Chloroplast Metabolism and Photosynthesis Meeting Abstracts

Faculté des Sciences UniMail, Emile-Argand 11, Aula Louis-Guillaume (ALG, 2nd floor)

Invited Speakers (IS)

Selected Speakers (SS)

26 June 2017

Session 1 – Mechanism of photosynthesis

Chair : Jean-David Rochaix

IS-1: BREAKING THE DOGMA: PHOTOSYNTHESIS WITHOUT CAROTENES.

Pengqi Xu, Vohla U. Chukhutsina, Gert Schansker, Ludwik W. Bielczynski, Yinghong Lu, Daniel Karcher, Ralph Bock and Roberta Croce

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Carotenoids play essential roles in plant photosynthesis: they stabilize the pigment-protein complexes, are active in harvesting sunlight and are especially involved in photoprotection via several different mechanisms. In plants, carotenoids are present as carotenes, mainly in the form of β-carotene, associated with the core complexes of Photosystem II and I, and as xanthophylls, a variety of oxygenated derivates, which are bound to the light-harvesting complexes. While mutant plants lacking xanthophylls show photoautotrophic grow, no plants lacking carotenes have been reported so far, leading to the conclusion that carotenes are essential for the survival of the plant. We have studied an N.tobacco plant modified to produce astaxanthin, a ketocarotenoid. We show that these plants do not contain any carotenes and that astaxanthin is the only xanthophyll present. Despite this, they grow photoautotrophically, showing that carotenes are not essential for photosynthesis. The effect of the absence of β-carotene on the components of the photosynthetic apparatus is discussed.

IS-2: PHOTOPROTECTION IN CYANOBACTERIA: THE ORANGE CAROTENOID PROTEIN AND THE OCP RELATED PROTEINS.

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The photoactive Orange Carotenoid Protein (OCP) photoprotects cyanobacteria cells by quenching excess energy absorbed by phycobilisomes (PBSs), the cyanobacterial antenna, and by directly interacting with singlet oxygen. Both, the dark orange form (OCP^o) and the photoactivated red light form (OCP^r) quench singlet oxygen. In contrast, only OCP^r is able to bind PBSs and to quench excess energy. Light absorption by the carotenoid provokes changes in carotenoid-protein interaction leading to protein opening and carotenoid translocation. In the opening of the OCP, the amino acid interactions in the central interface between the C-terminal (CTD) and the N-terminal domains (NTD) and those between the N-terminal arm and the CTD are broken. The carotenoid binding NTD of OCP^r binds to the core of PBS inducing energy

and fluorescence quenching. The Fluorescence Recovery Protein (FRP) by binding to the CTD of the bound OCP^r helps the detachment of the OCP from the PBS and then accelerates the dark reconversion OCP^r to OCP^o. In many cyanobacteria, multiple copies encoding homologues to OCP-NTD (named HCPs) and one homologue to the CTD-OCP (named CTDH) are dispersed in their genomes. Both HCPs and CTDHs are carotenoid proteins. HCPs are monomers while CTDHs are dimers stabilized by the binding of a carotenoid molecule. The subgroups of HCPs have distinct functions. Only one is able to bind PBSs and to quench energy; others are good singlet oxygen quenchers. The CTDHs are good singlet oxygen quenchers and have the ability to give their carotenoids to apo-HCPs and apo-OCPs. The importance of this activity in vivo remains to be demonstrated since holo-OCPs are able to be synthesized in the absence of CTDHs in *E. coli* and cyanobacteria. Moreover, we show that apo-OCP is able to take the carotenoid directly from the membranes.

IS-3: HIGH-RESOLUTION STRUCTURES OF PLANT AND CYANOBACTERIAL PHOTOSYSTEM I.

Yuval Mazor, Ido Caspy, Tirupathy, Malavath, Anna Borovikova, Sigal Netzer-El, Daniel Klaiman, Nidaa Herzallah, Maya Antoshvili and Nathan Nelson

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Plant Photosystem I (PSI) is one of the most intricate membrane complexes in Nature. It is comprised of two complexes, a reaction center and light-harvesting LHCI. We developed a method for obtaining better mass spectroscopy data from membrane complexes. Using the corrected amino acid sequences an improved plant PSI structure was obtained. An atomic-level structural model of higher plant PSI at 2.6 Å resolution has been constructed based on new crystal form. The crystal belongs to P212121 symmetry space group, with one protein complex in each asymmetric unit. The structure includes 16 subunits and more than 200 prosthetic groups, the majority of which are light harvesting pigments. The model reveals detailed interactions, providing mechanisms for excitation energy transfer and its modulation in one of Nature's most efficient photochemical machine. Recently we solved the structure of trimeric PSI from Synechocystis at 2.5 Å resolution. Several differences between the mesophilic and thermophilic PSI were reveiled and the position of lipids between the monomers was determined. Similarly the structure of monomeric PSI was determined. An operon encoding PSI was identified in cyanobacterial marine viruses. We generated a PSI that mimics the salient features of the viral complex containing PsaJ-F fusion subunit. The mutant is promiscuous for its electron donors and can accept electrons from respiratory cytochromes. We solved the structure of the PsaJ-F fusion mutant as well as a monomeric PSI at 2.8 Å resolution, with subunit composition similar to the viral PSI. The novel structures provided for the first time a detailed description of the reaction center and antenna system from mesophilic cyanobacteria, including red chlorophylls and cofactors of the electron transport chain. Our finding extends the understanding of PSI structure, function and evolution and suggests a unique function for the viral PSI.

IS-4: ENERGY LIMITATIONS IN PHOTOSYNTHETIC REACTION CENTERS.

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Oxygenic photosynthesis is driven by the photochemistry of chlorophyll *a* with PSI and PSII undergoing charge separation at 700 and 680nm respectively. PSII seems to be "energy squeezed". Out of the 1.82eV available in the 680nm photon, ~1 eV is used for the redox chemistry of water oxidation, O_2 formation and quinone reduction, and for the electrochemical gradient formation. The rest of the energy, ~0.82 eV, is released as heat to drive the reactions (for charge separation and stabilization, quinol release and as activation energy for water oxidation) and to minimise damaging back-reactions. The unique energy requirements of PSII mean that damaging back-reactions cannot be fully eliminated. This could be responsible for the "red limit", i.e. that oxygenic photosynthesis occurs at 680/700nm and not at longer wavelengths. However *Acaryochloris PSII,* using chlorophyll *d* at 720nm, represents an extension of the red limit. To rationalize this and the PSII energy accounting, we suggested that 720nm PSII must have sacrificed an expendable energy requirement, some sort of "energy slack" or "energy headroom", which is needed to deal with variations in environmental conditions, particularly light intensity. *Acaryochloris*, living under a green sea-squirt, seemed to fit the bill as occupying a low variation ecological niche where this "headroom" could be cut back. The PSII energy headroom was recently identified as being that needed to allow for transient decreases in energy gaps imposed by pulses of electric field (ΔΨ) induced by variable intensity light. Here biophysical studies will be presented that improve PSII energy accounting, show the redox tuning to minimize photodamage and provide data on far-red species where the red limit is further extended.

SS-1: DISSIPATIVE RESPONSE TO EXCESS LIGHT IS CATALYZED IN MONOMERIC AND TRIMERIC LIGHT-HARVESTING COMPLEXES BY TWO DISTINCT MECHANISMS.

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Oxygenic photoautotrophs require mechanisms for rapidly matching the level of chlorophyll excited states produced by light harvesting with the rate of photochemical reactions and electron transport from water to CO2. These photoprotective reactions prevent formation of reactive excited states and photoinhibition. The fastest response to excess illumination is the so-called Non-Photochemical Quenching (NPQ) which, in higher plants, requires the lumenal pH sensor PSBS and other yet unidentified components of the Photosystem II antenna system. Both trimeric LHCII and monomeric LHC proteins have been indicated as site(s) of the heat-dissipative reactions. Different mechanisms have been proposed: energy transfer to a lutein quencher in trimers, formation of a zeaxanthin radical cation in monomers. Here, we report on the construction of a mutant lacking all monomeric LHC proteins while retaining LHCII trimers. Its NPQ induction rate was substantially slower with respect to the wild type. A carotenoid radical cation signal was detected in wild type, while lost in the mutant. We conclude that NPQ is catalyzed by two independent mechanisms with the fastest activated response catalyzed within monomeric LHC proteins depending on both zeaxanthin and lutein and on the formation of radical cation. Trimeric LHCII, instead, is responsible for the slow activated quenching

component, which does not involve charge transfer events and depends on zeaxanthin while lutein is not required.

Session 2 – Chloroplast biogenesis

Chair : Felix Kessler

IS-5: NEW INSIGHTS INTO THE COMPOSITION AND FUNCTION OF THE 1-MDA TIC COMPLEX.

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The chloroplast protein import machinery is essential for chloroplast biogenesis as it imports several thousand proteins from the cytosol into the chloroplast. However, knowledge on its exact functioning and the subunits involved in protein translocation is incomplete. Using Tap-tagged Toc159, we reproducibly identified stable interaction partners of the outer envelope translocase among them several proteins with unknown function and a 1-MDa TIC complex. Tic56 is an essential subunit of the 1-MDa protein complex at the inner chloroplast envelope membrane that comprises Tic100, Tic214 and the protein conducting channel protein Tic20-I. The complex was designated the “general protein import translocase” because mutants in either of its subunits have a severe growth phenotype and fail to assemble a photosynthetic machinery. We show here that the albino phenotype of Tic56 null mutants results at least in part from a defect in ribosome assembly and a deficiency in plastid translation. Using specific enrichment of protein amino termini, we demonstrate a considerable degree of protein import activity into Tic56-deficient plastids indicating the existence of alternative translocases at the inner envelope membrane. Our data establish a previously unknown link between plastid protein import and the assembly of plastid ribosomes and suggest that the 1-MDa complex consists of subunits that have functions other than protein import.

