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Session 1
Regulation of pre-mRNA processing

Lecture 1.1

Genome-wide analysis reveals photoreceptors regulate alternative splicing in plants

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Light is one of the most important factors influencing plant growth and development. Changes of light condition affect many developmental programs throughout the life cycle of plants. Light-sensing photoreceptors play major roles in regulating photomorphogenic changes via signal transduction and gene regulation. Although various levels of gene expression are modulated by light, regulation at the pre-mRNA splicing step is less discussed. We performed mRNA sequencing to analyze transcriptome changes during light exposure in *Physcomitrella patens*. In addition to transcriptional regulation, light induces intensive alternative splicing. Light-responsive intron retention preferentially occurred in transcripts for photosynthesis and translation in *Physcomitrella*, which reveals that light-mediated splicing regulation has transcript specificity. Many splicing-related and light signaling gene transcripts were alternatively spliced in responding to light changes. Moreover, intron retention was rapidly induced by light but misregulated in phytochrome-deficient and -knockout mutants, suggesting the involvement of photoreceptors in splicing regulation. We also identified an exonic splicing element that potentially functions in the light-regulated intron retention regions. In summary, our results support that during photomorphogenesis, alternative splicing is rapidly fine-tuned by light to re-organize certain metabolic processes and modulate photomorphogenic gene regulation. Photoreceptors primarily participate in regulation of alternative splicing.

Lecture 1.2

Polypyrimidine tract binding proteins – regulators of alternative splicing & development in *Arabidopsis*

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Alternative splicing (AS) allows the formation of distinct transcript variants from one type of precursor mRNA and affects, according to recent studies, more than 60% of all multi-exon genes in *Arabidopsis*. Despite its prevalence, information on the regulatory factors constituting the splicing code and the functional implications of AS in plants is scarce. We have identified polypyrimidine tract binding proteins (PTBs), members of the group of heterogeneous ribonucleoprotein (hnRNP) proteins, as major regulators of AS in *Arabidopsis*. PTB-mediated AS control is linked to diverse biological processes, including seed germination and flowering regulation. PTB knockdown also results in a serrated leaf phenotype, stunted growth, and elevated drought resistance. Our current work aims, on one hand, at providing molecular links between these processes and PTB-mediated AS regulation. On the other hand, the interaction between plant PTB proteins and their target RNAs is further characterized. Our findings will help to decipher the plant splicing regulatory code and are expected to provide novel insight into the versatile functions of AS in plant biology.

Short talk 1.1

The SR45 splicing factor, involved in sugar and ABA signaling, targets alternative splicing of several *Arabidopsis* genes and modulates SnRK1.1 levels

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Alternative splicing is a versatile means of regulating gene expression and generating proteome diversity likely to be crucial in plant responses to external and endogenous cues. Higher plants are able to transduce sugar signals in order to adjust developmental programs, maintain energy homeostasis, and achieve stress tolerance. Glucose, being a carbon and energy source, also plays an important regulatory role as a central signaling molecule. The first enzyme in glycolysis, hexokinase (HXK), is the conserved glucose sensor in a wide range of organisms including plants, where both HXK-dependent and HXK-independent pathways appear to coexist. Furthermore, an *Arabidopsis thaliana* protein kinase, SnRK1.1, has been described as a sugar sensor and central integrator of a transcription network for stress and energy signaling. We previously reported that SR45, a plant-specific SR-related splicing factor, negatively regulates glucose signaling via downregulation of the ABA pathway during early seedling development in *Arabidopsis*. Surprisingly, we found that disruption of *HXK* does not supress the glucose phenotypes of the *sr45-1* mutant, indicating a mechanism independent of this sensor. However, glucose-treated *sr45-1* leaves contain significantly higher levels of the SnRK1.1 protein kinase due to lower degradation rates of this protein in the mutant. Importantly, the *sr45-1* mutation causes changes in alternative splicing of the *5PTase13* gene, encoding an inositol polyphosphate 5-phosphatase previously shown to interact with and regulate the stability of SnRK1.1 *in vitro* thus providing a mechanistic link between SR45's function and the modulation of SnRK1.1 levels in response to glucose. We used a high-resolution RT-PCR alternative splicing panel to identify additional SR45 endogenous targets. To pinpoint direct targets of this splicing factor, we performed electrophoretic mobility shift assays to test the binding of recombinant SR45 to the *5PTase13* mRNA as well as to transcripts of selected genes showing substantial alternative splicing pattern changes in the RT-PCR panel. Finally, using a protoplast transient expression system, we investigated whether the two isoforms of the 5PTase13 protein have a differential effect in the stability of the SnRK1.1 protein kinase.

