PGRP 2026

Post-transcriptional Gene Regulation in Plants

5 - 7 Oct 2026 MÁLAGA, SPAIN

ABSTRACT BOOK

Keynote

Hervé Vaucheret
Brian D. Gregory
Rosa Lozano-Durán
Federico Ariel
Polly Hsu

Invited Speakers

Javier Palatnik
Zofia Szweykowska-Kulinska
Rupert Fray
Peter Brodersen
Ykä Helariutta
Dominique Gagliardi
Pascal Genschik
Seong Wook-Yang
Remy Merret
Julia Bailey-Serres
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Monday, 5th October

8:30 Registration

9:30 Welcome remarks - Organizers

9:40 **The EMBO Lecture:** **Hervé Vaucheret**. Univ. Paris-Saclay, INRAE, AgroParisTech, JPB. France
Tracking the roots of posttranscriptional gene silencing events

Session 1: RNA Silencing
Chairs: Pablo Manavella & Ignacio Rubio-Somoza

10:25 **Javier Palatnik**. Instituto de Biologia Molecular y Celular de Rosario (IBR). Argentina
MicroRNA control of plant regeneration and stem-cell niche reconstitution

10:50 **Diego López-Márquez**. IHSM La Mayora (CSIC-UMA). Spain
The tryptophan-binding pockets of Arabidopsis AGO1 facilitate amplified RNA interference via SGS3

11:10 **Felipe Fenselau de Felippes**. Queensland University of Technology. Australia
The role of terminators in the regulation of gene expression and the onset of silencing

11:25 Coffee break

11:55 **Artur Jarmolowski**. Adam Mickiewicz University. Poland
D-bodies are nuclear storage structures accumulating DCL1 and HYL1 that are not engaged in co-transcriptional miRNA biogenesis

12:20 **Zofia Szweykowska-Kulinska**. Adam Mickiewicz University. Poland
Marchantia polymorpha miRNA1889 targets the mRNA of Dual Specificity Phosphatase 12 (DUSP12), a protein conserved across eukaryotes, and affects spermatogenesis

12:45 **Marco Incarbone**. Max-Planck-Institute of Molecular Plant Physiology. Germany
Vertical transmission of RNA viruses and its regulation in plants

13:05 **Pratheek Pandesha**. Donald Danforth Plant Science Center, Washington University. USA
Initiation of RNAi in plants

13:20 Lunch

14:45 **The Plant Cell Lecture:** **Brian Gregory**. University of Pennsylvania. USA
The effects of RNA cleavage on plant post-transcriptional regulation

Session 2: RNA Modifications & Structure
Chairs: Laura Arribas-Hernández & Monika Chodasiewicz

15:30 **Rupert Fray**. University of Nottingham. United Kingdom
Suppressors of m^{6}A writer and reader mutants

15:55 **Friedrich Kragler**. Max Planck Institute of Molecular Plant Physiology. Germany
Unravelling complex relationship between mRNAs long-distance transport and m^{5}C methylation

16:15 **Claudia Cortés de Felipe**. Swedish University of Agricultural Sciences (SLU). Sweden
Viral influence on RNA granules: Unravelling condensate dynamics in infection and immunity

16:30 **Flash Talks:** **Wil Prall**, **Marta Zimna**, **Mateusz Bajczyk**, **Camila Zlauvinen** and **Jesús Pascual Vázquez**

16:45 Poster Session 1, with coffee

18:15 **Peter Brodersen**. University of Copenhagen. Denmark
A proxy RNAi pathway for sensing of cytoplasmic double-stranded RNA in plants

18:40 **Ykä Helariutta**. University of Helsinki. Finland
Recruitment of bifunctional regulator thermospermine to methylated ribosomes directs xylem fate

19:05 **Andrew Nelson**. Boyce Thompson Institute, Cornell University. USA
The mRNA covalent modification dihydrouridine regulates transcript turnover and photosynthetic capacity during plant abiotic stress

19:25 **Arsheed Sheikh**. Utrecht University. Netherlands
Turnip mosaic virus co-opts host RNA methylation to orchestrate plant infection

19:40 Dinner

Tuesday, 6th October

8:30 Registration at the Engineering School

9:15 **Keynote:** **Rosa Lozano-Durán**. ZMBP, University of Tübingen. Germany
Another one bites the dust: Geminivirus co-option of plant RNA splicing

Session 3: RNA Processing and Turnover
Chairs: Diego López-Márquez & Misato Ohtani

10:00 **Dominique Gagliardi**. CNRS, University of Strasbourg. France
A core combination of post-transcriptional processes drives mRNA poly(A) profile diversity

10:25 **Dorothee Staiger**. Bielefeld University. Germany
The in vivo binding landscape of Arabidopsis CFI and its interplay with spliceosomal components in CPA site choice

10:45 **Rodrigo Siqueira Reis**. University of Bern. Switzerland
RNA turnover mediates the plant growth-defense tradeoff at warm temperatures

11:00 **Walk from the Engineering School to the Psychology Faculty**

11:15 Coffee break at the Psychology Faculty

11:45 **Pascal Genschik**. CNRS. France
DRB2 is required for efficient and accurate DCL4 processing in plant antiviral immunity

12:10 **Seong Wook Yang**. Yonsei University. South Korea
Proteolytic regulatory networks fine-tuning miRNA biogenetic components and silencing machinery: Proteostasis of HYL1, SE, DCL1, and AGO1

12:35 **Sarah Assmann**. Penn State University. USA
Temperature-associated RNA structural variants in Arabidopsis and rice

12:55 **Monika Zakrzewska-Placzek**. University of Warsaw. Poland
Functional synergy between DXO1 and XRN3 in Pol II transcription and cotranscriptional mRNA processing

13:10 Lunch

14:35 **Keynote:** **Polly Hsu**. Michigan State University. USA
Decoding translation: Hidden ORFs and a new rule for maximizing protein production

Session 4: Translation
Chairs: Catharina Merchante & David Honys

15:20 **Rémy Merret**. IBMP-CNRS. France
Endoplasmic reticulum as a regulator of translation-dependent mRNA fate under heat stress in plants

15:45 **Daniel Gibbs**. University of Birmingham. United Kingdom
TOR–NOT4 signalling couples translation to co-translational quality control in plants

16:05 **Jean-Marc Deragon**. Université de Perpignan. France
Loss of tRNA uridine thiolation affects mRNA translation, protein production, and metabolite accumulation in Arabidopsis

16:20 **Flash Talks:** **Vivek Kumar Raxwal**, **Tomas Tessi**, **Vi-An Ly**, **Manuel Gonzalez Fuente** and **Manvi Sharma**

16:35 Poster Session 2, with coffee

17:40 **Julia Bailey-Serres**. UC Riverside. USA
High resolution disome footprinting links translational stochasticity with mRNA turnover

18:05 **Mar Castellano**. CBGP (UPM-INIA/CSIC). Spain
Selective translation in response to combined stresses and coordination with protein folding in plants

18:30 **Albrecht von Arnim**. University of Tennessee. USA
The circadian clock supports translational control in the context of diel light and temperature cycles

18:50 **Neha Shukla**. Central European Institute of Technology. Czechia
Post-transcriptional regulation of meiotic transcripts by the RNA binding protein CDM1 is associated with cytoplasmic condensate assemblies

19:05 End of Session 4

20:30 Gala Dinner: *La Pérgola*

Wednesday, 7th October

8:30 Registration

9:30 **Keynote: Federico Ariel**. IFIBYNE (UBA-CONICET). Argentina
Warmth-responsive lncRNAs in chromatin dynamics

Session 5: Long non-coding RNAs
Chairs: María Eugenia Zanetti & Emilio Gutiérrez-Beltrán

10:15 **Martín Crespi**. Institute of Plant Sciences Paris-Saclay (IPS2). France
Long non-coding RNAs in alternative splicing regulation

10:40 **Josephin Herbst** . Martin-Luther University Halle-Wittenberg. Germany
The lncRNA LINDA safeguards meristematic integrity after stem cell loss

11:00 **Florencia Rodriguez**. IFIBYNE (UBA-CONICET). Argentina
Alternative splicing of a coding gene produces a nuclear regulatory long non-coding RNA

11:15 Coffee break

11:45 **Andreas Niebel**. LIPME, INRAE, CNRS, Université de Toulouse. France
NFYA1 and long non-coding RNAs regulate nodule development

12:10 **Mathew Parker**. Max Planck Institute for Plant Breeding Research. Germany
Single-gamete genetics resolves epistatic variants underlying transmission bias

12:35 **Thomas Blein**. Institute of Plant Sciences Paris-Saclay (IPS2). France
The interplay between transcription factors and long non-coding RNAs in the regulation of lateral root initiation in Arabidopsis thaliana

12:55 **Jeremie Bazin**. Institute of Plant Sciences Paris-Saclay (IPS2). France
Unveiling a dual function of NSRa: Linking splicing and polyadenylation

13:10 Lunch

Session 6: Epitranscriptomics and epigenetics
Chairs: Hervé Vaucheret & Arturo Mari-Ordóñez

14:35 **Craig Pikaard**. HHMI, Indiana University. USA
The biochemistry of RNA-Directed DNA Methylation: enzymatic reactions of RNA Polymerase V and its protein partners

15:00 **Xuehua Zhong**. Washington University in St. Louis. USA
Epigenetic regulation of development plasticity and environmental adaptation

15:25 **Julia Qüesta**. Centre for Research in Agricultural Genomics (CRAG). Spain
Transcription dynamics at early stages of plant development

15:45 **Kai Wang**. Max Planck Institute of Molecular Plant Physiology. Germany
Identifying the origin and function of Pol4-dependent small RNAs for endosperm development in Capsella rubella

16:00 Coffee break

16:30 **Rebecca Mosher**. University of Oxford. United Kingdom
Epigenomic changes following the evolution of selfing

16:55 **Sarah Rennie**. University of Copenhagen. Denmark
Decoding the principles of m⁶A deposition and recognition in Arabidopsis thaliana

17:20 **Byeong-ha Lee**. Sogang University. South Korea
Nuclear speckle organization and splicing robustness promoted by spliceosomal protein interactions

17:40 **Eduardo Mateo-Bonmatí**. CBGP (UPM-INIA/CSIC)
PNUTS-like scaffolding of CPF phosphatase coordinates 3'-end processing and FLC silencing

17:55 Best Poster and Best Talk awards
Closing remarks
Announcements of PGRP2028

THE EMBO LECTURE

Hervé Vaucheret

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Tracking the roots of posttranscriptional gene silencing events

Martin Lacroix, Nicolas Butel, Andana Barrios, Agnès Yu, Nathalie Bouteiller, Taline Elmayan, Hervé Vaucheret

In plants, invasive nucleic acids of endogenous (e.g. transposable elements) or exogenous origins (e.g. wildtype or engineered T-DNA) are controlled by transcriptional or post-transcriptional gene silencing. These processes (referred to as TGS and PTGS) are mediated by 24-nt and 21/22nt-short interfering RNA (siRNA), respectively. Because the movement of transposable elements or the integration of T-DNA involves double-strand DNA breaks (DSBs), we investigated if certain forms of DNA damage/repair could be responsible for the activation of TGS or PTGS. For this, we targeted DSBs nearby non-silenced loci using the CRISPR-Cas9 system. At p35S-driven transgene loci, we observed that transgenes became silenced by PTGS upon CRISPR-Cas9-induced DSBs. The efficiency of triggering decreased with the distance between the DSB and the target transgene but still occurred at up to 4 kbs. When inducing DSBs nearby endogenous genes, PTGS was observed in some cases, likely at genes that are prone to produce siRNA in RQC-deficient mutants. Guiding Nickase or deadCas9 to target loci did not induce PTGS, indicating that DSBs are actually required for inducing PTGS. Sequencing of nascent RNA produced by transgenes targeted by DSB revealed disturbed transcription termination, leading to the accumulation of long non-polyadenylated transcripts, which likely are converted to dsRNA by RDR6. Lastly, we observed that mutations in DNA repair pathways increase the triggering of PTGS, suggesting that the frequency at which PTGS naturally occurs at a given locus is conditioned by the balance between the frequency of spontaneous DNA damages and the efficiency of their repair. These results not only reveal a potential cause for PTGS events, but also shed light on unexpected effects triggered by CRISPR-Cas9, which could jeopardize the interpretation of gene editing events, calling for cautious analysis of the expression of genes adjacent to the targeted genes.

SESSION 1: RNA SILENCING

CHAIRS: Pablo Manavella & Ignacio Rubio-Somoza

Javier Palatnik

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MicroRNA control of plant regeneration and stem-cell niche reconstitution

The remarkable developmental plasticity of plants underpins diverse regenerative processes, ranging from tissue repair and de novo organogenesis to the regeneration of entire plants from single somatic cells. We assessed the contribution of small-RNA pathways to different plant regeneration systems using Arabidopsis mutants impaired in small-RNA biogenesis or effector function. These analyses revealed a prominent role for microRNA activity in regenerative responses. To dissect how a specific microRNA network controls regeneration at the cellular and molecular levels, we focused on the miR396 - GROWTH-REGULATING FACTOR (GRF) module during root repair following root-tip excision in Arabidopsis. This process requires non-stem cells to acquire stem-cell identity and rebuild a functional meristem. We found that miR396 promotes regenerative competence by restricting GRF activity, whereas GRF activity accelerates regeneration and contributes to stem-cell niche reconstitution. Consistent with the inhibitory effect of miR396 on GRF activity, a *mir396* mutant undergoes regeneration more rapidly. Conversely, ectopic miR396 accumulation uncouples post-injury root growth from proper stem-cell niche reconstitution, allowing growth to continue despite a disorganized stem-cell domain. Overall, the results reveal broad roles for microRNA pathways in plant regeneration and identify miR396 as a key regulator that balances regenerative competence, regeneration rate, and stem-cell niche reconstitution.

References:

Baulies, J. L. et al. Nature Plants 11, 531–542 (2025).

Acknowledgements:

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The tryptophan-binding pockets of Arabidopsis AGO1 facilitate amplified RNA interference via SGS3

Diego López-Márquez, Laura Arribas-Hernández, Christian Poulsen, Emilie Duus Oksbjerg, Nathalie Bouteiller, Mads Meier, Johannes Blanke, Ángel del Espino, Maria Louisa Vigh, Simon Bressendorff, Alberto Carbonell, Rune Daucke, Erwin M. Schoof, Hervé Vaucheret, Peter Brodersen

ARGONAUTE (AGO) proteins bind small RNAs to assemble RNA-induced silencing complexes (RISCs). In Arabidopsis, AGO1 mediates post-transcriptional gene silencing directed by both microRNAs (miRNAs) and small interfering RNAs (siRNAs). AGO1 is also required for siRNA amplification, a process in which RISC-targeted RNAs are converted into double-stranded RNA by the RNA-dependent RNA polymerase RDR6 together with its essential cofactors SGS3 and SDE5. A common feature of many AGO proteins is the presence of hydrophobic pockets that bind tryptophan residues, frequently embedded within glycine-rich GW/WG motifs located in intrinsically disordered regions (IDRs) of RISC-associated cofactors. Here, we use a comprehensive, structure-guided mutational approach to investigate the tryptophan-binding pockets of AGO1 and their associated structural features in *Arabidopsis thaliana*. Our results demonstrate that both the GW/WG dipeptides located in the SGS3 IDR and the hydrophobic pockets of AGO1 are necessary for full RDR6-dependent siRNA amplification. In addition, we show that this mechanism depends on AGO1-specific structural features, including positively charged residues surrounding the tryptophan-binding pockets, as well as a conserved negatively charged region within the SGS3 IDR. These findings show that a conserved protein-protein interaction interface has been adapted to distinct functions in different eukaryotic AGO proteins. Whereas GW/WG-dependent binding between TNRC6 and Ago2 is essential for miRNA-directed silencing in metazoans, the corresponding GW/WG-mediated interaction between SGS3 and AGO1 promotes RDR6-dependent siRNA amplification in plants.

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The role of terminators in the regulation of gene expression and the onset of silencing

Buddhini Ranawaka, Inakshi Weerasekara, Kylie Shand, Kaitlin Fallows, Johanna Tannhäuser, Peter Waterhouse, Felipe Fenselau de Felippes

Terminators are key elements for the polyadenylation and 3' end formation of nascent transcripts - processes that directly influence mRNA stability, localisation, and translation. However, their role in gene expression extends beyond mRNA maturation. The choice of terminator can markedly affect transgene expression levels, often due to unproductive transcription and transgene self-silencing. Changes in transcription termination efficiency can lead to the accumulation of aberrant transcripts arising from read-through transcription, which in turn promotes the production of small RNAs (sRNAs). We identified a cis-regulatory region within the Arabidopsis thaliana HSP18.2 terminator that enhances transgene expression by limiting read-through transcription and sRNA accumulation. We term this sequence the Read-Through and Silencing Suppressor (RTSS) motif. We further demonstrate that the RTSS motif is not unique to the HSP18.2 terminator but also found in the 3' UTR of multiple genes and is conserved across Brassicaceae and related eudicots. Our findings suggest that terminators, and specifically the RTSS motif, play an important role in protecting genes from silencing while maximising expression, particularly under conditions of dramatic changes in transcriptional activity such as stress responses.

Acknowledgements:

This work was funded by the ARC (Australian Research Council) Centre of Excellence (CoE) for Plant Success in Nature and Agriculture (CE200100015) and QUT's Faculty of Science funding to the Integrative Computational and Plant Genomics research group. We also acknowledge the support provided by the German Academic Exchange Service (DAAD) through the PROMOS scholarship program for student mobility (to J.S.T).

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D-bodies are nuclear storage structures accumulating DCL1 and HYL1 that are not engaged in co-transcriptional miRNA biogenesis

Tomasz Gulanicz, Anna Kasprowicz-Maluski, Agnieszka Zientkiewicz, Zofia Szweykowksa-Kulinska, Artur Jarmołowski

In plants, miRNA genes (MIRs) are transcribed by RNA Polymerase II (RNAPII) as independent transcriptional units, generating long primary transcripts (pri-miRNAs). These pri-miRNAs are processed entirely within the nucleus into mature miRNAs by the Microprocessor complex, which consists of three core proteins: the endoribonuclease Dicer-like 1 (DCL1), SERRATE (SE), and Hyponastic Leaves 1 (HYL1). We have previously shown that these proteins are either diffusely distributed throughout the nucleoplasm or concentrated in dynamic, membrane-less nuclear compartments known as dicing bodies (D-bodies). In this study, we focus on the dynamics of D-body formation and demonstrate that their assembly depends on both RNAPII transcription and efficient miRNA processing. We show that microprocessor assembly at MIR transcription sites is a coordinated process, with SE facilitating the recruitment of DCL1 to the RNAPII complex while the binding of HYL1 to pre-miRNAs is not mediated by SE. Notably, when the association of DCL1 with RNAPII is disrupted, DCL1 accumulates within D-bodies. This observation suggests that D-bodies in the nucleus primarily function as storage compartments for the microprocessor proteins DCL1 and HYL1 when they are not actively engaged in co-transcriptional miRNA processing, rather than serving as the main sites of miRNA biogenesis as previously proposed.

References:

Gulanicz et. al., 2026 (in preparation)

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NCN UMO-2024/55/B/NZ2/01154

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Marchantia polymorpha miRNA1889 targets the mRNA of DUAL SPECIFICITY PHOSPHATASE 12 (DUSP12), a protein conserved across eukaryotes, and affects spermatogenesis.

Zofia Szweykowska-Kulinska, Halina Pietrykowska, Artur Jarmolowski

MicroRNAs are master regulators of gene expression across eukaryotic species. In *Marchantia polymorpha*, both conserved and liverwort-specific miRNAs guide key developmental transitions. Several miRNAs show distinct expression patterns in reproductive organs compared to vegetative thalli, underscoring their critical roles in reproductive development. Among these, conserved across algae and liverworts, MpmiR11889 is exclusively expressed in Marchantia sperm cells. Its only validated target is the mRNA encoding MpDUSP12, a dual-specificity phosphatase evolutionarily conserved across eukaryotes. MpDUSP12 contains a unique, conserved C-terminal zinc-finger domain. Our findings demonstrate that the MpmiR11889–MpDUSP12 module is essential for proper sperm development and reproductive success. Plants lacking MpmiR11889 or overexpressing MpDUSP12 produced sperm with bead-like nuclei, elevated protamine-like protein (MpPRM), and abnormal flagellar structures. These mutants exhibited severely disrupted sperm motility and chaotic movement, and crosses involving them showed dramatically reduced sporophyte formation. Moreover, *Arabidopsis thaliana* plants overexpressing or having deleted DUSP12 gene show abnormalities in pollen grain formation and reduced seed production. Caenorhabditis elegans DUSP12 ko mutants exhibit strong brood size reduction. These findings highlight ancient, conserved mechanism controlling chromatin remodeling and gamete differentiation that at least in liverworts is controlled by microRNA.

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Vertical transmission of RNA viruses and its regulation in plants

Gesa Hoffmann, Marco Incarbone, Sri Pravallika Sadhu, Juan Camilo Diez Marulanda, Antonia Proschwitz, Beatrice Ferretti, Hania Leon Contreras, Heinrich Bente, Ruben Gutzat, Gabriele Bradamante

The propagation of a viral infection from a host parent to its progeny is known as vertical transmission, or seed transmission in plants. It allows viral infections to rapidly spread locally via pollen and worldwide through seeds. To be vertically transmitted through the germline a virus must pass through the tight bottleneck of at least one cell per parent – the gametes. Therefore, stopping infection during sexual reproduction is of vital importance to generate healthy offspring. Accordingly, vertical transmission of plant pathogenic RNA viruses often occurs at very low rates, if at all, suggesting the existence of highly effective – yet mostly unknown – antiviral defenses in pre-meiotic cells, gametes and/or embryos. We have recently shown that AGO5 drastically reduces the vertical transmission of Turnip yellow mosaic virus (TYMV) in A. thaliana. Through a series of controlled pollination experiments leveraging different zygosity of ago5 knock-out, cell type-specific rescue of ago5 and TYMV detection in whole-mount reproductive tissues, we provide evidence that AGO5 acts in pollen and sperm cells to restrict virus transmission to progeny. Furthermore, triggering antiviral RNA interference specifically in sperm cells leads to a significant reduction in TYMV vertical transmission. This is the first description of a gamete-specific antiviral defense mechanism restricting virus vertical transmission. Our research currently focuses on identifying and characterizing other molecular mechanisms regulating vertical transmission, which are expected to exist due to the widespread ability of plants to stop transmission across host and virus species. Finally, to obtain a more complete view of this phenomenon we are mechanistically investigating viruses that, unlike most crop-infecting pathogenic viruses, are asymptomatic, vertically transmitted at very high rates and probably widespread in the wild, suggesting very different virus-host interactions which remain essentially unknown.

References:

Gesa Hoffmann et al. Diez-Marulanda, Antonia Proschwitz, Tobias Wegscheider, Ilayda Turhan, Heinrich Bente, Ruben Gutzat and Marco Incarbone (2025). AGO5 restricts virus vertical transmission in plant gametophytes. bioRxiv DOI: 10.1101/2025.11.28.691182

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Initiation of RNAi in plants

Pratheek Pandesha, Seth Edwards, Vivek Hari Sundar Gandhivel, Sandaruwan Ratnayake, Nels O'Connell, David Bisaro, Andrea McCue, R. Keith Slotkin

Every genome must distinguish self from non-self genetic elements, silencing foreign DNA while preserving endogenous gene expression. RNAi is the pathway responsible for this surveillance, eventually directing de novo DNA methylation to transposable elements, viruses, and transgenes to maintain genome integrity in plants[1,2]. While the downstream machinery of RNAi is well characterized, the molecular event that initiates the pathway de novo has remained unknown. Central to this question is the identity of primary small interfering RNAs (siRNAs), the initiating signal for RNAi, which have been intractable to study because RNA-Dependent RNA Polymerase (RDR) proteins amplify secondary siRNAs that obscure the primary siRNA population during active silencing[3]. We addressed this by deploying CRISPR-induced deletions to generate the first complete knockout of all six Arabidopsis RDR genes. This hextuple mutant depletes the secondary siRNA background, revealing the primary siRNA landscape at the endogenous transposable elements, integrated viruses, and transgenes in their native genomic context. Using the transgene-transposon system 35S:EVADE as a model for de novo silencing[4], we discovered that primary siRNA biogenesis initiates through an RDR-independent mechanism at naive chromatin, revealing an unexpected trigger for epigenetic surveillance that operates upstream of all known RNAi machinery. This work reframes how epigenetic surveillance is established: foreign DNA is recognized through a mechanism intrinsic to naive, unmodified chromatin, prior to engagement of the canonical RNAi and RdDM pathways. Our findings address two fundamental and long-standing questions in biology: how the genome discriminates regions designated for expression from those targeted for silencing, and how self-perpetuating epigenetic silencing cycles are first initiated.

References:

[1] Cuerda-Gil & Slotkin (2016). Nature Plants

[2] Nuthikattu et al. (2013). Plant Physiology

[3] Aoki et al. (2007). The EMBO Journal.

[4] Marí-Ordóñez et al. (2013). Nature Genetics

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THE PLANT CELL LECTURE

Brian Gregory
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The effects of RNA cleavage on plant post-transcriptional regulation

RNA cleavage is essential for processing and regulating all classes of RNA. Current methods profiling genome-wide RNA cleavage are biased towards cytoplasmic events and ignore compartmentalized differences. We have recently coupled subcellular RNA fractionation with degradome profiling to detect genome-wide nucleoplasm- and cytoplasm-enriched RNA cleavage in *Arabidopsis thaliana*. Using this enhanced profiling data we are studying RNA processing, including microRNAs, as well as RNA modification-mediated effects of RNA cleavage-mediated post-transcriptional regulation of the plant transcriptome. Our most recent results on miRNA processing and RNA modification-mediated regulation of the plant transcriptome will be presented.

SESSION 2: RNA MODIFICATIONS & STRUCTURE

CHAIRS: Laura Arribas-Hernández & Monika Chodasiewicz

Rupert Fray
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Suppressors of m^{6}A writer and reader mutants

Arabidopsis plants with reduced levels of mRNA methylation (m^{6}A) show characteristic phenotypes of stunted growth, altered leaf morphology, reduced hormone sensitivity, a constitutive immune response, and increased trichome branching. The severity of these phenotypes corelates with the degree by which m^{6}A has been reduced, with complete loss of m^{6}A being lethal. We have used genetic crosses and chemical mutagenesis to carry out screens for mutations that are able to restore normal growth to plants with defects in the enzyme complex that “writes” the m^{6}A in mRNA. Suppressor mutations fall into two classes; i) those that partially restore methylation and ii) those that restore aspects of normal growth, despite the low m^{6}A level. Suppressors that restore methylation include "compensatory" mutations in other writer complex members. Suppressors that restore aspects of normal growth to both writer and reader mutants reveal the importance of the induced constitutive immune response in promoting growth and developmental defects.

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Unravelling complex relationship between mRNAs long-distance transport and m^{5}C methylation

A substantial number of mRNAs are systematically exchanged between shoot and root tissues. However, the mechanism(s) governing their transport, the identity of transport mediators/regulators, and the functional significance of most mobile transcripts in recipient tissues remains largely unknown. Our recent work on mobile mRNAs revealed that they are not only transported to distinct plant parts but also translated and functional in recipient tissues. The complexity of transport regulation is further underlined by the presence of distinct transport motifs and structural features, including m^{5}C mRNA methylation that control their mobility. For example, transport of TCTP1 and HSC70.1 mRNAs in grafted plants depends on distinct short sequence stretches that are m^{5}C-methylated. Their transport is impaired in methylation deficient *nsun2b dnmt2b* double mutants in the vegetative phase but is restored during the flowering phase, highlighting a developmental regulation of mRNA transport. In contrast, GRP7 mRNA moves efficiently regardless of m^{5}C methylation status across all developmental stages suggesting that other factors facilitate their transport. Furthermore, an analysis of variants of tRNA-like sequence (TLS) transport motifs known to mediate transport of heterologous mRNAs revealed that they can function dependent or independent of m^{5}C-modifications depending on their sequence. In summary, our findings reveal a previously unrecognized layer of complexity in long-distance mRNA trafficking regulation, suggesting that transport of mRNAs can be dynamically regulated by i) development, ii) dependent on growth conditions, iii) shw tissue specificity, and iv) exhibiting (in)dependencies on m^{5}C-modifications.

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Viral influence on RNA granules: Unravelling condensate dynamics in infection and immunity

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Viruses evolve rapidly, developing diverse virulence strategies to exploit host cells and evade immunity. The collective viral diversity, or “virome,” provides a valuable framework for studying how host processes are subverted (Shukla et al., 2019). Currently, a significant knowledge gap remains in understanding how plants and viruses reprogram RNA metabolism during infection. The plant post-transcriptional regulatory network encompasses multiple mechanisms that influence RNA stability and translation. One central focus is how these pathways are modulated by various membrane-less biomolecular condensates that assemble through liquid-liquid phase separation in an RNA-binding-protein (RBP) and RNA-dependent manner. Among these, stress granules (SGs) and processing bodies have emerged as potential key hubs for RNA regulation during viral infection (Hoffmann et al., 2022; Hoffmann et al., 2023). An initial screen across different viruses for RNA granule responses revealed one that strongly induces condensation of a specific RBP. Although previously linked to SGs, the resulting condensates do not resemble canonical SGs. Rather, they appear to be a tailored product formed upon infection by this virus, associated with double-stranded RNA (dsRNA) and the viral RNA-dependent RNA polymerase. We hypothesize that these condensates constitute replication-related structures in which the identified RBP acts as a scaffold to organize dsRNA and associated factors within the network. Elucidating their composition and dynamics throughout the viral infection cycle may uncover a new layer of condensate-mediated regulation in host–virus interactions.

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A proxy RNAi pathway for sensing of cytoplasmic double-stranded RNA in plants

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Sensors of intracellular double-stranded RNA are central components of metazoan innate antiviral immunity, but such sensors have not been identified in plants. RNA interference (RNAi) constitutes a potent plant antiviral defense mechanism that relies on conversion of viral RNA into small interfering RNAs by two DICER-LIKE (DCL) ribonucleases, DCL4 and DCL2. I will present results that indicate that while DCL4 and DCL2 are both required for antiviral resistance, their roles are completely different. DCL4 is dedicated to RNAi in the classical sense. On the contrary, cytoplasmic dicing by DCL2 also triggers RNAi-independent defense gene expression via at least two intracellular nucleotide-binding domain/leucine-rich repeat (NLR) immune receptors. Remarkably, the DCL2-mediated catalytic turnover rather than silencing activity of the siRNAs produced by DCL2 underlies NLR activation, and I will discuss a plausible biochemical framework for how such a kinetic sensor may work. The DCL2-mediated sensing pathway is crucial for basal antiviral resistance, because deficiencies in both RNAi and intracellular dsRNA sensing obtained by DCL4/NLR inactivation abrogates basal resistance to a positive strand RNA virus. I will discuss how these results redefine the basis of plant antiviral immunity, and introduce autoimmunity as an explanation for DCL2-dependent growth arrest in dcl and RNA decay mutants in several plant species.

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Recruitment of bifunctional regulator thermospermine to methylated ribosomes directs xylem fate

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Polyamines are often associated with ribosomes and are thought to stabilize their integrity. In Arabidopsis, the polyamine thermospermine (tSpm) affects xylem cell fate. tSpm induces translation of SUPPRESSOR-OF-ACAULIS51 (SAC51) and SAC51-LIKEs (SACLs), which inhibit heterodimerization of the xylem development proteins LONESOME-HIGHWAY (LHW) and TARGET-OF-MONOPTEROS5. We have recently reported about a methyltransferase, OVERACHIEVER/ITS1, that methylates the peptidyl transferase center of the 25S ribosomal RNA (rRNA). Residue m3U2952 promotes functional tSpm binding to a specific site connecting the P-site transfer RNA (tRNA) with rRNA residues in the peptidyl transferase center. This interaction enhances the translation of SACLs but inhibits that of LHW. Our study uncovers the dependency between a conserved rRNA base methylation and a polyamine in orchestrating cell fate decisions, highlighting a role for the ribosome chemical landscape in translational regulation. I will also present how this Arabidopsis model applies in some other plant species.

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The mRNA covalent modification dihydrouridine regulates transcript turnover and photosynthetic capacity during plant abiotic stress

Andrew Nelson, Li'ang Yu, Anna Nelson Dittrich, Diep Ganguly, Hillary Fischer, Aleksandra Skirycz, Susan Schroeder, Brian Gregory, Duke Pauli

RNA covalent modifications (RCMs) are post-transcriptional chemical alterations that influence RNA stability and translation efficiency, and thus play critical roles in eukaryotic growth and development. However, their role in regulating plant performance under abiotic stress remain largely unexplored. Here, we integrated multi-omics data in six Sorghum bicolor accessions under water-limiting conditions in the field to explore the relationship between RCMs and drought response. Within a stress and photosynthesis-associated gene co-expression module, we identified SbDUS2, a member of a family of enzymes conserved across eukaryotes, that catalyzes the reduction of uracil to dihydrouridine (DHU) on RNA molecules. DHU-modified transcripts in this module were enriched for photosynthetic functions and showed strong correlation with photosynthetic traits. To elucidate the function of this RCM, we characterized loss of function *dus2* mutants in the genetic model, Arabidopsis thaliana. Under control conditions, these DHU-deficient mutants exhibited impaired germination and delayed development. Furthermore, when exposed to heat or water-limiting conditions, these mutants showed significantly reduced net CO2 assimilation and survival. Using multiple transcriptome-wide RNA stability assays, we demonstrated that transcripts associated with lower DHU levels in a *dus2* background generally exhibited increased stability compared to Col-0 controls. Particularly, lack of DUS2 led to the hyperstability of photosynthesis-related transcripts, impeding their turnover and likely preventing proper photosynthetic acclimation during stress. Using Arabidopsis *dus2* mutant lines complemented with DUS2-3xFLAG, we then performed immunoprecipitation of DUS2 followed by mass spectrometry, identifying a suite of protein interactors that highlight how it is targeted to specific mRNAs during stress and control conditions. We propose a model based on these data where DHU acts as a critical post-transcriptional regulator marking mRNAs for rapid turnover under stress, highlighting an overlooked regulatory layer contributing to plant resilience.

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Turnip mosaic virus co-opts host RNA methylation to orchestrate plant infection

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N6-methyladenosine (m^6A) is a key RNA modification that regulates transcript stability and translation. However, its function in plant viruses remains largely unclear. Here, we show that the positive-sense single-stranded +ssRNA Turnip mosaic virus (TuMV) relies on the host m^6A machinery to support efficient infection. Our findings uncover a previously unrecognized nuclear phase in the TuMV life cycle, during which viral RNA undergoes extensive methylation by host enzymes. We identify a complex and non-canonical methylation landscape on the TuMV genome, where canonical DRACH motifs are embedded within clusters of additional virus-specific non-canonical m^6A sites. Notably, we also detect the presence of another RNA modification, m^5C (5-methylcytosine), in close proximity to m^6A-marked regions. This coordinated methylation landscape appears to be critical for efficient viral polyprotein synthesis. In its absence, the virus displays aberrant methylation and reduced infectivity as observed in m^6A writer and reader mutants. Based on this, we propose a “seeding” model in which initial m^6A deposition at canonical sites nucleates the formation of a broader network of m^6A and m^5C marks, likely guided by RNA structure rather than sequence motifs alone.

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KEYNOTE

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Another one bites the dust: Geminivirus co-option of plant RNA splicing

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Viruses with compact genomes must maximize their functional output from limited coding capacity, yet how they reconcile conflicting protein activities remains poorly understood. While the contribution of RNA splicing to the regulation of plant immunity is well established, its hijacking by plant viruses is considered rare. We have recently shown, however, that splicing of viral transcripts is unexpectedly prevalent in the geminivirus tomato yellow leaf curl virus (TYLCV) and contributes to viral infectivity, uncovering RNA splicing as another cellular process co-opted by plant viruses to promote infection. Transcriptome analysis revealed eight viral splicing events generating novel protein isoforms. Focusing on the replication-associated protein (Rep), we show that TYLCV exploits host alternative splicing to partition two opposing Rep activities: viral DNA replication and autorepression of the Rep promoter. Splicing generates Rep isoforms lacking the central oligomerization domain that are unable to support replication but act as potent transcriptional repressors. Conversely, splicing-deficient Rep variants efficiently promote replication but fail to repress transcription. Thus, alternative splicing enables a single viral gene to generate protein isoforms with distinct and functionally incompatible activities. Disruption of Rep splicing perturbs viral gene expression dynamics, reduces viral accumulation, and attenuates symptom development, demonstrating that splicing is an important component of TYLCV infectivity. The presence of analogous splicing patterns in related single-stranded DNA viruses suggests that this strategy may be broadly used to expand regulatory capacity and functional diversity in compact viral genomes. Our findings reveal a role for RNA splicing in plant virus biology and suggest that plant virus transcriptomes may contain substantially more functional complexity than previously recognized.

