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PERSPECTIVE

Acclimation in plants – the Green Hub consortium

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SUMMARY

Acclimation is the capacity to adapt to environmental changes within the lifetime of an individual. This ability allows plants to cope with the continuous variation in ambient conditions to which they are exposed as sessile organisms. Because environmental changes and extremes are becoming even more pronounced due to the current period of climate change, enhancing the efficacy of plant acclimation is a promising strategy for mitigating the consequences of global warming on crop yields. At the cellular level, the chloroplast plays a central role in many acclimation responses, acting both as a sensor of environmental change and as a target of cellular acclimation responses. In this Perspective article, we outline the activities of the Green Hub consortium funded by the German Science Foundation. The main aim of this research collaboration is to understand and strategically modify the cellular networks that mediate plant acclimation to adverse environments, employing Arabidopsis, tobacco (*Nicotiana tabacum*) and *Chlamydomonas* as model organisms. These efforts will contribute to 'smart breeding' methods designed to create crop plants with improved acclimation properties. To this end, the model oilseed crop *Camelina sativa* is being used to test modulators of acclimation for their potential to enhance crop yield under adverse environmental conditions. Here we highlight the current state of research on the role of gene expression, metabolism and signalling in

acclimation, with a focus on chloroplast-related processes. In addition, further approaches to uncovering acclimation mechanisms derived from systems and computational biology, as well as adaptive laboratory evolution with photosynthetic microbes, are highlighted.

Keywords: acclimation, Arabidopsis, *Chlamydomonas*, *Camelina*, Tobacco, gene expression, signalling, metabolism, systems biology, adaptive laboratory evolution.

INTRODUCTION

In this Perspective article, we outline the activities of the Green Hub consortium funded by the German Science Foundation (DFG) as Transregional Collaborative Research Center 175 (TR175). The consortium was launched in 2016, and received funding for an additional 4 years in the spring of 2020. The collaboration currently comprises 20 research groups based in Munich, Berlin, Kaiserslautern and Golm, Germany, whose members are well acquainted with the metabolic, genetic and cell biological functions of chloroplasts and their interactions with other cell compartments. The aim of the Green Hub consortium is to understand and where possible reconfigure the cellular networks that mediate acclimation processes in plants with a view to increasing their efficacy. A long-term goal is to contribute to 'smart breeding' methods devoted to the creation of crop plants with improved acclimation properties.

Acclimation is the capacity to adapt rapidly at the physiological level to changes in the environment within the lifetime of an individual and involves changes in the expression of the genome, in contrast to adaptation, which involves heritable changes in a population and so changes to the genome. Plant acclimation is a consequence of the sessile lifestyle of land plants, which compels them to confront *in situ* a dynamic environment, in which many critical parameters are subject to rapid fluctuation. Frequent variations in ambient conditions have forced plants to develop uniquely flexible metabolic and genetic responses in order to maintain cellular function in the face of environmental instability. Chloroplasts play a central role in many acclimation responses (for review, see Crosatti *et al.*, 2013; Foyer, 2018; Liebthal *et al.*, 2018; Manavski *et al.*, 2018; Yang *et al.*, 2019; Morales and Kaiser, 2020). Therefore, the consortium focuses on acclimation processes that involve this organelle. Photosynthesis is the key process in chloroplasts and has a central position in various metabolic transformations and signalling pathways. However, photosynthetic reactions are also very sensitive to changes in incident light, environmental temperature and water supply. This makes photosynthesis (and the chloroplast as a whole) a cellular sensor, which triggers various acclimation responses in the plant cell (Bräutigam *et al.*, 2009; Pfannschmidt and Yang, 2012; Exposito-Rodriguez *et al.*, 2017; Havaux, 2020). Owing to its vital significance for plant metabolism, the chloroplast is also a primary target of various acclimation responses (Crosatti *et al.*, 2013). Hence, the organelle can be considered to be the 'hub of acclimation'.

Table 1 Key issues being explored by the Green Hub consortium

Feature	Description/comment
Environmental conditions	
Temperature	Cold and heat
Light	High light and fluctuating light
Water availability	Drought
Metal deficiency	Deficiency in copper and/or iron
Organisms	
Arabidopsis thaliana	Main workhorse of the consortium
Tobacco	Predominantly used in RA1 (Gene Expression) because of its accessibility to plastid transformation
Chlamydomonas reinhardtii	Used in RA1 and RA3 (Gene Expression and Signalling), as well as in systems biology and ALE experiments
Camelina sativa	Used as model crop to test impact of modulators on acclimation capacity of crops
Synechocystis PCC 6803	Used in ALE experiments
Acclimation levels	
RA1: Gene Expression	Focus on quantifying and modelling gene expression in chloroplasts
RA2: Metabolism	Focus on chloroplast metabolism and its links to other compartments
RA3: Signalling	Focus on retrograde signalling
RA4: Computational Biology	Focus on bioinformatics, statistics and mathematical modelling to foster functional data integration
Further approaches	
Systems biology	In addition to proteomics, metabolomics and RNA-Seq also more sophisticated approaches like Gro-, ChIP- and Ribo-Seq are used
ALE	Mutations underlying adaptation of photosynthetic microbes to the environmental conditions studied by the consortium will be tested for their capacity to enhance acclimation in plants

RA, research area (of the consortium); ALE, adaptive laboratory evolution; modulators, cellular components (e.g. proteins or metabolites) that are central to acclimation and impair it when they fail to function.

Table 2 Selection of modulators of acclimation identified by and/or worked on in the Green Hub consortium. Only proteins that show a mutant growth phenotype (relative to the wild type) under acclimation-relevant growth conditions are listed. The acclimation-relevant growth conditions considered here are heat, cold, high light (HL), fluctuating light (FL), drought, salt, flagellin treatment and metal stress (e.g. cadmium)

Gene/ metabolite	Accession code	Molecular function	Acclimation condition	Reference (from the consortium)
RA1: Gene Expression				
CP29A	At3g53460	RNA-binding protein	Cold	Kupsch *et al.*, 2012
CP31A	At4g24770	RNA-binding protein	Cold	Kupsch *et al.*, 2012; Lenzen *et al.*, 2020
RA2: Metabolism				
NTRC	At2g41680	NADPH-dependent thioredoxin reductase 3	FL	Thormählen *et al.*, 2017, Hou *et al.*, 2019
NTT	At1g80300	ATP importer	Cold	Trentmann *et al.*, 2020
MEX1	At5g17520	Maltose transporter	Cold	Trentmann *et al.*, 2020
JASSY	At1g70480	OPDA (jasmonate precursor) transporter	Cold	Guan *et al.*, 2019
PGR5	At2g05620	Thylakoid proteins involved in cyclic electron flow	FL	DalCorso *et al.*, 2008, Suorsa *et al.*, 2016; Dann and Leister, 2019
PGRL1	At4g22890/ At4g11960			
pSuT	At5g59250	Plastidial sugar transporter	Cold	Patzke *et al.*, 2019
Fumarate	—	Photosynthate storage in Brassicaceae	Simultaneous copper and iron deficiency	Garcia-Molina *et al.*, 2020b
RA3: Signalling				
FBN6	At5g19940	Affects sulphate metabolism	HL, cadmium	Lee *et al.*, 2020
FC1	At5g26030	Heme synthesis	Salt	Fan *et al.*, 2019
FC2	At2g30390	Heme synthesis	Salt, flagellin	Scharfenberg *et al.*, 2015
GUN1	At2g31400	PPR-SMR protein	Cold	Marino *et al.*, 2019
PP7L	At5g10900	Involved in transposable element silencing, located in cytosol and nucleus	HL, drought	Xu *et al.*, 2019
VEN4	At5g40270	dGTP catabolism in the nucleus	Cold, salt	Xu *et al.*, 2020b
VIPP1	Cre13.g583550	Coping together with VIPP2 with lipid packing stress at chloroplast membranes	HL	Nordhues *et al.*, 2012; Theis *et al.*, 2020

PPR-SMR, pentatricopeptide repeat–small MutS-related; OPDA, 12-oxo-phytodienoic acid.

The three environmental variables chosen by our consortium – light, temperature and water availability – represent omnipresent external challenges with major impacts on plant performance and productivity (see Table 1). Accordingly, fast, precise and efficient responses are required to enable plants to become acclimated to these stimuli. To reduce the biological and experimental complexity to a manageable level, we focused in the first funding period (2016–2020) on changes that affected a single environmental parameter at a time, but we have now moved on to consider more complex conditions in which multiple parameters are changing. Acclimation mechanisms act on timescales that vary from minutes to days and on processes ranging from gene regulation to metabolic reactions, and they ultimately involve all cellular compartments. This diversity of acclimation processes depends on a multiplicity of steps and mechanisms, and therefore the Green Hub consortium works on three major aspects of acclimation: (1) gene expression, (2) metabolism and (3) signalling. These three Research Areas (RAs) are complemented by the RA 'Computational Biology' that focuses on applying mathematics and informatics to analyse and structure data and by additional projects that focus (i) on systems biology approaches and (ii) on adaptive laboratory evolution (ALE) experiments using photosynthetic microbes as models (Table 1).

The diversity of levels on which acclimation processes operate is reflected in a plethora of underlying mechanisms, each of which includes steps that are central to acclimation and impair it when they fail to function. We refer to such steps – which may concern a specific protein or metabolite, or a transcriptional master switch that regulates batteries of genes relevant to acclimation – as 'modulators'. A list of representative modulators worked on or identified by the consortium can be found in Table 2. One important question that presents itself is whether such modulators can be used to enhance the abilities of crop plants to acclimate to challenging environments. Disappointingly, simple genetic manipulations using gain-of-function (overexpression) or loss-of-function (knockdown or knockout) approaches have generally failed to generate stress-resistant plants without marked trade-offs (see also

Concluding Remarks below). This strongly suggests that trial-and-error experiments are unlikely to sustainably improve plant performance. Hence, in order to improve plant acclimation with minimum trade-offs, a deep understanding of the function of modulators and their involvement in multiple cellular networks is required. The consortium therefore makes extensive use of systems biology and quantitative approaches to gain a comprehensive picture of cellular networks, and also plans to employ complementary ALE approaches with photosynthetic microbes to identify changes in protein sequence and function that have an impact on acclimation.

To ensure a high level of synergy between the various projects, at the beginning of the collaboration we chose to study only three different model systems – a representative green alga (*Chlamydomonas reinhardtii*) and two species of flowering plants (*Arabidopsis thaliana* and tobacco [*Nicotiana tabacum*]) (Table 1). Each system possesses specific advantages with respect to the focus of individual projects: *A. thaliana* is a superior system for genetic screens; *C. reinhardtii* (the 'green yeast') offers the advantages of a unicellular organism, is amenable to ALE experiments and represents the best-studied photoautotrophic system for acclimation to high light and increased temperature at the systems biology level (e.g. Hemme *et al.*, 2014; Mettler *et al.*, 2014); and tobacco is a model system for plastid transformation (e.g. Fuentes *et al.*, 2018). In the current funding period (2020–2024), we will also employ *Camelina sativa* (also known as gold of pleasure, false flax, wild flax, linseed dodder, German sesame or Siberian oilseed), which is closely related to *A. thaliana*, is accessible to efficient transformation technology and represents an oilseed plant that serves as both model system and commercial crop (Bansal and Durrett, 2016; Malik *et al.*, 2018). Moreover, the consortium uses standardised conditions for light, temperature and drought treatments, and grows organisms in only one location for large-scale quantitative biology measurements (transcriptomics, metabolomics, proteomics) in order to exclude unnecessary experimental noise.

In the following we shortly summarise the state of the art and the progress made by the consortium in the three RAs, and describe the further approaches (Table 1) that we are now employing or currently establishing.

