Jerzy Borysti

Polish-Japanese RNA Meeting

Poznań, Poland, June 17–18, 2019

under auspices of
the President of the Polish Academy of Sciences
Professor JERZY DUSZYŃKI

and

His Magnificence Rector of Adam Mickiewicz University,
Professor ANDRZEJ LESICKI

PROGRAM, ABSTRACTS & PARTICIPANTS

Organized by

Institute of Bioorganic Chemistry
Polish Academy of Sciences in Poznań

and

Faculty of Biology and Institute of Molecular Biology and Biotechnology
of Adam Mickiewicz University in Poznań

The Organizing Committee

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ZOFIA SZWEYKOWSKA-KULIŃSKA - Co-chair
YUICHIRO WATANABE - Co-chair

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Mikołaj Olejniczak, Patrick Perrigue,
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Program

June 16, 2019 (Sunday)

The arrival of Japanese participants at Poznań Main Station by 18.44 train

June 17, 2019 (Monday)

9.00–9.20 Opening
Marek Figlerowicz, Zofia Szweykowska-Kulińska and Yuichiro Watanabe

9.20–10.40 Scientific Session I Chair **Marek Figlerowicz**

(each talk is scheduled for 15–20 min and 5 min discussion)

Yuichiro Watanabe – *The liverwort Marchantia polymorpha demonstrates an early arising role of posttranscriptional regulation in land plants*
Halina Pietrykowska – *Liverwort-specific miRNAs control sexual reproduction in Marchantia polymorpha: a case study of miR8185*
Takashi Miki – *XRN2 ensures the integrity of gene expression in developing animals*
Barbara Nawrot – *The importance of being modified: sulfur and selenium-modified nucleosides in bacterial tRNA*

10.40–11.00 Coffee break (each coffee break is scheduled for 20 minutes)

11.00–13.00 Scientific Session II Chair **Zofia Szweykowska-Kulińska**

Takayuki Kohchi – *Sexual differentiation is regulated by a MYB transcription factor and a cis-acting antisense long non coding RNA in the liverwort, Marchantia polymorpha*
Agnieszka Fiszer – *Ribosome meets RISC at expanded CAG repeat tract – allele selective RNAi approach for therapy of polyglutamine diseases*
Agata Stępień – *The communication between the microprocessor, the spliceosome and RNA polymerase II in plants*
Nobuyoshi Akimitsu – *Identification of Functional Targets Reveals that the Suppression of Pumilio-mediated mRNA Decay Increases Cell Resistance to DNA Damage in Human Cells*
Patrick Perrigue – *Depletion of histone H3K27me3 mark and subsequent chromatin dysregulation manifests as senescence and loss of cell identity*
Elżbieta Kierzek – *Influenza virus. RNA secondary structure as a target for new antiviral therapies*

13.00–14.30 Lunch

15.00–17.00 Meeting of Japanese scientists with Rector Magnificus of the Adam Mickiewicz University

17.15–18.15 Visit to Poznan Supercomputing and Networking Center

18.30–19.00 Concert of the Institute of Bioorganic Chemistry Choir at Działyński Palace

19.30–21.00 Dinner for all participants

June 18, 2019 (Tuesday)

9.00–10.40 Scientific Session III — Chair **ARTUR JARMOŁOWSKI**

TSUTOMU SUZUKI – *Metabolic and chemical regulation of tRNA modification under physiological and pathological conditions*
DARIA SOBAŃSKA – *Regnase-1 and Roquin – evolutionarily conserved partners in RNA decay*
RUSLAN YATUSHEVICH – *Dormancy and drought – One antisense to rule them all?*
YUKIHIDE TOMARI – *Assembly and function of the RNA silencing Complex*
ANNA KURZYŃSKA-KOKORNIAK – *The RNA annealing activity of human ribonuclease Dicer*

10.40–11.00 Coffee break

11.00–13.00 Scientific Session IV — Chair **MICHAŁ SOBKOWSKI**

MASAYASU KUWAHARA – *Simple Test Methods for Various Biomarkers at Attomolar Level*
KRZYSZTOF SOBCZAK – *Activity of MBNL splicing factors and the mechanism of their sequestration on toxic RNA in myotonic dystrophy*
MARIA GÓRNA – *5' end modifications in RNA metabolism, innate immunity and their exploitation in biotechnology*
MAŁGORZATA BOROWIAK – *Exploring pancreatic transcriptional heterogeneity for better endocrine islet cell building*
SHINICHIRO AKICHIKA – *Cap-specific terminal N6-methylation of RNA by an RNA polymerase II-associated methyltransferase*
MIKOŁAJ OLEJNICZAK – *A new family of RNA chaperone proteins in bacteria?*

13.00–14.30 Lunch

14.00–15.20 Scientific session V — Chair **JERZY BORYSKI**

MISATO OHTANI – *Roles for pre-mRNA splicing regulation in environmental adaptation of plants*
K. DOROTA RACZYŃSKA – *U7 snRNA activity and replication-dependent histone gene expression is affected by ALS-linked FUS mutations*
OSAMU TAKEUCHI – *Control of Immune Responses via RNA Degradation*
MAREK ŻYWICKI – *Identification of 5'-capped tRNA halves in human cell lines*

15.20–15.40 Coffee break

15.40–17.00 Scientific Session VI — Chair **Przemysław Wojtaszek**

HIDEHITO KUROYANAGI – *Genome-Wide Kinetic Analysis of pre-mRNA Processing Reveals Action of Splicing Regulators*
AGNIESZKA KILISZEK – *Structural and biochemical insights into synthetic ligands targeting A-A pairs associated with disease-related CAG RNA repeats*
TAISUKE NAKAHAMA – *ADAR1 regulates early T cell development via MDA5- dependent and -independent pathways*
ZBIGNIEW WARKOCKI – *TUTs in mammalian RNA metabolism*

Concluding remarks
ZOFIA SZWEYKOWSKA-KULIŃSKA, YUICHIRO WATANABE and MAREK FIGLEROWICZ

17.00–18.00 Dinner for all participants

June 19, 2019 (Wednesday) – Departure

Abstracts

(in order of presentation)

The liverwort *Marchantia polymorpha* demonstrates an early arising role of posttranscriptional regulation in land plants

YUICHIRO WATANABE

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Graduate Program of Environmental Sciences

The liverwort *Marchantia polymorpha* is offering much information that is fundamentally common among land plants as a model plant. Recent study showed not so many miRNA families are conserved among land plants. Such miRNA families are encoded in a small number of loci in *M. polymorpha* genome compared to other land plants. We applied CRISPR-Cas9 system to see the phenotypes that would be triggered by disturbance of normal miRNA expression.

