Arabidopsis eIF4E1 protects the translational machinery during TuMV infection and restricts virus accumulation

Delyan Zafirov, Nathalie Giovinazzo, Cécile Lecampion, Ben Field, Julia Novion Ducassou, Yohann Couté, Karen S Browning, Christophe Robaglia, Jean-Luc Gallois

Post-transcriptional Gene Regulation in Plants 2024

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Table of contents

PGRP2024 Program

Location : Building B - Edouard Chatton - First Floor
20 Av. Pierre Fabre, 66650 Banyuls-sur-Mer

Tuesday 3 Morning

10:00 - 13:00 Registration Building B - Edouard Chatton - First Floor

Tuesday 3 Afternoon

13:30 - 13:45 Opening Rémy Merret

Session 1 mRNA translational control

13:45 - 14:45 Keynote Speaker Julia Bailey-Serres
Looking beyond transcription: post-transcriptional regulation and climate resilience

14:45 - 15:05 Talk 1 Polly Hsu
A Warning Note about 5'UTRs: Transcription Start Sites Have Profound Effects on uORF-Mediated Translational Regulation in Arabidopsis

15:05 - 15:25 Talk 2 Jeoffrey George
5'UTR cis-elements in soybean WLT1 fine-tune translation for waterlogging tolerance

15:25 - 15:45 Talk 3 Ming-Jung Liu
Using Machine Learning to Discover Translation Initiation Sites and Their Cis-Regulatory

15:45 - 16:20 Coffee break

16:20 - 16:40 Talk 4 Arnaud Dannfald
Plant response to intermittent heat stress involves modulation of mRNA translation efficiency

16:40 - 17:00 Talk 5 Annika Liefferink
PUMILIO RNA binding proteins and their role in seed germination

17:00 - 17:20 Talk 6 Sjors van der Horst
Ribosomal Traffic Jams: The Role and Regulation of Pausing in Plant mRNA Translation Under Hypoxia Stress

17:20 - 17:40 Talk 7 Catharina Merchante
Deciphering the role of ribosome heterogeneity in gene-specific translation

17:40 - 18:00 Talk 8 Said Hafidh
Characterising RNA Binding Proteins (RBPs) in plant fertility and response to heat stress

18:00 - 18:20 Talk 9 Jean-Luc Gallois
Arabidopsis eIF4E1 protects the translational machinery during TuMV infection and restricts virus accumulation

19:30 - 22:00 Dinner Building D - Accommodation Centre

Wednesday 4 Morning

Session 2 mRNA fate

08:45 - 09:25 Invited Speaker 1 Dominique Gagliardi
Processes shaping mRNA poly(A) tails in Arabidopsis

09:25 - 09:45 Talk 10 Michal Krzyszton
Untangling transcriptional and posttranscriptional regulation during early seed germination

09:45 - 10:05 Talk 11 Misato Ohtani
Early auxin response regulated by mRNA metabolism is key to environmental adaptation of Arabidopsis seedlings

10:05 - 10:40 Coffee break

10:40 - 11:00 Talk 12 Leslie Sieburth
RNA Decay typically contributes to mRNA abundance regulation by opposing the impact of transcription

11:00 - 11:20 Talk 13 Damien Garcia
A multi-transcriptomics approach identifies targets of the endoribonuclease DNE1 and provides insights on its coordination with decapping

11:20 - 11:40 Talk 14 David Alabadi
A cochaperone complex mediates high temperature-induced co-translational mRNA decay in Arabidopsis

11:40 - 12:00 Talk 15 Hélène Zuber
mRNA uridylation as a novel post-transcriptional process regulating seed maturation

12:00 - 13:40 Lunch Building D - Accommodation Centre

Wednesday 4 Afternoon

Session 3 RBPs identification and RNA splicing

13:50 - 14:30 Invited Speaker 2 Dorothee Staiger
Partners in time - Insights into the binding landscape of RNA-binding proteins in Arabidopsis

14:30 - 14:50 Talk 16 Christian Schmitz-Linneweber
A phase-separated compartment tunes cold acclimation of chloroplast RNA metabolism

14:50 - 15:10 Talk 17 Magdalena Weingartner
Analysing phase separation of conserved translation factors during heat stress response in Arabidopsis thaliana

15:10 - 15:30 Talk 18 Julieta Mateos
When posttranslational modifications and splicing shake hands to fine-tune stress responses

15:30 - 16:00 Coffee break

16:00 - 16:20 Talk 19 Shih-Long Tu
RNA-binding proteins selectively associate with pre-mRNAs for plant temperature responses

16:20 - 16:40 Talk 20 Chin-Mei Lee
MAC3A and MAC3B modulate FLM splicing to repress photoperiod-dependent floral transition

16:40 - 17:00 Talk 21 Matt Jones
REVEILLE2 thermosensitive splicing: A molecular basis for the integration of nocturnal temperature information by the Arabidopsis circadian clock

17:00 - 17:20 Talk 22 Kamil Ruzicka
CATSNAP - a machine-learning tool that reveals the plasticity of alternative splicing in plants and animals

18:30 - 22:00 Session Poster and Buffet Dinner - Biodiversarium - Building C

Thursday 5 Morning

Session 4 ncRNAs

08:45 - 09:25 Invited Speaker 3 Sebastian Marquardt
Languages of the Non-coding Genome

09:25 - 09:45 Talk 23 Tomas Maria Tessi
Connecting dots: how actin binding proteins link siRNA biogenesis and stress

09:45 - 10:05 Talk 24 Alberto Carbonell
Next-Generation Antiviral Vaccines Based on Synthetic Trans-Acting Small Interfering RNAs Produced from Viral Vectors

10:05 - 10:40 Coffee break

10:40 - 11:00 Talk 25 Aline Koch
RNA Sprays - harnessing the function of coding and non-coding RNAs for plant applications

11:00 - 11:20 Talk 26 Soledad Traubenik
The subunit 3 of the SUPERKILLER 3 complex mediates miR72-directed cleavage of Nodule Number Control 1 to control nodulation in Medicago truncatula

11:20 - 11:40 Talk 27 Mateusz Bajczyk
The cross-talk between PCF11-similar proteins and CstF64 in flower development in Arabidopsis thaliana

11:40 - 12:00 Talk 28 Bharti Aggarwal
MpmiR11887 and MpmiR11796 are involved in proper sexual organ formation in Marchantia polymorpha

12:00 - 13:40 Lunch Building D - Accommodation Centre

Thursday 5 Afternoon

Session 5 RNA structure, modifications and lncRNAs

13:50 - 14:30 Invited Speaker 4 Yiliang Ding
RNA structure, an important regulator in living cells

14:30 - 14:50 Talk 29 Philip Bevilacqua
Improving RNA structure determination by using multiple chemical probes

14:50 - 15:10 Talk 30 Rica Burgardt
The structured mRNA element DEAD can sense RNA helicase activity to regulate alternative splicing

15:10 - 15:30 Talk 31 Michel Heidecker
LncRNAs modulating alternative splicing in the regulation of the plant transcriptome

15:30 - 15:50 Talk 32 Caylyn Railey
The impact of a lncRNA on seedling development

15:50 - 16:20 Coffee break

16:20 - 16:50 Invited Speaker 5 Brian D. Gregory
The effects of RNA modifications on plant biology

16:50 - 17:10 Talk 33 Federico Ariel
Plant Long Noncoding RNAs - From Molecular Mechanisms to Agricultural Advancements

17:10 - 17:30 Talk 34 Kyle Palos
Diverse mRNA modifications influence stability, splicing, and stress responses across flowering plants

17:30 - 17:50 Talk 35 Jakub Dolata
small RNA biogenesis: co-transcriptional regulation and RNA modifications

17:50 - 18:10 Talk 36 Yuan Zhou
Viral RNA methylation and intercellular mobility

18:10 - 18:30 Talk 37 Cécile Bousquet-Antonelli
Unbiased identification of novel non-YTH m6A readers from Arabidopsis thaliana

18:30 - 18:45 Closing remarks Rémy Merret

Session 1 : mRNA translational control

Looking beyond transcription: post-transcriptional regulation and climate resilience

Julia Bailey-Serres *† [1]

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The Post-Transcriptional Gene Regulation of Plants meetings have been a long-running forum for presentations and discussions on the diverse RNA mechanisms of plants and their viruses. The first clues of post-transcriptional regulation of gene expression in plants came from study of changes in protein synthesis in response to heat and hypoxia. The proteins made during a sudden stress, as detected by labeling *in vivo* with radioactive amino acids, were far fewer than those synthesized *in vitro* from polyadenylated RNAs extracted from the same samples. Yet, the essential role of transcriptional activation of genes has largely overshadowed the post-transcriptional splicing, modification, turnover, and sequestration mechanisms that are essential for adaptation and plasticity. At the first PGRP meeting, held in California in 1997, we were just learning that certain mRNAs have 5' UTR sequences that determine their environmentally regulated translation and that *SMALL AUXIN UPREGULATED* (*SAUR*) mRNAs can have 3' UTR sequences sufficient to conditionally promote their turnover. These findings advanced the concept that mRNAs with shared features may be co-regulated. By 1999, progress reported at the meeting was astounding. This included mechanisms of splicing, 3' polyadenylation, the role of viral uORFs and the exoribonuclease XRN4, as well as pervasive RNA editing in mitochondria and chloroplasts. The meeting also included discoveries in posttranscriptional gene silencing, including the first glimpse of small interfering (si)mRNA. Twenty-five years later, our understanding of the mechanistic nuances of RNA mechanisms in plants is astounding. In my talk, I will highlight how methodologies have progressively advanced perspective on the continuum of gene regulation underlying abiotic responses. Now, genetic variation in mRNA sequences recognized in pan-genomes promise to aid improvement of climate resiliency in crops.

Keywords: PGRP, mRNA, post, transcriptional regulation, translation

*Speaker

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A Warning Note about 5 UTRs: Transcription Start Sites Have Profound Effects on uORF-Mediated Translational Regulation in Arabidopsis

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In eukaryotic mRNAs, upstream open reading frames (uORFs) in the 5 untranslated regions (5 UTRs) often attenuate the translation of downstream main ORFs (mORFs). While some uORFs are beneficial by playing important regulatory roles, uORFs are generally disfavored in evolution. Here we studied how uORF repression is suppressed in Arabidopsis. We found that the heterogeneous distribution of transcription start sites (TSSs) results in heterogeneous 5 UTRs that selectively exclude uORFs from mRNAs. Thus, only a subset of the transcripts from "uORF-containing" genes truly contain uORFs. Importantly, the fraction of uORFs remaining within transcripts determines uORF overall repressiveness. Interestingly, uORFs that encode conserved peptides are almost exclusively preserved within mRNAs, implying coevolution between TSSs and functional uORFs. Consistent with TSSs determining uORF presence, a sharp transition of AUG frequency between promoters and 5 UTRs was observed, and this pattern differentiates between genes lacking and carrying translated uORFs. Remarkably, while 55% of the genes are predicted to contain uORFs, upon accounting for the heterogeneous TSSs, only 9% of the transcripts within the mRNA pool genuinely contain uORFs. Our results highlight a profound effect of TSS distribution in determining uORF repressiveness, a factor that was previously overlooked. As a warning note, the TSS heterogeneity should be taken into consideration when studying various 5 UTR features, such as RNA structures and protein binding motifs, in post-transcriptional gene regulation. The uORFs and other features preferentially preserved in 5 UTRs (i.e., downstream of TSSs) are more likely to be functional as the result of natural selection.

Keywords: 5 UTR, uORF, translation, transcription start

*Speaker

5'UTR cis-elements in soybean WLT1 fine-tune translation for waterlogging tolerance

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Post-transcriptional mRNA regulation plays a crucial role in fine-tuning gene expression, particularly through the interplay between *cis*-acting elements within untranslated regions (UTRs) and *trans*-acting factors. The 5' untranslated region (5'UTR) is a key regulatory region for translation initiation, typically with a 5' m7pppG cap for stability and sometimes containing upstream open reading frames (uORFs) that can modulate translation efficiency. Here, we explore the regulatory potential of the remarkably long 5'UTR of the soybean *WATERLOGGING-TOLERANCE 1* (*WLT1*) mRNA. This 5'UTR harbors multiple regulatory elements, including six upstream uORFs. Sequence variations in the 5'UTR from soybean accessions, evaluated using near-isogenic lines, correlate with waterlogging tolerance. While uORF polymorphisms do not directly affect the main open reading frame (mORF) translation, they contribute to an overall reduction. Interestingly, an extended motif in the tolerant allele leads to a stronger negative impact on translation. Further investigation revealed cap-independent translation initiation within the 5'UTR, with the extended motif specifically repressing the mORF translation in a cap-dependent manner. This intricate regulatory network within the *WLT1* 5'UTR allows for precise control of protein abundance, ultimately impacting waterlogging tolerance, auxin levels, and lateral root emergence. Our findings highlight the critical role of 5'UTR elements in fine-tuning plant responses to environmental stress and offer novel insights into post-transcriptional regulation mechanisms.

Keywords: translation initiation, uORF, 5' cap, waterlogging, auxin

*Speaker

Using Machine Learning to Discover Translation Initiation Sites and Their Cis-Regulatory Controls

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The translation of mRNA into protein is at the heart of gene expression. A key factor in this process is the accurate identification of translation initiation sites (TISs) by ribosomes. This ensures the protein-coding potential of mRNA is regulated and allows for timely and precise protein production in plants. Our previous research demonstrated that over 30% of plant genes have novel TISs, which differ from the annotated AUG sites and can encode small peptides or play a regulatory role in protein diversity and subcellular localization. This leads us to the question of how plant ribosomes recognize these novel TISs? By incorporating machine learning, computational, and experimental techniques, we identified the hidden cis-regulatory sequence features of these novel TISs, including a novel CU-rich sequence that is critical and conserved across plant species and viruses. The presence of these cis-regulatory signatures across various organisms suggests their broad and significant roles in controlling protein synthesis. Furthermore, the TIS prediction models derived from humans and tomato provide global estimates of TISs, enabling the discovery of overlooked protein-coding genes across mammals, plant species, and viruses. By leveraging ML modeling into TIS identification, we introduce a comprehensive framework for users to reveal the translational landscape in eukaryotes and viruses.
Selected References:

T.-Y. Wu*, Y.-R. Li, K.-J. Chang, J.-C. Fang, D. Urano and M.-J. Liu*. (2024) Modeling alternative translation initiation sites in plants reveals evolutionarily conserved cis-regulatory codes in eukaryotes. *Genome Research 34, 272–285 (Featured as the cover story)* J.-C. Fang and M.-J. Liu*. (2023) Translation initiation at AUG and non-AUG triplets in plants. *Plant Science* 335 (2023) 111822 Y.-R. Li and M.-J. Liu*. (2020) Prevalence of alternative AUG and non-AUG translation initiators and their regulatory effects across plants. *Genome Research* 30, 1418-1433.

Keywords: Translation, RNA Biology, Molecular Biology, Predictive Biology, Data, Mining

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Plant response to intermittent heat stress involves modulation of mRNA translation efficiency

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Acquired thermotolerance (also known as priming) is the ability of cells or organisms to better survive an acute heat stress if it is preceded by a milder one. In plants, acquired thermotolerance has been studied mainly at the transcriptional level, including recent descriptions of sophisticated regulatory circuits that are essential for this learning capacity. In this work, we tested the involvement of polysome-related processes (translation and cotranslational mRNA decay (CTRD)) in plant thermotolerance using two heat stress regimes with and without a priming event. We found that priming is essential to restore the general translational potential of plants shortly after acute heat stress. We observed that mRNAs not involved in heat stress suffer from a reduction in translation efficiency at high temperature, whereas heat stress-related mRNAs are translated more efficiently under the same condition. We also show that the induction of the unfolded protein response (UPR) pathway in acute heat stress is favoured by a previous priming event and that, in the absence of priming, ER-translated mRNAs become preferential targets of CTRD. Finally, we present evidence that CTRD can specifically regulate more than a thousand genes during heat stress and should be considered as an independent gene regulatory mechanism.

Keywords: mRNA translation, heat stress, memory

*Speaker

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PUMILIO RNA binding proteins and their role in seed germination

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On a translational level, the seed is a unique phase of the plant's life cycle. Dry seeds contain many stored mRNAs, and the translation of these mRNAs is essential for seed germination. On the other hand, transcription is not necessary to complete germination, as was demonstrated by germination experiments in the presence of transcriptional and translational inhibitors. Moreover, it was revealed that seed germination is mainly regulated at the translational level. We aim at determining how the translation of those seed stored mRNAs is regulated during germination. We focused on mRNA binding proteins (RBPs) since they are known as important regulators of translation. In four timepoints of germination, we identified hundreds of RBPs through an mRNA interactome capture analysis. This led us to focus on the family of PUMILIO (PUM) RNA binding proteins. Through a RIPseq analysis we then identified the mRNAs that are bound by a seed specific Arabidopsis PUM protein. We found that the PUM protein changes its pool of target mRNAs upon salt stress, and this coincides with inhibition of germination when the protein is present. We also found that PUM knock-out mutants are hyposensitive to germination in salt stress. Based on these results we propose a role for PUM in the regulation of seed germination and germination under salt stress. We will present our latest results, which include information about the phosphorylation of the seed specific PUM protein that we are interested in.

Keywords: RNA binding protein, PUMILIO, seeds, germination, salt stress, RIPseq, phosphoproteome

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Ribosomal Traffic Jams: The Role and Regulation of Pausing in Plant mRNA Translation Under Hypoxia Stress.

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An mRNA can be decoded by several ribosomes at once, enhancing protein output. However, ribosome movement on mRNA isn't always smooth. Energy level fluctuations such as during hypoxia stress, and other factors, can cause ribosome pausing, potentially leading to collisions of ribosomes. Such stalling is not merely a roadblock; it serves vital regulatory functions. For instance, upstream (u)ORFs with specific amino acid sequences can induce stalling in the presence of certain metabolites, acting as a metabolic sensor in plants to regulate protein synthesis. Moreover, there is evidence of induced co-translational decay of mRNAs with stalled ribosomes. To investigate ribosome pausing on a genome-wide scale, di-ribosome-sequencing (disome-seq) was developed, a variant of monoribosome-seq (or ribosome-profiling). Instead of taking ¸˜30nt footprint of individual ribosomes, disome-seq captures the ¸˜60nt footprints of two collided ribosomes, highlighting pause sites. Thus far, large-scale analysis of collided ribosomes in plants remains absent, including its potential relation to co-translational decay. We have applied mono- and disome-seq in plants to hypoxia treated *Arabidopsis thaliana* seedlings and *Zea mays* (corn) stems. By capturing a wider range of footprint lengths we were able to identify 8 different types of ribosome conformations: ribosomes with empty or occupied A-sites as monosomes, or disomes that were tightly stacked or had 1 or 2 codons in between. Additionally, mRNA decay intermediates were analyzed using degradome-seq. Disome-seq confirmed stalling at known sites, and discovered many novel sites on both uORFs and mORFs. Similar to mammals and yeast, we observed slow decoding of polyprolines in plants. Interestingly, hypoxia stress resulted in slower decoding of aspartate codons in the ribosome A-site, likely caused by altered metabolism resulting in reduced cytosolic aspartate levels. While high co-translational decay was not observed on polyproline containing transcripts, it was induced on hypoxia-induced aspartate stall sites, indicating selective activation of co-translational decay by ribosome stalling. This study provides a comprehensive analysis of ribosome footprints, allowing us to compare positions of 8 different types of ribosome confirmations, and mRNA decay in plants. This provides a comprehensive list of conserved and stress-induced stall sites and reveals features involved in ribosome stalling.