Short talk 1.2

Critical roles of snRNP biogenesis for spliceosome formation in plant regeneration

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Pre-mRNA splicing is a critical process in gene expression in eukaryotic cells. This molecular process is executed by the spliceosome, which contains UsnRNPs (U small ribonucleoprotein particles) comprised of a specific kind of UsnRNA (uridine-rich small nuclear RNA) and its tightly associated proteins as core factors. *Arabidopsis* mutants *srd2* (*shoot redifferentiation defective2*) and *rid1* (*root initiation defective 1*) were shown to have severe defects in hypocotyl dedifferentiation and *de novo* meristem formation in tissue culture under high temperature conditions. *SRD2* encodes an activator of snRNA transcription, and in the *srd2* mutant, snRNAs were significantly decreased under the restrictive temperature. *RID1* encodes a DEAH-box RNA helicase similar to yeast pre-mRNA splicing factor Prp22. Transient expression analysis using intron-containing reporter genes and RT-PCR analysis on alternative splicing events showed that pre-mRNA splicing was affected by the *srd2* and *rid1* mutations during hypocotyl dedifferentiation. Interestingly, artificial introduction of RID1 could not recover the growth phenotype of yeast *prp22* mutant, indicating that RID1 is not replaceable to Prp22 but has a different function from Prp22 in pre-mRNA splicing at the molecular level; the fact that RID1-YFP was preferentially localized in the nucleolus, a place of snRNP biogenesis, suggested that RID1 would contribute to snRNP biogenesis and consequently spliceosome formation in the nucleolus. The results collectively suggest that robust levels of pre-mRNA splicing, which might be regulated through snRNP biogenesis, are critical for both of cell dedifferentiation and *de novo* meristem formation, elementary steps of plant regeneration.

Lecture 1.3

Decipher the RNA structural code: a transformative platform reveals novel regulatory features

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RNA structure plays critical roles in regulating various post-transcription events involved in translation, splicing, and polyadenylation. However, determining RNA structure *in vivo* has been very challenging, particularly for low abundance RNAs. In most cases RNA structures are based on *in vitro* synthesized RNAs or *in silico* predictions. Also lack of genome-wide *in vivo* RNA structural data limits our understanding of how RNAs fold and regulate gene expression globally *in vivo*. Here, we established a transformative platform to probe *in vivo* RNA structures at both the genome wide scale and in targeted individual cases. The genome wide study reveals native RNA structural features that relate to numerous biological processes including translation initiation and efficiency, alternative polyadenylation, and alternative splicing. The targeted individual RNA structure study has achieved attomolar (10^{-17}) sensitivity, which allows the analysis of very low abundance mRNAs *in vivo*. These novel and powerful methods for the investigation of RNA structure-function relationships in plants and should be applicable to any organism.

Lecture 1.4

Exitron splicing, a new type of alternative splicing event shaping the eukaryotic proteome

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Alternative splicing is a key mechanism for increasing transcriptome and proteome diversity. Here, we identify exitron splicing as a novel type of alternative splicing event in plants and humans. Exitrons (**ex**onic **in**trons) are internal regions of coding exons that can be removed by the spliceosome. Intriguingly, intronless genes can be also alternatively spliced via exitron usage, providing the first evidence of splicing in these genes. We show that exitron splicing is regulated in a tissue-specific manner, in response to stress and by splicing factors. Exitron-encoded sequences contain protein domains interacting with different molecules and are enriched in disordered regions and post-translational modifications; consequently their splicing impacts proteome dynamics and remodeling. We propose a "splicing memory" mechanism for the origin of exitrons whereby exitrons are derived from ancestral coding exons through a history of intron loss and maintenance of vestigial splicing regulatory elements that drives exitron evolution.

