DBG's Eduard Strasburger Workshop - Co- and posttranslational control in chloroplasts

Montag, 18. November 2024 - Mittwoch, 20. November 2024

Münster

Buch der Abstracts

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Chloroplast acetyltransferases in regulation of photosynthesis

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Acetylation is one of the most common chemical modifications affecting a variety of molecules ranging from metabolites to proteins. Recent development of enrichment techniques and mass spectrometry has revealed that acetylation is a prevalent modification also in plants, and that in addition to cytosolic and nuclear proteins also numerous chloroplast proteins are acetylated. We have characterized a chloroplasts-localized family of GNAT acetyltransferases in Arabidopsis thaliana. GNAT enzymes are unique among acetyltransferase enzymes as they possess dual protein acetylation activity, i.e. they catalyze both N-terminal and lysine acetylation. Our results show that each GNAT enzyme has distinct specificity in terms of favored substrates, and that they play unique roles in the accumulation of metabolites, e.g. ascorbate and oxylipins. Depletion of GNAT2 has marked effects on the acetylation level of several chloroplast proteins and on photosynthetic properties of plants. Specifically, formation of the Photosystem I-LHCII complex is prevented in the gnat2 knock-out plants, which results in impaired balancing of the light energy between the photosystems (state transitions). Moreover, loss of GNAT2 severely disturbs light-dependent dynamics of thylakoid stacking. Altogether, our results indicate that chloroplast acetyltransferases are new and important regulators of photosynthetic light harvesting with a marked impact on the metabolism of plants.

References: Koskela et al. 2018 Plant Cell 30 ; Koskela et al. 2020 Photosynth Res 145-; Bienvenut et al. 2020 Mol Syst Biol 16; Rantala et al. 2022 Plant Cell Physiol 63; Ivanauskaite et al. 2023 Plant Cell Physiol 64.

Poster session / **40**

A microRNA regulating targets massively up-regulated in response to stress in drought tolerant A. sagittata

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In plants, photosynthesis is a critical process for survival, and copper is one of the essential micronutrients required for this process. Under abiotic stress conditions such as drought, plants typically reduce photosynthesis to balance their competitive ability with stress tolerance, ensuring survival. This study investigates *Arabis nemorensi*s and *Arabis sagittata*, two species growing in competitive meadow environments, focusing on the molecular mechanisms that govern drought tolerance, with special focus on the role of miRNAs. We measured physiological parameters of both species during a dry-down experiment and performed RNA-seq analysis to explore molecular changes in response to drought stress. Our results revealed that both species tolerate extreme drought, wilting below 5% relative water content. However, *A. sagittata* showed a higher survival rate (90%) compared to *A. nemorensis* (49%). Notably, *A. nemorensis* formed larger rosettes that wilted more rapidly under stress. Structural equation modeling indicated that in *A. sagittata*, individuals with lower physiological responses to stress had higher survival rates, a pattern not observed in *A. nemorensis*. Gene expression analysis identified miR408 as a key regulator of drought response in both species. miR408 was upregulated in response to drought, especially in *A. sagittata*, and played a crucial role in modulating the expression of target genes involved in stress tolerance. One of the miR408 targets, AT5G50950 (FUM2), which is involved in the conversion of malate to fumarate in light-dependent reactions, was significantly downregulated during drought in *A. sagittata*. Malate, synthesized by malate dehydrogenase in the chloroplast, may be exported from the chloroplast and converted to fumarate. This study highlights miR408 as a pivotal factor in the drought stress response, with differences in gene expression contributing to the varying levels of drought tolerance observed between *A. sagittata* and *A. nemorensis*. Understanding the regulatory role of miR408 offers new insights into how plant species endure abiotic stress while competing in dense meadow habitats.

Tue 19 / 41

Regulation of Rubisco activity by light in tomato

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Regulation of Rubisco activity by light in tomato
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Rubisco is the most abundant protein in the chloroplast, fixing CO₂ into sugars during photosynthesis. Its activity is finely adjusted in response to changes in the environment. The regulation of Rubisco by its chaperone Rubisco activase (Rca) during shade-sun transitions in Arabidopsis is a good example of this fine-tuning. Rca promotes the ATP-dependent release of inhibitors that bind to Rubisco catalytic sites under shade. As most plants, Arabidopsis has a shorter redox-insensitive β isoform and a longer redox-sensitive α Rca isoform which shows higher sensitivity to ADP inhibition. Arabidopsis mutants expressing only Rcaβ showed faster induction of CO₂ assimilation after transition from low to high irradiance and enhanced growth under fluctuating light. In contrast, Solanaceae species such as tobacco and tomato naturally express only Rcaβ. Tobacco Rcaβ shows high sensitivity to ADP inhibition, but how other changes in the chloroplast environment affect Rubisco regulation in this species and Solanaceae crops is still enigmatic. In the framework of the CAPTALISE project, we are studying how Rca regulates Rubisco under changing chloroplast conditions in response to light in tomato. These studies advance our understanding of Rubisco regulation to inform the improvement of photosynthetic productivity in crop plants that experience dynamic light environments including shade-sun transitions.

Poster session / **42**

Towards fluorescent protein-based biosensing of thylakoid lumen pH

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The pH within the thylakoids is essential for photosynthesis. As part of the photosynthetic light reactions, proton translocation across the thylakoid membrane generates a proton motive force that consists of a pH gradient (ΔpH) and an electrical gradient (ΔΨ) to drive ATP synthase. While genetically encoded pH biosensors have been instrumental in dissecting pH dynamics of different plant cell compartments, in vivo pH biosensing in the subcompartments of the chloroplast had remained challenging. We recently established pH biosensing in response to photosynthetic activity and revealed a major impact on pH dynamics not only in the stroma but also in the cytosol and the mitochondria. While the observed pH dynamics clearly mirror photosynthetic proton pumping, measuring the bona fide pH gradient across the thylakoid membrane as the major determinant of the proton motive force, requires monitoring pH in the thylakoid lumen. Yet, establishing luminal pH monitoring by genetically-encoded biosensors presents unique challenges, such as import across three membrane systems, low pH values that may be adopted in the light, silencing of sensor expression and direct vicinity of photosynthetic pigments. To address those challenges and to optimize luminal pH biosensing in tobacco and Arabidopsis leaves, we have generated a collection of constructs that include different pH biosensors, signal peptides and promoters. Initial experiments show that illumination induces an inverse response between luminal and stromal targeted pH sensors, which provides evidence for correct subcellular targeting. I will present my recent progress in developing luminal pH sensors while highlighting remaining challenges.

Tue 19 / **43**

Cold-Triggered Phase Separation Forms a Chloroplast Compartment Modulating RNA Processing

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

Tue 19 / **44**

Diving into the unknowns of LHCII PTMs: the forgotten dark side of photosynthesis

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Post-translational modifications (PTMs) control protein stability, localization, turn-over and interactions, thus represent a fine-tuned regulatory mechanism to reversibly adjust cellular physiology.
In photosynthetic eukaryotes, light-dependent phosphorylation of thylakoid proteins dynamically regulates the light-harvesting apparatus in response to environmental fluctuations. In the chloroplast of land plants, threonine phosphorylation regulates the repair cycle of photodamaged photosystem II (PSII) reaction centers and controls state transitions (ST). ST equilibrate the excitation pressure between photosystem I and II (PSI and PSII) upon shifts in light quality to avoid redox imbalances along the electron transport chain. We previously showed that phosphorylation of Lhcb2 Thr40 by by the state transition 7 kinase (STN7) is necessary and sufficient to promote state 1 – state 2 transition in Arabidopsis thaliana, while Lhcb1 phosphorylation is dispensable. Furthermore, extensive phosphorylation was detected on serines of the Light harvesting Complex proteins of PSII (LHCII) independently from the STN7 kinase and light cues.
The plastid NUCLEAR SHUTTLE INTERACTING (NSI)/GNAT2 (general control non-repressible 5 (GCN5)-related N-acetyltransferase 2) GNAT acetylase was reported to regulate state transitions3,4, possibly under (light-dependent) redox regulation. Intriguingly, several phosphorylated LHCII isoforms are also acetylated by GNAT, suggesting a possible cross-talk between PTMs. However, it is currently not known whether LHCII phosphorylation and acetylation are mutually exclusive events and whether they play synergistic or antagonistic roles.
Our preliminary data show that removal of an experimentally described Lhcb2 acetylation site neighboring the major p-Thr does not impair state transitions, suggesting that other LHCII acetylation events, possibly of Lhcb1 isoforms, could be involved in GNAT/NSI-dependent regulation of state transitions. Overall, we suggest that the interplay between PTMs of chloroplast proteins is a rather overlooked biological process, which adds another layer of complexity to the understanding of photosynthetic regulation.

