Session 16: RNA biology

Lectures

L16.1

Transcriptome changes induced by toxic RNA in myotonic dystrophy

Krzysztof Sobczak

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland
Krzysztof Sobczak <ksobczak@amu.edu.pl>

RNA-binding proteins play critical roles in RNA alternative processing during tissue development and maintenance. A striking example of the importance of developmental functions of these proteins is provided by the muscleblind-like (MBNL) protein family, which function as alternative splicing factors at multiple developmental stages from embryonic stem cell differentiation to postnatal switches required for the expression of adult isoforms. Disruption of these critical developmental transitions occur in the neuromuscular disease myotonic dystrophy (DM) since the RNA processing functions of MBNL proteins are compromised by microsatellite instability and the expression of CUG or CCUG expansion (CUG^{exp}, $CCUG^{exp}$) RNAs. In DM, interactions between MBNL proteins and C(C) UG^{exp} RNA leads to the formation of nuclear complexes, or RNA foci. We demonstrated that MBNL-CUG^{exp} complexes are highly dynamic structures composed of tightly packed, although mobile, MBNL proteins that modulate RNA foci morphology. Using all-exon microarray platform we showed that sequestration of MBNL proteins in DM results in aberrant processing of hundreds of pre-mRNA. Among these, 20 events showed graded changes that correlated with strength of muscle weakness in patients. Alternative splicing changes of these exons may serve as biomarkers of disease severity and therapeutic response in DM. We also found that among alternative exons significantly misregulated in DM there are 3'-exons forming alternative 3' untranslated regions (3'UTRs). Although MBNLs bind to nascent transcripts to regulate alternative splicing during muscle and brain development, another major binding site for the MBNL protein family are 3'UTRs of target RNAs. Depletion of MBNL proteins leads to misregulation of thousands of alternative polyadenylation events. HITS-CLIP analysis indicate that these polyadenylation switches are a direct consequence of MBNL binding to target RNAs. These findings reveal an additional developmental function for MBNL proteins and demonstrate that DM is characterized by misregulation of pre-mRNA processing at multiple levels.

L16.2

Human exoribonucleases in health and disease

Andrzej Dziembowski[1,2]

[1]Institute of Biochemistry and Biophysics PAS, Warsaw, Poland;
[2]Department of Genetics & Biotechnology, Warsaw University, Warsaw, Poland
Andrzej Dziembowski <andrzejd@ibb.waw.pl>

The multisubunit RNA exosome complex is a major ribonuclease of eukaryotic cells that participates in the processing, quality control and degradation of virtually all classes of RNA in Eukaryota. All this is achieved by about a dozen proteins with only three ribonuclease activities between them. In this talk I will provide an overview of our recent results describing the role of the nuclear exosome complex in the regulation of transcriptome homeostasis. Moreover I will describe an interesting connection between the exosome and the pathogenesis of multiple myeloma, a cancer of plasma cells.

Oral presentations

O16.1

Revealing a new activity of the human Dicer DUF283 domain

Anna Kurzynska-Kokorniak[1], Maria Pokornowska[1], Natalia Koralewska[1], Weronika Hoffmann[2], Krystyna Bienkowska-Szewczyk[2], Marek Figlerowicz[1,3]

[1]Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznań, Poland; [2]Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, Gdańsk, Poland; [3]Institute of Computing Science, Poznan University of Technology, Poznań, Poland
Anna Kurzynska-Kokorniak <akurzyns@man.poznan.pl>

The ribonuclease III (RNase III) Dicer plays a fundamental role in the biogenesis of small regulatory RNAs by excising microRNAs (miRNAs) from their hairpin precursors (pre-miRNAs) and by producing small interfering RNAs (siRNAs) from long double-stranded RNAs (dsRNAs). The Dicer generated small regulatory RNAs can control gene expression by targeting complementary transcripts and inducing their cleavage or repressing their translation. RNase Dicer is a multidomain enzyme; for all its domains except one, named DUF283 (domain of unknown function), their involvement in RNA substrate recognition, binding or cleavage has been postulated. Accordingly, the N-terminal helicase domain is thought to be involved in discriminating between miRNA and siRNA precursors by interacting with the hairpin loop structures of pre-miRNAs. The PAZ domain has been shown to bind to the 3' end of a substrate. Further, it has been demonstrated that two RNase III domains of Dicer (IIIa and IIIb) form a single dsRNA cleavage center that binds miRNA/siRNA precursors and cleaves them in a specific manner. Finally, the C-terminal dsRNA-binding domain has been shown to play an auxiliary role in substrate binding and cleavage. In contrast, for DUF283 the interaction with Dicer protein partners has been the only function suggested thus far.
Interestingly, results of our recent studies revealed for the first time the involvement of DUF283 in interactions with nucleic acids. We demonstrated that the isolated DUF283 domain from human Dicer was capable of binding single-stranded RNAs and DNAs. More detailed analyses revealed that DUF283 acted as a nucleic acid annealer that facilitated hybridization between RNA or DNA complementary strands. Furthermore, we found that full length Dicer displayed an annealing activity as well. The overall results suggest that Dicer, presumably through its DUF283 domain, might facilitate hybridization between short RNAs and their targets. Thus, our results suggest that Dicer might function as a chaperone-like protein. These new findings reveal the complex nature of Dicer, whose functions may extend beyond the biogenesis of small regulatory RNAs [1].

