• Dinner at Atrium, building A35

CONFERENCE INFORMATION

Badges
Participants and accompanying persons will receive a name badge upon registration. Everyone is kindly requested to wear his name badge when attending the meeting. Only participants who are wearing their name badge will be admitted to the lecture hall.

Programme Changes
The organizers cannot assume liability for any changes in the programme due to external or unforeseen circumstances

Mobile phones
Participants are kindly requested to keep their mobile phones in the off position in the meeting room while the session is being held.

Photograph
Photography is not allowed during the workshop.
PROGRAMME

Friday 16th September

08:00 – 09:00  Registration

09:00 – 09:10  OFFICIAL OPENING

09:10 – 09:30  Lexogen presentation - miRNA quantification and analysis

09:30 – 09:50  Tomáš Bártta - miRNAsong: microRNA SpONge Generator and tester - high-throughput approach for miRNA sponge design and testing

09:50 – 10:10  Peter Androvič - New RT-qPCR system for miRNA analysis


10:30 – 11:00  COFFEE BREAK

PLENARY LECTURE I

11:00 – 11:50  Stefan Ameres - Mechanism and Biology of RNA Silencing

11:50 – 12:10  Martina Janoušková - Influence of major-groove chemical modifications of DNA on transcription by bacterial RNA polymerases

12:10 – 12:30  Sneha Kulkarni - Queuosine: The role of an essential tRNA modification in parasitic protist Trypanosoma brucei

12:30 – 12:50  Helena Covelo Molares - The mechanism of m6A demethylation in pre-mRNA processing regulation

12:50 – 15:00  LUNCH & POSTER SESSIONS
Programme

15:00 – 15:50
PLENARY LECTURE II
Eric Miska - RNA memories in C. elegans

15:50 – 16:10
Sravya Ganesh - Role of LncRNAs in Oocyte-to-Zygote transition

16:10 – 16:30
Lenka Veselovska - The rate of transcription, but not timing of its activation, defines dynamics of DNA methylation establishment in the oocytes

16:30 – 16:50
Denisa Jansova - RNA world in progress: Complex visualization of transcriptome in oocyte and embryo

16:50 – 17:20
COFFEE BREAK

17:20 – 17:40
Radek Sindelka - Identification of cell fate determinants and formation of organism body plan

17:40 – 18:00
Miroslav Krepl - Study of RRM Protein/RNA Complexes - The Synergy of NMR Spectroscopy and MD Simulations

18:00 – 18:20
Olga Jasnovidova - Assemblies of RNA polymerase II C-terminal domain with processing factors investigated using integrative structural biology

18:20 – 18:40
Darina Šítková - The human translation initiation factor 3 (eIF3) expression and integrity code

18:40 – 19:00
Maria Zlobina - Molecular aspects of CFTR exon9 aberrant splicing

19:00 – 19:20
Transfer to A35

19:20 – 19:30
CLOSING REMARKS AND AWARDS

19:30 – ??
DINNER & PARTY
The implementation of distinct gene expression profiles is essential for organismal development, physiological responses to external stimuli and pathogens, and defines a primary cause for disease. My lab is fascinated by the molecular events that control these processes at the post-transcriptional level by focusing on two major areas:

**Small RNA silencing.** Small silencing RNAs regulate gene expression in nearly all eukaryotes and have enormous biotechnological and therapeutic potential. Among these, microRNAs belong to the largest family of trans-acting gene regulatory molecules in multicellular organisms. In flies and mammals, they control more than half of the protein-coding transcriptome, and act as key regulators of organismal development, physiology, and disease. We are interested in molecular mechanisms that govern small RNA silencing pathways in flies and mammals. Our focus lies on processes that regulate the production of small RNAs, their assembly into functional ribonucleoprotein complexes, and the disassembly thereof in response to synthetic and natural triggers. Our goal is to unravel mechanistic principles of small RNA-mediated gene regulation, a phenomenon that impacts virtually every aspect of metazoan biology.

**The Epitranscriptome.** For RNA to fulfill its essential function within the cellular environment, numerous chemical modifications have evolved to sculpt its physical and functional interactions. Although more than hundred types of RNA modifications have built the descriptive foundation of what is referred to as the epitranscriptome, their mode of action remains largely unknown. We are studying the function of chemical RNA modifications, at the intersection of small RNA silencing pathways and general RNA metabolism. Our focus lies on the post-transcriptional addition of nucleotides to the 3’ end of RNA (i.e. tailing) by the only rudimentary characterized enzyme family of terminal nucleotidyltransferases in order to dissect the regulation of microRNA biogenesis and function; and the role of small RNA ribose methylation in order to gain insights into the antiviral immune response through the RNAi pathway in flies. The emerging concepts will inevitable impact our view on more general functions of post-transcriptional modifications in RNA metabolism. And we are applying our mechanistic insights to the development of novel epitranscriptomics approaches to probe post-transcriptional gene regulatory networks at the transcriptome-wide level.

Overall, our goal is to determine fundamental biological mechanisms of post-transcriptional gene regulation through pathways with enormous biological, biomedical, and technological impact.
MicroRNAs (miRNAs) are a class of small non-coding RNAs that serve as important regulators of gene expression at the posttranscriptional level in plants, animals and viruses. Importantly, miRNAs maintain their stability in circulating body fluids and therefore pose great potential to serve as non-invasive biomarkers of cancer and other diseases. Methods for accurate and sensitive quantification of miRNA expression are therefore highly needed. Although several approaches for the detection of miRNAs by RT-qPCR have been developed, precise and sensitive miRNA quantification remains challenging due to the small size of miRNAs, nature of their biogenesis and high degree of sequence homology between transcripts.

Here, we present a highly specific, sensitive and cost-effective system to quantify miRNA expression based on two-step RT-qPCR with SYBR-Green detection chemistry. It takes advantage of novel, target-specific primers for reverse transcription with stem-loop structure and two binding segments complementary to the different parts of the targeted miRNA. The new method has a dynamic range of 8 orders of magnitude and sensitivity to detect tens of target miRNA molecules. The introduction of the second binding sequence enables to discriminate highly homologous miRNAs regardless of the position of the mismatched nucleotide. Importantly, the reverse transcription can be multiplexed, which substantially increases throughput, saves on reagent costs and minimizes requirements for the RNA input amount. The expression profiles measured by the new method showed excellent correlation with the industry-verified TaqMan miRNA assays (R² = 0.985). Moreover, the system allows user modifications and rapid analysis with total time under 2 hours.
miRNAsong: microRNA SpONge Generator and tester - high-throughput approach for miRNA sponge design and testing

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MicroRNA (miRNA) sponges are RNA transcripts containing multiple tandem high affinity binding sites that bind and sequester specific miRNAs to prevent them from interaction with their target mRNAs. Due to their high specificity and strong inhibition of target miRNAs, these molecules have become increasingly applied in miRNA loss-of-function studies and also in miRNA based therapies. However, improperly designed miRNA sponge constructs may sequester off-target miRNAs, which may lead to false-positive results and/or off-target effects, therefore it has become increasingly important to develop a tool for in silico miRNA sponge construct design and testing.

In this study, we introduce miRNAsong: microRNA SpONge Generator and tester - freely available web-based tool for generation and in silico testing of microRNA sponge constructs. This tool generates miRNA sponge constructs for specific miRNAs and miRNA families/clusters and tests them for potential binding miRNAs in selected organism. Currently, miRNAsong allows to test sponge constructs in 219 species covering 35828 mature miRNA sequences.

Furthermore, in this study we also provide an example, supplemented with experimental data, how to use this tool. Using miRNAsong, we designed and optimised sponge for miR-145 inhibition and cloned generated the sequence into lentiviral inducible vector. Derived cell lines, expressing miR-145 sponge, strongly inhibited miR-145.