Wed 20 / 45

Protein import into chloroplasts and its regulation by the ubiquitin-proteasome system

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The development and operation of chloroplasts (or other members of the plastid family of plant organelles) requires the participation of thousands of different organellar proteins. Most chloroplast proteins are nucleus-encoded and synthesized in the cytosol in precursor form. These preproteins pass through multiprotein import machines in the organelle's outer and inner envelope membranes –these are called the TOC and TIC translocons, respectively. Receptor components of the TOC complex exist in multiple isoforms, and genetic studies in the model plant Arabidopsis have indicated that the isoforms have distinct preprotein recognition specificities and function in different import pathways. Operation of such client-specific import pathways controls the organelle's proteome and functions, and plays a role in the differentiation of different plastid types (e.g., chromoplast formation in ripening tomato fruit). Our work has shown that the chloroplast protein import machinery is proteolytically regulated by direct action of the ubiquitin-proteasome system (UPS), in a process termed "chloroplast-associated protein degradation"(CHLORAD). The CHLORAD machinery has three key components: the SP1 ubiquitin E3 ligase, the SP2 β-barrel channel protein, and the CDC48 AAA+ ATPase. The SP1 and SP2 proteins form a complex in the outer envelope membrane, and respectively mediate the ubiquitination and extraction (or retrotranslocation) of TOC protein targets. The CDC48 protein, located in the cytosol, provides the energy that drives the retrotranslocation step, delivering the target proteins to the cytosolic 26S proteasome for degradation. Recent advances in our understanding of the mechanisms and functions of CHLORAD will be discussed.

Tue 19 / **46**

The Lhcb8 antenna protein reshapes the functional architecture of the photosystem II supercomplex in Arabidopsis thaliana

Autor Roberto Caferri[1]

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The photosystem II (PSII) supercomplex (SC) of higher plants is a (multi)protein-pigment assembly performing the initial steps of photosynthetic electron transport consisting of water oxidation and photochemical plastoquinone reduction. In angiosperms, the PSII SC is equipped with three LHCB4-6 monomeric pigment-binding proteins which connect the dimeric core complex with the peripheral antennae composed of trimeric LHCB1-3 complexes. In Arabidopsis thaliana, LHCB4 is encoded by three gene isoforms: Lhcb4.1, Lhcb4.2, and Lhcb4.3. While LHCB4.1 and LHCB4.2 share high sequence similarity and are constitutively expressed, the latter is divergent both in terms of sequence similarity and for being exclusively expressed under persistent abiotic stress. Therefore, it has been renamed as LHCB8.
In this work, we constructed an Arabidopsis thaliana genotype depleted of the products of both Lhcb4.1 and 4.2 genes and exclusively accumulates the LHCB8 protein. The results demonstrate that LHCB8 replaces LHCB4.1 and LHCB4.2 in the CP29-binding site of the PSII SC, preventing the association of the LHCB6 subunit and leading to a reduction of LHCB3 levels. Upon biochemical investigation, the LHCB8-expressing plants displayed a smaller PSII SC compared to the wild type, which possibly results in a lower photon-harvesting capacity. Moreover, cryo-EM analysis revealed a loose arrangement of peripheral antenna complexes in the Lhcb8-containing PSII SC, potentially reducing the antenna-core energy transfer rate. The structural alterations are caused by a shorter C-terminal domain of LHCB8 compared with LHCB4.1/4.2 and the absence of two chlorophyll ligands. LHCB8-expressing plants displayed a smaller PSII functional antenna and lower non-photochemical quenching (NPQ) capacity than wild-type plants. Altogether, our results suggest that the induction of LHCB8 expression reduces photon harvesting in response to excess light. Therefore, the Lhcb8 gene may be considered a viable Lhcb target in plant biotechnology to optimize the balance between light-harvesting and photoprotection in crops.

Mon 18 / **47**

Stages of phase-separation during biogenesis of a bacterial micro-compartment

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Photosynthesis is a fundamental process in biology as it converts solar energy into chemical energy and thus, directly or indirectly, fuels all life on earth. The chemical energy is used to fix atmospheric CO2 and produce reduced carbon compounds in the Calvin-Benson-Bassham cycle. The key enzyme for this process in all photosynthetic organisms is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which is responsible for the conversion of an estimated amount of ~1011 tons of CO2 per annum into organic material. Rubisco is the most abundant enzyme in nature, owing in part to its low catalytic turnover rate and limited specificity for CO2 versus O2. Remarkably, Rubisco is also one of the most chaperone-dependent proteins, requiring the chaperonin system for folding and auxiliary factors for assembly.

To avoid the reaction with O2, cyanobacteria have evolved proteinaceous microcompartments called carboxysomes, in which the enzymes Rubisco and carbonic anhydrase (CA) are enclosed. Dissolved CO2 in the form of HCO3 diffuses through the proteinaceous carboxysome shell and is converted to CO2 by CA, generating a high concentration of CO2 for carbon fixation by Rubisco –the so-called

CO2-concentrating mechanism (CCM). The shell also prevents access to reducing agents, generating an oxidizing environment inside the carboxysome. In beta-cyanobacteria the assembly of the carboxysome first involves the aggregation of Rubisco and CA, followed by shell formation. Recent advances have shown that early in the process of pro-carboxysome assembly, a specialized scaffolding protein called CM initiates phase-separation of both Rubisco and CA into condensates. I will describe our present understanding of the complex multivalent interactions that result in the sequestration of several proteins into a pro-carboxysome. Understanding carboxysome biogenesis will be important for efforts to engineer a CCM into crop plants.

Tue 19 / **48**

Posttranslational control for Chlorophyll and Heme synthesis

Autor Bernhard Grimm[1]

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Tetrapyrrole biosynthesis (TPB) in plants consists of more than twenty enzymatic steps and is tightly controlled due to the synthesis of photoreactive intermediates and the different spatial and temporal requirements of their end products chlorophyll and heme. At the TPB hotspots, many complementary post-translational control mechanisms act on specific enzymes: The two enzymes glutamyl-tRNA reductase (GluTR) and glutamate-1-semialdehyde aminotransferase (GSAAT) provide the rate-limiting step of 5-aminolevulinic acid (ALA) synthesis at the beginning of the TPB pathway. At the branch point to chlorophyll and heme synthesis, Mg chelatase, a protein complex consisting of three different subunits, is responsible for the transfer of protoporphyrin to chlorophyll synthesis. The light-dependent protochlorophyllide oxidoreductase also requires several control mechanisms to prevent substrate accumulation in the dark and to ensure the activity and stability of the enzyme under unfavorable environmental conditions. Thus, several factors of these post-translational control mechanisms ensure a finely tuned metabolic flux in TPB at the level of activity, stability, oligomerization and subplastidal compartmentalization of the enzymes involved. In recent years, we have successfully contributed to elucidate several complex control processes at these foci during day, night and under changing conditions in nature. The talk will present some of the regulatory strategies recently investigated by our group for the post-translational control of TBP by regulatory and supporting factors

Mon 18 / **49**

The evolution of assembly complexity in Rubisco.

Autor Georg Hochberg[1]

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Rubisco is the central CO2 fixing enzyme of the Cavin cycle and responsible for the vast majority of all CO2 fixation on our planet today. In plants, Rubisco undergoes an elaborate set of steps involving the sequential action of at least 6 different dedicated folding and assembly chaperones to assemble into its enzymatically active form. This complexity evolved from much simpler Rubisco ancestors that functioned without any of these additional factors. In this talk I will summarize my lab's work on retracing the evolution of Rubisco's complex present-day assembly requirements. Using ancestral sequence reconstruction and the resurrection of billion-year-old Rubiscos, we are learning how this crucial enzyme gradually elaborated its structure and assembly mechanism. Some of these elaborations had history-changing effects on Rubisco's catalytic properties, whereas others appear to be

evolutionary accidents that simply became impossible to lose. This work is beginning to illuminate key events in Rubisco's history leading up to and following the evolution of oxygenic photosynthesis, one of the most consequential events in the history of life on earth. It also raises the possibility of learning from evolution to re-simplify and improve the assemblies of agriculturally important Rubiscos.