The loss of miR529c expression in *M. polymorpha* causes gametangium and gamete production without far-red light signaling normally needed for the reproductive transition because of the uncontrolled expression of MpSPL2 transcription factor. It is quietly likely that the miR156/529-*SPL* module generally controls the shift from the vegetative to the reproductive phase in land plants.

Arabidopsis miR319 and miR159 mainly target TCP and MYB transcription factors, respectively, whereas *M. polymorph*a has only miR319, which targets MpRKD transcription factor. The reduction of miR319 expression triggered severe decrease in gemma and gemma cup formation that are tightly involved in asexual reproduction in *M. polymorpha*.

Liverwort-specific miRNAs control sexual reproduction in *Marchantia polymorpha*: a case study of miR8185

HALINA PIETRYKOWSKA[1], WOJCIECH KARŁOWSKI[2], PRZEMYSŁAW NUC[1], ANETA ZABKA[3], JANUSZ MASZEWSKI[3], RAFAŁ MOL[4], ARTUR JARMOŁOWSKI[1], SHOHEI YAMAOKA[5], RYUICHI NISHIHAMA[5], TAKAYUKI KOHCHI[5] AND ZOFIA SZWEYKOWSKA-KULIŃSKA[1]

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MicroRNAs play a crucial role in eukaryotic regulatory networks by gene expression regulation. At least ten conserved miRNA families have been reported to play a key role during flower development in higher plants. However, the knowledge about the role of microRNAs in liverwort sexual organ development is still limited. To test whether sex-specific microRNAs are present in *M. polymorpha*, we isolated RNA and performed Illumina small RNA-seq from four different *M. polymorpha* thalli: vegetative female thalli, archegoniophores, vegetative male thalli and antheridiophores, respectively. Among them miR8185 was present only in antherdiophores. Using degradome sequencing technique and 5'RACE analysis, target gene coding DUSP12-like phosphatase was identified. This protein is known to be the negative regulator of the mitogen-activated protein (MAP) kinase superfamily. CRISPR/Cas9 Δ *miR8185* mutant lines exhibit defects in quantity and distribution of antheridia within male gametophore. Crossing of Δ *miR8185* mutant plants induces production of archegoniophores instead of normally developed sporophyte. Or results suggest that that this miRNA is pivotal for *Marchantia* sexual reproduction.

This work is supported by National Science Centre (UMO-2014/13/N/NZ3/00321; UMO-2017/24/T/NZ3/00134) and KNOW RNA Research Centre in Poznań (No. 01/KNOW2/2014).

XRN2 ensures the integrity of gene expression in developing animals

TAKASHI MIKI

Institute of Bioorganic Chemistry of the Polish Academy of Sciences

XRN2 is an essential eukaryotic exoribonuclease, which processes and degrades various classes of RNA in the nucleus. Although its cellular functions are well-described, its roles in whole organisms remain largely unknown. We investigated the impact of XRN2 inactivation on the transcriptome of developing *C. elegans* larvae and found its roles in transcription termination of a subset of protein-coding genes, regulation of polycistronic gene expression from specific operons, and suppression of a DNA transposon. A synthetic lethality screen of ~500 RNA-binding factors exclusively identified (pre-) mRNA regulators as genetic enhancers of an *xrn-2* hypomorphic allele. Thus, XRN2 ensures the integrity of gene expression for normal development by regulating (pre-) mRNAs as critical targets.

The importance of being modified: sulfur and selenium-modified nucleosides in bacterial tRNA

BARBARA NAWROT[1], MAŁGORZATA SIERANT[1], GRAŻYNA LESZCZYŃSKA[2], KLAUDIA SADOWSKA[2], KATARZYNA KULIK[1], PATRYCJA KOMAR[1], ELŻBIETA SOCHACKA[2]

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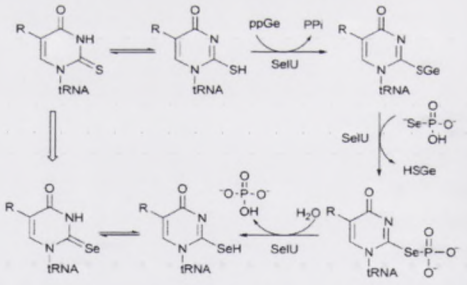
Sulfur- and selenium-modified uridines (5-substituted 2-thiouridines, R5S2U and 2--selenouridines) are present in the wobble position (position 34, the first one of anticodon) of transfer RNAs (tRNAs) in cells belonging to all domains of life. There are some data suggesting significant contribution of 5-substituted S2U units in fine tuning of translation process, and reading of genetic information. However, it is not clear why Nature introduced selenium into the wobble nucleosides of tRNAs, and to what extent the palette of functions of S2U was changed/extended by this modification. Recently, we have shown a possible SelU-catalyzed cellular mechanism of transformation of S2U-RNA to Se2U-RNA, via an S-geranyl-2-thiouridine-RNA intermediate identified in bacterial tRNAs (see Scheme).[1]



Scheme: A proposed chemical pathway of the R5S2U-tRNA to R5Se2U-tRNA transformation utilizing the geS2U-tRNA intermediate (ppGe - geranyl diphosphate, $SePO_3^{3-}$ selenophosphate, R – mnm or cmnm, SelU - tRNA 2-selenouridine synthase)

The lecture will focus on physico-chemical properties of S2U and Se2U, which may clarify the function of modified nucleosides of both types in regulation of gene expression.[2] Besides, the chemistry of oxidation of S2U and Se2U will be discussed in the context of survival of bacteria under oxidative stress conditions.[3]

This work was supported by The National Science Centre in Poland [projects UMO-2014/13/B/ST5/03979 and UMO-2018/29/B/ST5/02509] and by statutory funds of the Centre of Molecular and Macromolecular Studies, PAS.