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Keywords: ribosome stalling, co, translational decay, abiotic stress, ribosome profiling

Deciphering the role of ribosome heterogeneity in gene-specific translation

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Translational regulation is crucial for cellular responses to internal and environmental cues. However, the mechanisms underlying the selective translation of specific mRNAs remain poorly understood. Traditionally, ribosomes, the molecular machines that synthesize proteins, were viewed as passive entities in mRNA selection. This perspective is shifting, as recent studies indicate that ribosomes could be specialized and actively regulate the translation of distinct mRNA subpools (Genuth & Barna, 2019). In plants, the potential for specialization is more pronounced as each ribosomal protein (RP) is encoded by a gene family. Literature suggests these paralogs may have differential roles, though it is not yet confirmed whether this heterogeneity enables selective translation of specific mRNAs under certain cellular conditions. Our research aims to understand the biological significance of this extensive RP diversity and its potential link to adaptation. For this, we are studying various Arabidopsis RP families, focusing here on the eL24. eL24 comprises two paralogs that are ubiquitously expressed at similar levels in Arabidopsis. Previous studies identified significant growth retardation and auxin-defective phenotypes associated with the absence of eL24y, while eL24z was not studied (Nishimura et al., 2005; Park et al., 2017). By characterizing mutants of both paralogs, we found that eL24y and eL24z are involved in assembling the 80S ribosome, are components of actively translating ribosomes, and share common functions in translation. However, our sequencing studies reveal a more substantial impact on the translational machinery in el24y. Since overall translation remains unaffected in the mutants, the phenotypic differences likely stem from eL24y's specific role in translation reinitiation (Zhou et al, 2010), a process less influenced by eL24z according to our findings. Ongoing experiments aim to definitively determine whether these two paralogs within the eL24 family serve distinct functions in translation. Acknowledgments: Work funded by Grants BIO2017-82720-P, PID2021-123240NB-100, and RYC-2017-22323 from MINECO and UMA20-FEDERJA-100 grant from Junta de Andalucía to CM; PRE2018-083348 fellowship from MICIU to JADC, Plan Propio de investigación from the University of Málaga, Campus de Excelencia Andalucía Tech. We are grateful to Dr. Eduardo de la Peña (IHSM) for his help in statistical analyses.

Keywords: translation regulation, ribosomes, uORFs

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Characterising RNA Binding Proteins (RBPs) in plant fertility and response to heat stress

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Posttranscriptional gene regulation in flowering plant gametophyte plays a crucial role in regulating gametophyte development and plants fertility. Using genetic and transcriptomic approaches, a number of RNA binding proteins are now known to be central in the posttranscriptional control of RNA fate and their contribution towards pollen tube guidance, ovule competence to attract pollen tubes and successful fertilization. Their role under heat stress is yet to be fully elucidated. Here, I will exemplify three **RBPs** of diverse function, **LARP6C**, **PRP8** and **eIF3** as regulators of the aforementioned biological function and provide preliminary insight on their molecular dynamic in the gametophyte upon heat stress response.
Acknowledgement: The research receive financial support from the Czech Science Foundation and the European Regional Development Fund-Project "Centre for Experimental Plant Biology. No. CZ.02.1.01/0.0/0.0/16_019/0000738) and GACR grant No. 22-29717S
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Keywords: Posttranscriptional regulation, RNA binding protein, Pollen tube, VAHEAT, live cell imaging

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Arabidopsis eIF4E1 protects the translational machinery during TuMV infection and restricts virus accumulation

Delyan Zafirov [1,2], Nathalie Giovinazzo [3], Cécile Lecampion [2], Ben Field [2], Julia Novion-Ducassou [4], Yohann Couté [4], Karen S Browning [5], Christophe Robaglia [2], Jean-Luc Gallois * [3]

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Successful subversion of translation initiation factors eIF4E determines the infection success of potyviruses, the largest group of viruses affecting plants. In the natural variability of many plant species, resistance to potyvirus infection is provided by polymorphisms at *eIF4E* that renders them inadequate for virus hijacking but still functional in translation initiation. In crops where such natural resistance alleles are limited, the genetic inactivation of *eIF4E* has been proposed for the engineering of potyvirus resistance. However, recent findings indicate that knockout *eIF4E* alleles may be deleterious for plant health and could jeopardize resistance efficiency in comparison to functional resistance proteins. Here, we explored the cause of these adverse effects by studying the role of the *Arabidopsis eIF4E1*, whose inactivation was previously reported as conferring resistance to the potyvirus clover yellow vein virus (ClYVV) while also promoting susceptibility to another potyvirus turnip mosaic virus (TuMV). We report that *eIF4E1* is required to maintain global plant translation and to restrict TuMV accumulation during infection, and its absence is associated with a favoured virus multiplication over host translation. Furthermore, our findings show that, in the absence of eIF4E1, infection with TuMV results in the production of a truncated eIFiso4G1 protein. Finally, we demonstrate a role for eIFiso4G1 in TuMV accumulation and in supporting plant fitness during infection. These findings suggest that eIF4E1 counteracts the hijacking of the plant translational apparatus during TuMV infection and underscore the importance of preserving the functionality of translation initiation factors eIF4E when implementing potyvirus resistance strategies.

Keywords: Translation initiation, Potyvirus, eIF4E, eIF4G, Arabidopsis thaliana

*Speaker

Session 2 : mRNA fate

Processes shaping mRNA poly(A) tails in Arabidopsis

Quentin Simmonot [1], Pietro Giraudo [2], Hanzhang Yu [2], Jackson Peter [2], Léa Gerber [2], Elodie Ubrig [2], Alioua Abdelmalek [2], Hélène Zuber [2], Dominique Gagliardi *† [2]

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Gene expression is regulated at numerous post-transcriptional steps, and many of those processes are being characterized for decades in plants. Yet, how mRNA poly(A) tail metabolism contributes to regulating gene expression remains largely unexplored, and is even frequently misunderstood because of erroneous assumptions. The advent of new sequencing technologies developed by PacBio or Oxford Nanopore Technologies (ONT) has revolutionized our ability to investigate the metabolism of poly(A) tails. It is now possible to accurately profile the distribution of poly(A) tail sizes at the transcriptome level. We used Nanopore sequencing to investigate mRNA poly(A) tail regulation in Arabidopsis, and more precisely we compared global mRNA polyadenylation profiles between wild-type plants and mutants compromised for the expression of factors stabilizing or destabilizing mRNAs. By leveraging Nanopore data, we also analyzed the uridylation status of mRNAs, i. e. the presence of one or several uridines added to the 3' end of poly(A) tails. Our data reveal both gene-specific and general mechanisms regulating poly(A) tail profiles, and the intimate connection between deadenylation and uridylation processes. I will particularly discuss how the antagonistic effects of uridylation and deadenylation shape the poly(A) tail profiles of Arabidopsis mRNAs. Of note, the shift in mRNA poly(A) distribution between uridylation and deadenylation mutants is much more pronounced in rosette leaves than in flower buds, indicating that the equilibrium between uridylation and deadenylation is modulated during development.

Keywords: PolyA tail, Nanopore, mRNA polyadenylation

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Untangling transcriptional and posttranscriptional regulation during early seed germination

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Seed maturation and germination are accompanied by dramatic changes in mRNA levels, which are needed to ensure proper development and enabling responses to environmental conditions. Notably, seed maturation on the mother plants not only prepares the seed for desiccation but also for the first stages of germination immediately after water uptake. This includes the preproduction of multiple mRNAs stored in dry seeds. However, the extent of the posttranscriptional and transcriptional processes taking place during the most crucial first hours of germination is largely unknown. Here, using transcription inhibition and a transcript labelling method, I was able to decouple de novo transcription during the early stages of seed germination from posttranscriptional RNA processing. We show that the degradation of most maturation-associated mRNAs stored in seeds is a default program, as neither different seed maturation conditions nor exogenous ABA affects this early event. The transcript labelling method allowed us to select newly made transcripts and describe their kinetics during early seed germination. Surprisingly, newly produced mRNA of some genes are removed early during germination. This suggests that transcription during imbibition upon standard conditions produces excessive mRNAs, and this pool of transcripts is fine-tuned at the posttranscriptional level to adjust to external conditions during germination.

Keywords: seed germination, 4tU, ABA, mRNA degradation

*Speaker

Early auxin response regulated by mRNA metabolism is key to environmental adaptation of Arabidopsis seedlings

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Plants cannot escape from poor environments, therefore they constantly sense and respond to environmental changes, to adapt their growth and development to those environments. One of the key mechanisms for the gene regulation in response to the environment is active RNA metabolic regulation, such as pre-mRNA splicing and RNA degradation.
Nonsense-mediated mRNA decay (NMD) is a common RNA quality control mechanism in eukaryotes. We have been studying the NMD-deficient mutants *upf1-1/lba-1* and *upf3-1* mutants of *Arabidopsis thaliana*. These *upf* mutants show pleiotropic phenotypes, such as abnormal seed development, early flowering, pathogen response defect, and abnormal shoot regeneration. Here, our new sets of RNA-seq and nano-pore seq analyses of the *upf* seedlings focus on abnormalities in the expression and processing of mRNAs for certain sets of auxin-related genes. Indeed, we could recognize putative NMD-eliciting features in auxin-related genes, and especially in early auxin-responsive genes. Phenotypic analysis of *upf* mutants indicated their seedling developmental defects; we found that apical hook formation and root gravitropic response, typical auxin-mediated responses in seedling, were affected by the *upf* mutations, and these phenotypes can be partly attributed to abnormal regulation of auxin distributions.
The combination of alternative splicing (AS) and NMD has been known to assist the adaptation of plants to the various stresses by qualitatively controlling the homeostasis of functional and non-functional alternatively spliced isoforms. Together with our findings, we would like to propose that such AS-NMD-based adaptation can include the regulation of auxin response, for quick and effective environmental responses required in early seedling development.

Keywords: NMD, premRNA splicing, auxin, seedling development

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RNA Decay typically contributes to mRNA abundance regulation by opposing the impact of transcription

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To characterize the roles of mRNA decay in regulation of mRNA abundances, we induced widespread changes in gene expression by providing a vascular transdifferentiation signal to young leaves. We quantified mRNA half-lives in stimulus and mock-treated leaf samples and found that 22% of the mRNAs shifted their half-lives. To understand how these shifts in half-life affected mRNA abundances, we determined rates of RNA abundance change (RNAseq times series analysis) across the 8 h time point used for RNA decay analysis, and along with abundances and RNA half-lives, we calculated transcription rates. Analysis primarily focused on the 4,268 mRNAs with changing abundance. Transcription was a major driver of RNA abundance change, while decay (alone) regulated a small group of genes. For mRNA abundances regulated by transcription and decay, it was most common that shifts in RNA half-lives opposed the action of transcription (oppositional regulation), but mRNAs whose abundances were regulated by a synergistic interaction between decay and transcription were also found. Steady-abundance mRNAs also fell into four different regulatory groups, the most interesting of which was a set of mRNAs whose abundances were buffered (half-life and transcription rates were altered but abundances remained the same). How transcription and decay are coordinated in buffered RNAs is unknown but likely requires nuclear-cytoplasmic interaction. Kinetic features of RNA buffering and oppositional decay were strikingly similar (very large dynamic changes in half-life and transcription rates), suggesting that these two regulatory processes operate by a common mechanism.

Keywords: RNA abundances, RNA buffering, RNA decay

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A multi-transcriptomics approach identifies targets of the endoribonuclease DNE1 and provides insights on its coordination with decapping

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Decapping is a crucial step of mRNA degradation in eucaryotes and requires the formation of the holoenzyme complex between the decapping enzyme DCP2 and the decapping enhancer DCP1. In Arabidopsis, we recently identified DNE1, a NYN domain endoribonuclease, as a direct protein partner of DCP1. The function of both DNE1 and decapping are necessary to maintain phyllotaxis, the regularity of organ emergence in the apex. In this study we combined in vivo mRNA editing, RNA degradome, transcriptomics and small RNA-omics to identify targets of DNE1 and study how DNE1 and DCP2 cooperate in controlling mRNA fate. Our data reveal that DNE1 mainly contacts and cleaves mRNAs in the CDS and has sequence cleavage preferences. We found that DNE1 targets are also degraded through decapping, and that both RNA degradation pathways influence the production of mRNA-derived siRNAs. Finally, we detected mRNA features enriched in DNE1 targets including RNA G-quadruplexes and translated upstream-ORFs. Combining these four complementary high-throughput sequencing strategies greatly expands the range of DNE1 targets and allowed us to build a conceptual framework describing the influence of DNE1 and decapping on mRNA fate. These data will be crucial to unveil the specificity of DNE1 action and understand its importance for developmental patterning.

Keywords: mRNA degradation, endoribonuclease, DNE1, decapping, transcriptomics

*Speaker

A cochaperone complex mediates high temperature-induced co-translational mRNA decay in Arabidopsis

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Deciphering the molecular mechanisms that control the response of plants to adverse environmental conditions is key not only to understanding plant survival, but also to developing strategies to deal with these conditions. In the case of plant response to heat stress (HS), there is a wealth of information on transcriptional regulation that establishes the medium-term response. However, an important part of the response is the rapid down-regulation of hundreds of transcripts that are no longer needed under the new environmental conditions and for which transcriptional regulation cannot account for. This short-term response is controlled by post-transcriptional mechanisms. One such mechanism is co-translational mRNA decay (CTRD), in which the 5'-3' mRNA decay occurs in mRNAs associated to translating ribosomes, known as polysomes. The mRNAs are decapped while being translated and XRN4 degrades the mRNA following the last translating ribosome. Information on the functioning of CTRD and in particular in response to HS is still scarce. Here we show through genomic, biochemical and genetic approaches that the conserved cochaperone R2T/prefoldin-like complex (R2T/PFDL) contributes to CTRD in *Arabidopsis thaliana*. 5'P-Seq analyses reveal that CTRD is impaired in *tpr5-2* seedlings defective in the R2T/PFDL subunit RPAP3 to a similar extent as in *lsm1a lsm1b* and *xrn4* mutants respectively defective in decapping and mRNA degradation. The defect in CTRD is observed in both unstressed and stressed *tpr5-2* seedlings. In each condition, the transcripts targeted by RPAP3, LSM1 and XRN4 strongly overlap, although the overlapping transcripts are different in each condition. Biochemical analyses show an enrichment of subunits of the R2T/PFDL complex in polysomes in response to HS, although the total amount of each subunit is not affected by temperature. Importantly, the enrichment of LSM1A and XRN4 in polysomes in response to HS is reduced post-translationally in mutants defective in different subunits of the R2T/PFDL complex. We found that subunits of the R2T/PFDL and decapping complexes associate *in planta*, suggesting that R2T/PFDL may be directly involved in the recruitment of the decapping complex to polysomes. In accordance with the impaired CTRD in response to HS, *tpr5-2* mutants show altered thermotolerance.

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Keywords: cotranslational mRNA decay, 5'P Seq, polysomes, thermotolerance

mRNA uridylation as a novel post-transcriptional process regulating seed maturation

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Seed agronomic traits such as seed vigor and nutritional value are established during seed development under the control of complex and multi-layer processes including post-transcriptional regulation. Yet, the full extent of molecular processes involved in this post-transcriptional regulation remains to be elucidated.
In the last decade, mRNA uridylation, *i.e* uridine addition at 3' end of mRNAs, has emerged as a widespread modification destabilizing eukaryotic mRNAs, thus playing a key role in post-transcriptional regulation. In Arabidopsis, we showed that the main enzyme responsible for mRNA uridylation is UTP:RNA URIDYLYLTRANSFERASE 1 (URT1) and that mRNA uridylation prevents the accumulation of excessively deadenylated mRNAs. We proposed that uridylation regulates mRNA decay in two ways: firstly, by favoring 5' to 3' degradation and secondly, by hindering deadenylation. We have shown that preventing the accumulation of excessively deadenylated mRNAs is important to avoid the biogenesis of illegitimate siRNAs that can silence endogenous mRNAs and strongly impact plant growth and development.
Our new results highlight the contribution of URT1-dependent mRNA uridylation in shaping the transcriptome during seed maturation. In particular, the loss of URT1 has a prominent impact on the transcriptome of dry seeds when compared to other plant tissues. To generate a global inventory of mRNA tailing and uridylation events in Arabidopsis and to unravel the molecular mechanisms of mRNA uridylation in seeds, high-throughput approaches based on Oxford Nanopore Technologies (ONT) sequencing were used to analyze mRNA uridylation status in developing, dry mature and germinating seeds for wild-type plants and *urt1* mutants. Our results show both an accumulation and a lengthening of mRNA U-tails during seed maturation. This accumulation may reflect an increased activity of URT1 and/or a slowdown of the degradation of uridylated mRNAs. Interestingly, our findings also reveal the importance during a plant development process of mRNA uridylation dual roles: during seed maturation, URT1-mediated uridylation may promote the degradation of mRNAs encoding translation-associated proteins, while it hinders the deadenylation of mRNAs associated with the seed maturation program, thereby facilitating their accumulation.

Keywords: Uridylation, Seeds, Nanopore, RNA degradation

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Session 3 : RBPs identification and RNA splicing

Partners in time - Insights into the binding landscape of RNA-binding proteins in Arabidopsis

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RNA-based regulation plays a key role in plant responses to endogenous cues and environmental stimuli. To provide insights into *in vivo* RNA-protein interactions shaping the transcriptome, we improved our previous protocol for individual nucleotide resolution crosslinking and immunoprecipitation and established a bioinformatics pipeline to evaluate iCLIP-seq data (plant iCLIP2). We comprehensively profiled the binding landscape of a range of RNA binding proteins implicated in different steps of RNA processing and function in *Arabidopsis thaliana.* In the compendium of target transcripts, we delineated motifs preferentially associated with binding sites. Orthogonal transcriptomic data obtained from loss-of function mutants of the RNA binding proteins provide insights into the mode of action how the RBPs affect alternative splicing, RNA stability or mRNA 3' end formation. Collectively, our data begin to shed light on the organisation of networks in posttranscriptional regulation in Arabidopsis.