Wed 20 / 50

STIC2 selectively binds ribosome-nascent chain complexes in the cotranslational sorting of Arabidopsis thylakoid proteins

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Chloroplast-encoded multi-span thylakoid membrane proteins are crucial for photosynthetic complexes, yet the coordination of their biogenesis remains poorly understood. To identify factors that specifically support the cotranslational biogenesis of the reaction center protein D1 of photosystem (PS) II, we generated and affinity-purified stalled ribosome-nascent chain complexes (RNCs) bearing D1 nascent chains. Stalled RNCs translating the soluble ribosomal subunit uS2c were used for comparison. Quantitative tandem-mass spectrometry of the purified RNCs identified around 140 proteins specifically associated with D1 RNCs, mainly involved in protein and cofactor biogenesis, including chlorophyll biosynthesis, and other metabolic pathways. Functional analysis of STIC2, a newly identified D1 RNC interactor, revealed its cooperation with chloroplast protein SRP54 in the de novo biogenesis and repair of D1, and potentially other cotranslationally-targeted reaction center subunits of PSII and PSI. The primary binding interface between STIC2 and the thylakoid insertase Alb3 and its homolog Alb4 was mapped to STIC2's β-sheet region, and the conserved Motif III in the C-terminal regions of Alb3/4.

Tue 19 / 51

Plastid NAD-dependent Malate Dehydrogenase: Cysteine oxidation as a regulatory switch of a multitasking protein?

Autor Maike Cosse[1]

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Most plant cell compartments possess oxidoreductases that catalyze the interconversion between malate and oxaloacetate (OAA) in a reversible reaction. These malate dehydrogenases (MDHs) are either NAD- or NADP-specific.
Together with malate/OAA translocators, MDHs enable the indirect exchange of reducing equivalents across subcellular membranes. The exported malate can then be used as a substrate for mitochondrial ATP production or to supply NADH to the cytosol.
Chloroplasts contain two MDH isoenzymes that differ in their cofactor specificity and function: the redox-regulated NADP-dependent MDH restores NADP upon illumination to prevent acceptor site limitation at Photosystem I and is only active in the light, whereas the plastid NAD-dependent MDH (plNAD-MDH) has been described to be constitutively active and to restore NAD for plastidial glycolysis. In addition, it has been proposed that plNAD-MDH has a moonlighting function in plastid biogenesis and is involved in starch degradation, both of which are independent of its metabolic activity.
Since plNAD-MDH appears to have multiple functions in central metabolism, redox homeostasis and

plastid biogenesis, we were interested to find out if and how plNAD-MDH is regulated *in vitro* and found that plNAD-MDH is a target of redox regulation. Oxidation of recombinant plNAD-MDH inhibits its NAD-MDH activity and induces a conformational change of the protein visualized by non-reducing SDS-PAGE. Both redox effects were reversible upon re-reduction and prevented by its cofactors NAD and NADH. These results raise the possibility that plNAD-MDH is susceptible to redox regulation *in vivo* and provide an evidence for how plNAD-MDH might switch between different localizations and functions.

Wed 20 / **52**

Protein Mistranslation In Endosymbiotic Organelles Of Plant Cells

Autor Hans-Henning Kunz[None]

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Accurate protein translation is a hallmark for cell function. It guarantees an efficient proteome while minimizing detrimental unfolded proteins and concomitant energy loss. Surprisingly, protein mistranslation happens quite frequently in vivo (error rates 10-2 to 10-4) mainly due to transfer RNA (tRNA) mis-decoding and tRNA mis-acylation. Bacteria, mitochondria and plastids synthesize glutaminyl-tRNAs (Gln-tRNAGln) via an indirect pathway. Initially, an unspecific aminoacyl-tRNA synthetase charges tRNAGln with glutamate. Subsequently, glutamine is produced through transamidation by the aminoacyl-tRNA amido-transferase complex GatCAB. Bacteria can vary GatCAB activity. The resulting mistranslation increases proteome plasticity helping them to withstand adverse conditions. Conversely, fungi and animal mitochondria are highly sensitive to amino acid misincorporation. The role of GatCAB in plant organelles and the plant response to mistranslation is unknown. Our study of the Arabidopsis gatb-1 mutant provides global insights into GatCAB function in plants. Proteomics revealed varying degrees of Gln-to-Glu misincorporation in mutant plastid- and mitochondrially-expressed protein complexes of up to >90%. It appears that there are differences in nuclear, plastid, and mitochondrial control of mistranslation. Through transcriptomics and biochemical assays, we identified efficient compensatory mechanisms that yield only modest abundance changes of the steady-state plastid proteome and explain the surprisingly subtle phenotypes of gatb-1 plants. Hence, the gatb-1 mutant represents a premiere tool to dissect mistranslation effects in plant organelles, study their global effects, and pinpoint critical players in proteostasis and protein quality control events.

Tue 19 / **53**

Effect of photosynthetic cyclic electron transfer on the chloroplast redox proteome

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Fixing CO2 via photosynthesis requires ATP and NADPH. Linear electron transfer (LET) supplies both metabolites, yet depending on environmental conditions, additional ATP is required which can be generated by cyclic electron transfer (CET). Recent studies suggest that CET, which draws electrons from the ferredoxin pool affects the redox regulation of the Calvin-Benson cycle enzymes involved in CO2 fixation. Here we investigated this phenomenon further using an iodoTMT labelling and quantitative mass spectrometry approach. The results suggest a complex interplay between LET and CET in regulating the activation state of key chloroplast enzymes involved in reductant export, CO2 fixation and ATP synthesis.

Poster session / **54**

Investigating the role of Arabidopsis HISTONE DEACETYLASE 14 in chloroplasts

Autor Florian Kotnik[1]

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Lysine acetylation is an important post-translational protein modification that plays a vital role in plant development and in responses to different environmental stimuli. Histone deacetylases (HDACs) are responsible for removing lysine acetylation on various proteins. While most work has focussed on the role of Arabidopsis HDACs on histone acetylation, their role in the deacetylation of non-histone proteins is much less known, although proteins of many different organelles have been found to be lysine-acetylated. From the 18 HDACs found in Arabidopsis, only HDA14 has been found to be dual-localized in plastids and mitochondria. Here we performed a quantitative mass spectrometry-based approach, using isobaric TMT labelling, to profile the lysine acetylome of an Arabidopsis *hda14* mutant compared to WT. We identified 1509 acetylation sites on 881 Arabidopsis protein groups, of which 56 sites were de-regulated in the *hda14* mutant. Most of these sites were derived from chloroplast proteins. In addition, we used different co-immunoprecipitation approaches to identify possible interaction partners of HDA14 and to identify its function in the regulation of organellar metabolism.

Poster session / **55**

Proximity-based labelling of the HDA14 interactome

Autor Yannik Stichweh[None]

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Histone deacetylases (HDACs) are a pivotal enzyme in the removing of lysine acetylation on various proteins. While most work has focused on the role of Arabidopsis HDACs on histone acetylation, their role in the deacetylation of non-histone proteins is much less known, although proteins of many different organelles have been found to be lysine-acetylated. From the 18 HDACs found in Arabidopsis, only HDA14 has been found to be dual-localized in plastids and mitochondria. Considering the dynamic nature of these post-translational modifications across various cell types, growth conditions, and time points, our objective is to identify the interplay of protein modifications and regulatory proteins interacting with HDA14. For this, we employ pull-down methods and proximity labeling utilizing the biotin ligase Turbo-ID, fused to the open reading frame of HDA14 and stably integrated into Arabidopsis. Here, we will present our experimental approach and initial findings.