References

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[2] Sochacka E., Lodyga-Chruscinska E., Pawlak J. et al. C5-substituents of uridines and 2-thiouridines present at the wobble position of tRNA determine the formation of their keto-enol or zwitterionic forms - a factor important for accuracy of reading of guanosine at the 3'-end of the mRNA codons. *Nucleic Acids Res.* **2017**, 45, 4825.

[3] Sadowska K, Kulik K, Sochacka E & Nawrot B. in preparation.

Sexual differentiation is regulated by a MYB transcription factor and a cis-acting antisense long non coding RNA in the liverwort, *Marchantia polymorpha*

TAKAYUKI KOHCHI

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Mechanisms of sexual differentiation are poorly understood in plants, especially for gametophyte generation where germ cells with distinct morphologies and motility are generated. We here identified an autosomal MYB-type transcription factor gene Mp*FGMYB* as a key regulator of sexual differentiation in the liverwort *Marchantia polymorpha*. Mp*FGMYB* is expressed specifically in females and loss-of-function Mp*fgmyb* mutants produce male sexual organs in genetically female individuals. Antisense transcription of Mp*FGMYB* occurs specifically in males and its blockage led to misexpression of Mp*FGMYB* and acquisition of female morphologies in males. Our study suggests that members of the FGMYB subfamily represent evolutionarily conserved regulators of female sexual differentiation in the haploid growth phases of land plants, and that bidirectional transcription module at the Mp*FGMYB* locus functions as a switch between male and female differentiation in the liverwort.

References

Hisanaga, T.,† Okahashi, K.,† Yamaoka, S., Kajiwara, T., Nishihama, R., Shimamura, M., Yamato, K. T., Bowman, J. L., Kohchi, T.,* and Nakajima, K.* A cis-acting bidirectional transcription switch controls sexual dimorphism in the liverwort. *EMBO J.* **38**, e100240 (2019). doi: 10.15252/embj.2018100240 †Co-first authors, *Co-corresponding authors

Ribosome meets RISC at expanded CAG repeat tract- allele-selective RNAi approach for therapy of polyglutamine diseases

AGNIESZKA FISZER[1], ADAM CIESIOŁKA[1], ANNA STROYNOWSKA-CZERWIŃSKA[1], PAWEŁ JOACHIMIAK[1], AGATA ŁUŻNA[1], EMILIA KOZŁOWSKA[1], MICHAŁ MICHALAK[1], KATARZYNA D. RACZYŃSKA [2], DOMINIKA ZIELIŃSKA[1], WŁODZIMIERZ J. KRZYŻOSIAK[1]

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Polyglutamine (polyQ) diseases are neurological disorders caused by CAG repeat expansion in ORFs of specific genes. This group includes Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA) and several spinocerebellar ataxias (SCA). RNAi-based targeting of mutation site is an attractive therapeutic option for polyQ diseases. We designed CAG repeat-targeting siRNAs, with specific base substitutions that make them similar to miRNAs, and showed preferential decrease of mutant protein level in cellular models of HD, SCA3, SCA7 and DRPLA. Now we have investigated activity and mechanism of these allele-selective siRNAs in HEK293-based cell lines and HD iPSC-derived human neural progenitors.

In one type of constructs we placed CAG repeat tract at different locations of luciferase and also with specific sequence context. Silencing preference of mutant transcripts was obtained for its location within ORF but not for 3'UTR region, which is typical target location for microRNAs. Also, we found that specific huntingtin gene (*HTT*) sequence surrounding the repeat tract positively affects allele-selectivity of silencing.

Moreover, we developed Flp-In T-REx 293 inducible models with stable expression of a fragment of *HTT*, in which Nano-luciferase is expressed with the first exon of huntingtin with normal or mutant repeat tract. These cell lines were used for precise investigation of kinetics of the silencing process as well as to study translation inhibition initiated by CAG repeat-targeting siRNAs, also by polysome fractionation. We found that during the inhibition of mutant *HTT* these siRNAs cause translational repression, which precedes slight mRNA decay.

Additionally, to characterize targeted transcripts we performed their quantification in fibroblast cells using digital droplet PCR (ddPCR) and single-molecule fluorescent *in situ* hybridization (smFISH). *HTT* transcripts abundance and location was also investigated in HD neural progenitors after treatment of cells with selected siRNAs.

We show that activity of atypical CAG repeat-targeting siRNAs is more similar to the activity of miRNAs, especially those acting in a cooperative manner and targeting ORF regions. Moreover, these siRNAs offer allele-selective therapeutic strategy for several polyQ diseases.

The communication between the microprocessor, the spliceosome and RNA polymerase II in plants

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MicroRNAs (miRNAs) are small noncoding RNAs of about 21 nt in length, which regulate gene expression by cleavage or translation inhibition of target mRNAs. Plant miRNA biogenesis takes place in the nucleus, and DCL1 (Dicer Like 1), HYL1 (HYPONASTIC LEAVES 1) and SE (SERRATE) are key factors responsible for miRNA production. Interestingly, SE is also involved in pre-mRNA splicing. Many miRNA genes (*MIR*s) contain introns that have to be spliced from primary miRNA precursors (pri-miRNAs) by the spliceosome. We have already shown that splicing of intron containing pri-miRNAs influences the expression levels of mature miRNAs and that in the communication between the spliceosome and the microprocessor the interaction between SE and U1 snRNP is involved. We identified four binding partners of SE among U1 snRNP auxiliary proteins: PRP39b, PRP40a, PRP40b and LUC7rl. The interplay between SE and PRP40 has been found to be particularly important for the plant development since triple (*se/prp40a/prp40b*) knockout Arabidopsis plants are embryo-lethal. The goal of the study was to understand the role of PRP40 in miRNA biogenesis and Arabidopsis development.