Short talk 1.3

Unraveling post-transcriptional networks: Analysis of RNA-protein interactions with RNA immunoprecipitation

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RNA-BINDING PROTEINS (RBPs) control the fate of mRNAs including splicing, 3′ end formation, nuclear export and decay through dynamic interaction with *cis*-regulatory motifs, thereby forming messenger ribonucleoprotein complexes (mRNPs). The knowledge on post-transcriptional networks controlled by RBPs, especially in plants, is limited, not least due to the experimental challenge in defining RNA targets of candidate post-transcriptional regulators. We established a straightforward protocol for *in vivo* RNA immunoprecipitation (RIP) to isolate messenger ribonucleoprotein particles containing RBPs and associated RNAs in Arabidopsis (Köster and Staiger, 2014). Here, an *At*GRP7 (*Arabidopsis thaliana* GLYCINE RICH RNA-BINDING PROTEIN 7)-GFP fusion protein is precipitated via GFP-Trap beads from whole cell extracts. The associated RNAs are isolated and analyzed by quantitative Real Time PCR or high-throughput sequencing. Using this method, we showed that AtGRP7 regulates alternative splicing of downstream targets by direct *in vivo* binding. Additionally, RIP experiments showed that *At*GRP7 interacts with microRNA precursors, suggesting a role of *At*GRP7 in miRNA maturation. Future approaches will focus on the adaptation of iCLIP (individual nucleotide resolution crosslinking and immunoprecipitation; König et al., 2011) to the plant system to get a genome wide insight into the post-transcriptional networks controlled by *At*GRP7 and to identify the precise interaction sites.

Short talk 1.4

Deciphering the functions of P-class PPR proteins by combining bioinformatic and experimental methods

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Pentatricopeptide repeat (PPR) proteins are sequence-specific RNA binding proteins located in organelles that are involved in broad range of post-transcriptional RNA maturation processes. The plant PPR proteins are grouped into P and PLS classes which generally fulfil distinct functions. The majority of the PLS-class PPR proteins function as editing factors. P-class PPR proteins act in defining 5′ and 3′ RNA termini, splicing, and promoting or blocking initiation of translation. Thus P-type PPR proteins play key roles in determining the fate (protection or degradation) of their target RNAs. The recently developed "PPR code" suggests that it might be possible to predict the RNA target sequences of a particular PPR protein. Verifying editing site predictions is technically straightforward, but verification of binding sites of P-class PPR proteins, where no modification of the target RNA ensues, has been challenging. Comparing small RNA libraries between wild-type and mutants lacking specific P-type PPR proteins gives a rapid and effective way of identifying probable binding sites through analysis of putative "PPR footprints" – RNA remnants protected from degradation by a PPR protein. Here we present an example where a combination of bioinformatic and experimental methods have led to elucidation of the function of the SOT1 (suppressor of thylakoid formation 1) protein in maturation of the chloroplast 23S rRNA.

Short talk 1.5

Spliceosome disassembly factor NTR1 is involved in transcriptional pausing at alternative exons in *Arabidopsis*

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The interconnection between transcription and splicing is a subject of intense study in many different organisms. We have identified an *Arabidopsis* homologue of NTR1 (AtNTR1), a conserved spliceosomal disassembly factor, as a protein required for co-transcriptional pausing at alternative splice sites. We report that AtNTR1 is required for the correct expression and splicing of *DOG1*, a regulator of seed dormancy. Analyses of *DOG1* and other genes splicing defects, identification of NTR1 interactors and AtNTR1 co-immunolocalisation with PolII have shown that in addition to a well-conserved function in splicing, AtNTR1 plays a role in transcription elongation at alternative exons. In agreement with the altered elongation rate in *atntr1*, we demonstrate that the majority of splicing defects caused by the lack of AtNTR1 are opposite to changes observed in the TFIIS mutant, in which endonucleolytic cleavage by PolII is blocked. In addition, *atntr1* shows decreased PolII occupancy at the majority of the alternatively misspliced exons and introns tested. We provide evidence that the elongation defects observed in *atntr1* are not an indirect effect of splicing defects. We conclude that AtNTR1 is required for localised transcriptional pausing at the affected alternatively spliced exons and introns.