Poster session / **56**

A dynamin-like protein FZL shapes thylakoid membranes and maintains their integrity against enhanced proton motive force

Autor Yu Ogawa[None]

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A dynamin-like protein FZL is one of the few known thylakoid remodeling proteins and has been demonstrated to mediate thylakoid membrane fusion. However, its physiological functions have not been characterized well. First, to determine the sub-chloroplast localization of FZL, we subfractionated isolated chloroplasts/thylakoids and observed GFP-fused proteins, showing that FZL is enriched on curved grana edges. fzl knockout mutants displayed disorganized thylakoid morphologies; grana stacking was staggered, and the grana-stroma lamellae interconnection was scarce compared with that of wild type. Overall, we hypothesize that FZL fuses grana and stroma lamellae membranes at grana edges to shape the network-like thylakoids and that the lack of the FZL-mediated interconnections leads to the morphological disorder observed in the mutants. The thylakoid disturbance moderately altered the electron transfer but hardly affected the plant growth. However, in a series of double mutant analysis, we discovered that the fzl mutant plants were dwarfed when crossed with bfa1 and bfa3 mutants where chloroplast ATP synthase accumulation levels are reduced to 12.5 and 25 % of the WT levels, respectively. In particular, fzl bfa1 mutant exhibited swollen and damaged thylakoid membranes and dropped photosynthetic performance along with diminished photosystem accumulation. The declines in ATP synthase levels likely led to energy deficiency and incremented proton motive force (pmf) in thylakoid lumen; which factor so strongly enhanced the fzl phenotypes? To answer this question, we further crossed the fzl bfa1 double mutant with pgr1 and npq4 mutants and examined the effects of the additional mutations on the fzl bfa1 phenotypes. The former mutation is expected to restrict both energy production and pmf formation, and the latter vice versa. pgr1 suppressed the thylakoid collapse but enhanced the dwarfism, while npq4 recovered the plant growth without inhibiting the thylakoid swelling. This data suggests that the elevated pmf mainly led to the thylakoid explosion in the fzl mutant background, and we propose the novel function of FZL in protecting thylakoid membranes against high pmf. We discuss how one protein shapes and protects thylakoid membranes at the same time and also how membrane integrity is important to sustain the membrane proteins which are successfully translated.

Tue 19 / **57**

News on cyclic electron flow in plants

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Cyclic electron flow (CEF) in photosynthesis has been studied for decades, yet many aspects of its components, mechanisms, and regulation remain unclear. PROTON GRADIENT REGULATION5 (PGR5) is thought to play a crucial role in promoting CEF. The absence of PGR5 disrupts photosynthetic control and increases the sensitivity of photosystem I (PSI) to light damage, resulting in plant death under fluctuating light conditions. Two proteins, PGRL1 and PGRL2, have been identified as regulators of PGR5 activity. PGRL1 directs PGR5 activity, while PGRL2 triggers PGR5 degradation when PGRL1 is not present. This creates an interesting scenario where PGR5 cannot accumulate without PGRL1, but in the absence of both PGRL1 and PGRL2, PGR5 can accumulate and facilitate CEF. To further investigate the roles of PGR5 and PGRL1, researchers conducted a suppressor mutation screen to identify genetic alterations that could rescue *pgr5* or *pgrl1* plants from lethality under fluctuating light conditions. The screen revealed mutations in various genes affecting: PSII function, Cytochrome b6f assembly, Plastocyanin accumulation, Chloroplast FBPase, A regulator of chloroplast FBPase. Interestingly, in at least two cases, the *pgr5* (or *pgrl1*) mutation was found to suppress the effects of other mutations, revealing unexpected functional relationships between these genes and pathways. These findings provide new insights into the complex regulation of photosynthetic electron flow and the roles of PGR5 and PGRL1 in plant adaptation to varying light conditions.

Poster session / **58**

Investigating the function of Arabidopsis HISTONE DEACETYLASE 14 in chloroplasts

Autor Claudia Markiton[None]

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Lysine acetylation is a crucial post-translational modification involved in plant development and responses to environmental stimuli. While much attention has been focused on the role of HDACs in histone acetylation, their involvement in deacetylating non-histone proteins remains less explored. Among the 18 HDACs in Arabidopsis, HDA14 stands out for its dual localization in plastids and mitochondria. To investigate the role of HDA14, we used quantitative mass spectrometry and identified 1509 acetylation sites on 881 protein groups, with 56 sites deregulated in a hda14 mutant compared to WT. Most of the upregulated acetylation sites are associated with chloroplast proteins. Based on these results, proteins known for their substantial position in regulating organellar metabolic processes were chosen to perform interaction studies via enzymatic activity assays, BIFC and phenotyping. Moreover, HDA14 activity was further characterized upon changes in the amino acid code. The findings underline the importance of HDA14 in modulating lysine acetylation dynamics in Arabidopsis, revealing not only its influence on other proteins but also that it is modified itself by N-terminal acetylation through GNAT2, which is one of the N-acetyltransferases in chloroplasts. The function of the N-terminal acetylation of HDA14 is still unknown and requires further investigation.

Poster session / **59**

Searching for new concepts of N-Degron Pathway Mediated Proteostasis

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The N-degron pathway may relate stability of proteins to the biochemical features of its amino (N)terminal stretch or even only the very first residue at this end and its posttranslational modifications (PTMs). Often, these apparently crucial modifications have actually not been shown. To spotlight on these decisive biochemical events, we attempt to demonstrate their existence in vitro and in vivo by enzymatic assays. On top, novel concepts of the possible role of these PTMs in neofunctionalization
of proteins and/or deciding for their fate in the cell appear in the literature. One of them is the connection between N-terminal arginylation and autophagy where the modification can be sensed by adaptor proteins. Our candidate RESISTANCE TO DESICCATION 21A (RD21A) accumulates in N-degron pathway mutants (prt6 and ate) but also autophagy mutants (atg5). It is therefore a novel putative degradation target for one or both pathway branches.
PRT1 is a wellknown player of the plant N-degron pathway with still obscure roles (what are the substrates?) and peculiar functions (why does it autoubiquitinate itself?). We set out in our team to investigate PRT1 on multiple levels including new tools for better assessing its ubiquitination activities.

In the end, we aim to understand molecular functions and biological roles of the N-degron pathway by characterizing enzymatic components and physiological substrates and develop biotechnological tools based on targeted proteolysis.

Poster session / **61**

The function of N-terminal acetylation of plastid precursor proteins

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Chloroplast functionality requires the post-translational import of plastid-destined nuclear-encoded proteins. Chloroplast precursor protein availability in the cytosol and import into the plastid is tightly regulated to maintain chloroplast biogenesis and functionality, respectively. One of these regulatory mechanisms is the co-translational modification of the precursor proteins by N-terminal acetylation (NTA). NTA is a common protein modification and associated with the coordination of proteome stability. Thus, NTA is suggested to determine the half-life of chloroplast precursor proteins in the cytosol. We aim to further investigate and unravel the role of NTA and search for potential new players in the fate of chloroplast precursors. We therefore use Arabidopsis thaliana mutants with reduced N-terminal acetyltransferase A (NatA) complex function, to perform protein import analysis with native and non-acetylated chloroplast precursor substrates. Furthermore, the effects of precursor stability on the biogenesis of chloroplasts and their photosynthetic performance in plastid protein import-deficient plant lines will be investigated.

Mon 18 / **62**

Identification, characterization and function of the unique N-terminal modifiers of the large subunit of plant Rubisco

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Rubisco is a unique catalyst involved in biological carbon dioxide fixation. It is also the most abundant protein on Earth which accumulation rises up to 50% the protein content of leaves. As a major storage protein, nitrogen recycling from Rubisco is essential during degradative processes such as plant senescence or starvation. In land plants, Rubisco functions as a large multi-subunit complex that requires specialized auxiliary factors for the proper assembly of its subunits, RbcL and RbcS. [1]. The catalytic activity of Rubisco depends on the carbamylation of a lysine residue in RbcL and is sustained by a chaperone known as Rubisco activase (Rca). In addition to carbamylation, RbcL was suggested to undergo a number of putative modifications which might impact its overall overall activity [2,3]. Among them, RbcL features a conserved N-terminus starting with an acetylated proline [2,4]. This type of N-terminal acetylation has not been reported in any other protein [5].
I will present data on the identification and characterization of enzymes responsible for completing the N-terminal processing of RbcL and possible function lying behind this remarkably conserved pathway of plants.
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Mon 18 / **63**

Unveiling the Multifaceted Machinery of N-terminal Protein Modifications in Plant Plastids

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Protein modifications are emerging as key regulators of numerous essential cellular processes. Virtually all proteins undergo co- and/or post-translational modifications (CTMs, PTMs). However, a comprehensive understanding of the full range of CTMs and PTMs affecting a given protein throughout its life cycle remains elusive. This is particularly true for chloroplast-localized protein modifications, including N-terminal protein modifications (NPMs), which impact the N-terminus of proteins. The earliest NPM is the essential process of N-terminal methionine excision (NME), which involves the removal of the initial methionine (iMet) from nascent peptide chains. In plastids, NME is facilitated by methionine aminopeptidases (MetAPs), which work in conjunction with peptide deformylases (PDFs) to remove the formyl group attached to iMet. Despite the discovery of plastid NME modifiers in the early 2000s, the plastid-specific MetAPs (pMetAPs) have not been thoroughly characterized, nor have the plastid enzymes responsible for another critical NPM, N-α-acetylation (NTA).
In this study, I will present a comprehensive investigation of the plastid MetAPs and acetyltransferases families, revealing unexpected substrate flexibility and distinctive features that suggest a complex, multitasking protein modification machinery unique to plant plastids. I will also discuss how this machinery has evolved to meet the specific needs of this organelle.