We have found downregulation of 50% of all Arabidopsis pri-miRNAs in the *prp40ab* mutant, suggesting a role of PRP40 in *MIR* transcription regulation. Interestingly we have also observed that SE is localized in RNAPII containing nuclear foci and forms a complex with PRP40b and CTD of RNAPII. It raises a question about the co-transcriptional character of pri-miRNA processing in plants, and a special role of SE/PRP40 interaction in this crosstalk. The molecular mechanism of the interplay between U1 snRNP and the microprocessor in plants and its role in miRNA biogenesis will be discussed.

This work was supported by KNOW RNA Research Centre in Poznań (No. 01/KNOW2/2014).

Identification of Functional Targets Reveals that the Suppression of Pumilio-mediated mRNA Decay Increases Cell Resistance to DNA Damage in Human Cells

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RNA-binding proteins (RBPs) play a pivotal role in gene expression by modulating the stability of transcripts; however, the identification of functional targets of them remains difficult, even for RBPs with a high specific binding ability, such as mammalian PUMILIO proteins (PUMs). To understand the role of PUMs in biological processes and diseases, we present a systematic approach to define the functionnal targets of human PUMs and determine the stimulus that modulates PUM-mediated mRNA decay. By analyzing genome-wide physical interactions and mRNA stabilities, we identified 48 mRNAs that are bound to and degraded by PUM1. To determine biological conditions that modify PUM1-mediated decay, we analyzed fold change of PUM1 and it's targets in various RNA-seq deposited in an open source. The *in silico* screening revealed that DNA-damaging reagents, such as cisplatin, decrease PUM1 and increase targets of PUM1, suggesting that DNA-damaging reagents inhibit PUM1-mediated mRNA decay. We further found that addition of cisplatin stabilized *PCNA* and *UBE2A* mRNAs. The proteins encoded in these are key components of translesion synthesis (TLS) pathway after DNA interstrand crosslinks induced by cisplatin. Previous reports showed that UBE2A regulates DNA polymerase switching to Pol η during TLS through monoubiquitination of PCNA. Indeed, PUM1 decrease upon cisplatin treatment increased the monoubiquitination of PCNA. Further, TLS was inhibited by judging the formation of Pol η foci, DNA synthesis, and cell viability in the cells over-expressing PUM1. Our results indicated that cells activate translesion synthesis to repair damaged DNA after cisplatin treatment by suppressing PUM1-mediated mRNA decay.

Depletion of histone H3K27me3 mark and subsequent chromatin dysregulation manifests as senescence and loss of cell identity

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The aims of our studies is to elucidate the role of epigenetic factors that promote senescence and 'inflammaging' (increased inflammation with aging) in cancer. Recent evidence suggests that chromatin dysregulation is a major driver of cellular senescence. We show that overexpression of a histone H3K27 demethylase, jumonji domain-containing protein 3 (JMJD3), activates molecules involved in inflammation and recruitment of stem cells for regeneration. The removal of histone H3 tri-methyl (H3K27me3) by JMJD3 causes chromatin relaxation, a form of epigenetic stress leading to large-scale changes in chromatin landscape and growth arrest of cells. Paradoxically, the depletion of H3K27me3 contributes to the development of cancer cells. The finding suggests that upregulation of JMJD3 in senescent cells is an important signaling event that has context-dependent oncogenic and tumor suppressive functions.

Influenza virus. RNA secondary structure as a target for new antiviral therapies

ELŻBIETA KIERZEK

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The outbreak of epidemics and pandemics of influenza virus is a constant threat for human health and life. Every year, around billion people suffer from seasonal flu, and three to five million undergo severe infections with up to half million deaths. The influenza virus has a segmented RNA genome (vRNA), consisting of eight segments of negative polarity. The segmented genome allows for undergoing genetic reassortments which give rise to dangerous pandemics. It was showed that the secondary structure of influenza RNA plays important functions during virus cycle. However, this knowledge is still fragmentary and many questions remains unanswered. Revealing crucial influenza RNA structure motifs gives opportunity to develop new antiviral strategies targeting RNA.

We determined structurally conserved motifs of (+)RNA and (-)RNA of segments 8, 7 and 5 for type A influenza. Bioinformatic analysis was supported by secondary structure mapping of model RNAs. Secondary structure of entire vRNA7, vRNA8 and vRNA5 and (+)RNA5 segments were proposed on the basis of chemical mapping, and then validated with the use of microarray mapping, RNase H cleavage and comparative sequence analysis. Possible structure rearrangements, which permit or exclude long-range RNA interactions, were also proposed. Several structural motifs are highly thermodynamically stable, overlapping with vRNA genome packaging signals, which could be connected with the regulation of virion assembly. Other vRNA structural motifs may be involved in blocking the host immunity, regulation of the replication and/or transcription, whereas mRNA folding could influence translation.

Determined secondary structures of influenza RNA and microarrays mapping results provides a comprehensive view of the oligonucleotide-accessible regions in the RNA, leading to designing oligonucleotide tools and small molecules targeting influenza RNA. We tested these new inhibitors in infected MDCK cells showing success of that strategy.

Metabolic and chemical regulation of tRNA modification under physiological and pathological conditions

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It has been generally thought that tRNA modifications are stable and static, and their frequencies are rarely regulated. We previously reported that lack of tRNA modification causes major classes of mitochondrial diseases including MELAS and MERRF. Deficient tRNA modification results in defective protein synthesis, leading to mitochondrial dysfunction. These findings provided the first evidence of human disease caused by an RNA modification disorder. We call "RNA modopathy" as a new category of human diseases. I am going to show our recent studies on tRNA modifications associated with human diseases and their dynamic regulation by sensing intracellular metabolites under physiological condition.

