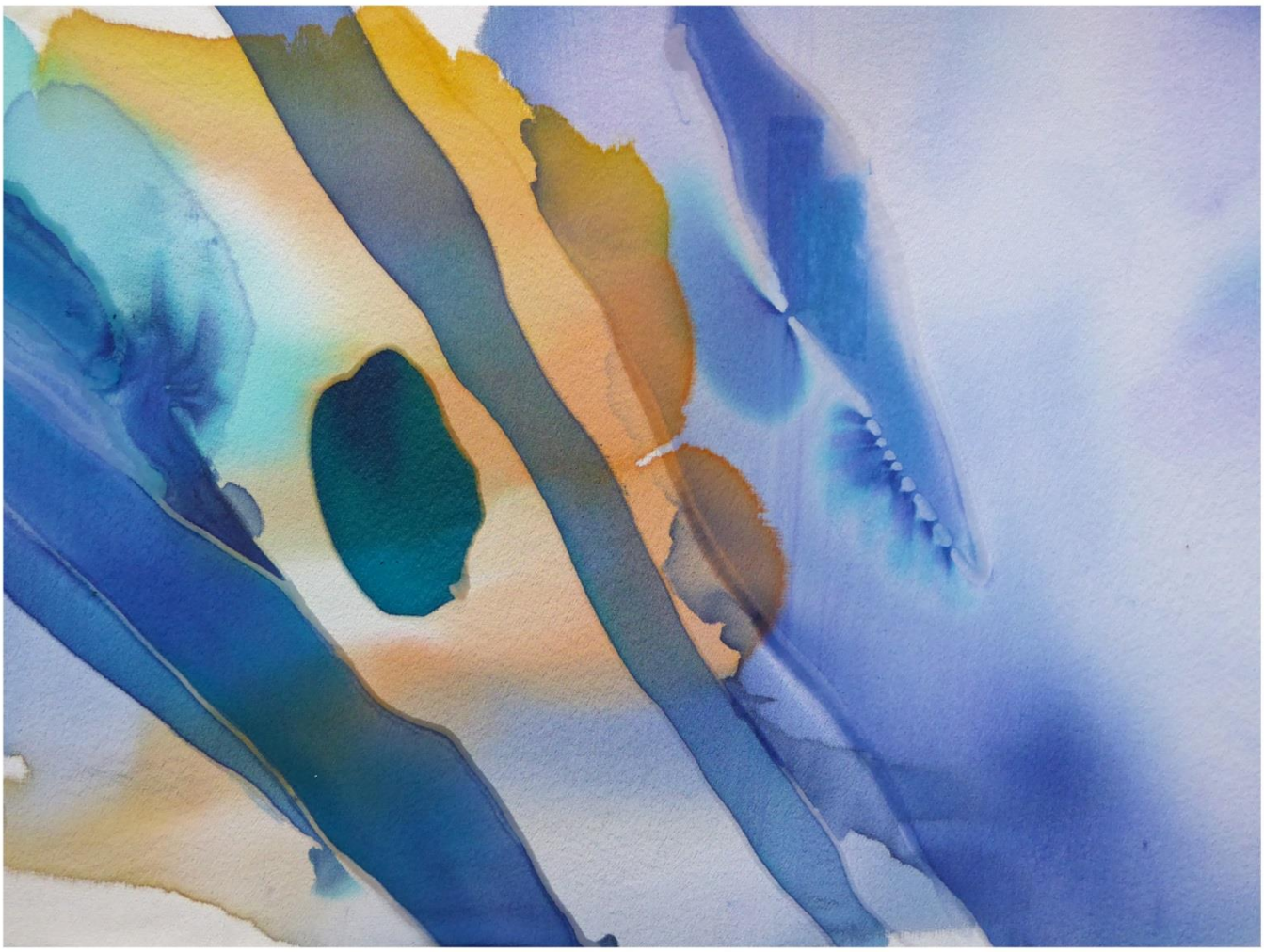

RNA Alternative Splicing 2016
4th Post-EURASNET Symposium

September 28th - 30th 2016
Poznan, Poland
Collegium Biologicum
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The Faculty of Biology is one of the fifteen faculties of Adam Mickiewicz University (AMU). It achieved its present institutional form in 1984, but natural sciences were always present at AMU since the very origin of the University in 1919. Today, the Faculty is organized into four institutes: Anthropology, Experimental Biology, Molecular Biology and Biotechnology, and Environmental Biology. These are further divided into 27 departments and five laboratories. In addition, there are also six central laboratories, four of which host core facilities of the Faculty (isotopic lab, electron and confocal microscopy lab, molecular biology techniques lab, and phytotron facility), one that groups all the natural collections, and one that specializes in educational issues. At present, the Faculty comprises more than 300 employees with roughly 200 academic staff. Its strength is built around ca. 40 full professors and 40 AMU professors; 1400 students, studying various full-time or extramural study programs, together with 150 PhD students, complement the whole community. The Faculty is seated in Collegium Biologicum, part of the state-funded state-of-the-art AMU Morasko campus located on the outskirts of Poznań. The Faculty of Biology AMU offers an outstanding research environment coupled with world-class state-of-the art infrastructure. The Faculty staff has been engaged in many EU funded research projects, such as AEROTOP, MONALISA, FUNGEN, CONTRASTRESS, EURASNET, EVOLGEN, HIALINE, FLORA ROBOTICA. Moreover our research projects were supported by the Norwegian financial mechanism, European Structural Funds, Ministry of Science and Higher Education, Ministry of Agriculture and Rural Development, National Science Centre and Foundation for Polish Science. In May 2014 “Poznań RNA Centre” formed by the Faculty of Biology AMU in Poznań together with the Institute of Bioorganic Chemistry, Polish Academy of Sciences received a status of Leading National Research Centre – first of only three such institutions in biological sciences in Poland. In August 2014 we opened a special international program for students wishing to acquire M.Sc. in Biotechnology. This program continues the successful study program established within a framework of the UNIKAT project co-funded by the European Union within the European Social Fund. These studies emphasize molecular biology, genetics, gene therapy and systems biology approaches, and aims to give practical training and advanced knowledge in bioeconomy and biotechnology legislation.

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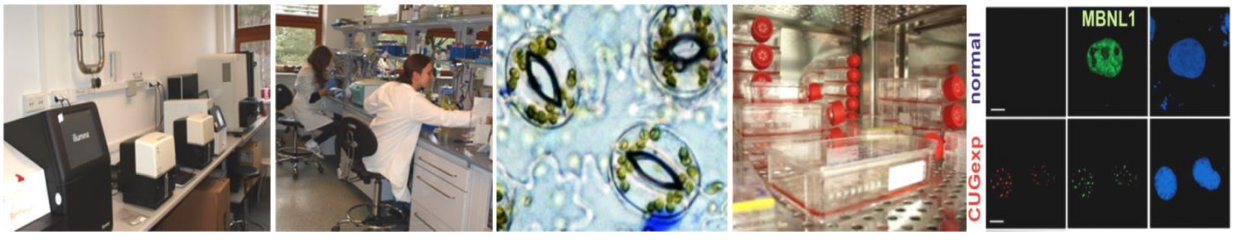

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Poznań RNA Research Centre

Leading National Research Centre – KNOW

In 2011, a new Act on Higher Education created a possibility to establish the most prestigious status of KNOW – Leading National Research Centre. This status, assigned through competition, can only be given to institutions carrying out scientific research at the highest level and providing PhD studies of the highest quality. Till now 6 KNOW centres have been named in exact sciences (mathematics, chemistry, physics) and medical sciences. In 2014, 4 new KNOW centres have been named in natural sciences, and in agriculture, forestry and veterinary sciences. On May 15th, The Poznań RNA Research Centre consisting of the Faculty of Biology, Adam Mickiewicz University in Poznań and the Institute of Bioorganic Chemistry, Polish Academy of Sciences, received KNOW status in biological sciences for 5 years. This status is closely related with additional state funds aimed at improving the quality of science and education. Up to 50 million Polish zlotys will be transferred from the Polish Ministry of Science and Higher Education to the Poznań RNA Research Centre. The money will support various aspects of RNA research from basic research on RNA metabolism, through more applied studies on RNA viruses and the role of RNA in cancer and inherited diseases and their therapies, up to the development of bioinformatics tools allowing advanced structure-function studies.

New RNA research groups will be created. Moreover, the Centre opened a new international school for PhD students. For young scientists from other scientific institutions a special fund was created to provide financial support for training in RNA research techniques. All information about the recruitment of new group leaders as well as details on our international PhD program can be found on the Poznań RNA Centre webpage (http://know-rna.amu.edu.pl).

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Wednesday, September 28th

09:00 – 09:05	*Welcome and Opening Remarks*	
Session 1	**Chair: Gil Ast**	**Mechanism of Splicing and its Regulation**
09:05 – 09:40	Reinhard Lührmann	Structural basis of pre-mRNA splicing
09:40 – 10:15	Magda Konarska	Similarities in the first- and second-step interactions at the spliceosome's active sites
10:15 – 11:00	*Coffee Break*	
11:00 – 11:20	Anabella Srebrow	Involvement of SUMO conjugation in pre-mRNA splicing
11:20 – 11:40	Andrea Pawellek	Characterisation of splicing modulator DDD00040800
11:40 – 12:00	Claudio Sette	An orchestrated intron retention program in meiosis controls timely usage of transcripts during germ cell differentiation
12:00 – 12:20	Elisa Monzón-Casanova	Ptbp1 is essential for the formation of germinal centre B cells and the generation of high affinity antibodies
12:20 – 12:40	Claudia Vivori	How alternative splicing changes during reprogramming - The case of C/EBPα poising B cells
12:40 – 13:00	Frédéric Allain	Structures of alternative-splicing factors: still new surprises
13:00 – 15:00	*Lunch*	
Session 2	**Chair: Christiane Branlant**	**Alternative Splicing: Insights from Genomics and Computational Approaches**
15:00 – 15:35	Benjamin Blencowe	Discovery and characterization of alternative splicing regulatory networks with important functions in development and neurological disorders
15:35 – 15:55	Manuel Irimia	Tiny changes, big effects: the impact of microexons on neuronal differentiation, function and evolution
15:55 – 16:15	Eduardo Eyras	Fast and accurate computation of differential splicing in plants and animals across multiple conditions
16:15 – 16:35	Estefania Mancini /Marcelo Yanovsky	ASpli: An integrative R package for analyzing alternative splicing using RNAseq
16:35 – 17:15	*Coffee Break*	
17:15 – 17:35	Julian König	High-throughput screening for splicing regulatory elements
17:35 – 17:55	Nuno Barbosa-Morais	A bioinformatics application for the analysis and visualisation of alternative splicing in cancer
17:55 – 18:15	Mateusz Dobrychłop	PyRy3D: a software tool for modelling of large macromolecular complexes and its application in prediction of Splicing Factor 3b's structure
18:15	**Poster Session with Buffet, Beer and Wine**	

Thursday, September 29th		
Session 3	**Chair: Frédéric Allain**	**Alternative Splicing: Disease and Therapy**
09:00 – 09:35	Adrian Krainer	Oncogenic splicing factors in breast-cancer pathogenesis
09:35 – 09:55	Gil Ast	Phosphatidylserine ameliorates neurodegenerative symptoms of Familial Dysautonomia
09:55 – 10:15	Christiane Branlant	SR and hnRNP proteins regulate HIV-1 multiplication by modulating mRNA splicing and translation
10:15 – 11:00	*Coffee Break*	
11:00 – 11:20	Giuseppe Biamonti	The Splicing Program of SRSF1 transcripts is finely tuned by cell metabolism
11:20 – 11:40	Katarzyna Gawęda-Walerych	Parkinson's disease-related gene variants influence pre-mRNA splicing processes
11:40 – 12:00	Paulina Ćwiek	Splicing correction in Erythropoietic Protoporphyria
12:00 – 12:20	Krzysztof Sobczak	Severe transcriptome changes induced by toxic RNA in myotonic dystrophy
12:20 – 12:40	David Elliott	The RNA binding protein SLM2 controls splicing patterns of synaptic proteins and its own expression required for neural network activity and mouse behaviour
12:40 – 13:00	Michael Ladomery	Effect of hypoxia on alternative splicing in prostate cancer cells
13:00 – 15:00	*Lunch*	
15:00 – 15:20	Sheila Graham	Targeting SR proteins to disrupt the Human Papillomavirus replication cycle
Session 4	**Chair: Andrea Barta**	**Alternative Splicing in Plants**
15:20 – 15:40	John Brown	Alternative splicing and the cold transcriptome of *Arabidopsis*
15:40 – 16:00	Ezequiel Petrillo	A chloroplast retrograde signal regulates nuclear alternative splicing… in the roots!
16:00 – 16:20	Paula Duque	The *Arabidopsis* SR protein SCL30a regulates drought and salt stress tolerance during seed germination
16:20 – 17:00	*Coffee Break*	
17:00 – 17:20	Dorothee Staiger	Posttranscriptional networks controlled by the hnRNP-like RNA-binding protein AtGRP7
17:20 – 17:40	Chueh-Ju Shih	hnRNP involvement in phytochrome-mediated alternative splicing in *Physcomitrella patens*
17:40 – 18:00	Andreas Wachter	Regulation of alternative splicing in *Arabidopsis* by polypyrimidine tract binding proteins
18:00 – 18:20	Maria Kalyna	The transcript survival guide: at the crossroads of alternative splicing and RNA interference
18:20	**Poster Session with Buffet, Beer and Wine**	

Friday, September 30th

Session 5	Chair: Paula Duque	Interconnections between RNA Processes
09:00 – 09:35	Witold Filipowicz	Mechanism and regulation of miRNA-mediated repression in cultured cells and mouse retina
09:35 – 09:55	Magdalena Maslon	Global biological consequences of the regulation of alternative splicing by Pol II elongation rate
09:55 – 10:15	Junaid Akhtar	The Exon junction complex (EJC) regulates promoter proximal pausing of RNA Pol II
10:15 – 11:00	*Coffee Break*	
11:00 – 11:20	Reini Luco	A chromatin signature for alternative splicing
11:20 – 11:40	Yael Mandel-Gutfreund	Dissecting the proteins bridging transcriptional and post-transcriptional regulation in human embryonic stem cells
11:40 – 12:00	Matthias Soller	m6A mRNA methylation regulates alternative splicing in *Drosophila* sex determination
12:00 – 12:20	Jean-Yves Roignant	Insights into the mechanisms of m^6A mRNA modification in alternative splicing regulation
12:20 – 12:40	Agata Stępień	The role of SERRATE/ U1 snRNP communication in microRNA biogenesis in *Arabidopsis thaliana*
12:40 – 13:00	Katarzyna Knop	Active 5' splice sites regulate intronic miRNA biogenesis efficiency in *A. thaliana*
13:00 – 13:05	*Concluding Remarks*	
13:05 – 14:30	*Lunch*	
14:30	***Poznań Sightseeing Tour!***	

Session 1
Mechanism of Splicing and its Regulation
Chair: Gil Ast

Session 1
Mechanism of Splicing and its Regulation

Lecture 1.1

Structural basis of pre-mRNA splicing

Reinhard Lührmann

Max-Planck-Institute for Biophysical Chemistry, Department of Cellular Biochemistry, Göttingen, Germany

The spliceosome catalyses the removal of the intron from nuclear pre-mRNAs and assembles initially into a pre-catalytic ensemble, termed complex B, which contains the snRNPs U1, U2 and the U4/U6.U5 tri-snRNP and numerous non-snRNP proteins. For catalytic activation the spliceosome undergoes a major structural rearrangement, mediated by the Brr2 RNA helicase, yielding the activated spliceosome (B^{act} complex). The final catalytic activation of the spliceosome requires an additional restructuring step by the RNA helicase Prp2, generating the B* complex which catalyses the first step of the splicing reaction, yielding the C complex. Subsequently the catalytic center of the spliceosome has to be remodeled by the RNA helicase Prp16 to generate the C* complex as a pre-requisite for second step catalysis. Using cryo electron microscopy (in collaboration with Holger Stark; MPIbpc, Göttingen) we have investigated the 3D structure of the human U4/U6.U5 tri-snRNP complex and several purified spliceosomal complexes. Our tri-snRNP model reveals how the spatial organization of Brr2 RNA helicase prevents premature U4/U6 RNA unwinding in isolated human tri-snRNPs and how the Sad1 protein likely tethers Brr2 to its pre-activation position. A medium-resolution structure of the human B complex reveals that Brr2 undergoes a large-scale movement during stable B complex formation where it is juxtaposed close to the U4 RNP domain. In the human B complex, Brr2 is still negatively regulated, as the U4/U6 base pairing is resistant to Brr2-mediated dissociation in the presence of ATP. The Cryo-EM structure of the purified *S. cerevisiae* Bact complex reveals how the first step reactants (i.e. the 5'splice site and the branch site adenosine) are sequestered by protein prior to catalysis and provide insights into the molecular remodeling events that must be facilitated by Prp2 in order to generate a catalytically active B* spliceosome. In addition, comparison of the B^{act} spatial organization with the cryo-EM structures of the tri-snRNP reveal how many spliceosomal components are rearranged during activation of the spliceosome. Finally, I will also present the Cryo-EM 3D structure of a human C* complex, which has undergone the Prp16-mediated remodeling step.

Session 1

Lecture 1.2

Similarities in the first- and second-step interactions at the spliceosome's active sites

Magda Konarska[1] and Charles Query[2]

[1] Centre for New Technologies, University of Warsaw, Warsaw, Poland
[2] Department of Cell Biology, Albert Einstein College of Medicine, New York, New York, United States of America

The two reactions of pre-mRNA splicing require transition between the first and the second step, which involves repositioning of the substrate effected by a conformational change in the enzyme. A variety of factors can affect relative stabilities of spliceosome's conformations, including both changes in protein factors and RNA-RNA interactions. Although models explaining the organization of the first step catalytic centre have been proposed – and now confirmed by the recent cryo-EM spliceosome structures – our understanding of the second step is much less advanced. The substrates need to be repositioned: the branch structure produced in the first step has to be moved out of the active site and the 3'SS has to be placed there – if the spliceosome uses a single active site for bor both steps – but details of these interactions have not been described. Using an orthogonal spliceosome system, where in addition to the authentic spliceosome we create a second spliceosome specific for a different set of substrates, we tested a model of the second step catalytic centre. We propose that after the first step, when the branch sequence is based-paired to U2-GUAGUA, the newly formed branch structure translocates by 3 nt on the GUA triplet repeats, opening the first GUA to provide a binding site for the 3'SS UAG. The UA of the 3'SS UAG base-pairs with U2, and the ultimate G of the intron is positioned in the same/similar purine-binding pocket to that used to bind the branch site-A for the first step.

Lecture 1.3

Involvement of SUMO conjugation in pre-mRNA splicing

Berta Pozzi[1], Laureano Bragado[1], Cindy Will[2], Reinhard Luhrmann[2], Anabella Srebrow[1]

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[2] Max Planck Institute for Biophysical Chemistry, Gottingen, Germany

Pre-mRNA splicing is catalyzed by the spliceosome, a multi-megadalton ribonucleoprotein machine comprised of five small nuclear ribonucleoprotein particles (snRNPs) and numerous associated proteins. While protein modification by phosphorylation, acetylation and ubiquitylation has been reported to influence spliceosome assembly and dynamics, hardly anything is known about the regulation of proteins involved in the splicing process by SUMO conjugation. This is indeed curious considering that proteomic studies have identified the RNA-binding proteins as one of the major groups among SUMO conjugation substrates, including splicing auxiliary factors and spliceosome components, and furthermore SUMO conjugation has been shown to regulate other aspects of mRNA maturation such as pre-mRNA 3'end processing. Previous work from our laboratory revealed a novel role for the splicing factor SRSF1 as a regulator of the SUMO conjugation pathway both in vitro and in living cells. We demonstrated that SRSF1 displays certain characteristics of SUMO E3 ligases and also affects the activity of an already known member of this group, PIAS1. These results lead us to explore a connection between the SUMO pathway and the splicing machinery. We have recently found that addition of a recombinant SUMO-protease decreases pre-mRNA splicing efficiency as evaluated by an in vitro splicing system. By performing anti-SUMO immunoprecipitation of purified splicing complexes formed at different time points of the splicing reaction, followed by mass spectrometry analysis, we have identified several spliceosomal proteins as SUMO conjugation substrates. We have focused on one of them, the spliceosomal component Prp3, mapping SUMO attachment sites within this protein and generating a Prp3 SUMOylation-deficient mutant by site directed mutagenesis. When overexpressed in cultured cells, this mutant fails to increase splicing efficiency, shows reduced interaction with certain snRNP components and a diminished recruitment to active spliceosomes, compared to the wt protein. Our findings clearly indicate that SUMO conjugation plays a role throughout the splicing process and suggest an involvement of Prp3 SUMOylation during tri-snRNP formation.

Lecture 1.4

Characterisation of splicing modulator DDD00040800

Andrea Pawellek[1], Ursula Ryder[1], Marek Gierlinski[2], Timur Samatov[3], Reinhard Luehrmann[4], Andrew Woodland[5], David Gray[5], Angus Lamond[1]

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[2] Computational Biology, College of Life Sciences, Dundee, United Kingdom
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[4] Department of Cellular Biochemistry, D-37077 Goettingen, Germany
[5] Drug Discovery Unit, College of Life Sciences, Dundee, United Kingdom

Pre-mRNA splicing is essential for the expression of most genes in higher eukaryotes. However, in contrast with the other essential transcription and translation steps in gene expression, there are very few well characterised small molecule inhibitors available. During the Eurasnet project we, together with the group of Reinhard Lührmann and the University of Dundee Drug Discovery Unit (DDU), screened a curated library of >75,000 small, drug-like compounds and identified several novel classes of small molecule splicing inhibitors, including madrasin (Pawellek et al., J.Biol. Chem. 2014). Here we describe the characterisation of DDD00040800 another small molecule identified in the screen, that both inhibits splicing *in vitro* and modulates the pattern of pre-mRNA splicing of endogenous transcripts in multiple human cancer cell lines, representing a different compound class to madrasin. DDD00040800, interfered *in vitro* with one or more early steps of spliceosome formation, stalling assembly at the E/H and A complex. RNA-seq analysis of HeLa and HEK293 cells treated with DDD00040800 identified ~3,000 altered splicing events, affecting transcripts including the viral oncogene E1A and key regulators of cancer cell survival MCL1 and Bclx. Treatment with DDD00040800 altered the pattern of isoforms expressed from these genes at both the mRNA and protein level. Treatment with DDD00040800 inhibited cell cycle progression, arresting cells in S/G2&M phase, as shown by FACS analysis. In contrast to other splicing modulators, DDD00040800 treatment did not lead to the formation of mega-speckles, but to the disruption of Cajal bodies. Work is ongoing to identify the direct targets of DDD00040800 and the other new small molecule splicing inhibitors identified.