URL: http://www.med.muni.cz/histology/miRNAsong/
Supported by European Regional Development Fund - Project FNUSA-ICRC (No.CZ.1.05/1.1.00/02.0123), by the project ICRC-ERA-HumanBridge (no. 316345) funded by the European Commission, by the project GJ16-24004Y funded by Czech Science Foundation, and by the project MINU/A/1352/2015.
Basic principles of genetic code were described several decades ago. We know general rules for base-pairing between individual coding triplets and aminoacyl-tRNAs as well as how the release factors recognize stop/nonsense codons. However, our understanding of what determines the difference in termination efficiencies among various stop codon tetranucleotides and how near-cognate (nc) tRNAs recode stop codons during programmed stop codon readthrough is still rather poor. Recently, we learnt that tyrosine or glutamine preferentially incorporates at UAA and UAG in vivo and tryptophan or cysteine at UGA. Here, we investigated what is the contribution of nucleotide following a given stop codon for selection of corresponding nc-tRNA. We show that the factor markedly contributing to the difference in termination efficiencies among all 12 tetranucleotides is given by their varying preferences for nc-tRNA. In particular, we revealed that: i) UGA-A allows increased incorporation of Trp-tRNA, whereas UGA-G and UGA-C favor Cys-tRNA; ii) UAG-C is the only tetranucleotide decoded by both tyrosine and glutamine tRNAs, whereas the remaining three UGA-N tetranucleotides are preferentially decoded only by glutamine nc-tRNA; and iii) that there is no specific preference among the UAA-N tetranucleotides as they are all decoded by tyrosine nc-tRNA. Our findings thus expand the repertoire of general decoding rules by showing that the +4 base determines the preferred selection of what we designate as readthrough inducing tRNAs (rti-tRNAs). Taken together we conclude that basal readthrough levels at particular stop codons are by a great deal influenced by natural cellular levels of individual rti-tRNAs. Furthermore, we will also present our current project where we investigate if and how specific compounds known to induce readthrough, such as aminoglycosides, affect incorporation of rti-tRNAs during readthrough in living cells.
P05 Recognition of pTyr1-CTD by the elongation factor Spt6

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The C-terminal domain (CTD) of RNA polymerase II (RNAPII) consists of heptad repeats with the consensus motif Y1-S2-P3-T4-S5-P6-S7. Dynamic post-transcriptional modifications of the CTD led to the formulation of the so-called “CTD code” more than a decade ago (1). It encodes recruitment of distinct processing factors for each specific position of RNAPII in the transcriptional cycle (2). However, how the CTD of RNAPII recruits, (de)activates, and displaces relevant processing factors, remains still poorly understood. Recent genome-wide mapping of CTD phosphorylation patterns revealed that Y1 and S2 phosphorylation levels are increased simultaneously during early elongation. Increased Y1 phosphorylation releases factors associated with RNAPII at the beginning of genes, and blocks recruitment of termination factors. In addition, phosphorylated Y1 mark stimulates binding to the tandem SH2 (tSH2) domain of elongation factor Spt6, consistent with Spt6 occupancy within Tyr1-phosphorylated region of genes in vivo (3). We will show our structural data, acquired via a combination of structural methods, supported by biochemical assays on the study of recognition Y1-phosphorylated CTD by the tSH2 domain of the elongation factor Spt6.
P06 In vitro selection of a DNA aptamer for glutamate carboxypeptidase III (GCPIII)

Edward Curtis[1], Juan Alfonso Redondo Marín[1]
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Following the discovery of catalytic RNA in the early 1980s, methods were developed to isolate RNA and DNA sequences with unusual properties, such as the ability to bind ligands with high affinity and specificity, from large random sequence pools. Such oligonucleotides, termed aptamers, have emerged as a new class of molecules that in some cases are superior to antibodies in therapeutic, imaging and diagnostic applications. In the present work we used in vitro selection to search for DNA sequences with high affinity and specificity for the metallopeptidase glutamate carboxypeptidase III (GCPIII). This enzyme is an interesting pharmacological target due to its evolutionary relationship to glutamate carboxypeptidase II (GCPII), which is involved in a variety of neuropathologies and malignancies, such as glutamatergic neurotoxicity and prostate cancer. The physiological role of GCPIII and its localization in both human and mouse are currently unknown due to a lack of GCPIII-specific antibodies/ligands. This is due to the high level of homology between these two enzymes at the level of both amino acid sequence and three-dimensional structure, making the development of specific antibodies a challenging process. We will overcome this difficulty by including a counterselection step in our protocol to remove pool members that bind to GCPII. The development of aptamers that specifically bind GCPIII would have applications in both basic research and medicine, allowing for the characterization and localization of this elusive enzyme.
G-quadruplexes are four-stranded nucleic acid structures stabilized by G-G-G-G tetrads. In some cases these structures are intrinsically fluorescent, a property which is potentially useful for applications in both biotechnology and basic research. Although the sequence requirements of the spacers in fluorescent G-quadruplexes have been characterized to a limited degree, the effect of mutations in tetrads has not been investigated. In this study we characterized the fluorescence of all possible single and double mutation variants of the central tetrad in a parallel-strand reference G-quadruplex structure. A surprising number of these variants were fluorescent, and several had shifted emission spectra relative to that of the reference construct. The sequence requirements for fluorescence were similar to those recently described for G-quadruplexes that bind GTP, but significantly different from G-quadruplexes that promote peroxidase reactions. A combination of chemical synthesis and rolling circle amplification was used to study the effects of concatemerization on G-quadruplex fluorescence. We also investigated the enhancement of G-quadruplex fluorescence in the presence of the lanthanide cation Tb3+. These experiments provide insight into the properties of fluorescent G-quadruplexes, and should aid in the development of improved label-free nucleic acid reporters.
P08 Sequence requirements and GTP-dependent formation of multimeric G-quadruplexes

Edward Curtis\(^{(1)}\), Sofia Kolesnikova\(^{(1)}\)

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G-quadruplexes can form different types of multimeric structures, but the sequence requirements of such higher-order structures are not well understood. Furthermore, conditions that promote formation of multimeric G-quadruplexes have not been systematically investigated. In this study we tested the ability of all possible variants of the central tetrad in a monomeric, parallel-strand G-quadruplex to form multimers. Although most of these variants existed primarily as monomers under the conditions of our screen, approximately 10% formed higher-order structures. These structures formed as a function of DNA concentration, and could be stabilized by a wide range of monovalent and divalent metal ions. In the presence of KCl the formation of such structures was highly cooperative. Several sequence variants were identified in which multimer formation was inhibited by GTP in a concentration-dependent manner. Inhibition no longer occurred when functional groups on the guanine base of the ligand were mutated, but was not sensitive to changes in either the sugar or the phosphate groups of GTP. Taken together, these experiments provide insight into the sequence requirements of multimeric G-quadruplex structures. They are also consistent with the intriguing possibility that GTP could regulate the formation of multimeric G-quadruplexes in cells.
P09 Functional analysis of human Prp8 mutations linked to retinitis pigmentosa

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Retinitis pigmentosa (RP) is hereditary retinal disorder leading to degeneration of photoreceptors. There are approximately 60 eye specific genes that are associated with RP. Surprisingly, RP-related alleles were also found in genes coding ubiquitously expressed pre-mRNA splicing factors (PRPF3, PRPF4, PRPF6, PRPF8, PRPF31 and SNRNP200).

Using BAC recombineering, we prepared five point RP-mutations of human hPrp8, tagged the mutants with GFP and expressed them stably in cultured cells. Under normal condition, wild-type hPrp8 is localized exclusively in the cell nucleus. Here we observed that some mutations affected hPrp8 nuclear localization. hPrp8 is a crucial component of the U5 snRNP. We showed that hPrp8 mutations inhibited maturation of the U5 snRNP and prevented incorporation of additional U5-specific proteins. However, each mutation inhibited U5 snRNP maturation to a different extent. In addition, we observed faster degradation of mutant proteins and the rate of degradation correlated with the effect the mutation had on U5 snRNP maturation. To examine effects of RP mutation on pre-mRNA splicing, we knocked down specifically the endogenous hPrp8 protein and analyzed splicing efficiency in cell lines expressing either wild-type hPrp8-GFP or RP mutants. None of the mutants was able to rescue the splicing defects to the extent observed in wild-type hPrp8 expressing cells.

Taking together, our data show that RP-related mutations of hPrp8 compromise hPrp8 stability, maturation of the U5 snRNP and reduce splicing efficiency. The extent to which individual mutants affect these events correlates with the severity of RP clinical phenotype.

Acknowledgement
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P10 The Role of DicerO during Mouse Oocyte Maturation

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Oocytes are one of the very few cells exhibiting RNA interference (RNAi). It has been shown that unlike somatic lineages, mouse oocytes express a shorter variant of Dicer, termed DicerO. This variant is driven by an intronic MT-C retrotransposon promoter and thus lacks the N-terminal helicase domain. DicerO is indispensable for fertility in female mice and more specifically, for correct chromosome segregation during the first meiotic division. DicerO deficient oocytes exhibit spindle defects that lead to incorrect chromosome content in the resulting metaphase II eggs. In our work, we have decided to analyse this phenomenon in more detail. In agreement with previous results, we found that approximately 75% of all oocytes analysed showed the presence of an abnormal meiotic spindle, thus resulting in an aneuploid female gamete. The most prevalent karyotype abnormality was the presence or absence of a single chromatid, followed by more serious numerical deviations (two or more chromatids missing or surplus). Cold Microtubule Assay analysis showed that these surplus chromatids are unattached to microtubules and often lie distant from the main spindle body. These results support the hypothesis that the spindle defect coupled with incorrect chromosomal content of DicerO-deficient oocytes may lie behind the observed female infertility.
P11 Age related differences in the translational landscape of mammalian oocytes

Michal Kubelka[1], Marketa Koncicka[1], Denisa Jansova[1], Anna Tetkova[1], Andrej Susor[1], Tomáš Mašek[2], Edgar Del LLano[1]

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Oocyte aneuploidy is the result of abnormal chromosome segregation during meiosis giving rise to a ready to be fertilized oocyte, which, however, possesses an aberrant number of chromosomes. These anomalies are inherited by the future embryo drastically reducing its developmental potential. Importantly, aneuploidy is not an infrequent event in mammalian oocytes but rather a common feature which increases in correlation with female age. Furthermore, maturing oocytes are transcriptionally silent and rely on the utilization of a pool of mRNAs synthesized and stored during the growth period.