Regnase-1 and Roquin - evolutionarily conserved partners in RNA decay

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Regnase-1 and Roquin are mammalian RNA binding proteins (RBPs) essential for many aspects of immune regulation. Regnase-1 acts as an endoribonuclease, being responsible for the degradation of various mRNAs involved in inflammatory responses. The set of mRNAs regulated by Regnase-1 overlaps with targets of another RBP, Roquin. However, the functional relationship between Regnase-1 and Roquin is not yet fully clear. The two proteins were originally suggested to cooperate in T cells, where Regnase-1 has been proposed to target specific mRNAs via the association with Roquin. More recent studies, in other cell types, showed that although Roquin and Regnase-1 control common mRNAs, they appear to do so in different sub-cellular compartments and through different mechanisms.

We have recently reported that the *C. elegans* homolog of Regnase-1, which we called REGE-1, contains the same functional domains as the human protein, and also acts as a cytoplasmic endonuclease. The key mRNA target of REGE-1, *ets-4* mRNA, encodes a transcription factor. Using *in vivo* studies in worms, we find that the nematode homologue of Roquin, called RLE-1, is, like REGE-1, essential for the silencing of *ets-4* mRNA. Using human cells, we found that the RNA elements, which direct *ets-4* degradation in worms, also mediate the silencing by the mammalian proteins. However, while REGE-1 and RLE-1 are both necessary for the silencing in worms, either Regnase-1 or Roquin are sufficient for the silencing in human cells. Thus, while our studies have uncovered an evolutionarily conserved functional relationship between the two proteins, they also hint at possible differences in the underlying molecular mechanism(s).

Dormancy and drought - one antisense to rule them all?

RUSLAN YATUSEVICH AND SZYMON SWIERZEWSKI

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Plants have developed multiple strategies to sense the external environment and to adapt growth accordingly. *Delay of germination 1* (*DOG1*) is a major quantitative trait locus (QTL) for seed dormancy strength in *Arabidopsis thaliana*. *DOG1* is extensively regulated, with an antisense non-coding transcript (*asDOG1*) suppressing its expression in seeds and in mature plants.

We showed that ABA elevates *DOG1* suppression by suppressing *asDOG1* in mature plants. This leads to the discovery of an unexpected role of *DOG1* gene in drought resistance. In this talk we will describe our efforts to understand *DOG1* antisense regulation by endogenous and exogenous signals.

Assembly and function of the RNA silencing complex

YUKIHIDE TOMARI

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siRNAs and microRNAs mediate posttranscriptional gene silencing of their target mRNAs via formation of the RNA-induced silencing complex (RISC). These small RNAs are born double-stranded and loaded into Argonaute proteins (Ago), the core component of RISC. Subsequently, the two strands of a small RNA duplex are separated and one of them is then discarded from Ago. Intriguingly, the Hsp70/Hsp90 chaperone machinery and their ATP hydrolysis are required for duplex loading—apparently simple binding between the RNA duplex and Ago—but not for strand separation—a process that disrupts ~20 base pairs between the two strands. Accordingly, we envision that the chaperone machinery mediates dynamic conformational changes of Ago so that they can accommodate bulky small RNA duplexes. I would like to discuss such actions of the chaperone machinery as the driving force for RNA silencing, in light of our recent progress in single-molecule imaging of RISC assembly and function.

The RNA annealing activity of human ribonuclease Dicer

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The ribonuclease III Dicer plays a fundamental role in the microRNA (miRNA) and small interfering RNA (siRNA) pathways by excising miRNAs from hairpin precursors (pre-miRNAs) and by producing siRNAs from long double-stranded RNAs. The Dicer generated small regulatory RNAs are handed over to Ago proteins to control gene expression by targeting complementary sequences within mRNA transcripts. A number of recent reports have demonstrated that the activity of Dicer may be associated not only with production of small regulatory RNAs. For example, it has been found that Dicer may participate in chromosome fragmentation during apoptosis, chromatin structure remodeling or inflammation. Interestingly, results of our latest studies indicate that human Dicer (hDicer) is capable of supporting base pairing between two complementary RNA molecules, which suggests that this enzyme might function as a nucleic acid annealer. During the talk, a comprehensive analysis on how RNA structure influences the RNA annealing activity of hDicer will be presented.

Simple Test Methods for Various Biomarkers at Attomolar Level

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We have developped a simple test method named signal amplification by ternary initiation complexes (SATIC)[1-5], which was achieved real-time visual observation and real-time quantitative analysis of genomic DNA (dsDNA), short RNA (e.g. miRNA), and their mutations, and non-nucleic acid targets (e.g., proteins and metabolites) as well as RNA transcripts (mRNA). The SATIC methd is a specific light-up system, which can serve as a one-tube-one-step real-time detection method for various biomarker detections, on the basis of the rolling circle amplification (RCA) mechanism. Of note, it does not need additional steps such as annealing, washing, or transferring of samples[1]. Therefore, we anticipated that the present method will be able to be applied to simple detection of various biomarkers, which relate to diseases, such as cancers and various circulatory system diseases.

Furthermore, we attempted to imporve the sensitibvity of this method. As a result, using a solid support immobilizing SATIC reagents, attomolar level detections of targets such as mRNA transcripts and RNA virus genome could be attained.