RA1. GENE EXPRESSION: ACCLIMATION OF CHLOROPLAST TRANSLATION TO TEMPERATURE AND MORE

Most of the genes in the chloroplast genome encode essential components of either the photosynthetic (PS) machinery or the plastid gene expression (PGE) system. The PGE apparatus, including the constituents of the major RNA polymerase and the ribosome and a set of RNases, is of bacterial origin, but there are also eukaryotic add-ons, many of which have a profound impact on gene regulation (Barkan, 2011). In this RA, we focus on the quantification of chloroplast-based genetic readouts and the characterisation of regulatory circuits during acclimation-associated adjustments. The tight and predominantly post-transcriptional regulation of PGE allows plant cells to quickly respond to sudden environmental changes, to adjust the composition of the PS machinery and to rapidly replace damaged proteins. Thus, PGE has been recognised as a major target of homeostatic adjustments during acclimation (Stitt and Hurry, 2002; Pfannschmidt and Yang, 2012; Crosatti *et al.*, 2013).

Plastid gene expression and acclimation: general remarks

Conceptually, PGE has two major roles in acclimation. It (i) generates signals that coordinate chloroplast activity with gene expression in the nucleus (retrograde signalling; covered by the RA Signalling, see below), and it (ii) senses and can be affected by environmental perturbations. For example, changing temperatures have a direct effect on the folding of chloroplast RNAs, as well as on the catalytic activities of key enzymes in gene expression, e.g. RNA polymerases, ribosomes and RNases. Unless actively opposed, this inevitably leads to changes in RNA production, RNA stability and translation efficiency. Appropriate responses to temperature shifts are essential to ensure the timely and appropriately balanced production of plastid-encoded proteins, e.g. to maintain the correct stoichiometry of the subunits in photosynthetic complexes. We are pursuing a two-pronged approach to understand acclimation responses of PGE. As a means of uncovering decisive regulatory steps and/or regulatory factors in PGE during acclimation, we have performed plastome-wide kinetic analyses of various steps in PGE. These experiments were designed to cover various stages of acclimation, including short- and medium-term responses. It was surprising to see that, apart from the known light-induced upregulation of *psbA* translation, PGE was only mildly affected during acclimation to higher or lower light intensities (Schuster *et al.*, 2020). Further kinetic analyses focusing on temperature changes are ongoing, and point to a far more important role of PGE during acclimation to heat and cold.

Molecular components that mediate acclimation of plastid gene expression

As a complement to such global and primarily descriptive efforts, this RA encompasses the identification of the molecular components that mediate acclimation of PGE. Obviously, responses of PGE and PGE-activated signalling require hard-wiring between signal receptors and gene expression readouts, i.e. modulators of PGE must translate acclimation signals into changes at the level of gene expression, which we aim to identify and characterise. Historically, transcription has been regarded as crucial for the regulation of PGE. Transcriptional activity indeed responds to diverse signals, e.g. to altered redox conditions in the

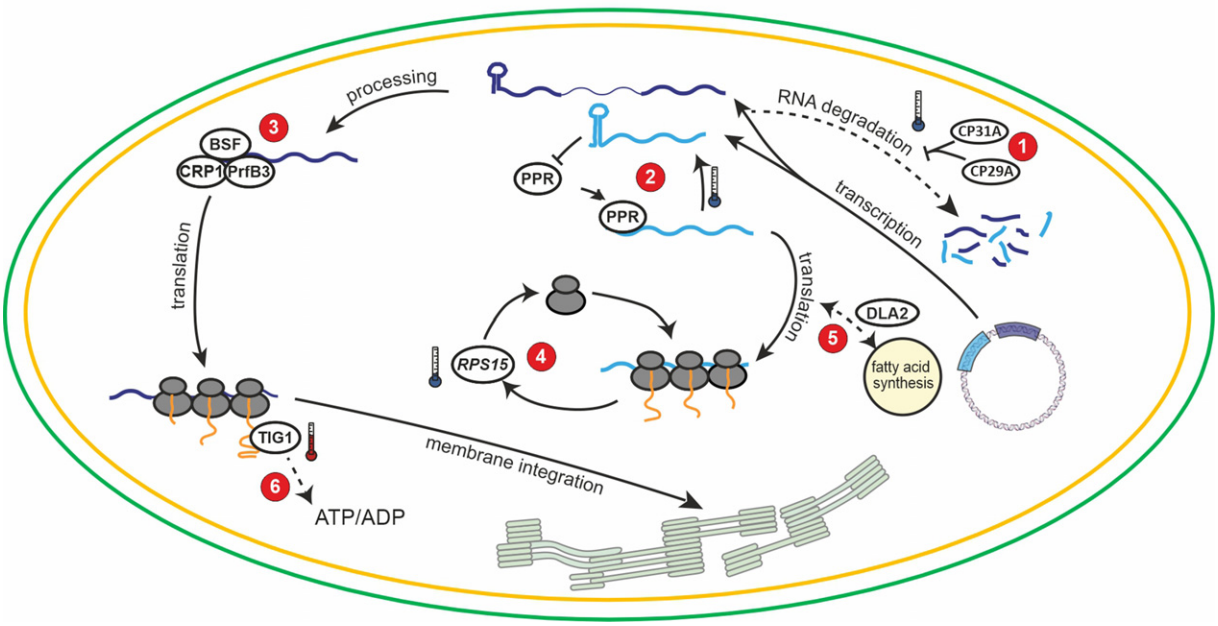

Figure 1. Acclimation-relevant components of plastid gene expression that are under investigation by the Green Hub consortium.
(1) RNA-binding proteins protect chloroplast mRNAs from degradation during cold acclimation (Kupsch *et al.*, 2012; Lenzen *et al.*, 2020). (2) PPR proteins protect RNAs from exonucleolytic degradation by binding to single-stranded UTR regions and hence facilitate the translation of their target mRNAs and the maturation of their target rRNAs (Wu *et al.*, 2016; Rojas *et al.*, 2018). (3) In the cold, UTRs are more likely to be inaccessible due to folding. Thus, specific mechanisms are expected to remove such structures, as exemplified by the ternary PrfB3 complex (Jiang *et al.*, 2019a). (4) Chloroplast-encoded components of the ribosome are differentially expressed in the cold to support ribosome biogenesis at low temperatures (Fleischmann *et al.*, 2011). (5) The moonlighting protein DLA2 connects fatty acid metabolism to the translation of selected mRNAs during acclimation (Bohne *et al.*, 2013; Bohne and Nickelsen, 2017). (6) Post-translational processes mediated by chaperones like TIG1 adapt protein production to changing environmental conditions (Rohr *et al.*, 2019; Ries *et al.*, 2020).

chloroplast (Pfannschmidt *et al.*, 1999), but it is expected to be of importance primarily for long-term changes in PGE. Therefore, transcription changes should be detectable during the intervals we have focused on (up to 4 days), together with post-transcriptional regulation that is likely to be dominant (Gruissem *et al.*, 1988; Zoschke and Bock, 2018). Three projects focus on RNA processing and stability, while three others analyse translational and post-translational processes. There is plenty of evidence for the adjustment of RNA levels in chloroplasts during acclimation to various environmental changes (Mentzen and Wurtele, 2008; Cho *et al.*, 2009; Castandet *et al.*, 2016) and, given the limited impact of transcriptional regulation on RNA levels (Eberhard *et al.*, 2002; Udy *et al.*, 2012), it is generally assumed that changing RNA levels reflect alterations in RNA turnover (Deng *et al.*, 1989; Germain *et al.*, 2013; Manavski *et al.*, 2018). Indeed, there is direct evidence that chloroplast mRNA half-lives respond to changing environmental conditions (Klaff and Gruissem, 1991; Germain *et al.*, 2013). We hypothesise that RNA stability and processing play an important role in chloroplast gene regulation, likely mediated by nucleus-encoded factors. A plethora of RNA-binding proteins (RBPs) are imported post-translationally into the chloroplast and modulate all aspects of gene expression, including RNA stability (Barkan, 2011). A surprisingly large number of these RBPs have been shown to be important during exposure to cold (e.g. Wang *et al.*, 2016; Nawaz and Kang, 2017; Paieri *et al.*, 2018; Pulido *et al.*, 2018). Promising candidates for the adjustment of RNA metabolism are, for example, the RNA recognition motif proteins CP29A and CP31A (Table 2), which are required for the accumulation of multiple chloroplast mRNAs in the cold and for cold resistance in *A. thaliana* (process 1 in Figure 1) (Kupsch *et al.*, 2012). A metabolic labelling approach was established recently (Szabo *et al.*, 2020) that allows one to quantitatively measure changes in the stability of RNAs during acclimation in wild type (WT) and *cp29a/cp31a* mutants on a genome-wide scale. More specific stabilisers of RNAs can be found in the pentatricopeptide repeat (PPR) protein family (Barkan and Small, 2014; Manavski *et al.*, 2018). This family includes PGR3 and SOT1, which are required for the expression and stability of ribosomal proteins and rRNA, respectively (Wu *et al.*, 2016; Rojas *et al.*, 2018). PPR proteins generally prefer single-stranded to double-stranded RNAs (Prikryl *et al.*, 2011). How they work at low temperatures, which stabilise many detrimental RNA structures and thus become an obstacle for RNA target binding, is another topic that is being investigated in this RA (process 2 in Figure 1). In addition to PPR proteins, HCF145, which

targets the 5′ UTR of the *psaA* mRNA and thus prevents its decay (Manavski *et al.*, 2015), is another transcript-specific binding protein and potential modulator of acclimation with a function in RNA stability. Likewise, PrfB3, a protein that exhibits RNA chaperone activity, is specifically required for the regulation of *petB* mRNA stability, acting as part of a ternary complex that includes the PPR protein CRP1 and the S1-domain-containing BSF protein (process 3 in Figure 1) (Jiang *et al.*, 2019a). Strikingly, HCF145, the PrfB3 complex and PGR3 all serve to link mRNA stability to translation, since they are all required for the recruitment of ribosomes to their target mRNAs. Interestingly, expression and RNA binding of PrfB3 and HCF145 are highly dependent on environmental conditions (Stoppel *et al.*, 2011). An impact of external signals on chloroplast translation has been suggested earlier, in particular in response to light and redox changes (Marin-Navarro *et al.*, 2007). For example, redox changes of regulatory proteins have been hypothesised to impact the translation of particularly the *psbA* mRNA in *Chlamydomonas*, which is mediated by pH-dependent reduction of cysteine residues in the protein RB47 or by light-dependent changes in the ATP/ADP ratio (for an overview, see Barnes and Mayfield, 2003). However, several aspects of this model are under debate (Zerges and Hauser, 2009; Nickelsen *et al.*, 2014). In general, translation has come to be recognised in recent years as the dominant target of chloroplast gene regulation, at least on short-to-medium timescales in several species (Trosch *et al.*, 2018; Zoschke and Bock, 2018). Therefore, this RA has devoted much effort to resolving the mechanism of translational regulation mediated by the aforementioned factors during acclimation. Key methods for assessing translation are proteomic analyses of active chloroplast ribosomes and chloroplast ribosome profiling, using protocols recently developed for different species (Zoschke *et al.*, 2013; Trosch *et al.*, 2018; Westrich *et al.*, 2020). Such methods are instrumental in testing hypotheses on the role of specific factors, such as HCF145 or the PrfB3 complex, in translational regulation during acclimation, in particular temperature acclimation. As well as analysing the roles of such specialised factors in translation initiation, we are studying how ribosome production itself becomes acclimated to cold. This includes the characterisation of the already mentioned PGR3 protein, but also an analysis of the role of the chloroplast-encoded ribosomal protein S15 (process 4 in Figure 1; Table 2), which was previously demonstrated to be required for cold resistance in tobacco (Fleischmann *et al.*, 2011). In addition, an exciting link between translation and carbon metabolism is being pursued by studying dihydrolipoamide acetyltransferase (DLA2). DLA2 acts as a subunit of the chloroplast pyruvate dehydrogenase complex, which provides acetyl-CoA for fatty acid synthesis. But it is also involved – in a light acclimation-dependent manner – in the synthesis of the chloroplast-encoded D1 protein, a core subunit of photosystem II (process 5 in Figure 1) (Bohne *et al.*, 2013; Bohne and Nickelsen, 2017). Hence, the moonlighting activity of DLA2 potentially links chloroplast lipid and protein synthesis during the early steps of light-driven thylakoid membrane biogenesis (Bohne and Nickelsen, 2017). Finally, processes downstream of translation could be relevant for acclimation as well. A case in point is trigger factor 1 (TIG1), which initiates protein homeostasis in plastids and is required for normal dark-to-light adaptation and energy homeostasis (process 6 in Figure 1) (Rohr *et al.*, 2019). TIG1 is a co-translationally acting molecular chaperone of a whole cascade of plastid chaperones dedicated to *de novo* folding and proteostasis under changing environments (Ries *et al.*, 2020).