Lecture 1.5

An orchestrated intron retention program in meiosis controls timely usage of transcripts during germ cell differentiation

Chiara Naro[1,2], Ariane Jolly[3], Sara Di Persio[4], Pamela Bielli[1,2], Niclas Setterblad[5], Antonio J Alberdi[5], Elena Vicini[4], Raffaele Geremia[1], Pierre De la Grange[3], Claudio Sette[1,2]

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[2] Laboratories of Neuroembryology, Fondazione Santa Lucia IRCCS, Rome, Italy
[3] Genosplice, Paris, France
[4] Fondazione Pasteur Cenci Bolognetti, Department of Anatomy, Histology, Forensic Medicine and Orthopedic, Section of Histology Sapienza University of Rome, Rome, Italy
[5] Saint-Louis Hospital, Paris, France

Global transcriptome reprogramming during spermatogenesis ensures timely expression of factors in each phase of male germ cell differentiation. Spermatocytes and spermatids require particularly extensive reprogramming of gene expression to switch from mitosis to meiosis and to support gamete morphogenesis. RNA-sequencing analyses uncovered an extensive alternative splicing program during this trans-meiotic differentiation. Notably, intron retention was largely the most enriched pattern, with spermatocytes showing generally higher levels of retention compared to spermatids. Retained introns are characterized by weak splice-sites and are enriched in genes with strong relevance for gamete function. Meiotic intron-retaining transcripts (IRTs) were exclusively localized in the nucleus. However, differently from other developmentally-regulated IRTs, they are stable RNAs, showing longer half-life than properly spliced transcripts. Strikingly, fate-mapping experiments revealed that IRTs are recruited onto polyribosomes days after synthesis. These studies reveal an unexpected function of regulated intron retention in modulation of the timely expression of select transcripts during spermatogenesis.

Lecture 1.6

Ptbp1 is essential for the formation of germinal centre B cells and the generation of high affinity antibodies

Elisa Monzón-Casanova[1, 2], Manuel Díaz-Munoz[1], Sarah E. Bell[1], Tomaz Curk[3], Jernej Ule[4], Michele Solimena[5], Christopher Smith[2], Martin Turner[1]

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Polypyrimidine tract binding proteins (Ptbp) regulate various aspects of RNA metabolism such as alternative splicing, mRNA stability, IRES-driven translation, mRNA localization and polyadenylation. During neuronal differentiation a switch between Ptbp1 and Ptbp2 promotes essential changes in a subset of neuronal alternative splicing events. In contrast, in the hematopoietic system a third paralog, Ptbp3, is co-expressed with Ptbp1 at high levels while Ptbp2 expression is not detected. We are particularly interested in the functions of Ptbp proteins during B cell activation, which results in the secretion of high affinity antibodies. These high affinity antibodies are produced by differentiated B cells that have undergone numerous rounds of proliferation in specialised anatomical locations in secondary lymphoid tissues called germinal centres. Interestingly the expression of Ptbp1 but not Ptbp3 is increased in germinal centre B cells compared to non-activated B cells. Ptbp1 and Ptbp3 iCLIP experiments showed that these proteins bind highly redundantly to the transcriptome of primary B cells. However, using conditional knockout (cKO) models that lack either Ptbp1 or Ptbp3 in B cells we have seen that only Ptbp1 is essential for the proper formation of germinal centre B cells. Accordingly, the secretion of high affinity antibodies is impaired in Ptbp1 cKO mice. In order to understand the molecular events that drive the defects of Ptbp1 KO B cells we have carried out RNAseq from primary germinal centre B cells. These experiments have identified hundreds of events that are likely to be regulated by Ptbp1. We are currently investigating which of these events are important for B cell function and responsible for the impaired antibody responses observed in the Ptbp1 cKO mice.

Lecture 1.7

How alternative splicing changes during reprogramming - The case of C/EBPα poising B cells

Claudia Vivori[1,2], Panagiotis Papasaikas[1,2], Bruno Di Stefano[1,2], Ralph Stadhouders[1,2], Thomas Graf[1,2], Juan Valcárcel[1,2,3]

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[2] Faculty of Health and Life Sciences, Universitat Pompeu Fabra (UPF), Barcelona, Spain

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Alternative splicing is a crucial mechanism of post-transcriptional regulation affecting virtually every biological process in higher eukaryotes. Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are pluripotent cells, capable of self-renewal and differentiation into the three germ layers, both *in vitro* and *in vivo*. While transcriptional regulation and epigenetic modifications have been the primary focus of research in the field, outstanding progress has been made in the last years in revealing how post-transcriptional mechanisms also play a fundamental role in controlling stem cell fate. Reprogramming of a somatic cell into an iPS by transient expression of OSKM factors is a highly inefficient process which can be divided into a stochastic and a deterministic phase. Transient expression of C/EBPα transcription factor during a 18h pulse prior to OSKM induction poises mouse B cells for rapid and efficient reprogramming into iPS [Di Stefano et al., Nature 2014; Di Stefano et al., Nat Cell Biol 2016]. Although the effects of C/EBPα are largely assumed to be due to the opening of chromatin in pluripotency genes promoters, involving DNA demethylation by Tet2, recent evidence showed that many splicing regulators are specifically interacting with C/EBPα. Therefore, to investigate whether C/EBPα pulse could also affect alternative splicing patterns additionally contributing to poise B cells for reprogramming, we analysed RNA sequencing data (unpublished data, Di Stefano, Stadhouders and Graf) examining alternative splicing events and following their changes during early steps of reprogramming after C/EBPα pulse. K means clustering analysis allowed not only to separate classes of alternative splicing events that behave differently during this process, but also to follow potential regulators featuring similar patterns in gene expression and RNA binding motifs showing specific enrichment on distinct clusters of events. Following appropriate validation, modulation of these selected events through antisense oligonucleotides will be carried out to determine whether inducing specific changes can contribute to reprogramming. The final aim will be to understand how C/EBPα could be affecting, directly or indirectly, alternative splicing and whether this phenomenon is independent of its chromatin-opening function, to potentially exploit alternative splicing modulation to reduce stochasticity during the process of reprogramming.

Lecture 1.8

Structures of alternative-splicing factors: still new surprises

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Although the structure of many alternative-splicing factors bound to RNA have already been solved including SR proteins, hnRNP protein and cell-specific proteins like RBFox, in investigating new factors and reinvestigating old factors we discovered novel modes of bindings. We recently solved the structure of the RRM of FUS bound to a stem-loop RNA. The structure revealed an unexpected mode of RRM binding due to the presence of a new feature in the RRM structure a beta-hairpin between alpha1 and beta2 that insert into the stem major groove. Although the RRM also binds the single-stranded loop region, the binding is not sequence-specific. The RRM of FUS is therefore the first example of an RRM recognizing a secondary structure rather than a sequence. Sequence-specificity is rather achieved by the zinc-finger domain which is C-terminal to the RRM. We also recently reinvestigated the RNA binding property of hnRNP A1 and SRSF1. We could show that depending on the RNA sequence, hnRNP A1 tandem RRMs can bind simultaneously the same RNA, however if RRM1 binds a high affinity sequence, binding to RRM2 is inhibited. Finally, we will present recent data showing that quite surprisingly SRSF1 interact with the RNA component of U1snRNP (U1snRNA) which raises questions on the mode of action of SRSF1 in splicing regulation.

Session 2
Alternative Splicing: Insights from Genomics and Computational Approaches
Chair: Christiane Branlant

Session 2
Alternative Splicing: Insights from Genomics and Computational Approaches

Lecture 2.1

Discovery and characterization of alternative splicing regulatory networks with important functions in development and neurological disorders

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In recent years, great strides have been made in the discovery and characterization of alternative splicing programs that function in development and human disease. A current major challenge is to define core splicing networks that have a broad impact on human disorders and diseases when misregulated. An important related challenge is to comprehensively identify trans-acting factors that control these and other critical RNA networks, as well as to define the specific functions of individual exons and introns in these networks. To address these challenges in the context of neurobiology, we have combined global-scale and focused approaches, including the development of new animal models, to discover and characterize an activity-dependent microexon network that underlies key features of autism spectrum disorders. We have also developed and employed a new high-throughput screening system to systematically define trans-acting factors that control this and other splicing programs. A surprising finding from our initial screens is that specific annotated transcription factors appear to dually regulate cell fate-dependent splicing networks through both indirect and direct mechanisms. Finally, we are investigating the function of individual alternative splicing events in these networks, including examples that appear to have a broad impact on biology by affecting the translational machinery. Progress in these areas will be presented.

Session 2

Lecture 2.2

Tiny changes, big effects: the impact of microexons on neuronal differentiation, function and evolution

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One of the major challenges for the development of complex multicellular organisms is to generate dozens of cell types from a single genomic sequence. Through differential processing of introns and exons, alternative splicing (AS) can produce cell type-specific protein isoforms that allow optimization of their specific cellular roles or even the emergence of novel functions. One of the most striking examples of this is provided by microexons in neurons. These tiny exons, which can encode as few as one or two amino acids, are switched on during neuronal differentiation and show the highest evolutionary conservation of all AS types. They are often located in structured domains of proteins, where they subtly sculpt their interaction surfaces thereby modulating protein-protein interactions in a neuronal-specific manner. Although we are still beginning to unveil their biological roles, we already know they are crucial for proper neuritogenesis, axon guidance, and neuronal function. Remarkably, most vertebrate microexons are regulated by a single splicing factor family, Srrm4/nSR100. This protein is specifically expressed in neurons where it directly promotes microexon recognition and inclusion. While this factor appeared to be vertebrate-specific, we have found that nSR100-like proteins are encoded in invertebrates as alternative isoforms of the pan-eukaryotic *Srrm2/SRm300* locus. Strikingly, the neural specificity of these isoforms in bilaterian animals is closely associated with the presence of neural-specific microexons across all studied species, and trans-phyletic swaps of nSR100-like proteins are sufficient to promote inclusion of endogenous neural microexons in human and fly cells. Our results thus suggest that the origin of a novel, cell type-specific, regulatory function in an ancestral splicing factor have allowed the origin of the program of microexons across animal nervous systems, which has in turn contributed to the origin of new molecular functions in this complex cell type.

Lecture 2.3

Fast and accurate computation of differential splicing in plants and animals across multiple conditions

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Alternative splicing plays an essential role in many cellular processes in eukaryotes and bears major relevance in development and disease. High-throughput RNA sequencing allows unbiased genome-wide studies of splicing across multiple conditions. However, the increasing number of available data sets in many different biological conditions represents a major challenge in terms of computation time, as well as for data analysis and interpretation. We describe SUPPA 2.0, a computational tool to calculate relative inclusion values of alternative splicing events [1]. We have extended SUPPA to make it suitable to study alternative splicing in plants, which present frequent overlapping events and different properties of exon-intron structures compared to animals [2]. We further show a new method to calculate differential splicing across multiple conditions with biological replicates, and a density-based clustering algorithm to determine regulatory modules across different conditions. We validated SUPPA high accuracy using multiple RT-PCR experiments for pairs of conditions in human and Arabidopsis. Further, we applied SUPPA to RNA-Seq data for a time-course of Arabidopsis plants transferred from 20°C to 4°C to examine the effects of low temperature and determine new splicing regulatory modules in circadian clock genes. SUPPA calculates alternative splicing profiles and differential splicing patterns across multiple conditions and from a large number of samples at a much higher speed than existing methods without compromising accuracy, thereby facilitating the streamlined analysis and interpretation of very large data sets without requiring large computational resources [3]. SUPPA is available at https://bitbucket.org/regulatorygenomicsupf/suppa.

[1] Alamancos et al. RNA 21(9):1521-31.
[2] Zhang et al. New Phytol 208(1):96-101
[3] Sebestyen et al. Genome Res, Apr 2016

Session 2

Lecture 2.4

ASpli: An integrative R package for analyzing alternative splicing using RNAseq

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Alternative splicing (AS) is a common mechanism of post-transcriptional gene regulation in eukaryotic organisms that expands the functional and regulatory diversity of a single gene by generating multiple mRNA isoforms that encode structurally and functionally distinct proteins. The development of novel high-throughput sequencing methods for RNA (RNA-Seq) has provided a powerful means of studying AS under multiple conditions and in a genome-wide manner [reviews]. However, using RNA-seq to study changes in AS under different experimental conditions is not trivial. Here we describe ASpli, an integrative and user-friendly R package that facilitates the analysis of changes in both annotated and novel AS events. This package combines statistical information from exon, intron, and splice junction differential usage, with information from splice junction reads to calculate differences in the percentage of exon inclusion (ΔPSI) and intron retention (ΔPIR), which reliably reflect the magnitude of changes in the relative abundance of different annotated and novel AS events. This method can be used to analyse both simple and complex experimental designs involving multiple experimental conditions ASpli is intended to facilitate the analysis of RNAseq data for the quantification and discovery of AS events. Results of the analysis are presented in a user friendly manner, including plots of the most relevant AS events discovered.

Lecture 2.5

High-throughput screening for splicing regulatory elements

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Alternative splicing constitutes a major step in eukaryotic gene expression and requires tight control of *trans*-acting factors that recognize *cis*-regulatory elements in the RNA sequence. However, the majority of *cis*-regulatory elements are poorly defined and the impact of intronic and exonic sequence variants on the splicing outcome remains elusive. Here, we established a high-throughput screen to comprehensively identify all *cis*-regulatory elements that determine a particular splicing decision. As a prototype example, we screened a minigene harbouring the cancer-relevant alternative exon of the RON receptor kinase gene. A library of thousands of randomly mutagenized minigene variants was transfected as a pool into human HEK293T cells, and the splicing products were subsequently analysed via RNA sequencing. In parallel, DNA sequencing enabled reliable point mutation discovery which were assigned to the corresponding splicing products via a unique barcode sequence. In addition to known *cis*-regulatory elements, this approach enabled us to identify numerous previously unknown regulatory sites. This approach proved particularly powerful when combined with knockdown experiments which allows to connect *trans*-acting factors with their corresponding *cis*-regulatory elements. In summary, this novel screening approach introduces a tool to study the relationship of *cis*-regulatory elements, sequence variants and their impact on the splicing outcome, offering new insights into alternative splicing regulation and the implication of mutations in human disease.

Session 2

Lecture 2.6

A bioinformatics application for the analysis and visualisation of alternative splicing in cancer

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The advent of next-generation sequencing has allowed the profiling of transcriptomes beyond gene expression, enabling genome-wide studies of AS. However, the currently available tools for the analysis of alternative splicing from RNA-Seq data are not very biologist-friendly and primarily focus on quantification, having limited downstream analysis features. To overcome these limitations, we have been developing an *R* application with a graphical interface for the integrated analysis of AS from large transcriptomic datasets, namely from The Cancer Genome Atlas (TCGA) project. The tool interactively performs clustering, principal component and other graphically-assisted exploratory analyses. It also enables, for example, the direct incorporation of clinical features (such as tumour stage or survival) associated with TCGA samples. Amongst its innovative aspects are the analysis of variance (which our research shows to be important in the detection of otherwise unnoticed putative targets) and the usage of more realistic distributions in the statistics of differential splicing. Interactive visual access to genomic mapping and functional annotation of selected alternative splicing events is also incorporated. We have successfully used the application in the revelation of cancer-specific alternative splicing signatures and associated novel putative prognostic factors. The application's architecture is modular and extensible, aiming to stimulate contributions from its users, as well as to gradually expand its support to other data sources and file formats and the scope of its analysis and visualisation tools without modifying its core functionalities. It will soon be freely available to the scientific community as open-source in *GitHub* and *Bioconductor*.

Lecture 2.7

PyRy3D: a software tool for modelling of large macromolecular complexes and its application in prediction of Splicing Factor 3b's structure

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One of the major challenges in structural biology is to determine the structures of macromolecular complexes and to understand their function and mechanism of action. However, compared to structure determination of the individual components, structural characterization of macromolecular assemblies is very difficult. To maximize completeness, accuracy and efficiency of structure determination for large macromolecular complexes, a hybrid computational approach is required that will be able to incorporate spatial information from a variety of experimental methods (like X-ray, NMR, cryo-EM, cross-linking and mass spectrometry, etc.) into modelling procedure. For many biological complexes such an approach might become the only possibility to retrieve structural details essential for planning further experiments. We developed PyRy3D, a method for building and visualizing low-resolution models of large macromolecular complexes. The components can be represented as rigid bodies (e.g. macromolecular structures determined by X-ray crystallography or NMR, theoretical models, or abstract shapes) or as flexible shapes (e.g. disordered regions or parts of protein or nucleic acid sequence with unknown structure). Spatial restraints are used to identify components interacting with each other, and to pack them tightly into contours of the entire complex (e.g. cryo-EM density maps or ab initio reconstructions from SAXS or SANS methods). Such an approach enables creation of low-resolution models even for very large macromolecular complexes with components of unknown 3D structure. Our model building procedure applies Monte Carlo approach to sample the space of solutions fulfilling experimental restraints. Splicing Factor 3b (SF3b) is a protein complex responsible for the recognition of the intron's branch site in U2- and U12-dependent introns. We applied the hybrid modelling approach implemented in PyRy3D software in order to build ensembles of structural models of the human SF3b complex that agree with currently available experimental and theoretical data.

Session 3
Alternative Splicing: Disease and Therapy
Chair: Frédéric Allain

Session 3
Alternative Splicing: Disease and Therapy

Lecture 3.1

Oncogenic splicing factors in breast-cancer pathogenesis

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Splicing factor SRSF1 is upregulated in human breast tumors, and its overexpression promotes transformation of mammary cells. Using RNA-seq, we identified SRSF1-regulated alternative-splicing targets in organotypic three-dimensional MCF-10A cell cultures, which undergo acinar morphogenesis and mimic a context relevant to breast cancer. We identified and validated hundreds of endogenous SRSF1-regulated alternative-splicing events, and determined the overlap with presumptive SRSF1-regulated events deregulated in human breast tumors. Overexpressing one such isoform, exon-9-included CASC4, increased acinar size and proliferation, and decreased apoptosis, partially recapitulating SRSF1's oncogenic effects. We then used the same experimental approach in a comparative analysis of several other SR-protein paralogs and the SR-like protein Tra2β, which are likewise deregulated in breast cancer. These factors had differential effects in various in vitro and in vivo oncogenic assays, correlating with changes in alternative splicing of their downstream targets. In particular, we found that Tra2β regulates cell migration and invasion, and plays a role in metastatic growth in vivo. One of our goals is to uncover oncogenic alternative-splicing events that represent potential targets for therapeutics development, e.g., using splice-switching antisense oligonucleotides.

Session 3

Lecture 3.2

Phosphatidylserine ameliorates neurodegenerative symptoms of Familial Dysautonomia

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Familial Dysautonomia (FD) is an autosomal recessive neurodegenerative disease. A point mutation at the 5' splice site of intron 20 alters the splicing pattern of *IKBKAP* exon 20 in a tissue-specific manner, leading to a reduction of IKAP protein in neuronal tissues. In order to examine the importance of the IKAP protein in neuronal development and survival, we generated a conditional knockout (CKO) mouse in which exon 20 of *IKBKAP* is deleted specifically in the nervous system. The CKO FD mice exhibit developmental delay, sensory abnormalities, and FD-like symptoms. Dorsal root ganglia (DRGs) from the CKO FD mice display less organized neuronal network, which exhibits excessive branches and neurite lengths. Reduction in IKAP levels led to attenuated microtubule with reduced acetylated α-tubulin levels. Furthermore, live cell imaging assays demonstrated impairment of retrograde nerve growth factor (NGF) transport along the axons of CKO FD DRGs. These abnormalities in DRGs properties due to lack of exon 20 of *IKBKAP* underlie neuronal degeneration and FD symptoms. Elevation of IKAP levels by treatment of phosphatidylserine increased acetylation of α-tubulin and enhanced retrograde transport of NGF along DRGs axons. Overall, these findings suggest that phosphatidylserine treatment stabilizes microtubules and facilitates axonal transport. Hence, our data indicate that phosphatidylserine has potential for treatment of FD and might effect on progression rates of neurodegeneration.

Lecture 3.3

SR and hnRNP proteins regulate HIV-1 multiplication by modulating mRNA splicing and translation

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Human Immunodeficiency Virus type 1 (HIV-1) is one of the major health concern. Deciphering mechanisms that regulate HIV-1 replication will facilitate the development of new therapy. Alternative splicing plays a crucial role in HIV-1 multiplication. Together with translation, it controls viral protein production at the various steps of the virus life cycle. HIV-1 primary transcripts contain five splicing donor sites (5'SSs) and nine splicing acceptor sites (3'SSs) and their combined and time scheduled utilizations generate more than 40 different mRNAs. In the early phase of the virus cycle, multiply spliced mRNAs coding for the Tat, Rev and Nef proteins are respectively produced. Then, singly spliced mRNAs coding for the Env, Vpu, Vif and Vpr proteins, respectively, and unspliced transcripts coding for the Gag and Pol protein precursors are synthesized. HIV-1 splicing regulations predominantly take place at the 3'SSs and involve SR and hnRNP proteins. We will present a summary of the present knowledge on HIV-1 splicing regulations and will show our data concerning the complex regulation of Tat protein production at the splicing and translation steps. This knowledge is as much important as the Tat transcription activator is essential for HIV-1 multiplication. In the absence of Tat only a tiny amount of full-length transcripts is produced, preventing new functional virus production. On the other hand, Tat production should be limited because of its strong toxicity for the host. The human immunodeficiency symptoms are mainly due to Tat protein production. We will show how, hnRNP A1, hnRNP H, DAZAP1, SRSF1, SRSF2, SRSF5, SRSF7 and the Tat protein itself each modulate negatively or positively *tat* mRNA production. *Tat* mRNA translation, largely depends upon internal ribosome entry sites (IRESs). We established the RNA 2D structure of *tat-1* mRNA and by in *cellulo* assays using a bi-cistronic RNAs, we identified the various *tat-1* mRNA regions showing an IRES activity. Then, we tested the capability of various SR and hnRNP proteins to stimulate or to inhibit the identified IRES activities. The conclusion is that some of the SR and hnRNP proteins which modulate *tat* mRNA splicing have similar modulation effects on *tat* mRNA translation, revealing a strong implication of these proteins in Tat protein production. Targeting these splicing and translation regulations open new possibilities for HIV-1 therapy.

