XVI. Annual Meeting
of the International Society of Endocytobiology
German Section (ISE-G)

Herzogenhorn, July 21st – 24th

http://plantco.de/ISE-G/

Abstract Book

Edited by Stefan A. Rensing
Marburg, Germany, June 2014

Venue: Leistungszentrum Herzogenhorn (Black Forest Highlands, Germany)
~1,300 mtrs above sea level

Acknowledgements

The XVI. Annual Meeting of the International Society of Endocytobiology - German Section (ISE-G) is organized by Plantco.de e.V. (Freiburg/Marburg, Germany) and supported by the German Botanical Society. Support by the companies shown below is gratefully acknowledged.

Thank you very much!

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Program

Venue: Leistungszentrum Herzogenhorn

Talks take place in the gymnasium. Poster sessions and industry exhibit take place in the gymnasium and the seminar room. Breakfast, lunch, supper, coffee breaks and evening entertainment are located in the dining rooms.

Please refer to the abstracts for speakers' affiliations. All welcome (W) and invited (I) talks are 17+3 minutes, all contributed (C) talks are 12+3 minutes.

Monday July 21st

15:15, 16:15, 17:15	Bus shuttles from Feldberg-Bärental train station to venue
15:15 - 19:00	Registration desk open *(open during the conference at all breaks as well)*
19:00	*Reception with food and beverages*
20:00 – 21:00	**Welcome and introduction (Stefan Rensing, Jörg Nickelsen)**
	W1 Wolfgang Loeffelhardt Welcome lecture: "The glaucophytes in the postgenomic era: insights and open questions"
later	*Welcome cnt'd : Moss cocktails fresh from the bench*

Tuesday July 22nd

7:00 – 8:45	*Breakfast*	
9:00 – 10:30	**Oral session I – Mitochondria** **Chair Volker Knoop**	
9:00	I1	Thomas Börner Do plant mitochondria contain a complete genome?
9:20	C1	Felix Grewe Unique evolution of mitochondrial genomes in the Geranium family: *nad1*-intron losses and RNA-mediated transfer of the intron-encoded *matR* gene into the nucleus
9:35	C2	Oren Ostersetzer-Biran Plant mitochondria: from gene expression to respiratory complex assembly
9:50	C3	Dietmar Funck Compartmentalisation of proline and arginine metabolism: a cross-kingdom comparison
10:05	C4	Sriram Garg N-terminal independent targeting into hydrogenosomes. Are we there ye(as)t?
10:30	*Coffee break*	
10:50 – 12:00	**Oral session II – Plastids II: Biotechnology and metabolism** **Chair Andreas Weber**	
10:50	I2	Ralph Bock Multigene engineering of metabolic pathways in plastids
11:10	C5	Janina Apitz Posttranslational control of glutamyl-tRNA reductase and dissection into functional domains for interaction to regulatory proteins
11:25	C6	Andreas Blatt Characterization of a lycopene cyclase-fusion protein from the green alga *Ostreococcus lucimarinus* catalyzing the simultaneous formation of alpha- and beta-carotene
11:40	C7	Oliver Dautermann A VDL protein of the violaxanthin de-epoxidase superfamily catalyzes the formation of neoxanthin, an intermediate in the biosynthesis of the algal light-harvesting carotenoids fucoxanthin and peridinin
12:00	*Lunch*	
13:00 – 14:00	**Poster session I with coffee (ODD numbers)**	
14:00 – 15:10	**Oral session III – Plastids I: Photosynthesis** **Chair Jörg Nickelsen**	
14:00	I3	Andreas Weber Towards engineering C4 photosynthesis: Lessons from transcriptomic and evolutionary analyses
14:20	C8	Bernhard Grimm Two auxiliary factors for the organization of plant tetrapyrrole biosynthesis in organellar compartments

14:35	C9	Björn Walter *In vitro* reconstitution of cotranslational D1 insertion using a chloroplast translation system reveals a role of the cpSec/Alb3 translocase and Vipp1 in PSII maintenance
14:50	C10	Jürgen M. Steiner A "c6-like" cytochrome in the muroplast of *Cyanophora paradoxa*

15:15 – 18:30 *Excursion**

18:00 *Supper (for those who are not on excursion)*

19:30 *ISE-G members meeting (non-members welcome to attend)*

**Excursion*

15:20	*Departure for those taking the longer but more comfortable path*
15:40	*Departure for those taking the steep and rocky alpine hiking path*
16:00 - 18:30	Glacial relict excursion (with Michael Scherer-Lorenzen, University of Freiburg) to scenic Feldsee. Supper with Black Forest specialties food at Raimartihof.
ca. 19:00	*Return*

Wednesday July 23rd

7:00 – 8:45 *Breakfast*

9:00 – 10:30 **Oral session IV – RNA stability and processing**
Chair Thomas Börner

9:00	I4	Volker Knoop RNA editing in the endosymbiotic organelles: new surprises
9:20	C11	Michael Tillich Angiosperm-wide analysis of chloroplast RNA editing sites and factors employing *Nicotiana tabacum* as experimental system
9:35	C12	Mizuki Takenaka Homo- and heteromers of MORF proteins mediate interactions with PPR type RNA editing factors in plant organelles
9:50	C13	Mareike Schallenberg-Rüdinger The first complete set of RNA editing factors in plant mitochondria: The DYW type PentatricoPeptide Repeat (PPR) protein family in the model moss *Physcomitrella patens*
10:05	C14	Alexandra-Viola Bohne A small multifunctional pentatricopeptide repeat protein in the chloroplast of *Chlamydomonas reinhardtii*

10:30 *Coffee break*

10:50 – 12:00 **Oral session V – Endosymbiosis and evolution**
Chair Uwe Maier

10:50	I5	Sven B. Gould When robust plastids meet sea slugs
11:10	C15	Jan de Vries Host response underpins plastid compatibility in animal cells
11:25	C16	Martin Lohr Transcriptomic and biochemical evidence for a cryptic plastid in a heterotrophic chrysophyte

11:40	C17	Steven Ball Dissecting the chlamydial connection to storage and polysaccharide metabolism
12:00	*Lunch*	
13:00 – 15:00	**Poster session II with coffee & industry exhibits** **13:00 – 14:00 EVEN numbers, 14:00 – 15:00 ALL posters**	
15:00 – 16:50	**Oral session VI – Plastids III: Control of expression and function** **Chair Ralph Bock**	
15:00	C18	Rudolf Hagemann How can nuclear genes influence the modes of plastid transmission?
15:15	C19	Elena Ulbricht-Jones Plastid encoded factors for leaf development in *Oenothera*
15:30	C20	Dirk Licht The role of *Nicotiana tabacum* chloroplast ribonucleoproteins in chloroplast gene expression
15:45	C21	Laura Kleinknecht RAP, the sole octotricopeptide repeat protein in Arabidopsis, is required for chloroplast 16S rRNA maturation
16:00	C22	Julia Legen Identification of mRNAs localized to chloroplast membranes
16:15	C23	Ayako Okuzaki The RNA binding domains of chloroplast CP31A are sufficient for stabilizing the chloroplast *ndhF* transcript
16:30	C24	Anna Schoenberg ChloroPhos1.0 identifies new phosphorylation targets of plastid casein kinase II (pCK II) in *Arabidopsis thaliana*
17:00	*ISE-G soccer tournament*	
18:00	*Supper*	
later	*Farewell party*	

Thursday July 24th

7:00 – 8:45	*Breakfast*	
9:00 – 10:30	**Oral session VII – Secondary plastids** **Chair Peter Kroth**	
9:00	I6	Uwe Maier Solar fueled microfactories from the oceans
9:20	C25	Ann-Kathrin Ludewig Apicomplexans and the fate of plastids
9:35	C26	Joern Petersen *Chromera velia*, endosymbioses and the rhodoplex hypothesis
9:50	C27	Julia Lau Ubiquitination is essential for protein translocation via a modified ERAD-system in complex plastids

10:05	C28	Ansgar Gruber Occurrence and significance of C-terminal targeting motifs in organisms with secondary plastids
10:30	*Coffee break*	
10:50 – 11:35	**Oral session VIII – Plastids IV: Protein transport** **Chair Sven Gould**	
10:50	C29	Beatrix Duenschede cpSRP54 was recruited to cpSRP43 mediated LHCP targeting during land plant evolution
11:05	C30	Daniel Köhler Characterization of plastid protein import in Tic56-deficient plants reveals functional import in the absence of the 1 MDa Tic20-complex
11:20	C31	Rena Isemer The plastid proteins WHIRLY1 and WHIRLY3 – twins or distant relatives?
11:45	**Announcement of prizes and closing remarks**	
12:00	*Lunch, end of meeting*	
13:30, 14:30	Bus shuttles to Feldberg-Bärental train station	

Talk Abstracts

All welcome (W) and invited (I) talks are 17+3 minutes.

Welcome and invited talks

W1: The glaucophytes in the postgenomic era: insights and open questions

Presenting author: Wolfgang Loeffelhardt, wolfgang.loeffelhardt@univie.ac.at

Wolfgang Loeffelhardt

University of Vienna

The species-poor glaucophytes (one of the three phyla constituting the archaeplastida) are considered by many researchers as the most ancient phototrophic eukaryotes, on the first branch after the single primary endosymbiotic event. Thus it is not surprising that their peculiar plastids (muroplasts) retain even more cyanobacterial characteristics than those of red algae. In 2012 the genome of the model glaucophyte, *Cyanophora paradoxa*, was sequenced providing answers to a number of issues studied in the past years. Experimental data suggesting a primordial plastid protein import mechanism were confirmed. The only documented and structurally proven eukaryotic peptidoglycan is now explained by the complete set of biosynthetic enzymes. The light harvesting antenna system is very close to that of cyanobacteria and lacks the Lhcr proteins found in rhodoplasts. The Rubisco-containing central body of the muroplasts is a pyrenoid rather than a carboxysome, at least in the case of *C. paradoxa*. Open questions are the astounding number of fermentative enzymes in an organism considered an obligate phototroph and the fact that no secondary endosymbioses are known where glaucophytes participated (in contrast to rhodophytes and chlorophytes). Possible reasons for that are discussed.

I1: Do plant mitochondria contain a complete genome?

Presenting author: Thomas Börner, thomas.boerner@rz.hu-berlin.de

Emilia Cincu, Tobias Preuten, Thomas Börner

Humboldt University Berlin

The existence of multiple genomes - not only in each cell but also in each individual organelle - is commonly regarded as a general phenomenon of mitochondria and chloroplasts. However, one has to draw another, at first glance irritating conclusion, if one combines the result of early investigation into the amount of mitochondrial (mt) DN with the more recent data on the size of mt genome in higher plants: the determined amount of mtDNA per cell or per organelle would be not sufficient to provide all individual mitochondria with a complete genome. To investigate whether the gene content in plant mitochondria is indeed such low we used quantitative real-time PCR in combination with flow cytometry to determine absolute copy numbers of four mitochondrial genes per cell in various Arabidopsis organs and leaves of tobacco and barley. To detect possible changes in mitochondrial gene copy numbers and their potential effects on gene function, we further compared the numbers of mitochondria, gene copy numbers and transcript levels of mitochondrial genes during the development of Arabidopsis leaves and included material with impaired chloroplast development. Our data demonstrates that individual mitochondria contain indeed only part of the genome or even no DNA at all. The consequences of this finding as well as factors determining gene copy numbers in plant mitochondria will be discussed.

I2: Multigene engineering of metabolic pathways in plastids

Presenting author: Ralph Bock, rbock@mpimp-golm.mpg.de

Ralph Bock

Max Planck Institut fuer Molekulare, Pflanzenphysiologie

The plastid genome represents an attractive target of genetic engineering in crop plants. Plastid transgenes often give high expression levels, can be stacked in operons and are largely excluded from pollen transmission, thus providing increased biosafety of transgenic crops. Recent research has greatly expanded our toolbox for plastid genome engineering and many new proof-of-principle applications have highlighted the enormous potential of the transplastomic technology in both crop improvement and the development of plants as bioreactors for the sustainable and cost-effective production of biopharmaceuticals, enzymes and raw materials for the chemical industry. In my talk, I will describe recent technological advances with plastid transformation in seed plants and summarize progress with harnessing the potential of plastid genetic engineering in selected areas of biotechnology. I will focus on the engineering of metabolic pathways that require the expression of multiple transgenes.