Reference:

1. Kurzynska-Kokorniak A *et al* (2016) *Sci Rep* **6**: 23989.

Acknowledgements:

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O16.2

Translational regulation of maternal transcripts during zebrafish mid-blastula transition

Cecilia Winata[1,2], Leszek Pryszcz[1], Maciej Łapiński[1], Vladimir Korzh[3], Sinnakaruppan Mathavan[4]

[1]International Institute of Molecular and Cell Biology, Poland; [2]Max Planck Institute for Heart and Lung Research, Germany; [3]Institute of Molecular and Cell Biology, Singapore; [4]Nanyang Technological University, Singapore
Cecilia Winata <cwinata@iimcb.gov.pl>

During the earliest stages of animal development, maternally deposited mRNAs direct embryogenesis without any contribution from the zygotic genome which is transcriptionally silent. Precise control of the expression of these maternal mRNAs is required to orchestrate development up to the point of mid-blastula transition (MBT) when zygotic genome is activated. We identified a large number of maternal mRNAs which undergo progressive cytoplasmic polyadenylation during pre-MBT development in the zebrafish, suggesting translational control mechanism of their expression. Inhibition of cytoplasmic polyadenylation by 3'-deoxyadenosine resulted in the failure of developmental progression past the MBT stage, suggesting the necessity of this mechanism for pre-MBT developmental control. To further characterize the relationship between cytoplasmic polyadenylation and translation, we performed profiling of polysome-associated mRNAs by next generation sequencing during several stages of pre-, during, and post-MBT. I will present results from the ongoing analysis of this data and show examples of how the dynamic translational regulation of maternal mRNAs correlate with early developmental events in the zebrafish.

Posters

P16.1

How anti-viral IFIT proteins discriminate self from non-self RNA

Joanna Boros-Majewska[1], Monika Kowalska[1], Krzysztof Młynarczyk[1], Giulio Superti-Furga[2], Maria W. Górna[1]

[1]Biological and Chemical Research Centre, Department of Chemistry, University of Warsaw, Warsaw, Poland; [2]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

Joanna Boros-Majewska <jboros@chem.uw.edu.pl>

Eukaryotic and viral messenger RNA are co-transcriptionally modified in the process of cap formation. This includes an incorporation of N7-methyl guanosine (m7G) to the *5'*-end of the transcript via a reverse *5'-5'* triphosphate linker and further methylation at the ribose 2'-hydroxyl position (2'-*O*) of the first (cap 1), the second (cap 2) or the following nucleotide (*e.g.* cap 4 in *Leishmania*) [1, 2]. Higher eukaryotes like humans produce mRNA cap 1 and cap 2 structures, however many viruses also generate these caps using viral 2'-*O*-methyltransferases or stealing the cap from the mRNA of the host [3]. Interestingly, other viral pathogens, like vesicular stomatitis virus, possess 2'*O*-unmethylated RNA (cap 0) or RNA completely uncapped, albeit bearing a triphosphate group at the *5'*-end (*5'*-ppp). Both cap 0 and *5'*-ppp RNAs are extremely attractive *'non-self'* patterns for the innate immune effectors, known as interferon-induced proteins with tetratricopeptide repeats (IFITs). The IFIT family members are evolutionarily conserved among vertebrates and reach very high copy numbers upon viral infection or stimulation with type I interferons. It was recently revealed that IFIT proteins can bind and neutralize single-stranded RNA of viruses by discrimination of the *5'*-end. Human IFIT1 preferentially recognizes cap 0 and to a lesser extent *5'*-ppp RNA with a higher (anti-viral) RNA-binding activity when it interacts with non-RNA binding protein IFIT3. IFIT5 works alone and exhibits the affinity for *5'*-ppp group, but IFIT2 might not have the capacity to distinguish '*self*' from '*non-self*' RNA. In our studies, we focus on understanding the basis of different specificity between human IFIT proteins for RNA ligands.