In summary, translational regulation, with a special emphasis on temperature acclimation, is the primary focus of the RA Gene Expression, combining candidate gene approaches with whole-transcriptome kinetic analyses to identify key modulators of and steps in chloroplast gene regulation. These approaches are tightly linked to signalling studies and metabolite analyses, which are in the focus of RA2 and RA3.

RA2. METABOLISM: METABOLIC CHANGES THAT PROMOTE ACCLIMATION TO ABIOTIC CHALLENGES

Chloroplasts are the metabolic powerhouses of the green plant cell. This organelle harbours the machineries required for (i) photosynthesis, (ii) production of fatty acids and aromatic amino acids and (iii) isoprenoid and tetrapyrrole synthesis, and provides energy and the reducing power required for the assimilation of nitrogen and sulphate (Finkemeier and Leister, 2010). Therefore, changes in light intensity, temperature, nutrient availability or water status that impact chloroplast metabolism directly alter the metabolism of the whole plant.

Multilayer control of photosynthesis contributes to acclimation to abiotic stimuli

To cope with excessive excitation energy, photoautotrophs can enhance metabolic sink capacity or induce photoprotective mechanisms (reviewed in Demming-Adams *et al.*, 2014; Hüner et. al., 2016). Given that photosynthetic cyclic electron flow (CEF) fulfils a critical role in the acclimation of plants to fluctuating light conditions – as demonstrated by the lethal phenotype of plants devoid of the CEF component PGR5 (Tikkanen *et al.*, 2010; Suorsa *et al.*, 2012) – we aimed in the RA Metabolism to understand the molecular details of PGR5-dependent CEF and its interplay with acclimation to altered light conditions (process 1 in Figure 2). CEF allows for the production of extra ATP and regulates, in conjunction with the 'malate valve', the ATP/NADPH ratio according to specific stromal demands (Shikanai, 2007; Selinski and Scheibe, 2019). In higher plants,

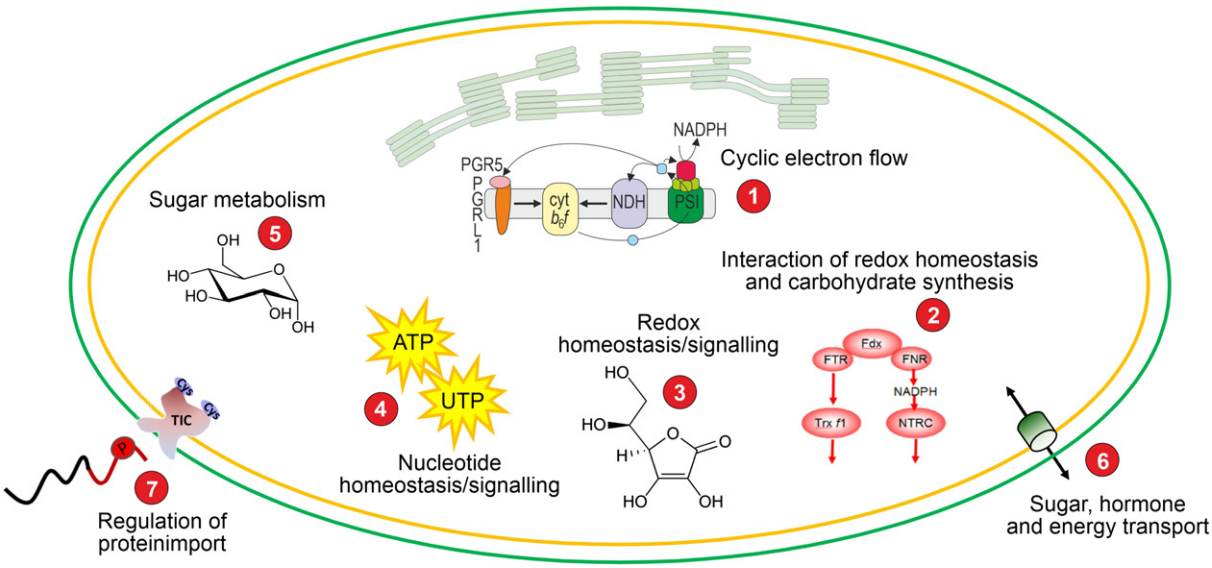

Figure 2. Acclimation-relevant pathways in chloroplast metabolism that are being studied by the Green Hub consortium.
(1) PGR5-dependent cyclic electron flow is a process that can be traced back to cyanobacteria, and becomes crucial under fluctuating light intensities (Suorsa *et al.*, 2016; Dann and Leister, 2019). (2) Chloroplast redox state and carbon assimilation are tightly interconnected during responses to alterations in light intensity and temperature (Geigenberger and Stitt, 1991; Thormählen *et al.*, 2015; Geigenberger *et al.*, 2017; Ojeda *et al.*, 2017; Thormählen *et al.*, 2017). (3) Ascorbate and thiamine are involved in the control of photosynthetic efficiency and coordinate acclimation-associated responses between chloroplasts and mitochondria (de Souza Chaves *et al.*, 2019; Rosado-Souza *et al.*, 2019; Feitosa-Araujo *et al.*, 2020; Obata *et al.*, 2020; Rosado-Souza *et al.*, 2020). (4) Enzymes involved in nucleotide biosynthesis/salvage perform moonlighting functions and contribute to the coordination of nuclear and organellar gene expression (Möhlmann *et al.*, 1994; Ohler *et al.*, 2019; Schmid *et al.*, 2019). (5) Cold-induced modulation of stromal sugar levels contributes to freezing tolerance (Nägele and Heyer, 2013; Weiszmann *et al.*, 2018; Patzke *et al.*, 2019). (6) Transport processes across the inner membrane of the chloroplast envelope are adjusted in response to low temperatures (Guan *et al.*, 2019; Trentmann *et al.*, 2020). (7) Fine-tuning of chloroplast protein import contributes to effective plant development and acclimation (Lamberti *et al.*, 2011; Eisa *et al.*, 2019a; Eisa *et al.*, 2019b).

CEF depends upon the physical interaction of the proteins PGRL1 and PGR5 (DalCorso *et al.*, 2008) (Table 2), and the consortium aims to identify further components involved in PGR5-dependent CEF. The evolutionary origin of CEF probably goes back to cyanobacteria, and we have used the cyanobacterium *Synechocystis* PCC6803 (in the following '*Synechocystis*') as an experimental test-bed for plant CEF components (Dann and Leister, 2019).

Effective acclimation to low temperatures or high light intensities requires that photosynthetic sugar synthesis, as well as partitioning of photoassimilate between starch and sucrose, is maintained (Wanner and Junttila, 1999; Mettler *et al.*, 2014; McCormick and Kruger, 2015). In fact, redox activation of its core enzymes is mandatory for the Calvin–Benson cycle (CBC) and is achieved via two thioredoxin (Trx)-dependent systems (Knuesting and Scheibe, 2018). The ferredoxin (Fd)-Trx system, consisting of various Trx isoforms, depends on photo-reduced Fd and thus on light, while the NADPH-dependent Trx reductase C (NTRC) (Table 2) system, comprising an NTR domain tethered to a Trx domain, depends on NADPH and may therefore be linked to stromal metabolism (Geigenberger *et al.*, 2017) (process 2 in Figure 2). Although the Fd-Trx and NTRC systems interact to jointly regulate CBC activity (Thormählen *et al.*, 2015) and are important for acclimation to fluctuating light intensities (Thormählen *et al.*, 2017), both systems exhibit specific features. For instance, NTRC does not directly activate the CBC core enzyme FBPase (Ojeda *et al.*, 2017), while independent *trxm1m2* mutants show lower photosynthetic efficiency in high light, but higher photosynthetic efficiency in low light (Thormählen *et al.*, 2017). The latter result suggests that the NADP-malate dehydrogenase involved in export of excess reductive power from the chloroplast via the malate valve is the main target of Trxs *m*1 and *m*2 in acclimation to fluctuating light (Thormählen *et al.*, 2017). Interestingly, NADP-MDH insertion mutants showed wild-type growth behaviour in constant light (Hebbelmann *et al.*, 2012), but increased ROS (H_2O_2) levels in high light conditions (Heyno *et al.*, 2014). Thus, the precise functions of the Fd-Trx and NTRC systems, as well as the physiological role of their interaction, deserve further analysis.

Mitochondrion–chloroplast interactions are involved in many biological processes, including acclimation. We provided evidence for the notion that acclimation-related mitochondrion–chloroplast interaction may be mediated by three factors, namely the essential redox components NAD^+, ascorbate and vitamin B_1 (thiamine) (process 3 in

Figure 2). Transporters that mediate NAD^+ import into mitochondria and chloroplasts were identified over 10 years ago (Palmieri *et al.*, 2009), and *A. thaliana* mutants deficient in the expression of either of the two NAD^+ transporters NDT1 and NDT2 displayed largely similar phenotypes, and – unexpectedly – both carriers were localised to mitochondria (de Souza Chaves *et al.*, 2019; Feitosa-Araujo *et al.*, 2020). The phenotypes of both mutants include plastid-mediated effects on photosynthesis but a proven link to acclimation is currently lacking. Another longstanding hypothesis from our own work is that ascorbate serves to coordinate photosynthetic and respiratory ATP production. This is based on the observation that downregulation of respiration results in an enhancement of ascorbate accumulation, since the terminal enzyme in its biosynthesis, L-GLDH, steps in to act as an alternative electron donor to the mitochondrial electron transport chain and to cause a 25% increase in the rate of photosynthesis (Rosado-Souza *et al.*, 2020). This observation was initially made in transgenic plants compromised in the expression of the TCA cycle enzyme malate dehydrogenase and these plants were characterised by upregulated expression of genes associated with photosynthesis (Nunes-Nesi *et al.*, 2005). In the same study, loading of leaves with ascorbate also resulted in an elevated rate of photosynthesis. This study showed that the reduction of ATP production in mitochondria resulted in its upregulation in the plastid; however, the route by which ascorbate biosynthesis elevates the rate of photosynthesis remains elusive. A further vitamin linking both organelles is thiamine, which regulates the rate of both photosynthesis and respiration, and clearly plays a role in day length acclimation in Arabidopsis, with *thic* mutants deficient in thiamine production being compromised in their acclimation to this condition (Rosado-Souza *et al.*, 2019).

Other nucleotides play an overwhelmingly important role in plant energy metabolism and also serve as signal molecules that regulate gene expression and development (Roux and Steinebrunner, 2007; Rieder and Neuhaus, 2011; Möhlmann *et al.*, 2014). Recently, we have obtained striking evidence for an interplay between chloroplastic nucleotide metabolism and the establishment of photosynthesis. This interplay is triggered by the stromal proteins uracil phosphoribosyl transferase (UPP) and (plastidic) UMP kinase (PUMPKIN) since both enzymes exhibit moonlighting functions. For example, apart from its catalytic activity, UPP is involved in chloroplast biogenesis and affects the expression of photosynthesis-associated nuclear genes (Ohler *et al.*, 2019), while PUMPKIN associates with plastid transcripts and its loss-of-function mutants exhibit altered PGE (Schmid *et al.*, 2019) (process 4 in Figure 2). These findings represent the starting point for further analysis of the interaction between PGE and nucleotide metabolism.