SESSION 3: mRNA PROCESSING AND TURNOVER

CHAIRS: Diego López-Márquez & Misato Ohtani

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A core combination of post-transcriptional processes drives mRNA poly(A) profile diversity

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Eukaryotic mRNAs display remarkably diverse and complex poly(A) tail size distributions, the origins of which remain largely unexplained. Intriguingly, poly(A) tail length correlates only weakly with the translational engagement of mRNA, its abundance or stability, except for early developmental stages in animals.I will discuss how poly(A) tail size heterogeneity in Arabidopsis thaliana can arise from an interplay of several processes, including gene-specific determinants, PABP loading, deadenylation, decapping, and remarkably, uridylation, which can counteract deadenylation. These combinatorial poly(A) tail-driven constraints can be modulated in a transcript-dependent manner, producing distinct polyadenylation profiles even among mRNAs with comparable abundance, stability, or translational output. By contrast, transcriptional output does not modulate poly(A) profiles.Computational modeling reveals that complex polyadenylation patterns can be accurately reproduced using a small number of parameters. Our model suggests that distinct poly(A) profiles can be predicted without substantially affecting steady-state mRNA levels. We propose that this dynamic interplay of constraints shaping poly(A) tails could confer robustness to gene expression under normal conditions while enabling regulatory flexibility in response to developmental or environmental stimuli.

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The in vivo binding landscape of Arabidopsis CFI and its interplay with spliceosomal components in CPA site choice

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3´end cleavage and polyadenylation (CPA) is highly regulated by protein complexes interacting with RNA sequence motifs. Whereas orthologues of the human CFI small (CFIm25) and large (CFIm59 and CFIm68 subunits) were assigned in the Arabidopsis genome, the molecular mode of action of the CFI complex remained elusive. Here, we determined in vivo binding of Arabidopsis CFI by iCLIP. All three subunits, CFI25a, CFI59, and CFI68, directly bind RNA with binding sites mostly located in the 3´UTR of protein-coding transcripts. The three subunits preferentially bound to transcripts which showed significant CPA site changes in cfi mutants with a predominant shift to proximal CPA sites. UGUA and UGUU motifs were enriched at CFI binding sites, indicating a shared sequence preference among all three CFI subunits. Furthermore, we show that in contrast to the distinct functions of CFm59 and CFI68m, Arabidopsis CFI59 and CFI68 act redundantly on CPA site choice. Co-immunoprecipitation of the CFI subunits has identified spliceosomal components[1]. To explore a potential involvement of U1-70K, and BRR2a in CPA, PAT-seq was performed on lines expressing artificial miRNAs against U1 snRNP proteins[2] or BRR2a. Reduced levels of U1-70K, U1C or BRR2a had much smaller effects on CPA site choice compared to the cfi mutants. Reduced levels of U1-70K or U1C led to a predominant shift to distal sites, i.e. opposite compared to the cfi mutants, whereas reduced levels of BRR2a caused a shift towards proximal CPA sites. iCLIP demonstrated that U1-70K apart from its interaction with U1 snRNA binds to mRNAs in the 3´UTR. The absence of a binding motif suggests that U1-70K may largely bind through U1 snRNP, requiring alternative methods to map snRNP binding in vivo. Collectively, the tested spliceosomal components may impact CPA in ways both, related and unrelated to their joined function in the spliceosome.

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RNA turnover mediates the plant growth-defense tradeoff at warm temperatures

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Plant response to warm temperatures involves coordinated morphological changes, including hypocotyl, root, and petiole elongation. This developmental reprogramming is a conserved acclimatisation mechanism to warming conditions in plants, with various negative impact on crop yield, food quality, and disease resistance. Here, we show that warm temperatures trigger selective, organ-specific changes in RNA turnover. We found that mutants in decay pathway genes are affected in plant response to warm temperature, showing that RNA turnover is critical in this process. Furthermore, various mutants for gene targets for selective decay show corresponding altered phenotype in the response to warm temperatures, such as higher thermosensitivity in mutants for genes that we observed mRNA destabilization in wild-type plants grown under warmer conditions. Transcriptome-wide analysis revealed that defense-related mRNAs are selectively destabilised by increased temperatures. Consistently, decay mutants displayed altered defence-related mRNA regulation and increased resistance to pathogen infection. Collectively, our work identifies RNA turnover as a central regulatory mechanism that suppresses immunity to prioritize growth under warming conditions.

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DRB2 Is required for efficient and accurate DCL4 processing in plant antiviral immunity

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In Arabidopsis thaliana, DICER-LIKE (DCL) enzymes associate with double-stranded RNA-binding (DRB) proteins to generate diverse classes of small RNAs. DRB1/HYL1 acts with DCL1 in microRNA (miRNA) production, whereas DRB4 functions with DCL4 in trans-acting and antiviral siRNA biogenesis. By contrast, the molecular function of DRB2 remains less understood. We will present our results showing that DRB2 and DRB4 contribute to the accumulation of specific miRNAs and cooperate in DCL4-dependent viral siRNA biogenesis. Using a DCL2-insensitive Tomato bushy stunt virus isolate, we found that the simultaneous loss of DRB2 and DRB4 reduces viral siRNA accumulation and alters their size distribution, indicating impaired DCL4 processing fidelity. We will also present evidence that DRB4 interacts directly with both DCL4 and DRB2, whereas the association of DRB2 with DCL4 requires DRB4. Together with AlphaFold3-based structural predictions, these findings support a model in which DRB4 recruits DRB2 into higher-order DCL4 complexes and establish DRB2 as a functional component of the plant antiviral RNA-silencing machinery.

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Proteolytic regulatory networks fine-tuning miRNA biogenetic components and silencing machinery: Proteostasis of HYL1, SE, DCL1, and AGO1

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MicroRNAs (miRNAs) serve as central post-transcriptional regulators in plants, relying on the core microprocessor complex—comprising DICER-LIKE 1 (DCL1), SERRATE (SE), and HYPONASTIC LEAVES 1 (HYL1)—and the RNA-induced silencing complex (RISC) kernel, ARGONAUTE 1 (AGO1). Although the proteostasis of many components has traditionally been viewed through the two slits of either the ubiquitin-proteasome system (UPS) or autophagy, emerging evidence indicates that far more complex regulatory networks are involved in the RNA-mediated silencing system. Here, we delineate integrated proteolytic and environmental networks that fine-tune the proteostasis and activity of HYL1, SE, DCL1, and AGO1.

We previously identified that HYL1 undergoes light-regulated turnover: in darkness, the cytoplasmic subtilase HYL1-CLEAVAGE SUBTILASE 1 (HCS1) cleaves HYL1, whereas light enables CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) to sterically inhibit HCS1, preserving HYL1 integrity. Extending this axis, we here report that HCS1 itself is negatively regulated by autophagy, forming a self-limiting feedback loop. Furthermore, AGO1 undergoes N-terminal processing by HCS1 and an unknown protease, after which its cleaved fragment lacking the N-terminal Prion-like domain (PrLD) is cleared via the coupled action of autophagy and the 20S proteasome. However, under drought stress, PrLD-driven liquid-liquid phase separation (LLPS) aggregation enables AGO1 to evade this combined proteolytic regulation. Because HCS1 plays a central role in the proteolytic regulation of both HYL1 and AGO1, these proteins are co-regulated in a forward manner. Proteostasis of the other microprocessor components is similarly multi-faceted: during photomorphogenic transitions, SE is physically safeguarded from 20S proteasomal cleavage by chromatin chaperones, while DCL1 is targeted by dedicated cytoplasmic proteases. Notably, both SE and DCL1 display an inverse regulatory correlation with HYL1, a crosstalk mechanism that warrants further investigation.

Essentially, our findings demonstrate that the fate of miRNA biogenesis components is not dictated merely by a simple binary choice between UPS and autophagy. Instead, plant miRNA machinery is governed by a highly interconnected control hub that integrates specific proteolytic cleavages, dual degradation pathways, LLPS, light signaling, and abiotic/biotic stress cues. These sophisticated, multi-factorial networks ensure precise, real-time recalibration of miRNA biogenesis and silencing efficiency in response to an ever-changing environment.

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Temperature-associated RNA structural variants in Arabidopsis and rice

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We developed a method, Structure-seq, to query RNA secondary structure transcriptome-wide (Ding et al., 2014). Application of Structure-seq to Arabidopsis reference accession Col-0 and rice (*Oryza sativa*) reference cultivar Nipponbare revealed that abiotic stresses affect RNA secondary structure, with associated impacts on transcript abundance (Tack et al., 2020; Su et al., 2018). For both Arabidopsis accessions and rice landraces, we have developed CLIMtools, a set of online resources for pan-genome prediction of climate-associated genetic variants (Ferrero-Serrano et al., 2022, 2024). CLIMtools includes computational predictions of riboSNitches, i.e. SNPs that change RNA secondary structure (Kirven et al., 2025). We are currently applying Structure-seq under control and heat stress conditions to experimentally assess the 61,013 predicted riboSNitches between the Arabidopsis accessions Col-0 and Vis-0, as well as the 18,444 predicted riboSNitches between the rice cultivars Nipponbare (japonica) and IR64 (indica). We will then ascertain which experimentally verified riboSNitches show significant climate correlations, which would implicate RNA secondary structure and stability in local adaptation. In addition, we will apply machine learning approaches to extract structural features associated with riboSNitches.

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Functional Synergy Between DXO1 and XRN3 in Pol II Transcription and Cotranscriptional mRNA Processing

Monika Zakrzewska-Placzek, Katarzyna Niedzwiecka, Michal Krzyszton, Magdalena Wrona, Agnieszka Gozdek, Joanna Kufel

The precise coordination of RNA polymerase II (Pol II) transcription and cotranscriptional processing is essential for maintaining the fidelity of the transcriptome. While the general mechanisms of transcript synthesis are well-established, the regulatory role of RNA-modifying enzymes in tuning the transcription cycle remains poorly understood. Our research defines the functional interaction between DXO1, a multifunctional deNADding enzyme, and XRN3, a 5'-to-3' exoribonuclease, which synchronize Pol II transcription in Arabidopsis thaliana.

XRN3 is a well-known Pol II termination and RNA quality control factor, acting as a 'torpedo' to displace the polymerase and degrade nascent transcripts. In contrast, plant DXO1 is a unique protein that differs significantly from its yeast and human orthologs. Beyond the RNA deNADding activity, DXO1, through its plant-specific N-terminal extension, interacts with and stimulates the activity of RNMT1 methyltransferase, contributing to the synthesis of the canonical m^7G cap. Our genetic analysis uncovered a surprising functional synergy between DXO1 and XRN3, associated with partial suppression of *dxo1-2* molecular and morphological defects by XRN3 knockdown, and vice versa. Moreover, we showed that DXO1 directly interacts with the C-terminal domain of Pol II and affects its phosphorylation status, which may be related to defects in capping. Using single and double mutant lines and plants expressing tagged variants of these enzymes, we observed that *xrn3-8* termination defects are less severe in the *dxo1-2* background, suggesting that DXO1-mediated capping contributes to the transition of Pol II from elongation to termination. Conversely, slower transcription due to XRN3 deficiency likely extends the 'kinetic window' for cap methylation, helping to rescue *dxo1-2* phenotypes. We propose a model in which DXO1 and XRN3 function as a coordinated regulatory axis that monitors Pol II processivity and transcript quality. Our findings reveal that the crosstalk between 5'-cap maturation and 3'-end processing represents a fundamental layer of gene expression control.

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KEYNOTE

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Decoding Translation: Hidden ORFs and a New Rule for Maximizing Protein Production

Translation from mRNAs to proteins is a fundamental step in gene expression, yet the mRNA features that determine what is translated and how efficiently remain poorly understood. In this talk, I will present our recent work uncovering several hidden layers of translational regulation in plants. Using improved Ribo-seq methods and new computational approaches, we identify previously difficult-to-detect noncanonical open reading frames (ORFs), including overlapping upstream ORFs and nested ORFs that overlap with annotated main ORFs in alternative reading frames, and reveal how they regulate translation and diversify protein products. Through comparative translatome analyses across plant species, we further uncover an unexpected relationship between codon usage and translation efficiency, identifying GC content at the third codon position (GC3) as a major feature associated with efficient translation across both GC- and AT-rich species. Together, our findings reveal new layers of information embedded within mRNAs that shape translational regulation and protein diversity and provide a new principle for engineering protein production.

SESSION 4: TRANSLATION

CHAIRS: Catharina Merchante & David Honys

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Endoplasmic reticulum as a regulator of translation-dependent mRNA fate under heat stress in plants

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Maintaining RNA integrity is essential for cellular homeostasis, particularly under environmental stress conditions. In plants, heat stress rapidly disrupts transcriptional and translational processes, requiring efficient mechanisms to preserve RNA homeostasis. Here, using Arabidopsis thaliana as a model system, we identify a conserved +1 ribosome frameshifting event that functionally couples the heat stress response to rapid RNA surveillance. We show that short-term heat stress induces widespread, temperature-dependent +1 ribosome frameshifting, resulting in out-of-frame translation that generates signals triggering RNA quality control. This response promotes extensive yet transient endonucleolytic mRNA cleavage at the surface of the endoplasmic reticulum (ER). Strikingly, we demonstrate that this mechanism is conserved across diverse plant species, suggesting that it represents an evolutionarily conserved adaptive strategy. Together, our findings uncover a previously unrecognized layer of post-transcriptional regulation in which translational fidelity is dynamically linked to RNA surveillance during heat stress, and further suggest that signaling at the ER surface plays a central role in coordinating this response.

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TOR–NOT4 signalling couples translation to co-translational quality control in plants

Protein synthesis must be tightly coordinated with quality control to prevent proteotoxic stress, yet the mechanisms underlying co-translational surveillance in plants, and how these processes are integrated with translational regulation, remain poorly understood. Here, we identify three NOT4-like E3 ubiquitin ligases in Arabidopsis thaliana as key regulators of co-translational protein quality control and uncover a functional link between NOT4 and TARGET OF RAPAMYCIN (TOR) signalling that couples translational output to quality-control capacity. Loss of NOT4 function increases basal TOR activity and global translation rates, resulting in the accumulation of polyubiquitylated proteins, as well as heightened sensitivity to proteasome and TOR inhibition, and protein misfolding stress. Consistent with NOT4 proteins being TOR-regulated phosphotargets, not4 mutants phenocopy TOR-inhibited wild-type plants for a subset of transcriptional and growth-related processes, revealing a requirement for NOT4s in normal TOR-dependent signalling. Furthermore, we show that elevated translation in NOT4-deficient plants confers enhanced resistance to *Pseudomonas syringae* pv. *tomato*, suggesting a functional interplay between translational control, co-translational quality control, and defence against pathogens that suppress host translation. Collectively, our findings uncover a functional connection between TOR signalling and NOT4 activity that may coordinate translational activity with protein quality-control capacity to safeguard proteome homeostasis, with potential evolutionary conservation across eukaryotes.

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Loss of tRNA uridine thiolation affects mRNA translation, protein production, and metabolite accumulation in Arabidopsis

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The uridine at position 34 of tRNA anticodon loops is always modified at variable levels depending on environmental conditions, but a physiological function for this highly conserved modification has not been firmly established. Using Arabidopsis thaliana, we show that the thiolation of U34 is a prerequisite for the subsequent modifications at position 32 and 37 of the tRNALys(UUU) anticodon loop, revealing a novel modification network. Furthermore, we observed that the ability of the plant ribosome to decode AAA, GAA and CAA codons is impaired when the thiol group is lost, leading to a reduction in protein production, especially for genes enriched in these codons. Finally, we demonstrate that loss of tRNA thiolation results in variations in the levels of numerous proteins involved in sulfur-compound metabolism and amino acid biosynthesis. These variations, in turn, affect the accumulation of several sulfur-containing metabolites and amino acids, suggesting that the level of tRNA thiolation has a direct impact on plant metabolism.

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High resolution disome footprinting links translational stochasticity with mRNA turnover

Ribosomes can slow or pause during mRNA translation, but what causes pausing, how pauses affect protein production, and whether they trigger mRNA decay is poorly understood in plants. To investigate the causes and consequences of ribosome pausing, the footprints of individual monoribosomes (monosomes) and closely spaced disomes were sized, mapped, and quantified at single-codon resolution on ORFs of Arabidopsis and maize. This resolved two monosome and three disome configurations: monosomes with a vacant or occupied A-site and disomes that had collided or were separated by one or two codons. Pausing is pronounced at initiation and termination codons, as well as di-Proline codons, which require eIF5A for decoding. Mapping free 5′-monoP ends of transcripts by “degradome-seq” provides strong evidence that rate limiting ribosome decoding can triggers cotranslational mRNA decay, but not at di-Proline codons or pause sites associated with processing Polyubiquitin into monomers. Notably, actively transcribed and translated hypoxia-response mRNAs accumulate 1-to 2-codon-separated disomes and are actively degraded, whereas known sequestered mRNAs are not. The dual species analyses reveal highly conserved pausing sites on CPuORFs, with some interesting exceptions. Thus, codon-specific ribosome stalling, coupled with ribosome A-site occupancy status, and disome spacing, modulate protein synthesis and translation coupled mRNA decay in plants.

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Selective translation in response to combined stresses and coordination with protein folding in plants

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Plants are continuously exposed to diverse environmental challenges. To cope with these conditions, they have evolved multiple adaptive mechanisms, including a tight regulation of translation. In addition, stress often leads to the accumulation of misfolded or unfolded proteins, requiring an efficient protein-folding machinery. Importantly, not all mRNAs or proteins are equally affected by stress in terms of translation efficiency or folding capacity, and the mechanisms coordinating both processes remain poorly understood in plants.

In our laboratory, we investigate how single and combined stresses reprogram translation. We identify the specific mRNAs that undergo selective translation under environmental constraints and characterize the molecular mechanisms underlying their regulation. Furthermore, part of our work focuses on uncovering the selective clients of distinct sets of chaperones and co-chaperones during stress conditions.

In this talk, we will present recent data highlighting the central role of translation in multistress responses and provide new insights into how plants coordinate translation and protein folding to maintain cellular homeostasis.

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The circadian clock supports translational control in the context of diel light and temperature cycles

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The endogenous timekeeper of the plant, the circadian clock, modulates the translation of mRNAs over the course of the day. We performed ribosome profiling (Ribo-Seq), a type of ribonuclease protection assay, to compare the patterns of translational control with earlier data obtained using polysome profiling, which measures mRNA-ribosome loading by sedimentation in a sucrose gradient. It was confirmed that (i) the circadian clock affects the phase, amplitude, and waveform of translational efficiency of hundreds of mRNAs; (ii) the clock suppresses swings in translation efficiency caused by changes between light and darkness. Polysome profiling and Ribo-Seq revealed different aspects of translational control. Ribo-Seq also defined new gene ontology networks of translational control.

Light and darkness regulate cellular metabolism in part through the target of rapamcin (TOR) kinase pathway, which controls the phosphorylation of the ribosomal protein eS6 (eS6-P). How does the plant integrate light and clock signals with diel swings in temperature? eS6-P is a suitable model system to address this because diel cycles of light and temperature influence eS6-P incoherently. Our data suggest that the clock is a proxy for temperature signals, rather than light - as is commonly the case -, in this system. Interestingly, realistic light and temperature conditions, as encountered by Arabidopsis in its natural environment, quell the typical diel cycle of eS6-P. eS6-P behaves like a cellular gauge that integrates light-dark and temperature signals with the assistance of the circadian clock. Therefore, eS6-P may be part of a homeostatic mechanism whereby plants sense and adapt to subtle changes in weather, seasons, or climate trends.

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Post-transcriptional regulation of meiotic transcripts by the RNA binding protein CDM1 is associated with cytoplasmic condensate assemblies

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The transition from diploid to haploid life phases requires extensive reprogramming of gene expression to coordinate meiotic progression and haploid cell differentiation. A hallmark of meiosis is widespread post-transcriptional regulation, including the storage of mRNAs and their delayed and timely utilization for translation to support post-meiotic development. However, the molecular mechanisms underlying this process remain unknown. Here, we describe a mechanism controlling the translation of meiotic transcripts during Arabidopsis microsporogenesis, mediated by CALLOSE DEFECTIVE MICROSPORES1 (CDM1). Biochemical and cellular analyses reveal that CDM1 is an RNA-binding protein with an intrinsic ability to form ribonucleoprotein condensates. Furthermore, we demonstrate that CDM1 binds mRNAs expressed in prophase I and represses their translation until later meiotic stages, coinciding with the disassembly of CDM1 condensates. These findings establish CDM1 as a key post-transcriptional regulator that fine-tunes the expression of meiotic genes to ensure proper progression of microsporogenesis. Loss of CDM1 disrupts multiple meiotic processes, including homologous chromosome pairing, condensation, and cytokinesis. We show that CDM1 forms dynamic, meiosis-specific cytoplasmic condensates that associate with components of processing bodies and stress granules, giving rise to three-phase ribonucleoprotein assemblies. Biochemical and cellular analyses further reveal that CDM1 is an RNA-binding protein with an intrinsic capacity to form condensates through phase separation. CDM1 binds a subset of mRNAs expressed during prophase I and represses their translation until later meiotic stages. Release of translational repression coincides with CDM1-condensate disassembly, enabling stage-specific protein accumulation. Together, our findings uncover a condensate-mediated mechanism for mRNA storage and translational repression during meiosis and establish CDM1 as a critical post-transcriptional regulator that coordinates meiotic gene expression and ensures faithful progression of microsporogenesis.

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KEYNOTE

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Warmth-responsive lncRNAs in chromatin dynamics

The noncoding genome plays a critical role in regulating gene expression in plants, yet its functions remain largely unexplored in plants. Thanks to advances in next-generation sequencing technologies, a growing number of long noncoding RNAs (lncRNAs) have been identified, with several already functionally characterized. These lncRNAs modulate gene expression through diverse mechanisms, including chromatin remodeling, alternative splicing, modulation of miRNA activity, and control of mRNA translation and stability. In our laboratory, we have uncovered the roles of several lncRNAs involved in alternative ribonucleoprotein complex formation. Among them, we characterized the lncRNA APOLO in Arabidopsis thaliana, which recognizes target genes via sequence complementarity and RNA–DNA hybrid (R-loop) formation, to epigenetically regulate their expression. Through interactions with DNA, transcription factors, and chromatin-associated proteins, APOLO mediates plant responses to temperature fluctuations and the shade avoidance syndrome. Our recent findings indicate that APOLO is a direct target of the master regulator PIF4 in response to warmth. Genome-wide Capture Hi-C analyses revealed that warmth triggers PIF4-dependent activating chromatin loops, whereas APOLO promotes repressive, R-loop-associated loop configurations, uncovering a dual regulatory module that coordinates thermomorphogenesis. Remarkably, exogenous application of APOLO was sufficient to modulate auxin homeostasis and alter Arabidopsis responses to environmental cues. Building on this expertise, we are now employing integrative multi-omics strategies to decode the regulatory functions of the noncoding genome in crop species. Our ultimate goal is to develop RNA-based foliar sprays to fine-tune plant development and enhance environmental adaptability in the face of climate change.

SESSION 5: LONG NON-CODING RNAS

CHAIRS: María Eugenia Zanetti & Emilio Gutiérrez-Beltrán

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Long non-coding RNAs in alternative splicing regulation

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The non-coding genome is the major part of the eukaryotic transcriptome and many long noncoding RNAs, or lncRNAs, were shown to act as positive or negative quantitative regulators of gene expression in last years. Multiple mechanisms involving chromatin, silencing, splicing, epigenetics, translation and the modulation of small si/miRNA action revealed new regulations that may be linked to evolutionary diversity of cell types and forms. We have studied the action of lncRNAs genome-wide acting through the regulation of alternative splicing (AS). This AS process enables eukaryotes to produce multiple RNA isoforms from one gene and we identified multiple lncRNAs bound by the splicing factors (SFs) Nuclear speckle RNA binding protein A (NSRa) and glycine-rich RNA-binding protein 7 (GRP7). We then tested their capacity to modulate AS using a transient expression screen in leaves and identified 4 active lncRNAs. Most lncRNA-induced AS events were unique for each lncRNA although 19% of identical AS events are shared between two NSRa-recognized lncRNAs. One of these lncRNAs was named ACHLYS and its deregulation in planta also modulates AS. Transcriptome-wide identification of NSRa binding sites by iCLIP revealed NSRa enrichment at ACHLYS-dependent intron retention events. A specific ACHLYS-induced AS event showed that the action of ACHLYS on this AS target requires NSRa. Interestingly, in vitro and in vivo assays indicate that NSRa phase separation is promoted by ACHLYS and results in increased accumulation of NSRa in Nuclear speckles. Hence, ACHLYS can modulate NSRa condensates in vivo and binds to NSRa to modify AS patterns. Direct lncRNA SF interactions in nuclear condensates may fine tune AS regulation in plants. LncRNAs may integrate a dynamic network interacting with spliceosomal proteins to modulate transcriptome reprogramming in eukaryotes.

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The lncRNA LINDA safeguards meristematic integrity after stem cell loss

Josephine Herbst, Solveig Henriette Nagy, Ilse Vercauteren, Lieven De Veylder, Reinhard Kunze

The genomic integrity of every living organism is endangered at all times by various intrinsic and extrinsic stresses that can cause a variety of DNA lesions. Those lesions, if unrepaired, affect plants growth, viability and yield. Fortunately, the sophisticated DNA damage response (DDR) network is rapidly activated to counteract those lesions. Although our knowledge on the regulation and fine-tuning of the DDR has been growing, many aspects still remain to be uncovered. One group of important, yet little understood, regulators of the DDR are the long non-coding RNAs (lncRNAs). Especially in humans, lncRNAs have gained increasing attention due to their oncogenic character. However, in plants only recently the impact of those transcripts onto DNA integrity has been proven. By meta-analysis, we identified the putative LONG INTERGENIC NON-CODING RNA INDUCED BY DNA DAMAGE (LINDA) in *Arabidopsis thaliana*. LINDA is rapidly activated in response to DNA double-strand breaks (DSBs) in an ATM- and SOG1- depended manner. Intriguingly, single-cell RNA sequencing and GFP-reporter lines revealed that LINDA is specifically upregulated in root initials and dividing cells following the induction of DSBs. We could further demonstrate that LINDA regulates its downstream located putative target gene in cis. Moreover, RNAseq experiments using a Δlinda deletion mutant, generated with CRISPR/Cas12a, indicated that the lack of LINDA lead to severe transcriptional changes in response to DSBs. Strikingly, Δlinda mutant meristems are unable to recover from DNA damage induced stem-cell death, most likely due to hyper-activation of cell death. Consequently, these results underline the potential of lncRNAs to be important players in the DDR pathway in plants, similar to humans.

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ALTERNATIVE SPLICING OF A CODING GENE PRODUCES A NUCLEAR REGULATORY LONG NON-CODING RNA

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Alternative splicing (AS) is a major mechanism expanding transcriptome complexity in eukaryotes and has traditionally been interpreted as a source of protein diversity. However, our analysis of available transcriptomic studies in plants reveals that a substantial proportion of AS-generated isoforms display limited or null coding potential. Hence, we decided to investigate whether non-coding isoforms derived from protein-coding genes can exert regulatory functions. Using an optimized and rapid subcellular fractionation protocol (SuB3), combined with RT-PCR, RT-qPCR, RNA immunoprecipitation assays, and transcriptomic analyses, we explored the subcellular localization and function of AS-derived isoforms in *Arabidopsis thaliana*. We found that multiple genes involved in splicing regulation, particularly members of the SR (Serine/Arginine-rich) protein family, consistently produce two major classes of isoforms: protein-coding transcripts distributed between nucleus and cytoplasm, and non-coding intron-retained isoforms preferentially accumulated in the nucleus, especially under dark conditions. Focusing on the SR protein gene At-RS31, we characterized mRNA3, a nuclear-retained non-coding isoform that accumulates in darkness and physically interacts with the RS31 protein. Functional analyses indicate that mRNA3 modulates splicing outcomes and contributes to gene expression regulation, likely through interaction with splicing regulators, including its own cognate protein. Notably, overexpression of mRNA3 restores phenotypic defects caused by overexpression of the coding isoform, supporting a functional role in trans. Together, our results reveal an additional regulatory layer in which AS of protein-coding genes could generate functional long non-coding RNAs that participate in feedback regulation of gene expression. These findings broaden the functional impact of AS beyond proteome diversification and suggest that coding loci may simultaneously generate proteins and regulatory RNAs. This mechanism can be of particular relevance in plants, where intron retention, that represents the predominant AS event, frequently leads to transcripts without coding capacity as they harbor premature termination codons (PTCs).

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NFYA1 and long non-coding RNAs regulate nodule development

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NF-Y are heterotrimeric CCAAT motif-binding transcription factors (TFs) that, as pioneer TFs, can also control chromatin accessibility to regulate transcription in both animals and plants [5,6]. The A1 subunit of the NF-Y complex in leguminous plants such as Medicago truncatula regulates nodule development during the legume-rhizobia symbiosis [1,2,3,4]. Our working hypothesis is that NF-YA1 also acts as a pioneer TF regulating the root to nodule transition in legumes such as Medicago truncatula. In recent years, long non-coding RNAs (lncRNAs) have emerged as a new class of gene expression regulators with notably roles in gene expression control via chromatin modifications [7, 8]. Comparative transcriptomics revealed numerous rhizobia-induced TFs and approximately 40 lncRNAs under the dependence of NF-YA1 in Medicago. In parallel, using an RNA-immunoprecipitation strategy, we have identified two additional lncRNAs interacting with NF-YA1. Hence, lncRNA interacting with or regulated by NF-YA1 may help to regulate gene expression and consolidate NF-YA1 pioneering activity in the context of nodule development. Functional and expression studies suggesting a role of these lncRNAs and TFs during the Medicago truncatula – Sinorhizobium meliloti symbiosis will be presented.

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Single-gamete genetics resolves epistatic variants underlying transmission bias

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The haploid phase offers a unique opportunity to study genetic variants that influence both gene regulation and their own transmission. Meiosis generates thousands of haploid gametes with unique recombinant genotypes, creating a natural single-cell mapping population in which inherited variation can be linked directly to transcriptional state.

We developed single-gamete eQTL (sg-eQTL) mapping, combining single-nucleus RNA sequencing with haplotype reconstruction to map regulatory variation directly in recombinant pollen. Applying this approach to Arabidopsis thaliana hybrids revealed extensive cell-type-specific regulatory variation in sperm and vegetative nuclei. We identified trans-eQTL hotspots that altered distinct transcriptional programmes, including DNA replication and S-phase entry in sperm, and stress-response, secretory and cell-wall pathways in vegetative nuclei. We also uncovered epistatic interactions in which combinations of inherited haplotypes produced regulatory states not apparent from either locus alone.

Genetic validation linked these transcriptional programmes to individual causal loci, some of which are associated with biased transmission through the male gametophyte. Single-gamete transcriptomics therefore provides a direct route from natural genetic variation to regulatory state, gametophytic function and reproductive success.

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The interplay between transcription factors and long non-coding RNAs in the regulation of lateral root initiation in *Arabidopsis thaliana*

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Lateral root initiation (LRI) is a complex process in plants, involving the coordinated action of multiple genes and regulatory elements. To gain insights into the underlying gene regulatory network (GRN), we performed a comprehensive analysis of LRI in Arabidopsis thaliana. By integrating publicly available annotations and large-scale prediction datasets, we established a quasi-exhaustive annotation of long non-coding RNAs (lncRNAs) in Arabidopsis. We then profiled gene expression along the LRI process, identifying distinct patterns of regulation for transcription factors, lncRNAs, and other coding genes. Using a GRN inference algorithm, we reconstructed a network comprising 50 driver clusters, including TF and lncRNAs, controlling 3520 target genes via 1833 edges. We further superclustered the driver nodes, revealing distinct regulatory patterns and highlighting the importance of lncRNAs in the network. Furthermore, we found that the LRI process is robust to single transcription factor mutations, and identifying a core network constituted of 85 regulators and 511 targets. Validation of the GRN using transcriptomic data from mutant lines and public datasets confirmed the predicted regulations, with a significant proportion of edges supported by independent evidence. Our study provides a comprehensive understanding of the LRI GRN in Arabidopsis, shedding light on the complex interplay between transcriptional regulators and highlighting the potential of lncRNAs as key players in this process.

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Unveiling a Dual Function of NSRa: Linking Splicing and Polyadenylation

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NSRa is a plant-specific single RNA recognition motif (RRM) RNA-binding protein (RBP) that regulates alternative splicing in *Arabidopsis thaliana*. It plays important roles in auxin-dependent developmental processes, including lateral root formation, and contributes to flowering time regulation. As a nuclear speckle-associated splicing factor, NSRa modulates transcript diversity in response to developmental and environmental cues. Previous work also showed that several long non-coding RNAs (lncRNAs) regulate NSRa activity and downstream splicing outcomes. However, the direct RNA targets and protein partners of NSRa have remained largely unknown thereby preventing a complete understanding of NSRa mode of action. To better understand NSRa function, we combined iCLIP to map RNA-binding sites, immunoprecipitation coupled to mass spectrometry (IP-MS) to identify protein interactors, and Nanopore long-read cDNA-PCR sequencing to characterize NSRa-dependent transcript isoforms. Our analyses reveal that NSRa exerts a dual role in RNA processing. Besides regulating alternative splicing, NSRa preferentially binds the 3′ regions of pre-mRNAs downstream of polyadenylation cleavage sites and controls alternative polyadenylation of numerous genes. NSRa-dependent events are strongly enriched for proximal intronic poly(A) site usage, indicating a specific role in coordinating splicing and 3′ end processing. Notably, several affected targets are regulators of thermomorphogenesis, and nsra mutants display altered thermomorphic growth. Consistent with these findings, IP-MS identified multiple components of the cleavage and polyadenylation machinery as NSRa interactors. Together, our results establish NSRa as a multifunctional RBP that links alternative splicing and polyadenylation. This coordinated regulation of pre-mRNA processing provides a mechanistic framework for understanding how NSRa fine-tunes gene expression programs underlying plant development and environmental responses.

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SESSION 6: EPIGENETICS AND EPITRANSCRIPTOMICS

CHAIRS: Hervé Vaucheret & Arturo Mari-Ordóñez

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The biochemistry of RNA-Directed DNA Methylation: enzymatic reactions of RNA Polymerase V and its protein partners

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RNA-directed DNA methylation (RdDM) is a critical process for silencing transposons and other selfish genetic elements throughout plant genomes. The process involves two plant-specific multi-subunit RNA polymerases, abbreviated as Pol IV and Pol V, that evolved as specialized forms of DNA-dependent RNA polymerase II. Pol IV collaborates with an RNA-dependent RNA polymerase, RDR2 to synthesize double-stranded RNAs that are then diced by DCL3 into short interfering RNAs (siRNAs). These siRNAs are loaded into the ARGONAUTE 4 protein and direct the cytosine methylation and silencing of complementary DNA sequences transcribed by Pol V. In recent years, we have recapitulated many of the enzymatic reactions of the RNA-directed DNA methylation pathway in the testtube, revealing reaction mechanisms of Pol IV, RDR2, DCL3 and AGO4 and how each of these enzymes generate RNAs that include sequence or structural codes that specify what happens in the next step of the pathway. Remaining challenges include understanding the dynamics of Pol V transcription, the length of its transcripts, the biochemical activities of helper proteins that influence Pol V function, and how chromatin modifying complexes are recruited to sites of Pol V transcription. We are developing biochemical assays to elucidate these Pol V-dependent steps of the RNA-directed DNA methylation pathway in vitro and will share our latest results.

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Epigenetic regulation of development plasticity and environmental adaptation

As the crucial interface between the genome and the environment, the epigenome plays a key role in plant survival and thriving. The field of plant epigenetics is advancing rapidly with groundbreaking discoveries that are transforming our knowledge of how plants regulate gene expression, adapt to environmental changes, and uphold genomic stability. Recent technological advancements have also dramatically enhanced our ability to study the epigenome with precision, offering insights into its role at an unprecedented scale. Here, we discuss our latest findings focusing on the intricate interplay of DNA methylation with RNA biology, the dynamic and multi-layered regulatory mechanisms, and the role and evolutionary significance of DNA methylation variations across diverse plant species. Mechanistic understanding DNA methylation establishment and inheritance offers crucial insights into the development of novel chromatin-based technology for biomass production and agricultural improvement.

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Transcription dynamics at early stages of plant development

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Seed germination and early seedling establishment requires a near-complete reprogramming of the plant transcriptome. However, when transcription itself resumes, and how the epigenome regulates this process, remains poorly explored. To start addressing these questions we profiled the nascent transcriptome of *Arabidopsis thaliana* undergoing germination and early seedling establishment. Our data revealed thousands of coding and non-coding Transcriptional Start Sites (TSSs) dynamically changing during the seed-to-seedling transition. We demonstrated that active transcription is detectable during the entire germination process, which we validated by single molecule RNA-FISH. In parallel, we performed Assay for Transposable-Accessible Chromatin using sequencing (ATAC-seq) to obtain a dynamic view of genome-wide accessible sites. Chromatin accessibility of promoters was found to be very dynamic over the course of germination. Furthermore, our data revealed a wealth of non-coding regulation taking place during germination including antisense and divergent transcription, as well as putative enhancer elements that are transcribed producing enhancer RNAs (eRNAs). Current work is aimed at characterizing the conservation and function of these eRNAs. Overall, our work defining the extent of coding and non-coding transcription during key stages of germination expands our understanding of transcriptional mechanisms underlying plant developmental transitions.