Acknowledgements

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Activity of MBNL splicing factors and the mechanism of their sequestration on toxic RNA in myotonic dystrophy

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RNA-binding proteins play critical roles in RNA alternative processing during tissue development and maintenance. One of them are muscleblind-like (MBNL) proteins that function as alternative splicing factors at multiple developmental stages. We found that MBNL1, MBNL2 and MBNL3 bind equally and effectively to the same consensus sequences located within similar RNA structures *in vitro* and in vivo and through which they modulate the alternative splicing of the same exons but with different strength. Recognized consensus sequence consists of three closely located YGCY motifs as a minimal target for efficient binding and functionality. Similarly to other RNA-binding proteins, the affinity and activity of all MBNL paralogs to RNA targets strongly depends on their structures. Similar structure determinants which modulate the effectiveness of proteins' binding and function may also partially explain various sensitivity of specific transcripts to different MBNL concentrations in cells. Disruption of critical developmental transitions in alternative splicing 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 expansion (CUGexp) RNAs. In DM, interactions between MBNL proteins and CUGexp RNA leads to the formation of nuclear complexes, or RNA foci. We showed that MBNL-CUGexp complexes are highly dynamic structures composed of tightly packed, although mobile, MBNL proteins that modulate RNA foci morphology. We also found that sequestration of MBNL proteins in DM results in aberrant processing of hundreds of pre-mRNAs on the level of alternative splicing and alternative polyadenylation.

5′ end modifications in RNA metabolism, innate immunity and their exploitation in biotechnology

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Virus-derived transcripts can often differ from the host RNA by several features, such as the presence of long stretches of exposed double-stranded RNA or the lack of some post-transcriptional modifications. These key differences are exploited by the vertebrate innate immune system: specialized receptors recognize the unusual RNA features as pathogen-associated molecular patterns, which leads to initiation of antiviral signaling cascades and mounting of the interferon response. A set of 5′ end modifications is important not only for proper translation or stability of RNA, but also for prevention of the recognition of the RNA as a pathogen-derived transcript. We investigate the family of Interferon-induced proteins with tetratricopeptide repeats and describe how their interactions with RNA and other proteins determine their ligand specificity. We also propose to employ components of the innate immune mechanisms to improve diagnostic procedures in detection of infectious diseases, and for use in molecular biology applications. This research is funded by the National Centre for Research and Development, Poland, under the grant agreement LIDER/039/L-6/14/NCBR/2015.

Exploring pancreatic transcriptional heterogeneity for better endocrine islet cell building

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Uncovering the molecular events, which are responsible for beta cell formation would greatly aid in the advancement of regenerative stem-cell-based methodlogies aimed at generating functional beta cells for treating diabetes. Here, through the characterization of murine pancreatic development at single cell resolution, we show that e14.5 and e16.5 endocrine progenitors (EPs) are composed of multiple subtypes and are temporally distinct. Unexpectedly, e14.5 alpha cell-biased EPs and e16.5 beta cell-biased EPs arise from separate types of bipotent progenitors (BPs), showing that cell fate is determined prior to the expression of Neurogenin3 (Ngn3). Moreover, BPs at e16.5 are already enriched for beta cell genes indicating fate priming before EP induction. Analysis of the ontological trajectories of single-cells further revealed gene regulatory circuits controlling endocrine cell fate decisions. We present transcriptional and epigenetic atlas of the pancreatic endocrine development, which can be used to propel the development of hormonal cell differentiation paradigms.

Cap-specific terminal N^6-methylation of RNA by an RNA polymerase II-associated methyltransferase

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RNA molecules are enzymatically modified, and more than 160 chemical modifications have been found in various RNAs across all domain of life. N^6-methyladenosine (m^6A) is a major modification of mRNAs, and plays critical roles in various biological events. Biogenesis and dynamics of m^6A have been studied extensively; the modification is introduced by the writers (METTL3 complex and METTL16), and demethylated by eraser proteins (ALKBH5 and FTO). Internal m^6As are decoded differently by several reader proteins including YTH family proteins, thereby leading to diverse fates of mRNAs. In addition to the internal m^6A, N^6,2'-*O*-dimethyladenosine (m^6Am) is present at the transcription start nucleotide of capped mRNAs in vertebrates. Although a recent study investigated the demethylation pathway of m^6Am and the link to mRNA destabilization, biogenesis and physiological significance of this modification have not been fully understood. Using a reverse genetics approach, we here identified cap-specific adenosine methyltransferase (CAPAM/PCIF1) responsible for N^6-methylation of m^6Am. CAPAM specifically interacts with the Ser5-phosphorylated C-terminal domain of RNA polymerase II, resulting in the formation of m^6Am at the early stage of transcription cycle. The crystal structure of CAPAM in complex with substrates revealed the cap-specific m^6A formation mediated by a novel helical domain of CAPAM. Furthermore, a transcriptome-wide analysis revealed that N^6-methylation of m^6Am promotes the translation of capped mRNAs, instead of stabilizing the A-starting capped mRNAs.

A new family of RNA chaperone proteins in bacteria?

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Small regulatory RNAs (sRNAs) are involved in numerous processes in bacterial cells. sRNAs act by base pairing with target mRNAs, in this way affecting their translation or stability. Those sRNAs which are *trans*-encoded bind to partly complementary mRNA sequences, and are mainly involved in the bacterial adaptation to environmental stress and maintenance of cellular homeostasis. On the other hand, *cis*-encoded sRNAs are fully complementary to regulated mRNAs, and several of them are involved in the control of plasmid replication or in the toxin-antitoxin systems. The main matchmaker protein involved in the regulation by *trans*-encoded sRNAs is the chaperone protein Hfq. However, it has been recently shown that another protein, ProQ, binds several *cis*-encoded and *trans*-encoded sRNAs in *E. coli* and *S. enterica*. ProQ is a member of a larger family of proteins, which share a ProQ/FinO domain connected to different N- or C-terminal extensions. To better understand the roles of the *E.coli* ProQ protein in the interactions between sRNA and mRNA molecules we used gelshift assays to compare the binding of ProQ to different RNA molecules and to test if ProQ can contribute to the sRNA pairing to target mRNAs. We expect that the results of these studies will elucidate the differences in RNA recognition and matchmaking properties of the Hfq and ProQ proteins, which may help to explain their complementary functions in *E.coli* cells.
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Roles for pre-mRNA splicing regulation in environmental adaptation of plants

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Plants with low mobility capability should adapt to the environmental issues by adjusting their growth and development, in response to various external stresses. Recent advances in plant molecular genetics have shown that RNA metabolic regulation, such as pre-mRNA splicing, plays an essential role in environmental adaptation processes of plants. For example, we have been demonstrating that pre-mRNA splicing-related mutants of *Arabidopsis thaliana* show abnormalities in light, temperature and salt stress responses. Interestingly, our recent data indicatesd that specific types of cell-stress inducer can affect splicing regulation in *Arabidopsis thaliana* seedlings, suggesting that specific cell stresses can change the expression of cell stress-response genes through alternatice splicing regulation. Based on these results, in this presentation, I would like to discuss possible plant-specific molecular strategy to adapt to environmental stresses, through pre-mRNA splicing regulation.