Transport processes across the chloroplast envelope are critical for acclimation efficiency

The compartmentation of solutes that act either as protective compounds or as signal molecules is key to the development of high tolerance against unfavourable environmental conditions (Nägele and Heyer, 2013, Hedrich *et al.*, 2015, Weiszmann *et al.*, 2018, Vu *et al.*, 2020) (process 5 in Figure 2). In the case of chloroplasts, the stroma is separated from the cytosol by an envelope consisting of an inner and an outer membrane. The inner envelope membrane harbours classical solute transporters, while the outer membrane contains pore-forming channels. Interestingly, both membranes exert control over the metabolite flux across the envelope system (Pottosin and Shabala, 2016).

It has long been known that sugars perform important functions in tolerance to cold and freezing temperatures (Wanner and Junttila, 1999; Pommerrenig *et al.*, 2018), and the set of sugar transporters in the chloroplast envelope comprises pSuT, pGlt and MEX1 (Weber *et al.*, 2000; Niittylä *et al.*, 2004; Patzke *et al.*, 2019) (Table 2). While pGlt and MEX1 are involved in export of the starch degradation products glucose and maltose, pSuT acts as a sucrose exporter during exposure to cold and contributes to the induction of flowering (Patzke *et al.*, 2019). During cold acclimation, amounts of the MEX1 transporter decrease, whereas the level of the envelope-located ATP importer NTT1 increases (Trentmann *et al.*, 2020) (Table 2; process 6 in Figure 2). Lower levels of MEX1 result in a cold-associated accumulation of maltose in the stroma, where this sugar might contribute to the protection of the delicate thylakoid-located proteins against frost (Nagler *et al.*, 2015). The increased capacity for ATP import into chloroplasts under cold conditions is expected to support the maintenance of energy-consuming reactions (Reinhold *et al.*, 2007) in order to promote cold acclimation (Trentmann *et al.*, 2020).

Jasmonates (JAs) fulfil an important function in various cellular responses, including reactions to biotic and abiotic stress stimuli, such as cold stress (Hu *et al.*, 2013; Ahmad *et al.*, 2016). In the outer envelope, a channel-forming protein named JASSY (Table 2; process 6 in Figure 2), which is able to export the JA precursor 12-oxophytodienoic acid (OPDA), has been identified by members of our consortium (Guan *et al.*, 2019). Remarkably, JASSY is a modulator of cold acclimation (Guan *et al.*, 2019). To date nothing is known about how OPDA crosses the inner envelope. However, we expect specific transporters to be involved in this, which are to be identified in future studies.

The chloroplast envelope is not only a barrier to the free exchange of metabolic substrates, it also controls the import of precursor proteins from the cytosol (Bölter and Soll, 2016). The efficiency of protein uptake is influenced

by phosphorylation of the precursor proteins, mediated by the cytosolic protein kinases STY8, 17 and 46 (Lamberti *et al.*, 2011). Our consortium recently showed that *S*-adenosyl methionine and isoleucine – both of which are synthesised in the chloroplast – bind to the highly conserved STY-specific aspartate kinase–chorismate mutase–tyrA domain, which leads to the inhibition of kinase activity (Eisa *et al.*, 2019a). In addition, rates of precursor protein import are reduced under high light conditions owing to a decrease in the expression of the STY kinase genes (Eisa *et al.*, 2019b). In summary, the regulation of chloroplast protein import by phosphorylation of precursor proteins is an interesting but incompletely understood facet of metabolic acclimation to challenging stimuli (process 7 in Figure 2).

RA3. SIGNALLING: COORDINATION OF DIFFERENT ACCLIMATION RESPONSES

In the RA Signalling we focus on signalling pathways that operate in, derive from or have an impact on chloroplasts. We are especially interested in the quantitative characterisation of these pathways. Our goal is to identify proteins or metabolites that transfer information between compartments and likewise modulate signals. In this context, we hypothesise that modification of the activity of these modulators has the potential to alter the acclimation capacity of plants. Following the perception of an environmental cue by proteins or pathways with receptor function in the chloroplast, signals are produced and conveyed to the nucleus, which serve to adjust the transcription of stimulus-specific genes and, in turn, allow for the complex metabolic reprogramming needed to cope with the changed environment.

The techniques employed to accomplish our goals include the mining of public databases in order to identify co-regulated factors (guilt-by-association approaches), genetic screens, environmental perturbation experiments combined with 'omics' technologies (transcriptomics, proteomics and metabolomics) and the analysis of molecular and gene regulatory networks. For example, FIBRILLIN6 (FBN6) (Table 2) was identified as a factor involved in high light acclimation and cadmium tolerance via a guilt-by-association approach (Lee *et al.*, 2020). Moreover, a screen for mutants that can cope better than WT with lincomycin, an inhibitor of organellar translation, revealed that a disturbance in the secondary cell wall promotes early chloroplast development (Xu *et al.*, 2020a). Another screen identified SAFEGUARD1 (SAFE1), which suppresses singlet oxygen-induced stress responses by protecting grana margins (Wang *et al.*, 2020).

GUN signalling

Chloroplast-derived retrograde signals have been classified into those originating from developed chloroplasts in fully expanded leaves in response to changing environmental conditions (operational control) and those that are activated during chloroplast and photosystem biogenesis (biogenic control, young seedlings) (Pogson *et al.*, 2008). The *genomes uncoupled* (*gun*) mutants, in which the expression of nuclear genes involved in photosynthesis is uncoupled from the developmental state of the chloroplast (Susek *et al.*, 1993), are the most extensively studied mutants in the biogenic control pathway. However, more than 25 years after their identification, the precise role of GUN proteins in the transmission of information from chloroplasts to the nucleus is far from understood. While GUN2–GUN6 are involved in tetrapyrrole biosynthesis, GUN1 is involved in PGE and is an integrator of diverse chloroplast signals (Kleine and Leister, 2016; Tadini *et al.*, 2016; Marino *et al.*, 2019; Richter *et al.*, 2020). Work by our consortium has contributed to a better understanding of the relationship between the *gun* and acclimation pathways and hints at entangled biogenic and operational control mechanisms. At the metabolic level, the comprehensive dissection of the physiological impact of GUN4 phosphorylation on plant tetrapyrrole biosynthesis and intracellular signalling during acclimation revealed a functional impact of protein phosphorylation on the control of chlorophyll biosynthesis (Richter *et al.*, 2016). Moreover, the cytosolic flavonoid biosynthesis pathway was identified as a target for the GUN signalling pathway (Richter *et al.*, 2020). Activation of flavonoid biosynthesis is a major trait of plants growing in high light or at low temperatures. It results in the accumulation of large amounts of anthocyanins, and is therefore crucial for plant acclimation. Although a lack of GUN1 provokes only very subtle phenotypes under normal growth conditions, the integrator function of GUN1 has mobilised many researchers to decipher its function, and an alleged breakthrough postulated the transcription factor ABSCISIC ACID-INSENSITIVE4 (ABI4) as an essential downstream component of GUN1-dependent signalling (Koussevitzky *et al.*, 2007). We teamed up with groups from the UK and Japan to demonstrate that ABI4 is not involved in this signalling pathway (Kacprzak *et al.*, 2019). Instead, using a guilt-by-association approach, we identified overexpressors of the Golden2-like transcription factors GLK1 and GLK2 as *gun* mutants (Leister and Kleine, 2016) (Figure 3). This is compatible with the finding that GLK1 is regulated antagonistically by retrograde and phytochrome signalling pathways (Martin *et al.*, 2016). Moreover, we demonstrated that GUN1 function becomes critical when chloroplast protein homeostasis (proteostasis) is perturbed by low temperatures (Table 2), decreased rates of protein synthesis or degradation of chloroplast proteins (Marino *et al.*, 2019).

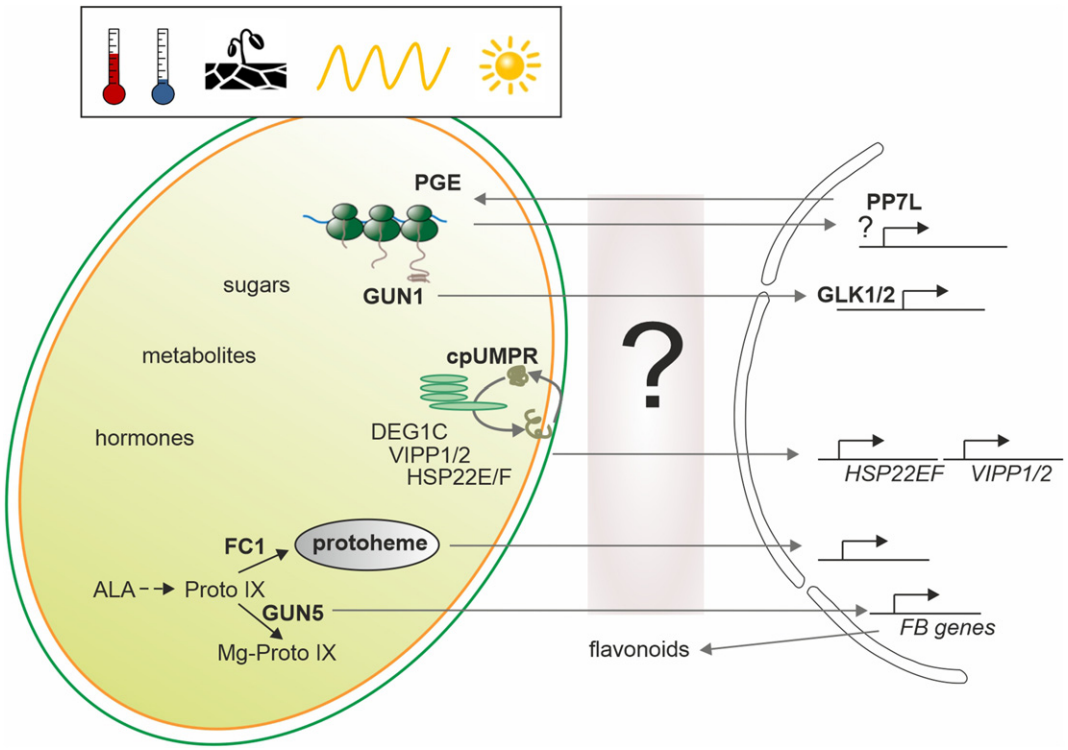

Figure 3. Overview of signalling pathways that are being investigated by the Green Hub consortium.
Temperature, drought, fluctuating light and high light levels (symbolised at the top) are at least partially sensed by the chloroplast (left side). Messenger molecules, the nature of which is largely elusive (symbolised by the question mark), convey information on the physiological state of the chloroplast to the nucleus, which results in altered nuclear gene expression.
ALA, 5-aminolevulinic acid; cpUMPR, chloroplast unfolded membrane protein response, DEG1C, DEGRADATION PROTEASE 1C; FB, flavonoid biosynthesis; FC1, FERROCHELATASE 1; GUN1, GENOMES UNCOUPLED 1; GLK1/2, GOLDEN2-LIKE 1/2; HSP22E/F, HEAT SHOCK PROTEIN E/F; PGE, plastid gene expression; PP7L, PROTEIN PHOSPHATASE 7; Proto IX, protoporphyrin IX; VIPP1/2, vesicle-inducing protein in plastids 1/2.

Other types of signalling

Recently published work indicates that retrograde signalling modulates nuclear gene expression not only by triggering differential transcript accumulation, but also by altering splicing patterns (Petrillo *et al.*, 2014), read-through events (Crisp *et al.*, 2018) and microRNA biogenesis (Fang *et al.*, 2019). Accordingly, our consortium has identified specific small RNAs (sRNAs) that respond to GUN signals (Habermann *et al.*, 2020) or exposure to cold (Tiwari *et al.*, 2020), and we have constructed a cold-specific miRNA and transcription factor-dependent gene regulatory network.