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Identifying the origin and function of Pol4-dependent small RNAs for endosperm development in *Capsella rubella*

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Seeds are composed of the seed coat, embryo and endosperm. The seed coat is a maternal tissue, while the embryo and endosperm are formed through fertilization. The endosperm is a nourishing tissue supporting embryo development. RNA polymerase IV (Pol4) generates 24-nt small RNAs (sRNAs) to regulate the activity of transposable elements and genes through DNA methylation. In *Capsella rubella* (Cr), loss of maternal Pol4 leads to depletion of 24-nt small RNAs at many sRNA-producing loci, connected with endosperm defects and deregulation of many genes. However, the origin of these sRNAs remains elusive. In this study, we found that NRPD1, the largest subunit of Pol4, is relatively strongly expressed in the ovule and seed coat, but only weakly expressed in the endosperm. To identify the origin of Pol4 function, NRPD1 was expressed in nrpd1 by using the Arabidopsis seed coat-specific promoter (pAtSTK) or the endosperm-specific promoter (pAtPHE1). pAtSTK rescued the nrpd1 endosperm defects while pAtPHE1 did not, confirming the sporophytic function of Pol4 during endosperm development. To investigate the movement of sRNAs, sRNAs were isolated from the ovule, seed coat and endosperm of wild type, nrpd1 and transgenic lines. We found that a large number of 24-nt dominant sRNA loci are shared among all three tissues in wild type, and sRNAs are largely depleted at many loci in nrpd1, indicating that Pol4 is crucial for the biogenesis of 24-nt sRNAs in all three tissues. Importantly, pAtSTK restored the accumulation of these sRNAs in both the seed coat and the endosperm, suggesting that Pol4-dependent sRNAs are generated in the seed coat and transported into the endosperm. Our findings reveal that sRNAs represent another maternal influence on offspring development, in addition to nutrients and hormones. How gene expression in the endosperm is affected by them will be discussed further at this conference.

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Epigenomic changes following the evolution of selfing

The evolution of inbreeding – one of the most common transitions across the plant tree of life – fundamentally alters the nature of genomic conflicts and is predicted to affect multiple epigenetic phenomena, including genomic imprinting, effective ploidy, and host defense against transposons. RNA-directed DNA methylation (RdDM) has an important role in epigenomic regulation and is linked to genomic conflicts, suggesting that the nature or function of RdDM might be altered following the evolution of selfing. Indeed, we have discovered that RdDM is required for seed production in outbreeding species in the Brassicaceae, but is dispensable in inbreeders. We therefore hypothesize that there is a common pattern of epigenomic change following the transition to inbreeding and that these changes are mediated by RdDM. To investigate the impacting of mating system on RdDM and epigenomes, we are performing comparative genetics in *Capsella grandiflora* (and outbreeder) and *Capsella rubella* (a closely related inbreeder). This presentation will discuss our latest findings on the differences between these species with respect to transposon defense, effective ploidy, and the role of RdDM during reproduction.

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Decoding the principles of m^{6}A deposition and recognition in *Arabidopsis thaliana*

N^6-methyladenosine (m^{6}A) is an abundant mRNA modification conserved across eukaryotes, with important roles in plant growth, stress adaptation and antiviral defence. However, the principles governing its transcriptome-wide deposition and recognition remain incompletely understood. In this talk, I will discuss our work in *Arabidopsis thaliana* investigating the sequence features that guide m^{6}A deposition and how local sequence context and interactions with RNA-binding proteins influence its recognition. We show that m^{6}A deposition in Arabidopsis occurs within a broader range of sequence motifs than is typically observed in mammals, where DRACH motifs predominate. We also show that U-rich sequences are enriched in the flanking regions around m^{6}A sites and play an important role in stabilising m^{6}A reader binding. Finally, I will present our ongoing efforts to use nanopore direct RNA sequencing to study plant m^{6}A at single-molecule resolution, including some of the technical challenges associated with applying this approach in plants.

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Nuclear speckle organization and splicing robustness promoted by spliceosomal protein interactions

Byeong-ha Lee

Pre-mRNA splicing is carried out by the spliceosome, a dynamic ribonucleoprotein complex enriched in nuclear speckles (NS), subnuclear compartments increasingly recognized as active splicing hubs. However, how spliceosomal protein–protein interactions are functionally linked to NS organization to maintain splicing robustness in plants remains poorly understood. Here, we show that the interaction between two U4/U6·U5 tri-snRNP components, STA1 and DOT2, plays an important role in NS organization, pre-mRNA splicing, and heat stress responses in Arabidopsis. A genetic suppressor screen of the PRP6 homolog mutant *sta1-1* identified a missense allele of DOT2 (a Snu66/SART1 homolog) in the suppressor mutant S307 that restored the *sta1-1* small size. The mutation restored the weakened STA1–DOT2 interaction in *sta1-1*. Genetic, biochemical, and cell biological analyses revealed that the strength of this interaction correlates with NS formation, splicing efficiency, plant growth, and thermotolerance. Pharmacological perturbation of STA1-associated NS by tubercidin phenocopied *sta1-1*, further supporting a functional link between NS organization and splicing activity. Furthermore, heat stress weakened the STA1–DOT2 interaction, reduced NS formation, and increased transcriptome-wide intron retention, including in key heat-responsive transcripts. We propose that the STA1–DOT2 interaction, which likely reflects the tri-snRNP assembly state, may represent one of the heat-sensitive interaction nodes linking spliceosome assembly to NS organization and splicing robustness during stress. Our findings further highlight an alternative strategy for improving plant stress tolerance: reinforcing non-stress-specific mechanisms that sustain basic cellular processes, such as pre-mRNA splicing.

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PNUTS-like scaffolding of CPF phosphatase coordinates 3′-end processing and FLC silencing

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Understanding how transcription shapes local chromatin states remains a major challenge in eukaryotic gene regulation. The Arabidopsis flowering repressor FLC (FLOWERING LOCUS C) provides a powerful framework to study this complexity. We previously identified a transcription-coupled chromatin silencing mechanism mediated by interactions between chromatin modifiers and components of the Cleavage and Polyadenylation Factor (CPF)1. In particular, APRF1 and TOPP4 form a phosphatase module (CPFpm) that promotes RNA Polymerase II (RNAPII) termination. To further dissect CPFpm function, we performed IP-MS using FLAG-tagged APRF1 and TOPP4, revealing strong interactions with a previously uncharacterized group of CCCH-type zinc finger proteins, which we named PNUTS-like (PNL1–3). Reciprocal IP-MS with FLAG-tagged PNLs confirmed their association with CPFpm, as well as with splicing factors, chromatin remodelers, and RNAPII-associated proteins. Structural modelling suggests that PNLs act as scaffold proteins bridging APRF1 and TOPP4. We generated *pnl1 pnl2 pnl3* triple mutants, which exhibit a photoperiod-independent late-flowering phenotype. This is consistent with FLC derepression, where we detect transcriptional termination defects at the antisense lncRNA COOLAIR using 3′-end RNA-seq, along with local changes in histone marks by ChIP-qPCR. We further characterised termination defects genome-wide using FLEP-seq, a long-read sequencing approach. To investigate the transcriptional termination triggering mechanism, we analysed RNAPII CTD phosphoisoforms by ChIP-qPCR. Our data support a model in which CPFpm removes elongation-associated marks (Ser2P, Ser5P), facilitating the deposition of termination-associated marks (Tyr1P, Thr4P). Together, these findings define CPFpm architecture and uncover new factors coordinating transcription termination, chromatin silencing, and flowering time regulation.

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FLASH TALKS

FLASH TALKS - DAY 1

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Poster 123

Nascent Long Read Direct RNA Seq captures dynamic post transcriptional m^{6}A erasure in Arabidopsis

Wil Prall, Jeremie Bazin, Brian Gregory, Andrew Nelson, Kyle Palos

Single nucleotide modification on RNA transcripts governs nearly every aspect of an RNA molecules' life, from transcription to degradation. The importance of modification deposition and removal across the plant (epi)transcriptome has been made clear in recent years, particularly in crop species. While the influence of the epitranscriptome is apparent, we still do not have a complete understanding of how or why these modifications are added and removed, their influence on co-transcriptional RNA maturation, and the dynamic regulation of them during the stress response. I have developed a sequencing pipeline to capture nascent RNA intermediates in various states and detect the RNA modifications they harbor using Oxford Nanopore Technologies Direct RNA sequencing. Using this method and mutants in known RNA modification erasers, I am able to capture a more accurate RNA modification landscape by comparing the predicated RNA modifications within subcellular fractions of RNA. I describe a potent role of known m^{6}A eraser ALKBH10B in selectively removing methyl marks in nascent chromatin-associated RNA in Arabidopsis by contrasting the methylation signatures between chromatin-associated, cytosolic, and whole cell fractions. Using this method I am able to capture and relatively quantify the presence of m^{6}A on maturing transcripts with single nucleotide resolution. These analysis in combination with preliminary biochemical analysis of the interactors of known m^{6}A erasers suggests a complex regulation of methylation erasure co- and or post-transcriptionally. Mining the direct long read data has also revealed preliminary associations between RNA methylation and co- and post-transcriptional processing events. Further work is ongoing to perform similar analyses in the context of stress and in crop species.

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Poster 124

Characterizing the plant Pseudouridylation Complex to better understand miRNA regulation

Marta Zimna, Sebastian Glatt, Artur Jarmolowski, Jakub Dolata

MiRNAs are one of the key gene expression regulators in plants, acting at the post-transcriptional level by either cleaving the target mRNA or blocking its translation [1]. The biogenesis and action of miRNAs are complex processes that still leave us with many unanswered questions. Only in last couple of years the technological advances allowed us to study how RNA modifications, such as N6-methyladenosine (m^6A) or pseudouridine (Ψ) influence small non-coding RNAs, including miRNAs [2]. Recent studies indicate that naturally occurring RNA modifications may play important roles on many stages of plant miRNA biogenesis, from maintaining the proper secondary structures of precursors to regulating miRNA transport [2], [3], [4]. In our laboratory, we have proven that Ψ is present in both precursors and mature miRNAs in Arabidopsis [3]. Additionally, we suspect that NAP57 protein (a homolog of human Dyskerin/DKC1 and yeast Cbf5) is responsible for small RNA pseudouridylation in plants [3]. To better understand how miRNA precursors are modified, we first focused on describing the NAP57 complex in Arabidopsis. We have used confocal microscopy techniques to visualize the intricate network between involved proteins: NAP57, NHP2, GAR1 and NOP10. Those results served us as a scaffold for more advanced in vitro studies that will allow us to describe how the complex is formed and how it interacts with substrate RNAs. Together, our work establishes a foundation for uncovering the mechanism of plant small RNA pseudouridylation, expanding our understanding of biogenesis of miRNAs and their influence on post-transcriptional gene expression regulation.

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Poster 125

DRH1, RH46 and RH40 form dynamic complexes with SERRATE and AGO1 to modulate miRNA biogenesis in Arabidopsis

Monika Jozwiak, Oskar Kaminski, Artur Jarmolowski, Mateusz Bajczyk

DEAD-box helicases have emerged as important players in gene expression regulation. Unlike typical helicases, they bind short dsRNA fragments and unwind them without translocation — strand separation results from conformational changes upon ATP binding. They show no sequence specificity; rather, their RNA targets are determined by the protein complexes in which they participate. Of 56 Arabidopsis DEAD-box helicases, only DRH1, RH46 and RH40 contain WW domains, identified by in silico analysis as potential interaction sites for the CTD domain of RNA polymerase II (RNAPII). DRH1 is known to be involved in rRNA processing, NMD and mRNA export. However, the role of these helicases in miRNA biogenesis remains poorly understood. To investigate their function, we generated mutant combinations of DRH1, RH46 and RH40. The triple mutant drh1 rh46 rh40 was indistinguishable from wild-type plants at 22°C but displayed a developmental phenotype at 16°C. The absence of all three helicases affected miRNA levels at 22°C. At 16°C, these changes were more pronounced, with most affected miRNAs being upregulated. Using co-IP coupled with LC-MS/MS analysis, we characterized the DRH1 in vivo interactome, revealing connections with miRNA biogenesis-associated proteins. Using FRET-FLIM analysis, we confirmed direct interactions between the three studied helicases and two key proteins in the miRNA biogenesis pathway: SERRATE (SE) and ARGONAUTE1 (AGO1). Strikingly, crossing the triple helicase mutant with the miRNA biogenesis mutant hyl1-2 reversed its strong phenotype to nearly wild-type plants. We also investigated their cellular localization. DRH1 and RH46 localized in both the nucleoplasm and the nucleolus, whereas RH40 localized only in the nucleoplasm. Notably, all three helicases form foci in the nucleoplasm. We further showed that DRH1, RH46 and RH40 form homodimers and heterodimers, and can also assemble into trimeric complexes. Interestingly, the subnuclear localization of SE depends on the composition of the helicase complex it associates with.

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Poster 126

Uncovering the molecular machinery of transposable element-triggered chromatin loop formation in plants

Camila Zlauvinen, Santiago Priego-Cubero, David Latrasse, Moussa Benhamed, Pablo Andrés Manavella

How organisms rapidly adapt to changing environments remains a central question in evolutionary biology. Transposable elements (TEs), once considered genomic parasites, are now recognized as powerful drivers of genome innovation and gene regulation. In plants, Miniature Inverted-repeat Transposable Elements (MITEs), TEs commonly located near genes, produce 24-nt siRNAs that trigger RNA-directed DNA methylation (RdDM), leading to local chromatin remodeling. Furthermore, recent studies from our lab revealed that MITEs also act as anchor points for short-range chromatin loops (SRCLs) that modulate the expression of neighboring genes. Yet, the molecular machinery that establishes and maintains these TE-mediated chromatin interactions remains unknown. Here, we combine genetics, dCas9-TurboID proximity labeling, Micro-C, and RNA-seq to uncover key regulators of MITE-associated chromatin looping in Arabidopsis thaliana. We show that a methyl-DNA reader complex is specifically enriched at MITE loci and is required for loop formation, as mutant plants display impaired chromatin interactions and altered expression of stress-responsive genes. To systematically identify additional components, we developed a locus-specific dCas9-TurboID strategy that revealed an unexpected repertoire of chromatin-associated proteins enriched at MITE-containing regions. Genome-wide Micro-C and transcriptomic analyses demonstrate extensive rewiring of chromatin architecture and gene expression in mutant backgrounds, with MITE-associated genes among the most strongly affected targets. These findings support a model in which transposable elements recruit dedicated protein complexes that actively shape local genome topology to regulate environmentally responsive genes.

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Poster 34

Mitochondrial retrograde signalling modulates the epitranscriptomic landscape in *Arabidopsis thaliana*

Jesús Pascual, Jesús Carballo, Ignacio Díaz-Floriano, Luis Valledor, María Jesús Cañal, Mónica Meijón

Chemical modifications of RNA have emerged as a widespread and functionally important layer of gene expression regulation, with increasing evidence pointing to their role in plant stress responses. Yet the signalling and regulatory pathways that control epitranscriptomic reprogramming under stress remain largely unknown. Here, we show that mitochondrial redox state is a key determinant of the RNA epitranscriptomic landscape in Arabidopsis thaliana. Using antimycin A to induce mitochondrial oxidative stress and Nanopore direct RNA-Seq to detect and quantify RNA modifications, we uncover that the transcription factor ANAC017, a master regulator of mitochondrial retrograde signaling, orchestrates the stress-induced epitranscriptomic response. Focusing on m^6A, the most prevalent mRNA modification, we show that ANAC017 drives stress-dependent changes in m^6A deposition by modulating the expression of associated enzymatic machinery. This work provide evidence for a mechanistic link between mitochondrial stress signalling and RNA epitranscriptomic regulation, revealing a previously unrecognized pathway through which mitochondrial redox state shapes the epitranscriptome during plant stress.

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FLASH TALKS - DAY 2

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Poster 127

Beyond nonsense-mediated decay: UPF1 regulates R-loop homeostasis and splicing in Arabidopsis

Vivek Kumar Raxwal, Jaroslav Fulneček, Karel Říha

UPF1 is an evolutionarily conserved SF1 RNA helicase and the principal ATP-dependent effector of nonsense-mediated mRNA decay (NMD)[1]. Although its cytoplasmic surveillance role is well established, the broader biological functions of UPF1 are poorly understood because complete UPF1 loss is lethal in several major multicellular systems, including Arabidopsis, Drosophila, and mouse[2-4]. This has limited whole-organism analysis of how UPF1 contributes to growth, development, and physiology, and has obscured studying potential functions beyond RNA decay. To overcome this barrier, we suppressed the defense signaling that drives lethality in the Arabidopsis *upf1* mutant[2]. Removing EDS1, the shared upstream component of the EDS1-PAD4-ADR1 and EDS1-SAG101-NRG1 immune modules[5, 6], blocked hypersensitive cell death and generated viable upf1 eds1 plants. This cleaner background enabled isoform-resolved transcriptome analysis with fewer secondary defense effects. As expected, UPF1 loss increased transcripts carrying canonical NMD-associated features. However, it also affected transcripts without obvious NMD features and caused differential isoform usage in genes with unchanged total mRNA abundance. Splice-aware analysis therefore separated RNA-processing defects from mRNA stabilization and showed that UPF1 contributes to isoform choice during nuclear RNA biogenesis, before transcripts enters cytoplasm. We then tested whether UPF1 has a nuclear function consistent with this splicing phenotype. UPF1 was detected in nuclei and accumulated after inhibition of transcription or nuclear export, indicating active nucleo-cytoplasmic trafficking. UPF1 was also enriched at R-loop-associated genomic regions, and this enrichment increased after transcription inhibition or RNase A treatment, suggesting association with DNA rather than nascent RNA. Mutational analysis separated RNA binding from R-loop control: an ATPase-defective, RNA-binding variant did not increase at these regions, whereas a helicase-defective variant accumulated more strongly. Together, these data suggest that UPF1 acts at R-loop-prone chromatin through its helicase activity to limit RNA-DNA hybrids and maintain co-transcriptional splice-site fidelity.

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Poster 128

Actin-crosslinkers regulate PTGS by integrating stress signals at a cellular level

Tomas Maria Tessi, Zhaoxue Ma, Alexis Maizel

Plants development and architecture is the result countless decisions taken based on the perception of environmental information and its integration into cellular processes. Increasing evidence indicates that biomolecular condensates formed by phase-separated macromolecular assemblies contribute to this regulation by organizing RNA processing and silencing pathways. In plants, siRNA bodies are condensates that host key components of secondary siRNA biogenesis, including Argonautes, RNA-binding proteins, and RNA polymerases. However, the mechanisms governing their assembly, dynamics, and regulation remain poorly understood. To investigate the molecular organization of siRNA bodies, we performed proximity labeling using ARGONAUTE7 (AGO7) as bait. Among AGO7-proximal proteins, the actin cross-linker FIMBRIN5 (FIM5) emerged as a promising candidate. Using transient and stable expression systems, we validated the partial colocalization of FIM5 with siRNA bodies and showed that FIM5 is stress-responsive, increasing its affinity for F-actin upon heat shock. We further found that the F-actin–dependent movement of siRNA bodies is rapidly arrested upon stress perception, coinciding with the relocalization of FIM5 to actin cables. Deletion analyses revealed that FIM5 specifically associates with siRNA bodies, but not with other cytosolic condensates such as P-bodies. Silencing reporter assays, RT-qPCR, and RNA-seq demonstrated that the overexpression of FIM5 strongly compromises AGO7-dependent silencing of auxin response factor (ARF) transcripts that are targets of tasiRNAs. Consistently, FIM5 acts as a negative regulator of PTGS. Finally, FRET-FLIM analyses revealed a physical interaction between FIM5 and the siRNA-body component SGS3 at contact sites along the actin cytoskeleton. Together, our results uncover a functional link between the actin cytoskeleton and siRNA-body dynamics, showing that the organization of the secondary siRNA biogenesis machinery is actively remodeled during abiotic stress. These findings highlight cytoskeletal regulation as a mechanism controlling RNA silencing and condensate function in plants.

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Poster 129

Conserved Eukaryotic Initiation Factor-gated translation initiation drives plant stress response

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Plant eukaryotic initiation factors (eIFs) and ribosomes are essential for reading mRNAs to locate translation initiation sites (TISs) for protein synthesis, yet nearly 50% of these sites in plants remain unannotated. The mechanisms by which plant eIFs and cis-regulatory mRNA elements orchestrate TIS selections, along with their biological and evolutionary importance, remain unclear. Here, by overexpressing plant eIFs in a transient reporter system, we found that eIF1 boosts initiation at AUG start codons in a strong Kozak context prefers initiation sites with favorable mRNA contexts, enhanced by AUG codon and strong Kozak/CU-rich motifs, while eIF5/eIF1A targets stimulates sites with unfavorable contexts influenced by non-AUG codons or weak Kozak motifs. eIF1 and eIF5 both translationally auto-repressed and cross-activated each other, whereas eIF1A promoted itself and also enhanced eIF5 translation, suggesting their antagonistic regulations to enable temporal translational changes. Both eIF1 and eIF5 modulate TIS usage of various physiological and stress-related plant genes, whereas eif mutants showed varying sensitivities to endoplasmic reticulum (ER) stress, underscoring their role in integrated stress responses. This regulated TIS selectivity selection shaped by eIFs was found across land plants, including Marchantia, dicots (tomato and Arabidopsis), and monocots (rice), highlighting its evolutionary and biological significance for land plant stress adaptation. Collectively, our findings illustrate how plant eIFs, alongside local mRNA contexts, antagonistically drive TIS selections to reprogram protein translation for plant stress tolerance.

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Poster 130

Bacteria use P-body condensates to attenuate host translation during infection

Manuel González-Fuente, Nico Schulz, Alibek Abdrakhmanov, Thorben Krüger, Gaiea Izzati, Shanshuo Zhu, Gautier Langin, Paul Gouguet, Mirita Franz-Wachtel, Boris Macek, Anders Hafrén, Yasin Dagdas, Suayib Üstün

Processing bodies (P-bodies) are dynamic ribonucleoprotein condensates formed through liquid–liquid phase separation in the cytosol. By controlling mRNA storage, translation, and decay, P-bodies enable rapid and cost-efficient cellular responses to environmental stimuli and regulate diverse developmental and stress-related processes in plants. Although pathogens are known to manipulate host protein degradation pathways to promote infection, little is understood about how they modulate translation. Given their key role in post-transcriptional gene regulation, P-bodies represent a potential target for pathogens seeking to reprogram their host cellular functions. Although pathogens are widely known to manipulate multiple host protein degradation pathways to promote infection, little is understood about how the modulate host translation. Here, we show that pathogenic bacteria *Pseudomonas syringae* pv. *tomato* (Pst) induces the formation of P-bodies to attenuate the host translation through two effectors with liquid-like properties. Moreover, we found that autophagy and repression of the ER stress response are required for the Pst-mediated induction of P-bodies and attenuation of translation. Altogether, our discoveries provide novel insights on how host translation is attenuated by bacteria to dampen plant immunity, and uncover unknown connections between ER stress responses and autophagy with P-body dynamics.

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Poster 131

Cell type specific translational landscapes of Arabidopsis root in response to temperature stress

Manvi Sharma, Amir Mahboubi, Dhruba Khakurel, Ling Cheng, Xuemin Ma, Johannes Hanson, Petra Marhava

Fluctuating temperature is one of the major factors affecting plant growth and development. Although there is abundant information about transcriptional responses, the contribution of cell type specific translational regulation remains poorly understood. In our study we used Translating Ribosome Affinity Purification sequencing (TRAP-seq) to investigate temperature stress–induced translatome dynamics across outer root cell populations in Arabidopsis. Comparative analysis of ribosome-bound transcripts reveals fundamentally different strategies observed during early and prolonged stress conditions. Our study also highlights that though cell layers have distinct translatomes, they exhibit a shared translational program of core stress responsive mRNAs. To identify coordinated regulatory mechanisms, we performed Weighted Gene Co-expression Network Analysis (WGCNA) that revealed multiple co-expression modules exhibiting strong associations with specific cell types and stress time points. Functional enrichment analyses revealed pathways involved in hormone signalling, cell wall integrity, and stress adaptation. Together our findings highlight the power of this technique to gain insights into root-stress interactions and identify potential targets that can be used to establish a framework for understanding how translational network remodelling contributes to plant temperature stress adaptation and bridging the gap between laboratory studies and field applications.

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POSTERS

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S1 Posters - RNA Silencing

Poster 1

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Translation potential of MIR169:: NF-YA:: ARGOS node in regulating biomass and yield enhancement from Arabidopsis to tomato

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Crop productivity is greatly affected by global increased biotic and abiotic stresses, resulting in yield reduction to plant death. Downstream molecular linkers between conserved miRNA: targets for plant growth and yield production have been poorly understood. Here we report how stress responsive conserved miRNA169 and its target NF-YAs regulate biomass and total yield in Arabidopsis and tomato. Arabidopsis transgenic plants overexpressing miR169d with reduced NF-YA2 and nf-ya2 mutants exhibit augmented vegetative growth and yield. Conversely, plants overexpressing MIM169defg (in which miR169d is chelated), NF-YA2 overexpression and NF-YA2-RT (non-cleavable form of NF-YA2) showed decreased biomass and yield. Further, we find increased auxin levels in MIR169d-OE and nf-ya2 mutant lines, supporting the enrichment of auxin terms in MIR169-OE transcriptome data. Plants with reduced NF-YA2 levels show upregulation of auxin-inducible genes like ARGOS, that correlates with elevated levels of the growth hormone auxin and thus, yield. Notably, we show ARGOS promoter contains conserved CCAAT-boxes and its direct regulation by NF-YA2. Genetic analysis show that neither miR169d overexpression in argos mutant nor nf-ya2 argos double mutant was able to rescue argos mutant phenotype, indicating MIR169::NF-YA2 node modulates ARGOS activity through an auxin independent pathway. To assess translation potential of this module in crop species, we show Sly-MIR169-OE plants with reduced level of its target Sly-NF-YA10 and increased Sly-ARGOS level, exhibit early flowering, early fruiting, higher fruit set, larger fruits and better shelf-life compared to wildtype tomato plants. In contrast, STTM169 plants with chelated Sly-miR169 show longer life cycle and reduction in overall yield. Thus, our finding uncovers a conserved MIR169:: NF-YA:: ARGOS module with strong translation potential can be exploited for engineering high yielding crops.

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Poster 2

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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, involved in the broad spectrum of post-transcriptional regulation of genes expression and proteins synthesis. In the process of miRNA biogenesis, SERRATE (SE) protein plays a crucial role. 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, showing a highly pleotropic phenotype of this mutant. Despite many years of intensive research, little is still known about the protein partners interacting with the end of the SERRATE protein. To determine the 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. Additionally, transgenic lines were prepared where, under the native promoter, both the wild-type and mutated (*se-1*) versions of the protein were expressed, in both cases fused with a FLAG epitope at the N-terminus. Protein identification has been performed using the co-immunoprecipitation assay. This experimental setup gave valuable information about the role of the SE C-terminus in the interactions between the proteins involved in microRNA biogenesis.

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Poster 3

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Interaction between the Serrate protein and the THO complex in RNA biosynthesis

Eliza Maniak, Artur Jarmołowski, Mateusz Bajczyk

MiRNAs are short, non-coding RNA molecules (21–24 nt) that play a key role in regulating gene expression in eukaryotes at the post-transcriptional level. In plants, primary miRNA precursors (pri-miRNAs) are generated by the activity of RNA polymerase II and have a hairpin-like structure. Their maturation occurs in the cell nucleus with the involvement of a complex containing the DCL1 endonuclease, as well as the SE (SERRATE) and HYL1 proteins. Recent studies show that six subunits of the THO complex co-purify with FLAG-SE in Co-IP experiment. In plants, these six subunits form a core complex that can associate with additional proteins, such as ALY export factors, DDX39 splicing factors, and RNA helicases, thereby forming the THO/TREX complex. The role of the THO complex in plants is not yet fully understood; however, several studies suggest that it regulates the biogenesis and function of small RNAs.As part of the initial phase of my research, I focus on investigating the interaction between SE and selected subunits of the THO complex, while pondering the functional significance of these interactions. Furthermore, I outline the experimental strategies that will be applied to clarify the role of THO proteins in small RNA biogenesis, including their potential involvement in pri-miRNA processing and coordination with the miRNA processing machinery. During my research, I intend to visualise protein colocalisation using a confocal microscope, employing techniques such as FLIM-FRET and proximity ligation assay (PLA). I will also investigate protein interactions using a yeast two-hybrid system. Moreover, for future I plan to generate Arabidopsis thaliana lines with selected mutated THO genes and carry out small RNA sequencing analysis. This work will provide a framework for understanding how the THO/TREX complex contributes to post-transcriptional gene regulation in plants.

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Poster 4

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SirenScan reveals the conserved presence of siren RNAs and their evolutionary diversification across angiosperms

Haoran Peng, Aurélien Valentin, Yichun Qiu, Katarzyna Dziasek, Claudia Köhler

Small interfering RNAs (siRNAs) play central roles in the silencing of transposable elements (TEs) and in epigenetic regulation in plants1. Siren RNAs represent a class of siRNAs that exhibit extreme expression dominance in ovules and endosperm2-8; however, whether this expression pattern is conserved across angiosperms remains unclear. Here, we present SirenScan, a computational pipeline that integrates rank-abundance and density-based analyses to identify siren loci from small RNA sequencing data. Applying SirenScan to ovule and vegetative tissues across diverse angiosperm species, including the early-diverging angiosperm Nymphaea colorata, we show that the presence of siren RNAs is a conserved feature of ovule tissues, although the strength of expression dominance varies substantially among species. We further demonstrate that siren loci are consistently enriched in TEs, with lineage-specific preferences for distinct TE superfamilies. Motif analyses further identified conserved cis-regulatory signatures within Brassicaceae that are consistent with CLASSY3-mediated targeting, while other angiosperm clades exhibited substantial motif diversification. These findings demonstrate that SirenScan provides a robust and scalable pipeline for comparative siren RNA research and enables cross-species investigations of reproductive small RNA evolution. By combining sensitive locus detection with standardized comparative parameters to define siren loci, it facilitates the discovery of conserved and divergent regulatory features across angiosperms. Overall, this study expands our understanding of the possible interconnection between siren RNAs and TEs during plant reproduction.

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Poster 5

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Understanding the role and biogenesis of transfer RNA-derived small RNAs in plant stress

Joan Marquez-Molins, Germán Martínez

Transfer RNAs (tRNAs) are no longer viewed merely as passive adapters in the translation machinery. Emerging evidence suggests they are a source of regulatory molecules known as tRNA-derived small RNAs (tsRNAs). While these fragments are known to accumulate under stress in multiple organisms, the specific mechanisms governing their biogenesis and their downstream functional roles in plants remain poorly defined. In this study, we demonstrate that diverse abiotic stresses (heat stress, salinity and oxidative stress) trigger a significant overproduction of tsRNAs (even above 50% of total sRNA) in the model plant Arabidopsis thaliana. Interestingly we found a significant positive correlation between stress intensity and the fraction of tRNA halves (30-35 nt). Our results indicate that this stress-induced biogenesis is mainly due to RNS1 upregulation and the most abundant species are tRNA halves of 31-33 nucleotides. A differential expression meta-analysis of RNS-T2 family confirmed the distinctive and significant RNS1 overexpression in multiple abiotic and biotic stresses, evidencing its major role in stress-triggered tsRNA biogenesis in plants. However, constitutive overexpression of this enzyme did not increase the tsRNAs levels as much as that of RNS3, pointing towards additional factors regulating tsRNA biogenesis. Furthermore, we explored the physiological relevance of these fragments using overexpression transgenic lines of RNS1,2 and 3. Phenotypic analysis in salinity and oxidative stress revealed a slight increase in fresh weight in the lines over accumulating more fragments. Subsequently, PARE analysis revealed a lack of evidence for mRNA targeting, but pointed to translation alterations evidenced by differential ribosome stall peaks in the stop codon. Overall, our results indicate that high levels of tsRNAs do not merely represent degradation products; rather, they actively regulate the stress response in plants.

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Poster 6

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Distinct roles of terminal nucleotidyl transferases in plant secondary siRNA biogenesis

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In plants, RNA interference (RNAi) mediated by the endonucleolytic RNA-Induced Silencing Complex (RISC) defends against foreign RNA and regulates endogenous genes. Targeting of RISC to foreign RNA establishes amplification loops, wherein RNA-dependent RNA Polymerase 6 (RDR6) synthesizes double-stranded RNA (dsRNA) for secondary small interfering RNA (siRNA) biogenesis, using cleavage fragments of RNA targeted by RISC programmed with a primary siRNA as template. Secondary siRNA production from endogenous RISC targets requires a particular primary small RNA size or target site multiplicity. siRNA amplification in yeast and nematodes requires terminal nucleotidyl transferases (TNTases), but their roles in plants are unclear. Here, we provide evidence for distinct activities of TNTases in either limiting or promoting secondary siRNA production in *Arabidopsis thaliana*. We further identify HESO1-dependent 3′-end extensions of RDR6-generated dsRNA, suggesting that TNTases contribute to secondary siRNA biogenesis at distinct stages of the pathway.

Poster 7

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Next-generation RNAi-based fungicides for crop protection using artificial small RNAs delivered by viral vectors

Ariel Hernán Tomassi, Alberto Carbonell

Plants are severely threatened by fungal pathogens, which cause major crop and post-harvest losses worldwide. Current control strategies rely heavily on chemical fungicides; however, their extensive use under global warming conditions accelerates the emergence of resistant strains and raises environmental concerns. RNA interference (RNAi) has gained considerable attention for antifungal control, particularly through exogenous applications of double-stranded RNA. However, Dicer-mediated processing generates heterogeneous small RNA (sRNA) populations, limiting precise control over size and sequence specificity. Artificial sRNAs (art-sRNAs) provide a highly specific alternative but have so far been largely restricted to transgenic systems. Recently, transgene-free expression of art-sRNAs using viral vectors applied as crude extracts has been developed for endogenous gene silencing and antiviral resistance1-3.Here, we adapt and validate these methodologies for a new application: antifungal plant protection. Using *Botrytis cinerea* as a model, art-sRNAs targeting BcDCL1, BcDCL24, BcERG115, and BcVPS516 were computationally designed based on conserved sequences across 43 natural strains. Artificial microRNA (amiRNA) constructs were generated and initially evaluated by agroinfiltration in *Nicotiana benthamiana*, where no antifungal effect was observed. To address this limitation, we implemented an alternative screening strategy based on the exogenous application of total RNA extracted from plants expressing anti-BcERG11 amiRNAs. This approach revealed that the mixture of amiRNAs targeting BcERG11 reduces fungal growth in planta. Ongoing work aims to identify the most effective amiRNAs and incorporate them into viral vectors for GMO-free delivery as a new generation of RNA-based fungicides.

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Poster 8

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Characterizing Small RNA Transcriptome of the Arabidopsis thaliana Egg Cell

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Reproductive development in flowering plants involves extensive cellular reprogramming during the transition from diploid sporophytic tissues to specialized haploid gametophytes. Within the female gametophyte, the angiosperm egg cell represents a highly specialized cell that links female gametophyte development with the initiation of the next generation. Despite its central role in reproduction, the egg cell small RNA repertoire has remained largely unexplored due to the technical challenges associated with isolating highly purified egg cells. To investigate the small RNA profile of the female gamete, we isolated GFP-positive egg cells from *Arabidopsis thaliana* ovules using enzymatic digestion and micromanipulation procedures. Purified egg cells were subjected to low-input RNA extraction and small RNA library preparation followed by deep sequencing. Bioinformatic analyses revealed canonical 21- and 24-nucleotide small RNA populations together with a set of annotated and candidate regulatory small RNAs.Current analyses focus on identifying egg cell-enriched miRNAs and siRNAs and evaluating their potential contribution to the establishment of egg cell identity, developmental competence, and the molecular processes associated with fertilization and the transition to embryogenesis. This dataset provides a comprehensive small RNA microtranscriptome of the Arabidopsis thaliana egg cell and represents a valuable resource for investigating small RNA-mediated regulatory networks in the female germline. These findings offer new insights into the post-transcriptional regulatory mechanisms underlying female gametophyte development and the earliest stages of plant reproduction.