References:

1. Ghosh A, Lima CD (2010) Enzymology of RNA cap synthesis.*Wiley Interdiscip Rev RNA* **1**: 152-172.
2. Ramanathan A *et al* (2016) mRNA capping: biological functions and applications. *Nucleic Acids Res* [Epub ahead of print].
3. Daffis S *et al* (2010) 2'-*O* methylation of the viral mRNA cap evades host restriction by IFIT family members. *Nature* **468**: 452-456.

Acknowledgements:

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P16.2

How to end a viral RNA: IFIT proteins and their complexes in the innate immune response

Maria W. Górna[1,4], Joanna Boros-Majewska[1], Monika Kowalska[1], Krzysztof Młynarczyk[1], Gregory I. Vladimer[4], Yazan M. Abbas[2], Anna Gebhardt[3], Keiryn L. Bennett[4], Andreas Pichlmair[3], Bhushan Nagar[2], Giulio Superti-Furga[4]

[1]Structural Biology Group, Biological and Chemical Research Centre, Faculty of Chemistry, University of Warsaw, Warsaw, Poland; [2]Department of Biochemistry, and Groupe de Recherche Axé sur la Structure des Protéines, McGill University, Montreal, Canada; [3]Innate Immunity Laboratory, Max-Planck Institute of Biochemistry, Martinsried, Germany; [4]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

Maria Górna <mgorna@chem.uw.edu.pl>

The interferon-induced proteins with tetratricopeptide repeats (IFITs) have recently emerged as a potent innate immune effectors that bind non-self RNA, which results in the inhibition of translation of viral transcripts. The structure of IFIT5 [1] reveals the mode of recognition of the 5' triphosphate (PPP) group on RNA, whereas IFIT1 preferentially binds cap 0 groups. IFIT1 interacts with IFIT3, which has no known RNA binding capability on its own, and for which the role in the larger multi-IFIT complex is elusive. We undertake the dissection of the role of the higher-order IFIT complexes and demonstrate that the IFIT1-IFIT3 complex binds RNA with a higher affinity than IFIT1 alone. The IFIT1-IFIT3 interaction is mediated by the last tetratricopeptide repeat motifs in both proteins, and results in reorganization of the RNA-binding site in IFIT1. In cells, IFIT1 and IFIT3 associated together, and re-distributed and co-localized together with PPP-RNA. We propose a role for IFIT3 as an enhancer of IFIT1 activity. Regulation of the IFIT1-IFIT3 complex may provide additional possibility for signal integration in the antiviral response.

References:

1. Abbas YM, Pichlmair A, Górna MW, Superti-Furga G, Nagar B (2013) Structural basis for viral 5'-PPP-RNA recognition by human IFIT proteins. *Nature* **494**: 60-64.

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P16.3

Transcriptome analysis of Niemann-Pick type C fibroblasts: preliminary results of a pilot study

Katarzyna Hetmańczyk[1], Roksana Iwanicka-Nowicka[2,3], Anna Fogtman[2], Marta Koblowska[2, 3], Mirella Filocamo[4], Susanna Lualdi[4], Barbara Żyżyńska-Granica[5], Paweł Włodarski[6], Rafał Płoski[7], Agnieszka Ługowska[1]

[1]Institute of Psychiatry and Neurology, Department of Genetics, Warsaw, Poland; [2]Polish Academy of Sciences, Institute of Biochemistry and Biophysics, Laboratory of Microarray Analysis, Warsaw, Poland; [3]University of Warsaw, Faculty of Biology, Laboratory of Systems Biology, Warsaw, Poland; [4]Istituto G. Gaslini, Centro di Diagnostica Genetica e Biochimica delle Malattie Metaboliche, Genova, Italy; [5]Medical University of Warsaw, Department of Pharmacodynamics, Warsaw, Poland; [6]Medical University of Warsaw, Department of Histology and Embryology, Warsaw, Poland; [7]Medical University of Warsaw, Department of Medical Genetics, Warsaw, Poland
Katarzyna Hetmańczyk <khetmanczyk@gmail.com>