We aim to study age-related aneuploidy in mammalian oocytes by analysing that translational pattern resulting from the specific utilization of the transcriptome in oocytes isolated from young and aged mouse females. We have applied a polysome fractionation method to isolate RNA population involved in the active translational machinery. From the isolated RNA in polysomes, we have prepared a cDNA library ready to be sequenced by Illumina system through which we will be able to find differences in the translational program of the oocytes from young and older females. This approach should help to identify variations on the synthesis of the essential proteins which might lead to a reduced quality and/or genomic instability of oocytes from older females.
P12 Human Embryonic Stem Cell-derived Neural Stem Cells: Role of Cell Cycle and MicroRNAs in Shaping Cell Identity

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Regeneration and replacement of neurons and glia that undergo cell death are the main goals of stem cell-based therapies. Clinically-relevant methods for generation of neural stem cells (NSCs) from human embryonic stem cells (hESCs) only begin to emerge. We have previously derived such self-renewing NSC lines with the ability to differentiate into functional neurons and glia in vivo. Importantly no tumor formation was noted, therefore studying self-renewing mechanisms of NSCs with “non-tumorigenic nature” might shed a light on deregulated proliferation mechanisms often found in CNS tumors. Thus, the aim of the present study was to define molecular mechanisms responsible for the maintenance of unlimited self-renewal of NSCs. Since self-renewal and differentiation has been previously connected with the regulation of cell cycle partially via microRNA in hESCs, we hypothesize that differentiation-associated miRNAs contribute to cell cycle regulation in self-renewing NSCs as well. We initiated our experiments by analyzing high throughput gene expression data in undifferentiated hESCs, self-renewing NSCs, and non-self-renewing neural progenitor cells (NPCs). Results show that only 16 genes are significantly upregulated in NSCs in comparison to NPCs. These include LIN28B, which has been associated with self-renewal of NSCs before, thus confirming our data. However other proteins, such as SALL4, have been connected to pluripotency of hESCs but never studied in the context of NSC-self renewal. Altogether, our data point to several candidate genes responsible for maintenance of self-renewal of NSCs and functional studies are ongoing to reveal underlying regulatory pathways. Curiously, results from qPCR screen did not identify miRNAs which are specifically expressed in NSCs and could thus be implicated in the maintenance of NSC phenotype and/or control of self-renewal. Therefore, we are now initiating a complex study of miRNA expression by RNA deep sequencing. This analysis will help to identify miRNAs with putative role in NSC maintenance and self-renewal.

This study was supported by GACR GJ15-18316Y, GJ16-2400Y and by funds from Faculty of Medicine MUNI/A/1352/2015
The mitochondrial ATP synthase is a bi-directional nanomotor, which in majority of eukaryotes works in its forward mode and synthesizes ATP. The activity of this enzyme is negatively regulated by highly conserved inhibitory protein IF1. In pathogenic flagellate Trypanosoma brucei, the mitochondrial ATP synthase switches its activity from the ATP production to ATP hydrolysis as the parasite differentiates from the insect procyclic stage (PF) to the mammalian bloodstream stage (BF). We identified T. brucei homolog of IF1 (TbIF1) and showed that on the protein levels its is present only in PF cells and its expression is transiently upregulated during the differentiation from PF to BF cells. Upregulation of IF1 is also observed in cancer cells, which undergo a metabolic switch from oxidative phosphorylation to glycolysis. Thus, TbIF1 can be involved in similar metabolic rewiring during the parasite’s differentiation.

To comprehensively understand PF to BF metabolic transition, we study mechanisms underlying TbIF1 differential expression during the T. brucei life cycle. We showed that both steady state level and half-life of TbIF1 mRNA are dramatically increased in PF compared to BF, which in the context of absence of transcriptional regulation in T. brucei, indicates regulation by mRNA stability. Inhibition of translation results in TbIF1 mRNA accumulation in BF, which suggest existence of short-lived destabilizing RNA-binding protein. Using CAT-based reporters, we dissect TbIF1 UTRs to identify sequential and/or structural elements directing TbIF1 mRNA stability. The factors, which interact with the identified motifs, are being purified using Csy4, an inducible RNA hairpin-binding nuclease. Together, the data reveal mRNA-controlled mechanisms contributing to metabolic progression during the development of the infectious form of this parasite.
P14 Role of LncRNAs in Oocyte-to-Zygote transition

Petr Svoboda[1], Kristian Vlahovicek[2], Fugaku Aoki[3], Yutaka Suzuki[3], Jana Nejepinska[4], Eliska Svobodova[4], Vedran Franke[2], Rosa Karlic[2], Sravya Ganesh[4]

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The oocyte-to-embryo transition (OET) is the first important biological transition in life. Upon fertilization, massive gene expression reprogramming transforms a differentiated oocyte into a totipotent blastomeres of the embryo. While the contribution of protein coding genes has been considerably explored, role of non coding RNAs in OET remanis largely unknown. Long non-coding RNAs (lncRNAs) are a heterogeneous group of transcripts with diverse functions including regulation of transcription, post-transcriptional control of protein-coding transcripts or intermediates in biogenesis of other RNAs. Here, we defined and explored the core population of maternal and zygotic lncRNAs in mice. Using next generation sequencing of total RNA and de novo transcript assembly and intense filtering, we generated a list of 1600 most expressed maternal and zygotic lncRNAs, of which over 1200 non annotated in the genome databases. Among the novel lncRNAs, two were maternally expressed in paternally imprinted regions. LncRNAs expressed during preimplantation form two distinct populations – maternal and zygotic genome activation-associated. Importantly, the bulk of maternal lncRNAs were eliminated prior the zygotic genome activation suggesting that maternal lncRNA contribution to early development is minimal. Consistent with previous studies in somatic tissues, only a minority of lncRNAs were ubiquitously expressed. We observed that approximately a half of OET lncRNA promoters were provided by LTR from a small group of rodent-specific retrotransposons suggesting that many identified lncRNA emerged recently. Functional analysis of two conserved lncRNAs using CRISPR-mediated knock-out did not reveal any effect on fertility. Taken together, our data significantly expand our knowledge of transcriptome remodeling during early development of lncRNAs and provide an excellent source for further OET lncRNA studies.
P15 Functional analysis of mammalian translation initiation factor 3

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Mammalian translation initiation factor 3 (eIF3) is composed of 13 different subunits (a-m). Initial mass spectrometric analysis of human eIF3 suggested that human eIF3 comprises three modules. Module i contains all but one yeast eIF3 core subunits (a, b, g, i), module ii comprises eIF3c, d, e, k and l, and module iii contains subunits eIF3f, h, and m. Later it was proposed that the structural core of human eIF3 is formed by the so called PCI/MPN octameric complex composed of 8 subunits (a, c, e, f, k, l, m and h) to which other 5 subunits are more flexibly linked. Our recent biochemical work and a follow-up study in preparation suggested that at least two stable eIF3 subcomplexes may exist: the PCI/MPN octamer and the “yeast eIF3-like” a-b-g-i formation. However, how each of the eIF3 subunits contributes to the formation and stability of the entire complex is yet to be elucidated. We continue our study of eIF3 with detailed analysis of individual subunits to decipher their specific roles in general and/or mRNA-specific translation initiation during the normal as well as stress conditions.
Bacteria remain most of their life in the stationary phase of growth. Adaptation to the stationary phase is therefore necessary for their survival and survival of pathogenic bacteria in the host. Our goal is to see how transcription is regulated during stationary phase in *Mycobacterium smegmatis*. *M. smegmatis* is related to pathogenic species that cause severe human diseases (e.g. tuberculosis).

We discovered a new small RNA - Ms1, which is present in large quantities during stationary growth in *M. smegmatis*. We found that Ms1 binds to RNA polymerase (RNAP). This RNAP is without the main sigma factor or any other sigma factor; no such RNA has been described in bacteria so far. To clarify the function Ms1, we constructed a *M. smegmatis* strain with deleted Ms1 and characterized its transcriptome using RNA-seq. Ms1 regulates expression of approximately 1200 genes (out of 6700 *M. smegmatis* genes) in stationary phase.

Ms1 can affect the binding of different sigma factors to RNAP, but can also simultaneously have an effect on the stability of this enzyme. Ms1 is therefore a new type of small RNA interacting with RNAP.