Poster 9

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A Priming Mechanism Drives Co-transcriptional miRNA Processing in Plants

Candela Houriet, Lucia Gonzalo, Pablo Manavella

MicroRNA (miRNA) biogenesis is increasingly recognized as a dynamic process that can occur co-transcriptionally while primary miRNAs (pri-miRNAs) remain associated with RNA Polymerase II. However, this model presents a fundamental paradox: because the characteristic stem-loop must be completely transcribed before it can be recognized by the microprocessor, only a narrow temporal window exists for co-transcriptional processing to occur. Here, we propose that MIRNA loci overcome this constraint through a chromatin-based priming mechanism. Using the phosphate starvation-inducible MIR399 locus, we compared the first and subsequent rounds of transcriptional activation and found that reactivated loci are transcribed more efficiently and display a substantially higher proportion of co-transcriptionally processed pri-miRNAs. Consistent with this model, we found that the core microprocessor components DCL1 and HYL1 remain associated with MIR399 chromatin even after transcriptional shutdown, establishing a poised state that facilitates co-transcriptional processing on subsequent rounds of transcription. Genome-wide analyses using transcriptional inhibitors further revealed that persistent chromatin association of the microprocessor is a general property of MIRNA loci. To investigate how the microprocessor is retained at chromatin, we developed a locus-specific proximity-labeling strategy by targeting dCas9-TurboID to the R-loop region surrounding the transcription start site of MIR160b. This approach identified candidate proteins potentially responsible for anchoring the microprocessor to MIRNA loci. Together, our findings provide a mechanistic explanation for how co-transcriptional miRNA processing is achieved despite its temporal constraints and suggest that chromatin-associated microprocessor complexes establish a molecular memory that enables rapid and efficient miRNA reactivation.

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Poster 10

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Inhibition of transgene silencing in Nicotiana tabacum BY-2 cells

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Nicotiana tabacum Bright Yellow 2 (BY-2) cell suspensions are an established platform for the production of recombinant complex glycoproteins. However, protein yields remain relatively low, even when strong constitutive promoters, such as the cauliflower mosaic virus 35S promoter, are used. One limitation arises from RNA silencing processes, including transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS), which prevent high-level accumulation of transgene transcripts. Our project aims to develop strategies to counteract the production of transgene dsRNAs involved in silencing and to reverse DNA methylation established at the 35S promoter. First, RNA-dependent RNA polymerase (RDR) genes involved in transgene silencing were knocked-out in BY-2 cells using CRISPR/Cas9 technology. Several mutant combinations were generated: RDR2-KO to impair TGS and RDR1/2/6-KO lines to prevent both PTGS and TGS. RDR1/6-KO lines, targeting PTGS, had previously been established in the laboratory. Next, BY-2 cell lines (WT, RDR2-KO, RDR1/6-KO and RDR1/2/6-KO) expressing the dCas9-SunTag/TET1 system are being generated. This system recruits multiple copies of the catalytic domain of the demethylase TET1 to a specific genome locus via a nuclease-deactivated Cas9 fused to repetitive GCN4 peptide epitopes, as previously described [1,2]. We aim to demethylate the 35S promoter driving the expression of GUS reporter gene and a gene encoding the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. Finally, co-expression of the protein of interest with RNase three-like 1 (RTL1), a broad suppressor of siRNAs [3], is being tested both transiently in N. benthamiana and stably in BY-2 cells. The effect of RTL1 on RBD expression in the transient expression system is compared with that of P19, the well-known viral suppressor of RNA silencing. Together, these complementary approaches are expected to alleviate RNA silencing and promoter methylation, thereby enhancing recombinant protein production in transgenic BY-2 cells.

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Poster 11

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Dual arginine dimethylation of ARGONAUTE1: a new regulatory layer of small RNA activity in Arabidopsis

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Small RNAs are central regulators of gene expression in plants. They contribute to development, genome stability and responses to environmental changes. ARGONAUTE1 (AGO1) is one of the main effectors of these pathways and uses small RNAs as guides to regulate complementary transcripts. Although AGO1 activity is tightly controlled, the contribution of post-translational modifications to its regulation remains poorly understood.AGO1 contains an intrinsically disordered N-terminal region enriched in RGG and GRG motifs that are conserved across plant orthologues. Strikingly, the same arginine residues within these motifs can carry either symmetric (sdMA) or asymmetric (aDMA) dimethylation marks in Arabidopsis thaliana. This dual signature, unique among AGO/PIWI proteins, has previously been observed only in histone proteins in plants. Although we previously demonstrated the presence of these modifications in AGO1, the biological role of these modifications in the regulation and function of AGO1 remains unknown.In Arabidopsis thaliana, we abolished arginine dimethylation in these motifs and characterized its functional impact. This perturbation induced reproducible developmental and molecular alterations, supporting the functional importance of this region in AGO1 regulation. Overall, our results suggest that arginine dimethylation may fine-tune AGO1 activity. This work opens new perspectives on the regulation of plant AGO proteins and reveals an additional layer of complexity in the control of small RNA-mediated gene regulatory pathways.

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Poster 12

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Exploring Natural Variation of Arabidopsis MIRNA Genes

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MicroRNAs (miRNAs) are key regulators of post-transcriptional gene expression in plants. They are produced from MIRNA genes, whose primary transcripts (pri-miRNAs) fold into characteristic hairpin structures that are processed by DICER-LIKE1 (DCL1). Because pri-miRNA structures differ substantially between MIRNAs, DCL1 employs distinct processing modes to generate mature miRNAs. Although individual examples demonstrate that natural variation in MIRNA genes can alter miRNA biogenesis, the extent to which this represents a general feature of the Arabidopsis miRNAome remains unknown.To address this question, we reconstructed haplotypes for 332 *Arabidopsis thaliana* MIRNA genes using sequence variation from the 1001 Genomes Project. Comparative analyses revealed that sequence variation is unevenly distributed across pri-miRNA structures. Mature miRNA sequences are highly conserved, whereas the stem and terminal loop regions accumulate substantially more SNPs and indels. Despite these regional differences, we found no overall association between sequence variation and the different DCL1 processing modes, suggesting that natural variation is shaped primarily by functional constraints within pri-miRNA structures rather than by processing mechanism. Ongoing work combines haplotype-specific RNA structure prediction, modeling of pri-miRNA recognition by DCL1, and haplotype-specific in vitro dicing assays to determine how naturally occurring sequence variants influence miRNA biogenesis.

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Poster 13

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The N-terminal extension: a key regulatory hub for AGO1 function

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ARGONAUTE1 (AGO1) is the major effector of small RNA (sRNA)-mediated post-transcriptional gene silencing in Arabidopsis thaliana. While the conserved AGO domains are well characterized, AGO1 also contains a long, intrinsically disordered N-terminal extension (NTE) whose importance for AGO1 function has only recently emerged. The NTE has been implicated in AGO1 subcellular distribution, sRNA loading and interactions with regulatory factors, suggesting that this region may act as an important regulatory hub. However, how these different functions are integrated remains poorly understood. Here, we investigate the molecular features of the AGO1 NTE and their contribution to AGO1 regulation. Our results reveal that the NTE integrates multiple regulatory inputs that influence AGO1 behavior and function. We identify distinct sequence features within this region that contribute to AGO1 regulation at different levels, including its subcellular distribution, association with sRNAs and interactions with regulatory proteins. In addition, our analyses reveal features of the NTE that may contribute to AGO1 protein homeostasis, pointing to an additional layer of regulation of this region. Together, these findings support a model in which the NTE acts as a multifunctional regulatory platform rather than as a passive extension of the AGO1 core.
Our work establishes the AGO1 NTE as an important regulatory region that connects different aspects of AGO1 biology and may contribute to the dynamic control of AGO1 activity and pathway engagement. Understanding how the NTE integrates these regulatory mechanisms will provide new insights into the molecular regulation of AGO1 and sRNA-mediated gene silencing in Arabidopsis.

Poster 14

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Dissecting the Maternal Role of NRPD1 in Interploidy Seed Failure

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Seed development is tightly regulated by multiple factors that influence seed filling and the survival of the next generation. Crosses between species or between individuals of different ploidy levels often result in pronounced developmental defects, most notably seed abortion—a phenomenon commonly referred to as the 'triploid block'. Interploidy crosses are characterized by endosperm over-proliferation and delayed cellularization, ultimately leading to embryo arrest and seed failure. Several mutants have been identified that alleviate the triploid block, reducing seed abortion despite unbalanced ploidy. Notably, many of these rescue effects are observed only when the mutant allele is inherited paternally, despite the fact that nutrient supply to the developing embryo is primarily governed by maternal tissues. Among the characterized mutants, alleles of Nuclear RNA polymerase D1 (NRPD1)—the largest subunit of RNA Polymerase IV (Pol IV)—exhibit a strong paternal rescue of seed abortion. Pol IV is the key enzyme initiating the RNA-directed DNA methylation (RdDM) pathway, which mediates de novo DNA methylation via small RNAs. Intriguingly, the same nrpd1 allele also causes enhanced seed abortion when inherited maternally—a phenotype that remains poorly understood. Here, we investigate the maternal effects of nrpd1 and other core RdDM components. We demonstrate that only nrpd1, and not other tested RdDM components, confers a strong maternal seed abortion phenotype. Furthermore, we show that this maternal phenotype is unlikely to represent a classical triploid block. Finally, we reveal allelic variation among different nrpd1 mutants and provide evidence that the severe maternal seed phenotype is not directly attributable to the absence of small RNAs. These findings highlight the complex and context-dependent roles of RdDM components in seed development and imply careful handling of mutant alleles.

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Poster 15

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Role of maternal small RNAs (sirenRNAs) in endosperm development

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Successful seed development requires precise regulation of gene expression and genome stability1. RNA directed DNA methylation (RdDM) plays a central role in silencing transposable elements (TEs) and establishing epigenetic marks during reproduction2. In the maternal genome, a small number of loci (1-2% of the genome) produces a large amount of 24 nt small interfering RNAs (sirenRNAs). Those loci potentially function as major epigenetic regulatory hubs during seed development3,4. The chromatin remodelers CLSY3 and CLSY4 recruit RNA Polymerase IV (Pol IV) to sirenRNA loci, thereby initiate sirenRNA biogenesis5. SirenRNAs target TEs, repeats and genes by inducing cis and trans DNA methylation6. In rice, loss of OsCLSY3 and OsCLSY4 drastically reduces sirenRNA production, connected to the formation ofsmaller seeds7. In Capsella, sirenRNAs regulate endosperm cellularization and their loss connects to reproductive barriers during interspecies crosses8.We are interested in understanding how sirenRNAs connect to the establishment of endosperm-specific reproductive barriers in interspecies and interploidy crosses. Interestingly, we observed that the triploid block is enhanced in Arabidopsis interploidy crosses, when the sirenRNA deficient clsy34 mutant was used as maternal parent. We thus hypothesized that maternal sirenRNAs are required to established an epigenetic state that is required for endosperm development in genomic imbalanced conditions. To test this hypothesis, I will Investigate the role of sirenRNAs in interploidy endosperm using genetic and genomics approaches.

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Poster 16

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Cross-kingdom RNA interference promotes arbuscular mycorrhiza development

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The exchange of small RNAs (sRNAs) between organisms, known as cross-kingdom RNA interference (ckRNAi), has been extensively studied in host-pathogen interactions. Whether ckRNAi contributes to mutualistic plant-fungal interactions is largely unstudied. Here, we investigated the role of ckRNAi in the mutualistic interaction between the arbuscular mycorrhizal fungus (AMF) Rhizophagus irregularis and its host plant *Lotus japonicus*. We hypothesized that AMF may also utilize ckRNAi to modulate gene expression in their host plants. By profiling LjAGO1-associated small RNAs from colonized Lotus japonicus roots, we identified fungal sRNAs displaying characteristics of AGO1-binding preference. Following host target prediction, candidate target interactions were supported by in vitro cleavage assays and expression analyses. Using a Csy4-based ckRNAi reporter, we showed the translocation of fungal sRNAs into host root cells, where they mediate sequence-specific gene silencing. Furthermore, sequestration of fungal sRNAs by short tandem target mimicry (STTM) reduced fungal colonization, demonstrating that RisRNAs promote the establishment of AM symbiosis. Together, our findings identify Rhizophagus irregularis sRNAs as functional symbiotic effectors, that upon transfer into plant cells, promote arbuscular mycorrhiza symbiosis through cross-kingdom RNA interference. Our work offers a glimpse into the intricate, yet still largely unexplored sRNA-mediated communication between plants and their beneficial fungal partners.

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Poster 17

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Silencing the silencer : How plants clear unloaded AGO1 ?

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RNA silencing is a conserved mechanism in eukaryotes that plays a crucial role in development and adaptive responses to environmental stimuli. Central to this process is the RNA-induced silencing complex (RISC), wherein small RNAs guide Argonaute (AGO) proteins to target mRNAs for degradation or translational repression. Despite the significance of this pathway, the regulatory mechanisms governing the turnover of AGO proteins, particularly under stress conditions, remain poorly understood. In Arabidopsis, the F-box protein FBW2 act as a key ubiquitin E3 ligase involved in the degradation of the AGO1 protein, the main effector of RNA silencing in plants. We have previously demonstrated that FBW2 interacts directly with AGO1 by forming an SCF (SKP1-CUL1-F-box protein) complex that preferentially targets unloaded AGO1 for proteolysis. This mechanism ensures that unloaded AGO1 is efficiently degraded, thereby preventing its erroneous loading with illegitimate small RNAS and off-target cleavage. Currently, using microscopic, genetic, and proteomic approches, we have elucidated the molecular mechanism that controls FBW2-mediated AGO1 degradation.

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Poster 18

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Small RNA biogenesis in Duckweeds

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Small RNA silencing pathways in plants play crucial roles in development, antiviral resistance, and transposon control. There are two types of silencing: transcriptional gene silencing (TGS) and post-transcriptional (PTGS). TGS acts via the RNA-directed DNA methylation pathway, with 24nt-long small interfering RNAs (siRNAs) being the key siRNA type; it leads to heterochromatin formation and mostly affects transposons. PTGS functions at the mRNA level, where viral or transgene RNA transcripts trigger the formation of 21nt- and 22nt-long siRNAs that target AGO proteins toward these transcripts. 21nt siRNAs mostly lead to mRNA cleavage and degradation, whilst 22nt siRNAs recruit RNA-dependent RNA polymerases (RDRs) to the mRNA to produce more double-stranded RNA (dsRNA), leading to amplification of the PTGS.Duckweeds, or Lemnoideae, fast-growing small aquatic flowering plants, have simplified their TGS pathway and lost many antiviral elements, including AGO2, and challenge the current model of PTGS in several ways. Dicer-like protein 2 (DCL2), the protein responsible for the production of 22nt siRNA, conserved among plants, is lost in duckweeds, yet we observe 22nt siRNA in all three species studied in our lab. Transgenic silencing in Lemna minor is characterised by the equal presence of both 21nt- and 22nt-long siRNAs; transient expression of an inverted repeat (IR) in Spirodela polyrhiza results in the production of both 21nt and 22nt siRNA derived from this IR; 22nt sRNAs are also coming from transposons in Wolffia brasiliensis. These 22nt siRNAs, in turn, do not seem to trigger transitivity, the silencing amplification process, despite the presence of all the necessary elements that we know of. The goal of our project is to understand the biogenesis of 22nt-long siRNA and find the mechanisms responsible for the difference in PTGS functioning in duckweeds, including transitivity — a phenomenon that is not well understood even in conventional models such as Arabidopsis.

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Poster 19

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AGO5 restricts virus vertical transmission in plant gametophytes

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Viruses are intracellular parasites that rely on transmission to new hosts to ensure their continued existence. Vertical transmission—the passage of viral infection from parent to progeny—is a key mechanism for virus persistence across generations and geographical regions. This mode of transmission is especially significant in plants, where viruses spread both locally and globally via pollen and seeds. However, vertical transmission in plants is often inefficient or entirely absent, suggesting the existence of potent transgenerational antiviral barriers which remain unknown (Bradamante et al., 2021; Hoffmann & Incarbone, 2024). In this study, we demonstrate that AGO5, an RNA interference factor uniquely expressed in stem cells and gametes, plays a crucial role in restricting the vertical transmission of Turnip yellow mosaic virus (TYMV) in Arabidopsis thaliana. Using reciprocal crosses, we show that AGO5 suppresses viral transmission through both male and female parents. Analysis of heterozygous ago5 knock-out mutants revealed that AGO5 restricts vertical transmission post-meiosis and pre-fertilization during host reproduction, emphasizing its gamete-specific antiviral activity while excluding involvement in the zygote or embryo. Moreover, production of virus-specific small RNAs in sperm cells significantly reduced pollen-mediated virus transmission. Combined with detailed virus localization studies in plant reproductive tissues, our findings unveil the first known antiviral mechanism restricting RNA virus transmission from parent to progeny through gametes, a significant advance that will increase our toolkit in the management of viral diseases.

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Poster 20

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Structural reconstitution of the Arabidopsis microRNA dicing complex for cryo-EM analysis

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Plant microRNA (miRNA) biogenesis depends on the nuclear dicing complex comprising DICER-LIKE1 (DCL1), HYPONASTIC LEAVES1 (HYL1), and SERRATE (SE), which processes primary miRNAs (pri-miRNAs) into mature miRNAs [1,2]. Although the structure of DCL1 has recently been resolved by cryo-electron microscopy (cryo-EM) [3], the architecture of the full dicing complex and the molecular mechanisms underlying its regulation remain poorly understood. To enable structural characterization of the full Arabidopsis dicing complex, we established two complementary expression platforms to produce the trimeric DCL1-SE-HYL1 complex: Recombinant production in Sf9 insect cells provides a scalable system for reconstitution and purification of DCL1 together with HYL1 and SE. In parallel, transient expression in Nicotiana benthamiana enables isolation of the complex from a plant cellular environment, preserving plant-specific assembly states and post-translational modifications. Both systems reproducibly yield all three components and provide the foundation for purification while remaining functional to process pri-miRNAs. To further explore how dicing-complex activity is regulated, we will use naturally occurring DCL1 proteoforms as structural and functional perturbations. Six representative proteoforms were selected based on their allele frequencies and the distribution of amino acid substitutions across functionally important DCL1 domains. Together, our work establishes the basis for determining the structure of the complete DCL1–HYL1–SE–pri-miRNA complex by cryo-EM. Resolving this assembly can also reveal how natural genetic variation influences dicing complex function.

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Poster 21

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Small meets smaller: unique aspects of small RNA biology in duckweeds, the smallest angioperms

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Duckweeds (Lemnaceae) are the smallest, fastest-growing and structurally simplest flowering plants: free-floating aquatic monocots, often rootless, that propagate almost exclusively by clonal budding. The family spans a wide gradient of genome size and transposable-element (TE) content, from the TE-poor Spirodela polyrhiza (~138 Mb, ~20% TEs) to the TE-rich Wolffia (~850 Mb, ~75% TEs), making it a powerful comparative system to ask how a simplified body plan, a clonal lifestyle and contrasting genome organization shape plant RNA silencing.Duckweeds run a streamlined silencing machinery with uneven losses. The RdDM and transcriptional-silencing arm is simplified (loss of CMT2, CLASSY 1/2 and SHH1), while the antiviral/post-transcriptional arm shows striking losses of DICER-LIKE 2 (DCL2), ARGONAUTE 2 (AGO2) and RNASE THREE-LIKE 1 (RTL1). Despite lacking DCL2, duckweeds still accumulate abundant 22-nt siRNAs in Wolffia almost exclusively and siRNAs of all size classes are frequently produced from the same loci, indicating a shared pool of loci processed differently rather than distinct pathways acting on separate targets.The clearest difference lies in PTGS. In Arabidopsis, 22-nt siRNAs trigger transitivity, the RDR6/SGS3-dependent amplification of secondary siRNAs. In duckweeds this appears strongly restricted: transgenes stay expressed for weeks with no sense-PTGS and no transgene-derived siRNAs, and endogenous 22-nt siRNAs do not trigger canonical transitivity. Yet the machinery is not absent, duckweeds produce TAS3-derived tasiRNAs, showing the RDR6/SGS3/DCL4 route is functional. This suggests an apparent, possibly developmentally regulated, restriction of amplification rather than its loss. We will present our advances on silencing and small RNA pathways in duckweeds, and in particular this apparent, or regulated, absence of transitivity, which makes these minimal, clonal plants both a system to define the essential core of plant PTGS and an attractive chassis for silencing-free transgene expression.

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Poster 22

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Enhancing Spray-Induced Gene Silencing (SIGS) in Solanaceae by modulating host microRNAs

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Exogenous application of double-stranded RNA (dsRNA) through Spray-Induced Gene Silencing (SIGS) is a promising strategy for sequence-specific crop protection. Most SIGS approaches focus on silencing essential genes in pathogens or pests. Yet, endogenous host regulators, including microRNAs (miRNAs), are key determinants of plant immunity and may provide additional opportunities to improve protection. Here, we explored a general approach in which sequence-specific dsRNA is used not only to target pathogen essential genes, but also to modulate plant's miRNAs, thereby enhancing defense. When co-applied with the pathogen-targeting dsRNA, this dual-action approach significantly enhanced plant protection compared to standard SIGS. Our results support a framework in which host silencing modulation can increase the robustness of SIGS for crop protection.

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Poster 23

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Fine-tuning root architecture for drought resilience: a microRNA regulating cluster root development in white lupin

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Phosphorus deficiency and water limitation are among the major environmental constraints affecting crop productivity worldwide. White lupin (Lupinus albus) has evolved specialized cluster roots (CRs), dense clusters of short lateral roots that enhance soil exploration and nutrient acquisition under adverse conditions. Because root system architecture is central to plant adaptation to environmental stresses, understanding the regulatory mechanisms controlling CR development may provide novel opportunities to improve crop resilience.To identify regulatory factors involved in CR formation, we previously generated a comprehensive transcriptomic dataset covering distinct stages of cluster root development. Comparative analyses revealed several conserved and species-specific microRNAs displaying dynamic expression patterns during CR development. Among these candidates, we identified a microRNA whose expression profile and predicted targets suggested a potential role in both root developmental programs and drought responses. Functional modulation of this microRNA altered root architecture, including cluster root development, supporting its involvement in the regulation of specialized root traits in white lupin. In addition, expression analyses under water deficit conditions indicate that this regulatory pathway is responsive to drought stress, suggesting a broader role in environmental adaptation. Ongoing work aims to characterize the underlying molecular mechanisms and evaluate how manipulation of this pathway may contribute to enhanced stress resilience.These findings identify a microRNA-mediated regulatory module linking cluster root development and drought adaptation in White lupin and highlight the potential of RNA-based regulatory networks as targets for the improvement of climate-resilient crops.

Poster 24

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CRISPR-mediated targeted gene replacement at the *Arabidopsis thaliana* MIR390a locus enables artificial microRNA production and effective gene silencing

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Plant microRNAs (miRNAs) regulate gene expression through sequence-specific RNA degradation. Artificial miRNAs (amiRNAs) are engineered small RNAs designed to selectively silence target transcripts, serving as powerful RNA interference (RNAi) tools (Cisneros et al. 2021). Typically, amiRNAs are expressed from transgenic constructs driven by strong constitutive promoters, which can lead to pleiotropic effects, including cytotoxicity and phenotypic variability. Although CRISPR/Cas technology has been widely used to disrupt endogenous MIRNA loci for loss-of-function studies (Bravo-Vázquez et al. 2024), these loci have not yet been engineered to express amiRNAs. Here, we edited the Arabidopsis thaliana MIR390a locus using CRISPR/Cas9-mediated targeted gene replacement. This was achieved using an all-in-one construct containing a YAO-promoter-driven Cas9 cassette (Yan et al. 2015), a single guide RNA targeting the MIR390a locus, and a donor DNA template encoding the corresponding amiRNA/amiRNA* sequences. The native miR390a/miR390a* duplex was replaced with amiR-CH42/amiR-CH42* or amiR-FT/amiR-FT*, targeting CHLORINA 42 (AtCH42) and FLOWERING LOCUS T (AtFT), respectively. Edited amiR-CH42 plants exhibited strong bleaching, while amiR-FT plants displayed significantly delayed flowering. Molecular analyses confirmed high amiRNA accumulation, strong downregulation of target mRNAs and precise editing of the MIR390a locus. In conclusion, our results demonstrate that endogenous MIRNA loci can be precisely reprogrammed to express amiRNAs for selective gene silencing, providing a novel RNAi strategy for functional genomics and crop biotechnology.

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Poster 25

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Transcription elongation factor SPT6L recruits ARGONAUTE to guide mRNA cytosine methylation preventing premature termination of Polymerase II

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ARGONAUTE (AGO) proteins are key effectors of small RNA (sRNA) pathways and are well known for their ability to induce post-transcriptional gene silencing or to direct DNA methylation. Some proteins that interact with AGOs possess a specific AGO-binding domain called the AGO-hook. One of the largest predicted AGO-hooks in *Arabidopsis thaliana* is found in SPT6L, an essential transcription elongation factor and histone chaperone of Polymerase II (Pol II). This implies that AGO might bind to the Pol II complex as a part of yet undescribed sRNA pathway. In this work, we investigated the functions of AGO and its associated sRNAs within the Pol II complex. We demonstrate that SPT6L interacts with AGO4 via its AGO-hook domain in the Pol II complex. Direct RNA sequencing in a mutant lacking the AGO-hook (*spt6l*ΔAh) revealed a decrease in mRNA cytosine methylation (m^5C) which was even stronger in the ago4 mutant. This effect of sRNAs on m^5C in mRNA was further validated in tobacco BY-2 cells, where artificial targeting of sRNAs to the GFP transcripts resulted in increased m^5C levels. Interestingly, Pol II stalling was detected at the m^5C sites, and this stalling was more pronounced in the spt6lΔAh mutant at hypomethylated m^5C sites. Furthermore, in both spt6lΔAh and *ago4* mutants, increased premature termination was observed and affected a similar subset of genes as those prematurely terminated in the *trm4b* mutant (a major m^5C methyltransferase in *A. thaliana*). Taken together, we propose a model in which AGO4 co-transcriptionally guides m^5C deposition onto nascent Pol II transcripts. Disruption of this pathway causes Pol II stalling and premature termination, revealing a link between sRNA-mediated RNA methylation and transcription elongation fidelity. These results expand the myriad known functions of sRNAs in eukaryotes to include the control of RNA methylation.

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S2 Posters - RNA Modifications and Structure

Poster 26

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The YTHDF proteins ECT2/3/4, ECT5 and ECT7 are the main m^{6}A effectors in post-embryonic plant development

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N6-methyladenosine (m^{6}A) in mRNA is indispensable for plant development. The best understood mechanism of m^{6}A-dependent gene control involves the association of m^{6}A-containing mRNA with cytoplasmic RNA-binding proteins specialized for m^{6}A-recognition via a YT521-B homology (YTH) domain, the so-called YTHDF proteins. The flowering plant Arabidopsis thaliana encodes eleven YTHDF proteins, named EVOLUTIONARILY CONSERVED C-TERMINAL REGION (ECT)1-11. Simultaneous knockout of *ECT2, ECT3* and *ECT4* causes developmental defects reminiscent of those in plants defective in m^{6}A deposition[1], but weaker with respect to adult organ size, raising the question of whether additional YTHDF proteins mediate m^{6}A-dependent regulation of plant development.
We found that ECT2 can associate with ECT5 and ECT7 in addition to ECT3 in vivo, suggesting cooperative regulation of common targets. Indeed, additional mutation of *ECT5* or *ECT7* exacerbates developmental defects of *ect2 ect3* or *ect2 ect3 ect4* mutants. In particular, ECT5 is important for definition of stem height, and ECT7 for leaf blade expansion and overall rosette size, explaining much of the difference between *ect2 ect3 ect4* mutants and mutants defective in m^{6}A deposition. Our results establish that the cooperative action of cytoplasmic YTHDF proteins mediates m^{6}A-controlled post-embryonic development and provide new insight into the mechanistic basis for plant YTHDF action.

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Poster 27

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Small RNA modifications - mechanism and role

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RNA modifications are a crucial component of the multi-layered system that regulates the expression of genetic information. Modified nucleotides, which are detected in all classes of RNA, influence the structure, processing, transport, and stability of RNA molecules. We are focused on microRNAs, approximately 21 nt long molecules, deriving from long, hairpin precursors (pri-miRNA). Highly abundant RNA modifications like N6-methyladenosine (m^6A) and pseudouridine (Ψ) are important to maintain the proper structure of pri-miRNA, which has a direct impact on efficient production of mature miRNAs. Moreover, pseudouridine plays an important role in the transport of small RNAs within Arabidopsis pollen grains. Our data indicate that epitranscriptomic marks could serve as signals to load small RNAs into complexes with ARGONAUTE proteins or to promote small RNA decay. We are working to describe the small RNA epitranscriptome and how it determines the function and fate of small RNAs.

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Poster 28

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Benchmarking Nanopore basecallers for m⁶A detection in plant mRNA: towards context-aware epitranscriptomic profiling

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N6-methyladenosine (m⁶A) is the most abundant modification of eukaryotic mRNA and plays central roles in plant development, stress adaptation, and antiviral immunity. Oxford Nanopore direct RNA sequencing (DRS) offers a PCR-free, single-molecule route to detect m⁶A directly from native RNA, and recent basecallers increasingly incorporate modification-aware models to call this mark alongside sequence. However, these models have been developed and validated predominantly on mammalian datasets, where m⁶A deposition is strongly associated with the DRACH consensus motif. Whether such models generalize to plant transcriptomes, where m⁶A regulation, sequence context, and RNA metabolism differ substantially from mammalian systems, remains largely untested.Here, we present a systematic benchmarking framework to evaluate the performance of current Nanopore RNA004 m⁶A basecallers using high-depth *Arabidopsis thaliana* direct RNA sequencing datasets generated from wild-type and vir mutant plants, in which m⁶A deposition is strongly depleted. Incorporating available orthogonal high-quality single-nucleotide resolution m⁶A reference maps, we assess how well existing tools recover known methylation sites and their quantitative stoichiometries across diverse sequence contexts, expression levels and transcript regions. This benchmarking effort aims to clarify the extent to which mammalian-trained m⁶A models are transferable to plant biology, and to identify specific limitations and guide the development of improved, plant-adapted epitranscriptome profiling.

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Poster 29

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The role of pseudouridine in small RNA biogenesis in plants

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One layer of post-transcriptional gene expression regulation is mediated by RNA modifications, such as methylation and pseudouridylation. One of the most conserved and abundant chemical RNA modifications is the isomerization of uridine to pseudouridine (Ψ), catalyzed by a group of pseudouridine synthases (PUS) together with dyskerin (NAP57 in Arabidopsis thaliana). Pseudouridylation is thought to influence mRNA stability, structure, decay, and translational efficiency.In our study, we employ cutting-edge nanopore direct RNA sequencing in combination with established molecular biology techniques to provide a comprehensive view of changes in the epitranscriptome and translatome of an Arabidopsis NAP57 mutant. Particular attention is given to small RNA biogenesis, which may be altered as a consequence of impaired pseudouridylation. Our data provide novel insights into the role of RNA modifications in the regulation of small RNAs in plants.

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Poster 30

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VIR-dependent m^{6}A enables timely photomorphogenic transition by tuning translational output during early de-etiolation

Yen-Chiun Chen, Shu-Hsing Wu

N6-methyladenosine (m^{6}A) is a widespread mRNA modification that regulates multiple aspects of RNA fate in plants, yet its role during early seedling developmental transitions remains incompletely understood. Arabidopsis seedlings undergo a rapid transition from skotomorphogenesis to photomorphogenesis upon light exposure, requiring coordinated regulation of gene expression at various levels of the central dogma. Here, we investigated whether and how m^{6}A-mediated modification of mRNAs contributes to the transition from skotomorphogenic to photomorphogenic development during early de-etiolation. We found that a weak *vir-2* allele expressing only truncated transcripts of VIRILIZER (VIR), a component of the Arabidopsis m^{6}A writer complex, led to delayed cotyledon opening and onset of photosynthetic activation after white-light exposure. Nanopore direct RNA sequencing revealed approximately 30% reduction of m^{6}A methylation in *vir-2* under both etiolated and de-etiolating seedlings. Comparison with published VIR-dependent m^{6}A datasets further indicated that VIR-regulated m^{6}A landscapes differ among young, etiolated, and de-etiolating seedlings, supporting developmental context-dependent m^{6}A regulation. During de-etiolation, light-induced m^{6}A accumulation was strongly associated with photosynthesis-related transcripts and was largely dependent on VIR. Despite widespread VIR-dependent m^{6}A loss, steady-state mRNA abundance was only modestly affected under conditions examined. Instead, *vir-2* showed attenuated light-induced translational enhancement. Representative light-responsive transcripts, including CHLH, CHLI1, PSBS, and BIN2, exhibited reduced VIR-dependent m^{6}A and lower polysome association in *vir-2* after light treatment. These candidates were also associated with altered 3’ end usage, suggesting a potential link between VIR-dependent m^{6}A modification, 3’ UTR architecture, and translational engagement. Together, our findings support a model in which VIR-dependent m^{6}A acts as a post-transcriptional tuning signal that promotes timely photomorphogenic development by coordinating the translation of proteins involved in photomorphogenic development during early seedling establishment.

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Poster 31

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Pseudouridine synthases in Arabidopsis – The role in small RNA biogenesis

Emily Daniela Gallardo Andrade, Marta Zimna, Katarzyna Tutak, Mateusz Bajczyk, Jakub Dolata

RNA modifications have emerged as an important layer of post-transcriptional regulation, influencing RNA structure, stability, processing, transport, and interactions. In plants, epitranscriptomic marks such as pseudouridine (Ψ) and N6-methyladenosine (m^6A) regulate sRNA pathways, including biogenesis, and intracellular RNA movement. Although RNA modifications are increasingly studied, the machinery underlying their deposition remains poorly understood. Arabidopsis contains approximately 20 predicted PUS enzymes, suggesting potential functional redundancy. Among the best characterized are SVR1, required for plastid rRNAs pseudouridilation, and FCS1, which catalyzes this modification on mitochondrial rRNA. Additionally, the Arabidopsis homolog of human dyskerin, AtNAP57, is the catalytic component for the snoRNP complex implicated in rRNA modification, ribosome biogenesis and part of miRNAs regulation. Localization patterns reveal whether enzymes act in the nucleus, where pri-miRNA processing occurs, or in compartments involved in RNA maturation, transport, storage, or degradation. Moreover, their intracellular distribution may identify the context in which RNA modifications are established and suggest interactions with components of the sRNA biogenesis machinery. This project focuses on the subcellular localization of candidate RNA-modifying enzymes in Arabidopsis thaliana. The subcellular localization of RNA-modifying enzymes can reveal how RNA modifications occur, which molecules are involved in sRNA metabolism. To address this question, fluorescently tagged versions of candidate enzymes will be used. This approach will characterize their localization patterns and identify enzymes enriched in nuclear or subnuclear domains, linked with sRNA processing. Since RNA modifications are thought to be coordinated in space and time, localization data will help interpret the role of these enzymes in RNA regulation and fate. Our study provides a basis for understanding the positioning of RNA-modifying enzymes and their potential function in sRNA pathways. Therefore, the study of subcellular localization is a strategy to infer enzyme participation in the broader regulatory network controlling sRNA biogenesis and the role of RNA modifications in plants.

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Poster 32

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Dissecting the role of RNA m^{6}A modification machinery in plant growth and immunity

Anjil Kumar Srivastava

N6-methyladenosine (m^{6}A) is one of the most abundant and dynamic modifications of mRNA, playing critical roles in RNA metabolism and gene expression. In plants, mRNA m^{6}A modification is essential for the viability of Arabidopsis thaliana; however, the molecular consequences of m^{6}A depletion remain poorly understood. In this study, we investigate the impact of disrupting the Arabidopsis mRNA m^{6}A writer complex. Our findings demonstrate that depletion of m^{6}A significantly affects plant growth and modulates immune responses, highlighting the essential role of m^{6}A-mediated RNA regulation in plant development and physiology. We will discuss new insights into the biological functions of m^{6}A and present evidence for a previously unrecognized connection between mRNA methylation and immune signaling pathways in plants. These findings advance our understanding of the molecular mechanisms underlying m^{6}A function and provide a framework for interpreting the diverse developmental and physiological phenotypes associated with m^{6}A deficiency.

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Poster 33

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Germline small RNA modification in epigenetic inheritance in *Arabidopsis thaliana*

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In eukaryotes, epigenetic reprogramming takes place during gametogenesis to restore pluripotency and reset developmental programs. Plants are an exception, as some epigenetic modifications can be inherited despite reprogramming. Although epigenetically activated small interfering RNAs (easiRNA) are known to mediate the deposition of epigenetic marks at imprinted genes, their inheritance through gametogenesis and later fertilization remains poorly understood. Recently, easiRNAs with pseudouridine modifications were shown to be required for establishing hybridization barriers known as the triploid block in Arabidopsis thaliana, and easiRNA pseudouridylation was suggested to be key for their inheritance. We propose a molecular and computational approach to investigate pseudouridylated-easiRNA inheritance and their role in the establishment of the triploid block. To determine the function of pseudouridine for the inheritance of easiRNAs, we aim to develop a system for targeted easiRNA pseudouridylation in pollen combined with small RNA sequencing of the endosperm. Using a CRISPR-guided RNA editing approach, we intend to artificially induce pseudouridylation of selected microRNA, and to investigate whether this artificial pseudouridylation affects their mobility and, subsequently, their inheritance in the male germline. Additionally, we aim to explore whether endogenous pseudouridylated small RNA and their associated targets in *Arabidopsis thaliana* establish reproductive barriers in a similar way to the Teosinte Pollen Drive system described in maize. By investigating pseudouridylated easiRNA targets, we hope to also decipher their role in genomic imprinting. Understanding inheritance of easiRNAs in the establishment of hybridization barriers will provide a better understanding of plant evolution and domestication, and contribute to the development of new plant breeding tools to overcome hybrid incompatibilities in modern crops.