IS-6: PROTEIN IMPORT INTO CHLOROPLASTS – HOW, WHEN AND HOW MUCH?

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Short-term acclimation of chloroplasts to various environmental triggers requires drastic changes in its proteome composition. The amount of photosynthetic complexes needs to be adapted in response to varying light intensities or temperature to ensure optimal performance. Since the majority of chloroplast proteins is encoded in the nucleus and translated as preproteins in the cytosol, posttranslational targeting is a first crucial step towards providing these organelles with the required proteins. This involves tightly regulated posttranslational modifications, such as phosphorylation or acetylation of preproteins in the cytosol. To enter the chloroplast, preproteins have to cross the double envelope membrane, which is facilitated by two complex translocation machineries responsible for preprotein recognition and transport. The import process itself is highly regulated at different levels from the outside as well as the inside of the organelle where distinct signalling cascades lead to dynamic acclimation of import activity.

SS-2: CHLOROPLAST BIOGENESIS: TOWARDS THE ROLE OF ALTERNATIVE PROTEIN TARGETING PATHWAYS IN ARABIDOPSIS.

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Chloroplasts are a major component of plant cells. Their origin traces back to a cyanobacterial ancestor that was engulfed by an ancient eukaryotic cell and eventually integrated as an organelle during evolution. As a result, more than 95% of the ancestral cyanobacterial genes were transferred to the host cell nucleus. Proteins encoded by these relocated genes need to return to internal chloroplast compartments. This import is mainly achieved by the general TOC/TIC machinery located at the chloroplast surface. Until recently, all proteins destined to chloroplast were believed to possess an N-terminal and cleavable chloroplast targeting peptide, and to engage the TOC/TIC machinery. However, recent studies have revealed the existence of several non-canonical preproteins, lacking cleavable transit peptides. Furthermore, few such proteins were demonstrated to use alternative targeting pathways, independent of the TOC/TIC machinery. The aim of the project is to decipher the molecular nature of these alternative targeting machineries. For that, we initiated a targeted study combining affinity purification and mass spectrometry aiming to identify alternative receptors at the chloroplast surface. Alternatively, we revisited the envelope proteome composition and initiated a gene candidate approach. We are currently studying the role of putative import receptors using in planta techniques.

SS-3: GIBBERELLIN-REGULATED DELLA-TOC159 INTERACTION CONTROLS CHLOROPLAST BIOGENESIS.

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Chloroplast biogenesis and hence the onset of photoautotrophic growth are critical events in early plant development. The differentiation of photosynthetically active chloroplasts in seedlings requires the import of hundreds of different proteins from the cytosol. The protein import receptor Toc159, a core component of the TOC (translocon at the outer envelope of chloroplasts) complex, is essential for chloroplast biogenesis. The process has been studied in the transition of dark grown plants to the light but not during germination. Germination is triggered by the phytohormone gibberellin (GA) and ensues degradation of inhibitory DELLA proteins. Here, we show that DELLAs directly interact with TOC159 under low GA concentrations, leading to polyubiquitination of TOC159 and its degradation by the proteasome. In addition, highly expressed photosynthesis-associated proteins fail to accumulate in the chloroplast and the unimported preproteins are degraded in the cytosol. We conclude that the TOC159-DELLA interaction controls chloroplast biogenesis in a GA-dependent fashion during seed germination.

IS-7: MOLECULAR MECHANISMS OF CHLOROPLAST PROTEIN IMPORT AND THEIR CURIOUS EVOLUTIONARY HISTORY.

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Virtually all chloroplasts/plastids in today's photosynthetic/plastid-containing eukaryotes derive from one successful primary endosymbiotic event with a cyanobacterium-like ancestor. During evolution, massive transfer of genes from the endosymbiont to the host's nuclear genome occurred concomitant with establishment of a protein transport system that allows these nucleus-encoded proteins back into the endosymbiotic organelle. Two successive protein translocons at the outer and inner envelope membranes of chloroplast, termed TOC and TIC, respectively, are responsible for this protein transport. My group previously identified a novel complex consisting of Tic20, Tic56, Tic100, and Tic214 in Arabidopsis as a genuine TIC complex. Furthermore, more recently, we identified a completely novel AAA-type ATPase complex at the inner envelope which functions as the TIC-associated ATP-driven import motor for preprotein translocation across the inner envelope. This TIC and the associated-import motor system appears to have evolved dramatically during the evolution of the green lineage including green algae and are well conserved among most land plants, though with some interesting exceptions. Our recent data also reveal the existence of an alternative TIC complex where Tic20 is a central constituent. These discoveries now provide us an extensively revised view of the molecular mechanisms of chloroplast protein import and their curious evolutionary history.

27 June 2017

Session 3 – Chloroplast metabolism I

Chair : Sam Zeeman

IS-8: TOWARDS A MOLECULAR UNDERSTANDING OF THE EUKARYOTIC CARBON CONCENTRATING ORGANELLE.

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Approximately one-third of global carbon-fixation occurs in an overlooked algal organelle called the pyrenoid. The pyrenoid contains the CO_2-fixing enzyme Rubisco, and enhances carbon-fixation by supplying Rubisco with a high concentration of CO_2. Since the discovery of the pyrenoid over 130 years ago, the molecular structure and biogenesis of this ecologically fundamental organelle have remained enigmatic. To advance our understanding of the pyrenoid and of photosynthetic organisms more broadly, we have developed new tools for the unicellular model alga *Chlamydomonas reinhardtii*. These tools include the world's first

genome-wide collection of mapped mutants in any single-celled photosynthetic organism, as well as methods for high-throughput localization of proteins and identification of protein-protein interactions. By applying these tools, we have increased the number of known pyrenoid components from 6 to over 80, and discovered the existence of three new protein layers in the pyrenoid- a plate-like layer, a mesh layer, and a punctate layer. We discovered that an abundant pyrenoid protein, Essential Pyrenoid Component 1 (EPYC1), works as a molecular glue that binds Rubisco holoenzymes together to form the matrix at the core of the pyrenoid. Furthermore, contrary to longstanding belief that the pyrenoid matrix is a solid structure, we have discovered that the matrix behaves as a liquid droplet, which mixes internally, divides by fission, and dissolves and condenses during the cell cycle. Our efforts have provided fundamental insights into pyrenoid protein composition, structural organization and biogenesis. Working with our collaborators in the Combining Algal and Plant Photosynthesis project, we aim to transfer algal pyrenoid components into higher plants to enhance carbon fixation and yields in crops.

IS-9: PROGRESS TOWARDS ENGINEERING THE ALGAL CARBON DIOXIDE-CONCENTRATING MECHANISM INTO HIGHER PLANTS.

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Introduction of a biophysical algal carbon concentrating mechanism (CCM) into higher plants could increase photosynthetic productivity. Our initial work has focused on the introduction of CCM components that the drive CO_2 uptake in the green alga *Chlamydomonas reinhardtii* into the model C3 plant *Arabidopsis thaliana*. Aggregation of RuBisCO within a chloroplastic pyrenoid-like microcompartment would also be required, to minimise CO_2 retro-diffusion and satisfy modelled predictions for CCM operating efficiency. RuBisCO accumulation within the *Chlamydomonas* pyrenoid may depends on several components, including two α-helices on the RuBisCO small subunit (*RbcS*) and a putative Rubisco linker protein (EPYC1). We have investigated whether “pyrenoid-competent” *RbcS* could form active RuBisCO in Arabidopsis chloroplasts and found that expression of heterologous *RbcS* increased RuBisCO content and largely rescued the slow growth of *rbcs* mutants. Towards the goal of assembling a functional CCM *in planta*, we are currently exploring the nature of the interaction between EPYC1 and *RbcS*, and co-expressing combinations of CCM components in *Arabidopsis*.

IS-10: REDOX REGULATION OF PHOTOSYNTHETIC CARBON METABOLISM.

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Redox regulation is largely based on different types of post translational modifications of cysteines that are the most intrinsically nucleophilic amino acids in proteins. Cysteine reactivity can vary significantly with the protein micronenvironment, and this contributes to selectivity in redox regulation and signaling. Highly reactive cysteines tend to react with ROS like H_2O_2 and RNS like NO giving rise, directly or indirectly, to cysteine sulphenic acids (-SOH) that can either react again with H2O2 and form an irreversibly oxidized group (-SO2H), or react with a thiol compound like GSH to form a disulfide (-SSG). The two alternative reactions have completely different faiths in the cell: the first leads to protein degradation, while the second can be

reverted by the glutaredoxin system. In other cases redox regulation is based on dithiol/disulfide interchange reactions between target proteins and thioredoxins. This type of redox regulation is particularly important in chloroplasts where hundreds of different proteins oscillate between dithiolic (-SH HS-) and disulfuric states (-SS-) during the light/dark cycle. Some enzymes of the Calvin Benson (CB) cycle are hubs of redox regulation because of the different types of redox modifications that can affect their activity. One of these enzymes is GAPDH that can either form disulfide bridges, or undergo oxidation, nitrosylation and glutathionylation reactions. On top of that, GAPDH may be assembled in a supramolecular complex with PRK, a second CB-cycle enzyme, via CP12, itself a redox regulated protein. GAPDH/CP12/PRK complex formation is under strict control of thioredoxins and chloroplastic metabolites and is believed to play different roles in light/dark regulation and protection against oxidative stress.