I3: Towards engineering C4 photosynthesis: Lessons from transcriptomic and evolutionary analyses

Presenting author: Andreas P.M. Weber, Andreas.Weber@uni-duesseldorf.de

Andreas P.M. Weber

Cluster of Excellence on Plant Sciences (CEPLAS), Institute of Plant Biochemistry, Heinrich-Heine-University, Düsseldorf, Germany

One of the limitations on crop productivity is the efficiency with which inorganic carbon is converted to carbohydrates in photosynthesis. Carbon conversion efficiency, in addition to light harvesting and conversion and the harvest index is thus a prime target for engineering highly efficient crops. Carbon conversion efficiency is constrained by the biochemical properties of the carbon-assimilating enzyme RubisCO, which accepts both carbon dioxide and molecular oxygen as substrates for the carboxylation or oxygenation of ribulose 1,5-bisphosphate, respectively. The oxygenation reaction reduces carbon conversion efficiency and requires the process of photorespiration to detoxify the product of the oxygenation reaction, 2-phosphoglycolate. Cyanobacteria, algae, and some land plants have evolved mechanisms to concentrate carbon dioxide at the site of RubisCO, thereby reducing the magnitude of oxygenation and hence increasing the conversion efficiency. In land plants, C4 photosynthesis is the predominant carbon concentrating mechanism that has convergently and concurrently evolved over 60 times in monocotyledonous and dicotyledonous flowering plants. Some of our most efficient crops, such as corn and sugarcane are C4 plants; however, others, such as rice and wheat are C3 plants that display relatively low carbon conversion efficiency. To uncover the genetic architecture of the C4 trait, we are employing transcriptomic comparisons of closely related C3 and C4 species to identify those genes that are differently expressed between C3 and C4. Through multivariate statistics, we identify transcriptional regulons constituting the trait and through metabolic modeling we unravel the evolutionary trajectories leading from C3 to C4 via intermediate states of C2 photosynthesis. Eventually, this work will provide the blueprint for the reconstruction of C4 in a C3 background, using the tools provided by synthetic biology.

I4: RNA editing in the endosymbiotic organelles: new surprises

Presenting author: Volker Knoop, volker.knoop@uni-bonn.de

Volker Knoop

University Bonn

Over 20 years after the discovery of cytidine-to-uridine RNA editing in flowering plant organelles, new surprises emerge. It is generally assumed that much more editing exists in plant mitochondria than in chloroplasts, but we now report a record case of over 3,500 sites of editing, exclusively of the C-to-U type in the chloroplast of a lycophyte. Reverse U-to-C editing coexisting with C-to-U editing in at least some plants has remained a puzzling evolutionary enigma. We now identified plants with even higher amounts of U-to-C than C-to-U editing and present a likely “gained-once-lost-twice” scenario for U-to-C in a modern land plant phylogeny. Plant-type RNA editing is now found to also exist in certain protist mitochondria and a new example will be presented. Notably, unique RNA-binding pentatricopeptide repeat (PPR) proteins previously considered land plant-specific turn out to be present in such protists. Such “DYW-type” PPR proteins are key factors to identify RNA sequence targets upstream of editing sites. A PPR-RNA binding code has recently been proposed and further improving this recognition code is fundamental. We currently integrate data on characterized RNA editing co-factors into future versions of our “Plant RNA Editing Prediction and Analysis Computer Tool” PREPACT, already serving as a database of organelle editome references and as a WWW service to identify RNA editing candidate sites in new organelle queries.

I5: When robust plastids meet sea slugs

Presenting author: Sven B. Gould, gould@hhu.de

Jan de Vries, Gregor Christa, Sven B. Gould

Heinrich-Heine Universität Düsseldorf

While some animals enter symbiotic interactions with other organisms to profit from phototrophic biochemistry, what happens in some sacoglossan sea slugs is unparalleled in nature. These animals sequester only the plastids from their algal food and of the many hundred known species, a few can survive starvation periods lasting for months with photosynthesising stolen plastids (kleptoplasts) they retain in the cytosol of cells that envelope the digestive tubules. The research on “photosynthetic slugs” is currently at a turning point. Topical research shows that kleptoplast survival inthe cytosol of an animalcell is primarily an intrinsic property of the organelles sequestered, and the survival of the animals during starvation is not coupled to actively photosynthesising kleptoplasts. This tells us two things that I will discuss: (a) some kleptoplast are - by default - more autonomous than their land plant relatives, especially in regard to fighting photodamage, and (b) profiting from the acquisition of robust plastids, and enduring prolonged starvation periods, is achieved through the adaption of the slug’s physiology and not necessarily the maintenance of kleptoplasts. The slug-kleptoplast association is less well understood than we until recently anticipated, and newest developments highlight the system’s great potential to study “plastid symbiosis” and photobiology from an entire different - and currently unique - perspective: that of an animal host.

I6: Solar fueled microfactories from the oceans

Presenting author: Uwe Maier, maier@biologie.uni-marburg.de

Uwe Maier

Philipps University of Marburg

Microalgae have enormous potential for diverse biotechnological applications and currently attract much attention not only in the biofuel sector. Still underestimated is the idea of using microalgae as an expression system for the production of recombinant proteins. Microalgae combine rapid growth rates with all the advantages of eukaryotic expression systems, and offer great potential for solar-powered, low cost production of pharmaceutical proteins.In our projects, we use the diatom *Phaeodactylum tricornutum* as expression platform for the production of therapeutic proteins like antibodies and vaccines but also biopolymers such as the bioplastic PHB and spider silk. Altogether, our studies reveal the great potential of diatoms as efficient factory for protein and biopolymer production.

Contributed talks

All contributed (C) talks are 12+3 minutes.

C1: Unique Evolution of Mitochondrial Genomes in the Geranium Family: *nad1*-Intron Losses and RNA-Mediated Transfer of the Intron-Encoded *matR* Gene into the Nucleus

Presenting author: Felix Grewe, grewe@unl.edu

Felix Grewe, Emily A. Gubbels, and Jeffrey P. Mower

University of Nebraska-Lincoln

The nucleotide substitution rate of plant mitochondrial DNA (mtDNA) is generally very low compared to the nuclear genome or its plastid counterpart. Mitochondrial sequences from taxa of the geranium family, however, have an exceptional increase in their substitution rate. To further analyze this unusual finding, we used next-generation sequencing to obtain complete mtDNAs of seven representative Geraniales taxa. Our results show that the elevated substitution rates co-occur with an exceptional increase of mitochondrial genome size. This increase in size is coupled with a radical reduction of complexity reflected by a massive loss of genes, introns, and RNA editing sites. Of particular note was the loss of three *nad1* introns, including a trans-spliced intron that re-established continuity of *nad1* gene transcription. In parallel, the maturase matR (usually encoded within a *nad1* intron) evolved to be a free-standing mitochondrial gene in most Geraniaceae genera. In *Pelargonium*, individual protein re-import into the mitochondria. This exceptional arrangement of *matR* in the nuclear DNA of *Pelargonium* provides the first opportunity for knock-out/down studies to understand the function of this peculiar gene.

C2: Plant mitochondria: from gene expression to respiratory complex assembly

Presenting author: Oren Ostersetzer-Biran, oren.ostersetzer@mail.huj

Oren Ostersetzer-Biran

The Hebrew University of Jerusalem

Mitochondria in plants are the major sites for energy production and the biosynthesis of numerous essential metabolites for the cell. Recent studies suggest that mitochondria, like their plastid counterparts, serve as environmental sensors for the plant cell. The challenges of maintaining prokaryotic-type structures and functions are common to all eukaryotes. A miscommunication between these organelles and their host cell has been implicated in many diseases in humans, while in plants mitochondria dysfunctions often result with reduced germination and severe developmental defects. Our work focuses on plant cells. Plants possess some of the most complex organelle compositions of all eukaryotic cells. While the loss of different respiratory complexes is regarded as lethal in mammals and insects, plants harbor non-energy conserving bypasses of the electron transport chain, and are thus excellent model systems for studying mitochondria biogenesis in eukaryotes.The aims of our research are designed to investigate the nature of nuclear-organellar interactions, which link mtDNA expression and phenotypic responses in plants. Using reverse-genetic screens in Arabidopsis we identified several genes which are required in mitochondria genome expression. Mutations in these genes result with retarded germination and altered growth and development phenotypes, which are tightly associated with defects in the biogenesis of mitochondrial respiratory complexes.

C3: Compartmentalisation of Proline and Arginine Metabolism: A cross-Kingdom Comparison

Presenting author: Dietmar Funck, dietmar.funck@uni-konstanz.de

Dietmar Funck, Giuseppe Forlani and Gudrun Winter

University of Konstanz

Metabolic pathways can be effectively separated by localisation of contributing enzymes to different subcellular compartments. Mitochondria are key players in the metabolism of proline and arginine in plants, animals and fungi. Despite very similar enzymatic activities in all three kingdoms, differential compartmentalisation creates the potential to establish different metabolic routes, which may reflect the different lifestyles of plants, animals and fungi. We characterised ornithine aminotransferase (OAT) and pyrroline-5-carboxylate reductase (P5CR) from Arabidopsis, the two key enzymes for a potential conversion of arginine to proline. Localisation of OAT and P5CR in mitochondria or in the cytosol, respectively, is different in Arabidopsis compared to mammals and yeast. Our data indicate that higher plants cannot use arginine as a direct precursor for proline synthesis alternatively to glutamate. Both the degradation of proline that is accumulated during stress and the re-mobilisation of stored nitrogen from arginine converge in mitochondria in Arabidopsis. We conclude that plants have evolved a specific metabolic network by subcellular compartmentalisation to adapt to the needs of autotrophic and often nitrogen-limited growth.

C4: N-terminal independent targeting into hydrogenosomes. Are we there ye(as)t?

Presenting author: Sriram Garg, sriram.garg@hhu.de

Sriram Garg, Ömer Temirci, Verena Zimorski, Jan Tachezy, William F. Martin, Sven B. Gould

Heinrich-Heine Universität Düsseldorf

Our current understandings of protein import into mitochondria substantiate the presence of N-terminal targeting sequences on the majority of proteins that acts as a ligand for a receptor of the outer mitochondrial membrane. However, recent studies indicate that some proteins can be targeted into hydrogenosomes (anaerobic mitochondria) of the excavate parasite *Trichomonas vaginalis* even in the absence of an N-terminal targeting sequence. This has been corroborated by similar studies in the mitochondria-related organelles (MROs) of *Giardia* and *Trypanosoma*. Being an evolutionary early divergent eukaryote, protein targeting in *T. vaginalis* could either bear similarities to ancient mechanisms of protein translocation or could have evolved an independent mode of targeting. In order to explore these possibilities, hydrogenosomal and mitochondrial proteins like Succinyl coenzyme A synthetase (SCS) and Iron-sulfur cluster assembly protein A (ISCA) along with other metabolic enzymes of *T. vaginalis* were heterologously expressed in *S. cerevesiae* and *vice versa*. Our experiments show that heterologously expressed proteins are targeted to the mitochondrial matrix without an N-terminal targeting sequence, and conversely *S. cerevisiae* protein, when expressed in *T. vaginalis*, get targeted to the hydrogenosomes too. Understanding internal (cryptic) signals could help us to shed light on the evolution of import receptors such as TOM20 and TOM70 and their relation to the TOM40 translocon in general.