Introduction: Niemann-Pick type C (NPC) disease is a rare lysosomal disorder due to mutations in the genes *NPC1* or *NPC2*. Proteins NPC1 and NPC2 are essential for intracellular transport of free cholesterol. Affected individualsaccumulate free cholesterol and glycosphingolipids in late endosomes and early lysosomes. So far, the pathomechanism of NPC is not fully understood. Hypotheses proposed to elucidate the pathologic events occuring in NPC cells include activated autophagy resulting in cell stress and apoptosis, imbalanced calcium homeostasis leading to block in late endosome-lysosomes fusion, oxidative stress and additionally, several cellular pathways like inflammation, intoxication with lysolipids, secondary storage of macromolecular compounds and the lack of same by-products essential for synthesis or regulation in the cell.
Aim of our study was to examine the expression levels of genes engaged in cellular metabolic pathways in cell lines obtained of NPC patients and controls.
Materials and Methods: Total RNA was isolated from 10 NPC1 patients cell lines and 9 cell lines from control persons. Biotin-labeled cRNA samples were hybridized to HumanHT-12 v4.0 Expression Bead Chip. Row data obtained after microarray experiments were then analyzed with the Partek Genomic Suite v6.6 and Ingenuity Pathway Analysis program.
Results: Statistically significant alterations in expression were observed for three genes: *SOD1* coding for superoxide dismutase 1 (mean fold change, MFC, 2.5), *CTSK* coding for cathepsin K (MFC 2.5), and *CTSB* coding for cathepsin B (MFC 1.5). We have found that in NPC cells the up-regulated genes were related to oxidative stress, autophagy, and apoptosis.
Conclusions: These preliminary results indicate that in humans activation of autophagy may enhance cell stress and eventually trigger the apoptotic pathway. This was already reported in NPC1 deficient mice as well as impaired proteolysis, which underlies autophagic dysfunction.
Our further work will include the validation of obtained data by qRT-PCR in human NPC fibroblasts.

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P16.4

Structural requirements of translational inhibition by tRNA fragments *in vitro*

Paulina Jackowiak[1,2], Anna Hojka-Osinska[1], Katarzyna Sowinska[1], Michał Stelmaszczuk[1], Marek Figlerowicz[1,3]

[1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland; [2]Institute of Chemical Technology and Engineering, Poznan University of Technology, Poznań, Poland; [3]Institute of Computing Science, Poznan University of Technology, Poznań, Poland
Paulina Jackowiak <paulinaj@ibch.poznan.pl>

Small RNAs derived from tRNA (tRNA fragments, tRFs) are an extensively studied group of non-coding RNAs with several proven regulatory functions in eukaryotic cells. Among other things, it has been demonstrated that tRFs can inhibit translation by a direct binding with the components of the translational machinery. Notably, the ability to repress translation is not a general feature of the tRFs – only a subset of them exerts the inhibitory effect. This observation implies that some of the small RNAs contain certain structural elements that are essential for the observed phenomenon. It was reported that the presence of the 5' oligoguanine motif is necessary for a tRF to efficiently impair translation. Recently, it has been shown that the formation of G-quadruplex structures by tRFs is required to inhibit the translation initiation step in a mammalian system. Interestingly, the results of our earlier studies [1] suggested that there are different structural determinants of translational inhibition by tRFs in mammalian and plant systems. To increase our understanding of these determinants, we selected several tRFs identified either in *Arabidopsis thaliana* or in human cells and evaluated their influence on *in vitro* translation in both rabbit reticulocyte lysate and wheat germ extract. Next, we assessed the potential of the examined tRFs to adopt G-quadruplex conformation. Moreover, we tested how the model oligonucleotides that fold into G-quadruplexes of various topologies influence the translation *in vitro*. Our results revealed that the inhibitory impact of tRFs on the translation in the mammalian system was dependent on their ability to form G-quadruplex structure, which was consistent with the previous report. In addition, we found that the efficiency of translational inhibition was associated with the type of G-quadruplex topology. In the case of the plant system, the ability of small RNA to adopt G-quadruplex conformation was required, but not sufficient to repress translation. These observations indicated that there exist considerable differences in the molecular mechanisms that underlie the inhibition of translation by small RNA in mammalian and plant system *in vitro*.