This work was supported by GAČR 13-27150P a P305/12/G034.
P17 Characterization of a molecular mechanism underlying translation reinitiation of mammalian transcriptional activator ATF4

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ATF4 (activating transcription factor 4) is a key player in the process called integrated stress response (ISR) which enables cells to cope with incoming stress stimuli. It has been shown that translational upregulation of ATF4 mRNA in stress is governed by a gene-specific regulatory mechanism called translation reinitiation exploiting the short uORFs (upstream open reading frames) present in some 5’UTRs and having an ability to retain the post-termination 40S ribosomal subunit on the mRNA. Notably, defective translational control of ATF4 as well as other uORF-containing mRNAs has already been linked to several human diseases including cancer. However, the requirements for reinitiation in mammals are still not known even though uORFs are present in up to 50% mammalian transcriptomes and thus might represent important cis-regulatory features in translational control of eukaryotic gene expression. So far, eukaryotic reinitiation has been deeply studied with the help of yeast GCN4 mRNA, the yeast homologue of mammalian ATF4 mRNA. The primary goal of this study is to study the molecular mechanism of reinitiation on ATF4 mRNA to able to determine the extent of conservation of the reinitiation mechanism between lower and higher eukaryotes. This study includes extensive mutagenesis and analysis of the constructs containing 5’UTR of human ATF4 mRNA as well as monitoring of the behavior of endogenous ATF4 mRNA after stress stimuli in order to get a comprehensive insight into ATF4 upregulation in stress conditions.
Natural DNA modifications are epigenetic signals that are vital for regulation of gene expression. Artificial DNA modifications possess immense potential with respect to modulate the way how RNA polymerase (RNAP) and/or transcription factors interact with DNA. We performed a systematic study focused on the effect of artificial DNA modifications on transcription by RNAP. The modifications were in the major groove: either 5-substituted pyrimidines or 7-substituted 7-deazapurines (H, methyl, vinyl, ethynyl or phenyl groups). The templates were prepared by PCR. The DNA contained always only one modified base (e. g. only adenines) with one modification (e. g. methyl). The base was modified throughout the template. In vitro transcription assays were performed with RNAP holoenzymes from Bacillus subtilis and Escherichia coli. In general, small modifications (H, Me in 7–deazapurines) had no or even a positive effect on transcription and bulky modifications (Ph at any nucleobase) strongly inhibited or blocked transcription. However, substituting U (uracil) instead of T (thymine) also resulted in a strong inhibition. Modifications on guanine had the most pronounced inhibitory effect on transcription. Additional detailed experiments revealed that the modifications affected transcription mainly at the level of initiation. In summary, this knowledge will pave the way to future research of biorthogonal switches of gene expression.
A hallmark of oocyte development in mammals is dependence on the translation of stored mRNAs to proteins and their posttranslational modifications rather than on the de novo transcription of genes in order to sustain meiotic progression and early embryo development. In the absence of transcription, the completion of meiosis and early embryo development in mammals relies on maternally synthesized RNAs. RNA localization followed by local translation is the mechanism for the spatial and temporal regulation of gene expression. This process might play a crucial role in the development of the female germ cells and embryos. Surprisingly, not much is known about mRNA localization and translation in the mammalian oocyte or early embryo.

To visualize RNA in the cells, oocytes and embryos we apply various protocols: single molecule Fluorescent In Situ Hybridization, Rolling Circle Amplification and fluorescently labeled oligo probes. To add information about localization of specific proteins and ribosomal RNAs in the cells the RNA was co-stained using immunofluorescence protocol. Here we present localization of the global transcriptome, as well as specific transcripts using combination of immunostaining, at the subcellular resolution in the NIH3T3 cells, oocytes and early embryos from mouse. Moreover, we detected specific mRNA coupled with its nascent translation in the live mammalian oocyte. In conclusion we developed a method to visualize transcriptome and nascent in situ translation, which should enable to enhance our knowledge of this terra incognita in this unique cell types.

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Posters

**P20 Assemblies of RNA polymerase II C-terminal domain with processing factors investigated using integrative structural biology**

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RNA polymerase II (RNAPII) is a eukaryotic enzyme responsible for transcription of protein coding and non-coding genes. RNAPII uses its long and flexible C-terminal domain (CTD) to recruit specific protein/RNA-binding factors for regulation of transcription. CTD consists of tandem repeats of the heptapeptide consensus YSPTSPS. The CTD sequence is post-translationally modified in a dynamic manner, yielding specific patterns that are recognized by appropriate factors in coordination with the transcription cycle events.

To follow the structural assemblies of CTD with different effector molecules involved in transcription, we have developed a model system that allows mimicking the full-length CTD with the specific phosphorylation pattern in vitro. By combining this system with advanced nuclear magnetic resonance spectroscopy (NMR), X-ray crystallography, small angle X-ray scattering (SAXS) and cryo-electron microscopy (CryoEM) we follow changes in structural behavior of both, the CTD and respective binding factor, at the atomic level resolution.

Here, we report a hybrid structure of a long CTD fragment bound to multiple copies of Rtt103, a protein that is related to the 3'-end processing and transcription termination processes. Reconstruction of multisubunit complex with Rtt103 shows that the CTD retains its highly flexible character upon binding, forming a beads-on-a-string topology no fixed contacts between the effectors molecules. However, the RNA 3'-end processing and transcription termination factor dimerizes using a previously unknown dimerization domain. This dimerization event creates topological and mobility restraints, which in turn tunes its affinity towards the CTD by increase of local concentration and governs exposure of the CTD sequence to other protein factors.
P21 Connecting the 3’ with the 5’: the dual role of Rtt103p in transcription termination and RNA processing

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In Saccharomyces cerevisiae, Rtt103p is a part of the complex that recognizes RNA polymerase II (RNAPII) C-terminal domain (CTD) phosphorylated at Serine 2 (pSer2), thus bridging 5’-3’ exoribonuclease, Rat1p/Rai1p, to RNAPII during transcription of mRNAs. Rat1p/Rai1p complex is believed to terminate the process of transcription by itself or with the help of Pcf11p. In contrast to mRNAs, transcription termination of non-coding RNAs is performed by the Nrd1p-Nab3p-Sen1p (NNS) complex, and is followed by 3’-5’ RNA degradation/processing by the nuclear exosome. For each termination pathway, the interaction between transcription termination machinery and CTD of RNAPII is crucial for the proper transcription termination. The interaction with CTD is achieved by the CTD-interacting domain (CID) of either Nrd1p or Rtt103p.

In our previous work, we have shown that transcription termination of ncRNAs is physically coupled to RNA degradation/processing. The coupling is achieved by the CID of Nrd1p that recognizes a CTD-mimic (Nrd1p-interactin motif, NIM) within the TRAMP (Trf4p-Air2p-Mtr4p) complex, a cofactor of the nuclear exosome [1]. Here, we show that the CID of Rtt103p also recognizes the NIM, and another region of Trf4p, the RIM (discovered as the „Rtt103-interacting motif“). Interestingly, the NIM and especially the RIM are also recognized by the CID of another mRNA termination factor, Pcf11p. Specifically, we report the structure of Rtt103p CID bound to Trf4p NIM and initial structural studies of the complex between Rtt103p CID and Trf4p RIM. We also show that Rtt103p CID utilizes the same pocket to bind all three partners, CTD pSer2, Trf4p NIM and Trf4p RIM, in a mutually exclusive manner. Furthermore, we show the genetic interaction between Rtt103p and the nuclear exosome, and that its loss leads to the accumulation of readthrough and to creation of elongated transcripts. Overall, our results unveil a novel role of the Rtt103p in not only transcription termination but also in RNA processing by the nuclear exosome.

P22 Study of RRM Protein/RNA Complexes - The Synergy of NMR Spectroscopy and MD Simulations

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The RNA recognition motif (RRM) is the most common RNA-binding domain motif in proteins. It is composed of four antiparallel beta-sheet strands packed against two alpha-helices. Despite this simple topology, it has displayed a remarkable variety of RNA binding modes. In our work we use the molecular dynamics (MD) simulations to study two biologically relevant RRM protein/RNA complexes - Fox-1 RRM and SRSF1 RRM2. We expand the previous structural data by identifying new interactions and dynamical modes of recognition. The simulations predict unanticipated specific participation of Arg142 at the protein–RNA interface of the SRFS1 complex, which is subsequently confirmed by NMR and ITC measurements. Several segments of the protein–RNA interface may involve competition between dynamical local substates rather than firmly formed interactions, which is indirectly consistent with the primary NMR data. We demonstrate that the simulations can be used to interpret the NMR atomistic models and can provide qualified predictions. Finally, we propose a protocol for ‘MD-adapted structure ensemble’ as a way to integrate the simulation predictions and expand upon the deposited NMR structures. Unbiased μs-scale atomistic MD could become a technique routinely complementing the NMR measurements of protein–RNA complexes.
P23 A Role of Introns in Function of Activating Non-Coding RNAs