It turns out that biogenic and operational/acclimation pathways are intertwined and merge with established intracellular pathways, such as those involving MAP kinases (Vogel *et al.*, 2014) or light signalling pathways (Jiang *et al.*, 2019b). Indeed, characterisation of the *protein phosphatase 7-like* (*pp7l*) mutant, in which PGE is disturbed, strongly suggests an association of PP7L with light signalling pathways. Strikingly, PP7L is an extra-chloroplastic protein (Figure 3) and functions in chloroplast development and acclimation responses to various adverse growth conditions, including cold and high light (Xu *et al.*, 2019; Xu *et al.*, 2020b) (Table 2). Further investigations of the interplay between photoreceptor and retrograde signalling pathways have used *phyB-9* seedlings as a control. During these experiments, we and others detected a second mutation in the *phyB-9* line, which was mapped to *VENOSA4* (*VEN4*) (Yoshida *et al.*, 2018). A more detailed characterisation of the *ven4* mutant revealed that lack of VEN4 reduces chloroplast translational capacity (Xu *et al.*, 2020b). Moreover, VEN4, like PP7L, is a nuclear protein, and VEN4 is involved in cold and salt stress tolerance (Table 2).

Stressed Arabidopsis chloroplasts can also signal to the nucleus via the endoplasmic reticulum (ER) through the unfolded protein response (UPR) (Walley *et al.*, 2015), or the UPR-like response, during which chloroplast proteins engaged in protein quality control accumulate (Dogra *et al.*, 2019). We have recently unravelled a chloroplast unfolded membrane protein response in *C. reinhardtii* which involves chloroplast vesicle-inducing protein in plastids 1 (VIPP1) and VIPP2, together with the small heat shock proteins HSP22E and F (Theis *et al.*, 2020). In chloroplasts challenged with high light or H_2O_2, VIPP2, VIPP1

(Table 2) and HSP22E/F bind to chloroplast membranes presumably suffering from lipid packing stress caused by accumulation of misfolded proteins (Figure 3). At the same time, VIPP2 modulates a retrograde signal for the expression of the *HSP22E/F* genes, in which the cytosolic Mutant affected in chloroplast-to-nucleus retrograde signalling 1 (Mars1) kinase is involved (Perlaza *et al.*, 2019). The stromal protease DEG1C likely participates with VIPP1/2 and HSP22E/F in the removal of misfolded proteins from chloroplast membranes, as *deg1c* mutants accumulated the protease FtsH, ROS scavengers and other proteins involved in high light acclimation under ambient conditions (Theis *et al.*, 2019).

Signalling: outlook

In summary, the results obtained in the RA Signalling corroborate the current view that the various chloroplast signalling and acclimation pathways do not operate independently, but are at least partially intertwined, and might exploit modules of nucleocytoplasmic pathways. Consequently, during the second funding period we will extend our field of view to the nucleocytoplasmic compartment (Figure 3) – which is not only the target of chloroplast signals, but is also in constant communication with the chloroplast. Here, the integration of chloroplast-derived signals with other environmental signalling mechanisms is of special interest, as is the elucidation of the response networks emanating from the nucleus. Another intriguing question concerns the molecules that transmit plastid-derived signals through the cytosol to the nucleus. Based on studies with *gun* mutants, heme is one promising candidate (Terry and Smith, 2013). Heme is synthesised in plastids by two functionally distinct ferrochelatase isoforms, FC1 and FC2, and overexpression of FC1 results in a *gun* phenotype (Woodson *et al.*, 2011; Page *et al.*, 2020). Moreover, previous reports have revealed temperature-, osmosis- and light stress-induced increases in the expression and/or activity of FC1 (Singh *et al.*, 2002; Mohanty *et al.*, 2006; Nagai *et al.*, 2007; Scharfenberg *et al.*, 2015; Zhao *et al.*, 2017; Fan *et al.*, 2019). Indeed, FC1 (Zhao *et al.*, 2017; Fan *et al.*, 2019) and FC2 (Scharfenberg *et al.*, 2015) are involved in the response to salt stress (Table 2). Therefore, we will further investigate the possible contributions of FC1 and FC2 to other stress responses. Moreover, it will be interesting and challenging to determine the amounts of heme delivered to the cytosol and other cellular compartments, as well as the low levels of free and regulatory heme potentially needed for retrograde signalling.

With the aid of the expertise of the whole consortium, we will continue to identify and characterise signalling pathways and molecules, with the ultimate aim of understanding entire signalling networks that underpin acclimation. This will put us in a position, in the long term, to modify acclimation in a targeted manner.

MATHEMATICAL MODELLING OF ACCLIMATION (RA4) AND SYSTEMS BIOLOGY

Owing to its intricacy, the study of plant acclimation needs sophisticated theoretical and quantitative methods to reveal regulatory elements, network properties and relevant signalling cascades. The intertwined and nested structure of plant metabolism comprises regulatory elements like feedback inhibitory loops, feed-forward activating loops and control circuits that link chloroplast metabolism with signalling, gene expression and photosynthesis (Figure 4). The multidimensional character of high-throughput experimental data, together with this nested metabolic network structure, frequently prevents intuitive interpretation of biochemical and physiological processes (Schaber *et al.*, 2009). Hence, over the last decade, the development, application and optimisation of methods for data-driven exploration of biomolecular systems has become a fast-growing research area, which is frequently combined with omics approaches (see e.g. Töpfer *et al.*, 2013). Artificial intelligence routines and algorithms, together with methods from the fields of control theory and mathematical modelling, promise to reveal fundamental principles of metabolic regulation in biological networks (Zampieri *et al.*, 2019).

The groups devoted to the RA Computational Biology develop routines from the fields of bioinformatics, statistics and mathematical modelling to integrate high-dimensional functional data from the other three RAs. The aim is to formalise hypotheses relating to the nature, dynamics and internal logic of chloroplast-related acclimation processes so as to enable reliable prediction of its outcomes and regulatory key nodes. In a recent approach, the consortium systematically monitored the dynamics of transcript and metabolite levels during acclimation and de-acclimation to a changing temperature and light regime (Garcia-Molina *et al.*, 2020a). Dynamics were experimentally resolved across 11 different time points and a combination of surprisal and conditional network analyses identified ribosomes as conserved hubs for the control of acclimation and de-acclimation responses to changing abiotic factors. Statistical methods for enrichment analysis of gene ontologies enabled the identification of this central control hub. In a more specifically targeted experiment to identify modulators of acclimation to metal ion deficiency, the metabolite fumarate was identified as a modulator of acclimation (Table 2) (Garcia-Molina *et al.*, 2020b). Although useful for studying experimental large-scale data, theoretical concepts of enrichment analysis are typically based on a purely statistical context, which frequently complicates its biological interpretation. Therefore, we redesigned statistical enrichment analysis with a particular focus on its application to biological networks, and developed what we call Thermodynamically Motivated

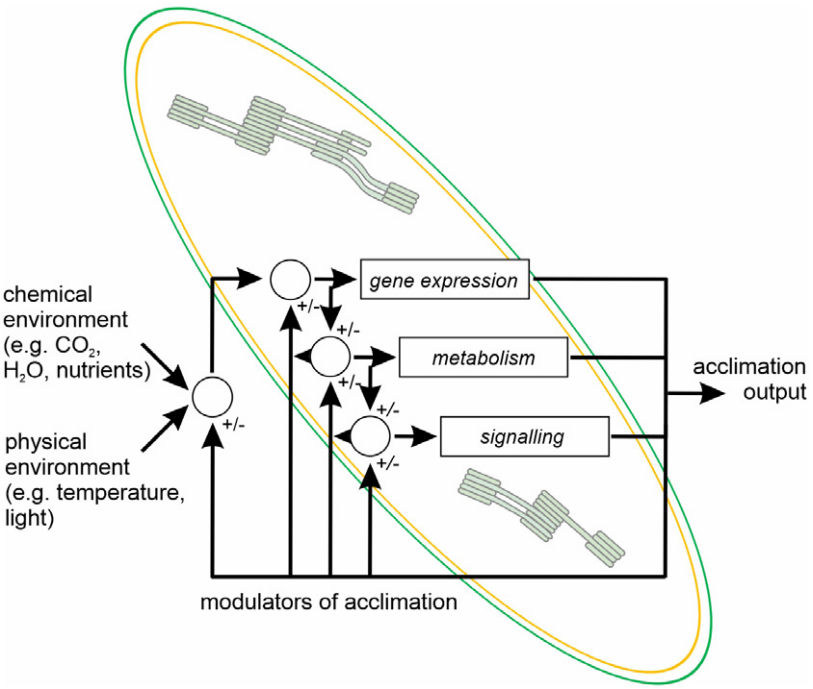

Figure 4. Chloroplast control circuits involved in plant acclimation.
Induced by dynamic alterations in the chemical and physical environment, modulators of acclimation affect gene expression, metabolism and signalling, which have mutual regulatory functions. Continuous signal integration and regulation modifies the observable acclimation output.

Enrichment Analysis, which accounts for energetic states and dynamics within a biological system (Schneider *et al.*, 2020). This methodology allows the unbiased functional description of thermodynamic constraints in a biochemical reaction network. This makes it possible to reduce the results of large-scale enrichment analysis to a set of biochemically feasible and physiologically most relevant terms.

In addition to its multidimensionality, the non-linearity of metabolic reprogramming during acclimation makes the interpretation of experimental findings and the development of predictive mathematical models of plant metabolism difficult (Fürtauer and Nägele, 2020). This non-linear system behaviour is attributable to non-linear enzyme kinetics and the interlinked circuits that control transcriptional, translational and metabolic processes. Further non-linearity arises from thermodynamic constraints on enzymes, metabolite transporters and transcription factors during acclimation to a changing temperature regime. Recently, we studied non-linear system behaviour in a biochemical network focusing on simple network motifs comprising linear and cyclic reaction sequences (Adler and Klipp, 2020). This analysis revealed reversible fluxes within a linear reaction sequence as a mechanism for temperature compensation within a biochemical reaction network. Furthermore, the design and simulation of a simple network indicated that branch points of metabolic networks represent potential control points, which redirect metabolic fluxes in a temperature-dependent manner and can also link pathways located in different subcellular compartments. We recently employed quantitative kinetic modelling to analyse invertase-driven hydrolytic sucrose cleavage, and found it to be differentially shunted between cytosol and vacuole during cold exposure of freezing-sensitive and -tolerant Arabidopsis accessions (Weiszmann *et al.*, 2018). Invertases are inhibited by their reaction products, i.e. glucose and fructose; thus, in order to study compartment-specific reaction rates, multiple kinetic parameters, e.g. substrate affinity (K_M) and inhibitory constants (K_i), must be estimated together with subcellular metabolite concentrations. Such time-dynamic regulatory interactions frequently prevent the intuitive prediction of system behaviour, and in our study a computational model was required for quantitative simulation, although the model comprised only eight reactions (Weiszmann *et al.*, 2018), which represents <1% of reactions of a genome-scale model of *A. thaliana*'s leaf metabolism (Mintz-Oron *et al.*, 2012). Thus, expanding metabolic networks to central primary metabolism and its interface with stress-induced secondary metabolism is an experimentally laborious and theoretically challenging task, which needs a tightly linked research platform to bring together analytical techniques and methodologies, both in theory and in practice. Based on the solid data basis on acclimation and its kinetics generated during the first funding period of our consortium, analysis of metabolism will now be expanded to the subcellular level. Kinetic modelling, together with network analysis and time-series statistics, will be applied to identify signalling cascades and quantify metabolic regulation of chloroplast metabolism, signalling and gene expression in plant acclimation. By combining experimental techniques for subcellular fractionation with omics

techniques, we will further deepen our understanding of the metabolic pathways, transport processes and signalling cascades that affect the chloroplast and connect it to the cytosol, nucleus and other compartments. Moreover, it will promote the integration into metabolic networks of information emerging from signalling cascades, derived by interaction network mapping (Habermann *et al.*, 2020). Integrating signalling processes into metabolic reprogramming is of particular interest for chloroplast-related research due to the significant impact of retrograde signalling on acclimation (Leister, 2019; Marino *et al.*, 2019) and adaptation (Zhao *et al.*, 2019). Finally, with its research-driven acquisition of contextualised data for plant biology and its close collaboration with the National Research Data Infrastructure (NFDI), the Green Hub consortium brings together multiple future-oriented interdisciplinary research areas.