Reference:

1. Nowacka M *et al* (2013) *Plant Mol Biol* **83**: 191–204.

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P16.5

Structurally defined RNA nanoparticles for gene expression regulation in a model GFP system

Dominika Jedrzejczyk, Arkadiusz Chworos

Centre of Molecular and Macromolecular Studies Polish Academy of Sciences, Department of Bioorganic Chemistry, Łódź, Poland
Dominika Jedrzejczyk <djedrzej@cbmm.lodz.pl>

The gene expression regulation is highly controlled process. The complex central molecular dogma depicts steps when the gene expression is naturally governed and those processes are likely windows for enforced alteration of protein biosynthesis. The RNA interference (RNAi) phenomena [1] is one of the most common mechanisms used for specific gene targeting and silencing. Despite the fact that transcript is created, the translation process is inhibited, mRNA is specifically degraded and does not result in protein production. The RNAi can be triggered by a number of artificial RNA structures, which can be designed, synthetized and engineered to target a chosen gene, leading to the expression inhibition in the treated cells [2]. Our approach is to apply RNA architectonics [3] to design and create structurally stable triangular RNA nanoobjects, which can be processed by nucleases involved in RNAi.
The novel structural RNA motif, 3wj-nRA [4], was chosen for the construct and the functionalized with regulatory RNA fragments. The nanoobjects were designed *in silico*, synthetized and used to deliver ribonucleic acid fragments and trigger the RNAi machinery to silence the Green Fluorescence Protein gene expression in two mammalian cell lines: HeLa and MDA-MB-231/GFP-RFP. Cellular tests showed no toxicity of monomeric and triangular RNA structures in tested conditions followed by expected biological effect – fluorescence inhibition. We found that the biological effect was stronger for multimeric particles compared to the same effective concentrations of single monomers. In the initial, model system, GFP expression was induced by GFP-coding plasmid transfection and almost completely silenced 48h after nanoobjects transfection. The second cell line has endogenous CopGFP protein, which has a different sequence and therefore requires to design a different set of siRNA to regulate it. The library of siRNA molecules targeting *copGFP* gene was designed, synthetized and examined for their silencing efficiency. The most effective siRNAs were selected for a new multifunctional nanoparticle design.

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P16.6

Comparative analysis of microRNA-target gene interaction prediction algorithms

Anna Krawczyk[1], Joanna Polańska[2]

[1]Silesian University of Technology, Faculty of Automatic Control Electronics and Computer Science, Poland; [2]Silesian University of Technology, Faculty of Automatic Control Electronics and Computer Science, Poland
Anna Krawczyk <anna.krawczyk@polsl.pl>

MicroRNAs are small non-coding molecules, involved in the process of gene expression regulation by either the translational repression of the protein or by the downregulation of the target gene. Since they play a key role in the cancer growth, the knowledge on microRNA's target genes is crucial from the contemporary oncology point of view.
Nowadays, the sustainable growth of microRNA-target gene interaction prediction algorithms is observed. There is a plethora of such kind of algorithms but each of them is based on different approach of seeking the possible gene target for the specific microRNA. It causes the problem with a choice of an algorithm that provides the most probable and significant results.
We propose a bioinformatics tool which takes into account results from three the most popular prediction algorithms: Diana-microT, MiRWalk, and TargetScan. In order to juxtapose the mentioned algorithms, for each of them we developed a statistical test verifying the hypothesis on gene not being a possible target for particular miRNA and applied p-value Fisher method to integrate them into one probability space.
The proposed method was evaluated on let-7 miRNA family with 19 members. While single algorithms were considered, 1298, 3450, and 1108 hypothetical targets with $p<0.05$ were found. Only 748 of them were common targets across all algorithms. After p-value integration, 3692 targets were identified. If significance level was down to 0.00001, the list of possible targets limits to 15 genes. Genes HERC4 (p=7.44e-5, hsa-let-7c-3p), KCTD16 (p=0.063, hsa-let-7a-3p) and HDLBP (p=0.0055, hsa-let-7a-5p) were designated as the targets after p-value integration only. KCTD16 is strongly connected with kinetics of the receptor response, whilst HERC4 is reported as related to breast, lung and multiple myeloma cancers. Also for HDLBP one can find reports on its direct impact on the growth of hepatocellular carcinoma and its indirect relation to the development of breast and prostate cancers. Well known oncogenes, as RAS (p=7.67e-4, hsa-let-7a-5p) and HMGA2 (p=0.0034, hsa-let-7e-5p) were also among our findings. In general, the targets genes of let-7 miRNA family are involved in regulation of cell cycle, proliferation and immunity.
We conclude that supporting miRNA targets detection by statistical inference and p-value integration is essential for reliable identification of its targets.