Session 3

Lecture 3.4

The Splicing Program of SRSF1transcripts is finely tuned by cell metabolism

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Splicing factor SRSF1 has an essential role in gene expression regulation and in cancer progression. We have previously shown that this factor controls epithelial to mesenchymal cell transition (EMT) driven by cell density. SRSF1 levels are finely regulated through a complex series of alternative splicing (AS) events. Six SRSF1 transcripts (called Is1 to 6) are generated through AS involving intron 3, the last coding exon (Ex4) and 2 introns in the 3'UTR: Is1 encodes for SRSF1; Is2, Is3 and Is4 are exclusively nuclear, Is5 and Is6 are exported to the cytoplasm and degraded by non-sense mediated RNA decay (NMD) pathway. Moreover, Is1 is the precursor of Is4 and Is5, while Is2, produced by retention of intron 3, is the precursor of Is3 and Is6. Splicing of SRSF1 transcripts is modulated in a tissue specific manner. We have found that AS of SRSF1 transcript is controlled by cell density; while Is1 prevails in Low-Density cells (LD, which display a typical mesenchymal phenotype) the abundance of remaining 5 RNA molecules, particularly of Is3, drastically increases in High-Density cells (HD, exhibiting an epithelial phenotype). This switch in splicing profile driven by cell density occurs in all the cell lines tested so far, from mouse to man. Similarly to cells in the inner mass of the primary tumor, HD cells undergo nutrient stress, such as depletion of Glucose and Glutamine, leading to a drop in ATP levels. This affects the expression of a large number of genes (about 2000), 50% of which are up-regulated in HD cells. We have found that the splicing profile of SRSF1 is controlled both by glucose metabolism and by extracellular factors Finally, Is6 encodes a novel variant of SRSF1 that lack the entire RS domain involved in protein-interactions. This variant is mainly detectable during the recovery from stress.

Lecture 3.5

Parkinson's disease-related gene variants influence pre-mRNA splicing processes

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We have analyzed the impact of Parkinson's disease (PD) - related mutations/polymorphisms on splicing using dedicated minigene assays. Out of fourteen putative splicing variants in five genes (*PINK1*, PTEN induced kinase 1; *LRPPRC*, leucine-rich pentatricopeptide repeat containing protein; *TFAM,* mitochondrial transcription factor A; *PARK2*, and *HSPA9*) four *LRPPRC* variants, (IVS32-3C>T, IVS35+14C>T, IVS35+15C>T, IVS9+30A>G) influenced pre-mRNA splicing by modulating the inclusion of the respective exons. In addition, MPP+-induced splicing changes of endogenous *LRPPRC* mRNA reproduced the effect of the *LRPPRC* IVS35+14C>T mutation. Using silencing and overexpression methods, we show that *LRPPRC* exon 33 splicing is negatively regulated by heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) both in a minigene and endogenous context. Furthermore, exon 33 exclusion due to PD-associated mutation IVS32-3C>T or hnRNPA1 overexpression and exon 35 exclusion due to IVS35+14C>T can be rescued by co-expression of modified U1snRNAs, providing a potentially useful therapeutic strategy. Our results indicate for the first time that *LRPPRC* intronic variants can affect normal splicing of this gene and may influence disease risk in PD and related disorders.

Lecture 3.6

Splicing correction in Erythropoietic Protoporphyria

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Erythropoietic Protoporphyria (EPP) is a disorder of heme metabolism caused by partial deficiency of the last enzyme of the heme biosynthesis pathway. Ferrochelatase (FECH) catalyzes the insertion of iron into protoporphyrin IX (PPIX) to form heme. FECH deficiency leads to accumulation of PPIX in erythrocytes followed by leakage of PPIX into the plasma, skin and bile. Upon activation by visible light,the accumulated PPIX causes photodamage of the skin resulting in extreme clinical symptoms: pain, itching, burning, ulceration, erythema, and edema. Moreover, ~5% of the patients suffer from cholestasis caused by accumulation of PPIX in the liver. Clinical symptoms become apparent when FECH activity drops below a critical threshold of ~35%. The vast majority of EPP patients (~97%) carry an intronic polymorphism (IVS3–48C) in *trans* to a mutated *FECH* allele (Mut) and produce only ~25% of active enzyme. In contrast, people carrying a *FECH* mutation combined with the normal IVS3-48T allele (T) or homozygotic for IVS3–48C(C) do not develop the disease. The C allele lowers the expression level by stimulating the utilization of a cryptic 3' splice site (ss) 63 nt upstream of the normal 3' ss. Due to the fact that most EPP patients have a C/Mut allele combination, correcting the aberrant splicing of intron 3 is a potential target for therapeutic approaches. The splicing defect can be corrected in cell lines by specially designed bifunctional U7 small nuclear RNAs (snRNAs) that carry both an antisense sequence allowing specific binding to intron 3 and a splicing repressor sequence that will additionally weaken the aberrant ss. To implement this therapeutic strategy, we have developed a humanized mouse model. By crossing two inbred lines carrying IVS3–48C and a severe *FECH* mutation, respectively, we obtain F1 hybrids containing the desired C/Mut combination. These mice have elevated PPIX in the blood as well as enlarged livers and spleens in comparison to F1 hybrids of a T/Mut genotype. Moreover, exposure to light causes irritation and pain on the exposed skin areas of C/Mut mice. These mice will serve as a disease model to test the U7-based therapeutic strategy.

Lecture 3.7

Severe transcriptome changes induced by toxic RNA in myotonic dystrophy

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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) proteins that function as alternative splicing factors at multiple developmental stages. 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 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. 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. 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.

Lecture 3.8

The RNA binding protein SLM2 controls splicing patterns of synaptic proteins and its own expression required for neural network activity and mouse behaviour

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SLM2 is a member of a conserved family of RNA binding proteins including Sam68 and SLM1 that control splicing of the *Neurexin1-3* genes encoding synaptic proteins, but the global targets of SLM2 or its phenotypic importance are unknown. The brain is made up of trillions of synaptic connections that together form neural networks needed for normal brain function and behaviour. Here we find SLM2 additionally controls splicing of the *Tomosyn2*, *LysoPLD/ATX*, *Dgkb*, *Kif21a* and *Cask* genes each important for synapse function. Furthermore, SLM2 levels are maintained by a homeostatic feedback control pathway that predates divergence of SLM2 and Sam68. Cortical neural network activity dependent on synaptic connections between normally SLM2-expressing-pyramidal neurons and interneurons was decreased within *Slm2* null mice, which also showed decreased ability to recognise novel objects and anxiety. Our data indicate SLM2 gene conservation and expression control because of roles in brain network activity and behaviour.

Lecture 3.9

Effect of hypoxia on alternative splicing in prostate cancer cells

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Hypoxia is defined as the state in which the availability or delivery of oxygen is insufficient to meet tissue demand. It is particularly significant in aggressive, fast-growing tumours in which the rate of new blood vessel formation (angiogenesis) cannot match the growth rate of tumour cells. Tumour hypoxia has been linked to poor prognosis and to greater resistance to existing cancer therapies. We hypothesize that one of the ways that tumour cells adapt to hypoxia is by changing the alternative splicing of key genes. A high throughput PCR analysis provided evidence of significant changes in rates of inclusion of several cassette exons in cancer-associated genes in hypoxic PC3 prostate cancer cells. We noted increased expression of splice factors (including SRSF1) and splice factor kinases (CLK1) in hypoxia. Chemical inhibition of CLK1 with the benzothiazole TG003 also altered exon inclusion rates. We suggest that CLK1 is involved in the modulation of alternative splicing in response to hypoxia.

Session 3

Lecture 3.10

Targeting SR proteins to disrupt the Human Papillomavirus replication cycle

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Constitutive and alternatively splicing is positively controlled by serine-arginine-rich splicing factors (SRSFs), otherwise called SR proteins. Phosphorylation controls the activity and alters the subcellular location of SR proteins, which is important for their function. For example, nuclear SRSF1 regulates splicing and nucleocytoplasmic transport while cytoplasmic SRSF1 can control RNA stability and translation. SR proteins are as essential for viral as they are for cellular mRNA processing and viruses such as herpes simplex virus and HIV are known to require SR proteins for efficient viral replication. Human papillomaviruses (HPVs) are tumour viruses that infect epithelial cells (keratinocytes). They cause mainly benign lesions (warts) but can also cause cancers such as cervical and head and neck cancers. New antiviral drugs are required to stop disease progression in the millions worldwide who are infected. HPV genotype 16 is the most prevalent HPV worldwide and causes the largest burden of cancers. During HPV16 infection at least 20 different viral mRNAs are produce through alternative promoter usage, differential polyadenylation and in particular, alternative splicing. Production of the mRNA isoforms is tightly linked to differentiation of the infected epithelial cell. We demonstrate here that the HPV replication/transcription factor E2 controls expression of SR protein during viral infection in a keratinocyte differentiation stage-specific manner. Further, we show that HPV regulates SR protein phosphorylation and subcellular location. Conversely, we have evidence that SRSFs1, 2 and 3 are required for expression of viral proteins and completion of the viral replication cycle. SRPIN small molecule drugs have been shown to inhibit replication of medically important viruses including HIV and HCV. SRPINs inhibit SRSF phosphorylation through inhibiting SR protein kinases (SRPK) 1 & 2. Because we have shown that SR proteins are required for completion of the HPV replication cycle, we tested whether SRPIN drugs could inhibit HPV gene expression. SRPINs were found to be nontoxic to HPV-negative and positive epithelial cells and did not significantly alter their proliferation. SRPIN drugs had a significant effect on HPV mRNA production. Our data reveal a specific reduction in transcripts encoding the key viral E2 replication/transcription factor and a concomitant loss of E2 protein suggesting that SRPINs could inhibit the HPV replication cycle. SRPINs also caused a reduction in cytoplasmic SRSF1 levels that we have demonstrated is required for viral RNA association with the translation apparatus. Collectively, these data suggest that SRPIN drugs could potentially inhibit HPV replication through a feedback loop centred on HPV E2.

Session 4
Alternative Splicing in Plants
Chair: Andrea Barta

Session 4
Alternative Splicing in Plants

Lecture 4.1

AS and the cold transcriptome of Arabidopsis

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Alternative splicing (AS) occurs in >60% of protein-coding genes and is involved in development and responses to environmental conditions. To address the regulation of low temperature-induced AS in circadian clock genes we performed a genome-wide study using ultra-deep RNA-seq of a high resolution time-course of plants moved from 20°C to 4°C. We analysed the RNA-seq data with Salmon which measures the abundance of individual transcripts. For this, we made a comprehensive Reference Transcript Dataset (AtRTD2) for Arabidopsis. We applied quality and redundancy filters to generate 82k transcripts where each represents a unique splice isoform. AtRTD2 significantly outperforms other currently available transcriptome sets in quantification of transcripts and AS for AS/expression analysis in Arabidopsis. Analysis of the temperature shift RNA-seq time-course with Salmon/AtRTD2 gave transcript-specific expression profiles for the over 20k which were expressed. We identified genes which are regulated only by transcription, by AS and by both. Therefore, in response to low temperature the Arabidopsis transcriptome is re-programmed at both the transcriptional and AS levels and that AS is a major component of the cold transcriptome. Many hundreds of genes show significant low temperature-induced AS, often occurring rapidly after transfer to the cold. Around half of these genes have isoform switches and a number of genes show changes in rhythmicity of expression of transcripts suggesting coupling or uncoupling of expression to the circadian clock. The AS-regulated genes cover multiple functions but include many transcription factors and are enriched in splicing factors. The genes which are regulated only by AS have significant changes in AS but no overall change in expression and therefore represent novel genes involved in the cold response.

Session 4

Lecture 4.2

A chloroplast retrograde signal regulates nuclear alternative splicing… in the roots!

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Light is a source of energy and also a regulator of plant physiological adaptations. We have previously shown that light/dark transitions affect alternative splicing of a subset of Arabidopsis genes, preferentially those encoding proteins involved in RNA processing. These effects require functional chloroplasts and are also observed in the roots when the communication with the photosynthetic tissues is not interrupted, suggesting that a signaling molecule travels through the plant. We are now aiming to identify the nature of the light signals that communicate the chloroplast status to the nuclei of leaf and of root cells. Focusing on the determination of the nature of the mobile signal that impacts in the roots, we have found evidence implying sugars as the main candidate to be responsible for the observed effects in the non-photosynthetic tissues, and we are currently dissecting the signaling pathway in the root cells.

Lecture 4.3

The Arabidopsis SR protein SCL30a regulates drought and salt stress tolerance during seed germination

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Abscisic acid (ABA), the major plant stress hormone, plays a crucial role in the response to drought and high salinity, the two most pervasive causes of loss of crop productivity worldwide. Consistent with a role for alternative splicing in plant responses to an adverse environment, our work has been providing functional links between SR proteins, established modulators of this posttranscriptional regulatory mechanism, and stress responses mediated by the ABA phytohormone. In one of these studies, we found that SCL30a, belonging to an SC35-like subfamily of *Arabidopsis thaliana* SR proteins, negatively regulates ABA signaling to control abiotic stress responses during seed germination. The *SCL30a* gene is upregulated during seed imbibition and germination, and loss of its function results in smaller seeds displaying enhanced dormancy. Moreover, mutant seeds show hypersensitivity to ABA as well as to high NaCl and mannitol concentrations, while transgenic plants overexpressing *SCL30a* exhibit reduced ABA sensitivity and enhanced tolerance to salt and osmotic stress during germination. The mutant's stress oversensitivity is rescued by an ABA biosynthesis inhibitor, and epistatic analyses confirm that SCL30a control of stress responses requires a functional ABA pathway. We are currently working on the identification of the mRNAs targeted by this SR protein to confer abiotic stress tolerance.

Session 4

Lecture 4.4

Posttranscriptional networks controlled by the hnRNP-like RNA-binding protein *At*GRP7

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The glycine-rich RRM-containing protein *At*GRP7 (*Arabidopsis thaliana* glycine-rich RNA-binding protein 7) negatively autoregulates its circadian oscillations via binding to its pre-mRNA followed by alternative splicing-Nonsense-mediated decay. To unravel posttranscriptional networks controlled by *At*GRP7 globally, *in vivo* RNA binding substrates were identified by RNA immunoprecipitation (RIP)-seq. Several hundred transcripts co-precipitated with *AtGRP7::AtGRP7:GFP* fusion protein upon precipitation with GFP Trap beads but not upon mock precipitation with RFP Trap beads. To resolve the impact of *At*GRP7 on its candidate targets, transcriptome profiling of *atgrp7* loss-of-function mutants and *At*GRP7 overexpressors was performed. A suite of transcripts bound by *At*GRP7 showed altered steady-state abundance in response to altered *At*GRP7 level. Furthermore, we identified transcripts aberrantly spliced in response to altered levels of *At*GRP7. Among those are several direct targets, indicating that *At*GRP7 regulates alternative splicing by direct binding to some transcripts *in vivo* and influences alternative splicing decisions of others indirectly. Currently, we are analysing how posttranslational modifications regulate the impact of *At*GRP7 on its downstream targets.

Lecture 4.5

hnRNPs involve in phytochrome-mediated alternative splicing in *Physcomitrella patens*

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Light is critically needed for the growth and development of plants. Therefore, plants have evolved photoreceptors to not only absorb light signals but also regulate gene expression to modulate photomorphological responses. Photoreceptor-mediated gene regulation at transcription level is largely studied, but research about regulation at pre-mRNA splicing step remains poor. Our lab has previously reported the involvement of photoreceptors, especially the red light photoreceptors, phytochrome, in light-responsive alternative splicing (AS). AS can be regulated by splicing regulators like serine/arginine-rich (SR) proteins or heterogeneous nuclear ribonucleoproteins (hnRNPs). In order to further investigate how photoreceptors affect alternative splicing, we aimed to investigate the relationship between photoreceptors and splicing regulators in the moss *Physcomitrella patens*. Among the tested photoreceptors and splicing regulators, Physcomitrella phytochrome 4 displays a red light-dependent interaction with two hnRNPs *in vitro* and *in vivo*, suggesting that phytochrome 4 controls AS via hnRNPs. Phenotypes like colony size and phototropism of over-expression and knock-out lines of hnRNPs also show the involvement of hnRNPs in the red light-mediated photomorphogenesis. Moreover, over-expressing and knocking out the two hnRNPs in the moss differ the AS pattern of several genes, showing their participations in splicing regulation. We conclude that phytochrome 4 partakes in the control of AS through hnRNPs. Elucidating the mechanism of how phytochromes regulate splicing factors to alter the outcome of pre-mRNA splicing is undergoing.

Lecture 4.6

Regulation of Alternative Splicing in Arabidopsis by Polypyrimidine Tract Binding Proteins

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Alternative splicing (AS) is widespread in plants and has been demonstrated to be critically involved in the regulation of development as well as stress responses. The AS outcome is defined by the splicing code, referring to the interplay of varying sets of splicing regulators and their target precursor mRNAs. To gain a better understanding of this process in plants, we have analysed the splicing regulatory functions of Polypyrimidine tract binding proteins (PTBs) from *Arabidopsis thaliana*. We found several hundred AS events to be controlled by PTBs, and both developmental processes, such as seed germination and organ formation, and drought resistance were altered in *ptb* mutants. Combining *in vitro* interaction assays and *in vivo* splicing reporter studies, we have defined PTB binding motifs in target RNAs with cassette exons that are either included or skipped in a PTB-dependent manner. Furthermore, regulation of PTB activity during development and in response to stresses is analysed. Our work aims at deciphering central components of the plant splicing code, thereby providing a mechanistic basis for the control of AS and its links to fundamental biological functions in plants.

Lecture 4.7

The transcript survival guide: at the crossroads of alternative splicing and RNA interference

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Alternative splicing (AS) is a major mechanism to increase transcriptome and proteome diversity in eukaryotic genomes. About 60% of the protein-coding intron-containing genes in Arabidopsis undergo AS. Transcript isoforms generated from a single gene via AS can have different fates; some are coding for proteins, others are degraded by the nonsense-mediated RNA decay (NMD) machinery. Around 13% of intron-containing genes in Arabidopsis are regulated by AS coupled to NMD. Intracellular localization of transcript isoforms can also be influenced by AS, whereby transcripts with retained introns usually remain within the nucleus, which allows them to escape the NMD machinery despite containing premature termination codons and long faux 3'UTRs. Ser/Arg–rich (SR) proteins are key splicing factors that govern splice site selection and AS outcomes. Arabidopsis genome contains eighteen SR protein genes, which themselves are alternatively spliced. To study functions of Arabidopsis SR protein genes, we generated knock-down transgenic lines for three of them using artificial miRNAs (amiRNAs), which were designed to target all AS variants of a respective gene. Unexpectedly, we have found that analysis of total mRNA levels of a target gene does not always reflect the knock-down efficacy for an amiRNA when the target gene undergoes AS. We show that AS variants of the same gene are affected differentially by an amiRNA even though all of them contain an amiRNA target site. This can mask the effect of an amiRNA on protein-coding isoform(s) when amiRNA efficacy is determined only by total mRNA levels. We addressed the question why AS variants of the same gene have a differential sensitivity to an amiRNA. Consequently, we have established an interplay between RNA interference and AS transcripts which vary by their intracellular localization, targeting to NMD, and protein coding capacity. In addition, we demonstrate that an amiRNA can influence AS decisions in Arabidopsis, thus the effect of amiRNAs on expression of target genes is not limited to RNA degradation.

Session 5
Interconnections between RNA Processes
Chair: Paula Duque

Session 5
Interconnections between RNA Processes

Lecture 5.1

Mechanism and regulation of miRNA-mediated repression in cultured cells and mouse retina

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MiRNAs regulate genes post-transcriptionally, by base-pairing to mRNA 3'UTRs and causing translational repression and mRNA deadenylation/degradation. miRNAs function as part of miRNPs, with Ago and GW182 proteins being their important components. Function of GW182 proteins involves recruitment of the CCR4/NOT complex. Our biochemical and structural studies (with E. Conti) demonstrated that CCR4/NOT recruitment by GW182 occurs via Trp-containing motifs present in GW182 repressive regions. Further downstream, CCR4-NOT recruits the DEAD-box ATPase DDX6. This interaction induces a conformational change in DDX6 resulting in ATPase activation, which is required for DDX6 function in repression of translation. We also study (with B. Roska) role of miRNAs in mouse retina. During retina development, processing of the sensory neuron-specific pri-miR-183/96/182 cluster to mature miRs is tightly regulated by RNA helicase Ddx3x and a retina-specific lincRNA Rncr4. Precocious accumulation of mature miR-183/96/182 leads to disorganization of retinal architecture caused by miR-mediated inhibition of Crb1, a component of the adhesion belt between glial and photoreceptor cells. While investigating a role of miRNAs in the function of cones, photoreceptors for daylight vision, we found that depletion of miRNAs in adult cones results in loss of their outer segments. Reexpression of miR-183/96/182 prevented the loss. These miRNAs were also necessary and sufficient for formation of light-responding photoreceptors in retinal cultures derived from ES cells.