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Poster 35

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RNA quality control (RQC) regulates geminivirus accumulation by modulating antiviral RNA interference (RNAi)

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RNA quality control (RQC) pathways maintain transcriptome integrity by resolving stalled ribosomes and promoting the degradation of aberrant transcripts. However, how RQC interacts with other post-transcriptional RNA surveillance pathways, including RNA interference (RNAi), during viral infection remains poorly understood.

Here, we investigate the role of the ribosome-associated RQC factor Pelota and componentes of the RNA decay pathway in regulating geminivirus infection. The naturally occurring *ty-5* allele of *Pelota* confers broad-spectrum recessive resistance to tomato yellow leaf curl virus (TYLCV) and other geminiviruses (1), which correlates with increased RNAi activity in *ty-5* plants. We hypothesized that impaired Pelota function alters the fate of aberrant transcripts, both endogenous (2) and viral (this work), thereby affecting their availability as substrates for RNAi and ultimately modulating viral accumulation.

To investigate the interplay between RQC, RNA decay and RNAi, we combined genetic approaches using virus-induced gene silencing and mutant plants impaired in core components of these pathways. Our results suggest functional connections between ribosome-associated RNA quality control and antiviral RNAi, suggesting that the cellular handling of aberrant transcripts can influence antiviral responses and, consequently, geminivirus accumulation.

Keywords: RNA quality control, RNA decay, RNAi, Geminivirus, Pelota, plant defence

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Poster 36

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m^{6}A controls dormancy of plant branch primordia

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Axillary bud dormancy is a crucial adaptive trait that enables plants to survive unfavourable conditions, while timely activation is essential for rapid growth, reproductive success, and crop yield. Although the hormonal and transcriptional regulation of this process has been extensively characterized, post-transcriptional mechanisms are largely unexplored.
We are studying the role that N6-methyladenosine (m^{6}A), an internal chemical modification of messenger RNAs, plays in axillary bud development. m^{6}A may affect transcript stability and translation rate among other properties, and this regulatory pathway is known to control different aspects of plant development[1]. Mutants with severe m^{6}A deficiency are bushy plants with reduced apical dominance and over-proliferation of rosette branches[2], suggesting that axillary bud activation is directly controlled by this pathway. Using Arabidopsis thaliana and a combination of genetic, biochemical and transcriptomic approaches, we are analysing how m^{6}A-methylation of growth/dormancy-related transcripts controls this important developmental transition.
m^{6}A-mediated regulation of plant development is largely executed via the so-called m^{6}A readers3, RNA-binding proteins of increased binding affinity towards m^{6}A-mRNA. The best characterised plant m^{6}A readers belong to the YTHDF family, with 11 members called EVOLUTIONARILY CONSERVED C-TERMINAL REGIONS (ECTs) in Arabidopsis[1]. We have characterised aberrant branching in selected groups of ECT mutants, and identified a specific ECT that is directly regulated by BRANCHED1, a transcription factor that acts as master regulator of bud dormancy[4]. Furthermore, we seek to identify key m^{6}A/ECT-regulated targets involved in axillary bud growth control, and unveil the molecular mechanisms by which m^{6}A/ECTs contribute to growth during dormancy release by transcriptome reprogramming.

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Poster 37

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Can epitranscriptomic modifications regulate alternative splicing to induce and maintain plant heat stress memory?

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Acquired thermotolerance enables organisms (including plants) to better withstand acute heat stress when preceded by priming treatment. In plants, alternative splicing (AS) contributes to heat stress memory, yet mechanisms controlling this regulation remain poorly understood. m^6A epitranscriptomic modifications regulate multiple aspects of RNA metabolism, including splicing. m^6A AS regulation has been proposed in animal system, but remain mainly unexplored in plants. Heat stress induces major changes in m^6A patterns in plants but its role in thermomemory has never been investigated. Epitranscriptomic regulation of alternative splicing in response to internal or external cues could represent a new layer of regulation allowing the plant to quickly acclimate its transcriptome to face the stress. Using RNA-seq analyses in tomato, we found that priming induces a strong type II AS splicing memory. Interestingly, priming also significantly alters the stead-levels of m^6A writers and readers : the overexpression of key players the m^6A writer machinery suggests that priming will reprogramed the m^6A mRNA epitranscriptome of tomato. The integration of those results led us to formulate the hypothesis of a potential connection between m^6A mRNA modifications and AS-mediated heat memory in tomato. To test this hypothesis, we are currently performing Oxford Nanopore Technologies direct RNA sequencing (DRS ONT) (on the exact same RNA samples) to simultaneously characterize new transcript isoforms and native RNA modifications, providing insights into the role of RNA modifications in alternative splicing regulation during heat stress memory in tomato. Using DRS ONT will allow per-read co-mapping of m^6A and splice events – on every molecules – indicating wether a read carrying m^6A is more of less likely to show a specific splicing event, leading to the central question: on the same transcript, does the presence of an m^6A mark at a specific position can predict the splicing outcome of this transcript ?

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Poster 38

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Mobile mRNA motifs for short and long-distance transport in different species

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mRNA mobility is an important signalling system for plants. But what makes an mRNA mobile? There are specific motifs present in mobile mRNAs that lead to their mobility. It was shown that a tRNA-like structure resembling tRNAMet (TLS1) when fused to the 3'UTR can mediate long distance transport of non-mobile mRNAs from roots to shoot including flowers (Zhang et al. 2016). Also, a TLS1 derivate, namely tRNAΔD/T (TLS2), with two loops deleted was functional as a mobility motif. These two motifs, fused to Cas9 mRNA, led also to gene-editing of the germline and, consequently to gene-edited transgene-free offspring in grafted plants (Yang et al. 2023). Additionally, the short variable region (SVR) of HSC70.1 mediated mRNA mobility when fused to YFP or GFP. However, mobility of these fusion constructs stopped in juvenile m^5C methylation deficient *dmnt2 nsun2b* double mutant suggesting that RNA methylation is also a key factor required for mRNA transport (Yang et al. 2019). To address whether the TLS structure is the main determinant of its mobility motif, we created a synthetic TLS with the same predicted structure as tRNAMet, but the predicted m^5C methylation sites were replaced (TLS1m) and the methylation consensus sequence (m5cons), which has no predicted mRNA folding, but contains five predicted methylation motifs. All these novel motifs were capable to mediate Cas9 mRNA transport in hypocotyl grafted Arabidopsis over long distance and in grafted callus cultures over short distances. Currently we are analysing the methylation patterns of these variant transport motifs and test their capacity to mediate mRNA transport in Nicotiana, Solanaceae and Malus.

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Poster 39

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Coordinated Post-Transcriptional Regulation of the Rice Drought Response

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Gene expression during environmental stress is controlled by multiple post-transcriptional regulatory mechanisms, including RNA modification, poly(A) tail dynamics, translation, and RNA localization. However, how these regulatory layers are coordinated at the transcriptome level remains poorly understood. Here, we combined nanopore direct RNA sequencing with Illumina RNA-seq, TRAP-seq, and RNA granule-enriched RNA sequencing to simultaneously characterize transcript abundance, poly(A) tail length, N^6-methyladenosine (m^6A) modification, translational activity, codon optimality, and RNA granule association in rice exposed to drought stress and subsequent recovery.Transcriptome-wide integration revealed that these molecular features are not independently regulated but instead form a highly coordinated post-transcriptional landscape. Highly abundant transcripts were preferentially associated with efficient translation, high codon optimality, shorter poly(A) tails, reduced m^6A modification, and limited RNA granule enrichment, whereas low-abundance transcripts displayed the opposite characteristics. Although the overall organization of this regulatory landscape remained largely stable during drought, stress selectively altered the relationships among individual regulatory layers. Drought weakened the association between m^6A modification and RNA granule localization while strengthening the coupling between RNA granule enrichment and poly(A) tail shortening, indicating dynamic reconfiguration of post-transcriptional regulatory networks.Furthermore, drought-responsive transcripts occupied distinct regions within this multidimensional regulatory landscape, suggesting that stress-responsive gene expression is determined by coordinated combinations of post-transcriptional features rather than by individual regulatory mechanisms. Members of the PYL abscisic acid receptor family exemplified this coordinated regulation through concurrent changes in m^6A modification and RNA granule association, consistent with an evolutionarily conserved m^6A-dependent regulatory mechanism.Our study provides a systems-level framework for understanding how diverse post-transcriptional processes are integrated to shape transcript fate during drought stress and demonstrates the utility of nanopore direct RNA sequencing for comprehensive analyses of plant post-transcriptional gene regulation.

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Poster 40

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Copper-dependent RNA methylation in the roots of soybean seedlings

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Plants exposure to excess metals leads to various adverse effects, including altered mineral homeostasis, decreased photosynthetic efficiency, oxidative stress, and damage to genetic material. Altogether, these mechanisms of toxicity result in hampered growth and decreased yield, which are particularly critical for crop production and an adequate food supply [1-2].In order to withstand metal toxicity, plants need to adjust their gene expression and activate defense mechanisms. Although certain aspects of metal-dependent gene regulation in plants are relatively well studied, information on the impact of metals on the plant epitranscriptome remains scarce. Our current research aims to gain deeper insights into the effects of metals on RNA modifications.As part of this study, soybean seedlings were treated with copper (Cu) at two concentrations (15 and 30 mg/L) for short-term periods of 3, 24, and 48 hours. The levels of 5-methylcytosine (5mC) and N6-methyladenosine (m^6A) were assessed using specific ELISA kits. Regarding 5mC, only the shortest treatment time (3 hours) resulted in significant changes. A decrease in the 5mC level was observed in total RNA. In contrast, in poly(A)RNA, an accumulation of 5mC was noted in transcripts isolated from Cu-treated seedlings. In the case of m^6A, a Cu-dependent decrease in its level was observed in both total and poly(A)RNA.Further research will focus on identifying specific methylated transcripts using RNA sequencing techniques.

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Poster 41

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Over-expression of m^{6}A demethylase ALKBH10B confers drought tolerance by sustaining the expression of photosynthesis- and drought-related genes in Arabidopsis

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Epitranscriptomic RNA modifications have emerged as important regulatory layers of gene expression in plant development and stress adaptation. Among the around 170 known post-transcriptional mRNA modifications, N6-methyladenosine (m^{6}A) is the most abundant, dynamic, and reversible internal mRNA modification in eukaryotes. m^{6}A is installed by methyltransferases known as writers, removed by demethylases called erasers, and read by RNA-binding reader proteins. In recent years, the role of m^{6}A in various abiotic stress responses such as salinity, drought, light, heat and cold have been explored in great detail. At molecular level m^{6}A affects various aspects of RNA metabolism including stability, translation, splicing, degradation, nuclear to cytoplasm export and alternative polyadenylation. In our study, we performed expression profiling of the m^{6}A machinery in Arabidopsis thaliana under progressive drought stress and observed the demethylase ALKBH10B as the most strongly up-regulated component. Hormone-related expression analyses and promoter-driven luciferase assays suggest that ABA induces ALKBH10B transcription via ABRE and MYB cis-elements, whereas JA alleviates this activation. Moreover, ALKBH10B overexpression plants (OX-1) accumulated WT levels of ABA under drought stress but displayed markedly reduced JA content, resulting in enhanced stomatal closure, improved water-use efficiency and thus an enhanced drought tolerance. Transcriptome analysis revealed that drought stress induces fewer transcriptional changes in OX-1 plants compared to WT and the drought-sensitive *alkbh10b-1* mutant. Differential expression and GO enrichment analyses highlight that photosynthesis-related processes were most affected by ALKBH10B activity, correlating with preserved chlorophyll content and PSII efficiency in OX-1. m^{6}A-IP-qPCR showed that ALKBH10B directly demethylates specific photosynthesis-related and drought-responsive genes, thus promoting their sustained expression during stress.Together, our results reveal that ALKBH10B enhances drought tolerance by coordinating m^{6}A-dependent post-transcriptional regulation to maintain photosynthetic capacity and mitochondrial ATP homeostasis, as well as fine-tuning ABA-JA crosstalk to optimize drought response.

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Dynamic remodeling of stress granules composition during prolonged hypoxia

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Stress granules (SGs) are dynamic cytoplasmic ribonucleoprotein complexes that form in response to stress and sequester non-translating mRNAs. Although SGs are considered dynamic structures, it remains unclear how their transcript composition changes during prolonged stress and what happens to transcripts released from SGs. In roots of Lupinus angustifolius, prolonged hypoxia induces SG remodeling through granule fusion, resulting in fewer but larger SGs that accumulate approximately 20% of the cytoplasmic poly(A) RNA pool after 15h of stress (Kubiak et al. 2025). Here, we investigated changes in SG transcript composition during prolonged hypoxia and their relationship with functional remodeling and P-body association.SGs were isolated by immunoprecipitation after 6 and 15h of hypoxia. SG-associated transcripts were preferentially longer than the average mRNA, but their mean length decreased with prolonged hypoxia, indicating dynamic changes in transcript recruitment. SG composition was highly dynamic, with more than 1,000 transcripts enriched after 6h compared with approximately 450 after 15h. Many transcripts that accumulated in SGs during early hypoxia were subsequently depleted, whereas only a relatively small subset remained consistently associated with SGs during prolonged stress. These findings indicate that SGs are not static mRNA storage compartments but undergo continuous transcript exchange during stress.GO enrichment analysis revealed a shift in the functional profile of SG-associated transcripts, from RNA metabolism, protein folding, and cellular organization at 6h toward energy metabolism, ATP synthesis, and electron transport at 15h. Notably, the P-body proteins DCP2 and DCP5 progressively accumulated within SGs, suggesting increasing functional coupling between SGs and P-bodies. Together, our results demonstrate extensive SG remodeling during prolonged hypoxia, with transcripts released from SGs potentially re-entering the translational pool or being targeted for P-body-associated degradation.

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D. J. Kubiak et al. Impact of m^{6}A modification and transcript quantity on mRNA composition in plant stress granules under hypoxia. Journal of Experimental Botany, Volume 76, Issue 8, 27 May 2025, Pages 2338–2355

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Poster 43

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Post-transcriptional regulation via calcium-modulated ribonucleotide structures

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Calcium ion (Ca^{2+}) is a central second messenger in plant development and immunity. The latter is characterized by rapid and transient cytosolic Ca^{2+} influx upon pathogen perception1. While Ca^{2+} is known to function through binding to protein sensors, recent studies in mammals revealed that Ca^{2+} influx can also trigger RNA G-quadruplex assembly, evidencing that cytosolic increases in Ca^{2+} concentration can alter functional RNA structure2. However, whether cytosolic Ca^{2+} influx can directly affect RNA structure in vivo and contributes to post-transcriptional regulation in plants remain unknown. Here, we demonstrated that Ca^{2+} plays a critical role in post-transcriptional regulation during pattern-triggered immunity (PTI) in plants. Using in vivo and in vitro RNA structure probing (DMS-MaP), we found that Ca^{2+} directly interacts with RNA and induces widespread RNA structural changes. By integrating RNA structure profiling, identification of conserved RNA structure, RNA decay analysis, and Ribo-seq data, we found that Ca^{2+}-dependent RNA structural remodeling is associated with altered RNA stability and translational efficiency in a subset of stress-responsive genes. Phenotyping analyses further revealed that mutants of these genes show impaired PTI-associated phenotypes, including flg22-induced root growth inhibition, bacterial resistance, and ROS burst response. Together, our findings uncover a novel regulatory layer in Ca^{2+} signaling in plant immunity, expanding the role of Ca^{2+} from protein-mediated signal transduction to include RNA-mediated post-transcriptional regulation.

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Poster 44

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Upstream cues regulating AS of auxin-dependent processes

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Alternative splicing (AS) affects most genes with the potential to generate physiologically distinct protein isoforms. Yet, the direct functional role of AS in plant development remains largely unknown. The gradients of the phytohormone auxin implement nearly all facets of plant growth and development. Their formation is highly dependent on the activity of the PIN-FORMED (PIN) auxin efflux carriers that facilitate polar auxin transport from cell to cell. Our group has revealed the AS isoforms of PIN7, PIN7a and PIN7b, in Arabidopsis thaliana coordinatively operate together (Kashkan et al., 2021). PIN7a is capable of auxin transport at accelerated rates, while the inactive PIN7b interacts with PIN7a and hampers its auxin transport potential. In a follow-up project, we are seeking the upstream mechanisms and pathways controlling this AS event. We analyzed the published transcriptomic datasets asking whether there are splicing-related factors that affect the expression of the individual PIN7 variants. Interestingly, based on ~70 transcriptomes, we identified two mutant groups affecting PIN7 AS: (1) Those involved in m^6A methylation, U6 snRNA m^6A writer FIO1 and m^6A reader FLK. Although both proteins participate in m^6A-mediated regulation, the functional relationship between FIO1 and FLK remains unknown, even in animal systems. (2) Those of U1 snRNP spliceosomal components PRP39a and RBP45D, which have previously been shown to interact both physically and genetically. We independently confirmed that *fio1, flk-1, prp39a,* and *rbp45d-3* mutants consistently exhibit elevated PIN7a levels over PIN7b. Furthermore, they display phenotypes that are consistent with a misregulated PIN7a/b ratio in these lines as well. In addition, we tested the effect of different environmental cues on PIN7 AS and found that, among the conditions tested, only cold treatment alters the PIN7a/b ratio. .

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Poster 45

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Identification of mRNA targets of m^{5}C methyltransferases and their role in mRNA mobility

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In plants, subsets of mRNAs are transported to distant tissues, contributing to the intercellular communication and coordination of growth and development. Although, more than 2,000 transcripts have been identified as graft-mobile in *Arabidopsis thaliana*, the mechanisms regulating RNA mobility remain largely unknown. We have shown that specific tRNA-like structures (TLS) trigger transport of otherwise non-mobile transcripts such as GUS or Cas9 and of endogenous CHOLINE KINASE 1 transcripts. As tRNA structures are known to be targeted by many ribonucleotide modification enzymes - for example, the RNA methyltransferases DNMT2 and NSUN2B, the role of m^{5}C methylation in the transport of RNA molecules was analysed. Significant overlap of up to 70% between m^{5}C methylation and mobile mRNAs was established using methylated RNA immunoprecipitation (MeRIP) and Nanopore sequencing. Further analysis identified two additional potential m5C methylation consensus motifs on mRNAs. The two methyltransferases, DNMT2 and NSUN2B, are known to target both tRNAs and mRNAs. In the double knock-out (dko) mutant of *A. thaliana*, a mobile GUS-TLS fusion and endogenous HSC70.1 and TCTP1 transcripts lose their mobility. As both enzymes are expressed in different tissues and function in different cell compartments, it is assumed that they methylate different mRNA transcripts. Cross-linking immunoprecipitation (CLIP), followed by sequencing, will be performed to further identify and characterise their targets. This will reveal similarities between transcripts and potential m^{5}C recognition sites, as well as features related to mobility. Nanopore sequencing focusing on m^{5}C modification of Col-0 WT and the DNMT2 and NSUN2B dko mutants could additionally strengthen the data obtained by CLIP.

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Poster 46

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Discovering alternative polyadenylation in plant transcriptomes from 3'end sequencing data

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Alternative polyadenylation (APA) expands transcript diversity but can also terminate transcription prematurely through the use of cryptic intronic polyadenylation sites. In metazoans, U1 snRNP suppresses such premature cleavage and polyadenylation (pCPA) through telescripting, a function distinct from its canonical role in pre-mRNA splicing. Recent work indicates that a related mechanism operates in Arabidopsis thaliana. However, how broadly telescripting is conserved across plants, particularly in species with substantially different gene architectures, remains unknown.To identify and quantify alternative and intronic polyadenylation events in diverse plant transcriptomes, we developed a flexible Nextflow workflow for the analysis of 3'end sequencing data. The workflow enables identification and quantification of terminal and intronic polyadenylation events alongside complementary transcript abundance analysis.Application of this workflow to the fern Ceratopteris richardii, containing genes with exceptionally large introns, presents a particularly informative system for investigating how full-length transcription is maintained across extensive intronic regions, which potentially contain numerous polyadenylation signals. Preliminary findings from C. richardii provide first insights into the relationship between intron architecture and pCPA, identifying candidate transcripts that may be subject to protection by a telescripting-like mechanism.Comparative analysis in Arabidopsis thaliana and crop species under stress will test whether these patterns are evolutionary conserved and environmentally regulated.

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Poster 47

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The epitranscriptomic mark m^{6}A influences stress granule formation and transcriptome composition under hypoxic stress in Arabidopsis thaliana

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Hypoxia is a stress condition characterised by severe oxygen shortage, which disrupts oxidative phosphorylation during flooding or waterlogging. To conserve ATP, Arabidopsis thaliana restricts energy-intensive processes such as transcription and translation, partly by storing non-translating mRNAs in cytoplasmic stress granules (SGs), biocondensates formed via liquid–liquid phase separation. The mechanisms governing selective transcript recruitment to SGs remain unclear, although preliminary data implicate the epitranscriptomic modification m^{6}A. This study therefore aimed to investigate the role of m^{6}A in SG dynamics and transcriptome composition. We first analysed SGs in pABI3::MTA-mta × Rpb47-GFP (~80% reduced m^{6}A; Rpb47 marks SGs) and pABI3::MTA-mta × ECT2-GFP (ECT2 is a m^{6}A reader present in SGs in WT) versus WT, suggesting that m^{6}A contributes to early SG formation and mRNA content. We then examined three m^{6}A demethylases: ALKBH10B, ALKBH9A, ALKBH6 and showed using knockout lines that increased m^{6}A significantly alters SG dynamics and morphology under hypoxia. Next we compare SG transcriptome in WT and pABI3::MTA-mta x Rpb47-GFP. In SG, 13,819 mRNAs were observed in WT, and 414 fewer in mta. The analysis showed that SGs are enriched in transcripts of the translational machinery (RNA-binding proteins, ribosomal components, translation) and glycolysis enzymes. However, when comparing the two genotypes, mta exhibits a depletion of stress pathways (including the response to hypoxia), while WT shows an enrichment of the translational machinery.Collectively, m^{6}A modulates several aspects of SG biology under hypoxia: morphology and dynamics (altered by both reduced [mta] and increased [*alkbh10b/9a/6*] m^{6}A), mRNA quantity (mta granules contained fewer poly(A) transcripts, implicating m^{6}A in early SG formation and RNA loading), and selective transcriptome composition. Together, these findings indicate that m^{6}A is a selective determinant of which transcripts are recruited to and retained within SGs, rather than merely a general regulator of their assembly.

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Poster 48

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The role of TTL3 in integrating brassinosteroid signaling, plant immunity, and stress response.

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TTL3 protein is a member of the TETRATRICOPEPTIDE THIOREDOXIN-LIKE (TTL) gene family, specific to land plants. It is involved in brassinosteroid (BR) signaling and directly interacts with several BR-related factors. We have recently shown that a deNADding enzyme DXO1, involved in RNA metabolism, interacts via its C-terminal domain with TTL3 in the yeast two-hybrid (Y2H) system. DXO1 directly or indirectly regulates several cellular processes, including ribosomal RNA processing and mRNA decay, stress responses and immunity to bacterial pathogens. In particular, plants lacking DXO1 are resistant to infection by Pseudomonas syringae pv. tomato (Pst) DC3000. In turn, mutations in TTL3 result in reduced plant growth in abiotic stresses, such as salinity and drought, but their role in biotic stress remains poorly understood. Since BRs are known to modulate plant response to pathogens, we investigated the susceptibility of the ttl3 mutant to Pst infection, as well as treatment with its effector flg22. Our results show that ttl3 mutant exhibit enhanced resistance to Pst, accompanied by marked changes in the expression of key pathogenesis-related markers. Furthermore, flg22 treatment increased production of reactive oxygen species (ROS) in ttl3 plants, indicating an altered immune response. Interestingly, heat stress in TTL3-GFP seedlings leads to the accumulation of stress granules, pointing to its role in RNA metabolism. Notably, cordycepin treatment revealed accelerated mRNA degradation in the ttl3 mutant compared to wild-type plants. Given the involvement of TTL3 in BR signaling, we also examined the expression of BR-responsive markers under stress conditions. Taken together, our findings suggest a functional link between BR signaling and plant immune responses to Pst, and point to a potential role of TTL3 in RNA metabolism, likely through interaction with DXO1.

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S3 Posters - mRNA Processing and Turnover

Poster 49

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Transcriptomic analysis of stress granules in *Arabidopsis thaliana* and *Caenorhabditis elegans*: identification of conserved and stress-specific components

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Nowadays, the world is facing the effects of climate changes and environmental pollution that represent a serious threat to plant and animal life and to the ecosystems. To cope with abiotic and biotic stress, cells activate conserved stress-related pathways, including the formation of Stress Granules (SGs), biomolecular condensates composed by RNAs, proteins and metabolites, which play a pivotal role in translational reprogramming throughout stress and post-stress recovery. Although the biology of SGs has been extensively investigated in mammalian systems due to its relevance to human diseases, interest in understanding SGs dynamics in model organisms widely used in climate change and environmental toxicity research, such as *Arabidopsis thaliana* and *Caenorhabditis elegans*, is rapidly growing. Several studies have characterized SGs formation, dynamics, and protein composition in these organisms; however, the transcriptomic composition of SGs remains largely unexplored, despite the central role of RNA in SGs assembly and function. To address this gap of knowledge, we aim to characterize the SGs transcriptome in both *A. thaliana* and *C. elegans* by combining biochemical purification of SGs with RNA-seq. Specifically, SGs will be isolated from: (i) A. thaliana Pro35S:GFP-Rbp47b seedlings, and (ii) pharyngeal muscle tissue of the *C. elegans* Pmyo-2:tagRFP-Pab-1 strain, following exposure to heat stress and arsenate (AsO43−), two environmental stressors closely linked to climate change and anthropogenic pollution. These lines bear very well-known SGs marker proteins, RBP47B and PAB1 respectively, fused to a fluorescent protein which allow the SGs visualization under confocal microscopy and their enrichment by Immunoprecipitation (IP). Updated results of this ongoing project will be presented.

Poster 50

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Impact of redox regulation on mRNP granule dynamics and mRNA fate in *Arabidopsis thaliana*

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Messenger ribonucleoprotein (mRNP) granules are dynamic biomolecular condensates formed through liquid–liquid phase separation (LLPS). These granules are thought to regulate mRNA fate by sequestering specific mRNAs and proteins. However, the molecular mechanisms governing mRNP granule formation and function remain poorly understood. Recently, post-translational modifications (PTMs) of mRNP granule components have emerged as a potential regulatory mechanism1. As mRNP granules are highly dynamic under stress conditions, we hypothesised that PTMs of key proteins could trigger granule formation. Under stress, reactive oxygen species (ROS) alter the redox state of cysteine thiol residues and can promote the formation of disulphide bonds between proteins, leading to their aggregation. Given that mRNP granules consist mainly of mRNAs and aggregated proteins, we investigated the role of cysteine modifications in their transient assembly dynamics. To address this question, we performed in vivo analyses in Arabidopsis seedlings using mRNP granule reporter lines under alkylating conditions that prevent cysteine modifications. We demonstrated that alkylating reagents inhibit both the formation and dissociation of mRNP granules in a dose-dependent manner during heat stress. Remarkably, even short pretreatments were efficient, as only a few minutes of exposure prior to stress completely prevented granule formation. This treatment therefore establishes a granule-free condition during stress. We took advantage of this condition to investigate the fate of mRNAs during stress using an orthogonal transcriptomic approach to monitor global changes in mRNA abundance, degradation dynamics, and translational engagement. Our results suggest that the absence of mRNP granules remodels mRNA fate both during and after stress. These findings highlight a key role for PTMs in coordinating mRNP granule dynamics and mRNA fate during stress.

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Poster 51

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Early response to warm temperature in Arabidopsis

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Temperature influences plant development and fitness. Warm temperature (24–30 °C) promote growth. and cooling-related morphology [1], whereas temperatures above 30 °C cause growth arrest and a. shortened life cycle [2,3]. How plants distinguish these ranges remains unclear. Because RNA structure. and stability are temperature-sensitive [4], we propose that warm conditions reshape mRNA stability. and secondary structure, selectively routing transcripts toward translation or decay/sequestration [5],. unlike the global translational stalling triggered by heat stress [6,7]. We show that Arabidopsis thaliana. exhibits rapid, selective mRNA-stability changes at 28 °C, arising within 10 minutes and preceding. transcriptional responses. Whereas heat stress destabilizes ~25% of transcripts [8], warm temperature. affects a smaller, targeted subset (13% destabilized; 8% stabilized). Destabilized mRNAs encode defence. functions, while stabilized ones relate to growth. This selectivity apears linked with conserved RNA. structural motifs across 29 Brassicaceae species, UTR GC-content differences, and temperature-. dependent RNA-binding protein interactions. The dcp5-1 mutant shows delayed warm-induced. hyponasty, indicating that the mRNA-decay machinery, particularly DCP5 [9], contributes to early warm-. temperature responses. Together, these findings link mRNA features, decay factors, and physiological. outcomes, revealing how plants fine-tune gene expression under warm conditions.

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Poster 52

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Processing Bodies as sites of ABA-induced post-transcriptional regulation

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Processing bodies (PBs) are condensates containing protein–RNA complexes that assemble via liquid–liquid phase separation (LLPS). In eukaryotes, PBs act as conserved hubs of post-transcriptional regulation and translational repression. Cellular mRNAs are selectively recruited to PBs, where they are sequestered, modified, or degraded. PBs form constitutively, but their assembly, composition, and function are highly dynamic and rapidly remodelled in response to diverse stimuli. Recent studies have highlighted the multifaceted roles of PBs in plant responses to abiotic stress and pathogen infection. The post-translational regulation of PB proteins represents a means to modulate PB function and composition. Core PB components include RNA-binding and RNA-modifying factors involved in mRNA deadenylation, decapping, and 5′–3′ decay, such as the exoribonuclease XRN4 and the decapping proteins DCP1, DCP2, DCP5, and VARICOSE (VCS), serving as markers of PB formation and function. Loss of these factors impacts PB formation and diminishes decapping activity, leading to severe developmental defects, postembryonic lethality and altered stress responses. Previous studies and our own data indicate that PBs contribute to the post-transcriptional regulation of responses to the stress hormone abscisic acid (ABA), which mediates plant adaptation to biotic and abiotic stress and induces extensive translatome reprogramming. We observed that genotypes affected in PB formation exhibit increased sensitivity to ABA and related abiotic stressors at different stages. Further, we found that the phytohormone negatively affects PB formation through its canonical signalling pathway and that key proteins of ABA signalling machinery directly interact with the decapping enhancer DCP1. We aim to elucidate how PBs and the decapping machinery shape ABA responses and to identify molecular mechanisms and regulatory switches that control PB function under stress conditions.

Poster 53

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NSRa Interacts with RNA Processing Machinery and Regulators of Light and Temperature Responses

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Nuclear speckle RNA-binding protein (NSRa) is regulated by lncRNAs and modulates alternative splicing during the auxin response. Analysis of the NSRa interactome revealed interactions with splicing factors, cleavage and polyadenylation machinery (such as CFT59), and m^6A-methylation writers (e.g. FIP37 and MTA).

Additionally, the interactome identified proteins related to photomorphogenesis and thermomorphogenesis, two processes influenced by auxin. Based on these findings, we investigated whether *nsra* exhibits a temperature-dependent phenotype. Furthermore, we performed Oxford Nanopore sequencing (cDNA and direct RNA) to analyze alternative splicing, polyadenylation, and m^6A modification. Finally, using published temperature dataset bioinformatic analysis, we selected target genes to evaluate their response under various light and temperature conditions.

Altogether, our findings reveal a pivotal role for NSRa in integrating co-transcriptional RNA regulation with environmental light and temperature signaling.

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ANR Ribores and ANR MASH

Poster 54

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Nuclear mRNA storage in seeds

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Seed maturation prepares both for extreme desiccation and the swift restart of metabolism after water uptake by the dry seed. A decrease in water content during late maturation gradually shuts down seed metabolic activities, including transcription. However, parallel blockage of mRNA turnover ensures the creation of a stored mRNA pool in dry seed that can be used for translation immediately upon imbibition. Several lines of evidence suggest that this pool of mRNAs is critical for successful germination and that its deterioration during seed storage contributes to decreased seed longevity. Initial biochemical analysis suggested that a significant fraction of the mRNAs in dry seeds is associated with ribosomes. Using microscopic techniques: oligo(dT) FISH and single-molecule FISH, we observed a gradual accumulation of mRNAs in the cell nucleus of maturing Arabidopsis thaliana seeds and their quick release during imbibition. Intriguingly, nuclear mRNA accumulation is stronger in the pab double mutant, which lacks two of the three Arabidopsis poly(A)-binding proteins. Phenotypic analysis of double pab mutant seeds revealed low dormancy and high germination kinetics. In agreement, transcriptomic analysis of seeds revealed altered gene expression, with upregulation of mRNAs typically observed early in germination. We hypothesise that the physiological changes in germination of double pab mutant seeds are linked to nuclear mRNA accumulation.

Poster 55

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RNA Fate Control by Nuclear Export and mRNA Decay Shapes Root Thermomorphogenesis

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Rising global temperatures increasingly threaten plant growth and crop productivity, necessitating a deeper understanding of how plants adapt to elevated ambient conditions. Thermomorphogenesis, the growth response to non-stressful warm temperatures, exhibits pronounced organ specificity: while hypocotyl elongation is progressively enhanced, primary root growth peaks at intermediate temperatures and declines under supra-optimal conditions. The molecular basis of this differential response, particularly in roots, remains poorly understood. While transcriptional regulation has been extensively characterized, how temperature reshapes gene expression through post-transcriptional control remains largely unexplored. Here, we investigate how RNA fate regulation contributes to temperature adaptation in Arabidopsis thaliana. We show that warm temperature (28°C) induces widespread, tissue-specific remodeling of mRNA stability, with roots exhibiting the most pronounced changes. These responses require an intact RNA decay machinery, indicating that mRNA turnover is integral to thermal acclimation. We identify the decapping factor DCP5 as a key regulator that restrains growth responses under normal conditions(21˚C), as its knockdown enhances temperature sensitivity. Consistent with this, DCP5-containing cytoplasmic condensates undergo dynamic, temperature-dependent reorganization and exert a dual role in co-translational mRNA decay, selectively protecting or promoting transcript degradation. In parallel, we uncover temperature-dependent changes in mRNA localization. The aquaporin transcript TIP2;1 shows reduced stability at 28°C and shifts from nuclear enrichment at 21°C to increased cytoplasmic accumulation at 28°C, while export-defective mutants exhibit enhanced nuclear mRNA retention. These findings suggest that nuclear export defines the pool of transcripts accessible to cytoplasmic decay and coordinates with RNA turnover to shape temperature-responsive gene expression. Together, our results reveal that temperature adaptation involves the integration of nuclear export with cytoplasmic decapping to fine-tune mRNA stability, highlighting RNA fate control as a key regulatory layer underlying developmental plasticity in plants.

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Poster 56

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Exploring a novel role of HYL1 in RNA Polymerase II transcript maturation

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HYL1 (Hyponastic Leaves 1) is a well-characterized component of the plant microprocessor complex that promotes the biogenesis of microRNAs. In our previous work, we demonstrated that HYL1 also performs a microprocessor-independent function by directly associating with RNA Polymerase II (Pol II), where it regulates transcription initiation and elongation. These findings suggested that HYL1 functions much earlier in gene expression than previously appreciated.Building on these observations, we are currently investigating whether HYL1 may also participate in the co-transcriptional maturation of RNA Polymerase II transcripts, with a particular focus on processes associated with transcript 3′-end formation. Given its close association with Pol II and newly synthesized RNAs, HYL1 represents an attractive candidate for coordinating transcription with downstream RNA processing events.To address this question, we are combining genome-wide transcriptomic approaches with molecular and genetic analyses in Arabidopsis thaliana to examine whether the loss of HYL1 affects RNA 3′-end homeostasis and transcript maturation. Our ongoing work aims to determine whether HYL1 contributes to the coordination between transcription and RNA processing, thereby extending its functional role beyond transcriptional regulation and microRNA biogenesis.