SS-4: HIGH-THROUGHPUT PHENOTYPING AND GENOTYPING OF VARIATION IN PHOTOSYNTHESIS TRAITS FOR INCREASED CROP YIELDS.

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In many cereals, yield improvements are slowing down and the lower hanging fruit, such as maximising the amount of biomass invested in grain, have generally been picked. There has previously been little active selection for processes to do with primary plant productivity, or photosynthesis, in crop improvement programs, making this a worthwhile target for further yield increases. For this to be viable we need to a) find allelic variation in genes related to leaf-level photosynthetic processes, b) discern whether leaf-level processes actually lead to net productivity gains at the whole-crop level, and c) identify chromosomal regions associated with increased growth efficiencies to enable transfer of genes of interest from donor material to breeding lines. Association mapping is the method of choice to study the genetic basis of complex traits in large natural populations or diverse germplasm collections. Cost-effective, high-throughput, and accurate genotyping and phenotyping methods are a prerequisite for association mapping. While high-throughput genotyping has become affordable, field phenotyping of large germplasm collections has generally been very time consuming. We developed remote and proximal-sensing platforms (UAV and tractor based) and image analysis pipelines to phenotype large field trials. We describe these methods and discuss how we use them to phenotype large and diverse germplasm collections of the food, feed and biofuel crop sorghum for variation in traits associated with increased photosynthetic capacity. The use of the acquired phenotypic information in association mapping studies to identify candidate genes for photosynthetic capacity and the potential to exploit the C4 species sorghum as a model for other cereals are also discussed.

IS-11: PHOTOSYNTHESIS 2.0, PROGRESS TOWARDS ESTABLISHING A EUROPEAN PROGRAMME ON IMPROVING CROP YIELD.

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Over the last 18 months a consortium of European photosynthesis specialists have been working on a large-scale European research programme that would make use of Europe's expertise in photosynthesis as a route to improving crop yields and so meet future demands for the food and non-food bioeconomies. the working title for this programme is 'Photosynthesis 2.0'. In my talk I will describe something of the history and current state of play of the programme and how we see things developing over the coming months and years. There has been, and continues to be, progress towards our goal and we expect that in the coming months there will be an upsurge in various activities we see as being necessary to create a permissive environment for the establishment of the kind of programme we would like to have. We see improving photosynthesis as an indispensable route to crop yield improvement. Major (in the order of 100%) sustainable increases in crop yields will be necessary for global food security, to which must the added the demands of the transition from a fossil-carbon to a sustainable bio-carbon economy. Meeting these needs will be a challenge, demanding creative intelligence, focus, organisation and determination.

Session 4 – Chlorophyll biosynthesis and catabolism

Chair : Stefan Hörtensteiner

IS-12: EXPLORING THE MOLECULAR LANDSCAPE OF *CHLAMYDOMONAS* WITH IN SITU CRYO-ELECTRON TOMOGRAPHY.

Benjamin D. Engel, Miroslava Schaffer, Sahradha Albert, Stefan Pfeffer, Tilak Gupta, Antonio Martinez-Sanchez, Luis Kuhn Cuellar, Dimitry Tegunov, Mike Strauss, Jürgen M. Plitzko, Wolfgang Baumeister

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We are leveraging new advances in cryo-electron tomography (cryo-ET) to investigate macromolecular complexes within the native cellular environment. Thin slices of vitrified Chlamydomonas reinhardtii cells are prepared by cryo-focused ion beam (cryo-FIB) milling and then imaged by state-of-the-art cryo-ET. The resulting 3D views of cellular volumes have provided new insights into the molecular organization of organelles. Within the chloroplast, we identified fine membrane tubules that likely serve as conduits for the directed diffusion of metabolites between the pyrenoid and the chloroplast stroma. We were also able to directly visualize photosynthetic complexes within native thylakoid membranes, stromal VIPP1 rods that function in thylakoid biogenesis, a novel structure bound to the small subunit of chloroplast ribosomes, as well as the liquid-like organization of RuBisCO complexes in the pyrenoid. In addition to the in situ characterization of individual macromolecular complexes, in the future we will aim for a visual proteomics approach to identify and classify every macromolecule within the cellular volumes.

IS-13: ASSEMBLY OF PHYCOBILIPROTEINS IN THE CHLOROPLAST OF CRYPTOPHYTES.

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Cryptophytes, cyanobacteria, red algae and glaucophytes perform oxygenic photosynthesis using chlorophyll containing antenna complexes and phycobiliproteins for light-harvesting. The latter consist of an apo-protein covalently associated with open-chain tetrapyrroles as light-harvesting chromophores. Cryptophytes evolved by secondary endosymbiosis when a heterotrophic eukaryote engulfed a red alga. This event led to the reduction of the red algal phycobilisome to a specialized, unusual phycobiliprotein which is found soluble in the thylakoid lumen. The cryptophyte *Guillardia theta* uses phycoerythrin PE545 as its phycobiliprotein, whose *α*-subunits carry one molecule of 15,16-dihydrobiliverdin (DHBV), whereas the *β*-subunits are associated with three phycoerythrobilin (PEB) molecules. The seminar will give an overview of cryptophycean chromophore biosynthesis and their subsequent attachment to apo-phycobiliproteins. Recombinant plastid encoded heme oxygenase *Gt*Ho was found to cleave heme yielding biliverdin IX*α* (BV) in *E. coli* providing the substrate for further reduction steps by ferredoxin-dependent bilin reductases (FDBR). BV is thought to be converted to DHBV by one of the nucleus encoded FDBRs of *G. theta*, *Gt*PEBA. In a next step, DHBV is further reduced by a second FDBR *Gt*PEBB to the pink product PEB. Once synthesized, the pigments need to be covalently attached to the apo-phycobiliprotein with the help of phycobiliprotein lyases. BLAST searches and sequence alignments identified several nucleus- and one nucleomorph-encoded putative phycobiliprotein lyases. Among those identified, *Gt*CPES was further investigated. GtCPES is an S-type lyase specific for binding of phycobilins with reduced C15-C16 double bonds (DHBV, PEB). The X-ray structure of *Gt*CPES was solved at 2.0 Å revealing a 10 stranded β-barrel with a modified lipocalin-fold. Based on the crystal structure, a model for the interaction of *Gt*CPES with the apo-phycobiliprotein CpeB is proposed and will be discussed.

SS-5: A TRANSCRIPTOME-BASED AND GENE CO-EXPRESSION NETWORK APPROACH TO UNDERSTAND THE REGULATION OF CHLOROPHYLL DEGRADATION PATHWAY.

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Detoxification of chlorophyll during leaf senescence in higher plants is a complex and tightly regulated process. It aims at opening the chlorophyll porphyrin ring to produce non-photoreactive degradation products, termed phyllobilins, which are stored in the vacuole. Despite a good understanding of most of the enzymatic steps of the pathway, its regulation remains unclear. Here we compared transcriptome of various Arabidopsis lines mutated in key enzymes of the pathway: pheophorbide a oxygenase (pao1), staygreen (sgr/nye1) and pheophytinase (pph1) during dark induced senescence. Our data revealed interesting cross-talk between the PAO pathway and hormonal signalling, especially jasmonate signalling. To get a new insight into the genetic basis of the PAO pathway regulation, we used weighted gene coexpression network analyses (WGCNA) and identified modules of tightly coexpressed genes. Characterization of these modules and their hub genes will allow new insights into the regulation of the PAO pathway during senescence.

IS-14: HLIPS – SMALL (STICKY) AND GREEN ANCESTORS OF PLANT ANTENNA COMPLEXES.

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Cyanobacteria contain a family of one-helix High-light inducible proteins (Hlips) that are homologous to third helix of light harvesting proteins (LHC) of plants and algae; the chlorophyll(Chl)-binding motif is particularly well conserved. Hlips are therefore widely accepted as ancestors of plant antenna. A spectrum of functions that includes photoprotection, regulation of Chl biosynthesis or transient Chl binding has been ascribed to these proteins, however these functions had not been supported by experimental evidence. In this talk I will summarize our recent work on the function of Hlips in the cyanobacterium *Synechocystis* 6803. This organism contains four small Hlips (HliA-D) but only HliD is detectable in cells cultivated under non-stress conditions. Other three Hlips accumulate promptly once the growth conditions become less favourable. We purified and biochemically characterized all four Hlips as pure proteins (HliC/D) or as components of different protein complexes. Obtained data show that most likely all four Hlips bind Chl and carotenoids and are able to quench Chl fluorescence. However, each Hlip has a specific role and associates with a different set of protein partners, which includes formation of Hlip homo- and hetero-oligomers. We identified HliD dimers and HliD/C heterodimers bound to Chl synthase enzyme, Ycf39 assembly factor and PSII assembly intermediate RCII*, whereas a HliA/B heteromer associates with the CP47 protein during PSII assembly. The presence of HliD is essential for the attachment of Ycf39 to RCII* and to Chl-synthase as well as for the dimerization of this enzyme. Interestingly, the smallest HliC expressed under stress conditions binds to HliD (and to HliB as well) and re-organizes the Chl-synthase complex. The possible model is that the stress-induced Hlips work like a regulatory pigmented 'glue' that re-organizes and protects the machinery for PSII biogenesis.