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In the last years, long non-coding RNAs (lncRNAs) have become one of the leading topics of RNA research because of versatile cellular functions such as nuclear structure organization, chromatin arrangement or transcription regulation. Under normal condition, the amount of lncRNAs vary from 1-2 copy per cell to several thousand copies depending on lncRNA species. Despite their low protein-coding potential, many lncRNAs undergo the same maturation pathway as protein-coding mRNAs including capping, splicing and/or polyadenylation. This project is focused on the activating type of long non-coding RNAs (ncRNA-a) which were shown to regulate expression of protein-coding target genes. The exact molecular mechanism how the ncRNA-a promotes the expression of target genes is unclear. We hypothesize that lncRNA splicing plays an important role in ncRNA-a function. First, we confirmed previous studies and showed that siRNA knock-down of selected ncRNA-as resulted in reduced transcription of their target protein-coding genes. Next, we have examined splicing efficiencies of these ncRNA-as and showed that all studied ncRNA-as are less efficiently spliced than pre-mRNAs transcribed from protein-coding genes. We also provide evidence that unspliced ncRNA-as are preferentially found in chromatin and nuclear fractions where they presumably function. Our data show that chromatin fraction contains preferentially unspliced ncRN-as, which suggests that unspliced ncRNA-as are the active form in transcription regulation.
Transfer RNAs (tRNAs) are extensively post-transcriptionally modified to increase their structural stability or fidelity maintenance. In particular, the modifications in the anti-codon loop, have a crucial role in accurate codon selection and translational frameshifting prevention. Queuosine (Q) is a hyper modified guanosine and may be found at the wobble position 34 of a 5′-GUN-3′ anticodon sequence-containing tRNAs (His, Asp, Asn, Tyr). Though Q is present in nearly all forms of life, its exact physiological role remains unclear. In bacteria, queuosine is obtained by modifying GTP through five enzymatic steps; it is then added to the tRNA by a tRNA-guanine transglycosylase (TGT) activity. However, eukaryotes lack the enzymes required for de novo synthesis of queuosine and hence rely on their environment or gut microbiome to obtain queuine (free base of queuosine), which is recognised by the eukaryotic TGT. In mice and humans, TGT exists as a mitochondrion-localised heterodimer with queuine tRNA ribotransferase 1 (QRT1), and its splice variant queuine tRNA ribotransferase domain containing 1 (QRTTD1). The T. brucei genome encodes two TGT paralogs, TbTGT1 and TbTGT2. TbTGT1 displays a nuclear localization in the procyclic form (insect gut) meanwhile in the bloodstream form (in mammalian host) the protein is extra-nuclear. Both TbTGT1 and TbTGT2 are involved in Q formation in tRNAs and TbTGT1 is essential for the growth of the bloodstream form of the parasite. This essentiality has been observed exclusively in trypanosomes so far, as no noticeable phenotype has been observed in the downregulation of the protein in the mammals. Consequently, TGT becomes an ideal target for drug development against diseases caused by trypanosomatid parasites.
P25 Intron mediated intergenic regulation of RPL22 paralogs in Saccharomyces cerevisiae

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Ribosomal protein coding genes (RPGs) are duplicated in various organisms including human. In *Saccharomyces cerevisiae*, paralogous ribosomal proteins have different localization and assembly requirements and can affect specific aspects of cell physiology. Recent genome-wide studies documented the existence of inter-genic loops controlling the relative transcript concentrations of RPG paralogs. We have analyzed the effects of intron deletions in several pairs of paralogous RPGs that contain secondary structures or atypical features in one of the paralogous introns. Here, we report the intergenic regulatory relationship between *RPL22A* and *RPL22B*, which code for the nonessential part of large ribosomal subunit. RT qPCR analyses of *RPL22A/B* intron manipulated strains and null mutants showed that introns in both A and B genes inhibit expression. The overexpression of any of *RPL22* genes without introns led to a decrease of both endogenous *RPL22* mRNAs, albeit the extent of the inhibition was much higher in *RPL22B*. Importantly, mutant version of either paralog had no effect on the mRNA levels of its counterpart. Using splicing reporter derived from *RPL22B*, we observed intermittent level of splicing efficiency in strains with WT *RPL22A*, while the level of spliced mRNA was further decreased in strains with intron deletion in *RPL22A* (i.e., with increased *RPL22* mRNA). Splicing efficiency was highest in strains where A paralog was missing, in accordance with the hypothesis that Rpl22 stalls the production of B by blocking the splicing step. In contrast to *RPL22B*, the relative changes of *RPL22A* mRNA accumulation were less pronounced. Increased pre mRNAs of *RPL22A/B* in mutants of RNA degradation pathways did not significantly affect the outcome of *RPL22* manipulations on the mRNA level, suggesting that once the pre-mRNA is diverted from the splicing trajectory, it is not competing for splicing. We conclude that the introns of *RPL22A/B* are differentially regulated by Rpl22 levels and discuss the role of splicing of genes encoding RNA-binding proteins in mediating intergenic regulatory circuits. Supported by the Grant Agency of the Czech Republic (14-190025).
P26 Transgenerational epigenetic inheritance and RNAe

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Since August Weismann (1834-1914) formulated the distinction between innate and acquired characteristics at the end of the 19th century, the debate relating to the inheritance of acquired traits has raised many controversies in the scientific community. Following convincing arguments against (e.g. William Bateson) this debate was then set aside by the majority of the scientific community. However, a number of epigenetic phenomena involving RNA, histone modification or DNA methylation in many organisms have renewed interest in this area. Transgenerational effects likely have wide-ranging implications for human health, biological adaptation and evolution, however their mechanism and biology remain poorly understood. We recently demonstrated that a germline nuclear small RNA/chromatin pathway can maintain epi-allelic inheritance for many generations in C. elegans. This is a first in animals. We named this phenomenon RNA-induced epigenetic silencing (RNAe). We are currently further characterizing the mechanism of RNAe. In addition, we are testing the hypothesis that RNAe provides a transgenerational memory of the environment (“Lamarckism”). We are currently exploring related phenomena in mice. We are also working towards establishing iPS cells differentiating into germ cells as a model to study the mechanism of transgenerational epigenetic inheritance.
P27 The mechanism of m6A demethylation in pre-mRNA processing regulation

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N6-methyladenosine (m6A) is the most prevalent internal messenger RNA (mRNA) modification in higher eukaryotes and it regulates diverse processes of RNA metabolism. The m6A is deposited on the RNA by a multicomponent methyltransferase complex and, interestingly, it can be removed by the action of two demethylases: FTO and AlkBH5. Despite their key role, the demethylases are still the least studied components of the methylation/demethylation pathway. By using cross-linking and immunoprecipitation coupled to high-throughput RNA sequencing (CLIPseq), we identified the RNA targets of the demethylase FTO transcriptome-wide. Additionally, the RNAseq analysis of a FTO knockout cell line uncovered quantitative and qualitative changes at the mRNA processing level, revealing that FTO is an important regulator of pre-mRNA alternative splicing. In summary, our study provides a comprehensive set of data describing the function of m6A demethylation pathway and proposing a novel role of FTO and m6A in regulation of nuclear pre-mRNA processing events.
P28 RPS3 and translational termination

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Eukaryotic translational initiation requires interaction of many protein factors with the 40S ribosomal subunit. These factors are called eukaryotic initiation factors (eIFs) and numerous studies have been published over the years describing their specific roles not only in translational initiation, but also in termination and ribosomal recycling. Small ribosomal proteins are key players in anchoring the eIFs to the ribosomal surface and perhaps even in modulating their function, but their exact roles remain largely unexplored. In this study, we focused on small ribosomal protein RPS3, which lies near the mRNA entry channel and is known to be a part of the latch mechanism modulating mRNA recruitment and movement of the ribosome along mRNA. In addition, RPS3 interacts with the TIF32 and TIF35 subunits of eIF3, recently implicated in controlling translation termination and promoting programmed stop codon readthrough. Indeed, our experiments identified two RPS3 variants with altered readthrough levels. Interestingly, both mutants seem to have an opposite effect on the efficiency of readthrough (increase vs. decrease), which most likely reflects their specific orientation towards other constituents of the latch mechanism.
P29 Ms 1, a small RNA in Mycobacterium smegmatis

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In recent years, the interest in small RNAs (sRNA) is increasing. These molecules are important for regulation of gene expression at various levels. One of them is transcription by RNA polymerase (RNAP). RNAP is regulated by various auxiliary factors. Here, we describe a newly discovered sRNA in mycobacteria, termed Ms 1 that interacts with the RNAP core in stationary phase. RNA sequencing showed that deletion of Ms 1 caused deregulation of about one thousand genes, including genes important for redox homeostasis and heat shock response of the cell. Subsequently, we performed phenotypic experiments where we confirmed an impaired ability of the ΔMs 1 mutant to resist oxidative stress and elevated temperatures. Importantly, Ms 1 was discovered by in silico approach also in pathogens (Mycobacterium tuberculosis or Mycobacterium leprae). Thus, this project contributes to our understanding of the transcription machinery in mycobacteria, a medicinally important group of bacteria.
P30 σI regulon from Bacillus subtilis

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σ factors play essential role in bacterial transcription initiation. Bacillus subtilis contains one main and 17 alternative σ factors. σI is one of the less investigated alternative sigma factors. It is known to be involved in heat shock response. Our main aim was to define the σI regulon. Based on RNA-sequencing data we determined over 70 genes regulated by σI. Among them we found genes involved in iron metabolism, amino acid biosynthesis, and cell shape determination. Moreover, we successfully purified the σI protein, and set up an in vitro transcription system with σI and its dependent promoters. Additionally, we tested binding properties of σI to DNA in the presence and absence of the RNAP core. In summary, the results define the σI regulon and the transcription properties of RNAP holoenzyme with this factor.