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Poster 57

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Loss of RBP47 enhances photosynthetic resilience and jasmonate-mediated heat stress tolerance in Arabidopsis

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Stress granules (SGs) are membraneless organelles that form during stress and have been widely studied as markers of the cellular stress response (1,2). However, their functional role in plant stress adaptation remains poorly understood. RBP47b, an RNA-binding protein of the RBP47 family, is one of the most widely used SG markers in plants. Here, we investigated the functional contribution of the four Arabidopsis thaliana RBP47 paralogues (RBP47a, b, c, and c') to thermotolerance. Plants lacking all four paralogues (rbp47abcc') exhibit enhanced survival and accelerated recovery of photosynthetic efficiency after heat stress, revealing an unexpected role for this protein family in stress adaptation. To define the molecular basis of this phenotype, we combined biotin-labeled oligo(dT) affinity purification with proximity labeling (BioID) to map the RBP47b-associated SG proximity proteome. This revealed enrichment of proteins linked to photosynthesis, lipid metabolism, redox homeostasis, and translational control. Integrating transcriptomic, proteomic, and metabolomic datasets, we uncovered a constitutive accumulation of jasmonic acid (JA) and jasmonate-derived oxylipins in the mutant, alongside reduced reactive oxygen species (ROS) accumulation under both control and heat-stress conditions. This points to a preconditioned redox and hormonal state that primes rbp47abcc'; plants for thermotolerance. Consistently, heat-induced proteome reprogramming is dampened in the mutant, suggesting altered mRNP dynamics. Collectively, our findings reposition RBP47-dependent SGs from passive stress markers to active regulators of stress adaptation, whose loss confers acute heat resilience through coordinated modulation of jasmonate signaling and redox balance — opening new avenues for engineering thermotolerant crops.

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Poster 58

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A reporter-based dissection of U1-dependent telescripting in Arabidopsis

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Telescripting is a U1 snRNP-dependent mechanism that protects nascent transcripts from premature cleavage and polyadenylation (pCPA), thereby promoting full-length mRNA synthesis. We recently showed that reduced U1 snRNP (U1) activity in Arabidopsis thaliana activates intronic polyadenylation sites and generates prematurely terminated transcripts, suggesting that U1 suppresses intronic pCPA in plants. However, the cis-regulatory features that distinguish pCPA-prone from protected/ non-pCPA introns remain unknown.To define these features, we established a GFP–intron–RFP reporter system based on a pCPA-prone intron. This reporter generated both full-length and prematurely terminated transcripts, giving rise to GFP-RFP and GFP expression, respectively, whereas a matched non-pCPA intron produces only the full-length transcript and protein. Using this system, we first investigated the contribution of U1 to pCPA suppression by modifying the 5′ and 3′ splice sites. We then systematically tested candidate cleavage and polyadenylation elements, including the Far Upstream Element (UGUA), Near Upstream Element (AAUAAA), and the Cleavage Element/ Cleavage Site (U/UG-rich motif/ YA). Our analyses define the sequence requirements that promote intronic pCPA, revealing that plant pCPA is governed by the local arrangement of CPA-promoting elements and 5′ splice-site-associated U1 binding. Furthermore, they imply that pCPA-prone introns, in their native genetic context, give rise to translated protein isoforms. This reporter provides an experimental platform for dissecting the cis-regulatory basis of plant telescripting and the sequence principles that determine susceptibility to pCPA.

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Poster 59

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Alternative splicing dynamics associated with heat stress

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As rising temperatures threaten global crop productivity, it is critical to understand how plants respond to heat stress. Recent studies have shown that, in parallel to gene expression changes, heat stress alters splicing patterns genome-wide [1,2]. However, while the physiological consequences of heat have been extensively studied at the gene expression level [3], how alternative splicing reshapes the transcriptome and contributes to heat responses remains largely underexplored [4,5]. To address this, we established an experimental framework combining phenotypic characterisation with transcriptome-wide analyses of gene expression and alternative splicing in Arabidopsis thaliana. Seven-day-old seedlings were exposed to moderate (32°C), severe (37°C), or extreme (42°C) heat stress. Samples were collected after 1 hour and 4 hours of stress, and after 24 hours of recovery at 21°C. Phenotypic analyses revealed a progressive shift in plant performance, from acclimatory responses at moderate and severe temperatures to damage and lethality under extreme heat. At the molecular level, RNA-seq analyses revealed consistent gene expression responses across the different stress conditions. Moreover, the data revealed a strong temperature-dependent increase in alternative splicing, being intron retention the predominant event across all conditions. Splicing changes were most pronounced under severe and extreme heat, whereas moderate stress induced comparatively limited effects. Importantly, intron retention dynamics, as well as gene expression changes, were reversible within the temperature range in which plants remained viable (32-37°C), but remained elevated under extreme heat, indicating a stress-intensity-dependent and reversible transcriptomic response associated with acclimation capacity. Together, our results link physiological plant performance to coordinated transcriptional and alternative splicing responses across a gradient of heat stress. In particular, the dynamic and reversible nature of intron retention within the viable temperature range supports a model in which alternative splicing contributes to transcriptome reprogramming during heat acclimation, potentially representing a regulated mechanism of plant adaptation to elevated temperatures.

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Poster 60

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The STA1–DOT2 interaction drives nuclear speckle formation for efficient splicing in growth and heat stress responses

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Pre-mRNA splicing is mediated by the spliceosome, a dynamic ribonucleoprotein complex. The spliceosome is known to be enriched in nuclear speckles (NS), which are now recognized as active subnuclear organelles for splicing. However, how spliceosomal protein–protein interactions are functionally linked to NS organization and splicing robustness in plants remains unclear. Here, we identify a specific interaction between two U4/U6·U5 tri-snRNP components, STA1 and DOT2, as a key determinant of NS organization, pre-mRNA splicing, and heat stress responses in Arabidopsis. A missense mutation in DOT2 (a Snu66/SART1 homolog), isolated as a genetic suppressor of the PRP6 homolog mutant *sta1-1* (named S307), restores the weakened interaction between STA1 and DOT2 in the *sta1-1* mutant background. Genetic, biochemical, and cell biological analyses indicate that variation in the strength of the STA1–DOT2 interaction is closely associated with changes in NS formation, splicing efficiency, growth, and heat tolerance. Pharmacological disruption of STA1-associated NS formation by tubercidin phenocopies the *sta1-1* mutant, supporting a functional link between NS organization and splicing outcomes. Furthermore, heat stress weakens the STA1–DOT2 interaction, accompanied by reduced NS formation and increased intron retention at the transcriptome-wide level, including those of key heat-responsive transcripts. Together, these findings suggest that the STA1–DOT2 interaction, likely reflecting the assembly state of the U4/U6·U5 tri-snRNP, functions as a heat-sensitive interaction node that couples spliceosome assembly to NS organization and splicing robustness under stress conditions.

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Poster 61

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Alternative splicing of PIF4 regulates plant development under heat stress

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The Phytochrome-Interacting Factor 4 (PIF4) is a key player in the integration of multiple internal and external stimuli to optimize different aspects of plant development (1). While both the DNA encoding this transcription factor and its protein are known to be under tight control, no regulation at the RNA level has been previously reported (2). Our genomic analysis revealed that the exon/intron structure of the basic Helix-Loop-Helix (bHLH) DNA binding domain of PIF4 is conserved and pointed to skipping of an exon in this region specifically in response to heat stress. We then showed that this alternative splicing event downregulates PIF4 function under heat, which in etiolated seedlings induces photomorphogenic-related traits. Our results disclose a role for PIFs in plant responses to heat and reveal a new regulatory layer for the control of PIF4 function, underscoring the critical role of posttranscriptional regulatory processes in the molecular integration of environmental cues.

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Poster 62

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Transcriptional co-regulator RCD1 influences RNA splicing through interaction with the pre-spliceosome

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Plants face constant fluctuations in environmental conditions, and various stress signalling pathways aid them in coping with these fluctuations through influencing the expression of nuclear genes. The nuclear protein RCD1 integrates multiple stress signalling pathways by interacting with over 30 transcription factors (TFs), yet its role as a transcriptional co-regulator has remained unclear. Our study reveals that the rcd1 mutant exhibits genome-wide defects in alternative splicing, indicating that the mechanism by which RCD1 regulates gene expression is more complex than was previously thought. We show that RCD1 physically interacts with components of the spliceosomal machinery, and that the majority of these interactors are part of the pre-spliceosomal U1 and U2 small nuclear ribonucleoprotein (snRNP) complexes. Moreover, subnuclear distribution of RCD1, namely the size and abundance of RCD1-containing nuclear bodies, change upon pharmacological inhibition of the pre-spliceosome but not upon inhibition of the downstream precatalytic spliceosome. Domain deletion analysis indicates that RCD1's TF-binding domain is not required for interaction with spliceosomal components in vivo. These findings suggest that RCD1 regulates gene expression not only by modifying transcription, but also through influencing RNA splicing. We hypothesise that RCD1 regulates co-transcriptional splicing by recruiting splicing factors to active chromatin through its interaction with DNA-bound TFs.

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Poster 63

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Repression of ABA-responsive alternative splicing by an Arabidopsis SR protein relieving ABA inhibition of early plant growth

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The phytohormone abscisic acid (ABA) inhibits postgermination growth under unfavorable conditions, delaying the seedling's transition to the autotrophic stage and thereby enhancing plant survival. Although stress-induced ABA accumulation is well known to trigger extensive transcriptional reprogramming, accumulating evidence indicates that alternative splicing also contributes significantly to plant stress tolerance. However, the molecular factors governing posttranscriptional regulation of ABA-mediated control of early plant growth remain largely unexplored. Here, we show that loss of function of Arabidopsis SR34a, a member of the highly conserved serine/arginine-rich (SR) family of RNA-binding proteins, results in increased ABA sensitivity during seedling establishment. At ABA concentrations that allow greening of wild-type seedlings, loss of SR34a function causes growth arrest following seed germination. Consistent with this phenotype, the *sr34a-1* mutant exhibits hypersensitivity to both drought and salt stress during postgermination growth. By combining individual-nucleotide resolution crosslinking and immunoprecipitation with RNA-sequencing analysis of the *sr34a-1* mutant, we show that SR34a modulates all major classes of alternative splicing events through preferential association with specific exonic regions near splice sites. Our results further uncover an important role for SR34a as a negative regulator of ABA-induced alternative splicing events, notably by binding on RNAs encoding key regulators of ABA signaling. Together, these findings provide mechanistic insight into how plant SR proteins coordinate alternative splicing and ABA-dependent developmental responses during early seedling establishment.

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Poster 64

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Etiolated seedlings reveal a link between sugar metabolism and splicing regulators

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The transition from skotomorphogenesis to photomorphogenesis is crucial for plant establishment as a photoautotrophic organism. Although skotomorphogenesis has traditionally been described as a developmental state that is maintained throughout seedling growth in darkness and terminated upon exposure to light [1], we observe that it becomes a dynamic process when the dark period preceding light exposure is extended. Arabidopsis thaliana seedlings germinated and grown under prolonged darkness progressively exhibit morphological traits associated with photomorphogenesis, including cotyledon opening, apical hook unfolding, and inhibition of hypocotyl elongation. We show that this phenomenon is not driven by light signaling but rather by the management of energy resources. During skotomorphogenesis, seedlings are heterotrophic and rely entirely on the nutrients stored in the seed to survive [2]. Quantification of starch and soluble sugars in this study reveals that, over time in darkness, starch is degraded and D-glucose and D-fructose are consumed. Through the integration of phenotypic, gene expression, and carbohydrate analysis of dark-grown seedlings, we demonstrate that the emergence of photomorphogenic traits in the dark is associated with a progressive depletion of sugar reserves. Furthermore, we reveal that this response is regulated by two members of the SR (serine/arginine-rich splicing factors) family of splicing factors [3]: RS40 and RS41. By analyzing loss- and gain-of-function mutants of these proteins, we report that these SR splicing factors act as positive regulators of this process. Finally, we uncover an interplay between sugars and RS40 and RS41 that fine-tunes the developmental status of etiolated seedlings according to endogenous sugar availability.

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Poster 65

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Plastid activity-dependent pre-mRNA splicing regulates lateral root morphology in Arabidopsis

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Plastid (Chloroplast) is a place for metabolic processes including photosynthesis, and the alteration of metabolic activity of plastids make retrograde signals from plastid to nucleus to regulate the nuclear gene expression. Previously, it has been reported that the strong inhibition of plastid activity, as well as the aberrant splicing activity, resulted in the formation of knob-like lateral roots (LRs) in *Arabidopsis thaliana*. These suggested that the retrograde signal can regulate LR morphology via pre-mRNA splicing. To test this hypothesis, we examined LR morphology of Arabidopsis splicing-related mutants and a plastid-signaling defective mutant. In wild type, the width of LRs was increased by the treatment of plastid inhibitor, as well as photosynthetic inhibitor, whereas not in the splicing-related mutants. Our RNA-seq analysis revealed that splicing patterns, but not gene expression levels, were altered by the plastid inhibitor treatment: especially in roots, a specific set of auxin-related genes were differentially spliced. In particular, the specific alternative splicing was observed in the transcription factor gene associated with LR. The protein variants produced by the alternative splicing repressed the transcriptional activity of its functional variants, and affected the expression of PIN genes, leading to abnormal auxin distribution in roots. Taken together, our findings indicate the importance of plastid retrograde signal-mediated nuclear pre-mRNA splicing for the regulation of LR morphogenesis. Considering the fact that wider LRs show distinct properties in nutrient uptake, this would be a mechanism that regulates the metabolic balance of the entire body by reflecting the aboveground photosynthetic activity in the underground structures.

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Poster 66

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An Arabidopsis SR splicing regulator in pattern- and effector-triggered immunity

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Plant immunity is a highly plastic response that enables plants to counter a wide range of pathogens. Alternative splicing has emerged as a key posttranscriptional regulatory mechanism to enhance the versatility and effectiveness of immune responses. Pathogen infection triggers distinct immune outputs associated with pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), representing two major layers of plant immune responses initiated by cell surface pattern recognition and intracellular effector recognition, respectively. However, how specific regulatory components are integrated into these immune layers remains incompletely understood. Serine/arginine-rich (SR) proteins are highly conserved RNA-binding proteins that function as central regulators of pre-mRNA splicing and are known to modulate stress-responsive alternative splicing under abiotic stress conditions. A potential role of SR proteins in plant-pathogen interactions remains largely unexplored, although the SR-like SR45 has been previously implicated in plant immunity. This suggests a link between splicing regulation and immune function, while its role within distinct immune contexts, namely whether it modulates responses in PTI, ETI or both, remains unclear. Here, we examine SR45 in plant immunity using the Arabidopsis–Pseudomonas syringae pathosystem. We assess disease symptoms and PTI-associated responses triggered by flagellin perception as well as ETI-related immune outputs induced by different Pseudomonas strains with defined virulence properties. This framework enables comparison of SR45-associated effects across distinct immune activation contexts. Together, this work provides insight into how a splicing regulator functions in PTI and ETI immune responses and establishes a basis for understanding how RNA processing components are integrated into plant immune signaling.

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Poster 67

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RSZ SR Proteins Modulate Immune Responses in Arabidopsis

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Alternative splicing is a fundamental posttranscriptional mechanism that contributes to plant development and adaptation to environmental stimuli[1]. While alternative splicing has been implicated in responses to abiotic stress, its role in plant immune regulation remains poorly understood[2]. Serine/arginine-rich (SR) proteins are key determinants of splice-site selection and thus alternative splicing, yet their specific contribution to plant immunity remains largely unexplored. Here, we focused on members of the RSZ subfamily of SR proteins and investigated their role in modulating immune responses in Arabidopsis thaliana during *Pseudomonas syringae* infection. Molecular and phenotypic analyses of rsz loss-of-function mutants revealed altered responses to bacterial infection. Single mutants of the three RSZ family members displayed similar immune phenotypes. Based on this, we focused our analysis on a higher-order mutant combining these loci, which exhibited an infection-resistant phenotype. Mutant plants showed reduced disease symptoms and decreased bacterial proliferation, supporting a role for RSZ proteins as negative regulators of plant immunity. Additional analyses of immune-associated responses further supported the involvement of these proteins in defense regulation. In addition, growth inhibition assays using immune elicitors, in the absence of bacteria, revealed altered responses in the rsz triple mutant compared to wild-type plants, suggesting that RSZ proteins also contribute to the coordination of growth- and defense-associated processes. Together, these findings identify a previously uncharacterized role for the RSZ subfamily of SR proteins in plant immunity, supporting the emerging notion that alternative splicing contributes to shaping defense responses in *Arabidopsis thaliana*.

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Poster 68

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Molecular properties underlying specificity of the splicing regulatory proteins RS31a and RS31

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During early seedling development, light exposure triggers the switch from skotomorphogenesis to photomorphogenesis, characterized by reduced hypocotyl elongation and cotyledon opening. This morphogenic shift is accompanied by rapid alternative splicing (AS) responses affecting numerous genes. Our group has identified members of the plant-specific RS subfamily of serine/arginine-rich (SR) proteins as regulators of light-dependent AS. Our previous findings uncovered an intricate regulatory network involving the four RS proteins from *Arabidopsis thaliana*, RS31a, RS31, RS40, and RS41. Within this system, RS31 can act as a negative regulator of photomorphogenesis – in an opposing role to the other three RS proteins[1]. Our current findings further support functional specialization of the two smaller RS proteins, RS31a and RS31, based on their impact on light-dependent hypocotyl elongation and flowering time. On a molecular level, RS31a does not influence the AS pattern of an RS31 splicing reporter, while the other three RS proteins induce a shift towards non-coding variants. Furthermore, RNA-seq analysis of inducible triple and quadruple mutants revealed specific as well as common AS regulatory functions of the RS proteins. By examining the splicing function of chimeric variants of RS31a and RS31, we could narrow down the functional characteristics to certain protein regions. With regard to their subcellular localization, GFP-tagged RS31a and RS31 show a distinct pattern of differently sized speckle-like structures in the nucleus. These observations are in line with in vitro turbidity assays and microscopic evaluations, which suggest individual molecular features of RS31a and RS31 that result in dissimilar condensation behavior. Interestingly, specific phosphorylation by the kinase AFC2 from *A. thaliana* has a differential effect on these condensation properties. Altogether, our study provides a multilevel characterization of the two highly similar splicing regulators RS31a and RS31 and their molecular specificity determining their roles within the splicing regulatory network modulating light-dependent plant development.

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Poster 69

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UBL1 regulates freezing tolerance in Arabidopsis by controlling the splicing of genes involved in the cold stress response

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Pre-mRNA splicing constitutes a critical step in the regulation of gene expression. The catalytic core of the spliceosome is mainly composed of the U6 snRNP complex, whose activity depends on various auxiliary factors that facilitate its assembly and function. Previously, our laboratory demonstrated that one of these factors, the LSM2-8 complex, regulates Arabidopsis tolerance to abiotic stresses (Carrasco-López et al., 2017). However, the involvement of other auxiliary factors in plant responses to such stresses remains largely unexplored. In this study, we analyzed the role of U6 BIOGENESIS LIKE 1 (UBL1)—a factor associated with the LSM2-8 complex—in freezing tolerance and splicing control under cold stress in Arabidopsis. Physiological characterization of ubl1 mutants revealed that this protein acts as a negative regulator of the cold acclimation process. Furthermore, transcriptomic analysis of these mutants exposed to 4°C demonstrated that UBL1 controls the correct splicing of a high number of messengers (2819), many of which correspond to genes involved in cold acclimation. This number is significantly higher than that regulated by the LSM2-8 complex under cold conditions (1642), indicating that UBL1 confers a high degree of functional specificity to the spliceosome in this environmental scenario. Finally, it is worth noting that among the transcripts whose splicing is coordinated by UBL1, we identified several described as essential for chloroplast development. Analysis of ubl1 mutants via electron microscopy under both control and 4°C conditions confirmed that UBL1 is indeed required for proper chloroplast development in Arabidopsis.

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Poster 70

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Splice to Survive: How RS proteins guide salt stress responses of plants

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Soil salinity is a major abiotic stress that severely limits plant growth and crop productivity worldwide. Although the transcriptional response to salt stress has been extensively studied, the contribution of post-transcriptional regulation remains poorly understood. Alternative splicing (AS) has emerged as an important mechanism for plant acclimation to environmental stress, however, the regulators of salt-responsive AS remain largely unknown. Here, we investigate the role of the RS family of serine/arginine-rich splicing factors in plant salt stress tolerance. Preliminary phenotypic analyses revealed that rs mutants are hypersensitive to salt stress, suggesting that RS proteins positively regulate salt tolerance. To elucidate the molecular basis of their function in cellular signaling in response to salt, we are examining both their upstream regulators and RNA targets. Using phosphoproteomics, we identified multiple serine residues that are specifically phosphorylated in response to salt and osmotic stress. Furthermore, to uncover the protein interaction partners that may regulate RS function, we are using TurboID-based proximity labelling under control and salt stress conditions. In parallel, we are establishing complementary approaches, including TRIBE and iCLIP, to identify the direct RNA targets of RS proteins and determine their impact on AS regulation. Together, this work aims to elucidate how post-translational modifications and protein interactors of RS proteins contribute to plant responses to saline conditions.

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Poster 71

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The function of alternative splicing in mediating temperature responses in *Arabidopsis thaliana*

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Alternative splicing (AS) constitutes an important layer of gene regulation that enables plants to adapt to changing environmental conditions. Temperature is a major environmental cue regulating plant growth and development, a process known as thermomorphogenesis. A central regulator of this process is the basic helix-loop-helix (bHLH) transcription factor PHYTOCHROME INTERACTING FACTOR 4 (PIF4), which integrates multiple endogenous and environmental signals to coordinate plant growth responses. While PIF4 is known to be tightly regulated at both the transcriptional and protein levels, little is known about its regulation at the RNA level. A recent study identified a heat-stress-induced alternative splicing event of PIF4 under dark-grown conditions, generating an exon 5-skipped (PIF4-ΔE5) mRNA encoding a protein with an in-frame deletion of 22 amino acids within the bHLH domain. Here, we show that this splicing event is regulated by both temperature and photoperiod and examined its biological functions. We found that PIF4-ΔE5 was induced by heat-stress under all photoperiod conditions, whereas moderate warm temperature triggered the PIF4-ΔE5 only under continuous light, indicating that both temperature and light influence PIF4 alternative splicing. Functional analyses using transgenic lines expressing different PIF4 isoforms revealed that the PIF4-ΔE5 was less functional than the full-length PIF4 in promoting thermoresponsive hypocotyl elongation and its function depends on the presence of other PIF proteins. These findings suggest that alternative splicing of PIF4 provides an additional regulatory layer that fine-tunes PIF activity during thermomorphogenesis.

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Value Plant

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The RNA polymerase II CTD kinase CDKC;2 couples transcription kinetics to cold sensitivity of Arabidopsis splicing mutants

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Alternative splicing (AS) allows plants to remodel their transcriptomes in response to ambient temperature, and because AS occurs largely co-transcriptionally, it is shaped by the activity of RNA polymerase II (RNAPII). RNAPII is in turn regulated by phosphorylation of its C-terminal domain (CTD) by cyclin-dependent kinases (CDKs), yet nearly all mechanistic models of this coupling derive from yeast and animal systems. To identify regulators of the low-temperature response, we performed an EMS suppressor screen on porcupine (*pcp-1*), an Arabidopsis splicing mutant with severe shoot and root defects at 16°C. Mapping-by-sequencing of two independent suppressors identified alleles of CDKC;2 (AT5G64960). *cdkc2-11* carries a T69I substitution in the PITAIRE cyclin-binding motif, and cdkc2-12 is an intron 8 splice-site mutation causing constitutive intron retention and a premature stop codon predicted to trigger nonsense-mediated decay. Crossing *pcp-1* to the *cdkc2-2* T-DNA null allele confirmed that loss of CDKC;2 is sufficient to partially rescue the cold-sensitive *pcp-1* phenotype, and it likewise suppressed cold sensitivity of the Sm-ring mutants *smd3b-1* and *ruxf*, indicating a general interaction with spliceosome function. Chemical inhibition with BML-259 phenocopied the rescue seen in *pcp-1 cdkc2-2*, and transcriptome analysis revealed that the splicing defects of *pcp-1* were largely restored in the double mutant. Using smFISH and 5-EU labeling, we found that low temperature slows transcription overall, yet *cdkc2-2* transcribes significantly faster than wild type at 16°C, while *pcp-1 cdkc2-2* restores wild-type rates at both temperatures. Notably, these differences are also true for intronless genes. Higher transcriptional output despite reduced CTD phosphorylation is not predicted by current RNAPII models, arguing that transcription–splicing coupling in plants requires dedicated, plant-specific frameworks. We are now asking whether these changes in transcription dynamics influence downstream RNA fate, combining long-read isoform quantification with cordycepin-based measurements of transcript stability across genotypes and temperatures.

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S4 Posters - Translation

Poster 73

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Genome-wide profiling of mRNA dynamics along pollen tube growth of *Solanum lycopersicum*

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Post-transcriptional regulations represent an important layer of gene expression control during plant reproductive development. Pollen tube growth is a highly polarized process that requires tight regulation of mRNA dynamics to sustain cellular activity and ensure successful fertilization. In mature pollen, where transcription is thought to be largely inactive, gene expression is hypothesized to be primarily controlled through translation, mRNA storage, and degradation during germination. However, global mRNA dynamics during pollen germination and pollen tube growth remain largely unexplored. Here, to investigate transcriptional activity, RNA degradation, and translation during these processes, we applied RNA-seq and 5′P-seq across defined growth stages. Samples were collected from dry pollen, hydrated pollen, germinating pollen, and early and late pollen tube elongation stages of tomato. To enable this analysis, we established and optimized an in vitro pollen tube growth system and defined distinct developmental stages based on time points, allowing reliable sampling across transitions. Preliminary RNA-seq analysis identified strong differential gene expression between dry and hydrated pollen, with comparatively reduced changes in subsequent stages. To investigate co-translational mRNA decay (CTRD), we generated 5′P-seq libraries, which capture 5′-monophosphate RNA intermediates produced during translation-coupled degradation and track the last translating ribosome. As a hallmark feature, 5′P-seq profiles typically show enrichment ~16–17 nucleotides upstream of stop codons, reflecting slow translation termination. However, previous studies in mature pollen grains (MPG) did not resolve this clear decay signature, suggesting possible context-dependent variation in 5′P-seq signals. Therefore, we aim to (1) refine metagene analysis through read-depth filtering to enhance detection of localized 5′P enrichment patterns, and (2) characterize transcripts exhibiting CTRD signatures and determine how their dynamics change during the transition from dry pollen to pollen tube growth. Understanding these dynamics will provide insight into how pollen reprograms gene expression by coupling translation and mRNA decay during germination and tube formation.

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Poster 74

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Target of Rapamycin (TOR) mediates translational regulation in Arabidopsis

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Plants have developed intricate mechanisms to sense environmental signals and internal energy status. Target of rapamycin (TOR) complex has a central role in fine-tuning the plant cell responses to nutrient availability and external cues. TOR controls ribosome biogenesis and protein synthesis, thereby reprogramming metabolism and allowing cells to cope with nutritional and external stresses. Precise assessment of TOR-mediated translational regulation has been challenging due to limited approaches and tissue complexity across cell types and stages. In this study, we combined an Arabidopsis cell culture system with Ribo-seq to globally investigate translational control in plant cells and determine how TOR-mediated regulation contributes to metabolic changes. Our results show AZD-8055-mediated TOR inhibition represses gene expression and translation of genes encoding ribosome biogenesis related proteins, consistent with reduced protein synthesis and amino acids accumulation. We examined whether TOR uses upstream regulatory motifs such as 5’ terminal oligopyrimidine (5’ TOP) as translational mechanisms to boost the synthesis of its downstream target proteins. Our analysis identifies Translationally-Controlled Tumor Protein 1 (TCTP1) and ADP-ribosylation factor A1E (ARFA1E) as TOR-responsive transcripts and shows that TOR positively regulates their translation through elements in the 5’ UTR. Overall, this study provides a framework for understanding how TOR-mediated translational control reshapes metabolism in response to nutrient and environmental signals.

Poster 75

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Meiotic bodies and their role in meiosis progression

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Recent research has highlighted the importance of RNP granules in the temporal control of gene expression. These condensates, such as P-bodies and stress granules, modulate the translatability of mRNAs, and play an important role during cellular reprograming. Our laboratory has discovered that during meiosis, specialized RNP granules assemble and orchestrate the progression through the cycle and the transition from meiosis to mitosis. Meiotic bodies (M-bodies) are cytosolic granules with a P-body core surrounded by a shell enriched in stress granules components. They assemble in early stages of prophase, a period characterised by a transcription burst, and they remain nucleated through the rest of the cycle. M-bodies dynamically modify their composition and activity across the different stages. For instance, the protein CDM1 modulates the storage and protection of specific mRNAs during meiotic prophase, by forming CDM1 condensates and associating with M-bodies. The cyclin TAM also associates with M-bodies during prophase, and regulates the recruitment of the meiotic specific protein TDM1. At the end of the cycle, TDM1 sequesters the translation initiation complex eIF4F into M-bodies, thereby promoting a cellular reprogramming that is essential for meiotic exit. Mutations in these factors or deficient condensation of M-bodies result in aberrant meiotic progression, impacting plant reproduction.

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Poster 76

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Alternative splicing likely drives functional diversification within the MATH-BTB family in Arabidopsis thaliana

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MATH-BTB proteins play essential roles in diverse plant developmental processes and responses to environmental stresses. These proteins function as substrate adaptors in CUL3-based E3 ubiquitin ligase complexes. Through their MATH domain they recognize and bind target proteins, while their BTB domain mediates interaction with CUL3, thereby promoting target protein ubiquitination and subsequent proteasomal degradation. Although MATH-BTB proteins are encoded by a limited number of genes in most of species, they exhibit remarkable functional diversity. We investigated alternative splicing and its potential role in the functional diversification of MATH-BTB (BPM) proteins in Arabidopsis thaliana. Bioinformatic analysis revealed numerous alternatively spliced BPM transcripts, most of which are predicted to encode proteins containing an intact MATH domain but a truncated BTB domain. This structural organization suggests that truncated MATH-BTB protein isoforms retain the ability to recognize target proteins but are likely unable to interact with the E3 ubiquitin ligase, thereby preventing target protein ubiquitination and proteasomal degradation. Focusing on BPM2, which exhibited the highest number of splice variants, we cloned and sequenced four full-length alternative BPM2 transcripts from diverse tissues. We then tested interactions of the canonical and putative alternative BPM2 protein isoforms with CUL3 and previously identified BPM target proteins (WRI1, HB6 and MYC2) using yeast two-hybrid assays, and analysed their subcellular colocalization in planta. Whereas two BPM2 isoforms likely retain the ability to interact with CUL3 and mediate target protein ubiquitination, the other isoforms appear to influence target protein function by altering their subcellular localization rather than promoting their ubiquitination. Our results demonstrate that alternative splicing may contribute to the functional diversification of the MATH-BTB protein family in *A. thaliana*.

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Poster 77

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Dynamics of translational machinery composition in *Arabidopsis thaliana* pollen and pollen tubes

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Translation regulation plays a critical role in plant development and reproduction, yet the proteomic composition of the translational machinery and its specialization across tissues and developmental transitions remain largely uncharacterized. The male gametophyte is an ideal model system to study specialized translation regulators and ribosomes due to dynamic shifts in translational activity between inactive mature pollen and rapid pollen tube growth. Here, we report the first proteome-scale characterization of monosome- and polysome-associated fractions from Arabidopsis thaliana mature pollen grains (MPG) and pollen tubes (PT). Interestingly, the polysome profile of Arabidopsis MPG was more active than anticipated based on previous reports in Nicotiana tabacum, suggesting species-specific strategies of translational regulation in the male gametophyte. Using quantitative proteomics, we identified a conserved core of translation machinery proteins shared across all fractions, alongside ribosomal proteins uS15y, eL15z, and uL22y exclusively associated with inactive monosomes, suggesting specialized ribosome subpopulations in the dormant pollen stage. Several ribosomal proteins, most notably uS11z, transitioned from MPG monosomes to PT polysomes upon germination, together with shifts in ribosomal protein paralog composition between developmental stages. Additionally, eukaryotic initiation factors (eIFs) and RNA-binding proteins were detected in the polysome fractions upon pollen activation, suggesting a coordinated mRNA regulatory network operating during germination. Together, these findings reveal multiple layers of translational control in pollen, from ribosome heterogeneity to RNA-binding protein dynamics, advancing our understanding of post-transcriptional regulation in plant reproduction.

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Poster 78

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Ribosome profiling reveals novel translated genes and a shade-induced microprotein regulating phytochrome signalling in Arabidopsis

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Microproteins encoded by previously overlooked small open reading frames are emerging as important regulators of eukaryotic signalling and development, yet their functions in plant environmental responses remain largely unexplored. Here, we used ribosome profiling to uncover extensive hidden coding potential in white light- and shade-treated Arabidopsis thaliana seedlings, identifying more than 2,000 previously unannotated translated genes. Among these, 283 high-confidence novel genes encoded translated small open reading frames associated with light-responsive growth. We further characterized one translated 12-amino-acid microprotein that promotes shade avoidance responses. Application of the synthetic microprotein, but not a scrambled control peptide, enhanced hypocotyl elongation and seed germination specifically under shade conditions, with minimal effects in white light. The microprotein stimulated hypocotyl growth across a range of red:far-red (R:FR) ratios in wild-type seedlings, but this activity was abolished in phytochrome and PIF signalling mutants, demonstrating that its function depends on an intact phy–PIF signalling pathway. Remarkably, the microprotein also promoted hypocotyl elongation in shade-treated Cardamine hirsuta, suggesting evolutionary conservation of this regulatory mechanism across species. Furthermore, CRISPR-induced frameshift mutants disrupting the microprotein-coding sequence in Arabidopsis displayed shorter hypocotyls under shade but elongated hypocotyls in white light, uncovering a dual and light-dependent role in growth regulation. Together, our findings reveal a previously unrecognized microprotein-mediated layer of phytochrome signalling, uncover novel translated genes involved in adaptive light responses, and establish ribosome profiling as a powerful approach for discovering hidden regulators of plant development.

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Poster 79

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Translational stress-response module controls pollen tube integrity under high temperature

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Pollen germination and pollen tube (PT) growth are highly sensitive to elevated temperatures. Under heat stress (HS), global translation is suppressed to prioritize synthesis of proteins essential for cellular protection and protein homeostasis. The eukaryotic translation initiation factor eIF3 plays a key role in selectively regulating stress-responsive mRNAs. Using T-DNA insertion lines, RNA interference, and CRISPR-Cas9 mutants, we show that eIF3M1 and eIF3M2 are stage-specific regulators of male gametophyte development in *Arabidopsis thaliana*. Under normal conditions, the *eif3m1/eif3m2* double mutant shows impaired pollen germination, loss of PT integrity, and increased seed abortion. Unexpectedly, under HS (37°C), *eif3m1* pollen shows enhanced germination and improved PT integrity compared to wild type. This increased thermotolerance is associated with upregulation of its paralog eIF3M2, which elevates HSP70 mRNA and protein levels. Overexpression of eIF3M2 enhances HSP70 expression, whereas *eif3m2* knockdown reduces HSP70 promoter activity and increases PT bursting under HS. Additionally, eIF3M2 interacts with cytoplasmic HSP70 and eIF4G. Polysome profiling further indicates that eIF3M2 selectively regulates stress-adaptive mRNAs. Together, these findings identify the eIF3M2–HSP70 module as a key mechanism maintaining PT integrity under heat stress.

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Poster 80

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Tuning gene expression with 5'UTR sequence in *Nicotiana benthamiana*

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How the 5' Untranslated Region (UTR) impacts gene expression is key to understanding the regulatory information encoded in the sequence of a gene. In many species, varying the sequence of a 5'UTR alone can have a substantial impact on the expression of a gene, demonstrating the importance of this region in tuning the exact expression patterns of genes. Prior work has identified several key regulatory features that connect 5'UTR sequence to its regulatory function. For example, the five nucleotides directly preceding the start codon (Kozak sequence) strongly influence ribosomal loading and thus ultimate protein translation. Recent work has utilized Massively Parallel Reporter Assays (MPRAs) to test thousands of 5' UTR variants in a single experiment, but this work has been largely limited to a few species. In this work, we conducted an MPRA with random 5'UTR variants in Nicotiana benthamiana to uncover the principles of 5'UTR gene expression regulation in plants. We performed Translating Ribosome Affinity Purification (TRAP)-seq to identify which 5'UTR variants exhibited enriched ribosomal occupancy. Using this approach, we identified sequences across a large dynamic range of gene expression and ribosomal occupancy, indicating that the 5'UTR is a potent way of tuning gene expression in plants. We identify sequence preferences proximal to the start codon and find that upstream open reading frames (uORFs) are associated with decreased ribosome association. Further, uAUGs with strong Kozak sequences exhibit lower ribosomal occupancy than uAUGs with weak Kozak sequences. The results of this study will provide a foundation for designing synthetic 5′UTRs that precisely control gene expression in plants.