IS-15: MG-DECHELATASE CONTRIBUTES TO THE FORMATION AND THE DEGRADATION OF PHOTOSYSTEMS.

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SGR encodes Mg-dechelatase which catalyzes the conversion of chlorophyll *a* to pheophytin *a*. This reaction is the first step of chlorophyll degradation and the sgr mutants show a strong stay green phenotype in land plants. Many studies indicate that SGR plays a central role in the regulation of chlorophyll degradation. For example, when SGR is transiently expressed, chlorophyll and photosystems are immediately degraded. On the other hand, pheophytin *a* is an indispensable molecule of photosystem II, which suggests the involvement of SGR in the formation of photosystem II. Therefore, SGR can be potentially involved in the opposite processes, the degradation and the formation of photosystems. In order to elucidate the physiological function of SGR, we isolated three independent Chlamydomonas *sgr* mutants in which SGR gene was completely deleted. These mutants had low Fv/Fm ratio and reduced level of pheophytin *a*. Blue-native polyacrylamide gel electrophoresis and western blotting analysis showed that the level of photosystem II was low but that of photosystem I and Light-harvesting chlorophyll *a*/*b* complex did not decreased in these mutants. However, chlorophyll

degradation under nitrogen starvation condition normally proceeded in the sgr mutant as in wild type, indicating that Chlamydomonas SGR is not involved in chlorophyll degradation and contributes mainly to the formation of photosystem II. In contrast, photosystem II is normally synthesized and chlorophyll degradation was delayed in the Arabidopsis SGR triple mutant (sgr1 sgr2 sgrL) which completely lack SGR activity. These results indicate that Arabidopsis SGR has evolved to be responsible for the chlorophyll degradation, however, Chlamydomonas SGR become specific for photosystem II formation.

SS-6: THE TETRAPYRROLE BINDING GUN4 PROTEIN MEDIATES INTERPLAY BETWEEN CHLOROPHYLL BIOSYNTHESIS AND ARGININE METABOLISM.

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Amongst the 21 proteigenic amino acids arginine has the highest nitrogen to carbon ratio (6/4) that implies a particular importance for this amino acid in cell metabolism. Both, in chloroplast and in the cyanobacterium *Synechocystis* the single way to produce this amino acid is via L-ornithine formed by transamination reaction catalyzed by the aminotransferase ArgD. Its tightly regulated synthesis begins with the amination of 2-oxoglutarate to form glutamate. However, glutamate also serves as precursor for tetrapyrrole molecules such as chlorophyll. Efficient chlorophyll biosynthesis in oxygenic phototrophs requires the tetrapyrrole binding protein Gun4. Previous studies revealed physical interaction of Gun4 with manganase chelatase, the enzyme catalyzing the first committed step of chlorophyll biosynthesis. Using the model cyanobacterium *Synechocystis* 6803 we identified a protein complex containing Gun4 and two key enzymes responsible for arginine production in cyanobacteria: ArgD and cyanophycinase (CphB). We found that additional L-ornithine was primarily utilized to build arginine pool along with decreasing the levels of chlorophyll precursors, indicating an antagonistic nature for the arginine and chlorophyll biosynthetic pathways. We also observed slower growth and lower chlorophyll / cell ratios in the absence of the Gun4-CphB-ArgD complex under 12 hours / day light regime. Our data imply a yet unrevealed regulatory interplay between chlorophyll biosynthesis and arginine metabolism that is likely to be advantageous for the cell when it has to maintain growth and chlorophyll biosynthesis during dark periods.

IS-16: SEVERAL WAYS TO CONTROL ALA SYNTHESIS BY POSTTRANSLATIONAL REGULATION OF GLUTAMYL-TRNA REDUCTASE.

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In angiosperms, 5-aminolevulinic acid (ALA) synthesis is rapidly suppressed in darkness, while it is immediately light-stimulated after transition from dark to light. In leaves, the ‘closure’ of the Mg branch of tetrapyrrole biosynthesis for Chl synthesis in darkness is obligatory to avoid accumulation of photoreactive tetrapyrrole intermediates (i.e., Pchlide) and subsequent subcellular photooxidative damage. However, synthesis of heme and other tetrapyrroles needs to be continuously guaranteed in light and darkness. Apart from the light-induced transcriptional control of genes in tetrapyrrole biosynthesis, it is expected that posttranslational control rapidly balances the metabolism with the demands of chlorophyll and heme. It is

hypothesized that the rate limiting step of ALA synthesis takes place at the level of glutamyl-tRNA reductase (GluTR), the first committed enzyme of tetrapyrrole biosynthesis. Several post translational mechanisms (e.g. redox control, proteolysis, heme) and interacting factors (e.g. FLU, GBP, cpSRP43) contribute in concert with other interacting enzymes of the tetrapyrrole biosynthesis pathway to the stability, activity, oligomerisation and localization of GluTR in chloroplasts.

28 June 2017

Session 5 – Chloroplast metabolism II

Chair : Diana Santelia

IS-17: EFFECTS OF FLUCTUATING LIGHT ON PHOTOSYNTHESIS AND STOMATAL BEHAVIOUR: IMPACTS ON CARBON GAIN AND WATER USE EFFICIENCY.

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Photosynthetic carbon gain and stomatal behaviour is dependent upon light intensity. In the field plants experience a range of light intensities and over the long term modify their growth through changes in leaf anatomy and morphology (e.g. sun and shade leaves). In a naturally fluctuating environment, stomata and photosynthesis are continually experiencing and adjusting to a variable light intensity. However, these responses are not always synchronized, as stomatal movements can be an order of magnitude slower than the more rapid photosynthetic response. Therefore over the diel period, short term fluctuations in light (sun/shade flecks) drive temporal and spatial dynamics of carbon gain and water loss, meaning that under natural fluctuating environmental conditions water use efficiency is far from optimal. However, in the laboratory we generally grow plants under square wave lighting regimes that do not mimic the natural environment. Little is known about the effect of a fluctuating growth light environment on plant photosynthesis or stomatal behaviour; for example, do stomata respond faster and/or photosynthetic capacity differ when compared to conventionally grown square wave plants? Photosynthetic and stomatal response data from plants that have been grown under LEDs in a fluctuating or 'constant' square wave lighting regimes of identical photoperiods and total daily light intensity.

IS-18: REGULATION OF STOMATAL AND SOURCE LEAF METABOLISM BY TREHALOSE 6-PHOSPHATE.

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Trehalose 6-phosphate (Tre6P), the intermediate of trehalose biosynthesis, is as a signal metabolite in plants, which links their growth and development to the availability of sucrose. The close relationship between Tre6P and sucrose is described in the so-called nexus model, which postulates that Tre6P is both a signal and negative feedback regulator of sucrose levels. It is proposed that this regulation by Tre6P maintains *in-vivo* sucrose concentrations within an optimal range, and is functionally analogous to the homeostatic control of blood glucose levels

by the insulin-glucagon system in animals. During the day, Tre6P regulates the partitioning of photoassimilates into sucrose in source leaves, with high Tre6P diverting carbon away from sucrose and into organic and amino acids, via post-translational activation of phospho*enol*pyruvate carboxylase and nitrate reductase. At night, Tre6P controls the remobilization of transitory starch reserves into sucrose. By linking these processes to sucrose status, Tre6P coordinates sucrose production in source leaves with demand for sucrose from growing sink organs, and also coordinates carbon and nitrogen metabolism. In developing tissues, Tre6P controls the utilization of imported sucrose for growth. Studies of various *Arabidopsis thaliana* mutants have shown that trehalose metabolism is essential for normal regulation of stomatal conductance and the sensitivity of stomata to abscisic acid. The potential functions of Tre6P in regulation of guard cell metabolism and stomatal regulation will be discussed.

IS-19: STOMATAL BEHAVIOUR IN PHOTOSYNTHETIC MUTANTS.

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Earth system models require mechanistic models of photosynthetic CO_2 exchange and leaf water loss. While mechanistic models of C_3 and C_4 photosynthesis exist, models of stomatal function remain empirical due to incomplete mechanist understanding of guard cell function. A close relationship has been observed between leaf stomatal conductance and photosynthetic capacity in both C_3 and C_4 species with the result that the ratio of intercellular to ambient CO_2 partial pressure, C_i/C_a, is almost constant under many environmental perturbations and amongst species. This has led to the hypotheses that there may be some mechanistic link between guard cell function and leaf or guard cell photosynthesis. We have used transgenic tobacco (a C_3 species) and transgenic *Flaveria bidentis* (a C_4 dicot) and *Setaria viridis* (a C_4 monocot) with impaired photosynthesis to probe the relationship between leaf photosynthesis and stomatal function. We will discuss the effect of reduced Rubisco, electron transport, carbonic anhydrase and the C_4 PEPcarboxylase on the light and CO_2 response of stomatal opening and closing.

SS-7: CHLOROKB: A WEB-APPLICATION FOR THE INTEGRATION OF KNOWLEDGE RELATED TO CHLOROPLAST METABOLIC NETWORK.

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Context: Higher plants, as autotrophic organisms, are effective sources of molecules. They hold great promise for metabolic engineering, but the behavior of plant metabolism at the network level is still incompletely described. Although structural models (stoichiometry matrices) and pathway databases are extremely useful, they cannot describe the complexity of the metabolic context and new tools are required to visually represent integrated biocurated knowledge for use by both humans and computers.