Keywords: iCLIP, RNA, protein interactions, RBPs

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A phase-separated compartment tunes cold acclimation of chloroplast RNA metabolism.

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Arabidopsis plants can produce photosynthetic tissue with active chloroplasts at temperatures as low as 4∘C, and this process depends on the presence of the nuclear-encoded, chloroplast-localized RNA-binding protein CP29A. We demonstrate that CP29A undergoes phase separation in vitro and in vivo in a temperature-dependent manner, which is mediated by a prion-like domain (PLD) located between the two RNA recognition motif (RRM) domains of CP29A. The resulting droplets display liquid-like properties and are found near chloroplast nucleoids. The PLD is required to support chloroplast RNA splicing and translation in cold-treated tissue. Together, our findings suggest that plant chloroplast gene expression is compartmentalized by inducible condensation of CP29A at low temperatures, a mechanism that could play a crucial role in plant cold resistance.

Keywords: chloroplast, RNA splicing, phase separation, prion like domain, cold acclimation

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Analysing phase separation of conserved translation factors during heat stress response in Arabidopsis thaliana

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Plant heat stress response involves global inhibition of protein synthesis in order to prevent proteotoxic stress. At the same time, translation of stress-related transcripts needs to be sustained. How this translational reprogramming is regulated is still largely unknown. Heat stress triggers also transient formation of cytoplasmic condensates through a process called liquid-liquid phase separation (LLPS). These condensates, named stress granules, typically contain translation-stalled mRNAs, RNA-binding proteins and translation initiation factors and are believed to be important for post-transcriptional regulation of gene expression. Here we focused on translation factors belonging to the eEF1B complex and how their phase separation might influence translational control in plants. The eEF1B complex is the GTP exchange factor of the essential translation elongation factor eEF1A. It consists of three subunits, of which only eEF1Bα and eEF1Bβ harbor a GTP/GDP exchange domain, while the eEf1Bγ subunit seems to have a structural function. Using mutants with reduced eEF1B levels we showed the importance of this complex for efficient protein synthesis rates at all stages of development. Analysis of their intracellular localisation in the context of heat stress revealed a diffuse distribution at control conditions, while during heat stress each of the three eEF1B subunits accumulated in cytoplasmic foci, which overlapped with stress granules. Confocal live cell imaging showed that the eEF1Bβ subunit which mediates interaction of the two other subunits has the highest propensity to form condensates. Moreover, its co-expression influenced the localisation pattern of the other two subunits and promoted their condensation. In vitro phase separation assays confirmed that the eEF1B subunits underwent LLPS and that eEF1Bβ has increased phase separation ability. Currently, we are investigating the molecular properties of the eEF1Bβ protein that determine its distinct phase separation behaviour and how its alteration might influence thermotolerance in plants. In addition, we analyzed the interactome of the eEF1B complex under control and HS conditions. Mass-Spectrometry analysis revealed that at HS it associated with numerous additional proteins involved in mRNA binding, translation and protein folding and stability. Our data identify novel components of heat-induced condensates and provide insights into mechanisms regulating heat -induced phase separation in plants.

Keywords: heat stress, translation, stress granules

*Speaker

When posttranslational modifications and splicing shake hands to fine-tune stress responses

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Arabidopsis (*Arabidopsis thaliana*) PROTEIN ARGININE METHYLTRANSFERASE5 (PRMT5) post-translationally modifies RNA-binding proteins by arginine (R) methylation. However, the impact of this modification on the regulation of RNA processing is largely unknown. We used the spliceosome component, SM-LIKE PROTEIN 4 (LSM4), as a paradigm to study the role of R-methylation in RNA processing. We found that LSM4 regulates alternative splicing (AS) of a suite of its in vivo targets identified here. The *lsm4* and *prmt5* mutants show a considerable overlap of genes with altered AS raising the possibility that splicing of those genes could be regulated by PRMT5-dependent LSM4 methylation. Indeed, LSM4 methylation impacts AS, particularly of genes linked with stress response. Wild-type LSM4 and an unmethylable version complement the *lsm4-1* mutant, suggesting that methylation is not critical for growth in normal environments. However, LSM4 methylation increases with abscisic acid and is necessary for plants to grow under abiotic stress. Conversely, bacterial infection reduces LSM4 methylation, and plants that express unmethylable-LSM4 are more resistant to *Pseudomonas* than those expressing wild-type LSM4. This tolerance correlates with decreased intron retention of immune-response genes upon infection. Taken together, this provides direct evidence that R-methylation adjusts LSM4 function on pre-mRNA splicing in an antagonistic manner in response to biotic and abiotic stress.

Keywords: splicing, arginine methylation, arabidopsis

*Speaker

RNA-binding proteins selectively associate with pre-mRNAs for plant temperature responses

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Plants have evolved rapid and complex systems to sense the temperature signal and translate it into cellular responses for acquired tolerance, morphological adjustment, and developmental reprogramming. Understanding how plants adapt to temperature changes has been an important topic in improving thermotolerance in crops. RNA-binding proteins (RBPs) are critical regulatory factors controlling posttranscriptional gene expression. RBPs interact with their RNA targets via RNA-binding domains and these interactions are essential for modulating both RNA metabolism and protein expression for plant development and stress responses. We recently discovered a previously unidentified splicing factor in *Arabidopsis thaliana*, RNA BINDING PROTEIN 45d (RBP45d), which is a component of the U1 small nuclear ribonucleoprotein (U1 snRNP). RBP45d associates with the U1 snRNP by interacting with pre-mRNA-processing factor 39a (PRP39a) and directly regulates alternative splicing (AS) for a specific set of genes. Plants with loss of RBP45d and PRP39a function exhibit defects in thermomorphogenesis potentially due to the misregulation of temperature-sensitive AS. We also used genome-wide approaches, such as targets of RBPs identified by RNA editing (TRIBE) and RNA immunoprecipitation (RIP) to identify the targets of RBP45d. Taken together, we report that RBP45d is a novel U1 snRNP component in plants that functions with PRP39a to regulate AS for thermomorphogenesis. These findings provide new insights into the understanding of plant adaptation to changing environmental conditions and may have potential applications in crop improvement.

Keywords: RNA, binding protein, temperature, RBP45d, pre, mRNA splicing

*Speaker

MAC3A and MAC3B modulate FLM splicing to repress photoperiod-dependent floral transition

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Plants adjust their flowering time by integrating environmental cues through complex regulatory networks. RNA splicing plays a crucial role in modulating gene expression in response to flowering signals. The MOS4-associated complex (MAC), consisting of the evolutionarily conserved E3 ubiquitin ligases MAC3A and MAC3B, is pivotal in splicing regulation. However, the involvement of MAC3A and MAC3B in floral transition remained unclear. This study observed that *mac3a/mac3b* mutants flowered significantly earlier under short-day (SD) conditions, a phenotype absent under long-day (LD) conditions. This early flowering correlated with upregulation of *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) compared to wild-type plants. Transcriptomic analysis revealed alterations in transcript levels and splicing profiles of key floral regulators across different flowering pathways. Further exploration identified the thermosensory flowering regulator *FLOWERING LOCUS M* (*FLM*) as being influenced by *MAC3A* and *MAC3B*. Our findings showed that SPL9 bound to *MAC3A* and *MAC3B* promoters to induce their expression under a short photoperiod. Subsequently, MAC3A and MAC3B associated with *FLM* transcripts to modulate their splicing in SD. This study elucidates how the MAC complex, through RNA splicing regulation, integrates environmental signals to modulate flowering, unveiling a new layer of complexity in flowering pathway crosstalk under non-inductive photoperiods.

Keywords: alternative splicing, flowering time, photoperiod, MAC, FLM, FLC

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REVEILLE2 thermosensitive splicing: A molecular basis for the integration of nocturnal temperature information by the Arabidopsis circadian clock

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Cold stress is one of the major environmental factors that limit growth and yield of plants. However, it is still not fully understood how plants account for daily temperature fluctuations, nor how these temperature changes are integrated with other regulatory systems such as the circadian clock. We demonstrate that REVEILLE2 undergoes alternative splicing after chilling that increases accumulation of a transcript isoform encoding a MYB-like transcription factor. We explore the biological function of REVEILLE2 in Arabidopsis thaliana using a combination of molecular genetics, transcriptomics, and physiology. Disruption of REVEILLE2 alternative splicing alters regulatory gene expression, impairs circa- dian timing, and improves photosynthetic capacity. Changes in nuclear gene expression are particularly apparent in the initial hours following chilling, with chloroplast gene expression subsequently upregulated. The response of REVEILLE2 to chilling extends our understanding of plants immediate response to cooling. We propose that the circadian component REVEILLE2 restricts plants responses to nocturnal reductions in temperature, thereby enabling appropriate responses to daily environmental changes.

Keywords: alternative splicing, chilling, circadian, isoform switch

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CATSNAP – a machine-learning tool that reveals the plasticity of alternative splicing in plants and animals

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Alternative splicing (AS) expands the complexity of transcriptomes, regulates gene expression and creates different protein isoforms. It is largely debated which alternative transcripts carry a biologically relevant role. By common sense, functionally relevant alternative isoforms are more likely evolutionarily conserved. Previous studies assessing AS conservation primarily explored the types of AS events, linking them to the preservation of regulatory processes occurring at the RNA level. In contrast, we investigated the conservation of AS at the amino acid level. To this end, using the machine-learning approach, we developed a tool Catsnap (Conserved AlTernative SpliciNg in Animals and Plants), which evaluates plant and animal alternative proteins from the common sequence databases. The low-throughput validation revealed that the known experimentally characterized protein isoforms are indeed conserved. Surprisingly, in plants, we observed that the alternative protein isoforms frequently arise from various types of AS across different species. In contrast, most (but not all) animal alternative proteins displayed more stable patterns of AS types. We hypothesize that the emergence of similar protein changes resulting from various underlying mechanisms at the RNA level evidences the existence of specific hotspots that are more likely to produce advantageous traits through AS. We relate this observation to the frequent duplication events occurring in plants. The algorithm can be tested by any user at https://catsnap.cesnet.cz/.

Keywords: alternative splicing, evolution, bioinformatics, plants, animals

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Session 4 : ncRNAs

Languages of the Non-coding Genome

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Genomes are the blueprints of life: the genomic DNA sequences contain the instructions for the formation of a given species. DNA sequence polymorphisms between individuals of the same species offer a hypothesis to understand the molecular bases of fitness differences. However, many polymorphisms are located in parts of the genome that we poorly understand: the non-coding genome. While polymorphisms that change the protein sequence offer a sufficiently clear path forward, my research addresses approaches and solutions to understand the non-coding genome. Results from my laboratory illustrate different molecular mechanisms of how the non-coding genome promotes organismal fitness. The results from my lab highlight different ways of how the non-coding genome communicates functional information, including important roles as non-coding RNA molecules. Our findings have intriguing implications for current models explaining non-coding genome conservation and function.

Keywords: Non, coding genome, non, coding RNA

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Connecting dots: how actin binding proteins link siRNA biogenesis and stress

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In plants, ARGONAUTE7 (AGO7) is part of a highly conserved module that regulates plant morphogenesis via post-transcriptional gene silencing (PTGS). This module includes AGO7, miRNA390, TAS3, and requires the molecular machinery involved in the biogenesis of secondary small RNAs, such as the RNA-binding protein SGS3 and the polymerase RDR6. The processing of the TAS3 RNA precursor results in trans-acting short interfering RNAs (tasiRNAs), which target the mRNAs of AUXIN RESPONSE FACTORs 2, 3, and 4, thereby influencing plant morphology (Marin et al., 2010; Dastidar et al., 2018). Despite extensive research, key aspects such as the mechanism's responsiveness to the environment remain unclear. For instance, it is known that the subcellular localization of proteins involved in siRNA biogenesis is influenced by stress. AGO7, RDR6, and SGS3 partition between two localizations: free in the cytosol or within cytosolic liquid-liquid phase-separated (LLPS) siRNA bodies, with the latter being favoured upon stress (Jouannet et al., 2012). Despite these bodies being known for more than ten years, their role is poorly understood. They may enhance tasiRNA biogenesis efficiency by increasing the local concentration of enzymes and substrates or, alternatively, serve as storage sites where AGO7, RDR6, and SGS3 are sequestered.
To better understand the role of siRNA bodies, we employed a proximity labelling approach to elucidate their composition. By fusing the TurboID biotin ligase to AtAGO7, we characterised its proxiome. The most abundant protein identified was AtFIM5, an actin-associated protein. Given that siRNA bodies move along the actin cytoskeleton, we investigated AtFIM5's potential role in siRNA body dynamics. We found that AtFIM5 subcellular localization is stress-responsive, and changes in its behavior or sequence impact siRNA body shape and movement, but not those of similar bodies like P-bodies. Through the expression of different FIMBRIN deletions, we found that FIM5 diverges from the canonical FIMBRINs, as their actin-binding domains present higher affinity towards siRNA bodies than to actin filaments. Finally, the analysis of tasiRNA reporters in Arabidopsis suggests that ectopic expression of FIM5 may influence PTGS efficiency.

In conclusion, our research sheds light on the impact of stress on siRNA body dynamics, composition, and fluidity.

Keywords: tasiRNA, AGO7, siRNA bodies, miR390, stress, actin

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Next-Generation Antiviral Vaccines Based on Synthetic Trans-Acting Small Interfering RNAs Produced from Viral Vectors

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Plant viruses can benefit from the impact of global warming and spread to new geographical regions, eventually becoming a pandemic. They are responsible for huge economic crop losses worldwide and cause nearly half of all plant diseases. Thus, there is an urgent need to develop new, more efficient and modern antiviral tools such as those based on artificial small RNAs (art-sRNAs). Art-sRNAs are highly specific 21-nucleotide long sRNAs computationally designed to silence target transcripts with high specificity. Synthetic trans-acting small interfering RNAs (syn-tasiRNAs) are a class of art-sRNAs used to confer long-lasting antiviral plant protection, typically by overexpressing syn-tasiRNA precursors in transgenic plants. Interestingly, syn-tasiRNA precursors are engineered to produce multiple antiviral syn-tasiRNAs thus allowing the silencing of various target sites within one or more viral transcripts which minimizes the possibility that the virus simultaneously mutates all different target sites. Unfortunately, no transgene-free approaches for efficient syn-tasiRNA delivery to plants have been reported to date. Here, we describe the development of a new generation of sRNA-based vaccines to immunize plants against pathogenic viruses. Plant vaccination consisted in spraying infectious crude extracts into leaves for delivering modified RNA viral vectors such as potato virus X (PVX) or tobacco rattle virus (TRV) engineered to produce in planta four different syn-tasiRNAs targeting tomato spotted wilt virus (TSWV), an economically relevant pathogen affecting tomato and pepper crops worldwide. Importantly, viral vectors included artificially shortened "minimal" syn-tasiRNA precursors that remained stable upon viral vector replication. A few days after vaccination, Nicotiana benthamiana or Solanum lycopersicum plants were inoculated with TSWV and monitored for symptom appearance. The effect on TSWV infection of both PVX- or TRV-based vaccines were analyzed phenotypically by monitoring the appearance of TSWV symptoms and molecularly by measuring TSWV accumulation, while syn-tasiRNA biogenesis was confirmed by sRNA sequencing. Our results indicate that the majority of the vaccinated plants are resistant to TSWV, thus highlighting the potential of plant vaccination with antiviral syn-tasiRNAs produced from harmless viral vectors as a novel strategy for controlling viral diseases.

Keywords: vaccines, antiviral resistance, small RNAs, viral vector

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RNA Sprays - harnessing the function of coding and non-coding RNAs for plant applications

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The demonstration that plants are capable to take up spray-applied RNA will pave the way for various applications and innovations in both basic and applied research. For example, the discovery that foliar spraying of double-stranded RNA induces intra- and interspecies RNA interference (RNAi; post-transcriptional gene silencing) has led to the development of dsRNA-based pesticides. In addition, recent evidence suggests that even exogenously applied mRNA can enter plant cells for protein translation (spray-induced gene expression). These groundbreaking findings will allow the implementation of other RNA species (e.g., circRNA, CRISPR/Cas-guided sgRNA) as "sprayable RNAs" with distinct gene editing and regulation properties. However, the establishment of RNA sprays to mediate gene silencing, gene knockout, epigenetic gene editing and protein synthesis (avoiding the generation of negatively connotated GMOs) requires mechanistic knowledge of RNA uptake and transport. Understanding the routes, cell fate and how exogenously applied RNAs can overcome various external and internal barriers is a prerequisite for improving RNA uptake, stability and transport to ensure the efficacy of extracellular RNA applications in plant science.

Keywords: RNA Spray, RNAi, mRNA, dsRNA, RNA transport, spray, induced gene expression

*Speaker

The subunit 3 of the SUPERKILLER 3 complex mediates miR72-directed cleavage of Nodule Number Control 1 to control nodulation in Medicago truncatula

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Legumes and rhizobia establish a nitrogen-fixing symbiosis involving the formation of a lateral root organ, the nodule, and the suppression of the immune response of the plant, which allows the rhizobia to infect the root tissue. This involves significant gene expression changes regulated at the transcriptional and post-transcriptional levels. We have previously shown that a transcript encoding the Subunit 3 of the Superkiller Complex (SKI), which guides mRNAs to the exosome for 3'-5' degradation, is required for nodule formation, bacterial survival, and induction of early nodulation genes during the *Medicago truncatula* - *Sinorhizobium meliloti* symbiosis. Now, we revealed through degradome analysis that MtSKI3 affects the miR172-directed endonucleolytic cleavage of the *Nodule Number Control 1* mRNA (*MtNNC1*), an APETALA2 transcription factor negatively regulating nodulation. Specific silencing of *MtNNC1* enhanced nodule number and bacterial infection upon inoculation with *S. meliloti*, whereas overexpression of a miR172-resistant form of *MtNNC1* significantly reduces nodule formation, indicating that the miR172-MtNNC1 and its control by SKI3 form a new regulatory hub controlling indeterminate nodulation.