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P31 Sm-ring dependent targenting of U snRNA into Cajal bodies

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An essential component of the spliceosome, snRNPs consists of snRNA, a ring of seven conserved Sm proteins [B/B’, D1-3, E, F, G] and U snRNP-specific proteins. After transcription snRNA is exported to the cytoplasm where the ring of Sm proteins is formed around the Sm binding site in SMN complex-dependent manner. The core particle is reimported to the nucleus where associates with snRNP-specific proteins to form mature, splicing competent particle. It was suggested that final steps of snRNP biogenesis occur in the nuclear structure called the Cajal body (CB). However, it is unclear how the core of snRNPs is navigated to CBs. To analyze CB targeting sequences we microinjected fluorescently labeled snRNA deletion mutants into human cells and observed their accumulation in CBs. While deletion of sequences interacting with snRNP-specific proteins did not prevent CB accumulation, we provide several lines of evidence that Sm proteins are essential for targeting of the snRNP to CBs. 1) Deletion of the Sm binding site from snRNA efficiently prevents snRNA accumulation in CBs. 2) Preventing the interaction between snRNA and the SMN complex, which is essential for Sm ring assembly, inhibited snRNA localization in CBs. 3) Knockdown of several Sm proteins blocked the targeting of microinjected snRNA to CBs. 4) Expression of SmD3 deletion mutant reduced snRNP accumulation in CBs suggesting that SmD3 is important for CB localization. Surprisingly, we observed CB accumulation also after nuclear injection, which indicates that the nuclear SMN complex is also able to assemble the Sm ring.
P32 Identification of cell fate determinants and formation of organism body plan

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Starting from a single fertilized oocyte, through manifold of divisions a complex organism is developed that has distinct head-tail (bottom-up), left-right and dorsal-ventral (back-belly) asymmetries. One of the main challenges in developmental biology is to understand how and when these asymmetries are generated and how they are controlled. The African clawed frog (Xenopus laevis) is an ideal model for studies of early development thanks to their very large oocytes. We have developed a unique molecular tomography platform based on RT-qPCR, RNA-seq and iTRAQ UPLC-ESI-MS/MS to measure asymmetric localization of fate determining mRNAs, non-coding RNAs and proteins within the oocyte and among the early stage blastomeres. The first axis called animal-vegetal, is formed during oogenesis and we found mRNA and microRNA gradients determining its formation. First cell division following fertilization producing 2-cell stage embryo forms the left-right, and second cleavage generating 4-cell embryos specifies the dorsal-ventral axis.
P33 Structural studies of the extended RRM form of Nab3p

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In S. cerevisiae transcription termination is mediated through two major pathways, poly(A)-dependent and poly(A)-independent. In the latter pathway, RNA is processed by the Nab3p-Nrd1p-Sen1p transcription termination complex. Nab3p and Nrd1p function as a heterodimer, and each protein contains a so-called RNA recognition motif (RRM). RRMs share a typical β-α-β-β-α-β topology with α-helices located behind antiparallel β-sheet. The aforementioned β-sheet usually serves as a platform for the binding of single-stranded RNA, with consensus sequence UCUU [1, 2]. Nab3p RRM, however, was shown to be extended by two N-terminal α-helices (hence the name αRRM) that play an important role in the process of RNA binding, leading to an increased affinity. The exact mechanism of this effect still remains poorly understood.

In the present study, a spectrum of methods, such as NMR, SAXS or fluorescence anisotropy (FA), were used in order to elucidate the structure of αRRM of Nab3p and to investigate the exact topology of α-helical part and its effect on binding to RNA.


P34 Regulation of rRNA transcription by DNA topology in Bacillus subtilis

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Bacterial cells need to adapt to changing environment. In nutrient-rich environment the cells grow and divide and this requires a large amount of ribosomes. In nutrient-poor environment the number of ribosomes is strongly reduced. The amount of ribosomes is regulated on the level of transcription initiation of ribosomal RNA (rRNA). In Bacillus subtilis rRNA promoters are regulated by the concentration of GTP, the initiating NTP (iNTP).

Here, we will demonstrate that rRNA promoters are regulated also by another mechanism than responding to changes in [GTP]. Upon entry into stationary phase the intracellular GTP level decreases, but after ~60 min it levels off. However, the activity of rRNA promoters decreases even after these 60 min.

We investigated the role of DNA supercoiling on the activity of rRNA (regulated by [GTP]) as well as control promoters (not-regulated by [GTP]) by in vivo and in vitro approaches. We revealed that changes in supercoiling of rRNA promoters strongly affected the affinity of RNA polymerase (RNAP) for the DNA (initial binding) and iNTP (open complex formation) while in the case of the control Pveg promoter we observed only altered affinity for the iNTP. The ultimate goal is to determine DNA sequence elements required for promoter sensitivity to the level of supercoiling.

In summary, a new mechanism of regulation of rRNA synthesis is proposed here.

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P35 Endogenous RNAi in mammals: an update

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RNAi is an antiviral innate immunity and genome defense pathway in plants and invertebrates. Yet, in mammals, RNAi became a marginal pathway. Although mammalian genes constituting the RNAi pathway are present, their primary role is in the gene-repressing miRNA pathway. An exception of the rule are mouse oocytes, which have highly active RNAi because of an oocyte-specific truncated Dicer isoform (DicerO). A rodent-specific MT retrotransposon insertion in Dicer gene functions as an oocyte-specific promoter, which produces a highly active Dicer isoform lacking N-terminal DExD helicase domain. DicerO is essential for oocyte maturation; deletion of the MT insertion causes sterility due to meiotic spindle defects.

Here, we report on recent progress in several areas of mammalian RNAi. First, we continued phenotype analysis of mice lacking the MT insertion (denoted MT1); we found out that mice can recruit as an alternative promoter another downstream MT element insertion (denoted MT2). Expression of evolutionary younger MT2 partially rescues the sterile phenotype of MT1 KO females in an outbred background suggesting an additional layer of DicerO expression control.

Second, we generated mESC lines expressing DicerO. We observed variable levels of endoRNAi in different lineages, showing that DicerO expression is necessary but not sufficient for endoRNAi in mammals. These lines will help to define minimal conditions for physiologically relevant RNAi in mammals.

Third, analysis of other mammalian species revealed that bovine and porcine oocytes do not express DicerO while we detected DicerO expression in rat and golden hamster oocytes. Yet, it has been shown that long dsRNA microinjection is capable of inducing RNAi in bovine oocytes. Therefore, we are assessing kinetics of RNAi in mouse, bovine and porcine oocytes in order to determine how robust RNAi can be supported by the full-length Dicer protein.

Finally, we explore redundancy of small RNA pathways in mouse, in particular in retrotransposon control. We generated mice defective both, endoRNAi and piRNA pathways, and examined whether they would exhibit an earlier onset of phenotype than individual knock-outs.
Bacteria need to rapidly adapt to changing environment. One of the important enzymes for this process is RNA polymerase (RNAP). RNAP is an extensively studied multisubunit enzyme required for transcription of DNA into RNA. The RNAP core consists of α2ββ’ω subunits. Unlike gram-negative bacteria, the RNAP core from *Bacillus subtilis* and other gram-positive Firmicutes contain an additional subunit, delta. The delta subunit is composed of two domains: the structurally well-organized N-terminal and the unstructured highly acidic C-terminal domain. Recent studies mainly focused on functioning of the whole delta subunit. The mechanistic functioning of individual domains is still not fully defined. Here, we will present results of experiments that address functions of the two domains of delta studied by *in vitro* transcriptions and *in vivo* competitive cell growth assays. Implications of these results for the functioning of the delta protein and the transcription machinery will be discussed. This work is supported by grant No. 13-16842S from the Czech Science Foundation.
P37 Novel antisense RNAs in an antibiotic-producing bacterium Streptomyces coelicolor

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Streptomyces is a genus of gram-positive filamentous soil bacteria. They undergo complex developmental life cycle accompanied with an antibiotic production. High demand on fast, specific and cost-effective control of cell processes is ensured by an antisense regulatory mechanism, where the target double stranded asRNA-mRNA complex is subjected to subsequent ribonuclease degradation. RNase III is a double-strand specific endoribonuclease acting in prokaryotes. In an agreement, as the RNase III-deletion strains fail to produce antibiotics, we presume that the streptomycete antibiotic biosynthetic pathways include the antisense RNA expression control mechanisms. Recently published data revealed several mRNAs bound by RNaseIII. We confirmed an expression of 18 novel antisense RNAs, complementary to the mRNAs taken from the set. Differential expression analysis of chosen sRNAs and their corresponding mRNAs in wild type and RNaseIII-deletion strains showed noticeable distinctions. Further analysis of detected sRNAs is now being examined.
P38 YloH, an RNAP subunit of Bacillus subtilis