Tue 19 / **64**

Functional interaction of STN7/8 and pCK2 in photosynthetic acclimation

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In plant chloroplasts, protein kinases regulate photosynthetic acclimation by phosphorylation of thylakoid membrane proteins allowing rapid short-term acclimation to changing light conditions. This type of phosphorylation control is mediated by the light-regulated kinases STN7/STN8 at the thylakoid membrane system. Recent data suggested furthermore that STN7 may be involved in long-term acclimation affecting chloroplast and nuclear gene expression (Schönberg et al., 2017, Longoni and Goldschmidt-Clermont, 2021). The plastid kinase originally identified as a regulator of plastid gene expression is plastid casein kinase 2 (pCK2) that phosphorylates RNA binding proteins and components of the transcription apparatus (Rödiger et al., 2021). We have generated the triple mutant stn7/stn8/pck2 and characterized it phenotypically and biochemically. Our goal is to unravel functional crosstalk between these three protein kinases in photosynthetic acclimation. Since the stn7/stn8/pck2 phenotype is severe we hypothesized cooperativity between the different chloroplast kinases in the regulation of chloroplast functions. Phosphoproteome analyses revealed cooperation in the phosphorylation of at least two proteins of the thylakoid membrane system, i.e. PsbH and PSI-P.

Poster session / **66**

Plant Rubisco maturation requires a specific aminopeptidase

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During the oxygenic photosynthesis in plants, algae and cyanobacteria, atmospheric carbon dioxide (CO2) is assimilated into carbohydrates making photosynthetic organisms autotrophic. Rubisco catalyzes this step of carbon dioxide and oxygen uptake. Interestingly, the catalytic subunit of Rubisco (RbcL) undergoes a unique maturation pathway leading to unique N-terminal modifications. This mechanism is conserved in plants, and results in the formation of an N-terminal acetylated Pro3. Which protease(s) are in charge of N-terminal cleavage(s) is unknown so far, as is the impact of this maturation on Rubisco. Here, we present conserved aminopeptidase (AtAMPP and AtAARE) with in vitro experiments from the purified proteins and ad hoc knockout Arabidopsis plant lines. We show that AtAMPP is in charge of residue 2 release, while AtAARE is not involved neither in RbcL maturation or in any N-terminal protein maturation of the plastid. Next, we have established conditions that allow the production of a range of RbcL N-terminal variants in the presence or absence of the identified enzymes involved in its N-terminal maturation. Together, my data deal with a comprehensive characterization of the unique N-terminal Ser2 excision in RbcL processing.

Tue 19 / 67

Photosynthesis-triggered pH and NAD(P) redox signatures across plant cell compartments revealed by advanced illumination-imaging

Autor Ke Zheng[None]

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Plants undergo daily dark-light transitions, leading to dynamic changes of the metabolic and physiological status of their cells. Those changes, which include cofactors, ions and other small molecules such as ATP, NAD(P) redox status, ROS, Ca2+ and pH, are a prerequisite for tuning protein functions through post-translational modifications (PTMs). However, it has been notoriously difficult to monitor those changes in vivo with subcellular precision.
To address this limitation, we have been investigating how photosynthetic activity affects key parameters within and beyond the chloroplast. We developed a new standard of monitoring subcellular energy physiology live by combining confocal imaging of genetically encoded fluorescent protein biosensors with advanced on-stage illumination technology to investigate pH, NADPH/NADP+ and NADH/NAD+ dynamics at dark-light transitions in living Arabidopsis leaf tissues, focusing on mesophyll cells. Our findings reveal a stromal alkalinization signature induced by photosynthetic proton pumping, extending to the cytosol and mitochondria as a cellular 'alkalinization wave'. Moreover, we have been able to dissect the redox dynamics of the stromal and cytosolic NAD and NADP pools driven by photosynthesis-derived electron export.
I will discuss how both the technology as well as the novel insights into the subcellular physiology of photosynthesis will enhance our understanding of the intricate relationship between photosynthetic activity and the biochemical regulation of proteins in phototrophic cells.

Wed 20 / **68**

How a Tiny ATP Synthase Hairpin Loop Affects Dark Adaption in Plants and Algae

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Cysteine redox modulation in proteins is a reversible adaptation to changing environments. For example, activity adjustment of the chloroplast ATP synthase (CF1FO) ensure full activity in the light and deactivation during night. The latter is believed to prevent ATP hydrolysis and build-up of excessive proton motive force (pmf). The adjustment is realised by a cysteine couple in the central CF1FO stalk, the γ-subunit. While being oxidised in the dark, the disulphide is cleaved enzymatically upon illumination which decreases the activation threshold of ATP synthesis/hydrolysis in vascular plants. Although molecular details are known, the general role of CF1FO redox regulation is not understood; it is not vital and absent in non-green chloroplasts.
To revisit potential functions of the redox tuning, we performed reciprocal γ-subunit domain exchanges between Chlamydomonas reinhardtii and Arabidopsis thaliana. We did not swap the cysteines but the adjacent γ-hairpin loop that shows sequence variations between terrestrial and aquatic phototrophs. (i) The Chlamydomonas wild type enzyme remains active despite its disulphide, leading to an elevated dark-pmf. Introducing the plant-like hairpin loop restored the redox fine-tuning mechanism known from plants and the modified algal enzyme maintained a significantly lower dark-pmf. Photosynthesis was not changed in contrast to bioenergetics under heterotrophic dark conditions where various processes, such as chlororespiration, were more active. Only in wild type the photosystem II quantum yields remained stable in darkness for several days. The energetic coupling between mitochondria and the algal chloroplast is influenced by the circadian clock, and misregulated during extended dark when CF1FO is inactive. Consequently, the plant-like mutants had a more reduced plastoquinone pool. (ii) The dark-pmf and CF1FO activity are also important in vascular plants. Preventing CF1FO redox regulation in Arabidopsis increases ATPase activity and thus the dark-pmf. It also sustains high photosynthetic capacities, whereas wild type plants displayed dark-induced senescence.
Our findings link the CF1FO redox regulation to a genetic versatility of the γ-hairpin loop and reveal that the ATP saving mechanism in the dark upon CF1FO inactivation is an oversimplification: While switching off CF1FO activity has developmental implications in plants, this strategy restricts bioenergetics unicellular algae.

Wed 20 / **69**

tRNA sequencing uncovers a blend of ancestral and acquired characteristics in chloroplast tRNAs

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Chloroplasts, crucial organelles in autotrophic organisms, possess distinctive regulatory pathways to control molecular processes that respond dynamically to environmental cues. Protein synthesis in these organelles relies on transfer RNAs (tRNAs) encoded by the chloroplast genome, which undergo extensive post-transcriptional modifications. These modifications play critical roles in accurate mRNA decoding, structural stability of tRNAs, modulation of amino acid charging, and efficient ribosomal recognition. While the phenomenon is common across all genetic systems, the landscape of chloroplast tRNA modification is not well characterised.

In this study, we investigated the post-transcriptional modifications in chloroplast tRNAs of *Arabidopsis thaliana* using tRNA sequencing, liquid chromatography-mass spectrometry, and analysis of public data. Our results revealed similarities between chloroplast tRNAs and bacterial systems (e.g., *Escherichia coli*), such as modification patterns at the anticodon-adjacent position and the variable loop of tRNAs. Additionally, we identified structural features shared with eukaryotic systems that likely contribute to the proper folding and functionality of chloroplast tRNAs. Notably, our tRNA-seq analysis identified the presence of the recently discovered 2-aminovaleramididine modification at the wobble position of tRNA-IleCAU, which is crucial for differentiating isoleucine codons from methionine codons.
These findings suggest that the chloroplast translation machinery, through co-evolution with its eukaryotic host, has adopted features beyond those typically found in bacteria, reflecting a blend of ancestral and acquired characteristics. This dual adaptation likely enhances the efficiency and fidelity of chloroplast translation, providing insight into the evolutionary convergence of chloroplast and eukaryotic systems.