Content: ChloroKB is a web-application (http://chlorokb.fr/) for visual exploration and analysis of the *Arabidopsis thaliana* metabolic network in the chloroplast and related cellular pathways. The network was manually reconstructed through extensive biocuration to provide transparent traceability of experimental data. Proteins and metabolites were placed in their biological context (spatial distribution within cells, connectivity in the network, participation in supramolecular complexes, regulatory interactions) using CellDesigner software. The network contains 1147 reviewed proteins (559 localized exclusively in plastids, 68 in at least one additional compartment and 520 outside the plastid); 122 proteins awaiting biochemical/genetic characterization; and 228 proteins for which genes have not yet been identified. The visual presentation is intuitive and browsing is fluid, providing instant access to graphical representation of integrated processes and to a wealth of refined qualitative and quantitative data.

Conclusion: ChloroKB will be a significant support for structural and quantitative kinetic modeling, for biological reasoning, when comparing novel data to established knowledge, for computer analyses, and for educational purposes.

SS-8: PLASTIDIAL NAD-DEPENDENT MALATE DEHYDROGENASE PLAYS A CRUCIAL ROLE IN EARLY CHLOROPLAST DEVELOPMENT.

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Plant tissues contain multiple malate dehydrogenases (MDH) that catalyse the reversible interconversion of malate and oxaloacetate, using NAD(H) or NADP(H) as a cofactor. Arabidopsis chloroplasts contain an isoform that is NADP-dependent, and another that is NAD-dependent (pdNAD-MDH). The *pdnad-mdh* knockout mutant is embryo-lethal, and the constitutive silencing line, *miR-mdh-1*, has a pale, dwarfed phenotype with aberrant chloroplast ultrastructure. In this study, we investigated the role of pdNAD-MDH in chloroplast development. When grown in the dark, the etiolated *miR-mdh-1* seedlings were indistinguishable from the wild type, showing that skotomorphogenic growth was unaffected by pdNAD-MDH deficiency. Nevertheless, the examination of etioplast structure with electron microscopy revealed compromised or absent prolamellar body formation in *miR-mdh-1* seedlings. Levels of prolamellar body components, such as chlorophyll precursors, galactolipids, and carotenoids were also severely decreased in *miR-mdh-1*, suggesting a critical role of pdNAD-MDH in early chloroplast development. The embryo-specific expression of pdNAD-MDH under the ABI3 promoter could rescue the embryo lethality of the *pdnad-mdh* knockout mutant, but the seedlings had white leaves with severe defects in chloroplast ultrastructure, suggesting that aberrant chloroplast development is not due to defects gained during embryogenesis. Surprisingly, mutated forms of pdNAD-MDH with no detectable activity were also able to complement the embryo-lethal phenotype when expressed in *pdnad-mdh*, and the plants grew indistinguishably from the wild type. Heterologous expression of active, non-plastidial NAD-dependent MDH isoforms (At CYMDH1, At PMDH1, At MMDH1 and Sc MMDH1) within the plastids of *pdnad-mdh* did not complement the embryo lethality. Therefore, pdNAD-MDH protein plays an indispensable post-embryonic role in early etioplast and chloroplast development, but its enzymatic activity is not required. We suggest that pdNAD-

MDH is important because it is involved in protein-protein interactions with other key proteins involved in chloroplast development.

Session 6 – Regulation of photosynthesis

Chair : Michel Goldschmidt-Clermont

IS-20: REGULATION OF PHOTOSYNTHETIC LIGHT USE EFFICIENCY BY PH AND XANTHOPHYLLS IN LOWER AND HIGHER PLANTS.

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Light harvesting for oxygenic photosynthesis is regulated to prevent the formation of harmful photoproducts by safely dissipate excess energy. Excess light limits proton back transport to chloroplast stroma leading to lumen acidification which activates de-epoxidation of violaxanthin to zeaxanthin by the pH-dependent enzyme violaxanthin de-epoxidase. Zeaxanthin, in turn, activates quenching reactions in both lower and hgher plants depending on both PSBS and LHCSR1. With the aim to uncover the photoprotective functional states responsible for dissipation in green algae and mosses the fluorescence dynamic properties of the light-harvesting complex stress-related (LHCSR1) protein, which is essential for fast and reversible regulation of light use efficiency in lower plants, are compared to the major LHCII antenna protein which mainly fulfills light harvesting function. Both LHCII and LHCSR1 had a chlorophyll fluorescence yield and lifetime strongly dependent on detergent concentration and yet the transition from long- to short-living states was far more complete and rapid in the latter. Also, binding of zeaxanthin and low pH enhanced the relative amplitude of quenched states, which were characterized by the presence of 80 ps decay components with a red-shifted spectrum. This fast lifetime was the only decay component when Zea and acidic conditions were present together as it synergically occurs in the chloroplast membrane under excess light conditions. We suggest that energy dissipation occurs in the chloroplast by the activation of 80 ps quenching sites in the thylakoid membranes which spill over excitons from the photosystem II antenna system whose lifetime falls in the 1-2 ns range.

IS-21: TOPOLOGICAL CONSTRAINTS TO PHOTOSYNTHESIS IN DIATOMS.

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Photosynthesis is a unique process that allowed independent colonisation of the land by plants and of the oceans by phytoplankton. Although the photosynthesis process is well understood in plants, we are still unlocking the mechanisms evolved by phytoplankton to achieve extremely efficient photosynthesis. By combining *in vivo* spectroscopy, biochemical analysis and chloroplast tomography we have evaluated the main topological constraints to photosynthesis in diatoms, major components of the eukaryotic phytoplankton. We found that the "poorly" organized plastids of diatoms harbor a sophisticated thylakoid network that orchestrates light harvesting, photosynthetic electron flow and $C0_2$ assimilation[1]. Refined electron microscopy analysis reveals the existence of direct connections between the nucleus and the chloroplast[2], as well as between mitochondria and the chloroplast[3], explaining how information, reducing equivalents and ATP for CO_2 assimilation are exchanged between the main cellular compartments.

IS-22: Biochemical characterization and physiological role of the plastid terminal oxidase PTOX.

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Protein levels of the plastid terminal oxidase PTOX increase upon abiotic stress. PTOX may protect the photosynthetic apparatus when electron transport is limited. The active site of PTOX comprises a non-heme diiron centre that catalyses the oxidation of plastoquinol and the reduction of O_2 to H_2O. We have performed a biochemical characterization of purified PTOX. The activity of PTOX was found to be much higher than what had been previously estimated. The main reaction of PTOX is the reduction of O_2 to H_2O but PTOX generates $O_2^{\bullet-}$ in a side reaction in a substrate- and pH-dependent manner. PTOX activity *in planta* was investigated using *Nicotiana tabacum* expressing PTOX1 from *Chlamydomonas reinhardtii.* PTOX competed efficiently with photosynthetic electron flow. High CO_2 concentrations inactivated PTOX most likely because of an acidification of the stroma. Immunoblots showed that PTOX detached from the membrane in dark-adapted leaves or in the presence of uncouplers. Binding of PTOX to liposomes was studied at different pH and ion concentrations. A model is proposed in which the membrane association of PTOX is controlled by stromal pH. When the stromal pH is neutral, PTOX is soluble and inactive. When the stromal pH is alkaline and the photosynthetic electron chain is highly reduced under stress conditions, PTOX binds to the membrane, has access to its substrate and can serve as safety valve.

SS-9: REGULATION OF PHOTOPROTECTION BY PERCEPTION OF UV-B IN *CHLAMYDOMONAS REINHARDTII*.

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Sunlight is essential for phototrophs, being a substrate for photosynthesis and a developmental signal. When photosynthetic organisms are exposed to high light, a wide range of mechanisms are activated to dissipate excess absorbed energy and protect the photosynthetic machinery from photodamage. These processes, mainly associated with photosystem II (PSII), are referred to as Non-Photochemical Quenching mechanisms (or NPQ). The most prominent NPQ component in plants and green algae is qE, the energy-dependent component of NPQ,

which corresponds to a thermal dissipation of excess energy. UV is an intrinsic component of the sunlight spectrum, which severely impacts photosynthetic organisms mainly via its UV-B component. UV-B is sensed by the UV-B-specific photoreceptor UVR8 and regulates several biological functions. The impact of UV-B radiation on photosynthesis is becoming a major challenge in plant biology since it affects crop productivity but also aquatic ecosystems. Here, we report in the green alga Chlamydomonas reinhardtii that UVR8 induces accumulation of specific members of the light-harvesting complex (LHC) superfamily that contribute to qE, in particular LHC Stress-Related 1 (LHCSR1) and Photosystem II Subunit S (PSBS). The capacity for qE is strongly induced by UV-B, although the patterns of qE-related proteins accumulating in response to UV-B or to high light are clearly different. The competence for qE induced by acclimation to UV-B markedly contributes to photoprotection upon subsequent exposure to high light. Our study reveals an anterograde link between photoreceptor-mediated signaling in the nucleocytosolic compartment and the photoprotective regulation of photosynthetic activity in the chloroplast.

SS-10: THE ROLE OF THE NADPH-DEPENDENT CHLOROPLAST THIOREDOXIN SYSTEM IN ACCLIMATION OF PHOTOSYNTHETIC ELECTRON TRANSFER TO CHANGES IN LIGHT CONDITIONS.