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P16.7

Transcriptome analysis of hnRNPK and XRN2 proteins using new generation sequencing

U. Kuklińska, M. Kopczyński, M. Statkiewicz, K. Unrug-Bielawska, Zuzanna Sandowska-Markiewicz, M. Mikula, J. Ostrowski

Department of Genetics, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland
Urszula Kuklińska <ukuklinska@gmail.com>

Background: The heterogeneous nuclear ribonucleoprotein K (hnRNPK) is a DNA and RNA binding protein that acts as a docking platform integrating signal transduction pathways to nucleic acid-related processes. We previously revealed the direct interaction between hnRNPK and human 5'-3' exonuclease Xrn2, a factor involved in RNA degradation and transcription termination. In addition, we showed that both proteins, with similar dynamics, interact directly with transcripts of immediate early genes, including FOS [1].
Aim: To generate XRN2 and hnRNPK overexpressing cell lines models to further uncover the transcriptome under the control of either of these factors using RNA-Seq.
Methods and results: We obtained stable transfected cell lines inducibly expressing FLAG tagged hnRNPK (Flp-InTMT-RExTM293/hnRNPK) or XRN2 (Flp-InTMT-RExTM293/XRN2) using the Flp-In System (Life Technologies). To generate XRN2 stable knockout, Flp-InTMT-RExTM293/XRN2 cells were transfected with GeneArt® CRISPR Nuclease Vector including a U6-gRNA cassette (Life Technologies) and selected by fluorescence-activated cell sorting (FACS). We confirmed elevated level of XRN2 and hnRNPK proteins in transfected cells using Western Blotting. Cells treated or not with doxycycline after 24-h of serum starvation were stimulated with FBS for 30 minutes. Harvested pellets were subjected to RNA and protein isolation. hnRNPK, XRN2 and FOS transcript level was measured with qPCR. We showed that overexpression of hnRNPK but not Xrn2 in serum-stimulated cells increased FOS mRNA level.
Conclusions: We obtained stable hnRNPK and XRN2 expressing cell lines, which we believe will contribute to a better understanding of the hnRNPK and XRN2 interaction role in control of transcriptome.

Reference:

Mikula M *et al* (2013) *J Biol Chem* **288**: 24788-24798.

P16.8

Elucidating the role of cpeb1b and cpeb4 in translational control of maternal transcripts through cytoplasmic polyadenylation

Maciej Łapiński, Cecilia Winata

International Institute of Molecular and Cell Biology in Warsaw, Laboratory of Zebrafish Developmental Genomics, Warsaw, Poland
Maciej Łapiński <mlapinski@iimcb.gov.pl>

During the period of translational inactivity in the early animal embryo, developmental control relies on maternally deposited RNA messages. A surprisingly large number of those transcripts have been found to undergo cytoplasmic polyadenylation. This process progresses through the developmental stages leading to mid-blastula transition (MBT), at which point the first cohort of zygotic genes is believed to be activated. Blocking of cytoplasmic polyadenylation causes failure of MBT, which suggest that this process is crucial for proper embryonic development in the zebrafish. However, despite this knowledge, the exact mechanism of cytoplasmic polyadenylation is not thoroughly understood. Important to this mechanism are cis-acting elements in the 3' UTR of mRNAs and their associated factors - the Cytoplasmic Polyadenylation Element Binding proteins (CPEB).
Previous research from our laboratory showed that Morpholino (MO) induced gene knockdown in embryos injected with MO specific to zorba transcript typically causes delay in epiboly at 6 hours post fertilization (hpf), which proved to be lethal at later stages of development. Additionally embryos injected with anti-cpeb4 MO exhibited no characteristic phenotype at 6hpf, although at 30 hpf they displayed a phenotype characterized by missing yolk extension and axis deformities. Moreover embryos coinjected with both MOs exhibited high mortality rates at 30 hpf. Those observations indicate that both of those proteins play an important part in the early zebrafish development.
Here we present the results of the ongoing research conducted to elucidate the role of Zorba and Cpeb4 in translational control of maternal mRNAs through cytoplasmic polyadenylation.

P16.9

Copy number variation of genes involved in the hepatitis C virus-human interactome

Malgorzata Marcinkowska-Swojak[1], Lucyna Budzko[1], Paulina Jackowiak[1,2], Piotr Kozlowski[1,2], Marek Figlerowicz[1,3]

[1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland; [2]Institute of Chemical Technology and Engineering, Poznan University of Technology, Poznań, Poland; [3]Institute of Computing Science, Poznan University of Technology, Poznań, Poland
Malgorzata Marcinkowska-Swojak <m-marcinkowska@o2.pl>

The hepatitis C virus (HCV), is a hepatotropic, positive-sense, single-stranded (ss) RNA virus that belongs to the family *Flaviviridae*. The virus, displays an extremely high level of genetic variability (Jackowiak, 2013) which has been shown to influence patients' response to antiviral treatment. Moreover, human genetic variation is another factor that underlies the unique susceptibility of each person to HCV infection and treatment. The two main types of this variation are: single nucleotide polymorphism (SNP) (Ge 2009) and recently discovered copy number variation (CNV).