ACCLIMATION MEETS ADAPTIVE LABORATORY EVOLUTION

While acclimation refers to short-term physiological adjustments activated by organisms in response to altered environments, adaptation refers to genetic changes that allow organisms to cope more efficiently with their environments by enhancing their evolutionary fitness. For flowering plants, acclimation and adaptation ensues on very different timescales, with acclimation encompassing up to weeks, but adaptation occurring over many generations and usually requiring between millennia and millions of years.

Microbes have short generation times, which allows one to follow adaptation in real-time (over weeks to months) in the laboratory, using ALE experiments. ALE has been used to introduce and optimise various traits into model microbes, like *Escherichia coli* or yeast, but this approach has not yet been extensively exploited for photoautotrophic microbes (reviewed in Leister, 2018). Clearly, the power and potential of the ALE approach in microbes is that it provides a concept that is in principle capable of coping with the speed of ongoing climate change, which outpaces the evolution rate of long-living flowering plants.

The model cyanobacterium *Synechocystis* is, like algae and plants, capable of oxygenic photosynthesis, but it is much more amenable to genetic engineering and its genome can be sequenced much faster. In fact, *Synechocystis* has been subjected to ALE experiments to enhance its tolerance against heat and to identify the underlying genetic changes (Tillich *et al.*, 2012; Tillich *et al.*, 2014). Other ALE experiments with *Synechocystis* have included its adaptation to low pH, increased butanol concentrations or the presence of toxic amino acids (reviewed in Leister, 2018). *Synechocystis* is a unicellular freshwater cyanobacterium that is typically cultivated at low light intensities (50 μmol photons m^{-2} s^{-1}), and ALE experiments in our consortium are intended to generate strains capable of growing in extreme light conditions.

A more ambitious target organism for ALE experiments on photosynthetic functions is the green alga *C. reinhardtii*, which is technically more challenging because of its complex eukaryotic genome. Nevertheless, its closer relatedness to plants (compared to cyanobacteria) makes it the optimal ALE system for identifying mutations in proteins that might also be functional in flowering plants. Proof-of-principle for the utility of *C. reinhardtii* in ALE experiments with relevance for acclimation has been provided by the identification of a mutation in this species that is associated with enhanced tolerance to high light (Schierenbeck *et al.*, 2015).

Moreover, relative to the fundamental concept in our consortium – first identify key factors by system-wide analyses, then modify key factors and check for potentially beneficial effects on acclimation – this process is reversed in ALE approaches. During ALE, the desired trait is generated first and subsequently the genetic alteration responsible is identified. A further advantage is that the ALE approach can identify amino acid substitutions with high functional impact, which might represent dominant mutations. This is highly promising and complementary to the classical approaches which usually only alter gene dosage by altering their expression through knockout or overexpression. Whether ALE-identified protein changes in cyanobacteria like *Synechocystis* or green algae like *C. reinhardtii* that confer adaptation can also enhance acclimation in flowering plants – and especially crops – remains to be seen and makes ALE a high-risk/high-gain approach for enhancing acclimation in plants.

CONCLUDING REMARKS: THE BIOTECHNOLOGICAL OUTLOOK

Second-generation transgenic crops with enhanced capacity to acclimate to adverse environmental conditions – especially drought – might provide the backbone for a 'Second Green Revolution' (Chan *et al.*, 2020). However, lines with a demonstrably improved acclimation capacity under field conditions are virtually absent from the scientific literature (Araus *et al.*, 2019), largely because candidates tested in model organisms under laboratory conditions have failed to live up to their promise ('lab-to-field mishaps') (Chan *et al.*, 2020). For instance, overexpression of three photoprotective proteins violaxanthin de-epoxidase, PsbS and the zeaxanthin epoxidase in parallel in the so-called VPZ lines has been reported to result in increased photoprotection and biomass accumulation in tobacco (Kromdijk *et al.*, 2016), but this positive growth effect could not be reproduced in *A. thaliana* by our consortium (Garcia-Molina and Leister, 2020). On the contrary, the Arabidopsis VPZ lines underperformed relative to wild-type plants under conditions of illumination that mimicked

natural light (Garcia-Molina and Leister, 2020), which implies that interspecific diversity of acclimation mechanisms and their interplay with other cellular functions might well prevent the general use of this particular set of modifications in (crop) plants. This finding underlines the need to test such approaches in 'real' crops. Quantifying biomass accumulation in field-grown tobacco plants cannot substitute for a rigorous study of transgenic food crop plants. For this reason, our consortium has now included *C. sativa* in its experimental pipeline as a model crop in which to test the effects of the most promising modulators.

One instance of an apparent 'lab-to-field success' is a transgenic wheat (*Triticum aestivum L.*) that expresses the sunflower (*Helianthus annuus*) transcription factor HaHB4, which clearly outstrips the wild type in terms of yield in the field (Gonzalez *et al.*, 2019). Because overexpression of HaHB4 confers drought tolerance not only in wheat but also in Arabidopsis (Dezar *et al.*, 2005), this example highlights several issues that are important for our consortium's strategy. First, single factors can indeed enhance acclimation, which corroborates our 'modulator' concept. Second, these factors also function in model plants like Arabidopsis, our current workhorse plant. Third, how overexpression of HaHB4 enhances crop yield remains unclear (Gonzalez *et al.*, 2019) and the question requires further in-depth studies, for which a model species like Arabidopsis is well suited. This situation is reminiscent of the original Green Revolution in the 1960s and 1970s, insofar as the molecular mechanisms of the decisive mutations involved were characterised only decades later (e.g. Peng *et al.*, 1999).

Consequently, searching for key players in acclimation (our 'modulators') and testing their efficacy in a model crop like *C. sativa* is a promising approach to the discovery of additional candidates for 'lab-to-field successes'. Our ALE approach is clearly riskier, given the evolutionary distance between the photosynthetic microbes used in this approach and the ultimate target – crop plants. But because the ALE approach might identify adaptations that are attributable to amino acid substitutions in key proteins rather than changes in the dosage of endogenous genes (which was the starting point for the HaHB4 approach), results obtained by ALE might identify very different starting points for enhancement of acclimation compared to conventional approaches.

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AUTHOR CONTRIBUTIONS

All authors contributed to the writing of this manuscript. Writing was coordinated by TK, CSL, TN, EN and DL.

CONFLICT OF INTEREST

The authors of this manuscript claim no conflict of interest.

REFERENCES

Adler, S.O. and Klipp, E. (2020) Chemical reaction networks possess intrinsic, temperature-dependent functionality. *Entropy*, **22**, 117.

Ahmad, P., Rasool, S., Gul, A., Sheikh, S.A., Akram, N.A., Ashraf, M., Kazi, A.M. and Gucel, S. (2016) Jasmonates: multifunctional roles in stress tolerance. *Front. Plant Sci.* **7**, 813.

Araus, J.L., Serret, M.D. and Lopes, M.S. (2019) Transgenic solutions to increase yield and stability in wheat: shining hope or flash in the pan? *J. Exp. Bot.* **70**, 1419–1424.

Bansal, S. and Durrett, T.P. (2016) *Camelina sativa*: an ideal platform for the metabolic engineering and field production of industrial lipids. *Biochimie*, **120**, 9–16.

Barkan, A. (2011) Expression of plastid genes: organelle-specific elaborations on a prokaryotic scaffold. *Plant Physiol.* **155**, 1520–1532.

Barkan, A. and Small, I. (2014) Pentatricopeptide repeat proteins in plants. *Annu. Rev. Plant Biol.* **65**, 415–442.

Barnes, D. and Mayfield, S.P. (2003) Redox control of posttranscriptional processes in the chloroplast. *Antioxid Redox Signal.* **5**, 89–94.

Bohne, A.V. and Nickelsen, J. (2017) Metabolic control of chloroplast gene expression: an emerging theme. *Mol. Plant*, **10**, 1–3.

Bohne, A.V., Schwarz, C., Schottkowski, M., Lidschreiber, M., Piotrowski, M., Zerges, W. and Nickelsen, J. (2013) Reciprocal regulation of protein synthesis and carbon metabolism for thylakoid membrane biogenesis. *PLoS Biol.* **11**, e1001482.

Bölter, B. and Soll, J. (2016) Once upon a time – chloroplast protein import research from infancy to future challenges. *Mol. Plant*, **9**, 798–812.

Bräutigam, K., Dietzel, L., Kleine, T. *et al.* (2009) Dynamic plastid redox signals integrate gene expression and metabolism to induce distinct metabolic states in photosynthetic acclimation in Arabidopsis. *Plant Cell*, **21**, 2715–2732.

Castandet, B., Hotto, A.M., Strickler, S.R. and Stern, D.B. (2016) ChloroSeq, an optimized chloroplast RNA-Seq bioinformatic pipeline, reveals remodeling of the organellar transcriptome under heat stress. *G3: Genes - Genom – Genet.* **6**, 2817–2827.

Chan, R.L., Trucco, F. and Otegui, M.E. (2020) Why are second-generation transgenic crops not yet available in the market? *Journal of Experimental Botany*, **71**, 6876–6880.

Cho, W.K., Geimer, S. and Meurer, J. (2009) Cluster analysis and comparison of various chloroplast transcriptomes and genes in *Arabidopsis thaliana*. *DNA Res.* **16**, 31–44.

Crisp, P.A., Smith, A.B., Ganguly, D.R., Murray, K.D., Eichten, S.R., Millar, A.A. and Pogson, B.J. (2018) RNA polymerase II read-through promotes expression of neighboring genes in SAL1-PAP-XRN retrograde signaling. *Plant Physiol.* **178**, 1614–1630.

Crosatti, C., Rizza, F., Badeck, F.W., Mazzucotelli, E. and Cattivelli, L. (2013) Harden the chloroplast to protect the plant. *Physiol. Plant*, **147**, 55–63.

DalCorso, G., Pesaresi, P., Masiero, S., Aseeva, E., Schünemann, D., Finazzi, G., Joliot, P., Barbato, R. and Leister, D. (2008) A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in Arabidopsis. *Cell*, **132**, 273–285.

Dann, M. and Leister, D. (2019) Evidence that cyanobacterial Sll1217 functions analogously to PGRL1 in enhancing PGR5-dependent cyclic electron flow. *Nat. Commun.* **10**, 5299.

de Souza Chaves, I., Feitosa-Araújo, E., Florian, A. *et al.* (2019) The mitochondrial NAD^+ transporter (NDT1) plays important roles in cellular NAD^+ homeostasis in *Arabidopsis thaliana*. *Plant J.* **100**, 487–504.

Demmig-Adams, B., Stewart, J.J. and Adams 3rd, W.W. (2014) Multiple feedbacks between chloroplast and whole plant in the context of plant adaptation and acclimation to the environment. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **369**, 20130244.

Deng, X.W., Tonkyn, J.C., Peter, G.F., Thornber, J.P. and Gruissem, W. (1989) Post-transcriptional control of plastid mRNA accumulation during adaptation of chloroplasts to different light quality environments. *Plant Cell*, **1**, 645–654.