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U7 snRNA activity and replication-dependent histone gene expression is affected by ALS-linked FUS mutations

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U7 small nuclear RNA is a part of U7 snRNP, which is a major factor in correct 3'end processing of replication dependent core canonical histone pre-mRNAs. We have recently reported that U7 snRNP interacts in the nucleus with FUS (Fused in sarcoma), that results in increased efficiency of 3'end processing of histone pre-mRNAs during the S phase of the cell cycle. Moreover, we showed that FUS acts as a positive regulator of replication-dependent histone gene transcription (1).

FUS is a nuclear RNA-binding protein, wherein mutations are observed in patients with familial Amyotrophic Lateral Sclerosis (ALS). Most reported FUS-linked ALS causing mutations are missense mutations clustered in the nuclear localization signal. They lead to almost abolished or significantly reduced nuclear import of FUS and to cytoplasmic accumulation of FUS aggregates in neurons and glial cells of ALS patients. What is more, mislocalized FUS mutants sequester also RNA-binding proteins and some U snRNPs, resulting in extensive pre-mRNA processing defects in cells (2-6).

Our microscopic analysis revealed that ALS-linked mutations in FUS gene leads also to re-localization of U7 snRNA in the cytoplasmic aggregates. As a further consequence of FUS and U7 snRNA mislocalization, we observed impaired transcription and processing of histone pre-mRNAs. The results were obtained in different cell lines, including glioblastoma cells as well as undifferentiated and terminally differentiated neuroblastoma cells, transfected with FUS mutant as compared with wild type FUS. What is more we also used iNeurons differentiated from patients fibroblasts. Taken together, disturbed expression of histone gene expression can result in genome instability and may be the molecular mechanisms underlying altered motor neurons homeostasis in ALS.

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Control of Immune Responses via RNA Degradation

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Immune cells sense pathogen invasion and evoke immune responses by inducing a set of immune-related genes including proinflammatory cytokines. Their expression is controlled at the transcriptional and posttranscriptional levels. Degradation of cytokine mRNAs is critical for preventing persistent production of cytokines. We identified Regnase-1 as an endoribonuclease essential for the degradation of immune-related mRNAs such as *Interleukin-6* induced by pathogen sensing. Regnase-1 is also critical for suppressing T cell activation and maintenance of immune homeostasis in mice. Regnase-1 recognizes stem-loop structures in 3′ untranslated regions, and degrades translationally active mRNAs in the presence of UPF1, a helicase essential for the nonsense-mediated mRNA decay (NMD). Regnase-1-mediated mRNA decay is triggered by the phosphorylation of UPF1 by a kinase SMG1. Treatment of dendritic cells (DC) with a SMG1 inhibitor enhanced cytokine expression and DC maturation. These results demonstrate that RNA degradation is critical for the control of immunity by targeting host inflammatory mRNAs. In addition, RNases can degrade foreign RNAs such as viral genomic RNAs. We further tried to identify novel RNA binding proteins suppressing viral RNAs by using HIV-1 as the model. We discovered a novel RNase N4BP1 is critical for the restriction of HIV-1 infection in T cells and macrophages, by degrading viral mRNAs. Although the expression of N4BP1 is induced in response to type I IFN stimulation, T cells signaling resulted in the cleavage of N4BP1 by a MALT1 protease, which is potentially involved in the reactivation of HIV-1 latent cells. Collectively, RNA degradation controls immune reactions to pathogen infection at the levels of direct control of foreign RNAs as well as the regulation of host inflammatory mRNAs.

Identification of 5′-capped tRNA halves in human cell lines

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Small non-coding RNAs (sRNAs) play central regulatory roles in eukaryotic cells. Besides well-known classes like miRNAs or piRNAs, there is a growing number of reports describing functional sRNAs which are excised from other functional RNAs. The examples include 18 nt-long ribosome-associated RNA processed from ORF of TRM10 mRNA in yeast and tRNA-derived sRNAs observed in multiple organisms. Although most of those RNAs are revealed by cDNA sequencing, currently available computational tools for their identification are inaccurate.

Here we present a novel bioinformatic tool, missRNA (Method for Identification of Small Stable RNAs), which efficiently identifies novel small, stable RNAs from sRNA sequencing data by distinguishing them from unspecific degradation products. In contrast to currently available tools, it analyzes the distribution of both RNA ends simultaneously, resulting in superior accuracy of RNA ends estimation.

By application of our method to meta-analysis of human sRNA sequencing datasets from ENCODE project, we have identified a plethora of novel sRNA candidates. When we compared the repertoires of sRNAs among investigated samples, we have observed significant differences in content of tRNA-derived fragments. After a detailed analysis of cDNA libraries aimed at cloning of sRNAs with different 5′ ends (all 5′ end types or specific for 5′phosphate or 5′ cap), we have identified a set of tRNA halves which are specifically enriched in cell line-derived cDNA libraries specific for 5′-capped RNAs. Moreover, for tRNAs which were source of observed 5′ capped tRNA halves, we were able to confirm the presence of cap structure by analysis of matching CAGE-seq data, which revealed cap signatures corresponding to 5′ ends of tRNAs of origin. Our observations suggest significant contribution of 5′ cap to stability or processing of tRNA halves in human.