CNV is a form of intra-species genetic polymorphism that is defined as deletions or duplications of genome segments ranging from 1 kbp to several Mbp (Sebat, 2004, Iafrate 2004). CNV accounts for the majority of the genetic variation observed in humans (CNV regions cover more than 10% of the human genome); therefore, it may significantly influence both the phenotype and susceptibility to various diseases. Unfortunately, the impact of CNV on a number of diseases, including HCV infection, remains largely unexplored.

To address this issue, we analyzed 421 human genes encoding proteins that have been shown to interact with HCV proteins or genomic RNA (proteins from the HCV-human interactome) (de Chassey, 2008). We found that 19 of the 421 candidate genes are located in putative CNV regions. For all of these genes, copy numbers were determined for European, Asiatic and African populations using the multiplex ligation-dependent amplification (MLPA) method according to previously developed strategy of CNV genotyping (Marcinkowska-Swojak, 2013, Marcinkowska-Swojak, 2014). As a result, we identified 4 polymorphic genes, *IGLL1*, *MLLT4*, *PDPK1* and *PPP1R13L,* for which the copy number-genotype ranged from 1 to 6 copies. All of these genes are involved in host-virus interaction; thus, their polymorphism has a potential impact on the development of HCV infection and/or therapy outcome.

P16.10

Marked differences in the miR-17~92 miRNA expression pattern in non-Hodgkin lymphoma

Izabella Slezak-Prochazka[1,2], Jan-Lukas Robertus[1], Debora de Jong[1], Philip Kluin[1], Joost Kluiver[1], Anke van den Berg[1]

[1]Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands, [2]Biosystems Group, Institute of Automatic Control, Silesian University of Technology, Gliwice, Poland
Izabella Slezak-Prochazka <izabella.slezak-prochazka@polsl.pl>

The oncogenic miR-17~92 cluster contains six microRNAs: miR-17, miR-18a, miR-19a, miR-20a, miR-19b and miR-92a. These six miRNAs are expressed at variable levels in different normal and malignant cell types. Inactivation or deletion of miR-92a was shown to increase the oncogenic potential of the miR-17~92 cluster. This effect is mainly caused by miR-19 indicating that balance between miR-92a and miR-19 is important for oncogenesis.

We aimed to determine possible differences in the expression pattern of the six miRNAs members of the miR-17~92 cluster in B-cell lymphoma in comparison to normal B-cell subsets. We analyzed the levels of miR-17, miR-18a, miR-19a, miR-20a, miR-19b and miR-92a in three B-cell subsets, 117 NHL cases and 21 NHL cell lines using qRT-PCR. We show that miR-92a is the most abundant miRNA in three of the four subtypes of the NHL cases, all NHL cell lines and in the normal B-cell subsets. Only in diffuse large B-cell lymphoma, miR-19b levels were higher than miR-92a levels. Comparison of the levels of the six mature miR-17~92 miRNAs between the NHL cases and their normal B-cell counterparts indicated the highest induction of miR-19b in cases and cell lines of all analyzed NHL subtypes. The observed miR-19b induction is consistent with the oncogenic role of miR-19b in lymphomagenesis.

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P16.11

Identification and secondary structure characterization of the 5' non-coding regions of p53 mRNA transcripts in mouse

Joanna Sroka, Agnieszka Górska, Aleksandra Kasprowicz, Jerzy Ciesiołka

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland
Joanna Sroka <jsroka@ibch.poznan.pl>