C5: Posttranslational control of glutamyl-tRNA reductase and dissection into functional domains for interaction to regulatory proteins

Presenting author: Janina Apitz, janina_apitz@web.de

Janina Apitz, Boris Hedtke, Bernhard Grimm

Humboldt University Berlin

The Arabidopsis glutamyl-tRNA reductase (GluTR), the initial enzyme of tetrapyrrole biosynthesis, is encoded in the HEMA gene family with HEMA1 as its dominant member. Knockout mutants of HEMA1 are pale-green and grow heterotrophically on MS media, indicating that HEMA2-encoded GluTR2 does not sufficiently compensate for the extensive needs of the metabolic precursor, 5-aminolevulinic acid (ALA), for chlorophyll. GluTR1 is posttranslationally inactivated by the regulator FLU and heme. Bimolecular fluorescence complementation (BiFC) and the crystal structure of Arabidopsis GluTR reveal binding of GluTR-binding protein (GluBP) to the N-terminal putative heme-binding domain (HBD). Expression of GluTR lacking the HBD entirely complements the hema1 knockout mutant. Interestingly, this truncated GluTR did not show degradation during three days of darkness, whereas wild-type GluTR content was almost abolished. However, GluTR1 in GluBP knock-out plants showing a wild-type like phenotype displayed a higher degradation after dark incubation compared to wild type indicating a protective role of GluBP against protein turnover. BiFC experiments revealed binding of ClpS1, a substrate selector for the chloroplast Clp protease system in Arabidopsis, to GluTR1, but not to the truncated protein. ClpS1 may compete with GluBP for the same binding site in GluTR1. A model of the control of ALA synthesis will be presented including a potential role of heme for the degradation of GluTR.

C6: Characterization of a lycopene cyclase-fusion protein from the green alga *Ostreococcus lucimarinus* catalyzing the simultaneous formation of alpha- and beta-carotene

Presenting author: Andreas Blatt, aphidina@web.de

Andreas Blatt, Matthias Bauch, Yvonne Pörschke, Martin Lohr

Johannes Gutenberg-Universität Mainz

Biosynthesis of asymmetric carotenoids such as alpha-carotene and lutein in the plastids of plants and green algae involves two functionally different lycopene cyclases. The two cyclases are closely related and probably the result of an ancient gene duplication. While in most plants and algae investigated so far the two cyclases are encoded by separate genes, prasinophyte algae of the order Mamiellales contain a single gene encoding a fusion protein with two cyclase domains and a C-terminal light-harvesting complex (LHC) domain. We have cloned the gene and found that it catalyzes the simultaneous formation of alpha- and beta-carotene when expressed in *Escherichia coli*. Partial deletions of the linker region between the two cyclase domains resulted in an altered stoichiometry of the two products, suggesting that the overall structure of the fusion protein tunes the balance between the two enzymatic activities. The ratio between alpha- and beta-carotene could also be altered by gradual truncation of the C-terminus. This suggests the LHC domain to be involved in regulating the product stoichiometry in the algae, which would represent a novel mechanism for adjusting the carotenoidcomposition in response to changes in light intensity. The possibility to control the product ratio by C-terminal truncation makes the lycopene cyclase fusion protein a promising tool for the biotechnological production of the asymmetric carotenoids alpha-carotene and lutein in bacteria or fungi.

C7: A VDL protein of the violaxanthin de-epoxidase superfamily catalyzes the formation of neoxanthin, an intermediate in the biosynthesis of the algal light-harvesting carotenoids fucoxanthin and peridinin

Presenting author: Oliver Dautermann, oliverda@students.uni-mainz.de

Oliver Dautermann, Mirjana Becker, Janine Fröhlich-Nowoisky, Hans Gartmann, Dominik Pieper, Martin Lohr

Johannes Gutenberg-Universität Mainz

Although diatoms and dinophytes are important contributors to marine primary production, the biosynthesis of their major light-harvesting pigments fucoxanthin and peridinin is largely unexplored. Based on experimental data and the molecular structure of both pigments, violaxanthin and neoxanthin are likely intermediates in their biosynthesis. We have cloned two VDL (violaxanthin de-epoxidase-like) genes from the diatom *Phaeodactylum tricornutum* and the dinophyte *Amphidinium carterae* encoding proteins with similarity to the enzyme violaxanthin de-epoxidase (VDE) involved in the photoprotective xanthophyll cycle in land plants and many algae. In an *in vitro*-assay, we found VDL to isomerize violaxanthin to neoxanthin with a final ratio of 75% neoxanthin and 25% violaxanthin. Contrary to VDE, VDL required neither ascorbate nor any other cofactor. An *in silico*-analysis of the deduced protein sequences indicated the presence of tripartite targeting signals at their N-terminus suggesting that they localize to the thylakoid lumen. In line with this prediction, a biochemical characterization of the diatom VDL revealed the enzyme to be most active under acidic conditions between pH 5 and 6. Furthermore, expression of VDL from both algae in tobacco resulted in detectable activity only when the enzymes were targeted to the thylakoid lumen. Our results indicate that VDL is a novel enzyme catalyzing a central step in the formation of fucoxanthin in diatoms and peridinin in dinophytes.

C8: Two Auxiliary Factors for the Organization of Plant Tetrapyrrole Biosynthesis in Organellar Compartments

Presenting author: Bernhard Grimm, bernhard.grimm@rz.hu-berlin.de

Maxi Rothbart, Olaf Czarnecki and Bernhard Grimm

Humboldt University Berlin

Tetrapyrroles are one of the most ancient prosthetic groups in all organisms and includes the most abundant pigments on earth. In plants, chlorophyll and siroheme act in plastids, while heme is universally distributed to all cellular compartments. Phytochromobiline is associated with phytochrome in the cytoplasm. The appropriate allocation of tetrapyrroles follows the specific intracellular needs and is controlled by a network of processes including synthesis and degradation, membrane transport and assembly with different apoproteins. Following a concept of metabolic compartmentation, tetrapyrrole biosynthesis is suggested to be organized in protein complexes that enable metabolic channeling within this pathway. The new glutamyl-tRNA reductase (GluTR)-binding protein (GBP) contributes to the control of active ALA biosynthesis by assembling a portion of GluTR to plastid membranes, while bulk GluTR can be inactivated by FLU-mediated feedback inhibition. The LIL3 protein, a member of the light-harvesting protein family, stabilizes enzymes of chlorophyll synthesis. Recent findings on the interaction of LIL3 with multiple plastid proteins will be presented. A recent model describes how LIL3 is involved in the integration of different enzymatic steps of chlorophyll synthesis, including the synthesis of the long hydrophobic phytyl chain and protochlorophyllide reduction, with the assembly of pigments and chlorophyll-binding proteins of the photosynthetic complexes.

C9: *In vitro* reconstitution of cotranslational D1 insertion using a chloroplast translation system reveals a role of the cpSec/Alb3 translocase and Vipp1 in PSII maintenance

Presenting author: Björn Walter, Bjoern.Walter@rub.de

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Photosystem II (PS II) is a multi subunit complex localized in the thylakoid membrane. Since the PS II reaction center protein D1 is prone to light induced damage, an efficient PS II repair cycle has evolved. This repair cycle includes, amongst others, the cotranslational insertion of a newly synthesized D1. However, little is known about the mechanism of D1 insertion. Experimental data suggest an involvement of cpSecY and Alb3 in D1 insertion. To elucidate the process of D1 insertion, we used a previously described *in vitro* translation system derived from pea chloroplasts to reconstitute the D1 insertion. Truncated D1 versions were synthesized in the presence of isolated thylakoids. Subsequently, chloramphenicol stabilized D1 insertion intermediates were enriched from solubilized thylakoids by sucrose cushion centrifugation. Mass spectrometry and immunological analyses of the enriched D1 insertion intermediates revealed a role of the cpSec translocase, Alb3, cpFtsY and VIPP1 in D1 insertion. This assumption was supported by size exclusion chromatography and coimmunoprecipitation experiments that pointed to an interaction between cpSecY, Alb3, cpFtsY and VIPP1. These results suggest a cooperative function of these proteins in PS II biogenesis.

C10: A "c6-like" cytochrome in the muroplast of *Cyanophora paradoxa*

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Cyanophora paradoxa (Glaucocystophyta), the “coelocanth“ of the algal world, is an obligatory photoautotrophic biflagellated protist containing cyanelles (muroplasts), peculiar plastids surrounded by a peptidoglycan wall, apparently a relict from the cyanobacterial endosymbiotic partner. Glaucocystophyte algae serve as an ideal model system to study essential aspects of plastid evolution such as protein translocation and assembly processes of supramolecular structures such as phycobilisomes and carboxysomes. In *Cyanophora*, which does not contain plastocyanin, photosynthetic electron transport from the cytochrome b6/f complex to photosystem I is mediated by cytochrome c6 (PetJ). As we could show earlier (via homologous and heterologous import experiments), apocytochrome c6 is a Sec passenger in cyanelles. In the course of the genome project we found a gene coding for a “c6-like“ cytochrome harboring a twin-arginine (Tat) consensus motif in its signal peptide. Import experiments and localization studies will be presented and the enigmatic nature of the “c6-like“ cytochromes in cyanobacteria and higher plants will be discussed. A second topic will be the discovery and analysis of a gene coding for a nonribosomal peptide synthetase (NRPS) in *Cyanophora*.

C11: Angiosperm-wide analysis of chloroplast RNA editing sites and factors employing *Nicotiana tabacum* as experimental system

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Chloroplast C-to-U mRNA editing is essential for proper expression of chloroplast genes and, thus, for chloroplast biogenesis. The editing process is carried out by nuclear encoded trans-acting factors, which can be divided into two classes: i) specificity factors (PPR proteins) usually recognize one to two editing sites, whereas ii) co-factors (cpRNP, MORF and ORRM proteins) promote editing at many sites. So far, experimental analyses of editing factors have been mainly conducted in *Arabidopsis thaliana*. We set out to (re-)establish *Nicotiana tabacum* (tobacco) as prime model for the experimental analysis of chloroplast RNA editing. To this end, we established bioinformatic pipelines for the assembly of tobacco’s gene sequences and the accurate prediction of editing factor target sites across angiosperms. The great advantage of tobacco is the amenability of the chloroplast chromosome towards genetic manipulation. This, combined with our bioinformatic platforms, ultimately allows the manipulation of both chloroplast cis-elements and their cognate nuclear encoded trans-acting factors in a single experimental system. Now, we can perform a systematic angiosperm-wide analysis of editing site / factor combinations and, finally, address several yet unsolved riddles surrounding chloroplast RNA editing. Aside of presenting our approach, tools and individual experimental examples, the evolution of chloroplast RNA editing will be discussed.

C12: Homo- and heteromers of MORF proteins mediate interactions with PPR type RNA editing factors in plant organelles

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RNA editing in flowering plant mitochondria and plastids alters 400-500 and 30-40 nucleotides from C to U in mRNAs, respectively. Loss of individual RNA editing events often leads to severe defects in organellar function and plant development. Recent molecular-genetic approaches have assigned more than 40 PPR (Pentatricopeptide repeat) proteins to be involved in different RNA editing sites in plant mitochondria or chloroplasts. The PPR domains in these specific RNA editing factors vary in their numbers and structures. Computational analyses suggest that only few amino acids in a given PPR domain determine the nucleotide specificity. In addition to the PPR proteins, we identified another class of proteins to play a role in RNA editing in both plant organelles. The loss of one of these MORF proteins (multiple organellar RNA editing factor) abolishes or lowers editing at many sites that also require individual PPR proteins. Y2H and BiFC analyses document selective combinations of interactions between various PPR editing factors and MORF proteins. These homo- and heteromer assemblies are part of the apparently complex structure of the RNA editosomes in plant organelles.