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Cyclic electron flow (CEF) around photosystem I (PSI) is a process where electrons are shunted from Ferredoxin back to the PQ pool. In contrast to linear electron flow, NADPH is not produced. CEF does however contribute to generation of ΔpH and therefore to production of ATP and induction of non-photochemical quenching of excess excitation energy (NPQ). Two distinct pathways of CEF exist in plant chloroplasts: an antimycin A –sensitive pathway depends on the proteins PGR5 and PGRL1, while the other pathway involves the chloroplast NAD(P)H dehydrogenase complex (NDH). Another mechanism that plants use to acclimate to changing light conditions is a process called state transitions, where the association of light-harvesting complex I I to either PSII or PSI shifts according to changes in light conditions in order to balance excitation between the photosystems. Both CEF pathways as well as state transitions are highly dependent on stromal redox state and have been proposed to undergo thiol-regulation by stromal thioredoxins. Details about the physiological roles of each CEF pathway and about the regulatory involvement of thioredoxins in CEF and state transitions are still very much unclear, and therefore we investigated the potential role of the NADPH-thioredoxin reductase (NTRC) in regulating these processes in vivo by using an Arabidopsis knockout line of NTRC as well as a line overexpressing NTRC. Our results show that NTRC is an activator NDH-dependent CEF, significantly affects the generation of the proton motive force during dark-light transitions and is needed to downregulate NPQ and to effectively perform state transitions.

IS-23: MACHINERY FOR THE REGULATION OF PROTON MOTIVE FORCE IN CHLOROPLASTS.

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Coupled with linear and cyclic electron transport around photosystem I (PSI), protons are translocated across the thylakoid membrane. A part of resulting proton gradient (∆pH) is

transformed into membrane potential. Both factors of proton motive forces (*pmf*) contribute to ATP synthesis. In contrast, only ∆pH down-regulates electron transport via dissipation of excessively absorbed light energy from PSII (qE) and also down-regulating the electron transport through the cytochrome b_6f complex (photosynthetic control). The size of *pmf* is regulated by operation of PSI cyclic electron transport, which generates pmf without accumulation of NADPH. The partitioning of *pmf* components is determined through controlling the ion movement across the thylakoid membrane. KEA3 is a H^+/K^+ antiporter localized to the thylakoid membrane and transforms ∆pH to membrane potential to rapidly relax qE at low light intensity under fluctuating light. The chloroplast NDH complex mediates the minor pathway of PSI cyclic electron transport. The complex interacts with two copies of the PSI supercomplex to form the NDH-PSI supercomplex. Lhca5 and Lhca6 are linker proteins and intermediates this supercomplex formation. The stromal loop of Lhca6 is essential and sufficient for this linker function. I will discuss the structure of NDH-PSI supercomplex.

POSTERS

P1 : NEW CHLOROPLAST PHOSPHATASES NECESSARY FOR STATE TRANSITIONS IN CHLAMYDOMONAS.

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In the green alga *Chlamydomonas reinhardtii*, a major component of short-term acclimation of the photosynthetic apparatus to changing light is the ability to distribute energy between photosystem II (PSII) and photosystem I (PSI). In response to changes in the redox state of the photosynthetic chain, due to fluctuations in light quality or quantity, or in metabolic demands of the cell, the light harvesting complex (LHCII) can migrate between PSII and PSI in a process called “state transitions”. LHCII allocation to PSI is triggered by phosphorylation of specific threonine residues. The kinase responsible for this process, Stt7, was identified more than a decade ago. In *Arabidopsis thaliana* a kinase homologous to Stt7 (STN7) phosphorylates LHCII while a single phosphatase (PPH1/TAP38) is mainly responsible for LHCII de-phosphorylation which favors its association with PSII. Another antagonistic pair of kinase (STN8) and phosphatase (PBCP) is known in Arabidopsis, mainly involved in the phosphorylation / de phosphorylation of PS II core proteins. We are currently characterizing the orthologous phosphatases in Chlamydomonas, CrPPH1 and CrPBCP. Here we show that surprisingly both Cr*pph1* and Cr*pbcp* mutants are affected in state II to state I transition. While Crpph1 appears to be more specifically affected in the dephosphorylation of LHCII components during state transitions, in Crpbcp some PSII core proteins and some of LHCII components appear constitutively more phosphorylated. The double mutant *pph1/pbcp* shows constitutive LHCII hyper-phosphorylation and appears to be locked in a ST II - like condition. Combining Phos-Tag based immune-blot techniques and mass spectrometry analysis, we aim to identify the targets of each phosphatase and to elucidate the phospho-sites crucial to state transitions. Biochemical and spectroscopical approaches are used to investigate their effects *in vivo.* The roles of CrPPH1 and particularly CrPBCP for light acclimation in Chlamydomonas appear to be different from those of the plant orthologues. Our preliminary results indicate a different

scenario for the regulation of LHCII phosphorylation and of the state transition mechanism in the green algae.

P2: FUNCTIONAL ANALYSIS OF PHOTOSYSTEM II PHOSPHORYLATION IN *CHLAMYDOMONAS REINHARDTII.*

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Light is essential for photosynthesis and is also used as a signal, but excess light can also be deleterious. Light-induced phosphorylation of chloroplast proteins is involved in balancing light allocation to the photosystems (state transition), in repair of light-induced damage and in retrograde signaling toward the nucleus. Phosphorylation of the core subunits is thought to play a role in the repair cycle of Photosystem II (PSII), upon photo-damage. We have used site-directed mutagenesis and chloroplast transformation in *C. reinhardtii* to generate mutants of known stromal phosphorylation sites in PSII. The target serines or threonines in PsbH, PsbD (D2) and PsbC (CP43) were replaced with non-phosphorylatable alanine or negatively charged phosphomimetic aspartate residues. Aspartate mutations in PsbD and PsbC led to dramatically reduced or blocked accumulation of PSII. Initial results indicate that two of the three known phosphorylation sites of PsbH are redundant and essential for PSII accumulation. Using PhosTag gels, two PsbH alanine mutants could be characterized as having non-detectable and reduced PsbH phosphorylation. No increased sensitivity to high-light could be detected in algae strains with alanine mutation(s) in a single protein. However, stacking of those mutations provided preliminary data suggesting increased high-light sensitivity when phosphorylation sites of least two proteins are mutated.

P3: EVIDENCE FOR POSITIVE REGULATION OF PHEOPHORBIDE A OXYGENASE THROUGH DE-PHOSPHORYLATION.

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Every autumn, a colorful festival of green, red, yellow and brown is visible in most plants, when green chlorophyll pigments are degraded during senescence, which designates the end of annual plant's life cycle. During senescence when nutrients are remobilized and stored in sink organs, chlorophyll is detached from the thylakoid membrane, becomes phototoxic and quickly needs to be catabolized into non-toxic end products. This project focuses on the regulation of the key enzyme pheophorbide *a* oxygenase (PAO), which catalyzes the degradation of the highly toxic intermediate pheophorbide *a,* through opening of the porphyrin macrocycle and conversion to a linear form called phyllobilin, which is the precursor for all downstream catabolites. PAO knockout mutants (*pao1*) accumulate pheophorbide *a* and display a premature cell death phenotype due to the generation of reactive oxygen species. It has been proposed that PAO might be positively regulated through (de-)phosphorylation at a specific calcium dependent protein kinase site in the protein. By expressing a respective phospho or non-phospho mimic form of PAO in *pao1* background, we demonstrate that PAO is positively regulated through de-phosphorylation.

To identify the responsible phosphatase, we screened a library of 84 mutants of chloroplast-located phosphatases. The screen revealed a mutant in PROTEIN PHOSPHATASE 1, which is involved in state transition and consistently accumulates pheophorbide *a* in a senescence dependent manner, indicating this phosphatase to have a regulating role in chlorophyll breakdown.

P4: CHLOROPHYLL BINDING ON THE C-TERMINAL TRANSMEMBRANE SEGMENT OF THE PLASTIDIC TYPE II FERROCHELATASE.

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Ferrochelatase (FeCh) is an essential enzyme of the tetrapyrrole pathway. FeCh creates heme by inserting ferrous iron into protoporphyrin IX. In photosynthetic organisms, FeCh has to compete for its substrate with Mg-chelatase, which directs the protoporphyrin IX into chlorophyll. The type II plastidic FeCh, which is present in chloroplasts, algae and cyanobacteria, contains a C-terminal chlorophyll *a*/*b* binding (CAB) motif conserved in the family of light harvesting proteins. Whether the FeCh CAB domain binds chlorophyll was however unknown. To clarify this longstanding question, we expressed and purified the FeCh CAB domain as a small protein from the cyanobacterium *Synechocystis* 6803. By a combination of chromatographic techniques, we demonstrated that this FeCh segment binds chlorophyll *a*, β-carotene and zeaxanthin in 6:1:1 ratio respectively and the chlorophyll fluorescence is quenched. Whether the observed pigment binding to the CAB domain plays a role in the regulation of FeCh remains to be determined. However, using chlorophyll labelling by ^{14}C[Glu], we have discovered that an inhibition of FeCh leads to a significant increase in the rate of de novo chlorophyll formation. A well-tuned activity of this enzyme is thus crucial for the regulation of chlorophyll pathway and 'sensing' of chlorophyll molecules via FeCh CAB domain might be an important regulatory event.

P5: TOWARDS A 3D MODEL OF THYLAKOID BIOGENESIS.