Session 5

Lecture 5.2

Global biological consequences of the regulation of Alternative splicing by Pol II elongation rate

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Alternative splicing (AS) is a major contributor to protein isoform diversity. Its regulation depends not only on the interaction of trans-acting factors with cis-acting sequences but also on coupling with RNA polymerase II (Pol II) transcription. The kinetic model of AS regulation proposes that the rate of Pol II elongation influences the outcome of AS selection. In agreement, the use of a mutant form of Pol II with a lower elongation rate leads to increased inclusion of alternative cassette exons into mature mRNA. This mutant is equivalent to the C4 point mutation, which was identified in the *Drosophila* Pol II largest subunit and resulted in some non-lethal developmental defects in the heterozygous state. We have generated mouse embryonic stem cells (ESC) knocked-in for this slow Pol II mutant allele (R749H). Using RNA-seq we have identified transcriptome and splicing changes in these cells. The presence of a slow RNA Pol II resulted in gross changes in transcription and influenced AS, affecting both exon inclusion and intron retention. Mouse chimaeras derived from slow/slow ESCs fail to transmit through the male germ line suggesting that inappropriate processivity of RNA pol II could affect spermatogenesis. In addition introducing the R749H mutation into mouse embryos using the CRISPR/Cas9 system caused defects around the time of gastrulation. To dissect how the slow Pol II mutation might be affecting transcription and AS during differentiation we have focused on neuronal development. Neural stem cells (NSCs) can be generated from ESCs in culture and have the ability to self-renew and to differentiate into all three neural cell types. We found that slow/slow ESC-derived NSCs lost self-renewal capacity and showed signs of premature terminal differentiation instead. Importantly, this was correlated with significant changes in the expression and splicing of genes important for NSC, including components of the Notch and the EGF pathway, among others. We will discuss possible scenarios linking the observed transcriptional and phenotypic changes in the light of the kinetic model of AS regulation.

Lecture 5.3

The Exon junction complex (EJC) regulates promoter proximal pausing of RNA Pol II

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Alternative splicing is a regulated process that increases the number and the diversity of proteins that can be encoded by the genome. We and other groups have previously demonstrated that the exon junction complex (EJC) plays a critical role in this process. One mechanism underlying this function involves the initial deposition of the EJC at spliced junctions and the subsequent recruitment of the splicing machinery at adjacent introns via its splicing subunits. Here, we present evidences that the EJC modulates pre-mRNA splicing via an additional mechanism that relies on the modulation of transcription and nucleosome positioning. These effects required the core EJC subunits but are independent of both EJC splicing subunits and of prior binding to exon junctions. We found that EJC core components are enriched at promoter regions and regulates promoter proximal pausing via restricting binding of the positive transcription elongation complex (P-TEFb). Furthermore, reducing the entry of RNA Polymerase II (Pol II) into the elongation phase is sufficient to rescue exon skipping and the previously reported phenotypes associated with the lack of the EJC *in vivo*. These effects are specific to EJC function since depletion of other spliceosome components did not alter the dynamics of Pol II recruitment similarly and have different splicing defects. Altogether, our data support a mechanism in which the EJC influences pre-mRNA splicing through the modulation of transcriptional state and chromatin architecture, providing new insights into the mechanisms of co-transcriptional splicing and EJC function.

Lecture 5.4

A chromatin signature for alternative splicing

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Alternative splicing regulation depends on the combinatorial recruitment of splicing factors to the pre-mRNA. In the past few years, chromatin has been shown to play an important role in the recruitment of these splicing regulators to the RNA. In particular, others and our group have shown that histone modifications influence alternative splicing by modulating the elongation rate of the RNA polymerase II and/or by favouring the binding of the splicing regulators to the pre-mRNA via chromatin/splicing-adaptor complexes. We are now extending these studies to a genome-wide level by analysing available high-throughput sequencing data from the Roadmap Epigenomics consortium to map, along differentially spliced genes, 27 histone modifications and DNA methylation in H1 embryonic stem cells. Using Machine Learning techniques, we have identified specific combinations of histone and DNA methylation marks differentially enriched along alternatively spliced cassette exons depending on the level of exon inclusion. Importantly, these chromatin marks are distributed non-randomly along the alternatively spliced exons, with the same histone modification marking either inclusion or exclusion depending on where it is enriched along the cassette exon and depending with which other histone mark it is co-enriched, suggesting that there are specific chromatin signatures differentially marking alternative splicing. Moreover, when looking for distinctive features for this chromatin-marked splicing events, we found differential enrichment of A/T tracks and RNA binding motifs, suggesting that those chromatin signatures might favour recruitment of specific splicing regulators to the pre-mRNA. From our results we have been able to define subtypes of alternative exons that are differentially marked by specific combinations of chromatin marks and specific RNA motifs in H1. We are now building a model based on these features to predict alternative splicing in other cell types.

Lecture 5.5

Dissecting the proteins bridging transcriptional and post-transcriptional regulation in human embryonic stem cells

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DNA and RNA binding proteins are the main regulators of gene expression. In the context of tight interactions between the transcriptional and post-transcriptional processes, proteins that bind both DNA and RNA are highly likely to be key players in gene regulation. The unlimited self-renewal capacity of human embryonic stem cells (hESCs) provides a biologically valuable and experimentally tractable model system to study the unappreciated group of dual DNA and RNA binding proteins, which can bridge between the different steps of the gene expression pathway in these cells, such as between transcription and splicing. Using RNA-sequencing, we found that the vast majority of a literature-curated set of dual binders are highly expressed in hESCs. Notably, these proteins have higher mRNA abundance compared with non-DNA and non-RNA binding genes and, surprisingly, also when contrasted with DNA and RNA binding proteins with no detected dual-function. Differential expression analysis further showed that a large fraction of DNA and RNA binding proteins are significantly up-regulated in hESCs, suggesting that cross regulation is involved in shaping the stem cell state. We further employed an RNA-interactome experiment to capture the RNA bound proteome of hESCs, showing that the stem cell RNA-interactome is enriched for known RNA-binding proteins, including a substantial number of potential dual binding proteins. Moreover, among the protein captured in the experiment we identified novel RNA-binding proteins which have not been previously identified and are likely to have a unique role in gene expression regulation in human pluripotent cells.

Session 5

Lecture 5.6

m6A mRNA methylation regulates alternative splicing in Drosophila sex determination

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N6-methyladenosine (m6A) is the most prevalent internal modification of messenger RNA (mRNA) in eukaryotes including mammals, yeast and plants, as well as in nucleus-entering viruses. Internal m6A is formed by the nuclear mRNA methylosome, a multiprotein complex consisting of the catalytic activity MT-A70 (METTL3 in vertebrates and IME4 in yeast), KAR4 (METTL14), fl(2)d (Wilms tumor 1 associated protein, WTAP) and virilizer (vir, KIAA1429) and other factors. In Drosophila, fl(2)d and vir are required for alternative splicing regulation of the sex determination factor Sex-lethal (Sxl), an ELAV/Hu related RNA binding protein. Here, we show that loss of function dIME4 mutants in Drosophila are viable, but are flightless and show a sex bias. In females, m6A potentiates female-specific alternative splicing of Sxl during auto-regulation required for determining female physiognomy and for translational repression of male-specific lethal2 (msl-2) to prevent dosage compensation. Likewise, we show, that dIME4 genetically interacts with fl(2)d and vir alleles. In addition to its requirement for Sxl alternative splicing, loss of m6A affects alternative splicing of a subset of genes with predominant neuronal functions. m6A is rare in polyA mRNA of Drosophila, but enriched in polysomes. On the contrary, the dIME4 methylase co-localizes to sites of transcription and its knock-out globally impacts on expression of metabolic genes. Our results suggest that the m6A mRNA modification constitutes an ancient mechanism to gene-specifically adjust levels of expression.

Lecture 5.7

Insights into the mechanisms of m^{6}A mRNA modification in alternative splicing regulation

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N6-methyladenosine (m^{6}A) is the most abundant mRNA modification in vertebrates, enriched around stop codons, in the 3'UTR regions, within long internal and alternatively spliced exons. m^{6}A is catalysed by a multicomponent complex consisting of two methyltransferases (METTL3 and METTL14), WTAP, KIAA1429 and can be reverted to adenosine via two known demethylases: FTO and ALKBH5. The modification plays important roles in various physiological processes including metabolism, circadian clock, meiosis, stem cell differentiation and behaviour by regulating the posttranscriptional processing of methylated mRNAs. m^{6}A is recognized by YTH domain-containing reader proteins that were shown to regulate mRNA stability, translation efficiency and to enable cap independent translation under stress conditions. Few proteins were also implicated in the regulation of m^{6}A-dependent splicing; either by direct binding to m^{6}A or via structural changes of mRNA caused by the modification. We have carried out a comprehensive molecular and physiological characterization of the individual components of the methyltransferase complex as well as of the YTH reader proteins in *Drosophila melanogaster*. We identified the member of the split ends family proteins, Spenito, as a novel *bona fide* subunit of the methyltransferase complex. We further demonstrated important functions of this complex in neurogenesis and sex determination, and implicated the nuclear reader YT521-B as a main m^{6}A effector in these processes. YT521-B regulates alternative splicing of crucial targets including *Sex lethal*, which encodes a master regulator of sex determination and dosage compensation. We will present our latest data regarding the underlying mechanism of YT521-B in alternative splicing regulation.

Lecture 5.8

The role of SERRATE/U1 snRNP communication in microRNA biogenesis in *Arabidopsis thaliana*

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MicroRNAs (miRNAs) are small non-coding RNAs of about 21 nt in length, which regulate gene expression by cleavage or translation inhibition of target mRNAs. In plants, miRNAs are encoded mostly by independent transcription units or, less frequently, miRNAs are encoded within introns of protein-coding genes. 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. Moreover, we have demonstrated that the stimulatory effect of introns on miRNA biogenesis mostly resides in the 5'ss rather than depends on a genuine splicing event. The goal of this study was to identify the proteins that are involved in the communication between the microprocessor and the spliceosome. In plants miRNA biogenesis takes place in highly specialized nuclear foci, and DCL1 (Dicer Like 1), HYL1 (HYPONASTIC LEAVES 1) and SE (SERRATE) are key factors of the plant miRNA biogenesis machinery. Interestingly, SE is also involved in pre-mRNA splicing. Our results suggest 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*) knock-out *Arabidopsis* plants are embryo-lethal. PRP40 proteins have been shown to interact also with the CTD domain of RNAPII. We have found that SE is localized in RNAPII containing nuclear foci. 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 process. The molecular mechanism of the crosstalk between the spliceosome and the microprocessor in plants will be discussed. This work was supported by the KNOW RNA Research Centre in Poznan (No. 01/KNOW2/2014) and National Science Centre (2015/16/T/NZ1/00026 to A.S.).

Lecture 5.9

Active 5’ splice sites regulate intronic miRNA biogenesis efficiency in *A. thaliana*

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Recently published papers have shown that in *A. thaliana* splicing and active 5’ splice sites (SSs) stimulate the production of several exonic miRNAs derived from intron-containing genes. Moreover, it is already known, that splicing plays an important role in the processing of animal intronic pri-miRNAs. These evidences prompted us to answer the question what is the impact of splicing and 5’SS on plant intronic miRNA production, which is an interesting and yet not fully understood field in plant biology. To answer this question we selected miR402, located within the *At1g77230* first intron. In abiotic stress conditions, we observed that the upregulated miR402 level correlated with miR402-carrying intron splicing inhibition. Additionally, the strong interconnection between miR402 accumulation and activation of the newly-identified alternative proximal polyadenylation site (PAS), located within this intron, was identified. It suggests that miR402 is not processed from an intron, but rather from shorter transcript after selection of the intronic PAS. Thus, our experiments provide compelling evidences revealing a completely new mechanism, when splicing inhibition and selection of alternative PAS lead to strong upregulation of intronic miRNA biogenesis efficiency. Furthermore, we demonstrate that this effect for miR402 mostly resides in the 5’SS and that its inactivation results in mature miRNA accumulation. Interestingly, when the miR402 stem-loop structure was moved to the upstream exon, the effect of 5’SS was reversed: inactive 5’SS actually decreased exonic miR402 levels, which was in agreement with the results concerning exonic miRNA biogenesis. This indicates that the stimulatory or inhibitory effects of the 5’ss on the accumulation of miRNAs clearly depend on the reciprocal spatial relationship between positions of the miRNA stem-loop structure and the nearest active 5’SS. Our data provide a significant contribution to the fundamental knowledge of plant miRNA biogenesis and function. We show new important ways of miRNA production regulation and, consequently, their target mRNA levels.

Poster Session

Poster Session

Poster 1

A role of crosstalk between the NEXT complex and SERRATE in degradation of miRNA precursor fragments

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The *Arabidopsis thaliana* SERRATE protein (SE) is involved in two important pathways of RNA metabolism: miRNA biogenesis and pre-mRNA splicing. Originally, SE was characterized as a protein involved in miRNA biogenesis, where together with DCL1 (Dicer Like 1) and HYL1 (HYPONASTIC LEAVES 1) form a core of the plant microprocessor. In this complex SE influences the accuracy of pri-miRNA cleavages catalyzed by DCL1. The Arabidopsis *se* null mutants are embryonic lethal that proves a key role of SE in plant development and growth. SE together with another factor involved in miRNA biogenesis, the nuclear cap-binding protein complex (CBC), have been also ascribed to splicing of pre-mRNA. We have shown that SE interacts with CBC. In order to understand this dual role of SE and CBC in different pathways of RNA metabolism, we decided to search for novel proteins interacting with SE. To this end, we carried out co-immunoprecipitation of the FLAG:SERRATE fusion protein that were expressed in the *se-1* mutant genetic background. The SE-bound proteins were identified by mass spectrometry, and the putative protein interactions were confirmed by the yeast two hybrid system and pull-down experiments. Our results have clearly demonstrated that SE contacts directly the Nuclear Exosome Targeting complex (NEXT). Moreover, CBC is also part of this molecular assembly. Our study has shown that the NEXT complex is necessary for proper degradation of 5’ pri-miRNA fragments after excision of miRNA by DCL1. We suggest that molecular interactions between CBC, SE and the NEXT complex is important for the quality control of miRNA precursors and degradation by the exosome the 5’ pri-miRNA fragments produced during miRNA biogenesis in the plant cell nucleus. This work was supported by grants: from the National Science Center UMO-2014/13/N/NZ1/00049 and by GDWB-01/2015.

Poster 2

Arabidopsis thaliana microRNA162 level is posttranscriptionally regulated via splicing and polyadenylation site selection

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Arabidopsis microRNA162 expression was studied under abiotic stresses such as drought and salinity. The TaqMan® microRNA assay proved that *A. thaliana* miRNA162 level was elevated under these stresses confirming its salt and drought responsiveness. Consistently, the *MIR162a* and *MIR162b* gene promoter region analyses identified numerous salinity and drought responsive elements. However, our analyses show that generally *MIR162b* is rather weakly expressed, both in control and stress conditions. On the other site the stress-dependent regulation of the pri-miRNA162a alternative splicing pattern revealed the increase of functional pri-miR162a isoform and preferential distal polyA site selection in stress conditions. Apart from the potential transcriptional regulation of the miRNA genes (*MIRs*) expression the data obtained point to an essential role of posttranscriptional regulation of the microRNA162 level.

Poster 3

The role of Methyl Transferase (MTA70) and the implications of N6A modification in miRNA biogenesis in plants

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Among various mRNA modifications, methylation of adenosine at N6 position is the most abundant mRNA modification. It is present near the 3'UTR's and the stop codons within a specific motif –RRACH (R= G/A; H= A/C/U), and is present in almost 3-5 sites per mRNA molecule in plants as well as mammals. Although the modification was discovered decades ago, its function remained unclear. Recent discovery of a mammalian Fat Mass and Obesity associated enzyme (FTO) as a RNA de-methylase indicates that the N6A methylation may be under temporal or spatial control. The N6A modification is also present in microRNA transcripts. Knockdown of mammalian *FTO* gene has been shown to result in altered steady state level of miRNAs. The N6A modification has recently been shown as a mark for further processing of animal pri-miRNA. An enzyme, HNRNPA2B1, was shown to act as a reader that reads the N6A mark and recruits the micro-processor machinery. The knockdown of the *METTL3*gene that is responsible for the N6A methylase enzyme has been shown to be lethal in both animals and plants. In this study we aim to identify the role of N6A methylation in plants miRNA biogenesis. MTA70, is the ortholog of METTL3 in plants. *Arabidopsis* plants with reduced levels of MTA70 were used to compare the levels of 16 pri-miRNAs and 10 mature miRNAs with wild type plants. Using real time PCR technique we determined that for most examples of selected miRNAs, pri-miRNA were down regulated and mature miRNA were up regulated. MTA70 was also cloned in the activation and binding domain vectors for Yeast Two Hybrid system and potential protein interaction partners were tested. These experiments showed positive interactions of MTA70 with proteins involved in very early stages of miRNA biogenesis, namely Cycling DOF factor2 (CDF2), Negative on TATA less 2b (NOT2b) and its isoform, and TOUGH1 (TGH1). These findings indicate that MTA70 may act at very early stages of transcription and may be acting with a different mechanism as compared to animals. Further experiments including experiments like CHIP, RIP, Co-IP and FLIM-FRET will be done to understand the mechanism and influence of MTA70 and N6A modification in plant miRNA biogenesis. This work was supported by KNOW RNA Research Centre in Poznan, 01/KNOW2/2014 and Dzialanosc Statutowa of the Faculty of Biology, AMU, Poznan.

Poster 4

The role of PC4 factor in the expression of replication-dependent histone genes in HeLa cells

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The canonical histone genes encode four core histones - H2A, H2B, H3 and H4 which form the nucleosome and the linker histone H1. Their expression is cell cycle regulated and highly activated during DNA synthesis at the S phase. The replication-dependent histone (RDH) mRNAs do not contain introns and are not polyadenylated. Instead, their transcripts end in a conserved secondary stem loop structure and 3' end processing consist of a single cleavage mediated by endonuclease CPSF73. U7 small nuclear ribonucleoprotein (U7 snRNP) is an essential factor required for proper 3' end processing of RDH transcripts. It binds by base pairing to the sequence located downstream of the cleavage site and recruits other factors needed for histone transcripts maturation. Recently, using affinity chromatography based on tagged U7 snRNA we identified PC4 as a new factor interacting with U7 snRNP that might be involved in RDH gene expression. This protein is a well-known transcriptional co-activator and co-repressor and acts as chromatin remodelling protein interacting with histone H2B and H3. Moreover, its interaction with 3' end processing factor CstF64 is well documented. To investigate PC4 influence on histone gene expression we prepared HeLa cell lines with stable overexpression of FLAG-tagged PC4 protein. The cells were synchronized to S phase when RDH gene expression is highly activated and to G1 phase when their expression is at basal level. Then CHIP-seq assay (Chromatin and Immunoprecipitation followed by high throughput sequencing) using antibodies against RNA polymerase II (RNAP2) subunit RPB2 was performed. Interestingly, we observed decreased occupancy of RNAP2 at the promoters of 26 RDH genes at S phase in cells overexpressing PC4, which suggest that this protein may be involved in regulation of RHD genes transcription during the cell cycle. The negative effect of PC4 overexpression on RDH genes transcription in the S phase will be discussed.

Poster 5

Genome-wide search for regulators of cold-specific alternative splicing of Arabidopsis clock genes

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We have shown that alternative splicing (AS) is important in regulating expression of key circadian clock genes in response to changes in temperature (James et al., 2012). The circadian clock is able to organize many physiological processes in anticipation/preparation to daily and seasonal changes. AS may therefore have roles in temperature perception, entrainment, compensation, regulation of downstream physiological responses and acclimation to exposure to low temperature. To identify potential regulators of AS of Arabidopsis clock genes, we performed a genome-wide study using ultra-deep RNA-seq of the same high resolution time-course conditions as previously (James et al., 2012). We took a new approach to RNA-seq analysis by using Salmon (Patro et al., 2015). With this approach we are able to accurately quantify unique transcript isoforms and deliver transcript-specific expression. To use Salmon, we developed the most comprehensive Reference Transcript Datasets (RTDs) currently available (Zhang et al., 2015, 2016). Analyses so far have identified genes which are regulated only by transcription, only by AS and by both. In particular, we have identified splicing factor-related genes with major changes in AS in response to cold, including isoform switches that occur rapidly after transfer to cold. To investigate the role of these splicing-related factors, knock-out mutants were obtained and we have observed their involvement in the regulation of low temperature AS of several genes, including core clock genes *LHY* and *PRR7*. These knock-out mutants are currently being assessed for their cold-related phenotype. The RNA-seq data is currently being used to build transcription and splicing networks to determine novel candidate genes that may regulate AS of core clock genes at low temperature.

Poster 6

Regulation of spo11 gene alternative splicing during male meiosis

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Meiosis is a specialized cell division that generates haploid cells from diploid progenitors. A prominent feature of meiosis I is homologous recombination, comprising the formation and repair of programmed DNA double-strand breaks (DSBs) formed by the SPO11 endonuclease. The *Spo11* gene encodes for at least two splice variants, which differ for the inclusion (SPO11β) or exclusion (SPO11α) of exon 2 and are differentially expressed during the prophase I of spermatocytes. SPO11β peaks at leptonema, when most DSB are made, whereas SPO11α accumulates in late prophase, after the repair of early DSBs and achievement of chromosome pairing. It was proposed that SPO11α expression is required to guarantee the late phase of DSBs that occur in sex chromosomes in late prophase. However, in spite of the potential importance of this event for gametogenesis and fertility, the molecular mechanisms of *Spo11* alternative splicing regulation are still completely unknown. In vivo analysis of *Spo11* expression in developing testis and male isolated germ cell confirmed that SPO11β is prevalently expressed in mitotic spermatogonia and early meiotic cells, whereas SPO11α is expressed in mid- to late pachytene spermatocytes and in haploid spermatids. To investigate the splicing factors involved in the switch in *Spo11* splicing, we constructed a minigene comprising the whole alternatively spliced genomic region of *Spo11.* In vivo splicing assay with recombinant splicing factors and antisense oligonucleotides allowed us to identify trans- and cis-acting factors that modulate *Spo11* alternative splicing. In addition, we found that reduction of the transcription elongation rate promotes skipping of exon 2, indicating that regulation of RNAPII processivity might influence *Spo11* splicing during male meiosis. In line with this hypothesis, we also observed a strong reduction of the RNAPII elongation rate concomitantly with the switch toward the α variant. Notably, the reduced RNAPII processivity specifically occurred within the *Spo11* transcription unit and not in other meiotic genes. Our results provide the first hints on the regulatory sequences and factors involved in *Spo11* splicing during male meiosis and suggest a mechanism of regulation for the splicing of a gene that is essential for meiotic recombination and fertility.