C13: The first complete set of RNA editing factors in plant mitochondria: The DYW type PentatricoPeptide Repeat (PPR) protein family in the model moss *Physcomitrella patens*

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Plant-type RNA editing, the site-specific conversion of cytidine to uridine (C-to-U) nucleotides in organellar transcripts, is a phenomenon to correct genetic information. In angiosperms, like *Arabidopsis thaliana*, approximately 500 editing sites in the mitochondrial and 30 editing sites in the chloroplast transcriptomes are found. RNA binding pentatricopeptide repeat (PPR) proteins with unique carboxyterminal extensions (E-/DYW-type) have previously been characterized as specificity factors recognizing particular RNA editing sites. The evolution of the editing factors as well as the mechanism of RNA editing, however, still remain enigmatic. With only 13 organellar RNA editing sites and 10 DYW-type PPR proteins, the moss *Physcomitrella patens* is an attractive model to study the phenomenon of RNA editing. We generated DYW-type PPR gene knockouts (KO) to assign editing sites to their corresponding editing factors. Knock-out analysis and RNA protein binding studies finally allowed the assignment of all mitochondrial DYW-type PPR proteins to their specific RNA editing targets. Two of our KO lines with DYW genes disrupted, do not show significant growth retardation, although lacking RNA editing at crucial sites in core subunits of respiratory chain complexes. These lines are perfectly suited for complementation studies with truncated and mutated versions of DYW-type genes to ultimately characterize the still unknown functions of the C-terminal protein domains.

C14: A Small Multifunctional Pentatricopeptide Repeat Protein in the Chloroplast of *Chlamydomonas reinhardtii*

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Organellar biogenesis is regulated by nucleus-encoded factors which mainly act on post-transcriptional steps of gene expression. Among these factors, Pentatricopeptide Repeat (PPR) proteins form the largest family of RNA-binding proteins, with hundreds of members in plants. In striking contrast, the genome of the unicellular green alga *Chlamydomonas reinhardtii* encodes only 14 such proteins. We analyzed PPR7, the smallest and most highly expressed PPR protein in *Chlamydomonas*. GFP-based localization and gel-filtration analysis revealed that PPR7 forms part of a high-molecular-weight ribonucleoprotein complex in the chloroplast stroma. RIP-chip analysis of PPR7-bound RNAs demonstrated that the protein associates with a diverse set of seven chloroplast transcripts *in vivo*. Furthermore, depletion of PPR7 results in mildly altered levels of its target RNAs that are compatible with defects in their maturation or stabilization. Historically, forward genetic studies focusing on mutants with highly specific phenotypes have led to the notion that target binding must be gene-specific. The diverse functions of PPR7, and the observation that a substantial decrease in PPR7 level only slightly perturbs the stability or processing of its targets, together point to another theme that may become more prominent as reverse genetic studies develop: broad specificity, multifunctionality and cooperation within a network of interacting RNA stability, maturation and translation factors.

C15: Host response underpins plastid compatibility in animal cells

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Sacoglossan slugs are the only animals known to host plastids in their cells. Acquired through suctorial feeding on macroalgae, plastids are retained and rest freely within the cytosol of digestive gland cells. Some sacoglossans retain photosynthetically active kleptoplasts, while surviving months of starvation. These species are called long-term retention (LtR) species, as most Sacoglossa retain functional kleptoplasts only briefly (short-term, StR) or not at all (non-retention, NR). Deemed vulnerable outside of algal cells, plastids were thought to require elaborate servicing by the slugs. Algal plastids sequestered, however, appear to be robust due to intrinsic properties. If it is not plastid maintenance, what is it then that defines an LtR species? We found kleptoplasts of *Acetabularia acetabulum* to retain their photosynthetic capacity equally well in the congeneric species *Elysia cornigera* (StR) and *Elysia timida* (LtR) during starvation. Yet, *E. cornigera* accumulated significantly higher amounts of reactive oxygen species in its digestive system and surrounding connective tissue during starvation. Gene expression profiling uncovered that *E. timida*, in contrast to *E. cornigera*, enters a state of quiescence upon progressing starvation, and which reaches beyond shifts in metabolism. Profiting from functional kleptoplasty appears to be primarily determined by the adaption of the hosts physiology and not the maintenance of plastids that are robust by default.

C16: Transcriptomic and biochemical evidence for a cryptic plastid in a heterotrophic chrysophyte

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As heterokont algae in general, chrysophytes aquired their plastids by a secondary endosymbiosis involving an ancient red alga. Chrysophytes of the polyphyletic genus Spumella, however, have lost pigmentation and the ability of photosynthesis and became secondarily heterotrophic. We examined the transcriptome of a cosmopolitan strain of the genus Spumella for genes involved in the biosynthesis of tetrapyrroles and carotenoids. As expected, transcripts of genes involved exclusively in the formation of chlorophylls were absent, whereas we found transcripts of nearly all the genes necessary for the biosynthesis of heme and siroheme. Surprisingly, we also detected transcripts of genes involved in the formation of carotenoids up to ß-carotene. Biochemical analyses confirmed the presence of several carotenes in Spumella, albeit at much lower cellular concentrations than in photosynthetic chrysophytes. Moreover, inhibitor studies indicated that carotenoid biosynthesis is essential for growth. *In silico*-analyses of the deduced protein sequences predicted the presence of N-terminal bipartite targeting signals in most sequences which is typical for nucleus-encoded plastid proteins of heterokont algae. Taken together, our results argue for the presence of a cryptic plastid in the Spumella strain that still harbors vitally important metabolic processes besides heme biosynthesis.

C17: Dissecting the chlamydial, connection to storage, polysaccharide metabolism

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We have recently proposed that an intracellular chlamydia pathogen triggered plastid endosymbiosis by allowing incorporation of photosynthate through chlamydia controlled effectors of storage polysaccharide metabolism. We review the three possible cases of LGTs in the starch metabolism enzyme network of Archaeplastida. We show that the previously reported glucan synthase and debranching enzyme genes remain robust cases of LGT from Chlamydiales to the Archaeplastida ancestor despite the ever expanding databases. We further confirm the complex phylogeny of the glucan synthase symbiotic gene and the effector nature of the enzyme tested both in heterologous and homologous systems. In particular in the glucan synthase phylogeny, the Chlamydiales are split into two subgroups with the Parachlamydiaceae as suspected donors of the symbiotic gene to the Archaeplastida while the remainder Chlamydiales seem to have further evolved leading to a split of the otherwise monophyletic Chlamydiales. We undertook a biochemical investigation to uncover the reasons explaining this complexity. We report that the Chlamydiale glucan synthase has progressively evolved from ADP-glucose to UDP-glucose substrate preference. We infer that ADP-glucose is ancestral and demonstrate that this is indeed the case for *Protochlamydia amoebophila* which indeed defines the closest suspected donor of the Archaeplastida sequences.

C18: How can nuclear genes influence the modes of plastid transmission?

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There are three modes of plastid inheritance: uniparental maternal, biparental and uniparental paternal. In this contribution the question will be discussed: By which molecular and cytological means are nuclear genes able to influence the modes of plastid transmission? In cases of biparental plastid inheritance female and male gametes transmit plastids. However, in many taxa there is uniparental transmission. The majority of angiosperms exhibit uniparental maternal plastid inheritance. In many gymnosperms a uniparental paternal (or biparental) plastid transmission is observed. Different lower plants show all possible modes of plastid transmission. Thus, often plastid DNA of one parent is excluded. So far two principal mechanisms have been found: (1) One way is the exclusion of plastid DNA by biochemical/molecular biological means using specific nucleases which destroy the plastid DNA and the plastids, e.g. destruction of paternal plastid DNA in *Chlamydomonas*. (2) In angiosperms, crosses within the genera Medicago and Pelargonium have identified nuclear genes that influence the modes in which plastids are transmitted to the next generation. Of great interest are also the processes of asymmetric distribution of plastids during the first and second pollen mitoses (in Gasteria, Tulbaghia, Plumbago). These findings will be described and discussed.

C19: Plastid encoded factors for leaf development in Oenothera

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Plant development and function rely on the communication of three genetic compartments: the nuclear genome, the plastome and the chondriome. Their functional interdependence leads to subgenomic coevolution. Hence, foreign combinations of nuclear and cytoplasmic genomes can result in cytoplasmic incompatibilities, an important mechanism of species formation. Moreover, an influence of the organellar genome on plant morphology has been observed in classical breeding experiments, but underlying molecular determinants are completely unknown. Reciprocal crosses of *Oenothera villaricae* and *O. picensis* yield hybrids with identical nuclear genomes but distinct organelle genotypes. Hybrids hosting *O. villaricae* cytoplasm have green, broad and strongly serrated leaves, whereas *O. picensis* cytoplasm confers virescent (pale) and narrow leaves with less serration. We could prove the differences in leaf shape and color to be attributed by the plastids. To identify the plastidic factors responsible for leaf phenotype determination, a comparative sequencing approach has been performed, covering full plastomes of various *O. villaricae* and *O. picensis* accessions. They were further analyzed regarding their ability to alter leaf phenotype (plastome-wide association mapping). Additionally, molecular analyses of the hybrids have been conducted. The results point towards an involvement of plastid translation in the determination of leaf phenotype.

C20: The role of *Nicotiana tabacum* chloroplast ribonucleoproteins in chloroplast gene expression

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Chloroplast gene expression involves complex transcript maturation including intercistronic and end processing, splicing and C-to-U RNA editing. The chloroplast ribonucleoproteins (cpRNPs) are abundant nuclear encoded chloroplast RNA binding proteins and are related to the heterogeneous nuclear ribonucleoproteins (hnRNPs). Like their nuclear relatives, also the cpRNPs function in various steps of gene expression. Several studies implicated the cpRNPs in stabilization, 3' end formation and RNA editing of chloroplast transcripts. In recent years, several cpRNPs have been characterized in *Arabidopsis thaliana* (Arabidopsis) by reverse genetics. However, for detailed analyses of the impact and function of cpRNPs on specific RNA maturation events, the possibility to manipulate their chloroplast targets in a directed manner is desirable. Therefore, we chose *Nicotiana tabacum* (tobacco) as model organism and we bioinformatically identified 17 cpRNP genes in the tobacco genome. To elucidate their chloroplast targets by reverse genetics, we generated tobacco RNA silencing mutants that exhibit reduced levels of either a specific or multiple, closely related cpRNP proteins. Interestingly, the tobacco cpRNPs appear to promote editing at different sites than their Arabidopsis counterparts.

C21: RAP, the sole octotricopeptide repeat protein in Arabidopsis, is required for chloroplast 16S rRNA maturation

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The biogenesis and activity of chloroplasts depends on an intracellular network of nucleus encoded factors that control almost all aspects of organellar gene expression. Most of these regulatory factors belong to the helical repeat protein superfamily, which includes tetratricopeptide repeat, pentatricopeptide repeat, and the recently identified octotricopeptide repeat (OPR) proteins. Whereas green algae express many different OPR proteins, only a single orthologous OPR protein is encoded in the genomes of most land plants. Here, we report the characterization of the only OPR protein in *Arabidopsis thaliana*, RAP, which has previously been implicated in plant pathogen defense. Loss of RAP led to a severe defect in processing of chloroplast 16S rRNA resulting in impaired chloroplast translation and photosynthesis. *In vitro* RNA binding and RNase protection assays revealed that RAP has an intrinsic and specific RNA binding capacity, and the RAP binding site was mapped to the 5' region of the 16S rRNA precursor. Nucleoid localization of RAP was shown, implicating the nucleoid as the site of chloroplast rRNA processing. Taken together, our data indicate that the single OPR protein in Arabidopsis is important for a basic process of chloroplast biogenesis.

C22: Identification of mRNAs localized to chloroplast membranes

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Many chloroplast mRNAs encoded integral membrane proteins. Insertion of most of these proteins has been suggested to occur co-translationally. Thus, many mRNAs are suggested to be associated to thylakoid membranes via nascent peptides. This is supported by early studies that identified RNA co-fractionating with membranes – a detailed identification of the nature of these RNAs has not been carried out. We have purified chloroplast membranes from *Zea mays* using gradient centrifugation techniques and determined bound RNAs by microarray screens. We find surprisingly little global qualitative differences in membrane-bound RNAs between etiolated, de-etiolated and normal green leaf tissue. By contrast, selected RNA species show a massive bias towards either soluble or membrane-bound chloroplast as evidenced by quantitative Northern blot analysis of chloroplast fractionations.