Keywords: Post, transcriptional regulation, microRNAs, nitrogen, fixing symbiosis

*Speaker

The cross-talk between PCF11-similar proteins and CstF64 in flower development in Arabidopsis thaliana

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Eukaryotic messenger RNA (mRNA) is produced from the primary transcript (pre-mRNA) through extensive processing steps, including splicing, 5' cap, and 3' poly(A) tail addition. The poly(A) tail protects the mRNA from degradation and is required for translation initiation. About 70% of Arabidopsis genes have more than one polyadenylation site and alternative polyadenylation (APA) can alter the length and composition/information encoded in the mature transcript. In plants, one of the best-studied processes affected by APA is the control of flowering time. In Arabidopsis thaliana, PCF11-similar protein 4 (PCFS4), a homolog of yeast polyadenylation factor protein 11 (PCF11), is an important factor involved in this process. Knockout *pcfs4* mutants show a delay in flowering time. However, there are three other PCF11-like proteins in *A. thaliana*: PCFS1, PCFS2, and PCFS5, which have not been characterized. We have shown that the *pcfs2* mutant has an opposite effect to the *pcfs4* mutant - it accelerates flowering time. Interestingly, some of the double mutants (*pcfs1 x pcfs4*; *pcfs2 x pcfs4*) showed more severe phenotypes, including aberrant development of the male part of the flower, leading to strong herkogamy. This phenotype is similar to a mutant of another polyadenylation factor, CstF64. Using FRET-FLIM, we showed that all *A. thaliana* PCF11-like proteins interact with CstF64. Using PAT-seq (Poly(A)-Tag Deep Sequencing) we showed that in *pcfs* as well as in *cstf64* mutants the distal polyadenylation site is mostly selected compared to wild-type plants. We also found that the male part of the flower is significantly affected in plants with only slightly altered levels of CstF64. In the analyzed mutants, genes encoding pollen envelope proteins have altered polyadenylation. This strongly suggests that PCF11 similar proteins and CstF64 are important factors in flower and pollen development. These changes are consistent with our analysis of pollen by Alexander staining and scanning electron microscopy.

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Keywords: Polyadenylation, PCF11, CstF64, flower development, 3' end processing, herkogamy

MpmiR11887 and MpmiR11796 are involved in proper sexual organ formation in Marchantia polymorpha

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MicroRNAs (miRNAs) play crucial roles in the regulation of gene expression. In higher plants, male and female gametophytes employ miRNAs to ensure sexual organ formation and reproductive success. However, most of these roles remain unknown in the non-vascular plants. Therefore, we employed the liverwort *Marchantia polymorpha* to investigate novel liverwort-specific miRNAs, which we found to be differentially expressed in sexual organs in comparison to the vegetative tissues, since, these could play a role in sexual reproduction. MpmiR11887 and its precursor show exclusive expression in the antheridiophores.. 5' RLM, 3' RACE, and genome database analysis revealed that the *MIR11887* gene probably represents an independent transcriptional unit containing a short ORF (potential miPeP) upstream of miRNA bearing stem-loop structure. Using CRISPR/Cas9, we obtained three KO lines and confirmed the absence of miRNA. Mp*mir11887*ge plants showed a delay in the antheridiophore production. However, the observed antheridiophore size was similar to WT plants. We are investigating the potential targets for MpmiR11887 in the degradome sequencing data and analyzing more detailed phenotypic features. MpmiR11796 accumulates abundantly in the archegoniophores. Genome database analysis revealed that the pre-miR11796 sequence overlaps with 5'-UTR of a protein-coding gene and 5' RLM and 3' RACE experiments show that it represents an intron-less independent transcriptional unit. Using the CRISPR-Cas9 approach, we obtained one female mutant line and confirmed the absence of miRNA. The Mp*mir11796*ge plants showed reduced size of thallus and archegoniophores. Archegonial heads are significantly smaller and the stalk is shorter but of variable length compared to WT plants. Furthermore, we identified novel putative targets using degradome sequencing data. These include CYTH-domain-containing protein (involved in root development) and AAA+ type ATPase (associated with a variety of cellular activities including control of cell division and gene expression). These results and the further establishment of miRNA-mRNA modules will significantly enhance our understanding of Marchantia's developmental and reproductive processes.

Keywords: miRNAs, Marchantia polymorpha, pri, miRNAs, reproduction

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Session 5 : RNA structure, modifications and lncRNAs

RNA structure, an important regulator in living cells

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RNA structure plays an important role in the post-transcriptional regulations of gene expression. Using in vivo RNA structure profiling methods, we have determined the functional roles of RNA structure in diverse biological processes such as mRNA processing (splicing and polyadenylation), translation and RNA degradation in plants. We also developed a new method to reveal the existence of tertiary RNA G-quadruplex structures in eukaryotes and uncovered that RNA G-quadruplex structure serves as a molecular marker to facilitate plant adaptation to the cold during evolution. Additionally, we have developed the single-molecule RNA structure profiling method and revealed the functional importance of RNA structure in long noncoding RNAs. Recently, we established a powerful RNA foundation model, PlantRNA-FM, that facilitates the explorations of functional RNA structure motifs across transcriptomes.

Keywords: RNA structure, G, quadruplex, RNA motifs

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Improving RNA structure determination by using multiple chemical probes

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RNA can fold into complex structures that participate in diverse interactions with other biomolecules. These structural interactions have functional implications for coding and non-coding RNA transcripts involved in diverse RNA regulatory processes such as splicing, protein binding, or polyadenylation (1). To examine these dynamic RNA secondary structures in vivo, we previously developed and applied a diverse set of nucleotide-specific chemical probes, including DMS and EDC / ETC to differentiate between paired and unpaired regions. DMS and EDC / ETC modify the Watson-Crick-Franklin face of A and C or G and U, respectively. Another reagent, NAI, modifies the 2'-OH of all four nucleosides, providing complementary and comprehensive information on RNA. As such, combining RNA structural information derived from these different chemical probes should increase the density of in vivo structural data, resulting in an improved understanding of RNA structure inside the cell. To determine how to combine multi-chemical data, Structure-seq2 libraries of month-old rice seedlings treated with no chemical (control), DMS, EDC, or NAI were prepared, and the resulting Illumina reads were processed using several different bioinformatic tools. We have previously shown that tRNAs, which are a convenient benchmark of RNA probing, can be probed in vivo (2). Herein, tRNAs with sufficient data coverage validated our approach of predicting RNA structure by combining information from multiple chemicals. In particular, the combination of DMS and EDC information improves the overall prediction accuracy of the tRNAs when using thermodynamically based RNA structure prediction tools. We are now applying our combined chemical approach to query mRNA structure in rice seedlings in the presence or absence of drought stress.
(1)Bevilacqua, P.C., Ritchey, L.E., Su, Z., and Assmann S.M. (2016) Annu. Rev. Genet. 50:235-66.
(2)Yamagami R, Sieg JP, Assmann SM, Bevilacqua PC. (2022) Proc Natl Acad Sci U S A. 119:e2201237119.

Keywords: RNA structure determination, chemical probing, rice, tRNA

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The structured mRNA element DEAD can sense RNA helicase activity to regulate alternative splicing

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Every messenger RNA is capable of folding into secondary structures, and this ability can impact gene regulation at various steps, such as transcription, translation, RNA stability, or alternative splicing (AS). Although AS control by structured elements on the precursor mRNA has been described multiple times in animals, there are only very few examples known in plants. We identified a stem-loop motif, named *DEAD*, that is conserved across the land plant lineage and is associated almost exclusively with genes from the DEAD-box RNA helicase family. In the model plant *Arabidopsis thaliana*, it is found in two paralogs, *DEAD-BOX RNA HELICASE 1* (*DRH1*) and *RNA HELICASE 46* (*RH46*). We show that the structure of *DEAD* controls transcript levels of *DRH1* by obstructing an alternative splice site required for negative feedback regulation. Opening of the stem shifts the splicing pattern towards different non-coding isoforms, followed by degradation of the major one via the nonsense-mediated decay (NMD) pathway. This feedback regulation is restricted to DRH1 and RH46, related helicases are unable to induce usage of the splice site. Controlling DRH1 levels is essential for the plant, as overexpression of this helicase results in bleaching and growth arrest within days after induction. Consistently, transcriptome sequencing in inducible overexpression lines identified DRH1 as a major regulator of AS and gene expression with more than 5,000 target genes. Among them, there is an overrepresentation of genes involved in RNA metabolism, protein phosphorylation and oxidative stress, suggesting a function as an upstream regulator of splicing and stress-related signaling cascades. In addition, DRH1 might promote NMD based on reduced steady-state levels of certain NMD target transcripts. We propose that *DEAD* functions as a sensor for activity of DRH1 and possibly RH46 by preventing splicing factors from accessing the splice site unless unwound by one of the helicases. This system would provide fast and flexible adaptation to fluctuations in helicase production or the amount of target RNAs, ensuring balanced levels of DRH1 activity as an important regulator of gene expression.

Keywords: Alternative splicing, RNA structure, RNA helicase

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LncRNAs modulating alternative splicing in the regulation of the plant transcriptome

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In all eukaryotic model organisms, an increasing number of long non-coding RNAs (lncRNAs) have been found to regulate alternative splicing (AS), albeit little is known about their underlying mode of action. We have identified a first lncRNA, called ASCO, that regulates AS of specific targets. We are now exploring whether lncRNAs are a general component of AS networks and how they contribute to AS selectivity. In a bioinformatic screen we identified 10 lncRNAs which physically interact with the known splicing regulators NSRa (based on RIPseq) and GRP7 (using iCLIPseq) and are regulated during lateral root development. These 10 lncRNAs were transiently expressed in *A. thaliana* leaves and their effects on splicing pattern was tested by stranded RNAseq and splicing profile analysis. Transient expression of two NSRa-bound (called ACHLYS and FLAIL) and two GRP7-bound lncRNAs (called NPC43 and RHAPSO) affected the splicing of more than 200 genes. Interestingly, FLAIL has been recently described as a lncRNA that affects AS during flowering (Jin et al. 2023). In a reverse genetic approach, the phenotype of deregulated plants for ACHLYS (NSRa linked) and RHAPSO (GRP7-bound) were characterized *in planta* (using knockdown and overexpression lines). ACHLYS overexpression and RHAPSO knockdown and overexpression led to a negative effect on root development. Transcriptome analysis of these deregulated lines revealed differentially alternative splicing in hundreds of genes confirming their action in AS *in planta*. To test whether NSRa and GRP7 can regulate the identified lncRNA-dependent AS, we mapped the *in vitro* RNA binding sites of NSRa and GRP7 using RAPseq (RNA affinity purification followed by sequencing). In addition, we identified *in vivo* NSRa binding sites by NSRa-iCLIP. The combination of these approaches to define the potential binding sites for lncRNA and target AS mRNAs will be used to elaborate hypothesis about how lncRNAs regulate AS of specific mRNA targets.

Keywords: lncRNAs, splicing, protein, RNA interaction

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The impact of a lncRNA on seedling development

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Long non-coding RNAs (lncRNAs) have emerged as crucial modifiers of eukaryotic transcriptomes. In plant systems, lncRNAs have been demonstrated to act as transcriptional regulators and to greatly influence developmental programming. In a recent publication (Palos et al. 2022), the authors identified lncRNAs that exhibited considerable expression conservation in floral and seed tissues across the Brassicaceae. In efforts to assess functional relevance for these considerably conserved lncRNAs, we acquired T-DNA lines that disrupted these lncRNAs and performed phenotypic screens. From these screens we discovered a lncRNA that is involved in seedling establishment and subsequently named it LncRNA with Germination Defects 1 (LGD1). Two independent insertion lines exhibited reduced germination on soil and delayed development when grown on MS agar plates. However, supplementing growth media with sucrose alleviated germination defects, suggesting that the mobilization of energy reserves in developing seedlings is impacted by LGD1. In an effort to understand the transcriptomic impact of this lncRNA we re-analyzed available RNA-seq datasets and performed RNA-seq on floral and seedling tissues grown under nutrient deficient (no sucrose) and nutrient sufficient (plus sucrose) conditions. From these data we were able to develop a testable molecular mechanism for LGD1 and propose a framework in which LGD1 acts as a transcriptional regulator of genes critical for the switch from heterotrophic to autotrophic metabolism. Our work is positioned at the intersection of seedling and lncRNA biology, reiterating the importance of lncRNAs as genomic factors that have the capacity to inform and shape plant survival.

Keywords: seedling, development, lncRNA

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The effects of RNA modifications on plant biology

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At the heart of post-transcriptional regulatory pathways in eukaryotes are cis- and trans-acting features and factors including covalent chemical modifications to the RNA bases themselves. This is especially evident for RNA molecules whose functionality, maturation, and regulation requires proper addition of these important chemical moieties. However, the global influence of RNA modifications on plant gene expression is still largely unclear, especially in the context of plant abiotic and biotic stress response. We have recently found that RNA modifications play important roles in regulating the plant transcriptome on a global scale in the context of normal development as well as in response to various abiotic and biotic stress responses. Our most recent findings from these studies will be presented.

Keywords: chemical modifications, RNA modifications

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Plant Long Noncoding RNAs - From Molecular Mechanisms to Agricultural Advancements

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RNA-DNA hybrid (R-loop)-associated long noncoding RNAs (lncRNAs), including the Arabidopsis lncRNA *AUXIN-REGULATED PROMOTER LOOP* (*APOLO*), are emerging as important regulators of three-dimensional chromatin conformation and gene transcriptional activity. We showed that *APOLO* interacts with the PRC1-component LIKE-HETEROCHROMATIN PROTEIN 1 (LHP1) and the methylcytosine-binding protein VARIANT IN METHYLATION 1 (VIM1), a conserved homolog of the mammalian DNA methylation regulator UBIQUITIN-LIKE CONTAINING PHD AND RING FINGER DOMAINS 1 (UHRF1). The *APOLO*-VIM1-LHP1 complex directly regulates the transcription of the auxin biosynthesis gene *YUCCA2* by dynamically determining DNA methylation and H3K27me3 deposition over its promoter during the plant thermomorphogenic response. We established that the lncRNA *UHRF1 Protein Associated Transcript*(*UPAT*), a direct interactor of UHRF1 in humans, can be recognized by VIM1 and LHP1 within plant cells, despite the absence of sequence homology between *UPAT* and *APOLO*. In addition, we showed that increased levels of *APOLO* or *UPAT* hamper VIM1 and LHP1 binding to *YUCCA2* promoter. Strikingly, the application of exogenous *in vitro*-transcribed*APOLO* on Arabidopsis plants leads to alterations in auxin homeostasis and modifies the plant's shade avoidance syndrome. Collectively, our results uncover a new mechanism in which a plant lncRNA orchestrates the interplay between Polycomb-mediated repression and DNA methylation. Moreover, our study underscores the intriguing phenomenon that evolutionarily unrelated lncRNAs can serve analogous functions across diverse biological kingdoms. Beyond this, our research expands the horizons for leveraging lncRNAs as bioactive agents, presenting innovative prospects for devising sustainable agricultural strategies.

Keywords: LncRNAs, APOLO

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Diverse mRNA modifications influence stability, splicing, and stress responses across flowering plants

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Proper regulation of an RNA's lifecycle is a critical aspect of plant development and responses to environmental cues. Modifications on ribonucleotides represent a broad class of over 170 known moieties which have a profound effect on RNA structure, stability, lifecycle, and ultimately function. While mRNAs and lncRNAs are targeted by numerous types of modifications, their functional roles are largely unclear. In this study, we leveraged diverse sequencing strategies to characterize multiple modification classes in important representatives from across the flowering plant lineage. We first used Nanopore direct RNA-sequencing and *in silico* strategies to capture a wide range of modifications, including m6A and the lesser studied, non-m6A moieties (m5C, m1A, etc), which allowed us to characterize their conservation and the developmental context under which they are observed. We then performed a focused series of stress experiments in four Brassicaceae: *Arabidopsis thaliana*, *Camelina sativa*, *Brassica rapa*, *and Eutrema salsugineum*. Specifically, we investigated seedling responses to exogenous ABA or salt stress at numerous time points and then mapped changes in their modification profiles. This strategy utilized species with varying tolerances to abiotic stress and provided a comparative framework to directly investigate how mRNA modifications change in response to acute abiotic stresses and whether this phenomenon is conserved. These comparative approaches uncovered multiple important findings regarding RNA modifications: (1) a strong and remarkably conserved enrichment on nuclear RNAs involved in photosynthesis and translation, (2) a striking enrichment (non-m6A moieties) or depletion (m6A) at exon-exon junctions, (3) non-m6A modifications marked unusually stable mRNAs, shedding light on their role in RNA stability, and lastly (4) both m6A and non-m6A mRNA modifications were dynamically re-programmed during stress and marked numerous transcripts involved in genotype or species-specific tolerance mechanisms. Overall, our research reveals novel characteristics of RNA modifications and should provide a foundation for future molecular investigations of these pivotal moieties.

Keywords: RNA modifications, RNA stability, gene expression regulation, stress responses

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small RNA biogenesis: co-transcriptional regulation and RNA modifications

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The maturation of nascent RNA is tightly connected with the transcription process. It is well known for animals and plants that RNA polymerase II (RNAPII) elongation rate, pausing, and termination steps may affect the quality and quantity of its final product: mature mRNA. Pre-mRNA splicing, incorporation of RNA modifications as well as 3' end maturation are co-transcriptional. However, plant miRNA biogenesis was considered post-transcriptional and localized in so-called D-bodies (Dicing bodies) in the cell nucleus for years. Our studies clearly show that in Arabidopsis, miRNA production takes place already during transcription and is tightly regulated at multiple levels including RNA modifications. Small RNA precursors contain modified nucleotides including m6A and pseudouridine (Ψ). Both modifications are known to be important for maintaining the proper secondary structure of RNA. Moreover, we developed assays to detect Ψ in short RNA sequences, demonstrating its presence in Arabidopsis miRNAs and their precursors. We also detect substantial enrichment in germline small RNAs - epigenetically activated siRNAs (easiRNAs) in Arabidopsis pollen. Pseudouridylated easiRNAs are localized to sperm cells, and we found that PAUSED (PSD), the plant homolog of Exportin-t, interacts genetically with Ψ and is required for the transport of easiRNAs into sperm cells from the vegetative nucleus. We further show that Exportin-t is required for the triploid block: chromosome dosage-dependent seed lethality that is epigenetically inherited from pollen. Thus, Ψ has a conserved role in marking inherited small RNAs in the germline.

Keywords: small RNA, miRNA, co, transcriptional, pseudouridine, N6, Methyladenosine

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Viral RNA methylation and intercellular mobility

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Viruses systemically infect plants by their capacity to move locally between cells and via the vasculature to distant non-infected tissue. Long-distance movement takes place via the phloem, while short-distance movement and entrance into the phloem depends on intercellular pores named plasmodesmata (PD). RNA viruses produce so-called movement proteins, which bind to the viral RNA (vRNA) to mediate their transport through PD. Numerous RNA viruses harbour viral tRNA-like sequence (vTLS) motifs at the 3' end of their genomic RNA. These vTLS motifs can be amino-acylated and are similar to TLS motifs mediating long-distance transport of distinct mRNAs. Based on our previous findings that both m5C methylation and TLS sequences play a role in mRNA transport from shoot to root we ask whether vTLS sequences and/or methylation might facilitate viral RNA transport to distant cells. Our preliminary findings indicate that vRNAs are m5C modified and m5C modification play an unexpected role in viral spread. To further substantiate this relationship, we are currently analysing i) the potential of distinct viral TLS motifs found in different viruses to mediate RNA transport in grafted plants, and ii) the effect of m5C modifications on viral RNA replication and/or delivery to distant tissues.