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Genome-Wide Kinetic Analysis of pre-mRNA Processing Reveals Action of Splicing Regulators

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In previous studies, we analyzed specific order of intron excision in production of alternatively spliced mRNA isoforms for some genes in *Caenorhabditis elegans*. For instance, we demonstrated that a downstream intron is excised first in selection of neuron-specific exon 7a of the *unc-32* gene in the wild type, whereas an upstream intron is excised first when exon 7b is selected in a splicing regulator mutant *unc-75*. These kinds of examples suggested that the splicing regulators change the order of splicing by either accelerating or decelerating excision rates of specific intron(s) and consequently switch the splicing patterns. However, dynamics of pre-mRNA processing such as rates and order of intron excision are not well studied in living organisms and the action of the splicing regulators on the dynamics of pre-mRNA processing are to be elucidated.

Here, we aimed to analyze global kinetics of nascent pre-mRNA processing in two approaches. First, we massively sequenced metabolically labeled nascent RNAs (nascent RNA-seq). The processing rates of *cis*-splicing, *trans*-splicing and 3'-end cleavage were calculated based on declining patterns of unprocessed reads. We reveal that the splicing rates vary on a genome-wide scale and even within a gene. We validated predicted order of excision for some pairs of neighboring introns. Furthermore, action of splicing regulators were predicted by comparing nascent RNA-seq data from the wild-type and mutant worms. Second, we labeled RNAs being transcribed by Nuclear Run-On (NRO) assay and performed long-read sequencing. This approach enabled us to elucidate the timing of co-transcriptional splicing as a function of the Pol II position at single transcript levels. We unexpectedly found that neighboring introns were both spliced or both unspliced in most cases, suggesting coupling of the splicing events. The splicing rates correlated with splice site sequences and intron lengths. Interestingly, 'slow' introns were likely closer to the 5'-end of genes. This position effect might be explained by coupling of multiple splicing events implied in our long-read sequencing.

Nascent RNA-seq also enabled genome-wide estimation of RNA half-lives. We found that estimated mRNA stability significantly correlated with codon usage as has recently been demonstrated in *E. coli*, *S. cerevisiae* and zebrafish. Totally, our study revealed the genome-wide dynamics of pre-mRNA processing and mRNA stability in *C. elegans*.

Structural and biochemical insights into synthetic ligands targeting A-A pairs associated with disease-related CAG RNA repeats

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The presented work is a part of our research focusing on incurable human neurological disorders associated with repeated sequence expansions (TREDs). The pathogenic factor is a transcribed RNA or protein whose function in the cell is compromised. TREDs are progressive and incurable, and there are no drugs to treat it at the present. Consequently, many ongoing studies are oriented at developing therapies. We present the first crystal structures of synthetic ligands that target RNA containing CAG repeats. We also performed a biochemical evaluation of the RNA-ligand interactions. Structures of the complexes show a pleasing directness of interactions between the ligands and the RNA, specifically at the A-A pairs, which make them a promising starting points for serious biomedical refinements and trials. Considering the surprising mode of binding and the large extent of structural changes upon complex formation, it seems very unlikely that this could be predicted theoretically. The crystallographic data indicate how the compounds could be further refined in future biomedical studies.

ADAR1 regulates early T cell development via MDA5-dependent and -independent pathways

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ADAR1 is an RNA editing enzyme that converts adenosine to inosine in double-stranded RNA (dsRNA) and is highly expressed in thymus. We have recently reported that deletion of *Adar1* specifically from T cells at the double-positive (DP: $CD4^+CD8^+$) stage in mice impairs negative selection, which results in abrormal thymic T cell maturation and autoimmunity without reduction in the number of thymocytes. These abnormalities are rescued by concurrent deletion of MDA5, a cytosolic dsRNA sensor, which suggests that ADAR1-mediated RNA editing is required for the establishment of self-tolerance during the late stage of T cell development by preventing MDA5 sensing of endogenous dsRNA as non-self. However, the role of ADAR1 at the early stage of thymic T cell development remains unknown. Here, we demonstrate that the mutant mice in which *Adar1* is deleted at the double-negative (DN: $CD4^-CD8^-$) stage reveal severe thymic atrophy with excessive apoptosis and loss of the expression of T cell receptors (TCRs), which inhibit the DN3-to-DN4 transition. Intriguingly, concurrent deletion of MDA5 ameliorates apoptosis but not restores TCR expression. In contrast, forced expression of TCRs on thymocytes rescues transition from DN3 to DN4, revealing the contribution of ADAR1 on early T cell development in a MDA5-dependent and -independent manners. In accordance, we find that TCR transgene and concurrent knockout of MDA5 synergistically ameliorates the defects of T cell development in mice lacking ADAR1 expression at DN stage. These findings suggest that early stage of thymic T cell development requires not only ADAR1-mediated preventon of MDA5 activation to avoid excess apoptosis but also MDA5 pathway-independent ADAR1 function underlying the expression of TCRs.

TUTs in mammalian RNA metabolism

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RNA metabolism is a general term used to describe a multitude of different processes from RNA transcription to its decay. Proper RNA metabolism stands for physiological wellbeing of a cell, proper cellular differentiation and tissue formation and for ability of a cell to adequately respond to environmental threats and pathogens.

There is a growing experimental evidence of addition of non-templated U residues to RNA 3′ ends by terminal uridyltransferases (TUTs) in a process called uridylation. Uridylation has come into focus as an important player in regulation of RNA stabilities and functionalities. The scope of RNAs regulated by uridylation is broad and includes cytoplasmic coding RNAs, non-coding structured RNAs and retrotransposon RNAs. The latter are RNA intermediates in a life cycle of mobile genetic elements, retrotransposons, that can propagate in the human genome by a copy-and-paste mechanism called retrotransposition. Besides shaping human genome in an evolutionary time scale also retrotransposonal RNAs impact cellular homeostasis with confirmed roles in autoimmunity and senescence.

In this talk I will summarize knowledge on uridylation in RNA metabolism and highlight a newly discovered uridylation-mediated LINE-1 regulatory mechanism.

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