C23: The RNA binding domains of chloroplast CP31A are sufficient for stabilizing the chloroplast ndhF transcript

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Chloroplast gene expression is characterized by a puzzling multitude of post-transcriptional RNA processing events. A large number of nuclear-encoded RNA binding proteins have been identified that play a role for individual steps in RNA processing – however, none of them has been shown to be a true regulator of chloroplast gene expression. It was reported that one of the chloroplast RNA binding proteins, CP31A, is phosphorylated in a light-dependent fashion. CP31A has two RRM domains as well as an unstructured acidic domain, the latter being the phosphorylation site. CP31A binds to multiple chloroplast RNAs and has multiple roles in RNA stabilization and RNA editing. In sum, CP31A is a prime candidate for mediating light-activated global regulation of chloroplast RNA processing. In this study, we aimed to investigate the role of CP31A domains. We complemented Arabidopsis cp31a knock-out line with acidic domain deletion mutants, a phosphorylation-mimic and phosphorylation-deficient mutant. As cp31a KO mutants were reported to show a strong reduction in ndhF mRNA accumulation, we checked ndhF mRNA levels in our mutant lines. We found that acidic domain deletion mutants are still able to complement the loss of ndhF, suggesting that this domain is not necessary for stabilizing target mRNAs. Apparently, the remaining two RRM domains are sufficient for this task. A role of phosphorylation and the acidic domain on multiple RNA editing events is still under investigation.

C24: ChloroPhos1.0 identifies new phosphorylation targets of plastid casein kinase II (pCK II) in *Arabidopsis thaliana*

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During the last decade many phosphoproteomic studies have revealed a vast spectrum of phosphorylated chloroplast proteins in *Arabidopsis thaliana*. We will present the development of a peptide chip based on this knowledge. Altogether, 915 peptides were spotted as 15mers with the known phosphorylation site centered onto glass slides. We used this microarray for *in vitro* phosphorylation experiments and specifically assessed the peptide substrate spectrum of chloroplast casein kinase II (pCKII). Native pCKII from *Arabidopsis thaliana* and *Sinapis alba* chloroplasts was enriched by Heparin Sepharose and showed a similar activity on the chip to the recombinant form. The majority of the pCKII phosphorylation targets are involved in plastid gene expression, which supports i previous name: plastid transcription kinase (PTK). Among several exceptions is ALB3, which is essential for the integration of light harvesting complex components into the thylakoid membrane. The phosphorylation activity on ALB3 was characterized further by *in vitro* phosphorylation reactions on recombinant proteins, thereby the wild type and a phosphorylation site mutant Alb3 was used. Our data show that the peptide microarray ChloroPhos1.0 is a suitable tool for the identification of new kinase targets *in vitro*, expanding the knowledge of phosphorylation network nodes.

C25: Apicomplexans and the fate of plastids

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The superphylum Alveolata comprises ciliates, dinoflagellates and apicomplexans including the malaria pathogen *Plasmodium falciparum*. Apicomplexan parasites harbor a non-photosynthetic plastid that is essentially required for the biosynthesis of isoprenoids. However, the apicoplast is lacking in *Cryptosporidium* and gregarines and it is unclear if the organelle was secondary lost or if these lineages diverged prior to secondary endosymbiosis. The discovery of the free-living apicomplexan algae *Chromera velia* and *Vitrella brassicaformis* caused a scientific sensation, because they represent an important connecting link in alveolate evolution. We established high-quality transcriptomes of both chromerids in order to reveal the evolution of apicomplexan host cells and plastids. First, we accomplished a comprehensive phylogenomic analysis of orthologous nuclear genes based on >30,000 amino acid positions and showed that chromerids are the most basal apicomplexan lineage. Furthermore we performed phylogenetic analyses of essential nuclear-encoded plastid genes e.g. for the isoprenoid biosynthesis in order to investigate the number of plastid endosymbioses. Our phylogenies showed the monophyly of all plastid-containing apicomplexans, thus reflecting a single plastid endosymbiosis in a common ancestor of these lineages. Accordingly these results provide the first clear-cut evidence of plastid loss exemplified for Cryptosporidium and gregarines.

C26: *Chromera velia*, Endosymbioses and the Rhodoplex Hypothesis

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Plastid endosymbioses are the driving force for the emergence of novel organismal blueprints and thus the extant diversity of photosynthetic eukaryotes. The origin of complex algae with red plastids (cryptophytes, alveolates, stramenopiles, haptophytes [CASH lineages]) was discussed controversially, but their exact relationships are still unknown. The discovery of the apicomplexan alga *Chromera velia* provided the opportunity to study the photosynthetic ancestry of malaria parasites. We established its draft-genome via next-generation sequencing and identified the most reduced mitochondrial genome containing a single coxI gene. Based on our high-quality transcriptome, we investigated several "lucky genes" of the primary metabolism and the protein import machinery (SELMA, TIC) in order to validate our null hypothesis of a common plastid origin of *Chromera* and peridinin-containing dinoflagellates (PCDs). However, none of our markers supports the monophyly of their plastids. Moreover, the absence of SELMA orthologs in PCDs is supported by a deviating morphology of four *versus* three plastid membranes. Thus, we conclude that apicomplexans and PCDs obtained their plastids independently. Finally, we introduce the "rhodoplex hypothesis" proposing a common plastid origin of all CASH lineages via a single secondary endosymbiosis with a red alga and subsequent higher-order endosymbioses as a novel evolutionary framework.

C27: Ubiquitination is essential for protein translocation via a modified ERAD-system in complex plastids

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Pre-potein import into complex plastids of red algal origin is challenging as the proteins need to be transported across up to five membranes. The transport via the second outermost membrane, which evolved from the plasma membrane of a eukaryotic symbiont, occurs via an endoplasmic reticulum-associated degradation (ERAD) derived machinery named symbiont-specific ERAD-like machinery (SELMA). Core components of SELMA are a ubiquitination machinery, a Cdc48 complex and Derlin proteins. These components are present in all investigated organisms with four membrane bound complex plastids of red algal origin, suggesting a ubiquitin-dependent translocation process comparable to the retro-translocation process in ERAD which depends on cargo ubiquitination. Even if, according to the current model, retro-translocation via SELMA does not lead to a classical poly-ubiquitination of substrates, ubiquitination of pre-proteins might be required mechanistically for translocation. We investigated the import mechanism by *in silico*, *in vitro* and *in vivo* methods and show that protein transport across the periplastidal membrane (PPM) depends on N-terminal ubiquitination of the pre-proteins. Furthermore we present a "frozen intermediate" of the translocation process – i.e. a substrate that got stuck in the translocon - which was used to initially characterize the translocation complex.

C28: Occurrence and significance of C-terminal targeting motifs in organisms with secondary plastids

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In cells of algae with secondary plastids, protein trafficking to the plastid involves passage through the endoplasmic reticulum (ER), a transport step that is shared with proteins that are targeted to various other destinations (like the Golgi apparatus or the extracellular space) as well as with ER resident proteins. Most of these ER resident proteins are retained in the ER via a retrieval mechanism that is mediated by a short C-terminal sequence motif, also known as “KDEL”-motif according to the sequence of the first instances of the motif that have been discovered.
To improve the overall prediction of protein targeting in algae with secondary plastids we screened the available genomes of such organisms for possible C-terminal targeting signals. Our results indicate that the ER retention motif is generally more variable than expected, and that the occurrence and composition of sets of individual motifs highly depends on the organism.

C29: cpSRP54 was recruited to cpSRP43 mediated LHCP targeting during land plant evolution

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The abundant light harvesting chlorophyll binding proteins (LHCPs) are transported by the chloroplast signal recognition particle (cpSRP) pathway to the thylakoid membrane. This pathway evolved from the universally conserved cotranslational SRP transport. The higher plants cpSRP complex is composed of two protein subunits, cpSRP43 and cpSRP54, and forms the transit complex by binding LHCPs. *In vitro* both cpSRP subunits are indispensable for the LHCP insertion process. While cpSRP43 keeps the LHCPs soluble our data show that cpSRP54 is required for the formation of a low-molecular weight, insertion-competent transit complex. We also analyzed the cpSRP pathway in the green alga *Chlamydomonas reinhardtii*. Due to several amino acid alterations in cpSRP43 and cpSRP54 compared to land plant cpSRP proteins they do not interact or form a stable complex. These amino acid changes are conserved in chlorophytes whereas land plants show the typical interaction motifs. Therefore the exact role of cpSRP54 in the chlorophyte’s LHCP targeting remains elusive. The widespread assumption was that cpSRP43 co-evolved with LHCPs and was recruitedto the chloroplast adapted SRP transport mechanism to improve transport efficiency. Now our data support a view in which cpSRP54 was recruited to cpSRP43 mediated LHCP targeting during evolution probably also enhancing LHCP targeting efficiency.

C30: Characterization of plastid protein import in Tic56-deficient plants reveals functional import in the absence of the 1 MDa Tic20-complex

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The TOC-TIC (Translocon at the Outer/Inner envelope membrane of the Chloroplast)-import-machinery has evolved into the main import pathway for nucleus encoded plastid proteins. For the translocation of proteins at the inner envelope membrane, a 1 MDa Tic20-complex with new components including Tic214, Tic100 and Tic56 was described recently (Kikuchi et al., Science, 2013, 339, 571-574). The authors demonstrate that plants lacking Tic56 in the albino mutant line tic56-1 are also missing the 1 MDa TIC-complex. Here we report the characterization of Tic56. Plastids in tic56-1 fail to assemble thylakoids and remain at an early undifferentiated state. For a functional characterization of plastid protein import in tic56-1, we specifically analyzed protein N-termini by mass spectrometry to distinguish precursor from mature plastid proteins. Surprisingly, we find a considerable number of imported plastid proteins indicating the existence of alternative import pathways at the inner envelope membrane. Furthermore, we observe a large overlap of correctly processed stromal proteins in tic56-1 compared to ppi2, a mutant lacking the Toc159 import receptor at the outer envelope membrane. This suggests that both components are involved in the import of a specific subset of proteins and indicates a partial functional convergence of Toc159 and Tic56 potentially mediated by their joint association in a TOC-TIC supercomplex.

C31: The plastid proteins WHIRLY1 and WHIRLY3 – twins or distant relatives?

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WHIRLY proteins belong to a small protein family whose members are directed to organelles. Most species only possess one plastid located (WHIRLY1) and one mitochondrial located WHIRLY protein (WHIRLY2). It is not known why few species, e. g. Arabidopsis, have an additional plastid located WHIRLY protein (WHIRLY3). Eventually, the closely related WHIRLY1 and WHIRLY3 proteins have redundant functions. Arabidopsis WHIRLY1 and WHIRLY3 were both found in nucleoid preparations (Pfalz et al. 2006, Plant Cell). Accordingly, fluorescence tagged WHIRLY1 and WHIRLY3 proteins, respectively, were detected in speckles inside plastids resembling nucleoids and immunoprecipitation with an antibody directed against WHIRLY3 showed interaction of WHIRLY3 with several nucleoid proteins. Fluorescence microcopy analyses further indicated that a subpopulation of the WHIRLY1 and WHIRLY3 proteins interact in nucleoids. Accordingly, Yeast-two-Hybrid analyses showed interaction of the two proteins. Interestingly, only the fluorescence tagged WHIRLY3 was detected in plastid protrusions resembling stomules. In addition to its plastid localization, immunological analyses detected WHIRLY1 in the nucleus. The protein is believed to translocate from plastids to the nucleus (Isemer et al. 2012, FEBS Lett). On the contrary, no such dual localization was observed for the closely related WHIRLY3 protein. Our results suggest that WHIRLY1 and WHIRLY3 share some functions but are not completely redundant proteins.