Keywords: RNA virus, RNA structure

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Unbiased identification of novel non-YTH m6A readers from Arabidopsis thaliana

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Messenger RNA chemical modifications are recognized as crucial effectors of organism growth, development, and reproduction. Methylation at position N6 of internal adenosines (m6A) is the most abundant and widespread of epitranscriptomic marks in eukaryotes. It participates in all steps of mRNA metabolism from transcription, maturation and export to stability, storage and translation control. The downstream molecular and cellular effects of m6As are conferred by their interaction with "reader proteins," whose affinity for target mRNAs is dictated by this chemical modification. The canonical and most widely studied readers are those sharing the YTH domain, a globular domain forming an m6A binding pocket; the high sequence conservation among these domains makes them easy to identify across genomes. Non-YTH readers were found to exist in mammals and recently in *Arabidopsis thaliana*, yet their identification is serendipitous or relies on unbiased affinity capture approaches. Using methylated and non-methylated RNA probes as baits, we set up an affinity capture approach to purify m6A readers from crude extracts prepared from Arabidopsis seedlings. Using label free quantitative LC-MS/MS analyses of the affinity enriched fractions, we identified 70 proteins specifically captured by the m6A-RNA probes. Within our results, we identify twelve of the thirteen Arabidopsis YTH-domain proteins, providing a convenient internal control for specificity, validating the ability of our assay to capture bon fide m6A readers. We will present the method we set up for plant extracts, including its pitfalls, difficulties and importance for dissecting m6A-based regulatory pathways in plants. The list of putative non-YTH readers we captured will also be discussed.

Keywords: m6A modification, YTH proteins, RNA chemical modifications

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Poster Session and Buffet Diner

ECT2 Peptide Sequences Outside the YTH Domain Regulate m6A-RNA Binding

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The m6A epitranscriptomic mark is the most abundant and widespread internal RNA chemical modification, which through control of RNA processing acts as a crucial actor of eukaryote reproduction, growth, morphogenesis and stress response. The main m6A readers in eukaryotes consist of a super family of RNA binding proteins with hundreds of members that share a so-called YTH domain (YT-521B). The majority of eukaryotic YTH-proteins seem to carry no obvious additional domain except for the Intrinsically Disordered Region (IDR), which may participate in RNA binding or be involved in phase separation. Concerning Arabidopsis proteins (known as *E*volutionarily Conserved C-Terminal region 1-12), the IDR appears to play a role in the functional divergence of ECT proteins. Here we use the ECT2 protein and *in vitro* biochemical characterization to further decipher regions or domains that have key role in regulating m6A RNA binding. We show that ECT2 and its YTH domain have a distinct ability to bind m6A compared to other YTH readers, and that *cis* regions in the IDR and C-part of the protein are required for m6A binding and act also as regulatory domains on binding affinity. Furthermore, our results show that the consensus DRACH sequence is not sufficient for stable binding and that addition of U-rich sequences is required for specific and high affinity binding.

Keywords: m6A, YTH readers, RNA chemical modification

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Role of redox regulation of the biogenesis of mRNP granules under heat stress conditions in Arabidopsis thaliana.

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Messenger RiboNucleoProtein (mRNP) granules are dynamic structures formed by liquid-liquid phase separation (LLPS). These granules were proposed to regulate mRNA fate by sequestration of both mRNA molecules and proteins. However, the molecular mechanisms that trigger mRNP granules are still unclear. Post-translational modifications (PTM) of key actors of mRNP granules appear as a potential regulatory mechanism. As mRNP granules are highly dynamic upon stress, we hypothesized that PTM of some RNA Binding Proteins (RBPs) during stress conditions can trigger granules formation. During stress, reactive oxygen species (ROS) cause changes the redox state of cysteine thiol residues and can lead to the formation of disulfide bridges between proteins, leading to aggregation. The aim of my thesis is to understand the importance of cysteine modifications in the formation of mRNP granules. To do so, an in vivo analysis using granules reporter lines was performed under alkylating conditions as alkylating reagents block cysteine modifications. We demonstrated that alkylating agents affect both stress granules and p-bodies formation. In fact, while heat stress (HS) triggers mRNP granules formation, a treatment with an alkylating reagent during HS abolishes granules formation in a dose-dependent manner. These preliminary results suggest that cysteines modification can have an impact on mRNP granules formation.

Keywords: mRNP granules, LLPS, ROS, Arabidopsis thaliana, Heat stress

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A role of the SERRATE C-terminal fragment in microRNA biogenesis

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MicroRNA (miRNA) is a group of short ribonucleic acids, which are the final products of non-protein-coding *MIR* genes. MiRNA molecules are involved in the broad spectrum of post-transcriptional regulation of genes expression and proteins synthesis. In the process of miRNA biogenesis, a protein complex called Microprocessor plays a crucial role. Among the core components of Microprocessor, there are: the Dicer-like endonuclease 1 (DCL1), the double-stranded RNA-binding protein - Hyponastic leaves 1 (HYL1) and the protein with the C2H2 zinc finger domain - SERRATE (SE). SE is involved in almost all stages of miRNA biogenesis. Studies on SE showed its significant impact on transcription regulation, splicing and RNA degradation. Up to now 4 different *se* mutants were described: *se-1, se-2, se-3, se-4*. In the *se-1* mutant, a deletion of 7 nucleotides was introduced at the 3' end of the gene, depriving the final protein of 27 highly conserved amino acids at its C-terminus. Even though the deletion is introduced at the C-terminus of SE, a highly pleiotropic phenotype of the *se-1* mutant was noticed. This phenomenon is interesting because the deletion covers part of the SE unstructured region, which goes significantly beyond the so-called "protein core". The pleiotropic phenotype of the *se-1* mutant combined with the knowledge of the high conservation level of the C-terminus of SE suggests a potentially important influence of the above-mentioned region in the interaction of SE with other proteins. To determine a potential role of the C-terminus of SE, different length variants (27, 97 and 177aa) of the above-mentioned sequence were combined with the GFP protein to determine the interactions between the studied SE fragments and other proteins. Identification of proteins has been proceed using the coimmunoprecipitation assay. Moreover, the SE protein produced by the *se-1* mutant, which in turn was fused with the FLAG-tag, has also been used in this study. This experimental setup will give us valuable information about a role of the SE C-terminus in the interactions between the proteins involved in microRNA biogenesis.

Keywords: SERRATE, miRNA, Arabidopsis

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A whole genomic overlays reveal exciting interplay between proteins, chromatin state, and unusual nucleic acid structures in Arabidopsis thaliana

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Unusual nucleic acid structures comprise both DNA and RNA structures differing from their classical textbook forms and presenting a stunning diversity of various three-dimensional shapes and functional molecular epitopes, often finely tuning important epigenetics processes in all living organisms. Here, we computationally intersected previously published experimental datasets characterizing chromatin accessibility together with our sequence-based predictions of unusual nucleic acid structures in model plant *Arabidopsis thaliana*, comprising G-quadruplexes and left-handed Z-DNA. In the case of G-quadruplexes, we also used laboratory-validated sites obtained by *in vitro* G4-sequencing. Our results showed unexpectedly high and statistically significant enrichments of particular proteins within the sites of experimentally validated open chromatin and G-quadruplexes. For the clear graphical representation of resulting genomic overlays, we identified so-called UpSet diagrams to be very helpful and informative. Finally, we discuss possible mechanistic explanations of identified phenomena together with possible post-transcriptional interplay and implications for future research in the field.

Keywords: Genomics, Transcription factors, RNA regulation, Chromatin accessibility, Quadruplexes, Plant bioinformatics

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Alternative splicing of RNA binding proteins from the RBP45 group is controlled by a structured mRNA motif.

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Alternative splicing (AS) is a widely distributed type of gene regulation among eukaryotes. It is based on the variable definition of exons and introns and results in increased transcriptome diversity. In plants, AS plays an important role in developmental processes such as photomorphogenesis and stress responses. Accordingly, a precise coordination of gene activity and AS is crucial for these processes. So far, the understanding of regulation is limited to single AS events. These regulatory mechanisms can involve the action of RNA-binding proteins (RBPs), but also *cis*-acting elements on the precursor (pre) mRNA such as structured mRNA motifs. Our study intends to provide a better understanding of the impact of structural elements on the regulation of AS in plants. Using bioinformatic strategies based on covariation, we identified the potentially structured RNA motif *45ABC*, which is highly conserved among monocots and dicots and harbors two stem loops. In *Arabidopsis thaliana*, this motif was identified in *RBP45A*, *RBP45B*, and *RBP45C*. These three homologs have two major transcript variants resulting from cassette exon inclusion or skipping. Skipping of this exon generates a splicing variant encoding the full-length protein, while its inclusion introduces a premature termination codon and a long 3' untranslated region, typical features that trigger degradation via nonsense-mediated decay. Interestingly, the *45ABC* motif encompasses the alternative splice sites, suggesting a functional relationship. Using splicing reporters for the three *RBP45* genes in transient expression assays, we demonstrated negative feedback autoregulation of their pre-mRNAs via AS, along with cross-regulation among all homologs. Disruption of the motif's structure results in an increase in the cassette exon variant, while compensatory mutations can restore this effect. Beyond their molecular functions, our findings suggest significant physiological implications. Preliminary evidence indicates that these RBPs not only promote primary root growth but also play a crucial role in abiotic stress responses. Higher-order loss-of-function mutants exhibit significantly shorter primary roots compared to wild-type plants and display reduced resilience to environmental stressors. In our ongoing research, we would like to address how the highly conserved *45ABC* element is functionally connected to the physiological functions of the corresponding genes.

Keywords: RNA Structure, RNA binding protein, Alternative Splicing, Stress response

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Arabidopsis mRNA decay landscape shaped by XRN 5-3 exoribonucleases

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5-3 exoribonucleases (XRNs) play crucial roles in the control of RNA processing, quality, and quantity in eukaryotes. Although genome-wide profiling of RNA decay fragments is now feasible, how XRNs shape the plant mRNA degradome remains elusive. Here, we profiled and analyzed the RNA degradomes of Arabidopsis wild-type and mutant plants with defects in XRN activity. Deficiency of nuclear XRN3 or cytoplasmic XRN4 activity but not nuclear XRN2 activity greatly altered Arabidopsis mRNA decay profiles. Short excised linear introns and cleaved pre-mRNA fragments downstream of polyadenylation sites were polyadenylated and stabilized in the *xrn3* mutant, demonstrating the unique function of XRN3 in the removal of cleavage remnants from pre-mRNA processing. Further analysis of stabilized XRN3 substrates confirmed that pre-mRNA 3 end cleavage frequently occurs after adenosine. The most abundant decay intermediates in wild-type plants include not only the primary substrates of XRN4 but also the products of XRN4-mediated cytoplasmic decay. An increase in decay intermediates with 5 ends upstream of a consensus motif in the *xrn4* mutant suggests that there is an endonucleolytic cleavage mechanism targeting the 3 untranslated regions of many Arabidopsis mRNAs. However, analysis of decay fragments in the *xrn4* mutant indicated that, except for microRNA-directed slicing, endonucleolytic cleavage events in the coding sequence rarely result in major decay intermediates. Together, these findings reveal the major substrates and products of nuclear and cytoplasmic XRNs along Arabidopsis transcripts and provide a basis for precise interpretation of RNA degradome data.

Keywords: 5 to 3 RNA decay, Endocleavage, Protect fragments, 5P end, polyadenylation

*Speaker

ARGONAUTE1, ARGONAUTE3, and ARGONAUTE4 ARE INVOLVED IN HYPOXIA TOLERANCE IN ARABIDOPSIS THALIANA

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In plants, hypoxia can be induced by submergence and the lack of oxygen impairs mitochondrial respiration, thus affecting the plant's energy status. Hypoxia has major effects on gene expression; these changes induce key responses that help meet the needs of the stressed plant. However, little is known about the possible role of RNA signaling in the regulation of gene expression under limited oxygen availability. The results indicated that tolerance to submergence in Arabidopsis requires a functional AGO1 protein that, together with AGO4-dependent non-canonical RNA-directed DNA methylation (RdDM) pathway, influences gene expression under hypoxia. Nowadays, still little is known about the role of ARGONAUTE 3 (AGO3) in the context of TGS and/or PTGS. We found that *ago3-3* mutants are more sensitive to submergence and gene expression analyses show that *AGO3* expression is induced under hypoxia. On the other hand, AGO3 protein stability is negatively regulated by the lack of oxygen. Additionally, mutant and transgenic plants affected in different steps of the hypoxic response proved that *AGO3* expression is regulated by ERFVII transcription factors. AGO3 immunoprecipitation and high-throughput sequencing of co-immunoprecipitated will hopefully lead to the identification of AGO3 biological targets, shedding light on its function.

Keywords: Arabidopsis, AGO1, AGO3, AGO4

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Biochemical characterization of Dicer-like proteins DCL3 and DCL4 in cauliflower

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Dicer-like proteins (DCL3 and DCL4) produce small interfering RNAs (siRNAs) with different sizes that are essential for transcriptional and post-transcriptional gene silencing, respectively, and they play crucial roles in the regulation of transcriptional and post-transcriptional gene expression. We have established the simple method to detect dicing activity of DCL3 and DCL4 in various tissues and organs of various plant species. Using this method, we can detect the dicing activity from various plants, including the model plant *Arabidopsis thaliana*, rice, and *Nicotiana benthamiana*, as well as fungus *Neurospora crassa* and the insect silkworm *Bombyx mori*. In this study, we attempted to biochemically analyze the enzymatic property of DCL3 and DCL4 using a curd (edible part) of cauliflower (*Brassica oleracea* var. *botrytis*). We detected high dicing activities of DCL3 and DCL4, which were approximately 20 times higher than those in seedlings of *A. thaliana* and a shoot apex tissue containing immature leaves of *N. benthamiana*. These dicing activities of DCL3 and DCL4 were inhibited by the addition of flavonoid aglycones. Especially, cyanidin, quercetin and luteolin had strong inhibitor activity for plant Dicers. The enzymatic characteristics of DCL3 and DCL4 will be discussed.

Keywords: Dicer, DCL3, DCL4, cauliflower, flavonoid, dicing activity

*Speaker

Cajal Bodies Regulate Intron Retention Under Hypoxia Stress

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One of the major alternative splicing (AS) events in plants is intron retention (IR). IR isoforms can regulate the amount of fully mature mRNA by enhancing transcription and directing the mRNA to degradation in the cytoplasm. Our study examines the involvement of nuclear Cajal bodies (CBs) in IR and the nuclear retention of transcripts during hypoxia stress in *Arabidopsis thaliana*. PCA analysis of individual RNAs from normoxia, long-term hypoxia, and reoxygenation in the wild type (WT) and the *ncb-1* mutant (lacking CBs) revealed 2435 new splicing forms, 287 new antisense transcripts, 188 transcripts with new loci, and 2 new transcripts located in the intron of another gene.Analysis of AS events revealed that IR was the most frequently deregulated event in all stages.The greatest number of such changes was observed in the hypoxia/normoxia comparisons in the WT, normoxia WT/*ncb-1*, and hypoxia WT/*ncb-1*. Additionally, we demonstrate that in both WT and mutant, there are over twice as many transcripts with IR whose expression increased during hypoxia. When comparing intron retention between genotypes, a similar accumulation of IR in RNAs was observed in hypoxia, slightly less in *ncb-1* during normoxia and reoxygenation. Next, we examined IR isoform expression differences in the hypoxia/normoxia comparison between the WT and the mutant. Fold change (FC) analysis between hypoxia and normoxia in both genotypes revealed 241 differentially expressed isoforms with positive FC in the *ncb-1* and negative in WT. GO enrichment analysis identified terms associated with mRNA splicing and hypoxia stress. Among the hypoxia response genes were genes encoding cytochrome c oxidase, heat shock protein 70, and xyloglucan endotransglycosidase. The accumulation of introns in these genes, along with the reduction in the expression of fully spliced ones, may be responsible for the decreased tolerance of *ncb-1* to hypoxia. Additionally, we observed that some IR isoforms accumulate in hypoxia and that their number decreases during reoxygenation, suggesting that these isoforms are spliced after stress removal. In summary, the results indicate that IR isoforms, could be used during reoxygenation following intron removal. These findings highlight the involvement of CBs in the response to hypoxia by regulating IR.

Keywords: hypoxia, Cajal bodies, alternative splicing, intron retention

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Developing tools to examine repressive lncRNAs that are mechanistically conserved across eukaryotes

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Long non-coding RNAs (lncRNAs) play critical roles in many biological processes, yet their functions in plants remain poorly understood. By identifying Arabidopsis lncRNAs with conserved mechanistic features to human lncRNAs, we aim to use these conserved features to elucidate lncRNA function in plants. Unlike protein-coding genes, lncRNAs often show lower sequence conservation, complicating the identification of lncRNAs with similar functions based on sequence homology. To address this, we employed SEEKR, a method that evaluates lncRNA similarity based on k-mer abundance rather than linear sequence. This approach has revealed that Arabidopsis lncRNAs with similar k-mer profiles as seen in human lncRNAs associated with chromatin silencing. Based on these shared profiles, we anticipate that these Arabidopsis lncRNAs will also have a repressive function. To validate this hypothesis, we developed a system to test the chromatin silencing potential of these candidate lncRNAs. Our system involves a construct containing the lncRNA to be tested, an adjacent reporter gene (GFP) to assess silencing, and an inducible transcription factor that targets the lncRNA. This inducible system will allow us to monitor the impact of a lncRNA on chromatin silencing based on reduction of GFP abundance. Our system provides a powerful tool to validate the repressive functions of Arabidopsis lncRNAs and deepen our understanding of the mechanisms underlying lncRNA-mediated gene regulation in plants.

Keywords: Long noncoding RNAs (lncRNAs), Conserved mechanistic features, Transcriptional repression system

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DXO1 is required for effective rRNA processing in Arabidopsis

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The conserved family of decapping 5'-3' exoribonucleases DXO/Rai1 is involved in mRNA 5' end quality control, deNADing of non-canonical NAD-capped RNAs and, in yeast, rRNA maturation. Arabidopsis DXO1 (AtDXO1) exhibits plant-specific properties that are reflected in the functions of this enzyme in vivo, e.g. the N-terminal, unstructured extension (NTE), which most likely facilitates interactions with other proteins, but negatively affects biochemical activity.

Our RNA sequencing analyses show that the *dxo1* mutation deregulates the expression of many ribosomal protein (RP) genes, which is correlated with inefficient pre-rRNA maturation, both in the nucleolus and chloroplasts. It is possible that the mechanism of regulation of RP gene expression is the result of the cooperation of DXO1 with the mRNA cap methyltransferase RNMT1.

We investigated the role of DXO1 in combination with nuclear XRN2/3 exoribonucleases, known to be involved in rRNA processing. These analyses showed that some of the molecular and morphological phenotypes observed in *dxo1* plants were suppressed by XRN3 knockdown, providing evidence for the functional interaction between these proteins.