Poster 7

PURA syndrome: an investigation into potential splicing abnormalities

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Mutations in the *PURA* gene have recently been found to be a cause of neurodevelopmental delay in children. Affected individuals typically suffer from hypotonia, feeding difficulties and breathing abnormalities in infancy. Most children have severe developmental delay and intellectual disability. Almost all remain non-verbal, and many develop seizures in early childhood. The pathogenetic mechanism of PURA syndrome has yet to be determined. However, the product of *PURA*, Pur-alpha, is a DNA- and RNA-binding protein with multiple cellular functions. Very recently, Pur-alpha has been shown to regulate the SRSF1 (SF2/ASF) splicing factor, suggesting a possible role for abnormal splicing in the pathogenesis of PURA syndrome. We are therefore investigating this hypothesis through RNA-seq analysis of blood samples from affected patients. We will report on our preliminary results from this ongoing study and present our plans for future work in this area.

Poster 8

The use of a mutated version of the splicing regulator SRSF1 (ASF/SF2) as a new strategy to cure Spinal Muscular Atrophy (SMA)

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SMA is caused by a decrease in expression of SMN proteins in motor neurons due to deletions or mutations within SMN1 gene. Although all SMA patients still have at least one nearly identical copy of SMN1 (the SMN2 gene), it fails to prevent SMA mostly due to a single C-to-U change at position +6 of SMN2 exon7. This nucleotide change modifies the set of splicing regulators recruited on the exon leading to its exclusion from SMN2 transcripts, which are then translated into nonfunctional SMN proteins. The splicing regulator SRSF1 was previously shown to be primarily responsible for the activation of SMN1 exon7 inclusion [1]. This protein contains two RNA recognition motifs (RRM1 and RRM2). By solving the structure of SRSF1 RRMs bound to RNA using NMR, we found a highly specific interaction of RRM1 with a cytosine. This result explains the importance of the cytosine located at the position +6 of SMN1 exon7 for the recruitment of SRSF1 and the absence of interaction of the protein with SMN2 exon7, which contains a uridine instead of the cytosine [1]. Guided by our structural data, we then tried to modify the RNA-binding specificity of SRSF1 RRM1 to force its recognition of the uridine-containing SMN2 exon7 ESE. By mutating to an asparagine a single glutamate side chain involved in the recognition of the cytosine, we allowed the specific interaction of SRSF1 with uridines in addition to cytosines. Remarkably, this protein variant could then bind to SMN2 exon7 ESE and stimulate inclusion of this exon to almost 90% in human cells, which is of therapeutic interest to cure SMA.

[1] Cartegni, L. et al. *Nat. Genet.*, 2002

Poster 9

The role of core spliceosomal components in tissue heterogeneity

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Generation of mature mRNAs in metazoa typically involves the removal of pre-mRNA segments referred to as introns through the process of Splicing. Definition of intronic boundaries and catalysis of their removal relies on a highly ordered, multistep cycle of recruitment, rearrangement and release of over 150components that comprise one of the most complex molecular machineries known as the Spliceosome. Additional auxiliary factors can enhance or repress the interaction between the spliceosome and the pre-mRNA resulting in Alternative Splicing (AS), the phenomenon of shifting of intronic boundaries indifferent contexts. Alternative Splicing has a key role in establishing the differential expression profiles underlying cell differentiation and its misregulation is known to be involved in a wide array of human pathologies. Previous work in our lab identified a network of functional interactions among spliceosome components, auxiliary splicing factors and few chromatin modifiers based on their effects on the regulation of a small number of alternative splicing events (Papasaikas, Tejedor et al, Mol. Cell 2015). In addition this work highlighted the spliceosome components as potential AS regulators .Different tissues exhibit highly distinct patterns and varying prevalence of AS. Therefore, the conservation of these functional interactions and the contribution of spliceosome components in regulating AS in particular tissues are fundamental questions for understanding alternative splicing regulation. I am working with the large collection of data from the Genotype-Tissue Expression project (GTEx) in order to shed light on these questions. GTEx provides transcriptome quantifications for multiple samples across over 40 tissues, thus supplying a high-resolution view of AS across different cell types. The comprehensive nature of these data can also allow me to investigate functional AS interactions for different physiological states or pathological states and establish detailed connections between the presence of specific functional interaction and sequence characteristics of subsets of targets. Our work aim to shed light on the organization and versatility of the splicing regulatory circuitry across tissues by analysing a large array of high throughput data and has the potential to greatly expand our knowledge of the mechanisms that underlie AS regulation in cell differentiation and disease.

Poster 10

Identification of transcripts producing differential splice variants in spikelets of apomictic and sexual *Paspalum notatum*

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The function of the trimethylguanosine synthase PIMT/TGS1 was characterized in yeast, drosophila and mammals. It promotes the sn(o)RNA biogenesis by catalyzing the post-transcriptional conversion of 7-methylguanosine caps (m^7G) into 2,2,7-trimethylguanosine (m^3G), a process essential to the splicing of target RNA candidates. Moreover, the gene acts as a coactivator of PPAR-regulated gene expression. In eukaryotic non-plant model systems, the abolishment of *PIMT/TGS1* causes cold-sensitive splicing defects, pre-rRNA processing deficiency, loss of nucleolar structural organization and aberrant splicing of key regulators. These alterations are associated with a wide range of phenotypic variations, including growth delay at low temperatures and meiotic failure. Apomixis, an asexual reproductive mode described in more than 400 species of angiosperms, arises from alterations in the sexual developmental pathway. Recently, we compared the floral transcriptomes of sexual and apomictic *Paspalum notatum* genotypes and found a methyltransferase-coding candidate differentially expressed between the two plant types. It encoded a TGS1-like protein with an enlarged N-terminal domain including a WW protein binding site, which we called *PNTGS1*-like. *PNTGS1*-like is active in the nucellus of sexual *P. notatum* plants from early developmental stages until maturity, whereas expression is strongly reduced throughout reproductive development in apomictic genotypes. We hypothesized that apomicts plants show RNA splicing variations in flowers associated with *PNTGS1*-like down-regulation. Our objective was to identify splice variants between the two plant types. We carried out 454 sequencing of the sexual and apomictic floral transcriptomes, identified 3251 differentially expressed candidates. The 120 candidates showing the lowest FDR values were used to search all alleles/paralogues sequences present in the database, which were aligned with Clustal Omega. 17 candidate genes showing differential splicing between the apomictic and sexual genotypes were identified. In further work, the splicing of the identified candidates will be examined in *tgs1* defective as well as apomictic and sexual wild types.

Poster 11

Antisense oligomers as a tool for identification of functional RNA targets for MBNL proteins

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Application of antisense oligonucleotides (AONs) as an efficient tool for experimental verification of RNA/protein interactions in *in vitro* and cellular models. The RNA immunoprecipitation (i. e. cross-linking immunoprecipitation) combined with deep sequencing of enriched RNAs enables identification of multiple RNA sequences potentially recognized by RNA-binding proteins e. g. Muscleblind-like protein 1 (MBNL1) which is a known regulator of alternative splicing. In this study we described an efficient method to verify the CLIP-seq results in both *in vitro* and *in vivo* assays via inhibition of RNA/protein interaction by chemically modified short antisense oligonucleotides (AONs). *In vitro* studies based on RNA structure prediction and assessment of affinity competition to the target sequence between recombinant MBNL1 and designed AONs enable selection of MBNL1-binding sequences. These preliminary results are subsequently supported by cell transfection with particular AON against potential MBNL1-binding site and analysis of change in pre-mRNA alternative splicing of given transcript. *In vitro* studies showed that all tested AONs complementary to functional MBNL1-binding motifs within a fragment of *Atp2a1* pre-mRNA blocked interaction with MBNL1. However, only two of four tested chemical modifications of AONs complementary to these intronic sequences significantly enhanced the exclusion of alternative exon 22 of *Atp2a1* from both minigene and endogene. Then, based on CLIP-seq results we selected two potential MBNL1-binding regions from intron 6 of *Pphln1* and *NASP* as well as three exonic sequences from alternative exons of *Nfix* (ex7), *Ldb3* (ex10) and *Mbnl1* (ex1) to test the activity of designed AONs. *In vitro* application of several AONs blocking different YGCY motifs, which is minimal binding sequence for MBNL1, allows us to identify one or two MBNL1-binding sites in ~200-nucleotides long selected pre-mRNA fragments. Unexpectedly, in cell models the phosphorothioated 2'-*O*-Methyl version of selected AONs blocked only functional MBNL1-binding motif for endogenous intronic sequences of *Pphln1* and *NASP*, but not for exonic sequences of *Ldb3*, *Nfix* and *Mbnl1* for which opposite direction of splicing changes were observed. However, substitution of MBNL1-sensitive elements in intron 22 of *Atp2a1* minigene with all studied sequences coming from five tested genes reconstituted alternative splicing of exon 22 of *Atp2a1*. In these chimeric constructs exon 22 inclusion responds significantly on both MBNL1 overexpression and the presence of gene-specific AONs complementary to MBNL1-binding sites. Here we present an efficient molecular tool which allows to identify and confirm RNA sequences essential for interaction with RNA-binding protein. However, depending on location of such binding sites, there is a probability of disruption of other protein-binding sites located closely or co-localizing with analyzed sequences. Such obstacle might be omitted by application of MBNL1-dependent chimeric minigene with genuine MBNL1-binding cassette replaced with other potential MBNL1-binding sequence of interest. This work was supported by Foundation for Polish Science TEAM program (to K.S.), with additional support from the Polish National Science Centre: 2011/01/B/NZ1/01603 and 2014/15/B/NZ2/02453 (to K.S.) and Ministry of Science and Higher Education of the Republic of Poland, from the quality promoting subsidy, under the Leading National Research Centre (KNOW) programme for the years 2014–2019.

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Poster 12

The influence of alternative splicing on the circadian clock transcripts of field-grown sugarcane

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Alternative splicing (AS) is a mechanism that generates multiple mature transcripts from a single gene. In plants, AS is widespread with around 60% of intron-containing genes in Arabidopsis, 30% in rice and 38% in maize undergoing AS. It also influences the circadian clock, a 24 h endogenous timekeeper mechanism that anticipates the Earth's day/night and seasonal cycles. The circadian clock integrates environmental cues with internal signals in order to co-ordinate metabolic and physiologic outputs. Like AS, the circadian clock is associated with growth, photosynthesis and biomass in plants, so these two regulation mechanisms may act together, or even regulate each other. The circadian clock is highly conserved among different plant species, such as Arabidopsis, Populus, Brachypodium and rice, all of them C3 plants. Little is known about how AS influences the circadian clock of C4 plants, like maize or sugarcane, an economically important C4 crop with a complex genome, being a polyploid hybrid of different species. This work aims to identify AS in sugarcane core circadian clock genes and to investigate if AS has a role in modulating the expression of core clock genes in sugarcane and the function of the sugarcane circadian clock. Samples of leaves (the +1 leaf) of a commercial variety of sugarcane (SP80-3280) grown in a field for 12 months were harvested in winter (August/2012), when the plants were 4 months old, and in summer (January/2012), when the plants were 9 months old. Sampling started 1h before dawn and leaves were sampled every 2 hs, for 26 hs. Total RNA was extracted, cDNA was synthetized and RT-PCRs reactions using overlapping primers on each core clock gene were carried out in order to identify putative AS events. To improve the analysis, existing genomic annotations for the sugarcane clock genes were manually curated. Six out of nine analyzed clock genes showed at least one AS event, depending on the season and plant developmental stage. The most frequent AS event found was intron retention, observed in *Sc*LHY, *Sc*PRR37, *Sc*PRR73, *Sc*PRR95, *Sc*TOC1 and *Sc*ELF3. There was also alternative splice sites on 3' end of exon 4 and 5' end of exon 5 of *Sc*PRR73 and exon skipping in *Sc*TOC1, which might not affect their open reading frames. High-Resolution Real-Time PCR (HR RT-PCR) experiments will measure the AS transcripts in the sugarcane clock genes through the different time-course samples to examine examine how AS is altered during the diel cycle, the season and the developmental stage.

Poster 13

Systematic CRISPR/Cas9-mediated knock out of Lola isoforms reveals novel functions in the octopaminergic pathway

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Functional studies of complex loci have traditionally been hampered by the difficulty to manipulate their sequence in vivo. longitudinals lacking (lola) is among the most complex loci in Drosophila melanogaster that encodes at least 20 protein isoforms, each sharing a constitutive N-terminal BTB-domain, fused to C-terminal variable zing-finger-containing exons. Lola was shown to act as a transcription factor having pleitropic roles in vivo, including axon pathfinding and neural reprograming. Most of theses studies employed loss of function alleles that disrupt the common BTB domain, making it difficult to assign and understand the role of different isoforms in distinct processes. To better characterize Lola function in vivo we have used the CRISPR/Cas9 system to generate specific mutations in every isoform. Our targeted screen allows us to revisit the previously demonstrated roles for few described isoforms. In addition, it reveals additional functions, including a critical role for a yet undescribed isoform in the octopaminergic pathway. Together, our study reveal new functions for Lola and demonstrates that CRISPR/Cas9 approach is a valuable tool to systematically address the role of specific isoforms in vivo.

Poster 14

A new role for AGO1 in plants: The co-transcriptional regulation of miRNA biogenesis in *A. thaliana*

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MicroRNAs are short RNA molecules that control gene expression at the post-transcriptional level by cleavage of mRNA targets or by inhibition of their translation. The AGO1 protein is described as a key component of the plant RNA-induced Silencing Complex (RISC) that is required for the miRNA-mediated gene silencing. We found that in *Arabidopsis thaliana*, in addition to the well characterized cytoplasmic role of AGO1 as a negative regulator of gene expression, the AGO1 is also involved in the co-transcriptional regulation of miRNA genes expression in the cell nucleus. In this study we have described several *Arabidopsis* miRNAs (e.g. miRNA161 and miRNA173) that show in salt stress a negative correlation between the levels of their primary precursors (pri-miRNAs) and mature miRNAs. Such changes in miRNA expression profiles suggested intensive post-transcriptional regulation of miRNA biogenesis. Indeed, we found that in *A. thaliana* in salt stress the pri-miRNA161 and pri-miRNA173 levels decreased, while the levels of mature miRNA161 and miRNA173 were higher than in unstressed plants. We proved that the lower levels of pri-miRN161 and pri-miRNA173 were not due to stress-induced changes of transcription initiation, since we showed that the promoters of *MIR161* and *MIR173* were not salt stress responsive. We were able to localize AGO1 in *Arabidopsis* cells not only in the cytoplasm but also in the nucleus, using both immunolocalization and western blot detection of AGO1 in the nuclear fraction. Using ChIP approach, we found that AGO1 in the nucleus accumulates at *MIR*161 and *MIR*173 genes in RNA-dependent meaner, and that this accumulation increased under stress conditions. The accumulation of AGO1 on *MIR161* and *MIR173* in salinity stress correlated with the lower levels of RNAPII at both the *MIR* loci studied, and leaded to the production of premature terminated, unpolyadenylated *MIR161* and *MIR173* transcripts. Our results suggest that in *A. thaliana* the AGO1, most likely loaded with miRNA161 and miRNA173, co-transcriptionally regulates *MIR161* and *MIR173* expression by disassembly of the RNA Pol II transcription complex. Under the same salt stress conditions we showed that mature miRNA161 and miRNA173 were stabilized in salinity stress, and that AGO1 was responsible for this stabilization.

Poster 15

Splicing factors and circular RNAs: an intricate relationship

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Circular RNAs (circRNAs) are a novel class of highly stable RNAs demarcated by a closed-loop structure. CircRNAs mainly derive from internal exons of protein-coding and non-coding genes and their biogenesis is catalysed by the spliceosome and can be regulated by both cis- and trans-acting factors including splicing factors. Conversely, a splicing factor may also be regulated by circRNAs, as demonstrated for MBL in Drosophila. However, only a limited number of splicing factors have been investigated to date with regard to circRNA biogenesis and regulation by circRNAs. To expand our knowledge of the interplay between splicing factors and circRNAs, the splicing factor SRSF4 was chosen for further analysis seeing as the fourth exon of SRSF4 gives rise to a small circRNA, circSRSF4, and since SRSF4 can bind to its own transcript including exon four. Hence, this could indicate that SRSF4 is involved in regulation of circSRSF4 formation or that SRSF4 is subject to regulation through interaction with circSRSF4. To investigate this, a mini-gene setup was utilized which allowed overexpression of either circSRSF4 or SRSF4. This disclosed an intricate relationship between the splicing factor, and the circRNA: SRSF4 and circSRSF4 seemed to promote expression of each other as seen on Western and northern blots respectively and a modest interaction between SRSF4 and circSRSF4 was observed upon immunoprecipitation, indicating that circSRSF4 might function in an autoregulatory loop.

Poster 16

Alternative splicing in barley (*Hordeum vulgare*) organs

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The alternative transcript population of 16 different barley organs was assembled using Cufflinks and Trinity. Transcript abundances were calculated using Cufflinks and Salmon algorithms and alternatively spliced transcript abundances validated by high resolution RT-PCR (HR RT-PCR). Splicing accuracy correlated well between assembled transcripts and HR RT-PCR, but, due to the absence or mis-assembly of multiple (>2) gene transcripts, abundances of the different alternative transcripts correlated poorly with HR RT-PCR, while genes that showed two alternative transcripts correlated strongly. The 2810 genes that produced simple two alternatively spliced transcripts were used to estimate transcript abundance variation between barley organs. Nearly half of these simple two-transcript alternative splicing events showed no significant change across the 16 barley organs. The remaining genes clustered into 10 different tissue specific patterns of alternative splicing across the different barley tissues. 16 barley SR-protein gene orthologues were further identified and their expression and alternative splicing was also found to change across the 16 barley organs. The patterns of alternative splicing and SR-protein expression support a role for alternative splicing in barley development.

Poster 17

Novel approaches to rescue exon 2 skipping due to the common splicing mutation c.-32-13T>G of the GAA gene

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Glycogen storage disease type II (GSDII) is one of the most prevalent lysosomal storage disorder due to mutations of the *GAA* gene, which causes functional deficiency of the lysosomal alfa-glucosidase enzyme. Clinically, GSDII encompasses a continuous spectrum of phenotypes ranging from infantile (IO) to late-onset (LO) forms due to glycogen accumulation. The only treatment approved for GSDII is the enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA) but limitations of this approach are becoming evident in LO patients. Thus, innovative and more effective therapies are needed. Most LO patients shared the leaky splicing mutation c.-32-13T>G in intron1 of GAA gene. In a previous study we have demonstrated that this mutation abrogates the binding of the splicing factor U2AF65 to the polypyrimidine tract of exon 2 of *GAA*, leading to the partial or total exclusion of exon 2. Furthermore, we have shown normal splicing can be rescued by over expressing the splicing factor SFSR4 or by treating the cells with small molecules (Dardis et al, 2014). In this work we have identified a new possible therapeutic agent for the treatment of the late onset (LO) form of GSDII able to restore a correct splicing of GAA exon 2 carrying the mutation c.-32-13T>G and moreover to rescue protein expression and enzymatic activity. Three different antisense oligonucleotides (ASOs) have been designed against an exonic silencer element, within the GAA exon 2, in order to block its function and rescue the GAA exon2 carrying c.-32-13T>G splicing mutation. The partial recovery of GAA exon2 inclusion has been revealed at mRNA level both in Hela transfected with an hybrid minigene and in patients fibroblast carrying the mutation. In addition, in patients fibroblasts, a higher production of the protein together with an increase of the enzymatic activity has also been detected compared to not treated cells. Our results suggest that the ASOs approach tested in this study constitute a promising therapeutic strategy to rescue normal splicing, and normal function, of the transcripts carrying the c.-32-13T>G mutation.

Poster 18

Splicing factor during response to abiotic and biotic stresses in *Arabidopsis thaliana*

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The appropriate cellular signalling in each organism depends on the optimal control of gene expression, not only under normal conditions but also under stress. It appears that in plants defects in RNA processing and degradation cause developmental problems, attenuated hormonal signalling and modified resistance to abiotic and biotic stress. In particular, response to various stresses is linked with changes in alternative splicing of stress-related factors. To study these connections we have tested changes in the response to abiotic stress caused by ABA and biotic stress induced by pathogen infection in the *smd3-b Arabidopsis thaliana* mutant. SmD3 is a core protein of small nuclear ribonucleoproteins (snRNPs) essential for pre-mRNA splicing and *smd3-b* knock-out in *Arabidopsis* results in pleotropic phenotypes, including delayed flowering, reduced root growth, partially defective leaf venation and changed numbers of floral organs. These phenotypes correlated with altered splicing patterns of several pre-mRNAs (Swaraz et al., 2011). Our stress analyses show that *smd3-b* plants are sensitive to ABA during germination and, to a lesser extent, root development. To assess the impact of splicing defects on plant immunity we have also tested sensitivity to *Pseudomonas syringae* pv. *tomato* DC3000 as well as its effector flagellin (flg22) and analyzed the response on the level of pathogen markers. The *smd3-b* mutant displayed increased susceptibility to bacteria, together with marked changes in the expression of key pathogenesis markers (e.g. *PR1, PR5, GSTF6*). In addition, induction of some transcripts following infection was either increased or had altered kinetics in the mutant. Similarly to the response to bacteria, mRNA levels of major biotic factors, including *PR1, FRK1, PAL1, BAK1, WRKY29,* and *JAZ1,* were also changed in *smd3-b* upon treatment with flagellin. These results suggest that splicing factor may contribute to plant immune response via regulation of mRNA splicing and alternative forms of the key pathogenesis factors.

Poster 19

The alternative splicing generating tRNA fragments involved in RNA interference in plant pathogens fungi

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The citrus crop is permanently affected by several pathogens, special fungi, causing severe and limiting diseases. New technologies and their application can contribute to better understand the pathogens biology and control. Amongst them, the RNA interference (RNAi) is a prominent strategy to decrease gene expression. Therewith, we investigate the presence and functionality of RNAi machinery in the Post--Bloom Fruit Drop (PFD) causal agent, *Colletotrichum abscissum* (former *acututum*), and *Phyllosticta citricarpa* the Citrus Black spot agent. The tRNA--derived RNA fragments (tRFs) are generated from the 5' or 3' flanks splicing of tRNA genes. The tRFs are involved in growing and development process. In A. fumigatus the tRFs induce the negative regulation of protein synthesis and in M. oryzae they abundant in spores and apressorium. We used in silico test to identify the tRNA sequences in both genomes and the putative tRFs sequences. The spliced regions may be incorporated by the RISC complex and operate silencing the fungi genes. The initial identification steps were done allowing the investigation of tRFs function in citrus plant pathogens fungi.