Poster Abstracts

P1: Peroxisomes of diatoms

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Diatoms are unicellular photosynthetic active algae which evolved by secondary endosymbiosis. As a consequence, these organisms harbor a so called complex plastid surrounded by four membranes. Transport of nucleus-encoded plastid proteins across the four membranes is facilitated by different translocators. One of them, located in the second outermost membrane, is an ERAD (ER-associated degradation)-like translocation system which additionally shows clear homology to the peroxisomal importomer. As little information regarding diversity and function of peroxisomes in these organisms is present, we started a molecular and functional characterization of peroxisomes in diatoms. Recently, we were able to show the first *in vivo* localization studies of peroxisomal proteins in a representative member, the diatom *Phaeodactylum tricornutum* and demonstrated furthermore that targeting of peroxisomal matrix proteins is interestingly mediated only in a PTS1 dependent mode of action. Additionally, further data indicate that peroxisomes of diatoms differ regarding metabolic pathways as well, exemplified by the photorespiratory pathway. Thus, peroxisomes of diatoms enlighten a new model with characteristics not known from the current model organisms.

P2: Construction of artificial PPR chimeras to introduce novel RNA editing sites in plant mitochondria

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PPR type RNA editing factors are necessary for specific RNA sequence recognition. We previously reported a strong correlation between two amino acid residues in each PPR domain of RNA editing factors and its target nucleotide identity (Takenaka et.al. 2013). Recent structural studies of P-class PPR proteins (Ke et al., 2013) confirmed the direct interaction between these two amino acids and the target nucleotide. Based on this finding, we attempt to create artificial RNA editing factors with PPR chimeras targeting specific cytidines that are not edited in wild type *Arabidopsis thaliana* plants. At first, in order to introduce artificial stop codons in some mitochondrial genes by C to U RNA editing, we design chimeric PPR proteins based on the PPR repeats in known RNA editing factors, which show a clear preference for the respectively bound nucleotide. The chimeric PPR repeats are cloned between a mitochondrial targeting sequence and an E-DYW domain, which are necessary for RNA editing in plant mitochondria. We have presently constructed several such artificial RNA editing factors to transform and overexpress them in wild type Arabidopsis plants.

P3: The evolution of plastid RNA editing in land plants - Lessons from the liverwort *Pellia endiviifolia*

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RNA editing is a post-transcriptional process that can act upon transcripts from mitochondrial, nuclear and chloroplast genomes. In chloroplasts, single nucleotide conversions in mRNAs via RNA editing occur at different frequencies and types across the plant kingdom. These range from several hundred edited sites in some mosses and ferns, to lower frequencies in seed plants and a complete lack of RNA editing in the liverwort *Marchantia polymorpha* and the whole marchantiid subclade. In order to evaluate the evolution of plastid RNA editing in liverworts and land plants in general we sequenced the plastid genome of *Pellia endiviifolia*, a member of the jungermaniid subclade, and determined the plastid editotype. The type and relatively low frequency of chloroplast RNA editing displays a pattern highly similar to the one in seed plants. Additionally, we observed only C to U conversion type of RNA editing in transcripts of the *P. endiviifolia* plastid genome. RNA editing gives rise to considerable changes of encoded amino acids. Observed editing sites are predominantly embedded in a nucleotide context which is known for having a low mutation rate in seed plants. Our data provide evidence favoring the hypothesis that chloroplast RNA editing evolved to compensate mutations which appeared in the first land plants.

P4: *In vitro* structure and inheritance of the Oenothera mitochondrial genome

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In Oenothera plastids are transmitted biparentally, whereas the inheritance of mitochondria is unknown. There are two phenotypes, which point to a biparental inheritance of the chondriome, and hence are suitable for studying mitochondrial recombination. These phenotypes are the “falcifolia-sydrome“and the “Rosmon“. The falcifolia arises in reciprocal crosses between *O. glazioviana* and *O. biennis* with different frequency, leading to malformation of organs. The genetic behavior points to an interparental recombination of extranuclear components. To address the question of sexual mtDNA recombination in Oenothera, we initiated comparative mitochondrial genome sequencing, based on highly pure mitochondria isolations. NGS data from 454 and Illumina HiSeq were assembled with different de-novo assemblers (CLC,MIRA,Newbler,IDBA_UD). A circular mitochondrial genome could be accomplished with IDBA_UD. Bioinformatics data suggested a branched clover leaf like structure at the mt repeats, rather than the duplication of these repeats in a circular master molecule. This theoretical structural model of mtDNA is investigated *in vivo*. The whole mtDNA in Oenothera allowed mapping large scale populations. Our results show that mitochondria in falcifolia strains are transmitted maternally, which excludes them as determinant for our sickle-shaped phenotype. Studies on the mtDNA inheritance of the “Rosmon“ phenotype, as well as a wide range of intraspecific crosses are ongoing.

P5: Localization of the plastid encoded RNA polymerase (PEP) by *in situ* immunofluorescence analysis.

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Chloroplasts are derived from a cyanobacterial ancestor and possess their own gene expression machinery. It is known, that the bacterial gene expression is compartmentalized (Cisse et al., 2013). Whether such a compartmentalization exists in higher plants is largely unknown. We have used *in situ* localization studies to observe the sub-organellar localization of the plastid encoded plastid RNA polymerase. The plastid genome is transcribed by three different polymerases. Two nucleus encoded polymerases (NEP) and one plastid encoded plastid RNA polymerase (PEP). PEP is a eubacterial multimeric enzyme and its core subunits are encoded by the plastid rpoA, rpoB, rpoC1 and rpoC2 genes (Allison et al., 1996; Liere et al., 2007; Cardi et al., 2012; Weihe et al., 2012). An association of the plastid encoded plastid RNA polymerase with the DNA is obvious. On the other hand, there are both indications of the existence of DNA-bound RNA polymerase and free soluble RNA polymerase (Suck et al., 1996; Finster et al., 2013). To investigate the localisation of PEP the hemagglutinin-fused α-subunit RpoA was detected via immunofluorescence assays. Our results indicate that the plastid encoded plastid RNA polymerase is localised in Foci, which can vary in size and overlap mainly with the signals of a DNA marker indicating the plastid nucleoids.

P6: Identifying the chloroplast outer envelope proteome of the diatom *Phaeodactylum tricornutum*

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Diatoms are unicellular microalgae harboring secondarily evolved, complex plastids, surrounded by four membranes. The two innermost membranes corresponding to the envelope of primary plastids are of cyanobacterial origin. So far we know only very little about the third outermost membrane that is similar to the chloroplast outer envelope of primary plastids. Only a homologue of Omp85 has been published so far, which is involved in general protein import. With ABC1 another homologue to an outer envelope protein of *Arabidopsis thaliana* (WBC7) has been identified recently. ABC1 belongs to the ABC transporter G family and might be involved in lipid transport. By using the web-based application PSORTb 3.0, which was designed for prokaryotic sequences, we are underway to predict the proteome of the third outermost membrane. As beta-barrel proteins are known to be part of the outer membranes of mitochondria and plastids, we expect them to localize to this membrane and concentrated our search on this class of membrane proteins first. Several candidates have been identified *in silico* which are to be analyzed and localized.

P7: Deciphering the interaction interface of MORF proteins

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Almost 30 years ago C to U RNA editing was discovered in plant organelles. Recent genetic approaches revealed the involvement of two types of editing factors, E-class PPR (pentatricopeptide repeat) proteins and MORF (multiple orgenellar RNA editing factor) proteins. It becomes apparent that the loss of a MORF abolishes or decreases editing at multiple sites, implicating the importance of this protein family in RNA editing in both plant organelles. All MORF proteins contain a conserved central domain of 100 amino acids (the MORF box), which is supposed to mediate interaction between several proteins in the editosome. In order to elucidate the function of the MORF proteins in the editosome, the interaction between different parts of the MORF box and different RNA editing factors, including mitochondrial- and plastid- PPR type RNA editing factors as well as other MORF proteins, were analysed by yeast two-hybrid assays. So far, a preference in interactions between the N-terminus of the MORF box and parts of PPR proteins is observed. Furthermore, the results suggest that MORF homo- or heteromers connect between their respective N- and N-, N- and C- , and C- and C-terminal MORF box-sections.

P8: Ever more reports on RNA editing events and their site-specific recognition factors call for novel bioinformatic tools

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New discoveries and abundant RNA editing in plant and protist organelles call for bioinformatic tools to store, analyze and predict RNA editing sites. Our Plant RNA Editing Prediction and Analysis Computer Tool PREPACT allows reliable prognosis of both C-to-U and U-to-C mRNA editing for entire new organelle genomes, making use of its own curated reference database of known organelle editomes. We found reason to doubt that low numbers of 30-50 cpRNA editing sites are a general feature of all flowering plants and were indeed able to confirm significantly higher RNA editing in basal angiosperms such as Amborella. Some 60 RNA-binding pentatricopeptide repeat (PPR) proteins have already been identified as site-specific RNA editing factors in plants, mostly in the model systems Arabidopsis and *Physcomitrella*. Importantly, a PPR-RNA recognition code has recently been proposed, which allows bioinformatic testing and, ideally, further improvement. We will enhance PREPACT with an EDIFAC database module integrating data on characterized RNA editing factors and with a TargetScan module allowing to screen for potential sequence targets in the PREPACT organelle references. The plant RNA editing PPR factors contain so called “PLS-type” arrays of non-canonical PPRs with length variations. A bioinformatic tool to properly assign PLS-type PPRs is currently under development and should ultimately allow to extract relevant RNA target information from any user-provided PLS/PPR protein sequence.

P9: Interaction partners of the Photosystem II assembly factor PratA in *Synechocystis* sp. PCC 6803

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The protein PratA has been shown to play an important role in the early steps of Photosystem II (PSII) biogenesis. By interacting with the C-terminus of the precursor protein of the PSII subunit D1 (pD1), PratA provides manganese ions for the water splitting complex of PSII. Previous research revealed that PratA is part of a membrane-bound complex when interacting with pD1. Furthermore PratA is also part of a soluble complex located in the periplasm, which has a size of approximately 180 kDa. Two proteins have been identified as putative interaction partners of PratA within this complex by co-immunoprecipitation, namely the proteins Slr1277p and the Deg-protease HhoA. Here we present the initial characterization of the PratA/HhoA/Slr1277p complex by applying methods like co-immunoprecipitation, 2D BN/SDS-PAGE and the yeast two-hybrid system.

P10: The chloroplast ribonucleoprotein CP31A is a limiting factor for the accumulation of the chloroplast *ndhF* mRNA in *Arabidopsis thaliana*

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The RNA metabolism in plastids of higher plants depends largely on imported nuclear encoded proteins. Among those imported factors are a large number of RNA-binding proteins (RBPs). One of these RBP classes are the chloroplast ribonucleoproteins, a small protein family with ten members in *Arabidopsis thaliana*. These proteins function in various steps of RNA metabolism (e.g. RNA editing and RNA stability). Loss of CP31A, one member of this family, leads to loss of the chloroplast *ndhF* mRNA. We generated an allelic series based on T-DNA insertion lines in coding regions and in the 5'-UTR of CP31A to test for a correlation of CP31A protein and *ndhF* mRNA levels. Quantitative Western analysis demonstrates progressive loss of CP31A in various CP31A mutants. CP31A levels in these mutants are paralleled by *ndhF* mRNA levels as determined by RNA Gel blot analysis and digital PCR. Our findings suggest that CP31A is rate-limiting for the accumulation of the *ndhF* mRNA.

P11: Dramatic differences of RNA editing between taxa and organelles among ferns

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Cytidine to uridine RNA editing is a general hallmark of organelle gene expression in land plants. In contrast, the evolutionary whereabouts of the opposite process have remained mysterious with reverse U-to-C editing often referred to as “rare” or “occasional” in the literature. We here refute earlier claims of reverse U-to-C editing in flowering plants and suggest a single gain of reverse editing in the common ancestor of tracheophytes and hornworts followed by two independent losses later in plant evolution. The evolution of RNA editing is fundamentally different in ferns (monilophytes) and in seed plants, the two clades of euphyllophytes. Firstly, reverse U-to-C editing is particularly prominent in the ferns and we report cases of mitochondrial U-to-C editing even significantly outnumbering the more widely distributed “classic” C-to-U editing existing in parallel. Secondly, in complete contrast to flowering plant diversification, RNA editing overall increases rather than decreases in frequency among the more recently emerging fern lineages. Thirdly, the usually strong correlation of editing frequencies in chloroplasts and mitochondria is much more relaxed among ferns.