Keywords: 5', 3' exoribonucleases, rRNA maturation, ribosome biogenesis, deNADing

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eIF3E Domains Architecture and Phospho-Switches Control Pollen tube Growth and Membrane Morphology

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Controlled translation of mRNAs plays a pivotal role in regulating plant growth and development. The eIF3E subunit assembles on the octameric ribonucleoprotein complex to initiate mRNA translation and its PCI domain simultaneously engages in the assembly of 26S proteasome supercomplex for protein quality surveillance and degradation. While eIF3E is known to control translation initiation, how eIF3E is structurally regulated is unknown and the mechanisms governing its activity are poorly understood. We show here using a combination of RIPseq enrichment and experimental validation of genome-wide computational predictions that, in tobacco pollen tubes, eIF3E co-immunoprecipitate with a subset of mRNAs comprising tandem cis-elements, MC1-MC2-MC3 that function as both repressors and enhancers of mRNA translation in eIF3E-dependent manner. We demonstrate that the deletion of eIF3E PCI domain or modification of eIF3E Thr417 and Ser421 phosphosites located within the PCI domain that not only disrupt the eIF3E interaction with eIF3L, an initiation subunit, but also hinders eIF3E ability to repress or activate the MCs cis-elements and impairs eIF3E efficient dissociation from translating polyribosomes. Consequently, both PCI domain deletion and phosphomutated-eIF3E Thr417 and Ser421 misregulated the pollen tube growth rate and induced drastic membrane morphological abnormalities. Our findings provide new insights on cis-motifs guided eIF3E-mediated translational regulation of subset of mRNAs and inform of eIF3E mechanisms that impose protein homeostasis for precise pollen tube growth and maintenance of structural membrane morphology essential for successful plant fertilization. **Acknowledgments:** This work was financially supported from the Czech Science Foundation and the European Regional Development Fund-Project "Centre for Experimental Plant Biology. No. CZ.02.1.01/0.0/0.0/16_019/0000738) and GACR, grant No. 22-29717S

Keywords: Translation, RIPseq, Phosphorylation

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Eukaryotic Initiation Factors: Regulatory Insights into Selective Translation in Plant Developmental and Stress Responses

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The selection of translation initiation sites (TIS) on eukaryotic mRNAs plays a pivotal role in shaping the proteome in response to developmental or environmental signals. The intricate interplay between eukaryotic initiation factors (eIFs) and mRNA *cis*-regulatory sequences is crucial for ribosomes recognizing a TIS. Although our laboratory has previously identified numerous unannotated TISs in plants, particularly at AUG, CUG, and ACG codons, the underlying mechanisms of their recognition remain elusive. Intriguingly, we observed that specific eIFs regulate AUG and non-AUG initiation activities differently, with these regulations depending on specific flanking motifs in plants. This indicates a collaborative governance of TIS recognition by eIFs and *cis*-regulatory sequences. Moreover, these regulatory eIFs exhibit auto-repression and cross-activation of their protein synthesis. Importantly, our research has revealed that translation efficiencies of development- and stress-related genes are specifically regulated by distinct eIFs. Given that these eIFs are induced under specific stress conditions, our focus will be on examining plant stress tolerance and gene expression profiles associated with these specific eIFs. In summary, our findings shed light on a unique regulatory mechanism governing the recognition of start codons, emphasizing the delicate balance between constraint and flexibility in utilizing both AUG and non-AUG initiation sites for protein synthesis regulation under diverse stress and growth conditions in plants.

Keywords: alternative translation initiation sites, TISs selection, eukaryotic initiation factors, plant growth, stress tolerance.

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Multi-omics analysis reveals massive translational regulation during cold acclimation in tobacco

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As sessile organisms, plants must acclimate to suboptimal environments. While considerable research has elucidated the transcriptional and proteomic responses to low temperature, the mechanisms of translational regulation remained largely unexplored. In this study, we conducted a comprehensive time-series analysis, generating and integrating transcriptomics (RNA-seq), translatomics (Ribo-seq), and proteomics (mass spectrometry) data to investigate the dynamic responses of tobacco seedlings subjected to low temperature (12 ∘C). The proteome analysis on total protein revealed that photosynthesis-related proteins were strongly regulated. Given the vital role of chloroplasts in temperature sensing and acclimation, we analyzed both the expression of chloroplast genes and nuclear genes at the level of transcript accumulation and translation. Our data showed that chloroplast genes were primarily regulated at the translation level, responding within minutes after the temperature shift. In contrast, nuclear genes exhibited responses at both transcriptional level (mainly from one day after the temperature shift) and translational level (mainly from one hour). Notably, a subset of nuclear genes displayed exclusive translational responsiveness, implicating new modulators of cold acclimation that have gone undetected in previous transcript-based analyses. We currently characterize ribosomal elongation dynamics, such as ribosomal pausing, in response to low temperature. In sum, our work aims to achieve an integrated network of cold-responsive gene expression dynamics at the levels of transcript accumulation, translation, and protein accumulation, thereby facilitating a system-level understanding of plant cold acclimation and providing a rich resource for the identification of regulatory genes involved in cold acclimation.

Keywords: cold acclimation, translational regulation, RNAseq, ribo, seq, proteomics

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Characterization of a CERES interacting protein with a potential role in translation regulation and stress response

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Translation is a fundamental step in gene expression that regulates multiple developmental and stress responses. eIF4E is a key factor in translation initiation, which in non-plants eukaryotes is highly regulated through its interaction with other proteins. In our lab, we have recently identified CERES, a novel eIF4E-interacting protein that forms non-canonical translation initiation complexes that positively regulates the translation process (Toribio et al., 2019). CERES is an LRR protein that is expected to bind other proteins, apart from eIF4E; therefore, in an attempt to continue CERES characterization, we carried out immunoprecipitation assays and we identified a protein that interacts with CERES. This protein was named as C-INT (CERES interactor). C-INT belongs to a family of proteins with three members in Arabidopsis. This family of proteins display a potential role in translation in non-plant eukaryotes by forming part of different complexes; however, its function in plants is not completely understood. In this study, we attempt to characterize C-INT1, C-INT2 and C-INT3. We have demonstrated that C-INT1, C-INT2 and C-INT3 interact *in vivo* with CERES but not with eIF4E. We have generated loss- and gain-of-function- transgenic plants of each C-INT gene. Interestingly, *c-int2* mutants seem to be more sensitive to ABA than the wild type, suggesting that C-INT proteins could have a role in response to stress. Furthermore, these proteins accumulate in ribonucleoprotein complexes under specific stress conditions. The involvement of the C-INT proteins in regulation of translation and response to stress is being investigated.
Reference: Toribio, R., Muñoz, A., Castro-Sanz, A.B. et al. A novel eIF4E-interacting protein that forms non-canonical translation initiation complexes. Nat. Plants 5, 1283–1296 (2019).
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Keywords: Translation, eIF4E, Stress

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Multi-transcriptomics identifies targets of the endoribonuclease DNE1 and highlights its coordination with decapping

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Decapping is a crucial step in mRNA degradation in eucaryotes and requires the formation of a holoenzyme complex between the decapping enzyme DECAPPING 2 (DCP2) and the decapping enhancer DCP1. In Arabidopsis (*Arabidopsis thaliana*), DCP1-ASSOCIATED NYN ENDORIBONUCLEASE 1 (DNE1) is a direct protein partner of DCP1. The function of both DNE1 and decapping are necessary to maintain phyllotaxis, the regularity of organ emergence in the apex. In this study, we combined *in vivo* mRNA editing, RNA degradome sequencing, transcriptomics and small RNA-omics to identify targets of DNE1 and study how DNE1 and DCP2 cooperate in controlling mRNA fate. Our data reveal that DNE1 mainly contacts and cleaves mRNAs in the coding sequence and has sequence cleavage preferences. DNE1 targets are also degraded through decapping, and both RNA degradation pathways influence the production of mRNA-derived small interfering RNAs. Finally, we detected mRNA features enriched in DNE1 targets including RNA G-quadruplexes and translated upstream open reading frames. Combining these four complementary high-throughput sequencing strategies greatly expands the range of DNE1 targets and allowed us to build a conceptual framework describing the influence of DNE1 and decapping on mRNA fate. These data will be crucial to unveil the specificity of DNE1 action and understand its importance for developmental patterning.

Keywords: DNE1, RNA degradation, Endoribonuclease, Targets, Multi, transcriptomics, DCP1, Decapping

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N6-methyladenosine (m6A) of mRNAs mediates the growth fitness of young Arabidopsis seedlings

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For plants, environmental light is one prominent signal that reshapes the gene expression at various levels of the central dogma. At the level of post-transcriptional regulation, N6-methyladenosine (m6A) is the most prevalent modification in mRNAs and plays a key role in fine-tuning mRNA destinies in both plants and animals. Although evidence has indicated the essential roles of m6A in regulating several light-responsive genes, the broader impact of m6A modification in seedling growth and its functional roles during the transition from skotomorphogenesis to photomorphogenesis remain elusive. Here, we conducted epitranscriptomic profilings of both etiolated and de-etiolating wild-type Col-0 (WT) and m6A-writer mutant (vir) seedlings by Nanopore direct RNA sequencing. Comparative analyses of the collected m6A epitranscriptomes reveal the dynamic modulation of light- and VIR-dependent m6A modification of mRNAs in both skotomorphogenic and photomorphogenic development. The results showed light triggers the m6A modifications for mRNAs enriched in photosynthesis-related processes and represses those in translation-related processes after light exposure. Phenotypic analyses indicated that, compared to 4-d-old WT seedlings, vir mutant showed a significant delay in acquiring PSII efficiency during the 24-h light exposure. Additionally, increased de novo protein synthesis rates were observed in the vir mutant grown under both dark and light conditions. These results indicate that m6A modification is crucial for young seedlings to properly adapt to light/dark environments and to acquire timely autotrophic capacities during early photomorphogenesis. By integrating transcriptomic, epi-transcriptomic, and phenotypic analyses, we discovered that m6A abundance is associated with key biological processes in both photomorphogenesis and skotomorphogenesis. Our preliminary study also indicates that m6A modification contributes to the light-dependent regulation of chloroplast development and translation capacity in young Arabidopsis seedlings.

Keywords: N6.methyladenosine, m6A, photomorphogenesis, skotomorphogenesis

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Plant specific RS proteins possess opposing roles during light-dependent early seedling development in Arabidopsis thaliana

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Monitoring alterations in light conditions is crucial for plants to adapt their developmental program to the environment. One example is the transition from skoto- to photomorphogenesis upon illumination of etiolated seedlings. This transition is accompanied by transcriptome-wide changes in gene expression, including alternative splicing (AS) of precursor mRNAs. These light-induced splicing alterations are similar to AS changes which were observed upon exogenous sugar supply, indicating that AS is an important modulation point for different pathways which control energy availability during plant development. AS decisions are controlled by splicing regulatory proteins, including the family of serine-arginine rich (SR) proteins. Using phosphoproteomics, we observed that the SR proteins RS40 and RS41 were hyperphosphorylated when etiolated seedlings were treated with white light or sugar, suggesting its function as intersection point in monitoring energy availability. Therefore, we further investigated the role of the plant-specific subfamily of RS proteins which consists besides RS40 and RS41 also of RS31 and RS31a. We observed that overexpression of *RS41* causes premature cotyledon opening and a reduced hypocotyl length in seedlings grown in darkness which indicates the importance of RS41 for photomorphogenesis. In addition, loss of *RS31a, RS40* and *RS41* in higher order mutants induces hypocotyl elongation in continuous red light, further supporting our hypothesis that RS proteins play a positive role in the regulation of light-dependent early seedling development. Interestingly, we observed that loss of *RS31* function has an opposing effect compared to the knockout of *RS31a*, *RS40* and *RS41*, namely a decrease in hypocotyl elongation. Additionally, we showed in an inducible artificial micro-RNA system, where we downregulated *RS31* in the *rs31a rs40 rs41* mutant background, that downregulation of *RS31* reverted the elongated hypocotyls of the triple mutant back to wild type length. Moreover, we obtained evidence that the expression of the RS proteins and another splicing regulatory protein involved in photomorphogenesis is interlocked on the AS level. Taken together, our observations underline the importance of RS proteins during light dependent early seedling development, indicate an opposing effect of different members of the RS family, and suggest that their regulatory network contributes to fine-tuning this critical developmental process.

Keywords: development, photomorphogenesis, serine arginine rich (SR) proteins, alternative splicing

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pre-mRNA splicing links photosynthesis activity to lateral root morphogenesis

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Land plants always face to many kinds of stresses, without escaping from unsuitable situations. Thus, for surviving, plants should sense the surrounding environment and adjust their growth and development.
Plastid is an organelle for important metabolic processes such as photosynthesis. The metabolic activity is changed by the surrounding environment; thus, plastid is called environmental sensor. The environmental information sensed by plastid is informed to nucleus by plastid signal. Previous study revealed that the plastid signal regulates not only transcription but also pre-mRNA splicing in nucleus. Besides, a photosynthetic inhibitor causes the formation of knob-like lateral roots. Considering these knowledges, we hypothesized that photosynthetic activity influences lateral root morphogenesis via pre-mRNA splicing.
To test this hypothesis, we examined Arabidopsis mutants, heme-signaling (one of the plastid signaling) or pre-mRNA splicing defective mutants. The treatment with photosynthetic inhibitor increased the width of lateral roots in the wild type, but not in the mutants. Subsequent grafting experiment revealed that heme signaling and pre-mRNA splicing in aboveground tissues are important for the regulation of lateral root width in response to photosynthetic activity. The RNA-seq data indicated that changes in splicing events, but not in gene expression levels, were observed after the treatment with inhibitors. Especially, in underground tissues, abnormal splicing events were detected in genes related to auxin, a phytohormone crucial for lateral root formation. In addition, abnormal splicing events were also detected in heme-signaling defective mutant, suggesting that the heme signaling can influence the pre-mRNA splicing regulation. These results reveal a novel plant response strategy in which photosynthetic activity in the aboveground tissues can alter lateral root morphology via heme-signaling-mediated splicing control.

Keywords: splicing, lateral root, photosynthesis

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Pseudouridine - an epitranscriptomic regulator of plant miRNAs

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Pseudouridine (Ψ), an isomer of uridine, was the first naturally occurring, chemically modified ribonucleotide to be described. For over 50 years, it was extensively studied in rRNAs, tRNAs and snRNAs of different organism. However, technical constraints made it impossible to thoroughly investigate the role of pseudouridine in molecules such as mRNAs or small non-coding RNAs.
Observed in the last few years, significant development and improvement of high-throughput next-generation sequencing methods allowed us to revisit studies on various RNA modifications, including pseudouridine. Recent work proved that Ψ is present in a set of mature micro RNAs (miRNAs) of Arabidopsis thaliana, among which miRNA-159b was identified.
miRNAs are one of the key regulators of plant gene expression levels. Transcribed by RNA Polymerase II from *MIR* genes, they undergo a multi-step, complex biogenesis and maturation by the microprocessor complex. It is now clear that RNA modifications, for example N-6-methyladenosine, are involved in those processes. We set out to look at the potential role of pseudouridine both in plant miRNA processing and action, firstly focusing on deciphering whether the identified sites of pseudouridylation in mature ath-miR-159b can influence its biogenesis.

Keywords: pseudouridine, epitranscriptomics, miRNA, Arabidopsis thaliana, RNA modifications

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Regulation of Gene Expression in Trees: The Role of Transcription Start Site Selection

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The adaptation of perennial trees in temperate ecosystems relies on their remarkable plasticity, governed by a combination of endogenous and environmental factors. Until now, our understanding of these endogenous factors and their molecular mechanisms has been quite limited. In plants, gene expression is tightly controlled through various transcription-dependent RNA quality control pathways, with transcription initiation being a key regulatory step. Thus, understanding the dynamics of transcription initiation, the signals that specify it, and the underlying molecular mechanisms is crucial. In this study, we performed a genome-wide analysis of transcription start sites (TSSs) in Norway spruce, *Picea abies*, using Transcription Start Site-sequencing (TSS-seq). Our findings reveal numerous novel TSSs on both sense and antisense strands at single nucleotide resolution. By clustering these TSSs into different TSS clusters (TCs), we identified alternative transcriptional initiation (ATI) sites in several specific genes, highlighting variability in transcription initiation. Moreover, we discovered that different tissues in spruce-needles, wood, and a pooled sample including embryo, xylem, and pollen-exclusively utilize various TSSs. This tissue-specific usage of TSSs underscores the complexity and specificity of transcription regulation in different biological contexts. These findings provide a comprehensive overview of TSS usage as a critical endogenous control factor in spruce. A deeper understanding of this regulatory mechanism is essential for developing practical approaches to address urgent issues in agriculture, horticulture, and forestry, particularly those exacerbated by climate change.

Keywords: Transcription start sites, Trees, TSS, seq, Norway spruce.

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Regulation of RNA homeostasis and thermotolerance by plant specific splicing factors in tomato

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Given current global events, the increase of temperatures is defined as one of the major threats in food security. Even a slight increase above organism-specific thresholds, can cause heat stress (HS) which in turn will have a negative impact on its physiological function, e.g. reduced growth, reduced photosynthesis and reduced reproductive success. To withstand, survive and recover from a heat stress, plants depend on the synthesis of heat shock proteins (HSPs), which protect proteins from irreversible aggregation. The expression of HSPs is mainly regulated by a class of HS transcription factors (HSFs). HSFs are considered as the molecular rheostat, integrating information about the temperature to determine the optimal output. To achieve this, the activity and expression of HSFs and their partner proteins need to be tightly regulated. Recent studies shed a light on the role of alternative splicing as a central process in the regulation of HSFs. (Hu et al., 2020, Mesihovic et al., 2022).
In our research, we aimed to identify core splicing regulators in HS-sensitive alternative splicing. We discovered two plant specific Serine/Arginine-rich proteins (RS2Z35 & RS2Z36) with important roles in mediating RNA homeostasis under HS. We showed that mutating *RS2Z35* and *RS2Z36* by CRIPSR/Cas9 results in a higher sensitivity under higher temperatures. Furthermore, RNA-Seq analysis revealed that _˜33% of differential alternative splicing events that occur under HS, are regulated by RS2Z35 & RS2Z36. By performing individual nucleotide resolution UV-cross-linking and immunoprecipitation (iCLIP) in combination with high-throughput sequencing we were even able to identify the in vivo RNA binding sites of both proteins.