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The central enzyme of transcription is RNA polymerase (RNAP). In gram positive bacteria, the RNAP core is composed of α2ββ′ω1ω2 and δ subunits. By associating with yet another subunit (a sigma factor), the resulting RNAP holoenzyme (RNAP-σ) can recognize promoter sequences, and initiate transcription of specific genes. One of the core subunits, ω2 (YloH), encoded by the rpoZ gene, binds to β′. Previously, it was shown that ω2 is not essential. However, it was also demonstrated that a number genes in bacteria were down- or up-regulated in ΔrpoZ strains and ω2 was proposed to be important for the interaction of σA with RNAP. Here, we set out to investigate the mechanistic details of how ω2 affects the binding of σA to RNAP, and also how ω2 affects the interaction of RNAP with δ, and HelD. The latter protein is a recently discovered binding partner of RNAP that functions in a synergistic manner with δ. This work contributes to our understanding of the structural and functional architecture of RNAP. This work is supported by grant No.P305/12/G034, from the Czech Science Foundation.
P39 The human translation initiation factor 3 (eIF3) expression and integrity code

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Eukaryotic initiation factor 3 (eIF3) is the largest of all initiation factors comprising 12 + 1 subunits (termed a-m) and a critical player almost in each step of translational cycle. Moreover, misregulation of many eIF3 subunits was observed in several types of cancer, however, in most of the studies only one subunit of eIF3 was monitored. Our recent investigation showed that changes in the expression of one subunit can concomitantly affect protein levels of other eIF3 subunits. To have a complete picture of intersubunit dependencies in the eIF3 complex, we individually knocked down each subunit of eIF3 in HeLa cells and examined the effect of each knockdown on the eIF3 integrity and expression levels of the remaining non-targeted eIF3 subunits as well as translation rates and cell viability. This enabled us to propose for the first time the human eIF3 disassembly pathway resulting from destabilization of several critical joints holding the whole complex together. As a consequence of ordered, stepwise disassembly of eIF3, all subunits that cannot be incorporated into complex are most probably very rapidly degraded.
P40 Altered biochemical specificity of G-quadruplexes with mutated tetrads

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A fundamental motif in canonical nucleic acid structure is the base pair. Mutations that disrupt base pairs are typically destabilizing, but stability can often be restored by a second mutation that replaces the original base pair with an isosteric variant. Such concerted changes are a way to identify helical regions in secondary structures and to identify new functional motifs in sequenced genomes. In principle, such analysis can be extended to noncanonical nucleic acid structures, but this approach has not been utilized because the sequence requirements of such structures are not well understood. Here we investigate the sequence requirements of a G-quadruplex that can both bind GTP and promote peroxidase reactions. Characterization of all 256 variants of the central tetrad in this structure indicates that certain mutations can compensate for canonical G-G-G-G tetrads in the context of both GTP-binding and peroxidase activity. Furthermore, the sequence requirements of these motifs are significantly different, indicating that tetrad sequence plays a role in determining the biochemical specificity of G-quadruplex activity. Our results provide insight into the sequence requirements of G-quadruplexes, and should also facilitate the analysis of such motifs in sequenced genomes.
P41 RNA world in progress: Complex visualization transcriptome in oocyte and embryo

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mRNA localization followed by local translation is a fundamental mechanism for the gene expression. In case of mammalian oocyte these processes play a crucial role in transfer of genetic information to the next generation and in the development. Surprisingly, not much is known about mRNA localization to the compartments or structures in the mammalian oocyte. Single molecule RNA fluorescent in situ hybridization (smRNA FISH) is the most used RNA localization method and similar concept is also used for imaging proteins by fluorescent immunocytochemistry (ICC). Although these methods are simple separately, the existing protocols and materials used for ICC and smRNA FISH make both difficult to combine and may lead to artifacts and/or failure of experiments. However, the possibility of visualization of RNA and proteins together can bring a new insight into the regulation of gene expression during the development of the egg.

We present an overview of techniques for immunostaining, fluorescence in situ hybridization with subcellular resolution and a combination of both, that can be used for spatial analysis in mouse oocytes and early embryo. We show advantage of rolling circle amplification step for visualization of global RNA in oocyte. We used new protocol for identification of global RNA population and ribosomal RNA in the growing oocyte, fully grown oocyte and 2-cell embryo with connection to the physiological relevance. Our protocol will be valuable mainly in biological and medical research.
P42 Computational analysis of splicing pattern difference between human retina and other human tissues

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Retinitis Pigmentosa (RP) is a heterogeneous group of genetically inherited retinal diseases leading to progressive loss of vision due to impairment of rod and cone photoreceptors. Over 65 causative genes and/or loci have been so far identified, that however account for only ~60% of cases, leaving dozens of genes awaiting yet to be discovered. Recent studies suggested that mutations in genes encoding ubiquitously expressed proteins involved in pre-mRNA splicing lead to RP development, which depicts the important role of pre-mRNA splicing in the pathogenesis of retinal degeneration. Therefore, it is critical to understand the splicing complexity in between retina and other cell types. Herein, we performed comparative transcriptome analysis of retina and 14 other human tissues using publically available RNA-seq data. We report a unique set of 133 genes, which are exclusively expressed in retina; and in comparison, a core of 3103 genes ubiquitously expressed in all analyzed tissues. Moreover, we are currently running a computational splicing analysis, which may be helpful in understanding the difference between splicing patterns in retina and other tissues. Overall, the present study could assist in understanding the physiology of the visual system and can be used for downstream analyses.
Pre-rRNA processing in higher eukaryotes is a complex process, involving endonucleolytic cleavages of the large precursor molecule within external and internal spacer sequences, and their further trimming by exonucleases. Eventually, the series of coordinated nucleolytic events leads to separation and maturation of 18S, 5.8S and 28S rRNA species, which are incorporated into ribosomes during their biogenesis. In addition, quality control systems based on the action of nucleases ensure that the excised spacer sequences do not accumulate in the cells during pre-rRNA processing and are instead efficiently removed. The identity of endonucleases, such as UTP24 or LAS1, responsible for several steps of rRNA maturation in human cells has been revealed relatively recently. For other cleavages, endonucleolytic activites remain to be identified. On the other hand, the role of exonucleases in trimming precursor molecules and quality control, has not been studied in detail. For instance, exonucleolytic activities of the catalytic subunits of the major human 3'-5' exoribonuclease - the exosome complex, have been demonstrated so far to participate in the 3'-end maturation of 18S (hRRP6) and 5.8S (hDIS3 and hRRP6) molecules. hXRN2 5'-3' exoribonuclease was in turn shown to be responsible for trimming the 5'-ends of 5.8S and 28S precursors.

Here, we present the results of our studies concerning the role of hDIS3, hRRP6 and hXRN2 exoribonucleytic activities in the removal of 5'-ETS fragments, arising after cleavages at sites A', A0 and A1. To this end, we utilized cell lines with silenced expression of the respective endogenous protein and exogenously producing WT or catalytically-dead enzyme variants. RNA isolated from these cells was analyzed by northern-blot, primer extension and 3'-RACE techniques. Altogether, our preliminary data indicate that hDIS3, hRRP6 and hXRN2 contribute differentially to the degradation of +1-A', A'-A0 and A0-A1 fragments of the 5'-ETS.
P44 The rate of transcription, but not timing of its activation, defines dynamics of DNA methylation establishment in the oocytes

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Gametogenesis in mammals entails profound re-patterning of the epigenome, as well as changes in transcriptome. Early embryonic oocytes are mostly devoid of DNA methylation; it is established de novo postnatally during oocyte growth. DNA methylation in gametes marks a subset of genes for appropriate expression in the next generation, as in the case of imprinted genes. Therefore, its correct establishment is essential for the healthy development of the progeny. Imprinted genes were shown to acquire methylation at different times during oocyte growth. However, little is known about the kinetics of methylation gain genome-wide and the reasons for asynchrony in methylation at imprinted loci. In addition, changes in the activity of transcriptional start sites during the oocyte growth remain to be elucidated, as well as the mechanism of the interplay between transcription and DNA methylation.