P12: RNA processing of chloroplast mRNAs bound to plastid ribonucleoprotein complexes

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RNA metabolism in chloroplasts of land plants is very complex and transcripts are extensively processed post-transcriptionally – quite in contrast to the ancestral cyanobacterial RNA metabolism. Plastid RNA processing includes endo- and exonucleolytic trimming, group I and group II intron splicing as well as C-to-U and seldom U-to-C RNA editing. Nuclear-encoded RNA binding proteins such as the highly abundant chloroplast ribonucleoproteins (cpRNPs) have been shown to be involved in multiple RNA maturation events in chloroplasts. RNA processing has been identified as an essential regulatory step in the modulation of chloroplast gene expression, besides transcription and translation. In this study we intend to investigate the separation of transcription, mRNA processing and translation by analyzing the processing state of mRNA bound to different parts of the gene expression machinery in *Nicotiana tabacum*. Therefore, we aim to immunoprecipitate the plastid-encoded RNA polymerase (PEP) as well as chloroplast ribonucleoproteins and chloroplast ribosomes with their associated mRNAs. Editing and splicing status of co-precipitated mRNA will be determined using RT-PCR-based methods. This will allow to determine whether the different components of the chloroplast gene expression machinery discriminate between unprocessed and mature RNA.

P13: Different Dinoflagellates

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Peridinin-containing dinoflagellates are unicellular alveolates, which evolved via secondary endosymbiosis from an eukaryotic host with an endosymbiont of red algal origin. Co-evolution of the secondary host and the endosymbiont led to a complex plastid surrounded by three membranes. The reduction of the endosymbiont genome in addition to gene transfer from the plastid to the nucleus led to a unique endosymbiont genome organization in peridinin-containing dinoflagellates. Some of their genes normally found on plastid genomes have been organized into so-called minicircles.

P14: Fibrillin 11 of *Arabidopsis thaliana*

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Fibrillin (FBN) proteins are expressed in all organisms performing oxygenic photosynthesis and are involved in stress protection. While cyanobacterial genomes typically contain only few FBN genes, the gene family expanded during the evolution of photosynthetic eukaryotes. In *A. thaliana*, at least 14 FBN genes could be identified so far. The genes are encoded in the nuclear genome but the proteins are predicted to be targeted to plastids due to the presence of an N-terminal plastid targeting sequence. Among the *A. thaliana* FBN, FBN11 is outstanding because the common FBN domain is fused with a Ser/Thr kinase domain. Transcript level analyses indicate a role of FBN11 in drought and salt stress responses as well as in biotic interactions. Interestingly, an association with Calmodulins (CaM) was reported in an interactome screen for Arabidopsis proteins indicating a Ca2+/CaM regulation of kinase activity. Ca2+/CaM regulation has long time been considered to be a eukaryotic trait and untypical of organelles. Moreover, no plastid-localised CaM has been reported so far. However, there were some reports about CaM-modulated activities of plastid enzymes and preliminary results also suggest a Ca2+/CaM regulation of FBN11.

P15: Going against the flow: extension of chloroplast RNA editing to a novel site

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In seed plants, RNA editing converts about 30-40 highly specific Cytidines into Uridines on several chloroplast mRNAs. Generally, RNA editing generates codons which encode for evolutionary conserved amino acids and thereby ensures protein functionality. Editing sites are recognized by pentatricopeptide repeat (PPR) proteins that guide the yet unknown editing activity. A single PPR protein typically recognizes one to two chloroplast editing sites. Evolutionary, chloroplast RNA editing appeared with early land plants and seems to be on the way out in seed plants. Editing sites have been independently lost throughout the evolution of seed plants by C-to-T point mutations on DNA level. Loss of an editing site often co-occurs with the loss or the degeneration of the corresponding PPR gene. Here, we present the first case of a recent gain of a chloroplast RNA editing event in seed plants.

P16: The WHIRLY1 protein – a mediator between chloroplast nucleoid organization and regulation of replication

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WHIRLY1, a member of a small family of single-stranded DNA binding proteins, was shown to be dually localized to plastids and the nucleus. In chloroplasts WHIRLY1 has been found to be associated to the transcriptionally active chromosome and to unspecifically bind plastid DNA. During development of chloroplasts from proplastids in barley primary foliage leaves the amount of plastid DNA is known to decrease. In comparison, the plastid DNA level in leaves of transgenic barley plants with an RNAi mediated knockdown of the WHIRLY1 gene increases during chloroplast development reaching a two- to threefold higher level in chloroplasts compared to the wildtype. This observation correlated with the enhanced expression of the gene encoding the organellarDNA polymerase. Additionally, microscopic analyses of chloroplast nucleoids stained with YO-PRO revealed a reduced compaction of nucleoids in WHIRLY1-RNAi plantscompared to wildtype. Particularly, two populations of nucleoids can be observed in RNAi plants: small wildtype-like nucleoids tightly associated to thylakoids and large irregularly formed nucleoids in the periphery of chloroplasts. Overexpression of barley WHIRLY1 gene in *E. coli* led to a retarded cell growth. Staining of bacterial nucleoids wit DAPI after induction of WHIRLY1 overexpression showed a higher compaction of nucleoids. Taken together these results indicate that WHIRLY1 affects organization of plastid DNA and regulation of replication.

P17: Breaking common rules: Chloroplast outnumbering mitochondrial RNA editing at new record amounts in the lycophyte *Selaginella*.

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Plant organelle RNA editing is generally more abundant in mitochondria with some 200-500 cytidine-to-uridine conversions *vs.* only 30-50 such sites in chloroplasts of flowering plants. The reasons for this bias are unknown. Using our recently updated PREPACT tool to systematically extract protein-coding genes and predict RNA editing for organelle genome sequences, we identified more than 2,000 candidate sites of RNA editing in the chloroplast genomes of two *Selaginella* species. An extensive chloroplast transcriptome study in *S. uncinata* indeed revealed more than 3,500 events of RNA editing, exclusively of the C-to-U type. Not only is that 100-fold the amount of cpRNA editing in the model plant *Arabidopsis thaliana* but it even surpasses a previous record of plant organelle RNA editing in the mitochondria of *S. moellendorffii*. Among the notable detailed features of the *S. uncinata* chloroplast transcriptome is an unparalleled amount of intron RNA editing with up to 30 sites affecting individual group II introns and a surprising accumulation of silent editing sites in the immediate vicinity of non-silent sites within exons. Although lycophytes represent the most ancient surviving lineage of vascular plants dating back more than 400 million years, the diversification of extant *Selaginella* species is much more recent. Surprisingly, we nevertheless find a tremendous variability of chloroplast and mitochondrial gene loci and their RNA editing patterns in this single plant genus.

P18: The plastidic leaf shape mutant pm45

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The plastome mutator of *Oenothera elata* is a nuclear mutation that leads to an increased rate of chloroplast mutations. Typically, the generated mutants show decrease in chlorophyll content and photosynthesis capacity. However, also green variants, such as the line Cornell-1, can be isolated. Interestingly, the further mutagenesis of Cornell-1 revealed a leaf shape mutant (pm45). To identify the polymorphism responsible for this phenotype, wildtype, pm45 and Cornell-1 plastomes were sequenced. Comparison between the three plastomes revealed a pm45 specific single base pair (bp) deletion in the coding region of *ycf1* causing a frameshift, multiple INDELs in the 5´ UTR and 5' end of accD and a single pb insertion in ndhD. The latter causes an extension of 9 amino acids at the C-terminal of the protein, whereas the single bp deletion in *ycf1* leads to a truncated form of Ycf1. Among the three mentioned genes, *ycf1* and *accD* have been shown to be essential in *Nicotiana tabacum*. Indeed, *ycf1* has been recently suggested to be a component of the TIC/TOC complex. Instead, AccD codes for the β-carboxyl transferase subunit of the plastidial acetyl-coenzyme A carboxylase, which catalyzes the first step of the fatty acid biosynthesis. Both proteins have an important role in the cell formation and chloroplast function and maintenance. Further analysis on pm45 might help us to confirm the role of Ycf1 in higher plants and elucidate the function of chloroplast in leaf shape establishment.

P19: Interaction of non-seed plants with arbuscular mycorrhiza fungi

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Symbiosis between plants and arbuscular mycorrhiza fungi (AMF) probably evolved concomitant with the water-to-land-transition of plant life, at least 450 MYA, and is assumed to have been instrumental in the occupation of the terrestrial habitat by plants. Bryophytes as the earliest diverging land plants are an ideal showcase to infer how mutualistic plant-fungus interactions first occurred and subsequently (co-) evolved. Here we demonstrate associations of the moss *Physcomitrella patens*, the liverwort *Marchantia polymorpha* and the fern *Microlepia* sp. with the AM fungus *Rhizophagus irregulare*. To elucidate the nature of this interplay, *Physcomitrella patens* has been treated with fungal exudates and subjected to transcriptome profiling using microarray technology. The results give insights into early (1h) and late (24h) responses of *P. patens* to AMF signaling molecules. Comparison with microarray data of the pathogenic response to *Botrytis* sp. demonstrates that the AMF response is not akin to a pathogen response. Phylogenetic analyses of the symbiotic signaling pathway (SYM) genes across land plants were conducted and reveal that the full set of required genes seems to be present in *P. patens*. In order to further elucidate the AMF signaling response, knockin mutants are currently generated.

P20: Isolation of Diatom Organelles

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Recent analyses of diatom genomes revealed that in diatom cells, major metabolic pathways are apparently operated in other cellular compartments compared to cells of higher plants. This also suggests that organelles in diatom cells may fulfill unusual functions compared to plant organelles. In order to further characterize the cellular metabolism of diatoms, organelle isolation protocols are required. Up to date, there are no protocols available for any of the diatom species of which the genomes have been sequenced. We have established the first isolation protocol for intact mitochondria from the centric diatom *Thalassiosira pseudonana* and in addition set up an isolation protocol for plastids from this diatom. Furthermore we developed a method for enzymatical removal of the cell wall from cells of the pennate diatom *Phaeodactylum tricornutum*.

P21: Plastid Transmission, Gene Transfer and the Impact of the Environment

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Plastids are maternally inherited in most crops. Maternal inheritance excludes plastid genes and plastid-encoded transgenes from pollen transmission. Therefore, plastid transformation is considered a superb tool for ensuring transgene containment and improving the biosafety of transgenic plants. We have identified two mechanisms how plastid-encoded genes can be transmitted via pollen: (i) by low-level paternal inheritance of plastids or (ii) by gene transfer from the plastid genome to the nuclear genome. Experimental reconstruction of gene transfer processes in the laboratory showed that the transfer of plastid genes to the nuclear genome is a still ongoing process and that rearrangements in the nuclear genome can convert transferred plastid genes into functional nuclear genes. It is conceivable that plastid DNA released into the cytoplasm upon plastid decay plays a role in the mechanism of gene transfer. The rate of plastid degradation is likely to be dependent on environmental factors and abiotic stress conditions are believed to enhance the rate of chloroplast degradation. Likewise, it seems possible that paternal leakage is dependent on the environmental conditions during pollen development. We therefore have determined the influence of environmental factors on (i) the frequency of paternal leakage, (ii) the frequency of gene transfer from the plastid to the nuclear genome and (iii) the rate of functional activation of transferred plastid genes in the nucleus.