Lecture 1.5

Alternative splicing controls translation of a novel *Arabidopsis* transporter to promote plant zinc tolerance

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Root vacuolar sequestration is one of the best-conserved plant strategies to cope with heavy metal toxicity. We have found that zinc (Zn) tolerance in *Arabidopsis thaliana* requires the action of a novel membrane transporter belonging to the major facilitator superfamily (MFS). ZIF2 (ZINC-INDUCED FACILITATOR 2) localizes primarily at the tonoplast of root cortical cells and is a functional transporter able to mediate Zn efflux when heterologously expressed in yeast. In *Arabidopsis*, loss of *ZIF2* function exacerbates plant sensitivity to excess Zn, while its overexpression enhances Zn tolerance. Zn content analyses indicate that, although not affecting the overall plant Zn status, ZIF2 activity influences Zn tissue partitioning, driving root immobilization of the metal and thereby preventing its translocation to the shoot. The *ZIF2* gene is Zn-induced and an intron retention event in its 5′ UTR generates two splice variants (*ZIF2.1* and *ZIF2.2*) encoding the same protein. Importantly, high Zn favors production of the longer *ZIF2.2* transcript, which compared to *ZIF2.1* confers greater Zn tolerance to transgenic plants by promoting higher root Zn immobilization. We demonstrate that the retained intron in the *ZIF2* 5' UTR enhances translation in a Zn-responsive manner, markedly promoting ZIF2 protein expression under excess Zn. Moreover, Zn regulation of translation driven by the *ZIF2.2* 5′ UTR depends largely on a predicted stable stem loop immediately upstream of the start codon that is lost in *ZIF2.1.* Taken together, our findings show that alternative splicing controls the levels of a Zn-responsive mRNA variant of the ZIF2 transporter to regulate plant tolerance to the metal ion.

Lecture 1.6

A role for *LSM* genes in the regulation of circadian rhythms in plant and human cells

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Circadian clocks allow organisms to time biological processes to the most appropriate phases of the day and year. We are interested in deciphering the regulatory networks that control clock function in plants, since this knowledge could be used to manipulate flowering time, a key factor influencing crop productivity. There is increasing evidence that propper regulation of clock function involves alterations in alternative splicing (AS) of clock genes, but little is known about the mechanisms linking AS and the clock. We have recently shown that defects in PROTEIN ARGININE METHYL TRANSFERASE 5, which transfers methyl groups to arginine residues present in Sm and LSm spliceosomal proteins, impair circadian rhythms in *Arabidopsis*. Here we show that some *LSM* genes, encoding core components of the spliceosomal U6 SnRNP complex, play a regulatory role in the control of circadian rhythms in plants and mammals. We found the circadian clock regulates expression of *LSM5* in *Arabidopsis* plants and several *LSM* genes in mouse SCN. Further, mutations in *LSM5* or *LSM4* genes in *Arabidopsis*, or down-regulation of *LSM3, LSM5* or *LSM7* expression in human cells, lengthens circadian period. Changes in expression and alternative splicing of some core-clock genes were identified in *Arabidopsis lsm5* mutants, but the precise molecular mechanism causing period lengthening remains to be identified. Genome-wide expression analysis of either a weak *lsm5* or a strong *lsm4* mutant allele in *Arabidopsis* revealed larger effects on alternative compared to constitutive splicing. Remarkably, no statistically significant defects were observed in the majority of all introns evaluated using RNA-seq in the strong *lsm4* mutant allele used in this study. These findings support the idea that some *LSM* genes play regulatory rather than constitutive roles in RNA processing, and that clock regulation of *LSM* gene expression is one mechanism integrating transcriptional and post-transcriptional regulatory layers within plant and mammalian circadian networks.