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Keywords: Alternative splicing, SR, Proteins, Heat stress response, iCLIP2

Rider of the Plant's defense storm: Turnip Mosaic Virus modulates Plant's stress granule response

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Stress granules (SGs) are dynamic cytoplasmic biomolecular condensates critical in Plants' response to biotic and abiotic stresses by transiently sequestering mRNA, thus regulating post-transcriptionally its translation. Despite their importance, the role of SGs in plant viral infection remains poorly understood. This study employs a comparative virology approach to examine SG dynamics during Turnip mosaic virus (TuMV) and Cucumber mosaic virus (CMV) infections in *Arabidopsis thaliana*. Using heat shock and arsenite as inducers of SGs, we observed that TuMV, notoriously impairs SG assembly. This disruption is evidenced by the decreased formation of SGs monitored via several distinct SG-markers following stress induction in TuMV-infected plants. In contrast, CMV infection did not inhibit SG formation. Neither virus affected the abundance of functionally linked but distinct condensates termed Processing Bodies, indicating a TuMV-specific impact on SGs. Despite that RBP47b, a canonical SG marker, was transcriptionally upregulated under both heat shock and viral infection conditions, this upregulation did not correspond with enhanced SG formation with TuMV. Suggesting a TuMV specific repurpose of this marker. To identify potential components of the observed contrasting TuMV SG-dynamics, we performed comparative GFP-RBP47b pulldown proteomics for control, TuMV, CMV, and heat shock conditions. This revealed that TuMV alters RBP47b protein interactions, recruiting RNA-binding proteins like Poly-A Binding Proteins, a host proteins that supports TuMV infection as well as previously unknown SG-like proteins from the ZFN family. Reporter assays with the TuMV 5'UTR attached to Renilla luciferase and viral protein immunoblots from infected tissue showed that PAB and ZFN proteins increase TuMV protein accumulation. These findings suggest TuMV repurposes SGs to enhance viral translation, whereas CMV does not exhibit this effect.

Keywords: Stress granules, Turnip mosaic virus, RNA binding proteins, RBP47b, Viral Translation

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RNA quality control ensures the epigenome and function of Arabidopsis centromeres

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The centromere is an important chromatin domain that ensures accurate chromosome segregation during cell division, and centromeric heterochromatin has been shown to be essential for the centromere function in other organisms. However in plants, mutants defective in DNA methylation curisouly do not exhibit sever chromosome instability despite reduced centromeric heterochromatin, and the mechanism that maintains the centromere function remains largely unknown. Here in the model plant Arabidopsis, we show that CCR4, the catalytic subunit of the RNA deadenylation complex CCR4-NOT, cooperates with DNA methylation pathways to maintain the epigenome and function of centromeres. We first observed severe mitotic chromosome instability and developmental defects in the mutant defective in both CCR4 and DNA methylation, suggesting the possibility that poly(A)-tail processing by CCR4 controls epigenetic pathways and centromere function. Small RNA sequencing analysis discovered that CCR4 is required for small interfering RNA (siRNA) biogenesis mediated by RDR6 (RNA-dependent RNA polymerase). RDR6 is known to prefer short poly(A)-tailed template transcripts for double-stranded RNA synthesis, and consistently Nanopore Direct RNA sequencing analysis revealed that CCR4 shortens the template transcripts for RDR6 such as centromeric ATHILA transposon RNAs and centromere repeat-derived RNAs. These CCR4-dependent centromeric siRNAs promote non-CG DNA methylation over the centromere repeats via the RNA-directed DNA methylation pathway. Because DNA methylation is known to recruit histone H3K9 methyltransferases to form heterochromatin, we also assessed the impact of CCR4-dependent siRNAs on H3K9 methylation. As a result, we discovered that CCR4 is essential for ensuring H3K9 methylation at the centromere repeats in the absence of primary DNA methylation pathways. Our study unveils the importance of poly(A)-tail processing in RNA interference and highlights the elaborate mechanism protecting the plant centromere through epigenetic regulations.

Keywords: RNA interference, chromosome stability, Centromere, DNA methylation, Epigenetics, Heterochromatin

*Speaker

SD5/DROL1 protein, a subunit of Arabidopsis U5 snRNP, controls nutrient response through pre-mRNA splicing and jasmonic acid signaling

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Plants senses environmental conditions like a humidity, temperature, and nutrition. In response to such conditions, the gene expression should be adapted to fulfill their better growth and development. Recently, it has become clear that pre-mRNA splicing, one of post-transcriptional regulation of gene expression, is crucial for nutrient-dependent plant growth. Here, we report an *Arabidopsis thaliana* mutant, *segregation disorder 5* (*sd5*), showing abnormal seeding growth in response to nutrient condition. The *sd5* mutant showed poor growth under rich-nutrient condition, but their growth was recovered under poor-nutrient condition. The molecular cloning revealed that the *sd5* mutation is found in the gene encoding a homolog of Dim1, a subunit of U5 snRNPs, a core component of splicing machinery, spliceosome. RNA-seq analysis of *sd5* demonstrated that many of minor-type splicing events, as well as certain groups of major-type splicing events were affected by the *sd5* mutation. Moreover, the genes revealed to jasmonic acid (JA) biosynthesis and response were abnormally upregulated in *sd5.* Indeed, in *sd5*, the response to JA biosynthesis inhibitor was affected. Taken together, we propose that SD5 protein works to regulate nutrient response through pre-mRNA splicing and JA signaling regulation.

Keywords: splicing, transcription, nutrition

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Searching for interactors of SOAR1, a translational regulator, in response to light

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Translation is one of the most energy-demanding processes in the cell and, therefore, it is finely regulated to coordinate growth and metabolism. Plants primarily produce energy through photosynthesis, providing peaks of energy associated to the light diel cycle. Subsequently, plants have evolved specific mechanisms to regulate mRNA translation into proteins in response to these light fluctuations (Missra et al. 2015). Despite being well established that translation is coupled to light conditions and photosynthesis, experimental evidence elucidating the key molecular players involved in this regulation remains elusive. SOAR1 is a pentatricopeptide repeat protein, recently demonstrated to modulate mRNA translation (Bi et al. 2019). The binding of SOAR1 to the translation initiation factors eIFiso4G1 and eIFiso4G2 inhibits their association with the other partners of the eIF4F complex, hindering factor assembly and inhibiting global translation (Bi et al. 2019). Additionally, SOAR1 is known to negatively regulate abscisic acid (ABA) signalling (Mei et al. 2014). Importantly, SOAR1 directly binds to the mRNA of *ABI5*, a key ABA-responsive gene, and coope rates with eIFiso4Gs to repress *ABI5* translation (Bi et al. 2019). While the collaboration between ABA and light signalling is generally accepted, there is currently no evidence linking SOAR1 and light-dependent translation regulation. In the presented work, we performed a proteomic analysis revealing that numerous putative SOAR1-interacting proteins are located in the chloroplast, suggesting a potential role of SOAR1 in controlling translation in a light-dependent manner. We then demonstrate the *in vivo* interaction of SOAR1 with one chloroplast-located protein. Moreover, we examine how light differentially affects protein accumulation in wild-type and *soar1* mutant Arabidopsis plants and discuss the implications of SOAR1 in translation regulation in this light-dependent context.

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Keywords: translational regulation, light

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Mechanisms and impact of ribosomal RNA methylation in plants

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Ribose methylation (2-*O*-Me) is the most common modification of ribosomal RNAs (rRNA) in various life forms and are pivotal for ribosome assembly and translation. These 2-*O*-Me modifications are guided by small nucleolar C/D-box RNAs (C/D snoRNA) and are deposited by the methyltransferase FIBRILLARIN (FIB). Our work, performed in the plant model *Arabidopsis thaliana*, highlights that rRNA 2-*O*-Me is a dynamic process. To understand the impact of such modulation of rRNA 2-*O*-Me on translation, we examined Arabidopsis plants lacking the major nucleolar protein Nucleolin 1 (NUC1). Depletion of NUC1 (*nuc1*) resulted in decreased rRNA 2-*O*-Me levels(1) and, hence, in ribosome hypomethylation. Our findings suggest that reduced rRNA 2-*O*-Me in *nuc1* may be triggered by defects in the assembly of the C/D snoRNP complex. Ribosome hypomethylation leads to an accumulation of polysomes detectable in their polysome profiles and a stalling of ribosomes at the start codon shown in 5'Pseq analysis. We are now trying to elucidate if this stalling could be either inferred to the formation of the 80S complex or to difficulties in interacting with translation initiation factors. All in all, our results underscore the crucial role of rRNA methylation and the heterogeneity of ribosomes in their assembly and translation processes. *(1) Azevedo-Favory J et al. (2021) RNA Biology 18:11, 1–18*

Keywords: ribosomal RNA, Ribosome methylation, Ribosomes

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The role of RNA BINDING PROTEINS in seeds

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Dry orthodox seeds can be stored for years and decades without losing their ability to germinate and develop into a viable seedling. This seed longevity is vital for a species survival, ecological diversity as well as for commercial purposes. Dry seeds contain high amounts of protein, oil, sugar and mRNA. Especially the translation of these long-lived mRNAs inside dry seeds of Arabidopsis is essential for the ability to germinate. It is however unclear how these otherwise fragile mRNA molecules can be preserved inside dry seeds for such an extended amount of time.
In this study, we hypothesise that these long-lived mRNA molecules are bound, regulated and possibly protected by RNA-binding proteins (RBPs). Some of these proteins bind mRNA inside cytoplasmic granules that can form through phase separation. To test our hypotheses, I use seed trait phenotyping of transgenic and *rbp* mutant seeds combined with imaging techniques to determine the role, presence and (co-) localisation of RBPs and mRNA inside seeds. However, seeds have evolved a seed coat to withstand the test of time and many biotic and abiotic stresses, yet this hinders clear visualisation of the embryo inside the seeds. I have used and developed several imaging approaches to circumvent these issues. I show that one of these highly expressed RBPs inside seeds affects the decision to germinate under saline conditions and that the protein translocates differentially inside the cell in these circumstances.

Keywords: RBPs, RNA BINDING PROTEINS, processing bodies, stress granules, mRNA, translation, seeds, germination

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sRNA-mediated response to extreme daily temperature variations in the desert plant Nicotiana attenuata

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Global warming critically impacts plant life, requiring plants to adapt to temperature changes. Deserts, with extreme temperature variations, are ideal for studying plant adaptation. *Nicotiana attenuata*, native to the Great Basin Desert, endures over 30∘C daily temperature changes. It has a fully sequenced genome and a plethora of available transcriptomic and metabolomic datasets. Many studies have examined temperature-regulated processes seasonally, but the effects of daily temperature changes on biological processes remain largely unknown.
To get insights into *N. attenuata* response mechanisms to daily extreme temperature changes, we analyzed RNA extracted from aerial tissues of this plant exposed to temperature variation (dT – from 0 to 43ºC daily) and control conditions (26ºC constant), sampled every 4 hours during a complete day. Two Zeitgeber time points (ZT14 and ZT22) were chosen for RNA and small RNA (sRNA) next-generation sequencing. We re-annotated sRNAs, identifying over 36,000 clusters producing sRNAs. From the identified clusters, 150 were predicted to be miRNA precursors, producing 127 unique sequences: 72 evolutionarily conserved, 29 previously discovered exclusively in *N. attenuata* roots under biotic stress (Nat-R-PN), and 26 novel miRNAs. We then compared the expression levels between the two time points at either control or dT conditions. While mRNA levels were mutually affected by circadian and temperature oscillation, miRNA showed mainly a dependency on temperature fluctuation. Some conserved miRNAs already reported to be affected by temperature stress were found out in our datasets as well, and others seem to play a role exclusively in these extreme conditions. Additionally, Nat-R-PN miRNAs were detected varying in response to dT, suggesting that miRNAs involved in biotic stress in roots may have a role in aerial tissues in temperature response. Furthermore, novel miRNAs were as well involved in this response. On the other hand, target prediction identified known and novel putative targets for conserved miRNAs. mRNA levels in our data were in concordance with this regulation, suggesting these miRNAs could play diverse roles in *N. attenuata*'s response to extreme temperature variations.
Overall, our findings provide insights into the complex regulatory mechanisms desert plants use to adapt to extreme temperature variations.

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Keywords: small RNA, Nicotiana attenuata, desert, extreme temperatures

The circadian rhythm is affected by the defect of uORFs in Arabidopsis clock gene LHY

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The circadian clock is a molecular timekeeping mechanism that allows organisms to anticipate periodic changes in their surrounding environment. Synchronizing with the environment via the circadian clock is important for plants and affects their fitness. It is known that circadian oscillation is controlled precisely by multiple interlocked feedback loops consisting of clock genes mostly at the transcription level. Even though the involvement of translational regulation in the expression of clock genes has been suggested, the underlying mechanism is not fully elucidated yet.
One of the known *cis*-regulatory elements that modulate the translation in coding sequence is upstream open reading frames (uORFs) in the 5'-untranslated region (5'-UTRs). To investigate the translation status of clock genes, Ribo-seq (Ribosome profiling) was conducted using *Arabidopsis* shoot samples in several timepoints throughout the 12 h-light/ 12 h-dark cycle. Consequently, at the timepoint after dawn, a prominent ribosome occupancy was observed within the 5'-UTR of *LATE ELONGATED HYPOCOTYL* (*LHY*), a major component of the plant circadian clock. We found that the major splicing variant of *LHY* gene contained five uORFs in its 5'-UTR, and interestingly, the significant ribosome occupancy which was observed in 5'-UTR of *LHY* started adjacent to uORFs. Therefore, time- or light-dependent translational repression mediated by uORFs in *LHY* gene was suggested.

To investigate the function of uORFs in *LHY*, we established transgenic lines called *LHY* promoter series which have *LHY* own promoter and 5'-UTR (with WT or mutated uORFs) fused to reporter luciferase gene. Also, in order to reveal the significance of *LHY* uORFs in the feedback loops of circadian clock, we established *LHY* genomic clone series which *LHY* genomic sequence from its promoter to 3'-UTR were introduced into *lhy* knock-out mutant.
Here, we report the results using these lines that the defect of uORFs in *LHY* results in a signif-

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icantly higher translation level, and the translational control mediated by uORFs is important to maintain the circadian rhythm.

Keywords: Translational control, upstream open reading frame (uORF), Circadian clock, Circadian rhythm

The effects of seed maturation at high temperatures on translational regulation during seed germination

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Seed quality includes the ability of seeds to germinate uniformly and produce healthy seedlings even under adverse circumstances. High temperatures (HT) during seed maturation affect major seed quality traits, such as germination ability in HT conditions and dormancy induction. The effect of HT during seed maturation can vary within species in a genotype-dependent manner. For example, HT during maturation can increase the sensitivity to germination under HT for some genotypes, while for others it may enhance their tolerance to HT during germination. The germination process is under control of translational regulation, which is thought to allow a fast response to the environment in which the seeds are present. We hypothesize that the maturation environment affects which transcripts are translationally regulated during germination under temperature stress. To test this hypothesis, we will perform polysome profiling followed by RNAseq on the polysome and monosome-associated fractions during germination under elevated and control temperatures in seeds matured under HT and standard conditions. This allows us to assess which transcripts are differentially associated to polysomes and identify heat specific translational regulation induced by the maturation environment. Later, translationally regulated genes will be identified and further characterized using knock-out and reporter lines to identify their role in conferring sensitivity or tolerance to germination under HT.

Keywords: Seeds, germination, maturation, temperature, heat, translational regulation

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Transcriptomic insights into A.thaliana cold stress response

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Environmental stress negatively affects the growth and development of all organisms. RNA methylation at the N6 position of adenosine residues (m6A) has been demonstrated to be a vital epigenetic mark which accounts for more than 80% of RNA methylation modifications in eukaryotes. In plants it's involved in countless developmental and stress responsive pathways. Acclimatisation to challenging conditions frequently lead to reprogramming of the transcriptional output by RNA polymerase II (RNAPII), which reflects steady-state levels of messenger RNAs and non-coding RNAs in the cell. These responses are usually created by sequence-specific transcription factors.

Transcriptionally engaged RNAPII complexes can be visualised by Plant Native Elongating Transcript sequencing (plaNET-seq), which enables profiling genome-wide nascent RNAPII transcription with the single-nucleotide resolution. Capturing nascent RNA allows to detect RNAs which are often targeted of co-transcriptional RNA degradation. While splicing regulation is one of the most essential for the stress response in A. thaliana, plaNET-seq also provides insight into co-transcriptional RNA splicing, since some part of the spliceosome is co-purified with RNAPII complexes.

It was recently showed that cold induced changes in abundance of differentially methylated transcripts are at-least partly due to the differences in the rates of transcription of such transcripts. RNAPII stalling was observed only on RNA methylation motifs that were tagged with m6A, and those that had a specific nucleotide composition. Transcripts that gained m6A methylation under cold stress conditions, showed increased abundance and polysome association. Through the application of the plaNET-seq technique, it is possible to understand the molecular basics of the change in RNAPII kinetics in response to cold temperature stress in plants.

Keywords: cold stress, transcription, plants acclimation, gene expression

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The role of DEAD-box helicases: RH11, RH37, RH52 in miRNA biogenesis

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Over the last decades, there has been an increased interest in topics related to the importance of microRNA (miRNA) for the development and physiology of plants, as well as the biogenesis of these molecules in the plant cell. MiRNAs are a class of small non-coding RNAs that regulate gene expression in eukaryotes. They are crucial regulators of plant development. In plants, a complete blockage of miRNA biogenesis is lethal. Primary miRNA precursors (pri-miRNAs) have stem-loop structures in which miRNA sequences are embedded. In the first step of miRNA biogenesis, the stem-loop structures are cut out from pri-miRNAs, forming shorter precursors - pre-miRNAs. In the second step, short miRNA/miRNA* duplexes are generated from pre-miRNAs. In plants, both steps are catalyzed by the same endonuclease DCL1 (the Dicer-like 1 protein) and both take place in the cell nucleus. DCL1, however, requires two other factors for efficient and precise cleavages: the dsRNA-binding protein HYPONASTIC LEAVES 1 (HYL1) and SERRATE (SE), a zinc finger domain-containing protein.
In this study we investigate how three DEAD-box helicases: RH11, RH37, RH52 regulate miRNA biogenesis. Our preliminary results showed that knock-outs of these genes in Arabidopsis reveals phenotypes similar to plants with elevated levels of mature miRNAs. Interestingly, the plants overexpressing RH11-FLAG showed a phenotype similar to the *hyl1-2* mutant which has characteristic hyponastic leaves. We also showed that several proteins involved in miRNA biogenesis, including SE, were co-purified with the RH11-FLAG fusion protein. Furthermore, a significant delay in flowering was observed in the *rh11 rh37* and *rh37 rh52* double mutants that suggests the importance of these helicases in plant development. Based on our results, we proposed a model in which the studied DEAD-box helicases are negative regulators of miRNA biogenesis, by influencing the stem-loop structure that in consequence prevents DCL1/ HYL1 binding to miRNA precursors and their further processing.