Tue 19 / 70

Establishing fluorescent protein-based biosensing of NADPH:NADP+ dynamics in living plants

Autor Jan-Ole Peer Niemeier[None]

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The pyrimidine nucleotide cofactors NAD and NADP operate at the critical intersection between the cellular metabolic network, Cys-based systems and redox regulation. In plants, the redox states of the NAD and NADP pools of the cytosol and the chloroplast stroma are physically separated, but connected by metabolic shuttles, such as the malate/oxaloacetate and the triose-phosphate shuttle. Changes in photosynthetic activity trigger a major transition in subcellular redox dynamics going far beyond the chloroplast. While the biochemical importance of NAD(P) has been well established, its subcellular regulation and its dynamics in living cells have been difficult to explore. I will present my recent efforts of rationally engineering a fluorescent protein-based biosensor for NAD redox status to generate and characterize a novel biosensor family that specifically responds to NADPH:NADP+. The design of the new sensor family enables the monitoring of the biologically important NADP redox status and overcomes critical downsides of previous NADPH sensors. Plant lines expressing the sensor enable the dynamic mapping of NADP redox dynamics and the resolution of NADP responses down to the single cell. I will discuss the properties of the new sensor family for exploration of plant cell physiology and will highlight our attempts to dissect NADP redox dynamics at the interface of metabolism and signalling.

Mon 18 / 71

Dual lysine and N-terminal acetyltransferases as modifiers of Rubisco and CBB enzymes

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Acetylation on amino groups is a common modification seen in proteins across various organisms. This process involves N-terminal acetyltransferases (NATs) and lysine acetyltransferases (KATs) that

transfer acetyl groups from acetyl-Coenzyme A (acetyl-CoA) to the N-terminal amino groups and to the side chains of lysine residues, respectively. In the case of plants, the majority of plastid proteins are imported from the cytosol and are subject to cleavage of their N-terminal signal peptides. The plastid proteins that are encoded internally are processed at their N-termini by various aminopeptidases. It has been observed that many of the newly formed N-termini and the lysine residues on the side chains of proteins undergo acetylation. However, the specific NATs and KATs responsible for these acetylation processes remain largely unidentified. In recent studies, specifically in the model plant Arabidopsis thaliana, eight plastid-localized and dual-specific acetyltransferases (GNATs) have been discovered (Bienvenut et al. 2020). Especially, the large subunit of Rubisco which is plastid encoded, undergoes many posttranslational modifications. The initiator methionine gets deformylated and removed. Subsequently, the second amino acid at the N-terminus gets cleaved of, revealing a proline. Multiple studies found the Pro3 N-terminus of RbcL acetylated (Zybailov et al. 2008; Rowland et al. 2015; Soh et al. 2020). Beside of this up to 19 ε-lysine acetylation sites were found on RbcL (Hartl et al. 2017; Finkemeier et al. 2011).
We employed three N-terminal CoA-conjugated peptide probes (CoA-N) to selectively enrich active N-terminal acetyltransferases (NATs) from plant extracts based on substrate preferences. Additionally, HPLC based activity assays with synthetic fluorophore-labelled peptides mimicking RbcL N-terminal sequences were used to identify plastid-localized NATs and lysine acetyltransferases (KATs) capable of modifying Rubisco. Additionally, lysine acetylome profiling of two gnat7 knockout lines and co-immunoprecipitation of GNAT7-GFP from Arabidopsis leaf material revealed regulated lysine acetylation sites on Calvin-Benson-Bassham cycle enzymes. Notably, three enzymes, previously linked to N-terminal modifications of RbcL, were significantly enriched in the co-immunoprecipitation, indicating their potential involvement in a modification pathway.

Poster session / **72**

Molecular determinants of protein halflife in chloroplasts

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Proteolysis is an essential process to maintain cellular homeostasis. One pathway that mediates selective protein degradation is the N-degron pathway, which relates the in vivo half-life of a protein to its N-terminal amino acid residue. In the cytosol of eukaryotes and prokaryotes, N terminal residues are major determinants of protein stability. While the eukaryotic N-degron pathway depends on the ubiquitin-proteasome-system, the prokaryotic one is driven by the Clp protease system. The adapter protein ClpS recognizes proteins bearing N-terminal destabilizing residues and delivers the substrate to the ClpAP chaperone-protease complex for degradation. Chloroplasts also contain the Clp protease system, including the adapter protein ClpS1, the chloroplast homologue of the bacterial substrate selector ClpS. Since chloroplasts are bacterially-derived, it seems reasonable to speculate that chloroplasts contain an N-degron pathway similar to the one in prokaryotes.

The aim of my PhD thesis is to investigate the impact of different N-terminal amino acids on chloroplast protein stability. Studying the stability of a protein dependent on its N-terminal residue requires a mechanism, which selectively exposes the amino acid at the N-terminus. In our case, a tobacco etch virus (TEV) protease based approach is used to activate the dormant N degron of a reporter protein. Plastid transformation in tobacco was used to generate plants with a plastid-encoded TEV protease. The reporter protein is introduced into the transformed plants using a transient transformation approach. Following translocation into the chloroplast, the plastid localized TEV protease can cleave the reporter protein and expose the desired N terminal residue. The outlined reporter system is used to, for the first time, systematically and comprehensively challenge the effect of specific N terminal amino acids on protein stability in this organelle.

Tue 19 / **73**

The plastidial protein acetyltransferase GNAT1 forms a complex with GNAT2, yet their interaction is dispensable for state transitions

Autor Annika Brünje[1]

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Plants are exposed to a constantly changing environment, which requires fast acclimation strategies. Post-translational modifications (PTMs) of proteins allow cells to respond rapidly to varying environmental conditions and have the potential to alter localization, interactions, or enzymatic activities of proteins. Protein acetylation is one of the most abundant co- and post-translational modifications in eukaryotes and its occurrence extends to chloroplasts in vascular plants. In Arabidopsis, a novel plastidial enzyme family consisting of eight acetyltransferases with dual lysine and N-terminal acetylation activities has recently been unveiled. Among them, GNAT1, GNAT2, and GNAT3 reveal notable phylogenetic proximity, forming a subgroup termed NAA90.
Here, I will focus on GNAT1, the closest relative of GNAT2, which is known for its regulatory function in the state transition of photosynthetic antenna proteins. In contrast to GNAT2, GNAT1 was not found to be essential for state transitions. However, our results demonstrate a shared set of N-terminal substrate sites between GNAT1 and GNAT2 in vivo. Furthermore, co-immunoprecipitation coupled to mass spectrometry revealed a robust interaction between GNAT1 and GNAT2, as well as a significant association of GNAT2 with GNAT3, the third acetyltransferase within the NAA90 subfamily. Dimer models of GNAT1-GNAT2 and GNAT2-GNAT3 generated by AlphaFold 2 Multimer indicate that the same surface-exposed amino acid residues of GNAT2 are involved in both interactions. This leads to the conclusion that the formation of multimers containing more than two GNATs is rather unlikely to occur - a conclusion, which is also supported by the AF 2 Multimer confidence score index, according to that all multimeric structures of three or more GNATs decrease significantly in their confidence ranking. Our results point to complex formation as a novel layer of regulation that may fine-tune the activities of plastidial acetyltransferases. Elucidating the specific, functional impact of individual dimer formations will be a promising task for future research.

Poster session / 74

Developing functional and structural imaging of proton motive force determinants

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In photosynthesis, light drives electron transfer reactions that conserve reducing power and energy. Electron transfer is coupled to proton translocation into the chloroplast thylakoid lumen, building

up a proton motive force (PMF) that drives ATP synthesis. Regulating PMF is essential for rapidly adjusting photosynthesis to fluctuations in light intensity, enabling a dynamic balance between protective mechanisms, such as non-photochemical quenching, and efficient light utilization. Even though the general principles that underpin the PMF in photosynthesis are well-understood, how exactly the makeup of the PMF is regulated in vivo and at the level of the individual chloroplast remains unclear.
The PMF consists of two components: the membrane potential ($\Delta\Psi$) and the proton gradient (ΔpH). Both components can be measured –however the available approaches are indirect and are limited in spatial resolution, which is why a detailed picture of PMF heterogeneity between individual cells, chloroplasts or even thylakoid membranes is lacking. To address this knowledge gap, we are developing in vivo biosensing methodologies for real-time PMF monitoring and optimize high-resolution light microscopy techniques to visualize single chloroplasts and photosynthetic membranes. To this end we have expressed different genetically encoded biosensors in Arabidopsis thaliana to directly measure pH dynamics at either side of the thylakoid membranes in response to varying light conditions. This approach will be expanded to Chlamydomonas reinhardtii and is bolstered by the in-depth characterization of the biochemical and biophysical properties of each of the sensors in vitro and in vivo, ensuring specificity and minimizing measurement artifacts. Furthermore, we will advance super-resolution imaging techniques to investigate the thylakoid membrane in order to explore the structural basis of the PMF, its partitioning and potential heterogeneity. To this end we have started to develop advanced protocols for protein labeling and single-particle localization microscopy.
By integrating real-time biosensing with cutting-edge imaging technologies, we will contribute to a new level of understanding to PMF dynamics and photosynthetic efficiency. Additionally, once established these tools and techniques will be made available to the community to foster further discoveries.