Poster 20

Differences between exonic and intronic cryptic splice sites utilization help distinguish cryptic from pseudo splice sites

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Splicing affecting mutations are a frequent cause of human disorders. Yet the recognition of such mutations and assessment of their pathogenic potential is often a challenging task. The most prevalent effect of splicing affecting mutation is exon skipping, followed by cryptic splice site activation. So far, in silico prediction tools are very helpful in recognition of splicing affecting variants located at splice sites. However, particular effects of splice site disruption are much harder to forecast. E.g. there is not an efficient algorithm to discern cryptic splice sites from abundant pseudo splice sites (i.e. those that resemble real splice sites but are never used). Seeking for some rules that might help with predictions of real cryptic splice sites, we tested our set of 14 splice site mutations found in genes connected with the development of primary immunodeficiencies (BTK, CD40LG, IL2RG, SERPING1, STAT3, and WAS). Using minigene assays, we found that four of these mutations induced purely exon skipping, while other 10 variants activated in total 16 different cryptic splice sites (7 exonic and 9 intronic ones). Globally, exonic cryptic splice sites were much weaker than the intronic ones, as predicted by NNSplice and MaxEnt tools. More particularly, exonic cryptic sites tended to be the closest to their authentic counterparts, while the intronic sites were usually the strongest ones in the vicinity of authentic site. The only exception was a case of large deletion of donor splice site region which led to utilization of multiple extremely weak splice sites, further supporting the importance of exonic/intronic sequence identity. These results may contribute to the assessment of cryptic splice sites utilization after disruption of authentic splice sites. This project was supported by the internal grant of Centre for Cardiovascular Surgery and Transplantation No. 201401 and by Ministry of Health of the Czech Republic, grant nr. 16-3441A.

Poster 21

Pipeline for identification of alternative isoforms in the eukaryotic genome

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The annotation projects of large eukaryotic genomes often provide a broad but shallow view of the structure and function of genes as their fundamental perspective is to offer an overview of entire genome rather than defining individual genes. One of the major challenges remains in any annotation project is to correctly identify the splicing variants. In this scenario, mapping of transcriptome data to genome followed by gene prediction with highly optimized gene prediction parameters is valuable. Here, we present the pipeline for the prediction of genes encoding for alternative transcripts exemplified for carpenter ant *Camponotus floridanus* genome. The pipeline presented here uncovered 1928 genes affected by alternative splicing events coding for 4666 alternative transcripts in *C. floridanus*. Additionally, the changes caused by alternative isoforms in protein-protein interaction network-behaviour dynamics is discussed.

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Poster 22

Splice-switching oligonucleotides as therapeutic approach for Erythropoeitic Protoporphyria

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Erythropoeitic Protoporphyria (EPP) is a severe genetic disorder with a worldwide prevalence estimated between 1/75,000 - 1/200,000 [1]. Patients suffer from photosentivity and skin irritation when exposed to blue light, which is present in common natural and artificial sources. Symptomatic treatments are currently the only therapeutic option [2]. EPP onset requires genetic variations on both alleles of the ferrochelatase (*FECH*) gene [3]. One allele harbours a non-sense or missense mutation shutting down FECH production. Concurrently, an intronic single nucleotide polymorphism (SNP) on the other allele causes aberrant splicing of the pre-mRNA and consequent decay through NMD. This combination causes pathogenic depletion of FECH and accumulation of its photoreactive substrate protoporphyrin IX (PPIX) in cells from the erythroid lineage in the bone marrow, and also in the liver. Our strategy to restore functional levels of FECH is to produce splice-switching oligonucleotides (SSOs) able to block the recognition of the cryptic splice site and force canonical splicing to occur. These compounds require a special design and we use two clinically validated features: the MOE chemistry (all riboses 2'-*O*-(2-methoxyethyl) substituted) and the phosphorothioate backbone. This pattern in mind, we produced >40 SSOs of various lengths, performing a microwalk on FECH pre-mRNA, and screened their *in vitro* ability to correct splicing of a minigene system developed by the Schümperli group (Bern, Switzerland). A small number of SSOs was selected for their efficient splice correction compared to a previously published LNA oligonucleotide [4]. To counteract *in vivo* PPIX accumulation, the SSOs should be vectorized to hematopoiesis-linked compartments: mainly bone marrow and to less extent the liver. Although the latter is usually not a delivery challenge, uptake by erythroid progenitors in the marrow is anticipated to be drastically poor. We are therefore building up a library of peptide-, lipid-, and small molecule-SSO conjugates that will be screened in patient cell lines (from Triemli Hospital, Zurich) for identification of an uptake-driving moiety. We hope then such a finely-tailored SSO-conjugate to be tested in a murine model being developed within the Schümperli group.

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Poster 23

Investigation of the physiological roles of SRSF1-mediated translation

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The serine/arginine-rich (SR-) family proteins constitute a functionally complex and diverse group of pre-mRNA splicing factors that are essential for viability. They can be characterised based on the presence of one or two RRMs and an RS domain. A subset, of which SRSF1 is the prototype, are capable of nuclear-cytoplasmic shuttling; a process governed by continual cyclic phosphorylation of the RS domain. In contrast, SRSF2 is unable to shuttle due to the presence of a nuclear retention sequence (NRS) at the very C-terminus of its RS domain (1). When fused to SRSF1, this NRS prevents nuclear-cytoplasmic shuttling. Our lab has previously identified a role for SRSF1 in mRNA translation, in which it acts to enhance translation of specific mRNA transcripts, particularly those encoding RNA processing factors and cell-cycle proteins (2). Here, we aim to study SRSF1 translational and cytoplasmic functions in vivo. We have used CRISPR/Cas9 editing to knock-in the NRS naturally present in SRSF2 at the SRSF1 genomic locus, creating a SRSF1-NRS fusion protein. Using this approach in mouse ESCs, we have been unable to successfully generate viable homozygous clones, despite being able to easily tag the genomic SRSF1 locus. To investigate whether the nuclear-cytoplasmic shuttling of SRSF1 is essential for viability, we are extending our approach to differentiated cells, which have a distinct cell cycle profile from ES cells. In addition, we are using proteomics to identify both functional binding partners of endogenous cytoplasmic SRSF1 and those that bind the NRS of SRSF2 to understand a mechanism for nuclear retention. In summary, we will present our ongoing work to determine the physiological significance and mechanisms of cytoplasmic SRSF1 function, including those which may dictate cellular viability.

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Poster 24

Function and Regulation of the Cassette Exon 7b of the Oncogenic Transcription Factor *ERG*

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The transcription factor ERG is increasingly considered a key oncogene in prostate cancer, leukaemia and other malignancies (Adamo and Ladomery, 2015). In prostate cancer, expression of *ERG* is generally associated with aggressive disease, and in T-cell acute lymphoblastic leukaemia *ERG* is associated with poor prognosis. *ERG* is alternatively spliced. Of particular interest is the cassette exon 7b, whose inclusion is associated with more advanced disease in prostate cancer (Hagen *et al.*, 2014). Recently we have shown that splice switching oligonucleotides (SSOs) targeted to the 5' and 3' splice site of exon 7b cause skipping of exon 7b in a range of cancer cell lines. SSOs are being used to observe the effect of SSO-mediated exon 7b skipping on the biological function of *ERG* and the expression of ERG target genes.

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Poster 25

Impact of alternative splicing changes of *CELF1* on its activity

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Myotonic dystrophy type 1 and type 2 (DM1 and DM2) are the most common muscle dystrophies in adults. They are caused by expanded C(C)UG repeat RNA, which change activity of at least two alternative splicing factors. It was shown that the gain of function of CELF1 protein and functional inaccessibility of MBNL proteins result in global alternative splicing disruptions in DM. MBNL proteins are sequestrated on toxic RNA with expanded C(C)UG repeats in both DM1 and DM2. Whereas increased activity of CELF1 which is caused by hyperphosphorylation, stabilizing CELF1 protein in nucleus was reported in DM1 only. Our experiments showed, however, that activity of CELF1 is significantly misregulated also in DM2. We also determined that sequences of both 5' and 3' untranslated regions (UTRs) of *CELF1* mRNA undergo an abnormal alternative splicing in DM1 and DM2 skeletal muscles. These regions are also efficiently regulated during differentiation of skeletal muscles, brain and heart by MBNL proteins. Several alternative *CELF1* 5'-UTRs which vary in length and sequence were cloned into luciferase expression vectors to test their impact on translation. We did not, however, observed significant differences in translation efficacy of tested 5'-UTRs. Alternative splicing of different *CELF1* 5'-UTRs may also impact the sequence of encoded CELF1 protein by adding 27 amino acids to the N-terminus of the muscle-specific protein isoform. Shorter CELF1 protein isoform which is significantly increased in DM muscles showed higher splicing activity. Our results suggest an existence of additional posttranscriptional mechanism, which can influence CELF1 activity in developmental and disease-specific processes by alternative splicing regulation.

Poster 26

De novo transcriptome sequencing of *Saussurea lappa* to identify and characterize genes involved in costunolide biosynthesis

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Saussurea lappa (family Asteraceae) is an important medicinal plant possessing various pharmacological properties, for instance, it promotes qi-circulation, relieves pain and treats cardiovascular diseases. It is also known to have anti-inflammatory, anti-cancerous, anti-ulcer and anti-microbial properties. Majority of the medicinal properties of the plant can be attributed to Costunolide (a sesquiterpene lactone), an active compound of this plant. Despite of diverse pharmacological applications of the plant no significant attempt has been made till date to characterize the sesquiterpenoid biosynthetic pathway. Henceforth, the present study aims to identify and characterize various genes involved in costunolide biosynthesis. *De novo* transcriptome sequencing is a powerful and cost-effective tool for mining novel genes in non-model organisms. Therefore, Illumina Highseq 2000 sequencing platform was performed for 100 cycles paired end sequencing of cDNA library. As a result, 62,039,614 paired-end reads were generated.With average GC content 42% , 12.41GB bases were obtained.The raw reads were subjected to quality check by FastQC and NGSQC-Toolkit .Then, the illumina adapters were removed followed by low complexity read trimmimg .The average phred score of filtered reads was above 30 for 87% of reads (error-probability >= 0.01). The filtered reads were then assembled using Trinity into 122,434 contigs with an N50 of 1053bp.Subsequently ,the trimmed reads were aligned to the assembled contigs using Bowtie2 program and of all the filtered reads 90.48% readswere aligned back to the assembled contigs.Overall 114,049 contigs were found which have expression >= 1 FPKM ,hence for downstream annotation we focused only on these contigs.The assembled transcripts were compared to NR database using blastx program. Around 51% of the transcripts found using BLASTX have confidence level of at least 1E-50. The total number of GO terms identified in molecular function were 3,132 , biological process were 4,477 and in cellular component category were 1,927. The key genes involved in Sesquiterpenoid biosysthesis have been identified and to obtain the full length clones, RACE PCR will be performed. Genes, expressed sequence tags (ESTs) and unique sequences from this study provide an important resource for the scientific community, interested in the molecular genetics and functional genomics of *S. lappa*.

Poster 27

MBNLs autoregulate MBNL1 function by binding to the first coding exon of its transcript

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Muscleblind-like proteins (MBNLs) are regulators of RNA metabolism. During tissue differentiation expression of MBNLs increases, while their functional insufficiency causes myotonic dystrophy (DM). Deep sequencing of RNA molecules cross-linked to immunoprecipitated protein particles (CLIP-seq) revealed that MBNL1 binds to *MBNL1* exon 1 (e1) encoding both the major part of 5'UTR and an amino-terminal region of MBNL1 protein. We tested several hypotheses regarding the possible autoregulative function of MBNL1 binding to its own transcript and found that MBNLs induce skipping of e1 from precursor *MBNL1* mRNA, and that e1 exclusion has no impact on translation despite the preferential localization of e1-depleted mature mRNA on cytoplasmic ribosomes. Importantly, the translated e1-deficient isoform lacking the first two zinc fingers is highly unstable and its EGFP fusion protein has severely compromised splicing activity. We also show that *MBNL1* can be transcribed from three different promoters and that the transcription initiation site determines the mode of e1 regulation. Taken together, we reveal that MBNL proteins control steady-state levels of MBNL1 through an interaction with e1 in its precursor and mature mRNA. Insights from our study open a new avenue in therapies against DM based on manipulation of the transcription initiation site and e1 splicing of *MBNL1* mRNA.

Poster 28

The bridge between the two splice sites *in vivo*

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The first and critical step in mRNA splicing is identification of the two splice sites delimiting an intron and bring them into proximity – termed the commitment complex. Within the commitment complex U1 small nuclear ribonucleoprotein particle (snRNP) binds the 5' splice site and form a protein bridge with U2AF65 (also called U2AF2) that binds the 3' end of that intron. Although the commitment complex was study extensively *in vitro*, how the same pairing occurs *in vivo* for splice sites separated by long introns (more than 400 bases) is still obscure. Our laboratory proposed a new model, suggesting a dynamic pairing between the two splice sites involving interplay of RNA polymerase II, splicing factors and chromatin organization. We propose that U1 small nuclear ribonucleoprotein particle (snRNP) and U2AF65, associate with the CTD of RNA polymerase II (RNAP II) when that travels from one end of an intron downstream. We propose that U1 binds to 5' splice site while being associates to the CTD of RNAP II. When the 3' end of the intron is synthesize, U2AF65 binds the 3' splice site and the commitment complex is formed. In my research I will examine the validity of this model. I already demonstrated that *in vivo* U1 and U2AF65 are associated with RNAP II when the polymerase is in the elongating phase. I will further explore how the interaction of U1 and U2AF65 with RNAP II facilitate the formation of the commitment complex.

Poster 29

Physcomitrella patens hnRNPs regulate RNA splicing through a GAA-repetitive RNA motif on pre-mRNA

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Alternative splicing (AS) is a process for eukaryotic organisms to achieve protein diversity. Mechanism of AS has been well studied in animals. However, regulatory machinery for AS in plants remains elusive. Previous studies have indicated that *cis* elements on pre-mRNA play important roles in recruiting *trans*-acting factors either to promote or suppress pre-mRNA splicing. Currently, knowledge for the involvement of *cis* elements in splicing regulation is less discovered in plants. Introns of many genes are found retained rapidly after light irradiation according to our mRNA sequencing analysis in the moss *Physcomitrella patens*. A GAA-repetitive motif (6-8 nt) is enriched among the gene transcripts undergoing intron retention after light irradiation. We are wondering whether this GAA motif is a regulatory *cis* element that promotes intron retention and can recruit *trans*-acting partners to pre-mRNA transcripts. First, we confirmed the RNA sequence containing GAA motif possesses protein-binding activity from RNA-Electrophoresis Mobility Shift Assay (EMSA) with nuclear extracts from *P. patens* and synthesized RNA oligos. We further showed that the GAA-repetitive oligo pulled down several splicing regulators including serine-arginine rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) from moss cell lysates. These findings provide evidences that the GAA-repetitive motif potentially functions as a *cis* element during the regulation of AS in *P. patens.* Ultimately, we hope to find more concrete evidences in how this *cis* element and its binding factors influences plants to cope with the changing environment through regulating pre-mRNA splicing.

Poster 30

Alternative splicing of *At4g25290*, a putative photolyase, influences its subcellular localization

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At4g25290 encodes a protein which contains two domains: N-terminal photolyase and a C-terminal hydrolase. The photolyase domain is responsible for blue light mediated repair of DNA photoproducts induced by UVB. The analysis of mRNA isolated from leaves shows that alternative splicing results in expression of proteins lacking one of these domains, as was confirmed using N- and C- terminal specific antibodies. Several splicing isoforms are expressed in *Arabidopsis WT* plants, their expression is organ dependant. Three splicing isoforms of At4g25290 were chosen for a detailed analysis. The shortest one has only the photolyase domain. The longer isoform, may use an alternative start codon, as compared to full length At4g25290. Depending on the start of translation it encodes a hydrolase or a photolyase domain. Alternative splicing influences the localization of At4g25290. Full length At4g25290 is found predominantly in chloroplasts. The shortest isoform shows miss-localization and forms clumps at the plasma membrane and in the cytoplasm. The longer one containing the photolyase domain, localizes in the nucleus, at the plasma membrane and in chloroplasts. The hydrolase domain is observed at the plasma membrane and nucleus. The research was funded by NCN, UMO-2011/03/D/NZ3/00210. FBBiB is a partner of KNOW supported by the MSHE.

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Poster 31

Deep conservation of microexons across bilaterian nervous systems

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The vertebrate central nervous system (CNS) is a complex network of cells whose development and functioning depends on a finely orchestrated regulation of gene expression that includes the interplay of RNA-regulatory networks and the synthesis of a vast repertoire of protein variants. Alternative splicing (AS) is a major post-transcriptional process involved in regulating gene expression and generating transcript and proteome diversity. Notably, AS shows the highest prevalence and the lowest divergence evolutionary rates in vertebrate neural tissues. Moreover, a specific-class of very small exons from 3 to 27nt, termed microexons, shows a sharp neural inclusion and a high evolutionary conservation among vertebrates, indicating a role of these exons in the function and development of the vertebrate CNS. Despite these observations, little is known about the impact of AS on the evolution of the vertebrate CNS. Therefore, to start addressing this question; in the present work we explore the conservation of AS events using tissue-specific transcriptomes of 7 vertebrate (human, mouse, cow, chicken, frog, zebra fish and elephant shark) and 5 invertebrate species (amphioxus, sea urchin, centipede, octopus and fly). Results on microexon conservation show that neural microexons are widely conserved among vertebrate species. Remarkably, a fraction of them are also conserved and showing neural-specific inclusion in bilaterians. This suggests a general role of microexon AS in the evolution of the animal nervous system, where the appearance of the neural-specific microexon AS program precedes the separation of the invertebrate and vertebrate lineages followed by its expansion in the vertebrate one.

Poster 32

Effective connection network between hnRNP proteins and TDP-43 to abolish production of a truncated SORT1 receptor in humans

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Frontotemporal dementia (FTD) is a general name for a group of clinically, pathologically and genetically heterogeneous disorders with cerebral lobar degeneration. One of the key causes of this disease is RNA metabolism impairment that occurs following aggregation and mislocalization of regulatory RNA-binding proteins. For example splicing of exon 17b in SORT1 mRNA, is regulated by TDP-43 and it has been shown that depletion of TDP-43 leads to inclusion of this exon in humans and translation of a truncated and toxic SORT1 receptor. In this work, using a combination of minigene systems, pulldown, and knock down functional assays, we open a new window to the complexity of the RNA-binding protein network, showing that TDP-43 as the main regulator for exon 17b splicing, is highly affected by other RNA- binding proteins specially hnRNP L, PTB/nPTB and hnRNP A1/A2. We also provided data from patients' brain with TDP-43 positive aggregations, showing significantly high expression of hnRNP A1/A2 and PTB/nPTB compare to physiological control groups. In conclusion, possibly age and individual dependent expression levels of hnRNPs and the interaction network between them, could affect the consequences of FTD like the onset and progression. Therefore, there is a need to better understand these connection networks to find out some effective therapies.

Poster 33

Mapping of splicing regulatory elements in the *BTK* gene exon 15

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The process of splicing is secured by a precise recognition of exon-intron boundary and its consensus motifs with support of other regulators of splicing, choosing among different possibilities, some of which are splice sites creating alternative transcripts. This project focuses on mapping of cis-regulators in the exon 15 of the gene *BTK* coding Bruton´s tyrosine kinase, in order to reveal their importance in disease causing process and their possible role in alternative splicing. Mutations in the BTK can cause malfunction of this kinase leading to a rare immunodeficiency called X-linked agammaglobulinemia, thus results can be helpful in terms of diagnostics. For the analysis of splicing in vitro, minigene constructs were prepared, mutated, expressed in HeLa cells and mRNA was studied. Subsequent deletions with length of 35 to 10 nt showed no major effect on splice site utilization or exon splicing in these regions, with exception of a region at the 3´ end of exon 15. Deletion of this region spanning 10 nt caused 30 % of exon skipping, suggesting presence of splicing enhancers. These results were confirmed by ESE-dependent minigene analysis using a pcDNA-Dup minigene. Moreover, point mutations were done to further investigate these regulatory motifs. In conclusion, 3´ end of exon 15 plays a significant role in its splicing regulation in the minigene context. This study was supported by the Specific University Research (MUNI/A/1183/2015), provided at MEYS CR, and the Health Research Council, MH CR (grant no. 16-34414A).

Poster 34

Transcriptional and post-transcriptional regulation of *CCND1* gene in the pathogenesis of Ewing Sarcoma

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Ewing sarcomas are tumors of bones and soft tissues that mostly affect children and young adults. The most common mutation occurring in Ewing sarcoma is the chromosomal translocation t(11;22), which fuses the *EWSR1* gene with the *FLI1* gene and creates the *EWSR1/FLI1* chimeric oncogene. The resulting oncoprotein, EWS/FLI, affects transcription and processing of genes involved in proliferation and tumor development. One of the key targets of EWS-FLI1 is Cyclin D1, which is encoded by the *CCND1* gene and is required for progression through the G1 phase of the cell cycle. The *CCND1* gene is significantly upregulated in Ewing sarcoma and its dysregulation likely contributes to defective regulation of cell cycle progression. Cyclin D1 is expressed as two main isoforms, derived by alternative pre-mRNA splicing, termed D1a and D1b. Both isoforms are frequently upregulated in human cancers, but cyclin D1b, which differs for the inclusion of intron 4, displays relatively higher oncogenic potential. In our study we have investigated the mechanism controlling cyclin D1 expression and splicing in Ewing sarcoma cells. We found that EWS-FLI1 regulates both transcription and splicing of *CCND1* gene, and that its interaction with the RNA helicase DHX9 differently affects these activities. Moreover, we identified the RNA-binding protein SAM68 as a modulator of DHX9-EWS-FLI1 interaction, which affects EWS-FLI1 recruitment to the *CCND1* gene. Both SAM68 and EWS-FLI1 interact with DHX9, and impairment of DHX9-EWS-FLI1 interaction by treatment with the synthetic peptide YK-4-279 interferes with EWS-FLI1 recruitment to the *CCND1* gene while enhancing SAM68 binding to the promoter and suppressing Cyclin D1 expression. Collectively our experiments indicate SAM68 as a novel mediator of EWS-FLI1 activity in Ewing Sarcoma cells.