Keywords: miRNA biogenesis, RH11, RH37, RH52, DEAD, box helicases

*Speaker

Two plant mitochondrial long non-coding RNAs – structure, expression and function

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Long non-coding RNAs (lncRNAs) are RNA molecules that do not encode proteins and are more than 200 nucleotides in length. They are recognize as important regulatory molecules in most post-transcriptional regulation processes, both in animals and plants. However, the function of lncRNAs in plant mitochondria remains largely unexplored. Recently, we focus our attention on two mitochondrial-encoded lncRNAs in Arabidopsis, the previously identified by Holec et al. (2006), the NCO transcript, and a novel lncRNA named by us "the BP transcript". The NCO transcript was found in several isoforms, of which a significant portion are circular RNAs with diverse 3 ends and homogenous 5 termini. This 5' end is identical with the 5' end of M112 cosRNA (clustered organellar sRNAs)The BP transcript appears to be present as a linear molecule. Next, we evaluated the expression of the NCO and BP transcripts under stress conditions and found that level of both transcripts increased under short-term high temperature and drought stress conditions. Moreover, both transcripts accumulate significantly in mitochondrial mutants with dysregulated small mitoribosomal subunit biogenesis, most likely due to decreased efficiency of PNPase, an exonuclease responsible for NCO and BP degradation. Most important, to define the function of BP and NCO transcripts we disrupted their expression using the mitochondria-targeted transcription activator-like effector nucleases (mitoTALENs) approach. Whole-mutant genome sequencing was utilized to determine the extent of the deletion. The phenotypic analysis indicate that the NCO transcript may influence growth and development of the Arabdopsis rosette, but not its roots. We observe no changes in plant morphology and development after removal of the BP transcript.
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Keywords: non, coding RNA, lncRNA, mitochondria, circular transcript, mitoTALEN

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ULT1 is a PRC2 dependent RNA binding protein and regulates indeterminacy

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The Polycomb (PcG) and trithorax group (trxG) chromatin complexes play key antagonizing roles in developmental transitions, via the deposition of mutually exclusive post-translational modifications on Histone H3.
The plant-specific ULTRAPETALA1 (ULT1) protein induces essential cell fate transitions such as those involved in flowering and flower morphogenesis (Fletcher, 2001; Carles et al, 2005). ULT1 allows target genes to transit from a PcG-repressed to a trxG-activated state and vice versa, via interactions with both chromatin and transcription factors (Carles & Fletcher, 2009; Moreau et al, 2016; unpublished data). We employ a multidisciplinary approach including genetic, epigenomic, interactomic and structural methods to elucidate ULT1 molecular function.

ChIP experiments reveal that ULT1 regulates the abundance the PRC2-mediated H3K27me3 mark. *In vitro* and *in vivo* assays indicate that ULT1 interacts with nucleic acids from the *AGAMOUS* locus in a PRC2 dependent manner.
These results reveal a novel chromatin regulatory function and uncover new avenues for understanding trxG/PcG interplay in eukaryotes. I will discuss how ULT1 may have a multidimensional role in this interplay, including targeting through nucleic acid interactions.

Keywords: chromatin, polycomb, trithorax, lncRNA

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Writers and reader: shaping and decoding the epitranscriptome landscape during the development of lateral root organs in the legume Medicago truncatula

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Legumes plants form two types of latera organs in their roots: lateral roots, which function in water and nutrient uptake, and symbiotic nodules, which allow the plant to acquired reduced forms of nitrogen because of its interaction with nitrogen-fixing bacteria. Formation of both types of organs depends on the establishment of morphogenetic programs that are regulated at multiple levels, including chromatin-based, transcriptional, and post-transcriptional events. Dynamic RNA modifications, known as epitranscriptomic marks, have emerged as a new mechanism for post-transcriptional control of gene expression. The methylation of adenosines (m6A) is the most abundant modification found in RNA and its presence impacts on splicing, transport, localization, translation, and decay of RNAs. This mark is deposited by RNA methylases (MTs) known as writers, mainly MTA and MTB, and decoded by RNA binding proteins of the Evolutionary conserved C-Terminal domain (ECT) family referred to as readers. In a previous work, we have characterized the changes in the translatome and RNA stability (degradome) of *Medicago truncatula* roots. Here, we attempt to elucidate the influence and function of the epitranscriptomic mark m6A on the translatability and stability of mRNAs during the formation of lateral roots and nodules. To globally assess changes in m6A, we applied MeRIP on roots under symbiotic and non-symbiotic conditions. In addition, to characterize the role of members of the ECT family in decoding m6A during the establishment of the root nodule symbiosis, we have knocked down and overexpressed a member of the ECT family, *ECT2/4*, which is differentially expressed during nodule and lateral root formation. Knockdown of *ECT2/4* did not affect nodule formation or bacterial infection, presumably due to functional redundancy with other ECT members during symbiosis, but affected lateral root elongation. On the other hand, overexpression of ECT2/4 in *M. truncatula* roots enhanced bacterial infection and reduced nodule number. Since RNA methylases MTA and MTB expression is upregulated at early stages of the root nodule symbiosis, we are currently addressing the functional characterization of these two RNA methylases, as well as its impact on the m6A landscape during the root nodule symbiosis.

Keywords: epitranscriptome, legumes, nitrogen fixation, root development, symbiosis

*Speaker

The Arabidopsis deNADding enzyme DXO1 modulates the plant immunity response

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The DXO proteins are highly conserved among eukaryotes and are involved in the removal of non-canonical NAD+ cap (deNADding) and mRNA 5'-end quality control. In Arabidopsis, DXO1 is the only homolog of the DXO family proteins. To evaluate the role of DXO1 in response to biotic stress, we investigated the susceptibility of the *dxo1-2* mutant and transgenics *dxo1-2* lines expressing DXO1(WT), DXO1(E394A/D396A) catalytic mutant, DXO1(DN194) lacking the unstructured plant-specific N-terminal domain and DXO1(DN194/E394A/D396A) to *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 infection, as well as its effectors flg22 and elf18. Our results show that the tested lines exhibit enhanced resistance to *Pst* accompanied by marked changes in the expression of key pathogenesis markers. Our genome-wide transcriptome analysis of *dxo1-2* mutant infected with *Pst*, verified by northern blot, revealed that the lack of DXO1 protein deregulates defense against *Pst* DC3000 infection on the transcriptional level. Additionally, other markers of plant immunity such as callose deposition and production of reactive oxygen species were strongly induced by elf18 and flg22 in the tested lines. Moreover, we observed decreased activation of MAPKs in the absence of DXO1, which most likely affects the function of multiple pathogen-related transcription factors. All molecular and morphological changes observed so far in the *dxo1* mutant are mainly dependent on the N-terminal (NTE) domain and this is also the case for resistance to pathogen. However, our data also supports the contribution of the DXO1 catalytic centre to this process. This is the first observation showing the involvement of the DXO1 enzymatic activity in plant physiology.

Keywords: DXO1, Pseudomonas syringae, flg22

*Speaker

MicroRNA controls the level of evolutionarily conserved DUSP12 phosphatase involved in spermatogenesis in Marchantia polymorpha

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Dioecious liverwort *Marchantia polymorpha* encodes conserved and liverwort-specific miRNAs. Several of these miRNAs exhibit a differential expression pattern in generative organs when compared to vegetative thalli suggesting their role in sexual organ development and reproduction. MpmiR11889 is exclusively expressed in sperm cells. We identified the target of this miRNA that encodes the dual-specificity phosphatase DUSP12 mRNA. DUSP12 protein is evolutionarily conserved and found in all eucaryotic organisms studied so far. It is known that in humans DUSP12 is involved in cell proliferation, differentiation, and in fungi sporogenesis. The expression profile of DUSP12 is conserved in humans and plants: it is significantly upregulated in the early phases of spermatogenesis but is absent in mature sperm and pollen grains. We found that the module MpmiR11889-MpDUSP12 activity is critical for proper sperm cell development and reproduction efficiency. CRISPR/Cas9 generated ΔMpmiR11889ge plants display defects in the distribution of antheridia that are moved upward the antheridiophore surface and often protrude outside of it. A similar phenotype was observed in Marchantia mutants overexpressing the Mp*DUSP12* gene. The sperm cells of both types of mutants exhibit significantly elevated levels of protamine-like protein (MpPRM), resulting in the increased ratio of heterochromatin to euchromatin and bead-like nuclei. Moreover, quantitative proteomic analysis of sperm cells from WT, ΔMpmiR11889ge, and OE MpDUSP12 revealed the elevated

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level of proteins responsible for cellular growth and development, DNA repair, and chromatin architecture. The movement trajectory of ΔMpmiR11889ge and OE MpDUSP12 sperm cells significantly differs from WT sperm cells resulting in chaotic and aimless motion. Crossings of male and female WT, ΔMpmiR11889ge mutant plants, and OE MpDUSP12 plants were performed. Generally, crossings performed for ΔMpmiR11889ge and OE MpDUSP12 plants resulted in a dramatic reduction of sporophyte production efficiency (3-14%). Therefore, the MpDUSP12 protein, regulated by MpmiR11889 is crucial for efficient and successful reproduction. Considering the evolutionary conservation of this protein one may speculate that it is also crucial for higher plants in pollen formation and in human spermatogenesis. The work is supported by the National Science Center: UMO-2020/39/B/NZ3/00539.

Keywords: miRNAs, Marchantia polymorpha, sexual reproduction, spermatogenesis

SVALKA-POLYCOMB REPRESSIVE COMPLEX2 module controls C-REPEAT BINDING FACTOR3 induction during cold acclimation

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C-REPEAT BINDING FACTORS (CBFs) are highly conserved plant transcription factors that promote cold tolerance. In Arabidopsis (*Arabidopsis thaliana*), three CBFs (CBF1 to CBF3) play a critical role in cold acclimation, and the expression of their corresponding genes is rapidly and transiently induced during this adaptive response. Cold induction of *CBFs* has been extensively studied and shown to be tightly controlled, yet the molecular mechanisms that restrict the expression of each *CBF* after their induction during cold acclimation are poorly understood. Here, we present genetic and molecular evidence that the decline in the induction of *CBF3* during cold acclimation is epigenetically regulated through the Polycomb Repressive Complex (PRC) 2. We show that this complex promotes the deposition of the repressive mark H3K27me3 at the coding region of *CBF3*, silencing its expression. Our results indicate that the cold-inducible long noncoding RNA *SVALKA* is essential for this regulation by recruiting PRC2 to *CBF3*. These findings unveil a *SVALKA*-PRC2 regulatory module that ensures the precise timing of *CBF3* induction during cold acclimation and the correct development of this adaptive response.

Keywords: SVALKA, CBFs, epigenetic, lncRNA, cold acclimation, Arabidopsis

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The DEAD-box helicases DRH1, RH46 and RH40 remodel the secondary structure of miRNA precursors to regulate miRNA biogenesis

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MicroRNAs (miRNAs) are short, single-stranded, non-coding RNAs that regulate gene expression at the post-transcriptional level. The process of miRNA production is complex and involves many proteins. Recently, DEAD-box helicases have become one of the most studied groups of proteins involved in miRNA biogenesis. The Arabidopsis genome encodes more than 50 DEAD-box helicases. Of these, only DRH1, RH46 and RH40 contain WW domains, which have been identified by in silico analysis as a site of their interactions with the CTD domain of RNA polymerase II (RNAPII). DRH1 is known to be involved in rRNA processing, nonsense-mediated decay (NMD) and mRNA export. However, the role of DRH1, RH46 and RH40 in miRNA biogenesis is unknown.

We tested single, double and triple mutants of DRH1, RH46 and RH40 in Arabidopsis under standard (22∘C) and modified (16∘C) growth conditions. We observed that the absence of all the studied helicases affected miRNA accumulation levels when cultured under both temperature conditions. Furthermore, the phenotype of drh1 rh46 rh40 plants was severely altered compared to wild-type plants when grown at 16∘C. Using FRET-FLIM analysis, we showed that DRH1 directly interacts with SERRATE (SE) and ARGONAUTE1 (AGO1), two important proteins in miRNA biogenesis and function pathways. In addition, DRH1 interacts with the CTD domain of RNAPII, in agreement with in silico predictions. However, contrary to this prediction, we showed that the WW domain is not required for this interaction. We also investigated the secondary structure of selected pre-miRNAs in drh1 rh46 rh40 using the targeted DMS-MaPseq technique. Our studies showed changes in the structure of a few pre-miRNAs tested. Taken together, our data suggest that DRH1, RH46 and RH40 influence miRNA biogenesis, possibly by shaping the structure of a subset of miRNA precursors.

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Keywords: miRNA, miRNA biogenesis, DEAD box helicases, DMS MaPseq

Understanding plant translational reprograming in response to multi-stresses

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Plants are key determinants for terrestrial ecosystems, acting as the most important source of food and oxygen. Because of their sessile nature, plants are continuously exposed to abiotic and biotic stresses that impose numerous detrimental effects on their health and survival. One of the most concerning constraints for agricultural ecosystems are pest and pathogens attacks. A risk that is expected to be exacerbated with climate change advancement, as rising temperatures will affect pest behaviour, distribution, development, and reproduction. Among pest, the two-spotted spider mite *Tetranychus urticae* is considered a serious threat to agricultural productivity. This herbivore is a generalist species that can feed over 1,100 host plant species, including important crops such as fruit trees and vegetables. The mite-associated injuries cause growth arrestment, flowering delay, and reduction of crop yield, which translate in important economic losses. To overcome this problem, one of the most promising solutions is to improve plant resistance to pests. To do so, it is needed a profound knowledge of the molecular basis of plant immune responses.

The response of plants to environmental stresses entails the reprogramming of the gene expression process, including translation. Consequently, analysing the translatome, which refers to the entirety of mRNAs associated with ribosomes for protein synthesis, can unveil crucial regulatory cues linked to plant resistance to different stresses. In this study we used *Arabidopsis thaliana* and the agronomically important specie *Brassica rapa* to investigate how plants modulates translation in response to the combined action of a *T.urticae* attack and a moderate increase in temperature (29∘C). To asses global changes in mRNA translation, we utilized the polysome profiling method8, which facilitates the separation of actively translated mRNAs bound to several ribosomes, from other components such as "free" RNA, the small (40S), the large (60S) subunits, and the 80S monosome. Interestingly, our results demonstrated that *B.rapa* plants exposed to *T.urticae* for 4 days at 21 or 29 ∘C, do not display the growth-defense trade observed in *A. thaliana* under the same conditions. Moreover, we showed a significantly higher damage in *B. rapa* plants when mites are allowed to feeds at 29∘C, indicating an enhanced pest performance at higher temperatures. Finally, our findings revealed that plants subjected to a mite treatment for 1h at 29∘C experienced a significant down-regulation of global translation. In contrast, Arabidopsis plants exposed to the same conditions did not showed differences in

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translation, but we observed the presence of "half-mers" in monosomes and polysomes peaks, which indicates defects in the biogenesis or maturation of the 60S ribosome subunit. Importantly, our results underscore the diverse molecular strategies plants employ to cope with pest attacks.

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Keywords: Translation, Stress, Multi, Stress, Ribosomes

The impact of AtCAF1i/k deadenylases on de novo shoot organogenesis and poly(A) tail length in Arabidopsis

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In eukaryotic cells, the first step in mRNA degradation, deadenylation, is considered the rate-limiting step. CAF1 is one of two deadenylase subunits in CCR4-NOT, a widely conserved eukaryotic deadenylation complex. We have reported that AtCAF1i/k, yeast CAF1 homologs in *Arabidopsis thaliana*, are incorporated into the AtCCR4-NOT complex as like other eukaryotes. The physiological functions of AtCAF1i/k, however, have not yet been reported. Plants possess a remarkable regenerative capacity; their somatic cells can dedifferentiate to form pluripotent cell masses called callus. This callus can then re-differentiate into shoot organs under the balanced control of the phytohormones auxin and cytokinin. Recent studies have linked the CCR4-NOT complex to the cell differentiation from stem cells in some eukaryotes, including planaria, suggesting possible involvement of AtCAF1i/k in shoot regeneration. To elucidate the role of AtCAF1i/k in plant cell differentiation, we conducted tissue culture analyses on *atcaf1i/k* mutants. The results indicated that *atcaf1i/k* mutants were defective in the early stages of shoot regeneration, suggesting that mRNA degradation by the AtCAF1i/k plays a crucial role in stem cell establishment and/or cell differentiation from stem cells for shoot regeneration. To identify the target mRNAs of AtCAF1i/k, we performed high-throughput full-length cDNA sequencing on callus samples from wild type and the *atcaf1i/k* mutant, and then estimated the poly(A) tail length of each transcript. We identified hundreds of mRNAs that showed significantly longer poly(A) tails in the *atcaf1i/k* mutant compared to the wild type. Furthermore, we found dynamic regulation of poly(A) tail length during callus induction in the wild type. These findings indicate that the dynamic control of poly(A) tail through the AtCAF1i/k is important for the efficient *de novo* shoot organogenesis in Arabidopsis.

Keywords: mRNA decay, de novo shoot organogenesis, poly(A) tail

*Speaker

Role of phloem Cold Shock Proteins in mRNA mobility

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The plant phloem delivers specific proteins and RNAs from shoots to roots. In several distantly related plant species it was shown that the phloem exudate harbors many RNA binding proteins (RBPs) and RNAs that are delivered from shoot to roots. How mRNAs acquire mobility is still not fully understood, but this process is most likely facilitated by phloem RBPs. Aditionally, phloem-mediated mRNA transport is thought to depend on structural features of mRNA molecules, such as folding motifs and secondary 5-methylcytosine (m5C) modifications. In human, proteins containing a Cold Shock Domain (CSD) have been found to act as m5C readers. The Arabidopsis genome encodes four orthologous CSD-containing COLD SHOCK PROTEINS: CSP1, CSP2, CPS3 and CSP4 of which CSP2/CSP4 was found enriched in the phloem exudate of *B. napus.*

To address the role of all four CSPs in Arabidopsis, we generated transgenic lines expressing CSP:mNeonGreen fusion proteins expressed by their native promoters. In these lines *CSP2* and *CSP3* are mainly expressed in shoot and root meristems, whereas *CSP1* and *CSP4* show low and high expression in the vasculature, respectively. Upon grafting, we found that CSP1 and CSP4 proteins, but not their mRNAs, move from shoot to root. To address the potential role of the phloem-delivered CSP4 in mRNA transport we performed RNA immuno-precipitation (RIP) assays. It seems that CSP4 binds preferentially to dehydrin *HIRD11* mRNA, which was previously annotated as graft-mobile and it is thought to be involved in acclimation to abiotic stresses. In line, we were able to show that the overexpression of CSP1 or CSP4, confers drought tolerance to Arabidopsis plants. Notably, overexpression of CSP1/4 – but not of CSP2/3 - affects plant growth, as leaf area was significantly reduced and flowering time largely delayed, whereas *csp1/4* loss-of-function mutants show increased rosette leaf area when compared to wild-type plants.

We are currently further addressing CSP1 and CSP4 role in mRNA mobility, by testing the movement of *HIRD11* and other candidate mRNAs in *csp* mutants lines. We are also further characterizing CSPs RNA binding activity and its affinity towards methylated versus unmethylated transcripts.

Keywords: phloem, RBP, mRNA mobility, drought resistance, growth, flowering time

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