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The photochemical reaction of photosynthesis takes place in the chloroplast thylakoid membranes. This membrane bilayer is organized in stacks (grana) interconnected by unstacked stroma lamellae and is constituted largely of galactolipids: mono- and digalactosyl diacyl glycerol (MGDG and DGDG), sulfoquinovosyl diacylglycerol (SQDG), phosphatidyl Glycerol (PG) and contain other lipids and pigments such as prenylquinones (plasto- and phylloquinone, tocopherols), carotenoids (beta-carotene, lutein and xanthophylls), and chlorophylls in association with large protein complexes. Thylakoid formation takes place during chloroplast differentiation. Intricate genetic and metabolic processes are required to build the thylakoid network during chloroplast biogenesis but the dynamics of the process is not fully described. To fill this knowledge gap we are investigating the thylakoid biogenesis process in Arabidopsis seedlings using complementary approaches. Quantitative data are

obtained using A) 3D electron microscopy to record ultrastructural changes, B) lipidomics to determine the dynamics of lipid composition, and C) quantitative protein analysis to determine photosystems concentration. Our goal is to construct a set of 3D-models that illustrate the dynamics of thylakoid biogenesis over time and determine the link between thylakoid architecture and composition.

P6: PHYSIOLOGICAL AND MOLECULAR FUNCTIONS OF ABC1K1 KINASE DURING EARLY DEVELOPMENT OF *ARABIDOPSIS THALIANA.*

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Photosynthesis is the key bioenergetic mechanism for photosynthetic organisms. This process takes place entirely within the chloroplast, a specialized organelle present in cells of plants and eukaryotic algae. The chloroplast contains a system of internal thylakoid membranes in which the components of the photosynthetic machinery are inserted. Furthermore, these membranes, mainly composed of glycerolipids and prenyllipids, are fused to lipid droplets called plastoglobules which have been shown to play an important role in plastids biogenesis, stress response, senescence and prenylquinones metabolism. These lipid droplets contain many prenylquinones as well as carotenoids and phytylesters, and possess a specific set of associated proteins. Among the plastoglobular proteins, the most abundant are structural proteins called Fibrillin, and the members of the Activity of BC1 complex Kinase (ABC1K) family. Many studies suggest that at least some of these plastoglobular kinases, such as ABC1K1 and ABC1K3, play a role in red-light mediated development as well as in chloroplast functions, especially in photosynthesis regulation and chloroplast metabolism, but their phosphorylation targets and the signaling pathway(s) are still unknown. Moreover, the role of ABC1K1 was mostly investigated on adult plants, while little is known about its role in young seedling. The aim of this thesis is to investigate the role of ABC1K1 in physiological and molecular functions during the early development of *Arabidopsis thaliana,* particularly under red light when the role of this kinase appears to be even more relevant. This will allow to better understand the molecular mechanisms regulating chloroplast development and its functions.

P7: RESEARCH INTO THE FUNCTION OF RUBISCO IN GREEN SEEDS.

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Aim of the project is to test genetically a CO2 recycling pathway that was proposed to operate during the development of green seeds, including many that are agronomically important. RubisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) is responsible of CO2 assimilation by the Calvin Cycle in leaves, which generates carbohydrates that are exported to the rest of the plant. Highly energetic components like lipids are derived from transported carbohydrates and are stored in seeds. During lipid synthesis CO2 is released leading to a potential loss of carbon. Previous works on Brassica napus L. proposed that RubisCO and other enzymes, working outside of the context of the Calvin Cycle, could increase the efficiency

of oil synthesis by directly reincorporating this CO2. This seed-specific pathway was supported by models and radioactive labelling experiments, but not tested genetically. Mutants defected in Calvin cycle are available in Arabidopsis thaliana, a relative of B. napus L. which also has green seeds. I use these mutants to modulate flow of metabolites through RubisCO in a seed-specific way. I test the proposed CO2 reincorporation model by lipid quantification.

P8: STRUCTURE AND FUNCTION OF THE PSB29 PROTEIN, A CYANOBACTERIAL HOMOLOGUE OF THE PLANT THYLAKOID FORMATION 1 FACTOR.

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Photosystem II (PSII) is the multi-subunit pigment-protein complex responsible for photosynthetic oxygen evolution in the thylakoid membranes (TM) of plants, algae and cyanobacteria. The Psb29 protein belongs to a group of protein factors that have been suggested to participate in the biogenesis of PSII, although its effect on accumulation of Photosystem I (PSI) has also be reported. The aim of this study was to characterize the structure and function of Psb29 in cyanobacteria. The structure of the *Thermosynechococcus elongatus* Psb29 protein overexpressed in E. coli was determined by X-ray crystallography to 1.4 Å resolution. It contains a bundle of eight alpha helices with an additional extra-long helix at the C-terminus. The protein is encoded by the *sll1414* gene in *Synechocystis sp*. PCC 6803 and its deletion leads to slower autotrophic growth and slightly lower chlorophyll content. The mutant also exhibits a decrease in the amount of FtsH2 and FtsH3 proteases which form a heterohexamer responsible for D1 replacement during the PSII repair. Consequently, the psb29 deletion mutant is more photosensitive than wild type. Analysis of the transcript level by RT-PCR also showed increased levels of the *ftsH2* and *ftsH3* transcripts when compared with WT. A close relationship between Psb29 and the FtsH2/FtsH3 complex was further confirmed by a reciprocal co-isolation of the FtsH2/ FtsH3 complex with C-terminally FLAG-tagged Psb29 on the one hand and FtsH2-FLAG with Psb29 on the other hand. Our results indicate that Psb29 directly interacts with and stabilizes the FtsH2/FtsH3 protease complex and in this way plays just an indirect role in the PSII repair and PSI biogenesis.

P9: VIRUS MIMETIC PHOTOSYSTEM I EXPRESSED IN SYNECHOCYSTIS.

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Oxygenic photosynthesis supports virtually all life forms on earth. Light energy converted to chemical energy by two photosystems: photosystem I (PSI) and photosystem II (PSII). PSI is present in multiple forms including monomeric and trimeric complexes. In cyanobacteria both forms of PSI are present, while the trimeric one being the major fraction. We purified, crystallized and solved the structure of *Synechocystis* monomeric and trimeric PSI. An operon encoding PSI was identified in cyanobacterial marine viruses. This viral PSI has a unique gene composition compared to the PSI composition of its hosts. Subunits PsaL and PsaI are missing and a unique PsaJ-PsaF (PsaJF) fusion-protein exists. Previously we solved the structures of monomeric PSI lacking PsaL and PsaI subunit as well as trimeric PSI containing PsaJ-F fusion protein. In this work we generated PSI containing largely the viral encoded J-F fusion protein

in a mutant lacking PsaL and PsaI. We solved the structure of this PsaJF PSI and identified the viral encoded protein in the structure. The revealing of the fine alterations in the proteins and prosthetic groups awaits high-resolution structure. Our finding extends the understanding of PSI structure, function and evolution and suggests a unique function for the viral PSI.

P10: DYNAMICS OF THE LIGHT-HARVESTING COMPLEX II PHOSPHORYLATION.

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The major light-harvesting complex (LHCII) is a crucial component of the photosynthetic machinery, playing a central role in light capture and acclimation responses to changing light conditions. In Arabidopsis thaliana the major LHCII is constituted of homo- and hetero-trimers composed of three classes of isoforms: Lhcb1, Lhcb2, and Lhcb3. These have different relative abundance, with Lhcb1 estimated to represent two thirds of the total, Lhcb2 one fourth and Lhcb3 approximately one tenth. The presence of these isoforms varies in different LHCII trimers associated to photosystems I and II, suggesting a specific role for each of them. Moreover, the two phosphorylatable isoforms Lhcb1 and Lhcb2, display distinct patterns and extent of phosphorylation, and play different roles in photosynthesis acclimation. In particular, phosphorylated Lhcb2 plays a central role of in stabilizing the PSI-LHCII supercomplex. The phosphorylated fraction for each isoform can be determined via a Phos-Tag based gel system. This information allows to quantify the extent of phosphorylation of Lhcb1 and Lhcb2 in different mutant lines. To address the regulation of the (de)phosphorylation of each isoform, single and multiple knock-out mutants of the thylakoid's protein phosphatases PPH1/TAP38 and PBCP, and of the kinases STN7 and STN8 were analyzed along with mutants affected in the formation of photosynthetic supercomplexes. The results show that the regulation of LHCII phosphorylation level is partially independent of the presence of PPH1/TAP38 phosphatase and STN7 kinase and requires a fine modulation in order to re-equilibrate the electron transport chain.

P11: THE LOSS OF THE PROTEASOME SUBUNIT RPN8A PARTIALLY RESCUES THE PPI2 PLASTID PHENOTYPE.