Wed 20 / 75

DIA-HUNTER: Increased plant N-terminome coverage by library-free data independent mass spectrometry

Autor Pitter Huesgen[1]

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The N-termini of chloroplast proteins are a common site of co- and post-translational protein modifications, including N-terminal acetylation, transit peptide cleavage and subsequent proteolytic processing, that result in distinct proteoforms that may differ in activity, interactions and location. However, protein N-terminal peptides are often missed in standard shotgun proteomics experiments.
Over the last two decades, multiple enrichment methods have been developed that overcome this problem. Terminal Amine Isotope Labeling of Substrates (TAILS), for example, enabled us to characterize N-terminal determinats of transit peptide cleavage and subequent N-terminal modifications in chloroplasts of both Arabidopsis thaliana and Physcomitrium patens. However, dertermination of sub-stoichiometric changes have often been hampered by poor reproducibility and insufficient sensitivity.
In recent years, data independent acquisition (DIA) methods, combined with advances in mass spectrometry instrumentation and new computational tools, have massively improved the reproducibility and sensitivity of mass spectrometry-based proteomics. We have now established analysis of protein N-terminal peptides enriched by our improved Hypersensitive Undecanal-mediated N-TERmini enrichment (HUNTER) protocol in library-free DIA mode using FragPipe. We evaluated the performance of DIA-HUNTER by rigorous benchmarking to traditional DDA-analysis across multiple instruments. We consistently observe substantial increases in reproducibly quantified N-terminal peptides in DIA-mode compared to DDA-mode, independent of the mass spectrometry system used. This will improve future analysis of dynamic, regulatory N-terminal modifications in plastids.

Tue 19 / **76**

The Clp chaperone-protease is a central regulator of chloroplast proteostasis; the search for substrate selection and regulation

Autor Klaas J. van Wijk[1]

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ABSTRACT Different proteases and peptidases are present within chloroplasts and non-photosynthetic plastids to process precursor proteins and to degrade cleaved chloroplast transit peptides and damaged, misfolded, or otherwise unwanted proteins. Collectively, these proteases and peptidases form a proteolysis network, with complementary activities and hierarchies, and build-in redundancies [1]. The challenge is to determine the contributions of each peptidase (system) to this post-translational network. This will require an understanding of substrate recognition mechanisms, degrons, substrate and product size limitations, as well as the capacity and degradation kinetics of each protease. Extra-plastidial degradation pathways complement these intra-chloroplast proteases. Following a conceptual overview of this intra-plastid protease network, I will provide an update on our search for substrates, substrate selection and regulation of the essential and abundant chloroplast Clp chaperone-protease system and its adaptors in Arabidopsis [2]. This search includes loss-of-function analysis, in vivo trapping, protein half-life measurements, N-terminomics, in vitro interactome analysis, combined with protein mass spectrometry. I will also highly our recent proposal that UVR protein motifs play a regulatory role in substrate selection and Clp activity [3].
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Wed 20 / **78**

Light-dependent regulation of post-translational modifications in chloroplasts of Arabidopsis

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Photosynthesis must be dynamically regulated in response to environmental conditions to prevent photoinhibition and photodamage to the photosystems. Various post-translational modifications of proteins are known to play important roles in modulating photosynthetic efficiency during plant acclimation responses. Alongside phosphorylation and dithiol-based modifications, acetylation of amino groups within proteins is a major post-translational modification, particularly abundant on proteins involved in photosynthesis. The role of protein acetylation in photosynthesis regulation is only beginning to be uncovered. Additionally, very little is known about the interplay and kinetics of different post-translational modifications. Here, I will present a global overview of light-dependent modifications in chloroplasts, with a particular focus on lysine and N-terminal acetylation in conjunction with phosphorylation.

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Functional interaction of STN7/8 and pCK2 in photosynthetic acclimation

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In plant chloroplasts, protein kinases regulate photosynthetic acclimation by phosphorylation of thylakoid membrane proteins allowing rapid short-term acclimation to changing light conditions. This type of phosphorylation control is mediated by the light-regulated kinases STN7/STN8 at the thylakoid membrane system. Recent data suggested furthermore that STN7 may be involved in long-term acclimation affecting chloroplast and nuclear gene expression (Schönberg et al., 2017, Longoni and Goldschmidt-Clermont, 2021). The plastid kinase originally identified as a regulator of plastid gene expression is plastid casein kinase 2 (pCK2) that phosphorylates RNA binding proteins and components of the transcription apparatus (Rödiger et al., 2021). We have generated the triple mutant stn7/stn8/pck2 and characterized it phenotypically and biochemically. Our goal is to unravel functional crosstalk between these three protein kinases in photosynthetic acclimation. Since the stn7/stn8/pck2 phenotype is severe we hypothesized cooperativity between the different chloroplast kinases in the regulation of chloroplast functions. Phosphoproteome analyses revealed cooperation in the phosphorylation of at least two proteins of the thylakoid membrane system, i.e. PsbH and PSI-P.

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Light changes promote distinct responses of plastid protein acetylation marks

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Protein acetylation is a key co- and post-translational modification. How different types of acetylation respond to environmental stress is still unknown. A member of the newly discovered family of plastid acetyltransferases, which is featuring both lysine- and N-terminal acetyltransferase activities, was used to obtain a holistic multi-omics acetylation-dependent view of the acclimation of plants to short-term light changes.
We investigated the role of acetylation in the plant's response to changes in light intensity using mass spectrometry-based proteomic and acetylome profiling. We grew WT and *gnat2* plants under the same conditions and subjected them to high light, darkness, or standard growth conditions for two hours. This analysis revealed that the different types of acetylations, catalysed by GNAT2 in the chloroplast, distinctively respond to changes in light conditions.

Under high light treatment, the *gnat2* mutant showed a more pronounced de-regulation in the lysine acetylome, with 50 acK sites up-regulated compared to only nine acK sites in the WT. The *gnat2* mutant specifically downregulated diverse anabolic reactions and upregulated the base excision repair pathway in response to short-term high light treatment. The analysis also showed that the gnat2 mutant had a more pronounced de-regulation in the lysine acetylome under darkness, with 7 acK sites significantly changed compared to only 2 acK sites in the WT.

Furthermore, the analysis revealed that plastid NTA yield did not significantly change under different light conditions. However, the *gnat2* mutant displayed downregulation of transcripts involved in translation-related pathways under darkness, suggesting that GNAT2 might be involved in the light-dependent control of translation.

In conclusion, our study highlights the importance of lysine acetylation in the plant's response to changes in light intensity and suggests that plastid K- and N-terminal acetylations may respond differently to environmental or developmental stimuli. Our research provides valuable insights into the role of lysine acetylation in the plant's response to changes in light intensity and the interplay between genetic and environmental factors by mass spectrometry-based acetylome profiling.

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Regulative phosphorylation of plastocyanin and cytochrome b6f subunit IV: Insights into photosynthetic electron transfer and STT7 kinase feedback control

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In this study, we examined plastocyanin (PC) binding and electron transfer with both photosystem I (PSI) and cytochrome b6f (cyt b6f), and show the synergetic adaptations between these three enzymes. Furthermore, we explored the effects of PC phosphorylation on these interactions. To do so, we generated several recombinant variants of PC, in which we genetically engineered two of the phosphorylated residues (S10 & S49). We studied the kinetics of both Cytf oxidation and P700 re-reduction by measuring fast optical spectroscopy. We also conducted chemical protein crosslinking and structural proteomics to gain further insights on the interaction between PC and cyt b6f. Our results show that the phosphorylation mode of PC alters the conformation in which they establish binding and electron transfer, and generated new models which elaborate the mechanism of this adaptation. To address the role of STT7 dependent phosphorylation of Thr4 in the N-terminal domain of cyt b6f subunit IV, we generated site-directed mutants in the N-terminal domain of cyt b6f subunit IV by chloroplast transformation. The phosphomimic mutation PetD Thr4/Glu effectively inhibits STT7 kinase activity, as shown by phosphoproteomic analyses, resulting in the PetD Thr4/Glu strain being locked in State 1. These findings reveal a novel feedback regulation mechanism that controls the phosphorylation capacity of STT7 kinase. Similarly, deletion of five N-terminal amino acids cyt b6f subunit IV also disrupts STT7 function, retains cells in State 1, and, in contrast to the PetD Thr4/Glu mutation, significantly impairs electron transfer within cyt b6f. These data reveal that the PetD N-terminus has crucial functions in cyt b6f electron transfer and STT7 regulation.