Dezar, C.A., Gago, G.M., Gonzalez, D.H. and Chan, R.L. (2005) Hahb-4, a sunflower homeobox-leucine zipper gene, is a developmental regulator

and confers drought tolerance to *Arabidopsis thaliana* plants. *Transgenic Res.* **14**, 429–440.

Dogra, V., Duan, J., Lee, K.P. and Kim, C. (2019) Impaired PSII proteostasis triggers a UPR-like response in the var2 mutant of Arabidopsis. *J. Exp. Bot.* **70**, 3075–3088.

Eberhard, S., Drapier, D. and Wollman, F. (2002) Searching limiting steps in the expression of chloroplast-encoded proteins: relations between gene copy number, transcription, transcript abundance and translation rate in the chloroplast of *Chlamydomonas reinhardtii*. *Plant J.* **31**, 149–160.

Eisa, A., Bölter, B. and Schwenkert, S. (2019a) The ACT domain in chloroplast precursor-phosphorylating STY kinases binds metabolites and allosterically regulates kinase activity. *J. Biol. Chem.* **294**, 17278–17288.

Eisa, A., Malenica, K., Schwenkert, S. and Bölter, B. (2019b) High light acclimation induces chloroplast precursor phosphorylation and reduces import efficiency. *Plants*, **9**, 24.

Exposito-Rodriguez, M., Laissue, P.P., Yvon-Durocher, G., Smirnoff, N. and Mullineaux, P.M. (2017) Photosynthesis-dependent H_2O_2 transfer from chloroplasts to nuclei provides a high-light signalling mechanism. *Nat. Commun.* **8**, 49.

Fan, T., Roling, L., Meiers, A., Brings, L., Ortega-Rodes, P., Hedtke, B. and Grimm, B. (2019) Complementation studies of the Arabidopsis *fc1* mutant substantiate essential functions of ferrochelatase 1 during embryogenesis and salt stress. *Plant Cell Environ.* **42**, 618–632.

Fang, X., Zhao, G., Zhang, S., Li, Y., Gu, H., Li, Y., Zhao, Q. and Qi, Y. (2019) Chloroplast-to-nucleus signaling regulates microRNA biogenesis in Arabidopsis. *Dev. Cell*, **48**, 371–382.

Feitosa-Araujo, E., de Souza Chaves, I., Florian, A. *et al.* (2020) Downregulation of a mitochondrial NAD^+ transporter (NDT2) alters seed production and germination in Arabidopsis. *Plant Cell Physiol.* **61**, 897–908.

Finkemeier, I. and Leister, D. (2010) *Plant Chloroplasts and Other Plastids*. Hoboken, NJ: John Wiley & Sons. https://onlinelibrary.wiley.com/doi/abs/10.1002/9780470015902.a0001678.pub2.

Fleischmann, T.T., Scharff, L.B., Alkatib, S., Hasdorf, S., Schottler, M.A. and Bock, R. (2011) Nonessential plastid-encoded ribosomal proteins in tobacco: a developmental role for plastid translation and implications for reductive genome evolution. *Plant Cell*, **23**, 3137–3155.

Foyer, C.H. (2018) Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. *Environ. Exp. Bot.* **154**, 134–142.

Fuentes, P., Armarego-Marriott, T. and Bock, R. (2018) Plastid transformation and its application in metabolic engineering. *Curr. Opin. Biotechnol.* **49**, 10–15.

Fürtauer, L. and Nägele, T. (2020) Mathematical modeling of plant metabolism in a changing temperature regime. In *Plant Cold Acclimation: Methods and Protocols*. (Hincha, D.K. and Zuther, E., eds). New York, NY: Springer US, pp. 277–287.

Garcia-Molina, A., Kleine, T., Schneider, K., Mühlhaus, T., Lehmann, M. and Leister, D. (2020a) Translational components contribute to acclimation responses to high light, heat, and cold in Arabidopsis. *iScience*, **23**(7), 101331.

Garcia-Molina, A. and Leister, D. (2020) Accelerated relaxation of photoprotection impairs biomass accumulation in Arabidopsis. *Nat. Plants*, **6**, 9–12.

Garcia-Molina, A., Marino, G., Lehmann, M. and Leister, D. (2020b) Systems biology of responses to simultaneous copper and iron deficiency in Arabidopsis. *Plant J.* **103**(6), 2119–2138.

Geigenberger, P. and Stitt, M. (1991) Regulation of carbon partitioning between sucrose and nitrogen assimilation in cotyledons of germinating *Ricinus communis* L. seedlings. *Planta*, **185**, 563–568.

Geigenberger, P., Thormählen, I., Daloso, D.M. and Fernie, A.R. (2017) The unprecedented versatility of the plant thioredoxin system. *Trends Plant Sci.* **22**, 249–262.

Germain, A., Hotto, A.M., Barkan, A. and Stern, D.B. (2013) RNA processing and decay in plastids. *Wiley Interdiscip. Rev. RNA*, **4**, 295–316.

Gonzalez, F.G., Capella, M., Ribichich, K.F., Curin, F., Giacomelli, J.I., Ayala, F., Watson, G., Otegui, M.E. and Chan, R.L. (2019) Field-grown transgenic wheat expressing the sunflower gene HaHB4 significantly outyields the wild type. *J. Exp. Bot.* **70**, 1669–1681.

Gruissem, W., Barkan, A., Deng, X.W. and Stern, D. (1988) Transcriptional and post-transcriptional control of plastid mRNA levels in higher plants. *Trends Genet.* **4**, 258–263.

Guan, L., Denkert, N., Eisa, A., Lehmann, M., Sjuts, I., Weiberg, A., Soll, J., Meinecke, M. and Schwenkert, S. (2019) JASSY, a chloroplast outer membrane protein required for jasmonate biosynthesis. *Proc. Natl. Acad. Sci.* **116**, 10568–10575.

Habermann, K., Tiwari, B., Krantz, M., Adler, S.O., Klipp, E., Arif, M.A. and Frank, W. (2020) Identification of small non-coding RNAs responsive to GUN1 and GUN5 related retrograde signals in *Arabidopsis thaliana*. *Plant J.* **104**, 138–155.

Havaux, M. (2020) Plastoquinone in and beyond photosynthesis. *Trends Plant Sci.* **25**, 1252–1265.

Hebbelmann, I., Selinski, J., Wehmeyer, C. *et al.* (2012) Multiple strategies to prevent oxidative stress in Arabidopsis plants lacking the malate valve enzyme NADP-malate dehydrogenase. *J. Exp. Bot.* **63**, 1445–1459.

Hedrich, R., Sauer, N. and Neuhaus, H.E. (2015) Sugar transport across the plant vacuolar membrane: nature and regulation of carrier proteins. *Curr. Opin. Plant Biol.* **25**, 63–70.

Hemme, D., Veyel, D., Muhlhaus, T. *et al.* (2014) Systems-wide analysis of acclimation responses to long-term heat stress and recovery in the photosynthetic model organism *Chlamydomonas reinhardtii*. *Plant Cell*, **26**, 4270–4297.

Heyno, E., Innocenti, G., Lemaire, S.D., Issakidis-Bourguet, E. and Krieger-Liszkay, A. (2014) Putative role of the malate valve enzyme NADP-malate dehydrogenase in H_2O_2 signalling in Arabidopsis. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **369**, 20130228.

Hou, L.-Y., Ehrlich, M., Thormählen, I., Lehmann, M., Krahnert, I., Obata, T., Cejudo, F.J., Fernie, A.R. and Geigenberger, P. (2019) NTRC plays a crucial role in starch metabolism, redox balance, and tomato fruit growth. *Plant Physiol.* **181**, 976–992.

Hu, Y., Jiang, L., Wang, F. and Yu, D. (2013) Jasmonate regulates the inducer of cbf expression-C-repeat binding factor/DRE binding factor1 cascade and freezing tolerance in Arabidopsis. *Plant Cell*, **25**, 2907–2924.

Hüner, N.P.A., Dahal, K., Bode, R., Kurepin, L.V. and Ivanov, A.G. (2016) Photosynthetic acclimation, vernalization, crop productivity and 'the grand design of photosynthesis'. *J. Plant Physiol.* **203**, 29–43.

Jiang, J., Chai, X., Manavski, N. *et al.* (2019a) An RNA chaperone-like protein plays critical roles in chloroplast mRNA stability and translation in Arabidopsis and maize. *Plant Cell*, **31**, 1308–1327.

Jiang, J., Zeng, L., Ke, H., De La Cruz, B. and Dehesh, K. (2019b) Orthogonal regulation of phytochrome B abundance by stress-specific plastidial retrograde signaling metabolite. *Nat Commun*, **10**, 2904.

Kacprzak, S.M., Mochizuki, N., Naranjo, B., Xu, D., Leister, D., Kleine, T., Okamoto, H. and Terry, M.J. (2019) Plastid-to-nucleus retrograde signalling during chloroplast biogenesis does not require ABI4. *Plant Physiol.* **179**, 18–23.

Klaff, P. and Gruissem, W. (1991) Changes in chloroplast mRNA stability during leaf development. *Plant Cell*, **3**, 517–529.

Kleine, T. and Leister, D. (2016) Retrograde signaling: organelles go networking. *Biochim. Biophys. Acta*, **1857**, 1313–1325.

Knuesting, J. and Scheibe, R. (2018) Small molecules govern thiol redox switches. *Trends Plant Sci.* **23**, 769–782.

Koussevitzky, S., Nott, A., Mockler, T.C., Hong, F., Sachetto-Martins, G., Surpin, M., Lim, J., Mittler, R. and Chory, J. (2007) Signals from chloroplasts converge to regulate nuclear gene expression. *Science*, **316**, 715–719.

Kromdijk, J., Glowacka, K., Leonelli, L., Gabilly, S.T., Iwai, M., Niyogi, K.K. and Long, S.P. (2016) Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science*, **354**, 857–861.

Kupsch, C., Ruwe, H., Gusewski, S., Tillich, M., Small, I. and Schmitz-Linneweber, C. (2012) Arabidopsis chloroplast RNA binding proteins CP31A and CP29A associate with large transcript pools and confer cold stress tolerance by influencing multiple chloroplast RNA processing steps. *Plant Cell*, **10**, 4266–4280.

Lamberti, G., Gügel, I.L., Meurer, J., Soll, J. and Schwenkert, S. (2011) The cytosolic kinases STY8, STY17, and STY46 are involved in chloroplast differentiation in Arabidopsis. *Plant Physiol.* **157**, 70–85.

Lee, K., Lehmann, M., Paul, M.V., Wang, L., Luckner, M., Wanner, G., Geigenberger, P., Leister, D. and Kleine, T. (2020) Lack of FIBRILLIN6 in *Arabidopsis thaliana* affects light acclimation and sulfate metabolism. *New Phytol.* **225**, 1715–1731.

Leister, D. (2018) Experimental evolution in photoautotrophic microorganisms as a means of enhancing chloroplast functions. *Essays Biochem.* **62**, 77–84.

Leister, D. (2019) Piecing the puzzle together: the central role of reactive oxygen species and redox hubs in chloroplast retrograde signaling. *Antioxid. Redox Signal.* **30**, 1206–1219.

Leister, D. and Kleine, T. (2016) Definition of a core module for the nuclear retrograde response to altered organellar gene expression identifies GLK overexpressors as gun mutants. *Physiol. Plant*, **157**, 297–309.

Lenzen, B., Ruhle, T., Lehniger, M.K., Okuzaki, A., Labs, M., Muino, J.M., Ohler, U., Leister, D. and Schmitz-Linneweber, C. (2020) The chloroplast RNA binding protein CP31A has a preference for mRNAs encoding the subunits of the chloroplast NAD(P)H dehydrogenase complex and is required for their accumulation. *Int. J. Mol. Sci.* **21**, 5633.

Liebthal, M., Maynard, D. and Dietz, K.J. (2018) Peroxiredoxins and redox signaling in plants. *Antioxid. Redox Signal.* **28**, 609–624.