Poster 35

SAM68-dependent regulation of gene expression and alternative splicing in male germ cells

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Spermatogenesis is a dynamic cell differentiation process that remains active throughout the adult life. Germ cells require a specific repertoire of factors at each stage of differentiation and gene expression is modulated at multiple layers during spermatogenesis. SAM68 is a RNA-binding protein involved in several steps of RNA metabolism and it is strictly required for the correct progression of spermatogenesis and male fertility. To investigate the gene expression signature regulated by SAM68 in male germ cells, we have performed transcriptomic analyses of highly purified populations of meiotic spermatocytes and post-meiotic round spermatids obtained by *Sam68* knockout and wild type mice. Bioinformatics analyses identified more than two thousands genes whose expression levels were affected by SAM68 in either meiotic or post-meiotic germ cells. Furthermore, *Sam68* ablation affected splicing of thousands of exons in male germ cells. Interestingly, we also observed that the large majority (~70%) of the genes regulated by SAM68 in germ cells were also regulated during trans-meiotic differentiation, whereas the exons regulated at the splicing level were not. Notably, in most cases knockout of *Sam68* mimicked the up- or down-regulation observed in the transition from spermatocytes to spermatids. Since SAM68 is also strongly down-regulated during this transition, our results suggest that its high levels of expression in spermatocytes prevents premature induction of spermatid-specific genes in meiotic cells. Regarding the splicing-regulated genes, we found a high percentage of down-regulated exons in *Sam68* knockout germ cells, suggesting that SAM68 is strictly required for inclusion of these exons in male germ cells. The most represented splicing patterns were exon cassette and alternative last exons. Interestingly splicing-regulated genes were more enriched in functional categories related to spermatogenesis than genes regulated at the expression level. Our results identify the signature of genes regulated by SAM68 in male germ cells and provide the basis for mechanistic studies aimed at understanding the specific contribution of this protein to the spermatogenic process.

Poster 36

U12 intron is evolutionary conserved in plant CBP 20 (Cap Binding Protein 20) genes and is required for correct pre-mRNA splicing

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Eucaryotic RNA polymerase II transcripts are characterized by the presence of the cap structure and polyA tail at their 5' and 3' ends, respectively. Cap Binding Complex (CBC) is a nuclear complex composed of two cap-binding proteins: CBP20 and CBP80. It is known that binding of the CBC to the 5' cap is crucial for the proper mRNA maturation and transport. We show that the *CBP20* gene structure is highly conserved across land plants from liverwort to higher plants. The gene contains always seven introns with the fourth intron belonging to U12 class. Additionally the U12 intron divides the gene in two parts: one that encodes the core domain containing RNA recognition motif and the second one that encodes the tail domain containing Nuclear Localization Signal (NLS). In all investigated plants *CBP20* genes first four exons encoding the core domain have always the same length whereas exons coding the terminal domain differ considerably in length. To answer the question why the presence and location of the U12 intron in *CBP20* gene is preserved across all plant species we prepared constructs representing *CBP20* mini-genes and its mutated full versions. Mini-gene constructs containing 4^{th} and 5^{th} exons from *A. thaliana CBP20* gene and U12 introns derived from different plants and differing in length (from 134nt to 2733nt) were transfected to tobacco protoplasts and splicing was analyzed. Our results show that the longer the U12 intron the more efficient splicing was observed. Splicing analyses of mini-gene construct containing U2 intron in U12 intron natural position revealed that 37% of mRNAs undergo alternative splicing. Additionally we prepared five constructs containing *A. thaliana CBP20* gene in which (i) U12 intron was removed, (ii) replaced by U2 one, and (iii – v) U12 intron was moved to different locations within the gene body. These constructs were introduced into *A.thaliana cbp20* T-DNA insertion mutant. Our results show that transcripts derived from the mutated *CBP20* gene (in which the U12 intron was moved to the other gene locations) generate additional alternatively spliced isoforms containing retained intron and/or longer/shorter exons (because of alternative 3' and 5' splice site selection). All these data suggest that U12 intron in the proper position in plant *CBP20* gene is necessary for correct pre-mRNA splicing.

Poster 37

Sex-specific miRNAs in *Marchantia polymorpha*

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MicroRNAs are important regulatory elements of eukaryotic gene expression. At least ten conserved miRNA families have been reported to play a key role during flower development in the higher plants. To investigate whether sex-specific miRNAs are present in the *Marchantia polymorpha*, we applied the high-throughput sequencing technique and sequenced sRNAs from four different tissues: vegetative female thalli, archegoniophores, vegetative male thalli and antheridiophores. 13 families of conservative miRNAs were identified. Among them are also flower-specific miRNAs:miR172, miR529/156, miR160, miR166/165, miR319/159, miR167, miR169, miR390,miR399. The presence of these selected conservative miRNAs was verified by northern hybridization. The level of mature miR160 and miR166a is strongly higher in antheridiophores and archegoniophores as compared to vegetative thalli, whereas the level of miR529a,b,c in antheridiophores and archegoniophores is much lower. In our previous studies we identified 42 novel miRNA in the liverwort *Pellia endiviifolia*. Analysis of *M. polymorpha* sRNA sequencing revealed the presence of 18miRNA families which were previously described as *Pellia*-specific. miR8190,miR8170, miR8184 were highly expressed in archegoniophores and antheridiophores. miR8185 and miR8166 were present only in antheridiophores. Additionally miR8163 and miR8181 were present at the lower level in the reproductive organs than vegetative thalli. Using degradome sequencing technique, new targets for these miRNAs were identified. Moreover, using *Agrobacterium*-mediated transformation we obtained mutants overexpressing miR8185, miR8170 andmiR8163. Analyses of these mutants with respect to sex organ development will be discussed. This research is supported by National Science Centre grant-UMO-2014/13/N/NZ3/00321 and KNOW RNA Research Centre in Poznan -(No.01/KNOW2/2014).

Poster 38

Analysis of splicing efficiency of tRNA-hosting introns in *Arabidopsis*

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tRNA molecules are fundamental components of the translation machinery. Besides their canonical function, tRNAs have been reported to have additional functions such as acting as signaling molecules in the regulation of cellular and physiological processes or serving as a source of small regulatory RNAs. These unexpected findings prompted us to look at tRNAs from a different perspective. We globally examined the phenomenon of tRNA occurrence in *Arabidopsis* genes across the whole genome, and found an interesting example of tRNA(Glu)-like sequence present within the intron of noncoding RNA *locus* (*GUT15*, GENE WITH UNSTABLE TRANSCRIPT 15). Comprehensive characterization of the biogenesis and function of the tRNA-like sequence revealed that it can regulate both splicing of the *GUT15* gene and the production of short RNAs deriving from its flanking region. Interestingly, we showed that the biogenesis of this tRNA-resembling molecule is dependent on RNAPII transcript processing and that it can serve as a source for other short RNA sequences. Additionally, using the tobacco protoplasts system for splicing efficiency analysis we investigated the role of tRNAs and tRNA-like sequences in splicing of the sense tRNA-hosting introns. This work was supported by grants from the National Science Center UMO-2011/03/B/NZ2/01416 and UMO-2013/11/N/NZ2/02511.

Poster 39

FUS/TLS negatively regulates the level of snoRNAs in human cells

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FUS/TLS (named FUS thereafter) is a multifunctional protein involved in many pathways of RNA metabolism in human cells, including transcription, splicing, alternative splicing, RNA transport, miRNA processing, replication-dependent histone gene expression [1,2]. Several FUS mutations have been found in familial forms of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), implicating a pathogenic role of this protein in neurodegenerative diseases. Interestingly, we have recently found that FUS can also bind and affect the level of small nucleolar ribonucleoproteins (snoRNP) in human cells. We performed RNA immunoprecipitation based on FLAG-tagged FUS followed by high throughput sequencing (RIP-seq) and identified snoRNAs in immunoprecipitated fraction. The UGAUGA motif that is specific for conserved sequence element of box C/C' was one of the highly recognized motifs by FUS. Interestingly, results of RT-qPCR and Northern blot experiments revealed that FUS negatively influences the level of snoRNAs in the cell. We observed reduced level of selected snoRNAs when FUS was overexpressed and inversely, elevated level of snoRNAs was detected in cells with FUS depletion (FUS knockdown and FUS knockout cells). Surprisingly, although FUS is known to regulate splicing and alternative splicing we could not observe any influence of FUS on the excision of introns encoding snoRNA genes. EMSA is now being performed to elucidate whether FUS can directly interact with snoRNAs. Moreover, the effect of selected snoRNA bound by FUS on the splicing of other pre-mRNAs as described before [3] is being examined. The mechanism of negative regulation of snoRNAs expression by FUS and the biological significance of this process will be discussed.

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Poster 40

The influence of human miRNAs in the physiology of hematophagous mosquito *Aedes aegypti*

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In January 2012, Zhang et al, showed that plant miRNAs taken orally are present in the serum of various animal tissues. The MIR168a is a miRNA abundant in rice plants and is one of the most abundant exogenous miRNAs in the serum of Chinese subjects. Functional studies showed that MIR168a can bind itself with the gene that encodes the low-density lipoprotein receptor adaptor protein 1 (LDLRAP1) in humans and in mice (specifically exon 4), inhibiting expression in the liver and thereby decreasing the presence of LDL (low density lipoprotein) plasma. These findings suggest that there is an external regulation of gene expression mediated by organisms in other kingdoms. To determine whether microRNAs present in human peripheral blood ingested by blood-sucking mosquitoes have a regulatory role in the gene expression of these insects. We will feed bloodsucking mosquitoes (*Aedes aegypti*) two different diets based on human blood and synthetic formulation using artificial feeders. We will extract the total RNA at 6, 12 and 24 hours after feeding and will hybridize RNA on two types of microarrays: 1) microarray gene expression screened / transcripts mosquitoes and microarrays 2) sifting the presence and abundance of human microRNAs. Comparing both results, we will be able to infer if there are microRNAs of humans regulating the expression in mosquito's genes. The findings will be corroborated by qPCR. The genes corroborated by qPCR, will be amplified, cloned and sequenced in the 5 'and 3' regions where the expression levels is modified with mosquitoes fed with human blood in counterpart with those fed with synthetic diet devoid of microRNAs. Finally, in order to validate the findings, will be performed RNAi assays. Expected results**:** 1- Knowing the differential gene expression of bloodsucking mosquitoes fed two different diets, 2- determinate the presence of human microRNAs in bloodsucking mosquitoes (which ones and quantity), 3- explore if human microRNAs could regulate bloodsucking mosquitoes genes, 4– If there are human microRNAs regulating the transcriptional expression in mosquitoes – find out which genes are regulated by exogenous microRNAs, 5– Correlate the impact of genetic interactions between humans and mosquitoes with a phenotype or trait of the vector. This study is important in public health –if is confirmed the biological interaction and influence by organisms of different kingdoms besides plants. Also, is transcendental know the mechanisms of gene regulation affected by diet.

Poster 41

Antheridia and archegonia connected gene expression in simple thalloid liverwort *Pellia endiviifolia* sp B

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We have chosen *Pellia endiviifolia* species B, a dioecious liverwort from class *Jungermaniopsida,* to profile the differences in gene expression by RNA-seq approach between the male and female thalli producing or not producing sex organs. 72 DEGs were selected with the highest differences in expression. Out of ten genes up-regulated in sperm-producing male thalli, eight are also expressed in the vegetative male thalli; out of 62 up-regulated genes in archegonia-producing female thalli, 46 are also expressed in the vegetative female thalli. Additionally, the previously identified male- or female-specifically expressed genes by RDA-cDNA technique were also DEGs in the RNA-seq data. To check whether the expression of selected genes is restricted to the antheridia/archegonia bearing parts of thalli we isolated the sex organs separately from the vegetative parts and used for RT-qPCR analysis. Only ten genes were validated to be enriched in archegonia and two in antheridia of female and male thalli, respectively. To get the information about the regulatory motifs within the promoter sequences of identified genes genome walking technique was used for several cases. Interestingly, often encountered elements common to several promoters of female-specifically expressed genes are the light responsive elements and the motifs found in the plant storage protein genes. Our studies provide possibility to learn about the gene expression regulation within the representative of genus *Pellia*, which is recognized as the one of the most basal lineage of simple thalloid liverworts. The work was supported by the Foundation for Polish Science, grants no. POMOST/2012-5/7 and Mistrz 3/2014.

Poster 42

RNA-Seq mediated transcriptome analysis of *Chlorophytum borivilianum* for identification of genes involved in saponin biosynthesis

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Chlorophytum borivilianum is an important species of liliaceae family, owing to its vital medicinal properties. Plant roots are used for aphrodisiac, adaptogen, anti-ageing, health restorative and health promoting purposes. The transcriptome information of this species is limited and only few hundred expressed sequence tags (ESTs) were available in the public databases. To gain molecular insight of this plant, high throughput transcriptome sequencing of leaf and root was carried out using Illumina's HiSeq 2000 sequencing platform. Available (e.g., De-Bruijn/Eulerian graph) and in-house developed bioinformatics tools were used for assembly and annotation of transcriptome. Bioinformatics analysis, using non-redundant proteins, gene ontology (GO), enzyme commission (EC) and kyoto encyclopedia of genes and genomes (KEGG) databases, extracted all the known enzymes involved in saponin and flavonoid biosynthesis. Few genes of the alkaloid biosynthesis, along with anticancer and plant defense genes, were also discovered. Five full length genes namely *farnesyl pyrophosphate synthase, cycloartenol synthase, β-amyrin synthase, cytochrome p450* and *sterol-3-glucosyltransferase* involved in saponins biosynthesis were identified. Additionally, several cytochrome P450 (CYP450) and glycosyltransferase unique sequences were also found. We identified simple sequence repeat motifs in transcripts with an abundance of di-nucleotide simple sequence repeats. Read per exon kilobase per million (RPKM) based comparative expression profiling was done to study the differential regulation of the genes. *In silico* expression analysis of 7 selected genes of saponin biosynthetic pathway was validated by qRT-PCR. Genes, expressed sequence tags (ESTs) and unique sequences from this study provide an important resource for the scientific community, interested in the molecular genetics and functional genomics of *C. borivilianum*.

Poster 43

Targeting Myotonic Dystrophy with splice-switching antisense oligonucleotides

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Myotonic Dystrophy (DM), the most common form of muscular dystrophy in adults, is an autosomal dominant neuromuscular genetic disorder with clinical features primarily related to, but not restricted to, muscle pathology. The genetic background of DM type 1 (DM1) is the expansion of unstable microsatellite tri-nucleotide CTG repeats within the 3'UTR of DMPK gene (*dystrophia myotonica-protein kinase*) which, upon transcription into CUG-repeat RNA, form thermodynamically stable hairpin structures, so-called 'toxic RNAs (CUG^{exp})'. The robust sequestration of proteins, MBNL splicing regulators among others, to these nuclear RNA foci ultimately leads to alternative splicing dysregulation of many pre-mRNAs (spliceopathy) and results in pathological DM hallmarks such as myotonia, muscle weakness and wasting. As of today, there is no cure for DM, and patients' therapy is limited to symptomatic treatment. In this work, we rationally designed splice-switching antisense oligonucleotides (AONs) to promote exon-skipping in *DMPK* pre-mRNA, and tested whether this approach can eliminate toxic CUG^{exp} RNA and thus have a therapeutic potential against DM. Two types of experimental strategies were tested: one targeting internal, constitutive exons of *DMPK* within putative exonic splicing enhance (ESE) regions (strategy 1) and the other targeting the last, constitutive *DMPK* exon 15 harbouring CUG^{exp} (strategy 2). In strategy 1, steric blocking of ESEs within internal constitutive *DMPK* exons could potentially inhibit their inclusion into mature mRNA, and trigger an open reading frame (ORF) shift leading to generation of a premature stop codon (PTC) and as such, elimination of mis-spliced *DMPK* transcript via distinct molecular mRNA surveillance pathways. In DM1 patients cell lines, this could potentially lead to mutant CUG^{exp} RNA degradation and correction of DM related spliceopathy and toxic ribonuclear CUG^{exp} foci accumulation. In strategy 2, we presumed in our working hypothesis that AON-mediated inhibition of *DMPK* exon 15 inclusion could redirect the splicing towards a cryptic alternative 3'-splice site located downstream of the CUG-repeat tract, which in turn would lead to preferential expression of a novel mRNA isoform lacking CUG^{exp} (*DMPK* E16+) and a fully functional protein isoform (DMPK G). Both experimental strategies are hypothesized to induce the release of MBNL proteins from pathogenic sequestration by expanded CUG repeats and boost their availability to natural pre-mRNA targets, which could lead to correction of DM spliceopathy and amelioration of DM-related molecular abnormalities. This work reports preliminary results of our analyses of a therapeutic potential of *DMPK* splice-switching AONs in DM1 cell culture models.

Poster 44

New potential protein factors involved in plant NMD

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Nonsense-mediated mRNA decay (NMD) is a conserved mRNA surveillance mechanism that prevents the production of potentially harmful proteins by eliminating aberrant mRNAs carrying premature translation termination codons (PTC) that are often generated by alternative splicing. The key NMD effectors, ATP-dependent RNA helicase UPF1 together with UPF2 and UPF3 form a core of the NMD complex in all organisms. In addition, SMG1, SMG5-9, ribosome, the exon-exon junction complex (EJC) and eukaryotic release factors ERF1 and ERF3A were shown to be involved in degradation of nonsense mRNA in vertebrates. Extensive studies in yeast, *C.elegans,* flies and mammals established a whole set of additional and auxiliary NMD components. Most of these proteins, including RNA helicases, subunits of eukaryotic initiation factor 3 (eIF3), transcription-export (TREX) complex and nucleus-associated RNA-binding proteins, are conserved and essential for growth. In contrast, only a few major players, including UPF1-3 proteins, SMG7 and EJC components, were identified in plants. To identify new plant NMD factors we have analyzed UPF1-interacting proteins by affinity purification using a transgenic Arabidopsis line expressing tagged UPF1. Besides UPF2 and UPF3 we have identified several proteins including ribosomal and RNA-binding proteins, splicing factors, RNA helicases, subunits of eukaryotic initiation factor 3 and 4, and proteins involved in nuclear transport and proteolysis. To investigate the NMD-related function of the best and most interesting candidates we have applied the VIGS approach (Virus-Induced Gene Silencing agroinfiltration transient NMD assay), which allows for quick and efficient testing of many potential NMD factors using transient transfection of *N. benthamiana* leaves. We will present the results of these proteomic and functional analyses.

Poster 45

Barley response to drought and rehydration is regulated by microRNAs

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MicroRNAs (miRNAs) are small, regulatory RNAs, typically of 21nt length. They downregulate gene expression by targeting mRNAs to cleavage or translation inhibition. We studied the role of miRNAs in barley response to drought and rehydration. Changes in miRNAs expression pattern were analyzed globally with NGS approach and confirmed with northern. Drought stress altered expression of 60 miRNAs. The expression of 36 of them can be restored within six hours after re-watering. An example of drought-regulated molecules are miRNA172a-3p and miRNA172b-5p. Drought downregulates miRNA172b-5p level, while its partner miRNA172a-3p is upregulated. The miRNA172b-5p/172a-3p expression is restored after rehydration. MiRNA172a-3p is well described in mono- and dicotyledonous plants and targets APETALA2-like TFs. miRNA172b-5p is present only in monocotyledonous plants and originates from the same precursor with miRNA172a-3p. The level of pri-miRNA172b is not regulated by drought/rehydration suggesting drought-induced selection of miRNA or miRNA* as functional molecule. With Parallel Analysis of RNA Ends (PARE) approach we identified serine-threonine kinase (SnRK2) as a target of miRNA172b-5p. The SnRK2 mRNA level is upregulated in drought and drops down after rehydration. The SnRK2 is abiotic stress related and most likely ABA-regulated kinase. We showed that drought stress induces the activity of SnRK2. We provide data describing drought and rehydration induced changes in miRNAs expression. The rapid changes of miRNAs levels during drought/rehydration can be partly explained by posttranscriptional regulation of miRNAs maturation. Moreover, we provide first evidence of SnRK2 level regulation during drought and rehydration by microRNA. Work supported by the POLAPGEN-BD UDA.POIG.01.03.01-00-101/08 „Biotechnological tools for breeding cereals with increased resistance to drought”, subject 20: "The role of micro RNA in regulation of mechanisms leading to drought adaptation in plants", Innovative Economy 2007-2013, subject „Biological progress in agriculture and environment protection”; and by the KNOW RNA Research Centre in Poznan 01/KNOW2/2014.

Poster 46

Isoform-level gene expression profiles of human Y chromosome azoospermia factor genes and their X chromosome paralogs in the testicular tissue of non-obstructive azoospermia patients

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The human Y chromosome has an inevitable role in male fertility because it contains many genes critical for spermatogenesis and the development of the male gonads. Any genetic variation or epigenetic modification affecting the expression pattern of Y chromosome genes may thus lead to male infertility. In this study, we performed isoform-level gene expression profiling of Y chromosome genes within the azoospermia factor (AZF) regions, their X chromosome counterparts, and few autosomal paralogues in testicular biopsies of 12 men with preserved spermatogenesis and 68 men with nonobstructive azoospermia (NOA) (40 Sertoli-cell-only syndrome (SCOS) and 28 premiotic maturation arrest (MA)). This was undertaken using quantitative real-time PCR (qPCR) at the transcript level and Western blotting (WB) and immunohistochemistry (IHC) at the protein level. We profiled the expression of 41 alternative transcripts encoded by 14 AZFa, AZFb, and AZFc region genes (USP9Y, DDX3Y, XKRY, HSFY1, CYORF15A, CYORF15B, KDM5D, EIF1AY, RPS4Y2, RBMY1A1, PRY, BPY2, DAZ1, and CDY1) as well as their X chromosome homologue transcripts and a few autosomal homologues. Of the 41 transcripts, 18 were significantly down-regulated in men with NOA when compared with those of men with complete spermatogenesis. In contrast, the expression of five transcripts increased significantly in NOA patients. Furthermore, to confirm the qPCR results at the protein level, we performed immunoblotting and IHC experiments (based on 24 commercial and homemade antibodies) that detected 10 AZF-encoded proteins. In addition, their localization in testis cell types and organelles was determined. Interestingly, the two missing proteins, XKRY and CYORF15A, were detected for the first time. Finally, we focused on the expression patterns of the significantly altered genes in 12 MA patients with successful sperm retrieval compared to those of 12 MA patients with failed sperm retrieval to predict the success of sperm retrieval in azoospermic men. We showed that HSFY1-1, HSFY1-3, BPY2-1, KDM5C2, RBMX2, and DAZL1 transcripts could be used as potential molecular markers to predict the presence of spermatozoa in MA patients. In this study, we have identified isoform level signature that can be used to discriminate effectively between MA, SCOS, and normal testicular tissues and suggests the possibility of diagnosing the presence of mature sperm cell in azoospermic men to prevent additional testicular sperm extraction (TESE) surgery.