Lecture 1.7

Alternative splicing of conserved alternative exons and splice sites as a mechanism for gene expression regulation in plants

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Relatively few examples of conserved alternative splicing in plants are known to date. Analysis of the link between alternative splicing (AS) and nonsense-mediated decay (NMD) using a high resolution RT-PCR system identified NMD-sensitive transcripts that contain PTCs located within alternatively spliced exons (Kalyna et al., 2012). Many of these alternative exons (AE) were conserved among different plant species. We therefore performed a genome-wide analysis of alternative exons in Arabidopsis to identify conserved AEs focussing on PTC+ AEs using annotated transcripts from TAIR and assembled transcripts from our AS discovery RNA-seq analysis (Marquez et al., 2012). In addition, an unbiased computational screen for conserved regions within introns identified novel, conserved alternative exons and other alternative splicing events. Multiple sequence alignments have been generated and manually curated. We are currently validating the conserved AS events in both *Arabidopsis* and barley and are examining their AS under stress conditions. Extensive alternative splicing regulates *Arabidopsis* clock genes through dynamic changes in AS transcripts when plants are transferred to low temperatures (James et al., 2012). AS events in *AtLHY* and *AtPRR7*, in particular, are temperature-dependent and generate altered levels of productive mRNAs through AS/NMD. To examine whether clock genes have conserved modes of regulation in other plant species, we identified 21 barley core clock and clock-associated genes, 60% of which are true Arabidopsis orthologues. We have identified AS in some of these genes, including *HvLHY* and *HvPpd-H1* (orthologue of *AtPRR7*) and demonstrate temperature-specific AS. Although there is little conservation of AS events themselves or sequence conservation around splicing events, regulation of expression by AS at low temperatures may be functionally conserved. This novel layer of fine clock control observed in two different species, a model plant and a crop species, might help our understanding of plant adaptation in different environments and ultimately may offer a new range of targets for plant improvement.

Lecture 1.8

Global analysis of targets of a splicing regulator in *Arabidopsis*: Implications in stress responses

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Alternative splicing (AS) of pre-mRNAs has been implicated in modulating several developmental and stress responses in plants. Regulation of alternative splicing is a complex process involving many RNA binding regulatory proteins that modulate splice site choice. Serine/arginine-rich (SR) and SR-like RNA binding proteins are key regulators of both constitutive and alternative splicing of nuclear pre-mRNAs as well as other aspects of RNA metabolism in eukaryotes. SR45, one of the SR-like proteins that is similar to human RNPS1 in its RNA recognition motif and in its domain organization, has been shown to regulate AS of pre-mRNAs of *SR* genes and multiple developmental and stress responses. To gain mechanistic understanding of AS regulation by SR and SR-like proteins in plants it is necessary to i) identify RNAs associated with these proteins, ii) map *cis*-elements necessary for this association, and iii) determine the effect of this association on regulated pre-mRNA splicing. However, no global studies have been performed thus far to identify RNAs associated with any of the plant RNA binding proteins. To address this, we performed the first global study to identify SR45 associated RNAs (SARs) in the transcriptome of *Arabidopsis* seedlings using RNA-immunoprecipitation (RIP) followed by high throughput Illumina sequencing (RIP-seq). The *SR45* knockout mutant line (*sr45*) expressing *SR45-GFP* fusion was used for RIP analysis using a GFP antibody and a transgenic line expressing GFP alone was used as a control. The efficacy of RIP was monitored by measuring the enrichment of two previously known targets of SR45 using quantitative RT-PCR (qRT-PCR). Analyses of the RIP-seq data has revealed that there are >3000 SARs in seedlings. In independently performed RIP assays, the association of 22 out of 23 randomly selected SAR targets was further verified, confirming the validity of our RIP results. Further analyses of these SARs uncovered some unexpected roles of SR45 in RNA metabolism. Analysis of SRAs for potential RNA motifs has led to identification of four significantly over-represented RNA motifs in specific locations relative to splice sites. Two of these are similar to known splicing regulatory motifs whereas the other two are novel. Interestingly, all four motifs identified in SARs were also found in a recent study we performed for discovery of putative splicing regulatory motifs that are conserved across plant species. Finally, the SARs are significantly enriched in transcripts involved in stress responses, confirming the roles of SR45 in regulating stress responsive genes at the post-transcriptional level.