We previously showed that oocyte transcriptome markedly differs from the reference annotation, and that its DNA methylome is established as a consequence of transcription - hypermethylated domains match actively transcribed genes, while unmethylated domains are transcriptionally silent. Now we investigated whether the differences in timing of transcription events required for de novo methylation could contribute to differential timing of methylation. We generated genome-wide transcriptome, methylation and histone modification maps of size-selected, growing oocytes to capture the progression of methylation. We find that the reference genes are mostly already active in the early embryonic oocytes, while novel oocyte-specific genes and novel start sites of known genes are predominantly activated postnatally, at the beginning of the oocyte growth. These transcriptional changes occur well before the de novo methylation phase and do not appear to affect the methylation timing; nevertheless, transcription rate does correlate with rate of methylation. Conversely, timing of methylation of CpG islands correlates inversely with enrichment of histone modifications inhibitory to DNA methylation and with dependence on the histone 3 lysine-4 demethylases, identifying chromatin factors as major determinants of methylation timing.
Protein synthesis is one of the fundamental cellular processes and is mostly regulated through its initiation phase, which is controlled by numerous initiation factors. Among them, eIF3 is the largest and the most complex factor, in mammals comprising 12 non-identical subunits (named eIF3a through m), with a molecular weight of around 800 kDa. Eight subunits form a structural scaffold and the remaining 4 non-octameric subunits (d, b, g and i) are most probably rather flexible as nearly no additional cryo-EM density can be observed between the octamer and the whole 12-subunit complex. siRNA analysis of human eIF3 by knocking down all eIF3 subunits individually in HeLa cells and following co-immunoprecipitation showed that several stable eIF3 subcomplexes can form in vivo. We propose the in vivo assembly as well as disassembly pathways for human eIF3 and compile an array of expression dependencies among eIF3 subunits. Further, we try to understand the specific function of single eIF3 subunits or subcomplexes in translation initiation.
P46 Structure of the double-stranded RNA binding protein Staufen1 bound to dsRNA target involved in Staufen-mediated mRNA decay

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Multiple double-stranded (ds) RNA binding proteins are known to regulate gene regulation post-transcriptionally, but their target specificity for dsRNA is still unclear. Human Staufen1 controls mRNA transport of certain cytoplasmic mRNAs, translation and decay by the Staufen-mediated mRNA decay (SMD) pathway. Staufen1 regulates levels of the ADP-ribosylation factor1 (ARF1) mRNA via SMD mediated by a specific interaction of Staufen1 with a 19 base pair helix formed within the ARF1 3’UTR, but how this RNA helix is recognized is unclear.

To reveal the dsRNA target specificity of Staufen1, we are determining the solution structure of the ARF1 dsRNA - Staufen1 complex by nuclear magnetic resonance spectroscopy. We designed a protein comprising the Staufen1 dsRNA binding domains (dsRBD) 3 and 4 for expression in E. coli and purified the protein by affinity, anion-exchange and size-exclusion chromatography. The ARF1 dsRNA was prepared by in vitro transcription and purified by anion-exchange and size-exclusion chromatography. Electrophoretic mobility shift assay and fluorescent anisotropy measurement show that the interaction of ARF1 dsRNA with dsRBD3 and 4 results in a single complex with affinity in the nanomolar range. A complete set of 2D and 3D NMR experiments was measured for the assignment of the dsRNA - Staufen1 complex. The NMR data show that both dsRBDs are well folded in free form and in the presence of the interaction partner ARF1 dsRNA. We will present the preliminary structure of the ARF1 dsRNA - Staufen complex which reveals that Staufen is indeed a sequence-specific dsRNA binding protein which specifically binds dsRNA targets by sequence readout in the minor groove. The biological implications will be discussed.
Eukaryotic translation initiation factor 3 (eIF3) is a large multiprotein complex promoting binding of several other initiation factors to the 40S subunit, where it coordinates their actions during the initiation phase of translation. It comprises 13 proteins in mammals and 6 proteins in yeast. Perhaps due to a high degree of flexibility of multiple eIF3 subunits, a detailed crystal structure of neither mammalian nor yeast eIF3 complexes has been solved till now.

We previously examined the protein-protein interactions among yeast eIF3 subunits and built the eIF3 subunit interaction map. Here we purified all individual subunits of yeast eIF3 from bacteria and reconstructed the whole protein complex (lacking the non-essential j/HCR1 subunit) in vitro to determine an overall shape of the free 5-subunit eIF3 in solution. The reconstituted eIF3 was cross-linked with two different cross-linkers and the trypsin-digested samples were analyzed using advanced Mass spectrometry. The obtained cross-links not only support our aforementioned eIF3 subunit interaction map but also reveal a completely new geometry of eIF3. The whole complex seems to be very compactly packed when free in solution, which contrasts with the recently published eIF3-40S models, where the extended eIF3 appears to wrap around the 40S head. These dramatic differences suggest a robust structural rearrangement of the overall shape of eIF3 prior to its 40S-binding with functional implications that will be discussed.
Alternative splicing is a process which allows to obtain various proteins from one gene. This process is highly regulated and even small malfunction can cause diseases. The involvement of TAR DNA-binding protein (TDP-43) in alternative splicing of cystic fibrosis transmembrane conductance regulator (CFTR) gene results in severe forms of cystic fibrosis. TDP-43 binds with high affinity to a mutated, extended UG-rich region with a shortened polypyrimidine tract upstream of the 3' splice site (3'ss) of CFTR exon 9 and recruits another splicing regulator, hnRNP A1. Thus formed complex prevents the recognition of the 3'ss of exon 9 by the spliceosomal machinery and causes exon 9 skipping resulting in a non-functional CFTR protein. Functional implications of RNA recognition and binding by TDP-43 alone and in complex with other hnRNPs are vast, nevertheless, little is still known about molecular details of such interactions.

Our structural and biophysical studies of TDP-43 - CFTR exon 9 3'ss RNA (3'ss RNA) interactions reveal that two copies of TDP-43 bind to the extended, UG-rich sequence not independently, but create a new protein-protein interface upon dimerization. Binding affinity of TDP-43 to the RNA sequence, at the same time, increases as a function of UG-repeat length reaching an optimum at 10 repeats. We further reveal that two copies of hnRNP A1 which is recruited by TDP-43 to the intron 8 - exon 9 junction are involved in interaction with 3'ss RNA via their RRM2 domains while RRM1 domains presumably participate in protein dimerization. NMR monitoring of the TDP-43 - hnRNP A1 complex assembly confirmed that their interaction with 3'ss RNA is not masking the polypyrimidine tract on the pre-mRNA for binding of the canonical splicing factor U2AF65. Shortening of the polypyrimidine tract, on the other hand, lowers the affinity of U2AF65 and binding of hnRNP A1 at the 3'ss blocks the interaction of U2AF35. Thus a network of RNA-protein and protein-protein interactions which competes with the formation of the canonical splicing complex drives CFTR exon 9 skipping.
P49 Promoter sequence determinants of NAD+ utilization as transcription initiation substrate by RNA polymerase

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NAD+ was recently discovered to be covalently attached to the 5’ ends of some RNAs in Escherichia coli1, analogously to 7-methyl guanosine, the cap, in eukaryotes. Subsequent experiments revealed that it is RNA polymerase (RNAP) that can utilize NAD+ as transcription initiation substrate, and this modification increases the lifetime of the RNA in the cell2. Here, we investigated the sequence elements that determine the efficiency of NAD+ incorporation by RNAP. We show, that it is the sequence surrounding the +1 position (transcription start) that affects the incorporation, thereby shaping the epitranscriptome. Biological implications of this phenomenon will be discussed.

The yeast RNA polymerase II (RNAP II) synthesizes both protein-coding and non-coding RNAs (ncRNA). The transcription termination plays a significant role in the nascent transcripts metabolism and stability. RNAP II transcription termination is regulated by the dynamic modification of the C-terminal domain (CTD). CTD consists of multiple hexapeptide repeats with the sequence Y1S2P3T4S5P6S7. There are two different mechanisms for mRNAs and ncRNAs. The CTD phosphorylation at Ser2 position is recognized by the CTD-interacting domain (CID) of Rtt103p, which is believed to stimulate termination of coding transcripts. Rtt103p recruits Rat1p exonuclease and Rai1p pyrophosphatase, the so-called “torpedo,” which degrades the downstream cleavage product and displaces RNAPII from template DNA. Termination of ncRNA transcription, in turn, depends on the recognition of pSer5 by Nrd1p CID and consequent connection with Nrd1p-Nad3p-Sen1p (NNS) complex. NNS complex recruits the TRAMP-exosome machinery which trims the 3' ends of nascent transcripts. NNS complex is coupled with exosome through physical interaction between Nrd1p and TRAMP subunit, Trf4p. TRAMP complex was not previously expected to participate in mRNA processing. Our data shows that Trf4p can recognize CID of Rtt103p and bind to it. Moreover, depletion of TRF4 gene causes the accumulating of 3' extended mRNAs. Presented data shows that TRAMP complex involve in mRNA metabolism, along with its well-described role in ncRNA quality control.
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Extraordinary variation of floral organs in Phalaenopsis orchid is due to
differential expression of multiple genes in sepal, petal, and labellum.¹

¹ Hino, Y. et al. Genes 518, 97-100 (2013).

Differential gene expression creates beauty

Differential gene expression plays a significant role in development of many species including orchids.

Phalaenopsis orchids are important species for development and evolutionary studies. Moreover, they are well recognized for their exceptional beauty. The flowers have several organs (i.e. sepal, petal, and labellum), which are significantly different, however, together compose conspicuous and harmonious look (view photo). The unique shape of these flower organs is a result of differential expression of multiple genes involved in their development.

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