Poster 47

Exon-intron architecture in high and low GC-content genes affects alternative splicing

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The splicing machinery recognizes exons and introns by using multiple signals. Two splicing signals that delineate proper exon-intron boundaries are the 5' and 3' splice sites. Alternative splicing is a major mechanism that enhances transcriptomic diversity. My lab's previous studies revealed that gene architecture had evolved from an ancestral state of low GC-content, in exons flanked by short introns of lower GC-content. One group of genes maintained the low GC-content and their introns became longer. In another group mutations accumulated, leading to elevation of the GC-content, which revoked the differential GC-content between exons and introns. In this group introns are under selection to remain short. My research hypothesis is that the splicing machinery selects exons and introns differently between the two groups of exon-intron architecture. This hypothesis is based on my lab's previous findings that exon skipping predominantly associates with the low GC-content genes and intron retention with the higher GC-content genes. To examine the mode of selection of exons and introns by the splicing machinery in the high and the low GC-content genes I reduced the affinity of the splicing machinery to the 5’ splice-site using site-specific mutations. My results demonstrate different splicing patterns in the high and low GC-content genes. I thus conclude that the splicing machinery recognizes the exons and introns of the two GC-content gene groups differently. Also, our bioinformatics analysis revealed that the high GC-content genes harbor an even higher GC content region downstream of the 5’ splice-site. I examined the functionality of that sequence by replacing it with even higher or lower GC-content segments. My results show that this region is important for the inclusion of the upstream exon in the mature mRNA molecule. Furthermore, I checked how the splicing machinery can switch between intron definition and exon definition through the formation of exon-intron differential GC-content. And how intron lengthening impacts intron definition in high GC-content genes. Overall, my research shed light on genomic elements that contribute to the diversity in splicing recognition units in the high and low GC-content families.

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VAST-DB: an Atlas of Alternative Splicing Profiles in Vertebrate Tissues and Cell Types

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Alternative splicing (AS) is a widespread process facilitating the generation of regulatory and proteomic complexity. However, the function of the vast majority of AS events detected to date is unknown, and landscapes of regulated AS in different biological contexts remain to be identified. To contribute to address these challenges, we present VAST-DB, a massive resource of genome-wide quantitative profiles for all main classes of AS events across a wide range of human, mouse and chicken tissues, cell types and developmental stages. The resulting atlas of AS events reveals extensive new intergenic and intragenic regulatory and functional relationships involving different classes of AS events, as well as previously unknown conserved landscapes of tissue-regulated exons. We also report and validate hundreds of AS events that are alternatively spliced in virtually all profiled tissue and cell types. These AS events are highly enriched in genes that encode transcription factors and DNA binding proteins, and single cell RNA-seq data show that they are usually predicted to generate protein isoforms that co-exist in the same cell. Finally, we also provide mapping of these AS events to protein regions and experimentally determined or modeled protein structures. Our AS atlas thus provides a valuable basis for new explorations of splice isoform regulation and function in normal and disease contexts.

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Solution structure of the plant HSP90-SGT1 complex

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Heat Shock Protein 90kDa (HSP90) is an molecular chaperone that is involved in client protein folding, maturation and stabilization. For this purpose HSP90 uses energy from ATP hydrolysis. Thus HSP90 protein is important for maintaining cellular homeostasis in both normal and stress conditions. HSP90 client proteins include oncogenic kinases like v-Src, BCR-ABL and RAF1, p53 tumor suppressor, telomerase reverse transcriptase TERT, Myosin and viral proteins like HCV NS3 protein. HSP90 interacts with a specific diverse class of proteins called co-chaperones. Co-chaperones interact with HSP90 and modulate its ATPase activity and function in protein folding either accelerating or inhibiting the process. Some of the co-chaperones are specific for the one class of client proteins like CDC37 protein required for the HSP90-dependent maturation of several kinases. Other co-chaperones do not affect folding process having function in client delivery. HSP90 protein has three domains. N-terminal domain (NTD) is required for the ATP hydrolysis and interacts with some client proteins, middle domain (MD) is the main place of the interaction with client proteins, whereas C-terminal domain (CTD) is responsible for HSP90 dimerisation. At the very end of CTD the MEEVD is located that is involved in TPR domain containing co-chaperones binding. In solution HSP90 protein exists in equilibrium between open, apo state and closed, ATP bound-like state with additional dimerisation of N-terminal domains. Some co-chaperones like p23 stabilize closed state of HSP90 protein. In contrast HOP1 protein stabilizes open conformation of HSP90 protein. In plants HSP90 protein was found to be required for the R protein mediated immune response against various pathogens. For proper function in plant innate immunity HSP90 requires interaction with another protein called SGT1 (Suppressor of G2 allele of skp1). SGT1 was first discovered to be a member of E3 ubiqutin ligase complex and suppressor of the mutation of one of its component - Skp1 in yeasts. SGT1 has modular structure and consists of three domains with variable regions between them. At the N-terminus SGT1 protein possess tetratricopeptide repeats (TPR) domain that is required for dimerisation of plant and human homologs, CHORD and Sgt1 (CS) middle domain is responsible for the interaction with HSP90 NTD and SGT1 specific (SGS) domain at the C-terminus is thought to be involved in the interactions with various LRR domain containing proteins. Although crystal structure of HSP90 NTD in complex with SGT1 CS domain with ADP was solved there is lack of detailed description of the structure of the HSP90-SGT1 complex in the context of the full length proteins. In this study we investigated low resolution structure of the plant HSP90-SGT1 complex with ADP in solution using small angle X-ray scattering (SAXS) technique. SAXS technique is specially suitable for studying large and flexible proteins that are very hard to study using X-ray crystallography or NMR. Using SAXS we studied complex between full length HSP90 protein and SGT1 protein with deleted SGS domain (SGT1ΔSGS). In addition we also studied complex of SGT1ΔSGS with HSP90 without CTD (HSP90ΔC) and HSP90 NTD proteins. Using MCR-ALS analysis and molecular mass estimation we showed that complex between HSP90 and SGT1ΔSGS proteins has 2:1 stoichiometry and only one SGT1ΔSGS monomer is present within the complex even though SGT1ΔSGS protein exists as a dimer in solution and HSP90 dimer has two potential SGT1 binding sites. Similar to the full length protein, HSP90ΔC also caused SGT1ΔSGS monomerization. Finally using the same SAXS methodology we proved that HSP90 NTD is sufficient to monomerize SGT1. Using ab-initio modelling we obtained low resolution models of the studied complex and showed that HSP90 exist in open conformation in complex with SGT1ΔSGS. Acknowledgments: This work was supported by the grant (2012/05/N/ST3/03087) from National Science Center.

Poster 50

RNA structural properties for efficient binding and activity of MBNL splicing regulators

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The main aim of the studies was focused on the RNA structural preferences of three tissue specific alternative splicing regulators and zinc finger domains containing paralogs of **M**uscle**b**li**n**d-**l**ike proteins (MBNL1, MBNL2 and MBNL3). The RNA consensus motif, which is a GpC dinucleotide flanked by pyrimidines (YGCY), is well established (Warf et al., 2007, Goers et al., 2008, Lambert et al., 2014) contrarily to the protein preferences towards the RNA structure they are tangled within. The latest research pointed the necessity of embedded single stranded pyrimidines whereas the GpC step may stay single or double stranded (Lambert et al., 2014,). It is also obscure whether all paralogs equally and effectively bind to the same consensus motifs and favor the same structure determinants. To solve these problems we studied several MBNL-specific target structures and artificially designated short RNAs in order to determine which features highly recruit each paralog or cause the binding abort, leading to the protein activity distortion in both *in vitro* and *in cellulo* conditions. We also tested whether all three paralogs find the same consensus structure motifs significant and functional. The comparison of the binding affinity of three recombinant MBNL paralogs to the same binding sites and differently structured RNA molecules was studied via an *in vitro* filter binding assay. The functionality of those sites and the effect exerted on the splicing activity of MBNLs was tested *in cellulo* by utilizing an *Atp2a1* minigen with a natural regulatory site within an intron 22 replaced by studied RNA structures (artificial *Atp2a1* minigenes). We observed that MBNL1, MBNL2 and MBNL3 bind equally and effectively to the same consensus motifs located within similar RNA structures *in vitro* and through which they modulate the alternative splicing of exon 22 of artificial *Atp2a1* minigenes *in cellulo*. Our studies indicate three closely located YGCY motifs as a minimal target for efficient binding and functionality. The structure facilitating the binding and activity seems to be semi-stable or partially single-stranded including at least several nucleotides surrounding consensus motifs. The nucleotide distance between the motifs within a secondary structure on one side of the hairpin structure seems to be negligible but the motifs' position plays an important role and may entirely impede MBNLs binding. 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. This work was supported by Foundation for Polish Science TEAM program (to K.S.), the Polish National Science Centre: 2011/01/B/NZ1/01603 and 2014/15/B/NZ2/02453 (to K.S.) and Ministry of Science and Higher Education of the Republic of Poland under the Leading National Research Centre (KNOW programme).

Poster 51

Coupling alternative splicing with nonsense-mediated decay in potato

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Plants have evolved systems to defend against pathogens and adapted pathogens deliver effector proteins inside host cells to suppress these defences. Effectors are detected by immune receptors, the cytoplasmic nucleotide binding leucine-rich repeat (NB-LRR) proteins, leading to effector-triggered immunity (ETI). Using a NB-LRR gene enrichment and RenSeq, previous studies identified over 700 NB-LRR genes in potato. Most fall into two classes: the N-terminal toll/interleukin 1 receptor (TIR)-like domain (TNL) and N-terminal coiled-coil (CC) domain (CNL). TNLs show conserved gene nucleotide-binding domains and some show alternative splicing (AS). AS occurs in at least 60% of plant genes and is enriched in stress related genes, such as TNL and receptor-like kinase genes. Dynamic changes in ratios of AS of TNL transcripts have been shown in tobacco and Arabidopsis to be essential for the plant response to infection. AS is also associated with mRNA stability through the activation of nonsense-mediated decay (NMD); a conserved RNA surveillance mechanism. NMD targets endogenous mRNAs, aberrantly spliced transcripts and some AS transcripts for degradation. These transcripts contain features that allow them to be recognised by the NMD machinery: for example, premature termination codons (PTCs) and long 3' UTRs. In plant defence, some resistance genes such as TNLs are alternatively spliced such that transcripts are continually turned over by NMD. The hypothesis is that infection alters the efficiency of NMD leading to an increase in the alternatively spliced PTC-containing transcripts and thereby to efficient activation of the hypersensitive response. We aim to understand a role for AS and NMD in the interaction between potato and *Phytophthora infestans*, by focusing on the general post-transcriptional regulation and specifically of NB-LRR genes. We have started to develop a method to analyse NMD in potato so that we can follow the changes and effects of AS on NB-LRR genes. Furthermore by developing NMD and plant defence response signal defective mutants using a CRISPR/Cas9 gene editing system, we aim to understand the role of AS/NMD in potato plants. We will challenge plants of the doubled monoploid (DM) potato (used to generate the genome sequence) with *P. infestans* and analyse the AS/NMD changes during disease development.

Poster 52

Evolution of the neural splicing network regulated by nSR100/SRRM4

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Microexons are small exons as short as 3nt that present a striking neural-specific inclusion. Microexons are the most conserved type of AS events and have been found to be misregulated in autistic patients. Their inclusion is controlled by a vertebrate-specific, brain-specific splicing factor: SRRM4, aka nSR100. Importantly, recent transcriptomic data has shown that microexons are present in many invertebrate species, predating their appearance at least to the origin of bilaterians. Thus, an obvious initial question emerges: how is microexon inclusion regulated in invertebrates, given that they do not have the nSR100 gene? By studying the genome evolution of the srrm2/3/4 locus in invertebrates, we are currently investigating the origins of the nSR100-like function and the microexon splicing network. AS and a neural-specific promoter generate different isoforms from this locus in several invertebrate species; each isoform putatively encoding for the SRRM2-like or nSR100-like functions. This suggests a case of subfunctionalization of the ancestral gene within vertebrates. Identifying the origins of the master regulator of microexons could shed light on the molecular mechanisms involved in microexon recognition; and together with the comparative study of its targets, could inform us about the evolutionary impact that the appearance of this splicing network has had on metazoan nervous systems.

Poster 53

Characterising RBP expression patterns and alternative splicing in response to cold temperature

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Gene expression patterns in plants change dramatically in response to environmental stimuli. Alternative splicing is highly implicated in the regulation of such changes at the post-transcriptional level, mediated by RNA binding proteins (RBPs). Previous studies demonstrated the importance of AS in regulating the expression of core clock genes under low temperature. However, the underlying mechanisms and factors that control cold-sensitive AS are unknown. In Arabidopsis, more than 300 RBPs have been identified with serine/arginine rich proteins (SRs) and heterogeneous nuclear ribonucleoprotein particle proteins (hnRNPs) being the two main families. In order, ultimately, to build splicing networks to identify key splicing factors which are involved in the regulation of temperature-dependent AS, we have performed deep RNA-seq on a high resolution time-course of Arabidopsis plants transferred from 20°C to 4°C. To quantify alternatively spliced transcript variants, a comprehensive non-redundant reference transcript dataset was constructed to analyse the RNA-seq data. Dynamic data for the expression of individual transcripts have been generated, giving us the opportunity to study the effect of low temperature on the AS and expression changes of Arabidopsis RBPs, SFs and spliceosomal protein genes. So far, RBPs with either no change in expression and/or AS or major changes in both expression and AS in response to cold have been identified, including isoform switches that occur rapidly after transfer to cold. Furthermore, the dynamic RNA-seq dataset has allowed the identification of RBP genes which have not previously been associated with the cold response. The RNA-seq data is currently being prepared for construction of transcription and co-splicing networks aiming to identify genes that may regulate alternative splicing in response to low temperature.

Poster 54

The conformational flexibility and the oligomerization state of purified recombinant N-terminal fragment of the HYL1 protein in solution studied by the small-angle X-ray scattering and the circular dichroism spectroscopy

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The HYPONASTIC LEAVES 1 (HYL1) protein is one of the core components of miRNA biogenesis machinery in *Arabidopsis thaliana* alongside the SERRATE (SE) and the DICER-LIKE 1 (DCL1) proteins. It was shown to be required for accurate excision of mature miRNAs from the precursors (pri-and pre-miRNAs) and to interact with both SE and DCL1 *in vitro* and *in vivo*. The N-terminal fragment of HYL1 (residues 15-172, $HYL1_{15-172}$), possessing two double-stranded RNA-binding domains (dsRBD1 and dsRBD2) connected by the linker of 15 amino acids, was shown to be sufficient for rescue of the HYL1-deficient phenotype of *A. thaliana* plants, restoring the level of miRNA accumulation. The crystal structures of dsRBD1 and dsRBD2 showed the double-stranded RNA binding surface and dimerization interface, respectively. The dimerization was further confirmed by the *in vivo* and *in vitro* experiments and found crucial for proper selection of the DLC1 cleavage sites within the miRNA precursor. The dsRBD2 is also responsible for interaction with SE and DCL1 proteins. The in-solution structure of $HYL1_{15-172}$ was examined by the circular dichroism (CD) spectroscopy and the small-angle X-ray scattering (SAXS). As the Kratky plot suggested clear conformational flexibility of the protein, the Ensemble Optimization Method (EOM) was applied for the SAXS data analysis. EOM generates pool of models build up from the rigid bodies connected by linkers of random conformation. In the next step, the software uses the genetic algorithm to select the ensemble of models that fits best to the experimental data. In the modeling we used two pools of models - one generated for monomeric and second one generated for dimeric assembly of HYL_{15-172} - to check the oligomerization state of the protein. We performed EOM analysis using both pools individually and after mixing them both. The best fit (χ^2 = 0.951) was obtained for the ensemble consisting of seven models of the HYL_{15-172}, one of which being model for the dimeric HYL_{15-172} with an abundance of ~ 7% and radius of gyration (R_g) of ~ 42 Å and the rest being models for the monomeric protein with R_g ranging from 21 to 30 Å, thus indicating flexibility. The best χ^2 value obtained from EOM analysis for the exclusively monomeric ensemble was 0.958 with slight discrepancy of the fit at low scattering angles, prompting the conclusion that a small fraction of the dimer was indeed present in the studied sample. This stays in agreement with previously published data, indicating that the purified full-lenght HYL1 is monomeric and needs addition of the dsRNA to be shiftted towards the dimeric state. The results of EOM analysis were complemented by the CD spectroscopy measurements. Fitting the experimental CD spectrum with the Contin-LL algorithm revealed that nearly 40% of the HYL_{15-172} protein is disordered, pointing the low level of the ordered secondary structures and pointing to possible conformational disorder.These results together suggest that the dsRNA-binding domain of the HYL1 protein is connected with the second domain responsible for the dimerization and protein-protein interactions *via* flexible linker and that $HYL1_{15-172}$ protein has dynamic structure in solution. They also show the EOM algorithm as useful in determination of the oligomerization state of protein in the studied sample, as was also shown in the previous works. Hopefully, presented data together with future studies will provide a knowledge on the structural details of the miRNA biogenesis in plants. This work was supported by KNOW RNA Research Centre in Poznań (01/KNOW 2/2014).

Poster 55

Binary and Ternary Interactions of Cap-Binding Complex with Poly(A)-specific Ribonuclease and mRNA 5' Cap

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The 5’ terminal cap structure of the polymerase II transcripts is specifically recognized by different proteins in a variety of nuclear and cytoplasmatic regulatory processes. The nuclear Cap-Binding Complex (CBC) participates in mRNA splicing, in the export of the mature mRNA and U snRNA to the cytoplasm and in the nonsense-mediated mRNA decay (NMD). CBC is a heterodimer composed of a large CBP80 subunit and a smaller CBP20 component, which is an RRM domain responsible for the 5’ cap binding. On the other hand, the CBP80 subunit is an inhibitor of a homodimeric poly(A)-specific ribonuclease (PARN) which deadenylates the 3’ terminal mRNA tail i.a. during the NMD. Interestingly, PARN is an mRNA 3' end degrading enzyme while also simultaneously interacting with the mRNA 5’ cap. The processivity and rate of the deadenylation performed by PARN increase significantly for capped substrates. The CBC inhibitory influence on the PARN activity could be related to regulation of the PARN engagement in the NMD. Here, we present results of surface plasmon resonance binding studies showing the direct interactions in the binary and ternary complexes of CBC, PARN and the 5’ cap.

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Poster 56

To study siRNA associated suppression of Rabies Virus infection and assess its therapeutic potential

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Rabies virus (RABV) belonging to genus Lyssavirus of family Rhabdoviridae causes one of the oldest neurodegenerative diseases known to mankind. Rabies virus is an enveloped, non-segmented negative-stranded RNA virus. As per the WHO studies about 55,000 deaths are still an attribute of rabies infection. Rabies virus has approximately 12 kb long single stranded RNA genome consisting of a leader region followed by genes encoding Nucleoprotein (N), Larger Polymerase (L), Matrix Protein (M), Glycoprotein (G) and Phosphoprotein (P). Although the disease is 100% preventable by vaccines, once the clinical manifestations begin the condition is 100% fatal. The only success story of a survival after clinical management came in 2004 when the patient was put under ketamine induced coma followed by antiviral therapy of Ribavirin, amantadine and medazolam (Milwaukee Protocol). The appreciation of RNA Interference (RNAi) has advanced from basic discovery in lower organism to a powerful genetic tool in knockdown studies and also as a potential candidate for RNA-based therapeutics. The purpose of this study is to apply the concept of RNAi in suppressing rabies virus infection by targeting crucial viral genes like N, P & L with siRNA and observe the effect on the viral replication. The siRNA will be attached to a 29 amino-acid RVG peptide derived from rabies virus glycoprotein to facilitate delivery of siRNA. Briefly, for *in vitro* studies, Neuronal 2a (N2a) cell lines were infected with RABV followed by transfection with siRNA-peptide complex and for *in vivo* studies, mice infected with RABV were injected intravenously with the siRNA-Peptide complex and assessed for viral gene expression by Fluorescence Antibody Test (FAT) and qRT-PCR wherein we observed a reduction in gene expression of targeted viral genes N, P and L. These observations are in coherence with results reported in various studies where RNAi is used as a therapeutic tool for combating rabies. RNAi may therefore be considered as a potential component in the experimental management and therapy of rabies in the clinic.

Poster 57

Dynamic regulation of the *Medicago truncatula* translatome during root nodule symbiosis

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Regulation of gene expression occurs at multiple levels within eukaryotic cells, including chromatin-based, transcriptional and post-transcriptional events. The rise of the techniques associated with transcriptomics has led to the use of steady-state levels of mRNA as a criterion to select and study genes with a possible implication in agronomically important characters. This approach has excluded levels of post-transcriptional regulation, associated with a rapid response through the activation of pre-existing mRNAs. We have previously shown that genes involved in the legume root symbiosis are regulated at the level of their association with the translation machinery. Here, we used Translating Ribosome Affinity Purification (TRAP) combined with RNA-seq to allow the characterization of mRNA and sRNA populations associated to polysomes (referred as the translatome). The characterization of dynamic changes in the translatome of *Medicago truncatula* roots at early stages of the root nodule symbiosis led us to the identification of mRNAs that significantly increased or decreased their levels of association with polysomes, some of which play essential roles in nodulation (e.g., pectate lyase, SINA and NCR secreted peptides). We have also identified a group of genes either up- or down-regulated at translational levels that participate in pre-mRNA splicing, 3'-processing of the pre-RNA, RNA-mediated post-transcriptional gene silencing, miRNA-mediated repression and mRNA turnover, as well as genes involved in epigenetic and transcriptional regulation. The differential expression of these genes was confirmed by RT-PCR and they are being analyzed by functional genomics.

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