Short talk 1.6

A chloroplast retrograde signal regulates nuclear alternative splicing

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Light is a source of energy and also a regulator of plant physiological adaptations. We show that light/dark conditions affect alternative splicing of a subset of Arabidopsis genes preferentially encoding proteins involved in RNA processing. The effect requires functional chloroplasts and is also observed in roots when the communication with the photosynthetic tissues is not interrupted, suggesting that a signalling molecule travels through the plant. Using photosynthetic electron transfer inhibitors with different mechanisms of action we deduce that the reduced pool of plastoquinones initiates a chloroplast retrograde signalling that regulates nuclear alternative splicing and is necessary for proper plant responses to varying light conditions.

Short talk 1.7

The competition between splicing and microRNA processing machineries in the biogenesis of miRNAs located within the introns of protein-coding genes

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MicroRNAs are small non-coding RNAs of about 21 nt in length, which act by regulating expression of many genes in the cell. The target mRNAs are recognized by miRNAs in a sequence-specific manner and subsequently cleaved or blocked by inhibiting their translation. In plants, miRNAs are encoded mostly by independent transcription units, but it has been also reported that 11 plant miRNAs are embedded within introns of other genes. Our further bioinformatic analyses revealed the existence of the new additional 18 intronic microRNAs. 26 of them are located within protein-coding genes and the 5′ RLM RACE experiments confirmed that their transcription starts from host-gene promoters. Is there a crosstalk between spliceosome and miRNA microprocessor machineries during plant intronic microRNA biogenesis? By now three possible mechanisms of plant intronic pre-miRNA processing were proposed: 1) pre-splicing, 2) co-splicing or 3) post-splicing intronic microRNA cleavage. Yet none of them is experimentally confirmed. To outline the role of spliceosome and plant microprocessor complex during intronic miRNA maturation we selected *A. thaliana* miR402 located within the first intron of protein-coding host-gene – At1g77230. It was previously reported that expression level of the miR402 may vary in response to abiotic stresses. We found upregulation of mature miR402 level in heat-stress conditions and its accumulation was correlated with the inhibition of splicing of miR402-carrying intron. Additionally, within this intron we identified several alternative proximal polyadenylation sites using 3′ RACE PCR analysis. Their activation was strongly associated with splicing inhibition and consequently with mature miR402 accumulation after heat-stress treatment in comparison to normal conditions. To evaluate the exact role of splicing machinery during intronic miR402 processing we decided to generate constructs containing miR402 host-gene under control of the 35S promoter and transiently expressed them in *N. benthamiana* leaves. Identified conservative and alternative splice sites of miR402-bearing intron within host-gene sequence were mutated in all possible combinations. The strong accumulation of the mature miRNA was observed in each construct carrying mutated constitutive 5′SS. Real-time PCR results confirmed splicing inhibition after the constitutive 5'SS inactivation, which was in correlation with the activation of proximal polyadenylation sites within the first intron of the host-gene. These observations supported our results obtained for miR402 biogenesis regulation in *A. thaliana* upon heat-stress treatment. Performed experiments confirmed the strong competition between spliceosomal U1snRNP and plant microRNA biogenesis machinery in the case of miR402 processing: efficient splicing of miRNA-carrying intron results in lower accumulation of mature miR402. This is opposite to the results obtained for exonic miRNAs followed by an intron. We also revealed there may be some additional players, like polyadenylation machinery, involved in plant intronic microRNA biogenesis pathway. Our results show new ways of plant *MIR* genes expression regulation and consequently regulation of their target genes. Funding: This work was supported by the National Science Center (Grant no. 2012/05/N/NZ2/00955) and The Dean of the Faculty of Biology AMU (Grant no. GDWB-03/2013).