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Molecular strategies for thermotolerant pollen: A translational perspective

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Pollen germination and pollen tube growth are among the most heat-sensitive stages of plant reproduction. Elevated temperatures trigger a broad reduction in protein synthesis while promoting the selective translation of proteins required for stress protection, protein homeostasis, protection against protein misfolding, and cellular survival. Our work shows that components of the translational machinery play important roles in maintaining pollen performance under heat stress. Mutants affecting specific translation initiation factors display altered pollen germination, tube growth, and reproductive success, with some genotypes unexpectedly showing improved tolerance to elevated temperatures. This enhanced thermotolerance is associated with increased expression of protective chaperones and stress-responsive proteins. In particular, translation initiation factors appear to modulate the expression of molecular chaperones involved in maintaining protein folding, membrane integrity, and pollen tube stability during stress. Physical interactions between translation-related proteins and heat shock proteins further support the existence of a broader regulatory network linking translational control with pollen thermotolerance. Together, these findings highlight selective translation as a key mechanism safeguarding reproductive success under climate-induced temperature extremes.

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Poster 82

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

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Deciphering how plants respond to adverse environmental conditions is essential both for understanding plant survival and for developing strategies to improve stress tolerance. In the case of heat stress (HS), transcriptional regulation explains much of the medium-term response, but cannot fully account for the rapid down-regulation of hundreds of transcripts that occurs immediately after stress perception. This short-term response relies on post-transcriptional mechanisms such as co-translational mRNA decay (CTRD), in which mRNAs associated with translating ribosomes are degraded while translation is still ongoing 1. During CTRD, transcripts are decapped and subsequently degraded by the 5′–3′ exoribonuclease XRN4 following the last translating ribosome. Despite its importance, the mechanisms controlling CTRD during HS remain poorly understood.Here, using genetic, biochemical and genomic approaches, we identify the conserved cochaperone R2T complex 2 as a regulator of CTRD in Arabidopsis. We found that the interaction between the decapping factors LSM1A and PAT1 is enhanced in the tpr5 mutant, defective in the R2T subunit RPAP3, suggesting altered decapping complex function. Consistent with this, representative decapping target transcripts were stabilized in tpr5 under both control and HS conditions. Transcriptome-wide 5′P-Seq analyses further revealed a global impairment of CTRD in tpr5, comparable to that observed in lsm1a lsm1b and xrn4 mutants. Mechanistically, R2T subunits become enriched in polysomes during HS and promote the accumulation of LSM1A in translating ribosomes. Our results suggest that the CTRD defects observed in tpr5 under control conditions arise primarily from altered decapping complex assembly, whereas during HS they are associated with impaired recruitment of LSM1A to polysomes. Together, these findings identify R2T/PFDL as a key regulator of co-translational mRNA decay and reveal distinct mechanisms by which it supports CTRD under basal and heat stress conditions.

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Poster 83

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Alternative transcription start sites as a regulatory layer of salt stress response in Arabidopsis.

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Plants adapt to environmental stresses such as salinity by regulating gene expression at multiple levels. One emerging mechanism is alternative transcription start site (TSS) usage, which can generate transcript and protein isoforms with distinct regulatory or functional properties. Although genome-wide studies have shown that alternative TSS selection is widespread, its biological significance during abiotic stress in plants remains largely unexplored.To investigate alternative TSS usage, we performed CAGE-seq on *Arabidopsis thaliana* seedlings exposed to salt stress. Analysis of the sequencing data revealed that the overall TSS landscape changes in response to salt stress: 2.2% of the identified genes exhibited altered usage of canonical and alternative transcription start sites. Approximately one-third of these genes possess an alternative transcription start site within the coding sequence, probably resulting in the translation of a truncated protein isoform, likely affecting both protein function and its subcellular localization.Our study thus establishes a genome-wide resource of salt-responsive alternative transcription start sites and identifies candidate genes for functional characterization. Future work will determine how alternative TSS usage shapes protein diversity, subcellular localization, and regulatory networks during salt stress, providing new insights into the role of transcript isoform diversity in plant environmental adaptation.

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Poster 84

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Translatome dynamics during the thermomorphogenesis response

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Thermomorphogenesis — the developmental response through which plants adjust to elevated ambient temperature, including hypocotyl and petiole elongation — depends on rapid, selective changes in mRNA translation as well as transcription. The best-characterised example is the temperature-dependent translational enhancement of PHYTOCHROME INTERACTING FACTOR 7 (PIF7). Existing translatome data, however, capture only the first hours of warming, leaving unresolved how translational control is deployed as plants transition from acute response to sustained acclimation.We are generating a Ribo-seq time course in Arabidopsis, with matched RNA-seq, spanning hourly, daily and continuous exposure to elevated temperature. This will distinguish transcript-level from translational regulation and reveal whether the translational control seen in the first hours of warming is sustained, reversed, or replaced as plants acclimate.One route by which translation could be tuned is the conserved stress pathway in which the GCN1-activated kinase GCN2 phosphorylates the α-subunit of eukaryotic initiation factor 2 (eIF2α), attenuating initiation. Whether this module is engaged during thermomorphogenesis is unknown. We are assaying eIF2α phosphorylation across elevated temperature conditions, testing thermomorphogenic responses in T-DNA insertion lines, and developing phospho-mutant lines as an additional genetic resource.Together, this project aims to broadly characterises translatome dynamics and the contributions of known translational stress pathways during thermomorphogenesis in Arabidopsis.

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Poster 85

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VPS13 plays an indirect role in translational repression through its contribution to AGO1 localisation

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The VPS13 gene family is conserved across eukaryotes and encodes large lipid-transport proteins. VPS13 proteins play key roles in membrane dynamics by mediating non-vesicular lipid transfer between intracellular organelles. Although these proteins have been extensively studied in yeast and mammals, their functions in plants remain poorly understood. Recently, the Arabidopsis thaliana VPS13S protein, also known as SHRUBBY, was reported to be involved in tasiRNA-mediated post-transcriptional gene silencing during ovule development. In particular, VPS13S has been proposed to contribute to the homeostasis of membrane-associated siRNA bodies. In this work, we focus on the involvement of VPS13S in the regulation of AGO1 cellular localisation, providing new information regarding the molecular framework involved in AGO1-dependent post-transcriptional gene expression.

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Poster 86

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Translational regulation in the plant temperature response.

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Plants acclimate to elevated ambient temperature through thermomorphogenesis, a developmental response characterised by elongation growth that enhances cooling and survival. Although the transcriptional regulation of thermomorphogenesis has been widely explored, the contribution of translational regulation remains poorly understood. We previously found elevated temperature to significantly alter the translatome of Arabidopsis seedlings, independent of transcriptional regulation, and quantitative proteomic analysis also revealed extensive proteome remodelling by temperature. As translation initiation is considered the rate-limiting step of protein synthesis, eukaryotic translation initiation factors (eIFs) serve as crucial regulatory points for controlling protein synthesis in response to environmental cues. We hypothesise that specific eIFs contribute to thermomorphogenesis by mediating temperature-dependent translational regulation. A collection of eif T-DNA insertion mutants was identified and screened for altered thermomorphogenesis phenotypes under elevated temperature conditions. We are currently characterising selected eif mutants at the molecular level by analysing the expression of thermomorphogenesis marker genes, quantifying accumulation of the central thermomorphogenesis regulator, PHYTOCHROME INTERACTING FACTOR7 (PIF7) and monitoring overall proteome dynamics.This project aims to identify translation initiation factors that regulate thermomorphogenesis and provides new insight into how translational control contributes to plant adaptation to elevated ambient temperature.

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Poster 87

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One receptor, multiple initiation sites: Structural alternatives and translational control of Arabidopsis CRE1

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Cytokinins (CKs) are plant hormones that play a crucial role in plant growth and development. CKs are perceived by three Arabidopsis histidine kinase receptors (AHKs) that act as transmembrane sensor kinases, triggering a downstream signaling cascade. Among these, AHK4/CRE1 is central to controlling root meristem activity and vasculature development.We found that the first intron of CRE1 undergoes alternative splicing, producing several transcript variants (Králová et al., 2024). Moreover, the CRE1 gene carries four alternative ATGs distributed within the first exon and the beginning of the second. Consequently, these alternative CRE1 transcripts can encode up to three hypothetical proteins with altered N-termini, potentially affecting the receptor's function.To test our hypothesis, we developed a unique tool comprising four CRE1 reporter constructs, each containing a green fluorescent protein (GFP) seamlessly inserted at a distinct ATG in frame with the downstream receptor sequence. Microscopic analysis of these reporters revealed that the last ATG, located at the beginning of the second exon, is the most active. Moreover, this GFP signal completely matches the expression pattern and subcellular localization of the classic CRE1-GFP translational fusion, whereas the fluorescent signal in the first three ATG reporters was negligible.These results, together with our proteomic data, confirmed the existence of a single, genuine CRE1 receptor protein, whose translation initiates from the last ATG. We therefore conclude that the multiple ATGs located upstream of the main ORF serve a regulatory function, fine-tuning CRE1 receptor translation.

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Poster 88

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uORF-mediated translational control of flowering genes in *Lupinus albus*

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Regulation of flowering has been studied extensively at the transcriptional level, yet control acting directly on translation remains largely unexplored in legumes, a group of major relevance for sustainable agriculture. Upstream open reading frames (uORFs), short coding sequences located in the 5′ untranslated regions (UTRs) of mRNAs, can modulate translation of the downstream main ORF (mORF) through mechanisms such as ribosome reinitiation or ribosome stalling. Here we investigate putative uORF-mediated regulation of flowering-related gene translation in white lupin (*Lupinus albus*).To enable systematic uORF discovery, we refined the existing *L. albus* genome annotation using transcriptomic and proteomic data to improve 5′ UTR delimitation. Integration of RNA-Seq and Ribo-Seq data across three developmental stages, spanning vegetative and reproductive shoot apices, uncovered widespread translational regulation independent of mRNA abundance and RiboTaper and RibORF analyses identified thousands of actively translated uORFs within annotated transcripts; mass spectrometry-based proteomics confirmed that several of the corresponding peptides accumulate in shoot apices, validating the ribosome profiling data. Five uORFs located in the 5′ UTRs of flowering-related transcription factors, uORFLaATHB-16, uORFLaSVP, uORFLaLHY, uORFLaAS1 and uORFLaSPL, were selected as putative regulators for detailed characterisation. Transcription start site sequencing confirmed co-transcription of each uORF with its mORF and ribosome profiling revealed four distinct stage-dependent occupancy patterns across the sampled stages.Transient dual-luciferase reporter assays in *L. albus* showed that uORFLaATHB-16 enhances mORF translation without altering reporter transcript levels, whereas uORFLaSPL represses mORF translation and simultaneously reduces reporter mRNA abundance, revealing two mechanistically distinct modes of translational control. Together, these findings establish uORF-mediated translational control as a previously uncharacterised regulatory layer acting on flowering-related transcription factors in a legume crop.

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Poster 89

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Dissecting the role of the Arabidopsis translational regulator ILITHYIA in response to abiotic stress

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The yeast translational regulator General Control Nonderepressible 1 (GCN1) is a ribosome-associated protein that plays a central role in the cellular response to stress. Under starvation, GCN1 promotes the activation of the protein kinase GCN2, leading to phosphorylation of eIF2α and global translational repression while selectively enhancing the translation of stress-responsive mRNAs. This mechanism enables cells to reprogram gene expression and maintain proteostasis under adverse conditions[1]. In *Arabidopsis thaliana*, ILITHYIA (ILA) is the only homolog of yeast GCN1 and is thought to retain some conserved functions. However, unlike in yeast, eIF2α phosphorylation in Arabidopsis does not correlate with a global reduction in protein synthesis, and ila and gcn2 mutants display distinct phenotypes, suggesting that ILA also performs GCN2-independent functions. Consistent with this idea, ILA has been implicated in diverse biological processes, including translational regulation, immunity, stress responses, flowering and chloroplast biogenesis[2,3].To further investigate the role of ILA in plant stress, we analyzed the response of ila mutants to abscisic acid (ABA), a central phytohormone that coordinates molecular and physiological responses to abiotic stress. Our results reveal that ila mutants are hypersensitive to ABA at both the molecular and physiological levels. Furthermore, we discuss the impact of ABA on ILA-dependent translational regulation, providing new insights into the role of ILA in plant stress signaling.

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Poster 90

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Translational regulation during vernalization-induced flowering in *Lupinus albus*

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Vernalization, the promotion of flowering by prolonged exposure to cold, is a key adaptive process that enables plants to synchronize reproductive development with favourable seasonal conditions. In legumes, vernalization-dependent flowering has been studied extensively at the transcriptional level, whereas the contribution of translational regulation remains largely unexplored. To address this gap, we characterized the translational landscape of *Lupinus albus* during vernalization by integrating ribosome profiling (Ribo-seq) with RNA sequencing (RNA-seq).Comparative analysis of translational changes between vernalized and control plants revealed several functional groups associated with flowering regulation. These included hormone-related processes, particularly genes involved in gibberellin biosynthesis, as well as key flowering regulators belonging to the MADS-box and DOF transcription factor families. Furthermore, differential translation was observed among genes associated with epigenetic regulation, including factors involved in histone deacetylation and chromatin remodelling, suggesting that translational regulation of chromatin-related regulators may contribute to the control of gene expression during vernalization-induced flowering.To investigate translational regulation mechanisms, we used the RiboTaper pipeline to identify actively translated open reading frames (ORFs), including upstream (uORFs) and downstream (dORFs). We identified vernalization-responsive uORFs in putative homologues of TCP14 (LalbChr13g174590), a regulator of floral transition, and bHLH143 (LalbChr08g113930), a developmental transcription factor. The putative homologue of SHORT VEGETATIVE PHASE (SVP) (LalbChr09g123450), a MADS-box transcription factor involved in flowering repression, was translated under control and vernalized conditions. Ribo-seq analysis revealed condition-dependent differences in uORF translation, suggesting that SVP upstream regulatory regions may modulate translation during vernalization. Together, these findings suggest that uORF-mediated regulation within 5′ untranslated regions contributes to fine-tuning translation during flowering transition. Overall, our study shows that translational regulation represents an additional control layer during vernalization in *L. albus* and broadens understanding of flowering-time regulation in legumes, provides a foundation for future functional studies of translational control during plant developmental responses to environmental changes.

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Poster 91

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Investigating siRNA-mediated silencing in duckweeds, a potential model for translational repression in plants

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In eukaryotes, post-transcriptional gene silencing (PTGS) involves the production of small interfering RNAs (siRNAs) and their incorporation into ARGONAUTE (AGO) proteins. The resulting siRNA–AGO complex mediates mRNA cleavage and/or translational repression, leading to degradation via RNA decay pathways and ultimately resulting in reduced protein expression. In plants, mRNA cleavage is believed to be the main mode of action of siRNA-AGO complexes, and although translational repression has been shown, it is not yet well understood. Interestingly, in duckweeds, a group of small aquatic flowering plants, siRNAmediated transgene silencing causes strong loss of protein synthesis, while mRNA levels remain largely unchanged. On the contrary, loss of siRNA results in resoration of protein levels. This observation suggests that translational repression, rather than mRNA degradation, may be a predominant mechanism of gene regulation in this group of plants. To investigate this hypothesis, we are exploiting transgene silencing in duckweeds to analyze the fate and turnover of mRNAs, targeted or not by siRNAs, using a combination of polysome profiling, SLAM-seq, and ribosome footprinting. Understanding silecing mechanisms in duckweeds will expand the knowledge of silencing mechanisms, mostly investigated in a handful of plant models, and potentially provide further insights into their translational repression mechanisms.

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From RNA molecules to field trials: application of exogenous RNAs for improvement of the nitrogen fixing symbiosis in legume plants

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The RNA molecule has gained a central place in biotechnological approaches of the XXI century. Both small and long non-coding RNAs (sRNAs and lncRNAs, respectively) have been shown to fine tune modulate fundamental developmental processes as well as their adaptation to the environment. These RNAs have been used to modify important physiological traits in genetically modified and/or edited plants. However, genetic engineering of crops is expensive, time-consuming and should be applied each time to an individual species or varieties/cultivars. In the last decade, it has been evidenced that plants can take up RNAs from the environment, leading to the proposal that application of exogenous RNA can be used to manipulate the expression of endogenous genes, helping plants to adapt to changing environmental conditions. Our previous transcriptomic, translatomic and sRNA-seq approaches identified lncRNAs and sRNAs from legumes species that are regulated and/or play functions in symbiosis with nitrogen fixing bacteria. This symbiosis results in a new root organ, the nodule, where bacteria lodge and reduce atmospheric nitrogen to forms that can be assimilated by the plant. We will present data evidencing that application of nanostructured exogenous RNAs improves in 30% nodulation, increases shoot biomass and enhances the expression of symbiotic genes under controlled laboratory conditions. Moreover, it produces nearly 8% of grain yields (120-150 Kg/ha) in field trial experiments This non-transgenic strategy not only improves nitrogen fixation and grain yields in legume crops, but it is also beneficial to incorporate nitrogen in soils for subsequent crops.

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Comparative transcriptomic and translatomic profiling evidence the contribution of ethylene signaling at early stages of the nitrogen fixing symbiosis

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The establishment of nitrogen-fixing symbiosis between legumes and rhizobia requires extensive reprogramming of host cell developmental pathways. Although transcriptional responses to rhizobial infection have been extensively characterized (Roux et al., 2014; Schiessl et al., 2019), much less is known about how translational regulation contributes to the establishment of the symbiosis. To better understand the role of translational control during early stages of symbiosis and its relationship with ethylene signaling, we applied Translating Ribosome Affinity Purification (TRAP) combined with RNA sequencing to compare the transcriptome (total RNA) and translatome (TRAP-RNA) of wild-type *Medicago truncatula* plants and the *ethylene-insensitive2/sickle* (*ein2/skl*) mutant following inoculation with *Sinorhizobium meliloti* (Penmetsa and Cook, 1997). Differential expression analysis identified extensive transcriptional and translational reprogramming in response to rhizobial inoculation. In both genotypes, upregulated genes outnumbered downregulated genes, indicating that symbiotic establishment is predominantly associated with gene activation. The *skl* mutant exhibited a substantially larger number of differentially expressed genes than wild-type plants, consistent with the previously described hypersensitive response of *ein2*/skl mutants to Nod-factor signaling (Larrainzar et al., 2015). Comparison of transcriptomic and translatomic responses revealed genes whose regulation was specific to the translatome, supporting an important contribution of post-transcriptional mechanisms at early symbiosis. Moreover, specific mRNAs were translationally regulated exclusively in wild-type roots but not in *ein2*/*skl* mutants. Taken together, these results revealed that integrating transcriptomic and translatomic analyses not only provides a comprehensive view of the molecular programs activated during symbiosis but also highlights the contribution of ethylene signaling to the regulation of gene expression at the transcriptional and translational levels at early stages of the root nodule symbiosis.

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Poster 94

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Dual regulation of the receptor-like kinase BIR1 involves site-directed transcript cleavage and 5'-leader-mediated translational control

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Plants must tightly control immune responses to ensure effective defense while avoiding immune-associated self-damage. In Arabidopsis thaliana, the receptor-like kinase BIR1 functions as a negative regulator of multiple immune resistance pathways. Previous reports have shown that misregulation of BIR1 leads to cell-death-like phenotypes, underscoring the need for precise control of its expression. However, the mechanisms governing BIR1 expression have remained largely unexplored. Here, we show that BIR1 transcripts are subject to post-transcriptional gene silencing and, through mutational analysis, identify the precise cleavage sites involved. In addition, we demonstrate that virus infection compromises BIR1 translation efficiency and reveal a previously uncharacterized translational control mechanism mediated by the BIR1 5'- leader, involving the combined action of multiple upstream open reading frames and a virus-responsive long non-coding RNA derived from the antisense At4g39838 locus. These findings reveal an unexpected and sophisticated regulatory architecture controlling BIR1 expression, with potential relevance for the broader BIR receptor family and plant immune signaling networks.

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S5 Posters - Non-Coding RNAs and Other

Poster 95

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Engineering nanomaterials for regulatory RNA delivery in plants

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The ability to introduce exogenous regulatory RNAs, including small RNAs and other non-coding RNAs (ncRNAs), into plant cells provides a powerful means to investigate and manipulate RNA-mediated gene regulation. However, their delivery remains challenging due to RNA susceptibility to degradation and the extracellular and cellular barriers restricting access to intracellular targets.1 Materials science and nanotechnology offer a versatile toolbox to address these limitations.2 Polymers, peptides and other functional biomaterials can associate with RNA through electrostatic and non-covalent interactions, enabling its complexation and protection within nanoscale assemblies with tunable physicochemical properties. Parameters such as size, surface chemistry, binding affinity and stability can be engineered to modulate interactions with plant biological barriers and cargo release. The diversity of regulatory RNAs further highlights the need for adaptable delivery platforms.
Here, we present a materials-based framework for RNA delivery in plants using two complementary platforms as illustrative examples. The first relies on polyphenol–surfactant coacervates that form dynamic supramolecular nanoassemblies stabilized primarily by hydrogen-bonding interactions. Their polyphenol-rich environment could promote RNA association and protection through hydrogen bonding. The second platform is based on layer-by-layer (LbL) electrostatic assembly on silica nanoparticles, where sequential adsorption of oppositely charged species enables modular control over the particle interface. The polyanionic nature of RNA can be exploited to incorporate it directly as an anionic building block of the multilayer structure, enabling control over RNA loading, protection, surface properties and release.
Together, these examples illustrate how distinct interaction mechanisms and material architectures can be applied to address a central challenge in RNA delivery: achieving sufficiently strong RNA–material interactions to protect the cargo while retaining the reversibility required for the release of functional RNA. By integrating polymer and supramolecular chemistry, nanotechnology and plant RNA biology, these approaches could contribute to the development of new tools for the delivery of regulatory RNAs and facilitate the manipulation and investigation of RNA-mediated regulatory processes in plants.

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Poster 96

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The long non-coding RNA ACHLYS modulates biomolecular condensates and regulates alternative splicing

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Alternative splicing (AS) is a major source of transcriptome diversity and is tightly controlled by RNA-binding proteins. Increasing evidence suggests that long non-coding RNAs (lncRNAs) also contribute to AS regulation, although their underlying mechanisms are still poorly understood in plants. Using Arabidopsis thaliana as a model, we identified the lncRNA ACHLYS as a novel regulator of AS that interacts with the splicing factor NSRa. Our current work focuses on the molecular basis of this interaction, including how it influences splicing factor condensation and subnuclear organization. Together, our findings provide new mechanistic insight into how lncRNAs modulate splicing factor activity and expand the repertoire of regulatory mechanisms controlling post-transcriptional gene regulation in plants.

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Poster 97

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Role of the ALTERNATIVE SPLICING COMPETITOR (ASCO) lncRNA in ABA response.

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Long noncoding RNAs (lncRNAs) are emerging regulators of gene expression, modulating developmental programs and environmental responses in plants through diverse molecular mechanisms. In *Arabidopsis thaliana*, the ALTERNATIVE SPLICING COMPETITOR (ASCO) lncRNA has been shown to modulate the transcriptome through interactions with the Nuclear Speckle RNA binding proteins or NSRs which are splicing factors. Overexpression of the ASCO lncRNA in Arabidopsis induces significant changes in alternative splicing (AS) suggesting a link between ASCO deregulation and AS. However, many open questions remain regarding its precise mode of action and physiological roles.

In this work, we found that ASCO in addition to a low level of expression in several tissues, shows a particular strong accumulation during silique development and in dry seeds. As abscisic acid (ABA) is the primary phytohormone governing seed development, maturation, and dormancy, the spatiotemporal expression of ASCO in these tissues directly aligns with a potential role of the lncRNA in these ABA-mediated developmental processes. Using CRISPR/Cas9, we generated partial and full ASCO deletion lines, which exhibited a marked ABA-insensitive phenotype during seed germination. At the genome-wide level, the ASCO deletion induced widespread deregulation of core ABA pathway genes, including key biosynthetic enzymes (ABA1, AAO3), ABA receptors (PYL1), and major downstream responsive targets (PER1, ABI5). Interestingly, several splicing factors and AS events were shown to be modulated by ABA and we are exploring a potential link of ASCO expression in seeds with changes in AS. These results demonstrate a role for ASCO in the ABA response, further supporting novel regulatory functions of lncRNAs in hormone-mediated plant signaling.

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Poster 98

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Cold-responsive lncRNAs coordinate cold tolerance and hypocotyl development in Arabidopsis

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Long noncoding RNAs (lncRNAs) play important roles in regulating plant development and stress responses[1,2], including adaptation to low temperature environments[3,4]. Although increasing numbers of lncRNAs have been identified, the molecular mechanisms by which they coordinate plant development and stress adaptation remain largely unknown. Here, we identified three cold-responsive, intergenic lncRNAs in *Arabidopsis thaliana*. Loss of function of these lncRNAs resulted in increased freezing sensitivity and elongated hypocotyls under short-day conditions, indicating that they are involved in both cold tolerance and early plant development. Promoter-reporter assays demonstrated that lncRNA expression is predominantly restricted to root and shoot meristematic tissues. Furthermore, transcriptomic analyses revealed that the identified lncRNAs regulate expression of the transcription factor genes C-REPEAT/DEHYDRATION-RESPONSIVE ELEMENT BINDING FACTORS (CBFs) in a temperature-dependent manner. Together, our findings suggest that these lncRNAs coordinate cold response and hypocotyl development through the regulation of CBFs, providing new insights into the molecular mechanisms by which lncRNAs integrate environmental stress responses with plant development in Arabidopsis.

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Poster 99

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Functional conservation of long non-coding RNAs involved in lateral root development in Brassicaceae

Laura Chabes Pereira, Thomas Blein

Lateral roots are essential for root system architecture, optimizing water and nutrient uptake while allowing plants to adapt to changing environmental conditions. While the molecular mechanisms controlling lateral root initiation are highly conserved across Brassicaceae, little is known about the long non-coding RNAs (lncRNAs) regulating this process. Unlike protein-coding genes, lncRNAs generally exhibit low sequence conservation among species, presenting a challenge to identify homologues and understand their evolutionary conservation. However, lncRNA conservation extends beyond primary sequence to features such as secondary structure, genomic synteny and expression patterns. This work investigates the functional conservation of lncRNAs involved in lateral root development within the Brassicaceae family. We first identified conserved lncRNAs across eight Brassicaceae species using approaches based on synteny and sequence similarity. Candidate lncRNAs will then be overexpressed in *Arabidopsis thaliana* root protoplasts, and their effects on gene expression will be assessed by RNA sequencing to identify lncRNAs with potential regulatory functions and tissue-specific responses. Selected lncRNAs will be validated in A. thaliana mutant lines by evaluating their effects on root architecture. Finally, functional conservation will be tested through complementation of *A. thaliana* mutants with orthologous lncRNA sequences from different Brassicaceae species.

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ANR RESOLAT

Poster 100

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Phase separation compartmentalizes chloroplast RNA processing and translation during cold acclimation

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Plants are sessile organisms that cannot escape adverse environmental conditions and therefore rely on highly dynamic intracellular mechanisms to sense and adapt to temperature fluctuations. Biomolecular phase separation has emerged as a powerful principle for reorganizing cellular biochemistry in response to physicochemical change, yet its functional relevance in plants has only recently begun to be uncovered. We have shown that the chloroplast RNA-binding protein CP29A undergoes reversible, cold-induced phase separation, forming condensates that localize near chloroplast nucleoids and recruit RNAs (Legen et al., 2024). This condensation behavior is functionally important: CP29A is required to maintain splicing and thus, chloroplast development. In addition, outside granules, RuBisCo mRNA levels and protein accumulation is maintained in the cold via CP29A (Lenzen et al. 2025). These findings identify temperature-regulated condensation of CP29A as a plant-specific adaptation mechanism that supports localized RNA metabolism and chloroplast gene expression in the cold. We now present data on additional proteins that co-reside in CP29A granules, revealing a broader condensate-based platform coordinating chloroplast gene expression with a new function for photosystem II biogenesis during chloroplast development in the cold. Our work and work from other plant labs supports the idea that phase separation is likely to be particularly important in plants because, as immobile organisms, they must continuously remodel intracellular organization to cope with environmental stress, in particular temperature.

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Poster 101

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Characterization of an autophagy-related transcriptional regulator involved in strawberry fruit ripening

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Autophagy is a catabolic process required for the recycling of cellular components and essential for cell homeostasis. Autophagy is active at a basal level under normal conditions but can be induced in response to several stresses and during certain developmental programs[1]. In a previous work we have determined that autophagy is active during strawberry fruit ripening and that blocking autophagy either genetically or chemically produces a delay in ripening[2]. Now we have identified a strawberry b-zip transcription factor that is expressed in strawberry fruit receptacle during ripening and by Renilla/Luciferase assay we have determined that is able to directly bind to strawberry autophagy genes promoters, inducing their expression. We have confirmed its capacity to increase the amount of autophagosome formation in protoplasts of Arabidopsis. Finally, we have determined that transient silencing or over-expression of this transcription factor in strawberry receptacle affects fruit ripening in opposite ways and has an effect over autophagy genes expression, pointing towards a role as an autophagy-mediated strawberry ripening regulator.

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Poster 102

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A spatiotemporal transcriptome atlas reveals the tissue-specific regulatory landscape of strawberry fruit ripening

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Strawberry fruit ripening involves complex transcriptional programs controlling coloration, softening, hormone signaling, and specialized metabolism. However, most transcriptomic studies rely on whole-fruit analyses, masking tissue-specific regulatory processes. Here, we generated a high-resolution spatial transcriptome atlas of woodland strawberry (*Fragaria vesca*) receptacles using laser capture microdissection coupled with RNA sequencing. Four tissues (epidermis, cortex, vascular bundles, and pith) were profiled at green and red stages.
Transcriptomic analyses revealed tissue- and stage-specific expression patterns, with vascular bundles and epidermis showing the highest proportion of specific transcripts. WGCNA identified modules associated with tissues and stages, enriched in processes such as auxin signaling (vascular bundles), lipid metabolism (epidermis), phenylpropanoid biosynthesis (vascular bundles and epidermis), and wax biosynthesis (epidermis). These data were integrated into a “Microdissection Strawberry Atlas” available as an app in the StrawViz module of the PlantaeViz platform (https://plantaeviz.tomsbiolab.com/), which will provide, upon publication, a valuable resource to explore tissue-specific gene expression during strawberry fruit development and ripening.

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Poster 103

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Insights into the role of type 4 metallothioneins in the response of Arabidopsis thaliana to cadmium stress

Magdalena Michalak, Marcel Antoszewski, Agnieszka Pawełek, Grażyna B. Dąbrowska, Agnieszka Mierek-Adamska

Metallothioneins (MTs) are cysteine-rich intracellular proteins involved in the binding and homeostasis of heavy metals, particularly zinc (Zn), copper (Cu), and cadmium (Cd). Plant MTs exhibit greater structural diversity than those of other organisms and are classified into four types (pMT1–pMT4) based on the number and arrangement of cysteine residues. Cadmium is one of the most toxic environmental pollutants and a widely used model for studying plant responses to heavy metal stress. Although pMTs are involved in metal homeostasis, stress responses, and plant development, the specific role of type 4 MTs remains only partially understood[1]. This study investigated the response of the *Arabidopsis thaliana* mt4a/mt4b mutant lacking type 4 metallothioneins2 and wild-type (Col-0) plants to Cd exposure. Plants were grown in vitro on Murashige and Skoog (MS) medium or MS supplemented with $CdCl_2$ (10–100 µM). Germination was assessed using the germination index (GI), coefficient of velocity (CV), germination rate index (GRI), and mean germination time (MGT). Total antioxidant capacity via CUPRAC, phytohormone levels via LC-MS/MS, and expression of selected hormone-related genes via RNA sequencing were analysed. The *mt4a/mt4b* mutant exhibited improved germination under Cd stress, with higher GI, CV, and GRI values and lower MGT than wild-type plants, particularly at 60 µM Cd. No genotype-dependent differences in total antioxidant capacity were detected. Salicylic acid (SA), abscisic acid (ABA) and gibberellin 7 (GA_7) levels remained comparable between genotypes, while higher jasmonic acid (JA) levels were detected in the mutant under 60 µM and 100 µM Cd concentrations. In addition, 10 µM Cd exposure enhanced the expression of auxin-responsive genes in *mt4a/mt4b* plants, suggesting altered auxin signalling. These findings indicate that the physiological role of type 4 metallothioneins under cadmium stress is complex, and further studies are needed to clarify their contribution to plant adaptation to metal stress.

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Poster 104

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Multilayered post-transcriptional control of cytokinin signaling in Arabidopsis

David Zalabák, Ivona Kubalová, Jakub Hajný, Alžbeta Mičúchová

Cytokinins (CKs) are essential plant hormones controlling fundamental aspects of growth, development, and environmental responses. In Arabidopsis, the histidine kinase receptor AHK4/CRE1 is a key player in CK sensing, regulating root meristem activity and vasculature development. While downstream CK signaling components are well characterized, the precise mechanisms controlling the abundance and function of the CRE1 receptor remain poorly understood.Here, we demonstrate a novel multilayered regulatory mechanism that fine-tunes CRE1 expression at the post-transcriptional level. First, we show that the CRE1 gene undergoes extensive alternative splicing, yielding several transcript variants. Among these, a specific intron-retained variant encodes a truncated, non-canonical receptor, CRE1int7, which modulates active cytokinin signaling (Králová et al., 2024). Second, we identified a diverse set of transcript variants arising from first-intron splicing. Additionally, the CRE1 gene carries multiple alternative translation initiation codons. To decipher the role of these alternative initiation sites, we developed two novel GFP-reporter approaches. Our reporters revealed that only specific ATGs are actively translated into functional proteins, whereas the upstream initiation codons primarily contribute to the translational control of the canonical CRE1 receptor.In summary, our findings uncover sophisticated mechanisms governing cytokinin signaling at the post-transcriptional level. By precisely orchestrating receptor homeostasis, this regulation adds a crucial, previously uncharacterized layer of control to plant hormone signaling.

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Poster 105

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Insights into plant metal homeostasis: transcriptomic and hormonal reprogramming in *Arabidopsis thaliana* type 4 metallothionein knockout under zinc starvation

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Plant metallothioneins (pMTs) are cysteine-rich proteins essential for metal homeostasis and detoxification of toxic heavy metals. Among them, type 4 metallothioneins (pMT4) exhibit a unique structural feature: a conserved histidine residue that allows them to 'distinguish' essential zinc from other toxic metals. While zinc binding promotes correct protein folding, binding with other ions (e.g., cadmium) leads to aberrant protein folding. Traditionally, pMT4s have been viewed simply as seed-specific zinc storage proteins, but recent findings suggest they may play broader regulatory roles[1]. To test this hypothesis, we generated a double *Arabidopsis thaliana* mutant lacking the AtMT4a and AtMT4b genes (*mt4a/mt4b* mutant) using CRISPR/Cas9[2].Plants were grown in vitro on standard MS media and under zinc starvation for three weeks. Phenotypic analysis revealed that the *mt4a/mt4b* knockout stimulated seed germination but led to significantly reduced shoot length under control conditions. Transcriptomic profiling showed that the mutant's global transcriptional response to the tested conditions was less robust compared to the wild-type. However, we observed a strong, specific remodeling of ion homeostasis networks, with upregulated expression of key metal transporters, such as YSL, COPT, and ZIP, in mutant plants regardless of zinc availability. Most striking changes were found in the phytohormonal network. Lack of MT4 significantly reduced accumulation of jasmonic acid (JA) and indole-3-acetic acid (IAA) under control conditions. Interestingly, zinc deficiency stimulated accumulation of both hormones in the mutant, which directly correlated with an activated biosynthetic pathways. Additionally, while the overall antioxidant capacity (AC) decreased under zinc starvation, the mutant still displayed a distinct profile of SOD, CAT, GPX, and APX gene expression. In conclusion, our data redefine MT4s from simple zinc storage proteins to active regulatory components. The extensive transcriptomic and phytohormonal reprogramming in mt4a/mt4b mutant reveals the crucial role of type 4 pMTs in orchestrating plant adaptation to environmental ever-changing metal-bioavailability.

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Poster 106

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RTL1 orchestrates *NCR* gene expression and symbiosome integrity in *Medicago* nodules

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The development of symbiotic dinitrogen(N_2)-fixing nodules in legumes involves complex waves of transcriptomic and epigenetic reprogramming, accompanied by dynamic changes in small RNA populations and DNA methylation. Here, we identified a nodule-specific RNAse III-LIKE protein, MtRTL1b, in the legume species *Medicago truncatula*, homolog to the silencing suppressor AtRTL1 in *Arabidopsis thaliana*. Expression profiling highlighted the drastic induction of *MtRTL1b* expression upon infection by *Sinorhizobium meliloti. MtRTL1b* expression in mature nodules was confined to the interzone and the central zone, where bacteroid terminal differentiation and N_2 fixation take place respectively. *MtRTL1* knockdown in transgenic roots led to a higher proportion of ineffective white nodules, reduced N_2 fixation efficiency, and compromised symbiosome integrity and bacteroid viability. In contrast to the siRNA-suppressing effect of Arabidopsis AtRTL1, *MtRTL1* knockdown nodules showed no global changes in sRNA populations or DNA methylation levels, and *MtRTL1b* expression in a tobacco leaf system had little impact on hairpin-derived siRNA accumulation. Remarkably, transcriptome analyses of *MtRTL1* knockdown nodules revealed extensive downregulation of late NODULINS, including *Nodule-Specific Cysteine-Rich* (*NCR*) genes, many of which are essential for bacteroid terminal differentiation and persistence. Notably, we observed that the MtRTL1-regulated *NCR* genes have a greater potential to form long dsRNA structures, which are the known targets of RNase III enzymes. These findings suggest that, unlike AtRTL1, which is a general suppressor of siRNA pathways in Arabidopsis, MtRTL1b is a specialized RNAse III, orchestrating transcriptome shifts at the nodule differentiation stage, independently from siRNA silencing. This reveals a unique function of non-dicer RNase III enzymes in plant developmental processes.