The p53 protein is a transcription factor participating in cell cycle control, DNA damage response and induction of apoptosis by modulating cell response to stress. Recently, a secondary structure of the 5' non-coding region of human p53 mRNA encoding full-length p53 protein and its N-truncated isoform Δ40p53 has been determined in our laboratory. Earlier studies performed in our and in other laboratories have revealed high importance of this region in the translation initiation process. Here, we present the results of the structural analysis of the homologue region of p53 mRNA transcript in mouse. In the first step, the 5' ends of p53 mRNA transcripts derived from embryos, liver and thymus were identified using the 5'-RACE method. The 5' non-coding region comprising 113 nucleotides occurred most frequently. Nevertheless, its length distribution in the other mRNA transcripts was different in the analyzed mouse samples. Subsequently, we determined a secondary structure of the 5' non-coding regions of two major mRNA transcripts using SHAPE, Pb^{2+}-induced cleavage and DMS modification methods. Moreover, a secondary structure of the most abundant p53 mRNA transcript was analyzed in transfected mouse cell culture using DMS probing. It turned out that DMS modification sites *in vitro* and in the cell were very similar. However, there were some modification sites observed only in the cell, which occurred mainly in the regions of lower thermodynamic stability of RNA. Furthermore, some regions were modified only in *in vitro* conditions suggesting possible interactions between p53 mRNA and some intracellular components.

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P16.12

Inhibitors of human and viral methyltransferases involved in RNA cap biosynthesis – virtual screening and experimental validation

Diana Toczydłowska[1], Filip Stefaniak[1], Elżbieta Purta[1], Janusz M. Bujnicki[1,2]

[1]Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, 02-109 Warsaw, Poland; [2]Laboratory of Bioinformatics, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, 61-614 Poznań, Poland
Diana Toczydłowska <dtoczydlowska@genesilico.pl>

Human and some viral messenger RNAs (mRNAs) possess a cap structure at their 5' end. The cap0 consists of an inverted 7-methylguanosine linked to the first nucleoside by a unique 5'-5' triphosphate bond. Moreover, the 5' end of mRNA can be further modified with the 2'-*O*-ribose methylation of the first transcribed nucleotide, forming cap1 structure. The crystal structures of human (CMTr1) and some viral enzymes involved in this modification have been determined. They allow us to identify key differences between human and viral methyltransferases (MTases) in the context of their interactions with substrates. This knowledge provided the basis for the rational development of the compounds that selectively block the human or viral cap MTases.

The aim of our research is to find small molecule inhibitors of both human (CMTr1) and viral methyltransferases (VP39 from vaccinia virus and NS5 from Dengue virus). Inhibitors specific to human enzymes will help us study the importance of the cap methylation on human cell physiology, especially on the fate of human mRNAs. Moreover, inhibitors of particular viral enzymes could serve as lead compounds in antiviral drug development.

Here, we demonstrate the approach used at the initial stage of the human MTase inhibitors' development and its preliminary results. We applied various computational methods for a virtual screening of large databases of commercially available compounds. Top scoring compounds were chosen for the experimental validation that aimed at testing the influence of potential inhibitors on the whole protein and on the catalytic domain. That enabled us to find 10 compounds that significantly decreased activity of human methyltransferases.

P16.13

Investigating the potential roles of long non-coding RNAs as splicing modulators through RNA:RNA interactions

Elżbieta Wanowska, Michał Szcześniak, Izabela Makałowska

[1]Department of Bioinformatics, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

Elżbieta Wanowska <elzbieta.wanowska@amu.edu.pl>

Long non coding RNAs (lncRNAs) have been shown to play important roles in various biological processes and, in particular, they function as regulators of gene expression, both during the act of transcription and post-transcriptionally. They are also implicated in a number of human diseases, especially cancers. To date, however, only ca. 1% of lncRNAs have well established functions and still little is known about their engagement in gene expression regulation through lncRNA:RNA interactions. By hybridizing with other transcripts, lncRNAs could be involved in at least several regulatory mechanisms, including modulation of splicing, triggering RNA editing events, guiding protein-coding transcripts to degradation in a Staufen-mediated decay pathway, and abrogation of miRNA-induced repression by masking miRNA target sites.

Our goal is to investigate splicing-associated functions of lncRNAs that are exerted in the context of RNA:RNA duplexes. Recently, based on *in silico* predictions of lncRNA:RNA base-pairings across the human transcriptome, we discovered there is a great potential for lncRNAs to play a role of splicing modulators. This could be achieved by masking splice sites and other splicing signals. At the moment, we are experimentally testing the most promising candidates, including RNA Binding Motif Protein 8A (RBM8A) that itself is involved in splicing events. The first experiments involve cell fractionation and Real-Time PCRs in HEK293 and HeLa cell lines to determine the gene and lncRNA expressions in different cellular compartments. Then, silencing of nuclear lncRNAs will be performed with GapmeR technology and effect on splicing of potentially regulated genes will be determined. Our study should add up to better understanding of lncRNA biology and help decipher their functions in the context of RNA:RNA interactions.