Short talk 1.8

Genome-wide analysis of heat-sensitive alternative splicing in *Physcomitrella* patens

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As sessile organisms, plant growth and development are often influenced by temperature fluctuations of the environment. To respond to elevated temperature, the universal heat shock response (HSR) is rapidly activated for plants to prevent damage and enhance tolerance. Different levels of gene regulation are also modulated in the cell. Alternative splicing (AS) is well-known to increase transcriptome complexity and proteome diversity. Although genome-wide studies have revealed complex AS patterns among all kingdoms, there is still less information about whether temperature changes affect the dynamics of AS. Here we used heat shock (HS) treatments at nondamaging temperature and mRNA sequencing to obtain HS transcriptomes in the moss *Physcomitrella patens*. Data analysis identified significant amount of novel AS events in the moss protonema. Nearly 50% of genes are alternatively spliced. Intron retention (IR) is markedly repressed under elevated temperature but alternative donor/acceptor site and exon skipping are induced, indicating differential regulation of AS in response to heat. Transcripts undergoing heat-sensitive IR are mostly involved in specific functions, which suggest that plants regulate AS with transcript specificity under elevated temperature. An exonic GAG-repeat motif in these IR regions may function as a regulatory cis element in heat-mediated AS regulation. A conserved AS pattern for HS transcription factors in *Physcomitrella* and *Arabidopsis* reveals heat regulation for AS evolved early during land colonization of green plants. Our results support that AS of specific genes, including key HS regulators, is fine-tuned under elevated temperature to enhance gene regulation and reorganize metabolic processes.

Short talk 1.9

CBC and SERRATE cooperate in alternative splicing regulation of *Arabidopsis thaliana* gene transcripts

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Two subunits of the *Arabidopsis thaliana* nuclear cap-binding protein complex (AtCBC), AtCBP20 and AtCBP80, and the SERRATE protein (AtSE) have overlapping function in both miRNA biogenesis and pre-mRNA splicing. Recently, we have shown that AtCBC and AtSE can also regulate alternative splicing (AS) in *Arabidopsis thaliana*. Using an RT–PCR alternative splicing panel we analyzed alternative splicing events in selected *Arabidopsis* genes, encoding mainly transcription factors, splicing factors and stress-related proteins. The analyzed events included alternative 5′ and 3′ splice site selection, exon skipping and intron retention. Splicing profiles were determined in wild type plants, and the T-DNA insertion mutants: *cbp20*, *cbp80(abh1), se-1* and the *cbp20/80* double mutant. In the cases that showed significant changes in AS, the *cbc* mutants preferentially affect alternative splicing events located closest to the cap: within the first intron of the transcript, and particularly at the 5′ splice site. Most of these changes were common in all three *cbp* mutants suggesting that AtCBC is directly involved in the regulation of these alternative splicing events. Moreover, as we observed more changes in the *cbp80(abh1)* and *cbp20/80* mutants than in the *cbp20* mutant, we concluded that AtCBP80 plays a more significant role in alternative splicing than AtCBP20, probably being a platform for interactions with other splicing factors. Interestingly, many changes observed in the *cbp* mutants were common to those observed in the *se-1* mutant. Consequently, AtSE influences alternative splicing in a similar way to AtCBC – preferentially affecting selection of the 5′ splice site of first introns. However, the changes observed in *se-1* did not correspond with the changes observed in other mutants of plant miRNA biogenesis pathway, *hyl1-2* and *dcl1-7*, suggesting that the role of SERRATE in alternative splicing regulation is distinct from its role in miRNA biogenesis. In conclusion, the AtSE protein acts in cooperation with AtCBC in alternative splicing regulation of some transcripts in plants. Indeed, using BiFC and pull down techniques we showed that both AtCBP20 and AtCBP80 co-localize and directly interact with AtSE. Possibly, AtCBC and AtSE specify the binding of U1 snRNP to the 5′ splice site on the first intron of some pre-mRNAs. To confirm AtCBC/AtSE/U1 snRNP communication we will compare the distribution of AtCBC, AtSE and components of U1 snRNP throughout the fractions after size-fractionation of protein extract on continuous glycerol gradient.