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The Ubiquitin-26S-proteasome system (UPS) is the most important degradation system in eukaryotes and is involved in almost all cellular processes. Plastid precursor proteins and also several components of the Translocon in the Outer envelope of Chloroplasts (TOC) are thought to be regulated by the UPS suggesting an impact of the cytosolic degradation system on plastid processes. The structure of the 26S-proteasome is highly conserved and consists of a 20S catalytic particle (20S CP) capped by a 19S regulatory particle (19S RP) on one or both ends. The loss of the Regulatory Particle Non-ATPase subunit 8a (RPN8a) in *A. thaliana* leads to a higher abundance of 20S CP subunits and a lower abundance of 19S RP subunits supporting an effect on the 26S activity and/or specificity. We generated a double mutant between *ppi2* – a plastid protein import mutant deficient in Toc159- and *rpn8a* to further investigate the relation between UPS and plastids. Interestingly the chloroplasts of the double mutant *rpn8a ppi2* show more differentiated thylakoid structures compared to the *ppi2* single mutant. Correspondingly,

the chlorophyll and carotenoid content as well as the abundance of photosynthesis-related proteins (e.g. LHCs) is increased in the double mutant. Measuring the photosynthetic operating efficiency verified the improved photosynthetic functionality of *rpn8a ppi2* chloroplasts. The higher abundance of photosynthetic proteins, that can be both: nuclear or plastid encoded, is not related to higher transcript levels suggesting a regulation on protein level. We discuss here possible reasons for the observed partial complementation of the *ppi2* phenotype by a proteasome mutant.

P12: GIBBERELLIC ACID POSITIVELY REGULATES CHLOROPLAST BIOGENESIS.
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Plants show remarkable adaptability to their environments, and this is impart due to their ability to continue forming organs throughout their life. Responses to environmental factors are mediated through the action of various chemical messengers referred to as plant hormones. In particular, seed germination is modulated by the phytohormone gibberellin acid, which regulate growth and influence various developmental processes, including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction, and senescence. DELLA proteins are key regulators in the GA signaling pathway, largely known to regulate the transcriptional control, and they act as intracellular repressors of GA responses, inhibiting seed germination, seed growth, and other GA-dependent pathways. The outer envelope membrane of chloroplasts (TOC) initiates the recognition and import of thousands of nucleus-encoded photosynthetic proteins from the cytosol to plastid. The TOC-GTPase of TOC159 is essential for the initial stages of photosynthetic protein import into chloroplasts. TOC159 is required for the import of photosynthetic proteins and it is important for the development of the chloroplast. We report that DELLAs directly interact with TOC159-GTPase domain under low GA level and this interaction leads through reduction of TOC159 protein level. When GA reached optimal level, followed by GA receptors, directly binds with DELLA proteins, which allows the TOC159 protein to be released and active in the TOC complex. GA-regulated DELLA controls chloroplast biogenesis by targeting TOC159 for proteasome-mediated degradation and repressing its function at the translocon at the outer envelope of chloroplasts. Altogether, we conclude that GA-regulated TOC159-DELLA interaction is the unique mechanism to control chloroplast biogenesis under unfavorable condition during seed germination.

P13: ON THE IMPORTANCE OF THE 1 MDA TIC COMPLEX FOR PLASTID TRANSLATION.
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The main import route of nucleus encoded plastid proteins depends on translocons at the outer (TOC) and inner (TIC) envelope membranes of the chloroplast. Over recent years, knowledge on the import machinery, e.g. its components and regulatory factors has increased. One of the recent discoveries is a 1 MDa TIC complex that was identified at the inner chloroplast envelope membrane and suggested to constitute the general import pathway. This complex is composed

of Tic214, Tic100, Tic56 and Tic20-I. The only subunit with a known function in protein import is Tic20-I that forms the protein-conducting channel, while the function of the other constituents of this complex is elusive. We report here a characterization of Tic56 function by a detailed analysis of two mutant lines, *tic56-1* and *tic56-3*. We observed a reduced accumulation of plastid ribosomal proteins in *tic56-1* and the Toc159 mutant line *ppi2* and a processing defect of the plastid 23S rRNA in *tic56-1*, indicating a defect in the assembly of plastid ribosomes. Comparative proteome analyses of *tic56-1* and plants treated with the plastid translation inhibitor Spectinomycin revealed high similarity in plastid proteome accumulation supporting a defect in plastid translation as one cause for the albino phenotype. Thus, our findings suggest functional diversification of the 1-MDa complex subunits.

P14: NOVEL PROTEINS INVOLVED IN STARCH METABOLISM.

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Transitory starch is a plant storage compound produced as granules in the chloroplasts during the day, and degraded at night to provide energy and carbon sources to sustain metabolism. Starch consists of two glucose polymers that differ in structural complexity; amylopectin and amylose. Whereas amylose is synthesized by a single granule bound starch synthase (GBSS), amylopectin biosynthesis requires the activity of several soluble starch synthases (SSs), branching enzymes and debranching enzymes. In *Arabidopsis thaliana*, five starch synthases, GBSS and SS1-SS4, have been characterized so far. All of them transfer the glucose moiety of the substrate ADP-Glucose to a growing glucan chain. However, SS4 is special since it is also a major determinant of starch granule initiation. Recently, we have identified several, previously uncharacterized proteins that are involved in this process too. We are implementing a combination of approaches, including bioinformatics, *in vitro* and *in vivo* assays, and the analysis of T-DNA insertion lines, to characterize these proteins and their relations to each other. This will help us to shed light on those aspects of starch metabolism that have remained in the dark so far.

P15: IMPACT OF ELEVATED TEMPERATURES ON TOMATO CHROMOPLAST STRUCTURE AND METABOLISM.

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In tomato, the accumulation of carotenoids and other health-promoting compounds during fruit ripening is not only controlled by developmental program but also influenced by environmental signals and abiotic stresses. Although higher temperatures (a climate change-driven stress) have been demonstrated as one of the main factors controlling the fruit set and consequently yield in tomato, little is known about its consequences on fruit quality. In order to elucidate the cellular and molecular mechanisms associated with the response to elevated temperatures, transgenic tomato lines engineered with distinct carotenogenic-related genes (OE-*PSY1*, constitutively overexpressing tomato *PHYTOENE SYNTHASE1*; OE-*CrtI*, constitutively overexpressing bacterial phytoene desaturase *CrtI*; RNAi-*DET1*, with fruit-specific suppression of the transcription factor *DE-ETIOLATED1*, a component of the light signal transduction pathway), and producing different qualitative and quantitative carotenoid profiles, were exposed to short-term moderate heat stress (40°C day/ 30°C night) at mature green fruit stage in greenhouse. The preliminary results obtained from the comprehensive phenotyping

including analyses of fruit subplastidial fractions, metabolite and expression profiling will be shown.

P16: TARGETING THE GRANA MEMBRANES WITH CHIMERIC LIGHT HARVESTING STRESS RELATED PROTEINS.

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Plants and algae require light to survive, but excess light can be harmful. They have therefore developed a wide variety of protection mechanisms, commonly referred to as Non-Photochemical Quenching (NPQ). One of the most important and fastest components of NPQ is energy quenching (qE), this component is activated within seconds by a decreased pH in the thylakoid lumen. The precise mechanism of how the energy is quenched remains unknown, but there are several generally accepted theories that all depend on an interaction between chlorophylls and carotenoids. Two proteins are essential for the activation of NPQ, PSBS and LHCSR, which can be found in plants and algae respectively. The exception to this rule is the moss *Physcomitrella patens*, an evolutionary intermediate between algae and plants, which expresses both PSBS and LHCSR. The main difference between PSBS and LHCSR is that while LHCSR binds Chl a and xanthophylls, PSBS does not bind any pigments. Therefore PSBS itself cannot be the site of quenching. LHCSR on the other hand contains approximately 8 chlorophylls, 2 luteins and 1 or 2 violaxanthins, therefore making it likely that LHCSR is also the site of quenching. In this project we complement the quenching activity in *A. thaliana* npq4 strains by complementing them with a chimeric PpLHCSR1-AtLhcb1.1 construct. Initial expression of LHCSR1 in *A. thaliana* located the protein in stroma membranes only, hampering its interaction with PSII antenna and yielding a reduced level of functional complementation. The purpose of the chimeric construct is to direct LHCSR1 to the grana membranes where the PSII antenna system, the major substrate for quenching reactions, is located.

P17: ABC1K1 kinase function regulates tocopherol level to maintain photosynthesis under high light.

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Photosynthesis is one of the major reactions for life, during which water and carbon dioxide are transformed to sugar by light energy. This process takes place in plants in specialized green organelle called chloroplasts. Photosynthesis is the light dependant reactions and occurs on thylakoid membrane. During this process, reactive oxygen species (ROS) are generated, which could damage membranes and thus reduce the yield of the photosynthesis. Attached to the thylakoid outer membrane by a phospholipid monolayer, plastoglobules (PG) contain neutral lipids such as tocopherols, plastoquinone, phylloquinone, and plastochromanol, which act as antioxidant or electron transport. PG is surrounded by structural proteins, ABC1K kinases, and proteins which play role in lipid metabolism. Under high light, it was shown that ABC1K1/PGR6 contributes to tocopherol upregulation as well as thermal dissipation of the excessive absorbed light energy from photosystem II (NPQ) and electron transport. ABC1K1 phosphorylates tocopherol cyclase (VTE1), under high light to increase the level of tocopherols in order to protect thylakoid membranes from ROS. The ABC1K1 protein has highly conserved predicted active site of kinase at D400. The *abc1k1* mutant

complemented with ABC1K1$_{D400N}$ (Aspartate to Asparagine) have reduced amount of tocopherol and defective in photosynthetic parameters under high light compare to ABC1K1/*abc1k1* and WT. The double mutant of *abc1k1/abc1k3* recovers tocopherol and photosynthetic parameters under high light shows ABC1K3 act as the repressor of ABC1K1. In this study, we demonstrated the reduction of tocopherol level and the photosynthesis prg6 phenotype are due to the kinase function of ABC1K1.