P22: Separation of protein-unbound sRNAs and RBP-sRNA complexes from chloroplasts of *Arabidopsis thaliana*

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Chloroplast RNA metabolism is regulated by many nucleus-encoded RNA-binding proteins. The most abundant subfamily is represented by PPR (pentatricopeptide repeat) proteins that play crucial roles in many RNA-based processes. By binding non-cistronic regions of polycistronic precursors, they are hypothesized to serve as a barrier to RNA decay from either the 5' or 3' direction. Thus, PPR-directed 5' and 3' end maturation defines the termini of processed transcripts. When exoribonucleases converge on a bound PPR protein from both directions, a short non-coding RNA (sRNA) is generated. Deep sequencing identified 135 different sRNA clusters that were mapped to the chloroplast genome. It is at present unknown, whether RBP-sRNA complexes persist *in vivo*. If so, sRNAs could serve to titrate PPR proteins from their cognate mRNA targets. We used isopycnic gradient centrifugation to separate free from bound sRNAs. We identified a subpopulation of the ndhB 5'-UTR sRNA accumulating at a buoyant density similar to purified RNA, suggesting that it might represent protein-unbound sRNAs. Other subpopulations accumulate in various higher density fractions, likely representing protein-bound sRNAs. Future experiments will be directed at transcriptome-wide analysis of the distribution of sRNAs in low- and high-density fractions.

P23: Factors for biparental inheritance in the evening primrose

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Plastids in the model plant Oenothera are inherited biparentally and different types of plastids differ in their transmission efficiencies (strong, medium, weak). Factors for this organelle competition have not yet been uncovered. However, crossing studies showed that the competitive ability is mainly determined by the plastome itself and probably connected to different DNA replication speeds. To determine the plastid encoded factor, a strong plastome was mutagenized using a nuclear encoded plastome mutator allele. In a genetic screen 24 independent plastome variants were isolated with lower or higher chloroplast transmission efficiencies, but no defects in growth or leaf color. These variants were sequenced with Illumina to identify the loci responsible for the various inheritance strengths. The comparison of the variant plastomes with 9 strong, medium and weak wild type plastomes illustrated, that the action of the plastome mutator was biased towards three regions: the 16S-*trnI* spacer region, *accD* and *ycf2*. Interestingly, the occurrence of deletions in a certain repetitive region of *ycf2* correlates well with the different transmission efficiencies of variant and wild type plastomes. Therefore, this potential locus for inheritance strength and/or replication speed will be investigated further by crossing studies, measurements of ptDNA contents and replication activities, and replication origin mapping.

P24: How Membrane Proteins are imported into Secondary Plastids across the Sec61 Complex

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Protein import into complex plastids starts at the endoplasmic reticulum (ER) since the outermost of altogether four plastid membranes is connected to the host ER. Soluble as well as membrane proteins possess an N-terminal signal peptide which leads to co-translational translocation via the Sec61 channel. As there is so far no indication for vesicle transfer between individual plastid membranes, integral membrane proteins have to pass Sec61, and even more translocons, to be inserted at their final target membrane. Here, we address the question why the Sec61 channel of the diatom *Phaeodactylum tricornutum* does not recognize and insert the transmembrane domains (TMDs) of plastid membrane proteins. TMDs exchange experiments of marker proteins showed indeed a discrimination of secretory and plastid membrane proteins based on the TMD itself, represented by less hydrophobic plastid TMDs. A comparison of a larger dataset of secretory and plastid TMD sequences revealed a lower predicted biological hydrophobicity for single- as well as multi-spanning membrane proteins in general. In addition, we started to analyze if this observation is a result of evolutionary adaptation during primary or secondary plastid evolution

P25: The characterization of mitochondria encoded MatR protein in the splicing of group II introns in higher plants

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The mtDNA expression in plants is complex, particularly at the posttranscriptional level. RNA processing includes RNA editing and group II introns splicing, contributes to organellar genome expression. Introns excision is essential for mtDNA expression. Proteins required for the splicing of organellar introns have the capacity to control organellar gene expression, and thus to link mitochondrial functions with environmental or developmental signals. In bacteria the splicing of group II introns is facilitated by proteins encoded within the introns themselves, known as maturases. The plant mitochondrial introns, evolved from bacterial group-II introns, are degenerated and lost the vast majority of their intron-encoded ORFs. In fact, only a single ORF, matR, encoded within nad1 intron4, has retained in the angiosperms mtDNA. Its high conservation across the plant lineage and RNA-editing events, which restore conserved amino acids, suggest that matR encodes a functional protein. Despite these facts, a role for MatR in the splicing of mitochondrial introns has not yet been established. We analyzed the association of MatR with mitochondria transcripts by co immunoprecipitation. Our results suggest that MatR is associated with various introns, and suggested a role for MatR in the folding of its host nad1 intron4 pre mRNA *in vivo*. Interestingly, biochemical analyses indicated that MatR is associated mainly with matR 5'UTR regions and may regulates its own expression in plants.

P26: Strategy for the purification of RNA editing complexes from isolated mitochondria.

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The C to U RNA editing in flowering plant mitochondria specifically addresses hundreds of C-nucleotides and converts these to U by deamination. So far, the enzyme involved has not been identified. Site-specificity appears to be mediated by individual MEF proteins of the PPR family through sequence specific interaction with the target RNA molecule. The identification of the MORF proteins as essential co-factors shows that beyond selected specific MEF proteins additional proteins may be part of the editosome which performs the RNA editing. Several biochemical approaches have been initiated to identify the *in vitro* interaction between MEF, MORF proteins, respective RNA target and to investigate the overall sizes of the respective editosomes. Furthermore, the editosome complexes are being analysed to identify further components in Brassica oleracea . We will discuss our results from these analyses with respect to the composition of the RNA editing protein complexes in plant mitochondria.

P27: Relation between abundance of small RNA and mRNA in chloroplast

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PPR proteins protect chloroplast mRNAs against exonucleolytic decay. As a by-product, sRNA is generated, which represent the RNA footprint that a PPR leaves behind after the eventual demise of its target RNA. In the absence of its cognate PPR protein, neither the sRNA nor the mRNA will accumulate. Changes of sRNA abundance under different conditions could hint at changes in the stability of the corresponding mRNA. Here we used a technique called differential RNA-seq, which is able to distinguish primary and processed transcripts, and identified 44 accumulating clusters of sRNAs in chloroplasts of *Nicotiana tabacum*. Most of these sRNAs are mapped to a short distance upstream of start codon of the mature transcripts, while few sRNAs were found antisense to known chloroplast RNAs. All sRNAs are only abundant in processed libraries. An exception is Nt.*psbA*_5' and Nt.*ndhA*_5', which overlap with the 5' end of the respective primary transcripts. 7 out of 44 sRNAs were identified as differentially enriched among libraries prepared from tissue grown under different light conditions. To answer the question whether sRNA accumulation passively follows accumulation of their corresponding mature mRNAs, we analyzed steady-state levels of two sRNAs (Nt.*petB*_5', Nt.*psbC*_5') relative to their corresponding mature mRNAs (*petB* and *psbC*) under different light condition by Northern. Our data demonstrate that these two mRNAs share their expression pattern with their cognate sRNAs.

P28: Refinement and Verification of the RNA Recognition Code for PPR RNA Editing Factors

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The transcripts of plant organellar encoded genes undergo numerous posttranscriptional processing steps including 5'- and 3'-terminal processing, splicing and RNA editing during their maturation. Members of the PPR protein family (pentatricopeptide repeat proteins) consist of tandem arrangements of 31-40 amino acids long modules and are involved in all of these steps. The various tasks of PPR proteins require a potential for specific binding to the respective target RNAs at distinct nucleotide sequences. However, the specificity of the protein-RNA recognition machinery has been unclear until recently. Matching analysis found that amino acid positions 6 and 1' (1st of the next repeat) in the respective PPR module correlate with the respective nucleotide identity for two types of PPR modules, P (canonical: 35 aa) and S (short: 31 aa) (Barkan et al. 2012). These correlations allowed prediction of the target RNA sequence for a given PPR protein. In these analyses another type of PPR module, L (long: 35-40 aa), was not considered. We have now included the L module in the PPR nucleotide alignment and could thus improve the specific correlation between a given PPR type RNA editing factor and its target RNA sequence (Takenaka et al. 2013). Based on these findings, we developed a bioinformatic program tool not only to predict the potential target sites for a given PPR protein, but also to identify candidate PPR recognition factors for specific RNA editing sites.

P29: Plant mitochondria group-II intron splicing - an evolutionary step towards the spliceosome?

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Typical group II introns are large catalytic RNAs that are able to catalyze their own excision, under high salt and temperature. *In vivo* their splicing is facilitated by proteins (maturases) encoded within the introns themselves. Structural and mechanistic similarities between the spliceosome and group II introns led to the hypothesis that the spliceosome is a descendent of group II introns. In plants, the organellar introns lost the self splicing activities along with their maturase encoded ORFs. Therefore, their splicing is expected to be facilitated by nuclear encoded factors. Genetic analyses allowed us to establish the roles of two nuclear factors in the splicing of mitochondrial group II introns in Arabidopsis: mCSF1 (mitochondria CAF like splicing factor 1) and nMAT4 (nuclear encoded maturase 4). Analysis of the RNA profiles of mcsf1 or nmat4 mutants indicated that both mCSF1 and nMAT4 function in the splicing of several mitochondrial introns. This situation, in which the splicing of individual group II introns in plant mitochondria depend upon several splicing factors, is clearly different from the splicing of autocatalytic group II introns in bacteria, and may represent a step in the evolutionary transition from specific maturase facilitated splicing of group II introns in bacteria towards the more complex spliceosomal machinery in the nucleus of most eukaryotes. The homology of the core splicing factor Prp8 with group II intron maturases strongly supports this idea.

P30: Two loci, regulating Photosystem II and Cytochrome b6f- Genes, cause Plastome-Genome Incompatibility in Evening Primroses

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The model plant Oenothera (evening primrose) is perfectly suited to study molecular cause and evolutionary driving forces, connected with cytoplasmic elements in speciation. Hybrid offspring within the genus often displays so-called plastome-genome incompatibility (PGI). Within the Oenothera genus three basic nuclear genomes (A, B and C), and five distinguishable plastid chromosomes (I-V) were identified. All haploid nuclear genomes and plastome types are freely combinable in altogether 30 combinations which can lead to occurrence of inviable to various degree hybrids. In a pilot study the incompatible combination AB-I was chosen to identify molecular determinants causing PGI in Oenothera. From formal genetic data it appears, that plastome type I is incompatible in the AB background, but the combinations AB-II, AB-III, and AB-IV remain green. Sequence comparison of these four plastomes unveils two appealing candidate loci specific only for plastome I: a deletion of 148bp, and an insertion of 16bp, both in intergenic regions between genes involved in the photosynthetic apparatus. The deletion affects the promoter region of the *psbB* operon. The insertion, in turn, affects mRNA transcript stability/turnover of the *petN* and *psbM* genes. Concluding, the AB-I incompatibly in Oenothera is caused by an altered regulation of the mRNAs of PSII and cytochrome b6f subunits encoding genes.

P31: The How, When and Why of organelle shaping

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Biochemical processes of plant cells require a high degree of compartmentalization. Live cell imaging of fluorescent protein labelled cell compartments revealed that organelles are very dynamic and highly flexible in their morphology. Additionally dynamics and morphology of organelles change during the course of development and as response to stress. However to what extent shape and shape changes affect the functionality of organelles and how these processes are controlled is poorly understood. We are studying principles of organelle shape and their influence on organelle interaction by utilizing reporter lines that allow us to trace single and multiple organelles. To address the fundamental questions: 'How?', 'When?' and 'Why?' organelles change shape and move, we aim to identify genetic elements which control the ability to change organelle dynamics, morphology and behaviour, focusing initially on plastid stromules. In order to identify relevant genetic elements we combine forward genetic and reverse genetic approaches. In order to balance advantages and disadvantages of transient and classical screens we designed two complementary genetic screens, which are based on one hand on a classical EMS mutagenesis in *A. thaliana* and on the other hand on a transient Virus Induced Gene Silencing system in *N. benthamiana*. Based on previous experiments we study with reverse genetic approaches the role of sugar signalling and myosins in plastid shape changes.

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