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How do plants overcome the excess sugars-driven repression of photosynthesis in suc2 mutant leaves?

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With the energy from the sunlight, plants assimilate atmospheric CO2 via photosynthesis and produce sugars. However, photosynthesis-associated genes are downregulated when sugars accumulate beyond the storage or transport capacity in leaves to reduce carbon assimilation. While various sugar signalling pathways have been proposed to control this downregulation, the molecular mechanism of how plants cope with excessive sugar accumulation in photosynthetically active leaves remains elusive.
Here, we employed Arabidopsis thaliana mutants of *sucrose-proton symporter 2* (*suc2*), accumulating sugars in photosynthetically active leaves. In a new suppressor screen, we isolated novel mutants with larger biomass in the *suc2* mutant background. The physiological characteristics of the *suc2* mutant and the revertant, such as the starch accumulation pattern and photosynthesis performance, will be presented, and the hypothetical scenario of how the plants found a way to recover from the saturation of sugars will be discussed.

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Acetylated HY5 Mediates Chloroplast Gene Expression for Improved High Light Stress Defense in Arabidopsis thaliana

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Light is the primary energy source for photosynthesis and plays a crucial role in regulating numerous developmental processes in plants. However, high light (HL) conditions that exceed chloroplast energy requirements can trigger oxidative stress, necessitating protective responses within plant nuclei. The transcription factor ELONGATED HYPOCOTYL5 (HY5) operates downstream of multiple photoreceptor families and regulates diverse developmental pathways, including photomorphogenesis, anthocyanin biosynthesis, and chloroplast development. Despite its well-known functions, the molecular mechanisms underlying HY5-mediated transcriptional regulation of chloroplast-related genes under HL conditions remain largely unexplored. N-ε-lysine acetylation, a dynamic post-translational modification (PTM), is essential for modulating protein functions, particularly in transcriptional regulation. HY5 is known to interact with histone deacetylases, such as HDA15 (a negative regulator) and HDA19 (a positive regulator), to influence photomorphogenesis through co-regulated histone acetylation. Additionally, we identified an HL-induced acetylation site on the HY5 protein. Here, we aim to investigate the role of this acetylation in promoting phenotypic changes and explore its potential function in HY5-mediated transcriptional regulation. Our findings will provide insights into how acetylation of HY5 contributes to the plant's defense against HL-induced oxidative stress, ultimately supporting chloroplast protection and development.

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Challenging the role of plastid co-translational N-terminal modifications upon stress responses

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Co-translational N-terminal modifications of plastid genome-encoded proteins also belong to key mechanisms for the stability and function of these proteins. Previous studies have already shown that the plastid-localized methionine aminopeptidase isoforms MetAP1B, MetAP1C and MetAP1D remove the N-terminal methionine of several nascent peptides, while particular the role of MetAP1C

is crucial for the N-terminal processing of RbcL. The plastid proline aminopeptidase 2 (APP2) is proposed to be responsible for the cleavage of the second N-terminal amino acid residue serine in RbcL, which is positioned before a proline, while isoforms of GCN5-N-terminal acetyltransferases (GNAT) specifically acetylates different dedicated enzymes for the modification of either the N-termini of proteins or internal lysine residues. We are interested in investigating the specific role of these enzymes for the maturation of plastid-localised proteins, with particular emphasis on RuBisCO, and have used single and double mutants for MetAP1C, APP2 and GNAT7 (map1c, gnat7, app2-4, map1c x gnat7, map1c x app2-4, gnat7 x app2-4) to analyse the physiological and biochemical effects of the missing proteins for the N-terminal processing of plastid-encoded proteins under standard and stress conditions. In particular, single and double map1c mutants showed phenotypic changes in response to cold treatment, such as pale green leaves and slow growth compared to the WT plants. Altered photosynthetic capacities were detected due to reduced protein content of various proteins in the chloroplasts involved in photosynthesis and carbon assimilation.

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Occupancy of lysine acetylation in Arabidopsis proteome via chemical labelling and mass spectrometry measurements

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In course of the day, the environmental conditions are changing, so that the plants have different needs of proteins and their functions. As a result, the proteome needs to change constantly and in a dynamic way. Post-translational modifications (PTM´s) change the properties of present proteins and have a massive impact on their functions, structures and activities. Such a modified protein has a changing mass, that is detectible by mass spectrometry. We are looking at the acetylation of lysine residues over the whole proteome of Arabidopsis (e.g. adult leafs). Using a method for chemical labelling of lysine with heavy (D6-)acetic anhydride (Baeza et al. 2020), we want to define the occupancy from lysine residues, that were naturally acetylated in Arabidopsis, through to different stages of the day/night. Therefore, we use various proteases to generate lots of peptides, with different cleaving sites, measure them by mass spectrometry and determine the occupancy of lysine acetylation. For a better understanding which impact the site-specific acetylation of lysine has in plants and how big the changes are during different stages and environmental conditions.

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Characterization of a chloroplast Acetyltransferase in Arabidopsis thaliana

Autor Marie Stenkamp[1]

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Chloroplast Acetyltransferases in Arabidopsis thaliana are a part of the General control non-repressible 5-related N-Acetyltransferase superfamily (GNAT), which is characterized by a high structural conservancy. Among the acetylated chloroplast proteins, those involved in photosynthesis make up a large proportion, indicating that the GNATs may be important regulators of photosynthesis (Hartl et al. 2017). All chloroplast GNATs show N-terminal as well as lysine acetylation activity in different intensities. When expressed heterologous, 2 of the 8 acetyltransferases in the chloroplast show more relaxed acetylation activities compared to the remaining six, pointing to a putative redundancy in the plant on the one hand but give also hints on the dependency of these proteins on complex

partners on the other hand (Bienvenut et al. 2020). Previous research already highlighted the tendency of GNAT proteins to form complexes critical for their function in diverse organelles (Liszczak et al. 2013). Our research fucuses on one of the chloroplasts acetyltransferases with relaxed activity when expressed heterologous. It exists in 7 different splice forms and our goal is to shed light on the specific functions of these splice forms by investigating altered localisation or acetylation activities. Furthermore, we want to examine the physiological role of the acetyltransferase and its splice forms with a knockout mutant line and its respective complementations. Regarding the relaxed acetylation activity in heterologous expression studies, another focus is on the identification of putative interaction partners of the enzyme in planta and on the question whether the acetyltransferases activity in influenced by Co-Expression partners in vivo.

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Exploring the role of PSAG and PSAH in the modulation of PSI-LHCI composition and macromolecular organization in Chlamydomonas reinhardtii

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The structural plasticity of photosystem I –light harvesting complex I (PSI-LHCI) is reflected in the recent resolution of diverse PSI-LHCI macromolecular organization states in the green alga Chlamydomonas reinhardtii: PSI-LHCI1,2, PSI-LHCI –light-harvesting complex II (LHCII)3,4 and dimeric PSI-LHCI5. It has been proposed that dynamic changes in PSI subunit composition and LHCI configuration induce macromolecular PSI-LHCI remodelling: The small transmembrane PSI subunits PSAG and PSAH together with the antenna proteins LHCA2 und LHCA9 may comprise a structurally interlinked regulatory module controlling the macromolecular organization of PSI-LHCI. Adding another level of regulation, PSI-LHCI structural remodelling might be influenced by dynamic post-translational modifications: Both PSAG and PSAH have been observed phosphorylated in C. reinhardtii in response to high light or anoxia, with PSAG phosphorylation depending on STT76,7. This study investigates the impact of a CRISPR-mediated knockout of PSAG and PSAH on the composition and macromolecular organization of PSI-LHCI in C. reinhardtii, along with its potential implications on light harvesting and electron transfer. Furthermore, this study takes advantage of chemical protein crosslinking-coupled mass spectrometry to identify potential further interaction partners of PSI-LHCI in C. reinhardtii [8].

1: Suga et al., Nature Plants, 2019
2: Su et al., Nature Plants 2019
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6: Bergner et al, Plant Physiology 2017
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