Poster 107

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Tracing transcript mobility: RNA dynamics in the roots

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RNA functions as a mobile signal in plants, much like metabolites and proteins, moving cell-to-cell through plasmodesmata (PD) and long-distance from above ground tissues to roots via the phloem-dependent transport pathway. Grafting studies have revealed that certain RNAs can also travel against the phloem stream from root to shoot. Some siRNAs and mobile mRNAs have even been shown to move from the root into pollen forming meiocytes in flowers. However, the specific root cells these mobile RNAs are using to facilitate this transport are currently unknown. Here, we present an approach to visualise mRNA dynamics in the root by fusing the RNA aptamer Broccoli (3WJ-4xBro) to the highly mobile TRANSLATIONALLY CONTROLLED TUMOR PROTEIN 1 (TCTP1) mRNA. Using grafting assays with wild type plants, we aim to track long-distance movement at the mRNA level and identify cell types in which most mobile RNA is transported and/or unloaded. Similar to endogenous small RNAs and mRNAs, viral RNAs have long been known to be highly mobile and systemically infect distant plant tissues by moving locally between cells via the PD and long-distance via the vasculature to non-infected tissue, yet the dynamics of viral RNA movement within roots and from roots to shoots are still poorly described and understood. Our group utilised TURNIP MOSAIC VIRUS RNA tagged with GFP (TuMV-GFP) to visualise its spread from inoculated to systemic leaves. We aim to use TuMV-GFP to visualise movement and dynamics of viral RNA in and from roots and correlated it with movement of 3WJ-4xBro-tagged mobile mRNAs.

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Poster 108

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Proteolytically processing of the new characterized ScDef1 homologue AtDef1l promotes its nuclear localization

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Eukaryotes evolved complex mechanisms to achieve required protein levels though transcriptional, post-transcriptional and translational regulation. One fascinating regulator in these processes is the yeast protein Def1. The cytoplasmatic full lengths Def1 can be proteolytically processed, and its N-terminal part interacts with ubiquitinated small ribosomal subunit to promote translation dependent mRNA decay. During transcriptional stress the N-terminal Def1 part is imported into the nucleus and facilitates the degradation of ubiquitinated RNA-polymerase II. Despite its function at the core of transcription and translation, no Def1 homolog was identified outside of the fungal kingdom. We therefore set out to characterize a potential homolog of Def1 in plants. *Arabidopsis thaliana* encodes two Def1-like proteins (AtDef1l-A/B), which shared 35% amino acid (aa) similarity to *Saccharomyces cerevisiae* Def1. The first 75 aa are the most conserved and contain a ubiquitin-associated (UBA)-like domain, while the remaining 756 aa are intrinsically disordered and include polyglutamine (polyQ) tracs. Moreover, AtDef1l-A/B could be recognized by the ScDef1 antibody and are neighboring genes with 99.5% aa similarity. Promoter-reporter lines showed that AtDef1l-A/B are expressed in the vascular tissue. Partial trypsin digestion mass spectrometry and western blot showed that AtDef1l-A/B are like ScDef1 proteolytically processed, thereby separating the UBA-like domain from the polyQ tracs. Subcellular localization studies of fluorescence reporter lines of different AtDef1l-A parts indicate that the N-terminal part localizes with higher frequency to the nucleus, compared to full lengths protein and that the polyQ tracs promote cytoplasmatic localization. Overexpression of mCherry-AtDef1l-A-GFP, but not mCherry-AtDef1l, results in elongated petioles compared to wild type. This suggests that the C-terminal GFP-tag modulates the function of the polyQ rich C-terminal and thereby causes the phenotype.In Summary we identified ScDef1 homologues in plants, linked the proteolytical processing with nuclear localization and laid the foundation to study AtDef1l-A/B in the context of post-transcriptional gene regulation.

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S6 Posters - Epigenetics & Epitranscriptomics

Poster 109

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Mediator Recruitment Defines ARF Activation

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Auxin-dependent transcription relies on the antagonistic activities of AUXIN RESPONSE FACTORs (ARFs), yet how transcriptional activation is molecularly specified remains unclear. Here, we identify a minimal region within the intrinsically disordered middle region of an A-class ARF that is necessary and sufficient for transcriptional activation. This region mediates selective recruitment of the Mediator complex, thereby establishing a mechanistic link between ARF identity and co-activator engagement. Beyond simple recruitment, we show that ARFs form subnuclear condensates that selectively partition distinct cofactors: activator ARFs enrich Mediator, whereas repressor ARFs concentrate the co- repressor TOPLESS. This spatial segregation depends on the presence of our newly found activation domain and is consistent with some kind of phase separation-driven organization of transcriptional complexes. Strikingly, transfer of the activator region into a repressive B-class ARF is sufficient to reprogram it into an activator, indicating that cofactor recruitment operates as a dominant, modular switch. Our findings support a model in which biochemical features of ARFs—specifically intrinsically disordered regions—govern the nuclear partitioning of cofactors, thereby controlling transcriptional outputs. This work reveals a biophysical mechanism underpinning auxin-responsive gene regulation and provides insight into how activator functions may originate during evolution.

Poster 110

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Non-coding regulation of auxin canalization

Jakub Hajny

The formation of plant vascular networks depends on auxin canalization, a self-organizing process in which auxin transport becomes progressively restricted to narrow channels that later differentiate into vascular strands. This process requires the coordinated expression and polarity of PIN-FORMED1 (PIN1) auxin transporters, yet the mechanism that confines PIN1 expression to discrete canalization domains remains unclear. Here, we identify two long non-coding RNAs, RIVA1 and RIVA2, embedded in the Arabidopsis thaliana PIN1 promoter. RIVA1 and RIVA2 are expressed in actively forming vascular tissues and are rapidly induced by auxin, but their transcripts are highly unstable, suggesting a function associated with their genomic locus rather than with stable RNA products. CRISPR-mediated deletion of the RIVA1/2 region causes defects in leaf venation and vascular regeneration after wounding. At the cellular level, this deletion expands PIN1 expression domains and disrupts the formation of narrow, continuous PIN1-expressing canals. These results indicate that the RIVA1/2 genomic region functions as an enhancer-like element that restricts PIN1 expression during canalization. We further identify the transcription factors ATHB4 and UIF1 as candidate regulators acting at this region to fine-tune PIN1 expression and vascular pattern formation. Our study uncovers a non-coding enhancer module that spatially constrains PIN1 expression, providing a mechanism by which auxin canalization generates precise and adaptable vascular networks.

Poster 111

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From flowering time to genome-wide chromatin reprogramming: H3K27me3 demethylases orchestrate temperature responses

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Flowering time is a key crop trait, as premature or delayed flowering can severely reduce yield. Plants integrate endogenous and environmental cues to determine flowering onset. Although ambient temperature strongly influences flowering time, the role of epigenetic regulators in floral thermosensory pathways remains poorly understood. We found that the histone demethylases EARLY FLOWERING 6 (ELF6) and JUMONJI 13 (JMJ13) fine-tune temperature-dependent flowering in Arabidopsis. The loss of function *elf6-3 jmj13-1* double mutant shows reduced sensitivity to low ambient temperature, through a mechanism requiring the floral thermosensory regulator FLOWERING LOCUS M (FLM) and FLOWERING LOCUS C (FLC). Our results reveal that H3K27me3 demethylation is required for temperature-dependent regulation of FLM and FLC expression, with ELF6 and JMJ13 preventing excessive H3K27me3 accumulation at the FLM and FLC loci under low temperatures to maintain its transcriptional responsiveness. Furthermore, integrated transcriptomic and genome-wide H3K27me3 profiling reveal that H3K27me3 demethylases coordinate chromatin and transcriptional reprogramming in response to ambient temperature, extending their role from floral regulation to genome-wide control of temperature-responsive gene expression. Together, our results demonstrate a central role for H3K27me3 removal in environmental adaptation, providing insight into how plants integrate epigenetic regulation with ambient cues to optimize growth and reproductive timing under variable climatic conditions.

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Poster 112

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SUMOylation of CURLY LEAF modulates target binding activity of POLYCOMB REPRESSIVE COMPLEX 2 during photomorphogenesis in Arabidopsis

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The transition from skotomorphogenic to photomorphogenic development is a critical event in plant life triggered by light perception. This transition involves extensive chromatin reorganization and the establishment of a new transcriptional landscape. Polycomb Repressive Complex 2 (PRC2), which catalyzes the tri-methylation of Histone H3 at Lysine 27 (H3K27me3) to promote chromatin compaction and transcriptional repression, plays a central role in this process, influencing the expression of thousands of genes during development. Despite the importance of PRC2 in genome regulation, how its activity is modulated by environmental signals remains poorly understood. Our work demonstrates that SUMOylation is a key regulatory mechanism controlling PRC2 activity during the skoto-photomorphogenic transition. We show that CURLY LEAF (CLF), the main catalytic subunit of PRC2, is required for the induction of photomorphogenic development. CLF protein levels remain constant between dark and light conditions; however, CLF is activated through light-dependent SUMOylation mediated by the E2 conjugating enzyme SCE1, whose abundance is enhanced by light. Genetic and molecular analyses reveal that mutating all predicted SUMO sites in CLF impairs its function. While SUMOylation does not affect CLF interaction with other PRC2 components, it is required for proper H3K27me3 deposition at genes whose repression is essential for efficient photomorphogenic establishment. Together, our findings identify SUMOylation as a positive regulator of CLF activity that links light signaling to PRC2-mediated chromatin repression during the dark-to-light transition. .

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Poster 113

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Genetic and epigenetic regulation of winter cold responses in Arabidopsis

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Vernalization and cold acclimation are plant strategies that evolved to enhance their fitness during the winter season. The molecular mechanisms behind these processes in Arabidopsis have been intensively studied. However, how plants measure the duration of long-term cold exposure has not been fully understood. Our research shows that cytosolic chaperonin is crucial for appropriate long-term cold responses by regulating plant circadian clocks. Furthermore, one of the clock components, REVEILLE4 (RVE4) and RVE8 directly activate VERNALIZATION INSENSITIVE 3 (VIN3) and the C-REPEAT BINDING FACTOR (CBF)/DEHYDRATION-RESPONSIVE ELEMENT BINDING 1 (DREB1) family genes, which are key regulators of vernalization and cold acclimation, respectively. The activation of the VIN3 and CBF/DREB1 genes was specific to the time of day, indicating that cold exposure during the day is critical for long-term cold responses. In contrast, COOLAIR, an antisense RNA transcribed from FLC locus is not involved in the winter cold responses. Our research delves deeper into understanding the regulatory mechanism governing these two distinct long-term cold responses.

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Poster 114

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Insights into the structural features of epigenetically active MITEs

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Miniature inverted-repeat transposable elements (MITEs) are non-autonomous DNA transposon relics (i.e., they do not encode their own transposase) characterized by terminal inverted repeats (TIRs). Frequently located near or within genes, plant MITEs are a major source of 24-nt small RNAs (sRNAs). This sRNA production relies partially on their TIRs, which upon transcription allow MITEs to fold into hairpins. These MITE-derived harpins are recognized by DCL3, feeding directly into the RNA-directed DNA methylation (RdDM) pathway in a non-canonical manner. MITE insertions often drive the transcriptional rewiring of adjacent loci. Recently, our group demonstrated that MITEs and endogenous inverted repeats (IRs) act as methylation rheostats that alter local 3D chromatin architecture; MITE-derived epigenetically active sRNAs trigger self-methylation, promoting short-range chromatin loops that activate or repress neighboring genes.In this work, we sought to determine the structural features of MITEs that are associated with specific sRNA production pathways and their potential to be epigenetically active. To do so, we analyzed publicly available sRNA datasets from Arabidopsis, rice (*Oryza sativa*), and tomato (*Solanum lycopersicum*) mutants defective in RNA Polymerase IV (Pol IV), which lack canonical RdDM 24-nt sRNA. Our results suggest that while most MITEs produce sRNAs via the canonical RdDM pathway, a distinct subset continues to generate 24-nt sRNAs in mutants defective in Pol IV. Consistent with the formation of hairpin structures, a defining feature of this Pol IV-independent subset of MITEs and IRs is the presence of longer TIRs and shorter loop regions. Interestingly, this subgroup is enriched for the Mutator-like element (MULE) superfamily of MITEs and is preferentially located within introns. Together, these findings suggest that MITE transcripts derived from introns can be directly processed by DCL3 to produce 24-nt sRNAs, whose potential biological functions we aim to investigate in future work.

Poster 115

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Epigenetic regulation of transcriptome–metabolome uncoupling and symptom development during RNA virus infection in Arabidopsis thaliana

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Chromatin regulation is increasingly recognized as an important component of plant antiviral responses, yet its impact on downstream physiological and metabolic outcomes remains poorly understood. Here, we analyze *Arabidopsis thaliana* plants defective in an activation-associated chromatin mark that display reduced symptoms of turnip mosaic virus (*Potyvirus rapae*) despite wild type (WT)-like virus accumulation. An integrated transcriptomic and metabolomic profiling of mutant and WT plants under mock and infected conditions was performed. Upon infection, mutant plants exhibited repressed immunity, salicylic acid-, jasmonic acid-, and stress-responsive transcription processes, while maintaining growth- and development-associated ones. These transcriptional changes were infection-specific and not observed under mock conditions. In contrast, metabolomic profiling revealed a strong accumulation of defensive specialized metabolites in infected mutant leaves, including flavonoids, glucosinolates, phenylpropanoids, indolic compounds, organic acids, lipid-derived metabolites, and redox cofactors. This pronounced transcriptome-metabolome decoupling was substantially stronger than in wild-type plants and suggests regulation beyond transcriptional control. To assess the contribution of phenylpropanoid metabolism, plants were treated with the phenylalanine ammonia-lyase inhibitor 2-aminoindan-2-phosphonic acid (AIP). AIP treatment had little effect on viral accumulation but markedly increased symptom severity, particularly in mutant plants, effectively abolishing their low-symptom phenotype. Data integration supports a model in which the epigenetically impaired mutant reduces reliance on transcriptionally driven immunity and instead buffers infection-induced damage through enhanced metabolic defenses, structural remodeling, and redox stabilization. Interestingly, preliminary chromatin analyses suggest that genes associated with antiviral RNA-silencing pathways may also be affected in the mutant. Together with the accumulation of specific flavonoids previously reported to interfere with DICER-LIKE proteins, this raises the possibility that post-transcriptional antiviral mechanisms contribute to the observed phenotype, a hypothesis currently under investigation.

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Poster 116

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Regulation of alternative splicing by R-loop formation in plants

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Alternative precursor mRNA splicing (AS) modulates gene expression by generating transcript isoforms with distinct biological functions, thereby contributing to the fine regulation of cellular processes. While regulation by RNA-binding proteins and cis-elements is well established, the role of co-transcriptionally occurring RNA-DNA hybrids (R-loops) in shaping AS patterns remains poorly understood. R-loops consist of an RNA-DNA hybrid and a displaced single-stranded DNA and can influence transcription dynamics. Studies on the SEPALLATA3 gene in Arabidopsis thaliana suggested that an R-loop involving a circular RNA may affect AS by slowing transcription elongation and altering interactions with splicing regulators (Conn et al. 2017).In this project, we examine the potential role of R-loops in regulating AS during light-dependent developmental transitions in A. thaliana. Previous genome-wide analyses revealed differences in R-loop patterns between dark- and light-grown A. thaliana seedlings (Xu et al. 2020), alongside extensive light-dependent AS changes (Hartmann et al. 2016). To further address this, we are performing paired DRIP-seq (DNA-RNA Immunoprecipitation followed by sequencing) and RNA-seq analyses during the early light response, comparing dark-grown seedlings with seedlings exposed for few hours to light in order to identify dynamic changes in R-loop formation and connect them with light-induced changes in alternative splicing. Moreover, we use rnaseh1a knockout mutants and inducible overexpression systems to modify R-loop levels and assess their impact on splicing outcomes and light-dependent development. To support the functional characterization of RNase H1A, we employ Nicotiana benthamiana and heterologous yeast-based assays to assess its in vivo activity in resolving R-loops. Wild type and catalytically inactive versions of RNase H1A are used for cross-system validation.Together, this work examines a more widespread role of R-loops as regulatory elements in AS and their possible function in light-responsive gene regulation in plants. Understanding this may also provide new strategies to modulate plant development and stress adaptation.

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Poster 117

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Splice-site-associated DNA methylation and alternative splicing responses in contrasting soybean genotypes

Carla Valeria Filippi, Victoria Armstrong, Mariana Sotelo, Omar Borsani

Alternative splicing and DNA methylation are regulatory layers in plant responses to biotic stress, but their potential coordination in plant defense remains poorly understood[1, 2]. We analyzed RNA-seq and cytosine methylation data from resistant and susceptible Glycine max genotypes under soybean cyst nematode-inoculated and non-inoculated conditions, using three biological replicates per group[3]. RNA-seq data were analyzed with rMATS[4] to identify differential exon skipping, alternative 3' and 5' splice-site usage, mutually exclusive exons, and intron retention. Cytosine methylation profiles in CpG, CHG, and CHH contexts were integrated with splicing events by quantifying methylation within affected exons or introns and in windows surrounding donor and acceptor sites.Using stringent event-level filtering, we detected 270 infection-responsive alternative splicing events in the susceptible genotype and 188 in the resistant genotype. The susceptible response showed a higher contribution of exon-skipping and alternative splice-site events. Integration of DNA methylation and splicing profiles revealed context- and position-dependent trends between local methylation changes and exon inclusion. Candidate loci were prioritized based on concurrent splicing and splice-site-proximal methylation changes.Among prioritized loci, two strong candidates showed reduced exon inclusion following inoculation together with lower donor-proximal CpG methylation. These events occurred in transcripts from GLYMA_01G191100, encoding an SRFR1-like TPR protein, and GLYMA_18G240700, encoding a putative TGD3/NAP11-related ABC protein (dPSI = -0.168 and -0.184, respectively). Based on the reference transcript annotation, both affected exons were internal and fully coding, and their exclusion was predicted to disrupt the reading frame. GLYMA_18G240700 transcripts also showed increased inclusion of a distinct 108-nt in-frame exon, supporting inoculation-associated isoform remodeling. These results reveal genotype-dependent splicing responses to soybean cyst nematode infection and support splice-site-associated DNA methylation as a candidate regulatory layer. They also provide an event-level integrative framework for linking local methylation changes with transcript isoform selection and prioritizing loci for experimental validation.

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Poster 118

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MicroRNA, Macro Impact: Decoding NF-Y regulation during abiotic stress in rice

Arushi Jain, Dr. Ananda Mustafiz

Abiotic stresses pose a major challenge to crop productivity by triggering complex physiological, biochemical, and transcriptional responses. Nuclear transcription factor Y (NF-Y) complexes are emerging as key regulators of stress adaptation; however, their genome-wide characterization and post-transcriptional regulation remain insufficiently explored. In this study, a comprehensive genome-wide analysis of NF-Y family members across four plant genera identified 36 AtNF-Ys, 34 OsNF-Ys, 40 CaNF-Ys, and 42 SbNF-Ys. Structural analysis revealed conserved functional domains, including the CCAAT-binding factor domain in NF-YA subunits and histone fold domains in NF-YB/NF-YC subunits, essential for DNA binding and NF-Y complex formation. Cis-regulatory element analysis of OsNF-Y genes highlighted their potential involvement in stress and hormone-responsive pathways. Meta-analysis combined with qRT-PCR validation demonstrated differential expression patterns of OsNF-Y genes under drought and salinity stress, with OsNF-YA1, OsNF-YA4, OsNF-YA7, OsNF-YC1, and OsNF-YC4 showing strong induction in shoot and root tissues. Further, miRNA target prediction and validation revealed that Osa-miR169a targets the 3′ UTR of OsNF-YA1, OsNF-YA4, and OsNF-YA10. Importantly, RLM-RACE analysis experimentally confirmed the miRNA-guided cleavage of OsNF-YA1 by Osa-miR169a in rice, providing direct evidence of post-transcriptional regulation. These findings uncover NF-Y-miRNA regulatory networks contributing to stress resilience and provide potential targets for climate-smart crop improvement.

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Poster 119

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Unraveling an epidermis-specific MYB–bHLH complex in cuticle wax biosynthesis

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The strawberry fruit epidermis is the outermost cell layer and the first protective barrier against environmental stresses and pathogens. To better understand the transcriptional programs operating in this specialized tissue, our group previously combined Laser Capture Microdissection with RNA-seq to obtain tissue- and stage-specific transcriptomic profiles from *Fragaria vesca* receptacles. Gene Regulatory Network analyses of the ripe epidermis revealed an enrichment of genes related to lipid metabolism and cuticle biosynthesis, together with several specifically expressed transcription factors, including FvMYB29, and two bHLH proteins, FvbHLH22 and FvbHLH67.
To investigate their regulatory role, DAP-seq analysis of FvMYB29 identified binding sites enriched in promoters of genes involved in wax and cutin biosynthesis, including components of the fatty acid elongation complex, alkane- and alcohol-forming pathways, and ABC transporters associated with cuticular lipid export. Correlation analyses with transcriptomic datasets supported the coordinated regulation of cuticle-associated genes. Stable CRISPR/Cas9 knockout and overexpression lines of FvMYB29 were generated in F. vesca, revealing pleiotropic phenotypes affecting leaf morphology, flowering time, inflorescence architecture, and fruit development.
Consistent with the transcriptional data, FvMYB29-overexpressing plants accumulated higher levels of epicuticular waxes, particularly alkanes, aldehydes, primary alcohols, triterpenoids, and wax esters. In addition, protein interaction assays revealed that FvMYB29 associates with FvbHLH22 and FvbHLH67, forming heterodimeric or higher-order transcriptional complexes. Furthermore, transactivation assays in Nicotiana benthamiana showed that both bHLH proteins enhance FvMYB29-mediated activation of cuticle biosynthesis promoters.
Together, these findings support a role for the FvMYB29–FvbHLH complex in regulating cuticle wax biosynthesis in *F. vesca.*

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Poster 120

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MpSPL3 orchestrates the vegetative and reproductive developmental programs in the liverwort *Marchantia polymorpha*

Izabela Sierocka, Alisha Alisha, Artur Jarmolowski, Zofia Szweykowska-Kulinska

SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) genes encode plant-specific transcription factors that are widely distributed across the plant kingdom. In angiosperms, the multigene SPL family regulates various biological processes, including the vegetative-to-reproductive phase transition, inflorescence architecture, and lateral organ development. In contrast, the liverwort *Marchantia polymorpha* genome encodes only four SPLs, with functional studies available only for microRNA-targeted members, MpSPL1 and MpSPL2. While MpSPL1 was shown to control meristem dormancy to modulate the thallus architecture, MpSPL2 was found to promote the transition from vegetative-to-reproductive phase. Here, we show that the MpSPL3 gene is crucial for successful coordination between vegetative growth and the reproductive phase transition. The knockout of MpSPL3 leads to strong growth retardation with disordered thallus branching and reduced gemma cup number, which might be an implication of several genes' downregulation, like MpAINTEGUMENTA (MpANT), MpKARRIKIN INSENSITIVE2A (MpKAI2A), MpGOLDEN2-LIKE (MpGLK), and MpRho-like GTPase (MpROP) in the null Mpspl3 background. Additionally, MpSPL3 is responsible for the direct or indirect expression control of other MpSPL family members. Most profoundly, loss of MpSPL3 causes failure in germline initiation, leading to abolished gametangiophores production, which can result from reduced expression of several genes important for germ cell specification, like MpBONOBO (MpBNB), MpLOTUS JAPONICUS ROOTHAIRLESS-LIKE (MpLRL), MpBZR/BES3 (MpBZR3), and MpCYTOKININ-INDEPENDENT 1 (MpCKI1). Altogether, our findings indicate that MpSPL3 plays a crucial role in the regulation of gametophyte development and reproductive success in Marchantia.

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Poster 121

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Manipulation of Low-Complexity Domain in UBP1C stabilizes the core of stress granules leading to increased plant heat stress tolerance

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A hallmark of the plant heat-stress response is the selective sequestration of mRNAs into stress granules (SGs) and the arrest of translation. As stress subsides, SGs disassemble to release mRNAs and resume translation, yet how SG persistence is regulated and whether it dictates stress outcome remains poorly understood. In Arabidopsis, OLIGOURIDYLATE BINDING PROTEIN 1C (UBP1C) is a core SG RNA-binding protein carrying a low-complexity domain with a polyglutamine (polyQ) tract. Using UBP1C variants lacking the polyQ-containing region or carrying expanded, or substituted polyQ tracts, we show that this domain is dispensable for UBP1C recruitment to SGs, but at the same time it can tune the SG biophysical state. PolyQ expansion enlarged SGs, reduced molecular diffusion, and delayed SG disassembly during recovery, while broadening the protein and RNA interactomes of UBP1C. These stabilized SGs slowed translation restart after heat stress yet improved plant survival, chlorophyll retention, and growth. Tracing RNA regulation across cellular abundance, ribosome engagement, and UBP1C targets, together with proteome dynamics, we linked this resilience to the sustained translation of recovery-associated transcripts and the accumulation of ABA- and dehydration-protective proteins. By coupling SG material properties to this protective program, our findings suggest condensate dynamics as a tunable determinant of plant heat stress resilience.

Keywords: plant stress granules, low-complexity domain, polyQ repeat, TRAP, ribosome.

Poster 122

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Antisense transcription shapes the regulatory state of stress-responsive genes

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Plant genomes contain a relatively large proportion of non-coding regions, yet what determines where non-coding transcription occurs remains poorly understood. Among the diverse non-coding RNAs, Poly(A)-associated antisense transcripts (PASt), which initiate near the polyadenylation site (PAS) of protein-coding genes, are widespread in plants and can influence expression of their associated sense genes. However, what distinguishes PASt-hosting loci and how antisense transcription regulates host genes remain unclear. Here, we investigated PASt in Arabidopsis at a genome-wide level, with a particular focus on stress-responsive genes. Our previous work showed that antisense transcription at selected transcription factor loci positively influences sense transcription and contributes to cold acclimation. Building on this, we examined genomic architecture and chromatin features associated with PASt-hosting genes. We found that PASt-hosting genes occur within a distinct genomic environment and show higher levels of histone acetylation and other active chromatin marks. These differences are especially pronounced around the PAS and 3′ region of the sense gene, where antisense transcription originates. This suggests that the 3′ end is not simply a termination zone but also forms a regulatory region favourable for transcription in the opposite direction. Also, PASt is closely coordinated with sense transcription and contributes to maintaining an active chromatin state across the host gene. Together, our findings support a reciprocal relationship in which the local genomic and chromatin environment favours antisense transcription, while PASt helps sustain host-gene activity and stress-responsive transcription.

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Poster 132

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TISCalling: Leveraging Machine Learning to Identify Translational Initiation Sites in Plants and Viruses

Ming-Ren Yen, Chia-Yi Cheng, Ting-Ying Wu and Ming-Jung Liu

The recognition of translational initiation sites (TISs) offers complementary insights into identifying genes encoding novel proteins or small peptides. Conventional computational methods primarily identify Ribo-seq-supported TISs and lack the capacity of systematic and global identification of TIS, especially for non-AUG sites in plants. Additionally, these methods are often unsuitable for evaluating the importance of mRNA sequence features for TIS determination. In this study, we present *TISCalling*, a robust framework that combines machine learning (ML) models and statistical analysis to identify and rank novel TISs across eukaryotes. *TISCalling* generalized and ranks important features common to multiple plant and mammalian species while identifying kingdom-specific features such as mRNA secondary structures and G-contents. Furthermore, *TISCalling* achieved high predictive power for identifying novel viral TISs. Importantly, *TISCalling* provides prediction scores for putative TIS along plant transcripts, enabling prioritization of those of interest for further validation. We offer *TISCalling* as a command-line-based package [https://github.com/yenmr/TISCalling], capable of generating prediction models and identifying key sequence features. Additionally, we provide web tools [https://predict.southerngenomics.org/TISCalling/] for visualizing pre-computed potential TISs, making it accessible to users without programming experience. The *TISCalling* framework offers a sequence-aware and interpretable approach for decoding genome sequences and exploring functional proteins in plants and viruses.

Keywords: translational control, translation initiation site, open-reading frames, gene annotation, machine learning prediction

Poster 133

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Species-specific codon adaptation of CRISPR nucleases for genome editing in carrot and cabbage

Magdalena Klimek-Chodacka, Rafal Baranski

Efficient genome editing depends not only on guide RNA design and nuclease activity, but also on effective expression of the CRISPR-associated protein in the target host. Coding sequences described as plant-optimized are frequently designed using general preferences or data from model species and may therefore differ in their compatibility with individual crops.

We performed a comparative bioinformatic evaluation of 15 plasmid-derived Cas9 and Cas12a coding sequences for expression in carrot (*Daucus carota*) and cabbage (*Brassica oleracea var. capitata*). Host-specific codon reference sets were generated from representative coding sequences obtained from the corresponding reference genomes. Codon adaptation was assessed using the codon adaptation index, GC content at synonymous positions, the proportion of weakly adapted codons, relative synonymous codon usage and local CAI calculated in 30-codon sliding windows.

Marked differences were observed among the analysed constructs. The plant-optimized pcoCas9 showed the highest codon adaptation in both carrot and cabbage, with CAI values of 0.815 and 0.959, respectively. Arabidopsis-optimized Cas9 variants also ranked highly, particularly in cabbage. The strongest evidence for the influence of synonymous sequence design was obtained from six constructs encoding an identical SpCas9 protein. Their CAI values ranged from 0.623 to 0.815 in carrot and from 0.725 to 0.959 in cabbage, despite an unchanged amino-acid sequence. Highly GC-rich human- and maize-optimized variants generally showed lower host-specific adaptation and more local regions containing weakly preferred codons.

Although rankings were broadly conserved between the two crops, host-dependent differences were evident, demonstrating that broad plant optimization does not guarantee equivalent adaptation across species. Host-specific codon analysis can therefore support the selection or redesign of CRISPR nuclease coding sequences before experimental validation.

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Poster 134

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MITE-derived small RNAs in carrot abiotic stress responses

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Miniature inverted-repeat transposable elements (MITEs) are a potential source of various classes of small RNAs (sRNAs), including small interfering RNAs (siRNAs) and microRNAs (miRNAs). MITE-derived 24-nt siRNAs are involved in the RNA-directed DNA methylation (RdDM) pathway, which, in addition to transposon silencing, may affect the expression of nearby genes, while MITEs that have evolved into miRNA-producing loci can impact the post-transcriptional silencing of target transcripts.

To determine the extent to which carrot MITEs can influence the expression of nearby genes under abiotic stress by generating small RNAs, we analysed sRNA-seq, RNA-seq, and whole-genome bisulfite sequencing (WGBS) data obtained from plants grown under control conditions or exposed to heat (4 h at 40 °C), cold (4 h at 4 °C), or salt stress (plants flooded with a 150 mM NaCl solution for 15 min and then tested after 4 h).

Heat stress had the strongest effect on MITE-miRNA and MITE-siRNA expression. Of the eight carrot MITE-miRNAs (7% of all carrot miRNAs), three were differentially expressed under heat stress. For these miRNAs, 3–8% of the predicted target genes exhibited the expected change in expression. One of these genes codes for a protein called SUPPRESSOR OF MAX2 1-like, which may potentially be involved in improving plant tolerance to heat stress. We also identified 17 MITE copies that were likely to produce differentially expressed siRNAs under heat stress, associated with changes in MITE methylation and the expression of nearby genes. Notably, all but one of these MITE copies were inserted into the promoter regions of the associated genes (15 copies) or into an intron (one copy), suggesting their contribution to the observed changes in gene expression. The majority of these genes are not directly involved in responses to abiotic stress.

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PRP8 splicing subunit at the heart of pollen tube guidance

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Pre-mRNA PROCESSING factor 8 (PRP8) is a highly conserved core component of the U5 small nuclear ribonucleoprotein (snRNP) and is central to spliceosome assembly, activation and splice-site selection. Arabidopsis contains two PRP8 paralogs, PRP8A and PRP8B, with partially overlapping and reproductive-stage-specific functions. We show here that, *prp8a prp8b* plants showed impaired pollen-tube attraction, associated with misregulation of MYB98 and LURE expression, and mutant pollen tubes exhibited reduced ability to target ovules for fertilization. Pollen *prp8a prp8b* RNA-seq and TAPE-PCR revealed extensive intron-retention defects and differential expression of genes associated with RNA biosynthesis and RNA-processing pathways. Nuclear co-immunoprecipitation identified close association of PRP8A with U4/U6 tri-snRNP components and additional spliceosomal subunits, supporting its function within the core splicing machinery. Strikingly, deletion of its C-terminal JAB domain specifically abolished PRP8A accumulation in sperm nuclei while preserving vegetative-cell nuclear localization, and this alteration was accompanied by a pollen phenotype. The sperm-specific requirement for the JAB domain suggests that this region mediates gametophyte-specific nuclear targeting or retention, potentially through interaction with a sperm-enriched nuclear-import factor or a regulated transport/adaptor mechanism, thereby enabling PRP8A-dependent splicing during pollen function. Together, these findings identify PRP8A/PRP8B as important regulators of reproductive RNA processing and pollen–ovule communication.

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Cajal Bodies modulate nuclear mRNA retention, intron splicing efficiency, and snRNA biogenesis under hypoxic stress in *Arabidopsis thaliana*

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Cajal bodies (CBs) are evolutionarily conserved nuclear condensates involved in snRNP biogenesis and mRNA processing. Their role in stress-responsive transcriptome regulation remains poorly understood. Here, we show that *ncb1* mutants lacking functional CBs exhibit reduced hypoxia tolerance and profoundly diminished transcriptional dynamics. CBs regulate both total and nuclear transcriptomes: more genes are differentially expressed in WT under hypoxia and reoxygenation, and mRNA processing-related GO terms are exclusively enriched in WT implicating CBs directly in stress-responsive gene regulation.

FISH and nuclear RNA-seq confirm poly(A) RNA nuclear retention during hypoxia, more pronounced in WT. A specific transcript subset accumulates in the nucleus during hypoxia and declines upon reoxygenation, coinciding with increased total RNA levels — suggesting a reservoir of nucleus-retained transcripts mobilized for translation after stress relief. Conversely, transcripts sequestered in the nucleus under normoxia relocate to the cytoplasm during hypoxia; over 30 are stress-associated, implying CB-dependent pre-positioning that primes the cell for rapid response to oxygen deprivation.

Intron retention analysis revealed preferential retention of the last intron from the 3’ end and the first two from the 5’ end. During hypoxia, the number of transcripts with retained introns increases despite a lower per-intron retention index, indicating broader but less intense retention. Retained introns are characterized by reduced GC contrast with flanking exons, weaker 5’ splice site consensus, shorter polypyrimidine tracts, and enrichment for purine-rich and CG-containing motifs impeding spliceosome recognition.

The differences between the WT and *ncb1* transcriptomes may be influenced by the fact that CBs play a role in snRNA and snoRNA expression and promote the assembly of U2 snRNA with the U2B” protein, indicating that CBs coordinate transcription, nuclear retention, intron splicing, and snRNP assembly to enable efficient adaptation to hypoxic stress.

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Dehydration-induced condensation of AGO1 modulates miRNA functionality

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Water-deficit affects nearly every biological event in plants, and recent studies have shown that microRNA (miRNA) functionality is associated with plant responses to water-deficiency. Liquid-liquid phase separation (LLPS) facilitates the condensation of biomolecules, which is driven by intrinsically disordered proteins (IDPs) and plays diverse roles in cellular processes. Here, we demonstrate that the prion-like domain (PrLD) of ARGONAUTE 1 (AGO1) is responsible for topological changes under dehydration. These AGO1 condensates through LLPS in the cytoplasm influence miRNA regulatory activity, a process related to cytoplasmic calcium levels. We found that dehydration-induced AGO1 condensation influences AGO1 processing activity, while contributing to dehydration tolerance in plants. Upon rehydration, the condensation-driven accumulation of AGO1 is resolved, restoring their protein levels to normal. Overall, we propose that AGO1 phase separation acts as an emergent response for dehydration, serving to attenuate the energy-consuming miRNA regulatory pathway in young seedlings.