Malik, M.R., Tang, J., Sharma, N., Burkitt, C., Ji, Y., Mykytyshyn, M., Bohmert-Tatarev, K., Peoples, O. and Snell, K.D. (2018) Camelina sativa, an oilseed at the nexus between model system and commercial crop. *Plant Cell Rep.* **37**, 1367–1381.

Manavski, N., Schmid, L.M. and Meurer, J. (2018) RNA-stabilization factors in chloroplasts of vascular plants. *Essays Biochem.* **62**, 51–64.

Manavski, N., Torabi, S., Lezhneva, L., Arif, M.A., Frank, W. and Meurer, J. (2015) HIGH CHLOROPHYLL FLUORESCENCE145 binds to and stabilizes the *psaA* 5′ UTR via a newly defined repeat motif in embryophyta. *Plant Cell*, **27**, 2600–2615.

Marín-Navarro, J., Manuell, A.L., Wu, J. and Mayfield, S.P. (2007) Chloroplast translation regulation. *Photosynth. Res.* **94**, 359–374.

Marino, G., Naranjo, B., Wang, J., Penzler, J.F., Kleine, T. and Leister, D. (2019) Relationship of GUN1 to FUG1 in chloroplast protein homeostasis. *Plant J.*, **99**, 521–535.

Martin, G., Leivar, P., Ludevid, D., Tepperman, J.M., Quail, P.H. and Monte, E. (2016) Phytochrome and retrograde signalling pathways converge to antagonistically regulate a light-induced transcriptional network. *Nat. Commun.* **7**, 11431.

McCormick, A.J. and Kruger, N.J. (2015) Lack of fructose 2,6-bisphosphate compromises photosynthesis and growth in Arabidopsis in fluctuating environments. *Plant J.* **81**, 670–683.

Mentzen, W.I. and Wurtele, E.S. (2008) Regulon organization of Arabidopsis. *BMC Plant Biol.* **8**, 99.

Mettler, T., Muhlhaus, T., Hemme, D. *et al.* (2014) Systems analysis of the response of photosynthesis, metabolism, and growth to an increase in irradiance in the photosynthetic model organism *Chlamydomonas reinhardtii*. *Plant Cell*, **26**, 2310–2350.

Mintz-Oron, S., Meir, S., Malitsky, S., Ruppin, E., Aharoni, A. and Shlomi, T. (2012) Reconstruction of Arabidopsis metabolic network models accounting for subcellular compartmentalization and tissue-specificity. *Proc. Natl. Acad. Sci USA*, **109**, 339–344.

Mohanty, S., Grimm, B. and Tripathy, B.C. (2006) Light and dark modulation of chlorophyll biosynthetic genes in response to temperature. *Planta*, **224**, 692–699.

Möhlmann, T., Scheibe, R. and Neuhaus, H.E. (1994) Interaction between fatty-acid and starch synthesis in isolated amyloplasts from cauliflower floral buds. *Planta*, **194**, 492–497.

Möhlmann, T., Steinebrunner, I. and Neuhaus, H.E. (2014) Nucleotides and nucleosides: transport, metabolism and signaling function of extracellular ATP. In *Prog. Bot. 75* (Lüttge, U., Beyschlag, W. and Cushman, J.C. eds). Heidelberg, Germany: Springer, pp. 119–144.

Morales, A. and Kaiser, E. (2020) Photosynthetic acclimation to fluctuating irradiance in plants. *Front. Plant. Sci.* **11**, 268.

Nagai, S., Koide, M., Takahashi, S., Kikuta, A., Aono, M., Sasaki-Sekimoto, Y., Ohta, H., Takamiya, K. and Masuda, T. (2007) Induction of isoforms of tetrapyrrole biosynthetic enzymes, AtHEMA2 and AtFC1, under stress conditions and their physiological functions in Arabidopsis. *Plant Physiol.* **144**, 1039–1051.

Nägele, T. and Heyer, A.G. (2013) Approximating subcellular organisation of carbohydrate metabolism during cold acclimation in different natural accessions of *Arabidopsis thaliana*. *New Phytol.* **198**, 777–787.

Nagler, M., Nukarinen, E., Weckwerth, W. and Nägele, T. (2015) Integrative molecular profiling indicates a central role of transitory starch breakdown in establishing a stable C/N homeostasis during cold acclimation in two natural accessions of *Arabidopsis thaliana*. *BMC Plant Biol.* **15**, 284.

Nawaz, G. and Kang, H. (2017) Chloroplast- or mitochondria-targeted DEAD-box RNA helicases play essential roles in organellar RNA metabolism and abiotic stress responses. *Front. Plant. Sci.* **8**, 871.

Nickelsen, J., Bohne, A. and Westhoff, P. (2014) Chloroplast gene expression – translation. In *Plastid Biology, Advances in Plant Biology 5*. (Theg, S.M. and Wollman, F.-A., eds). New York: Springer Sciences, pp. 49–78.

Niittylä, T., Messerli, G., Trevisan, M., Chen, J., Smith, A.M. and Zeeman, S.C. (2004) A previously unknown maltose transporter essential for starch degradation in leaves. *Science*, **303**, 87–89.

Nordhues, A., Schottler, M.A., Unger, A.K. *et al.* (2012) Evidence for a role of VIPP1 in the structural organization of the photosynthetic apparatus in Chlamydomonas. *Plant Cell*, **24**, 637–659.

Nunes-Nesi, A., Carrari, F., Lytovchenko, A., Smith, A.M., Loureiro, M.E., Ratcliffe, R.G., Sweetlove, L.J. and Fernie, A.R. (2005) Enhanced photosynthetic performance and growth as a consequence of decreasing mitochondrial malate dehydrogenase activity in transgenic tomato plants. *Plant Physiol.* **137**, 611–622.

Obata, T., Klemens, P.A.W., Rosado-Souza, L. *et al.* (2020) Metabolic profiles of six African cultivars of cassava (*Manihot esculenta* Crantz) highlight bottlenecks of root yield. *Plant J.* **102**, 1202–1219.

Ohler, L., Niopek-Witz, S., Mainguet, S.E. and Möhlmann, T. (2019) Pyrimidine salvage: physiological functions and interaction with chloroplast biogenesis. *Plant Physiol.*, **180**, 1816–1828.

Ojeda, V., Pérez-Ruiz, J.M., González, M., Nájera, V.A., Sahrawy, M., Serrato, A.J., Geigenberger, P. and Cejudo, F.J. (2017) NADPH thioredoxin reductase C and thioredoxins act concertedly in seedling development. *Plant Physiol.*, **174**, 1436–1448.

Page, M.T., Garcia-Becerra, T., Smith, A.G. and Terry, M.J. (2020) Overexpression of chloroplast-targeted ferrochelatase 1 results in a genomes uncoupled chloroplast-to-nucleus retrograde signalling phenotype. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **375**, 20190401.

Paieri, F., Tadini, L., Manavski, N., Kleine, T., Ferrari, R., Morandini, P., Pesaresi, P., Meurer, J. and Leister, D. (2018) The DEAD-box RNA Helicase RH50 Is a 23S–4.5S rRNA maturation factor that functionally overlaps with the plastid signaling factor GUN1. *Plant Physiol.* **176**, 634–648.

Palmieri, F., Rieder, B., Ventrella, A. *et al.* (2009) Molecular identification and functional characterization of *Arabidopsis thaliana* mitochondrial and chloroplastic NAD^+ carrier proteins. *J. Biol. Chem.* **284**, 31249–31259.

Patzke, K., Prananingrum, P., Klemens, P.A.W. *et al.* (2019) The plastidic sugar transporter pSuT influences flowering and affects cold responses. *Plant Physiol.* **179**, 569–587.

Peng, J., Richards, D.E., Hartley, N.M. *et al.* (1999) 'Green revolution' genes encode mutant gibberellin response modulators. *Nature*, **400**, 256–261.

Perlaza, K., Toutkoushian, H., Boone, M., Lam, M., Iwai, M., Jonikas, M.C., Walter, P. and Ramundo, S. (2019) The Mars1 kinase confers photoprotection through signaling in the chloroplast unfolded protein response. *Elife*, **8**, e49577.

Petrillo, E., Godoy Herz, M.A., Fuchs, A. *et al.* (2014) A chloroplast retrograde signal regulates nuclear alternative splicing. *Science*, **344**, 427–430.

Pfannschmidt, T., Nilsson, A., Tullberg, A. and Allen, J.F. (1999) Photosynthetic control of chloroplast gene expression. *Nature*, **397**, 625–628.

Pfannschmidt, T. and Yang, C. (2012) The hidden function of photosynthesis: a sensing system for environmental conditions that regulates plant acclimation responses. *Protoplasma*, **249**(Suppl 2), S125–S136.

Pogson, B.J., Woo, N.S., Forster, B. and Small, I.D. (2008) Plastid signalling to the nucleus and beyond. *Trends Plant Sci.* **13**, 602–609.

Pommerrenig, B., Ludewig, F., Cvetkovic, J., Trentmann, O., Klemens, P.A.W. and Neuhaus, H.E. (2018) In concert: orchestrated changes in carbohydrate hmeostasis are critical for plant abiotic stress tolerance. *Plant Cell Physiol.* **59**, 1290–1299.

Pottosin, I. and Shabala, S. (2016) Transport across chloroplast membranes: optimizing photosynthesis for adverse environmental conditions. *Mol. Plant*, **9**, 356–370.

Prikryl, J., Rojas, M., Schuster, G. and Barkan, A. (2011) Mechanism of RNA stabilization and translational activation by a pentatricopeptide repeat protein. *Proc. Natl. Acad. Sci. USA*, **108**, 415–420.

Pulido, P., Zagari, N., Manavski, N., Gawronski, P., Matthes, A., Scharff, L.B., Meurer, J. and Leister, D. (2018) CHLOROPLAST RIBOSOME

ASSOCIATED supports translation under stress and interacts with the ribosomal 30S subunit. *Plant Physiol.* **177**, 1539–1554.

Reinhold, T., Alawady, A., Grimm, B. *et al.* (2007) Limitation of nocturnal import of ATP into Arabidopsis chloroplasts leads to photooxidative damage. *Plant J.* **50**, 293–304.

Richter, A.S., Hochheuser, C., Fufezan, C., Heinze, L., Kuhnert, F. and Grimm, B. (2016) Phosphorylation of GENOMES UNCOUPLED 4 alters stimulation of Mg chelatase activity in angiosperms. *Plant Physiol.* **172**, 1578–1595.

Richter, A.S., Tohge, T., Fernie, A.R. and Grimm, B. (2020) The genomes uncoupled-dependent signalling pathway coordinates plastid biogenesis with the synthesis of anthocyanins. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **375**, 20190403.

Rieder, B. and Neuhaus, H.E. (2011) Identification of an Arabidopsis plasma membrane located ATP transporter important for anther development. *Plant Cell*, **23**, 1932–1944.

Ries, F., Herkt, C. and Willmund, F. (2020) Co-translational protein folding and sorting in chloroplasts. *Plants (Basel)*, **9**, 214.

Rohr, M., Ries, F., Herkt, C. *et al.* (2019) The role of plastidic trigger factor serving protein biogenesis in green algae and land plants. *Plant Physiol.*, **179**, 1093–1110.

Rojas, M., Ruwe, H., Miranda, R.G., Zoschke, R., Hase, N., Schmitz-Linneweber, C. and Barkan, A. (2018) Unexpected functional versatility of the pentatricopeptide repeat proteins PGR3, PPR5 and PPR10. *Nucleic Acids Res.*, **46**, 10448–10459.

Rosado-Souza, L., Fernie, A.R. and Aarabi, F. (2020) Ascorbate and thiamin: metabolic modulators in plant acclimation responses. *Plants*, **9**, 101.

Rosado-Souza, L., Proost, S., Moulin, M., Bergmann, S., Bocobza, S.E., Aharoni, A., Fitzpatrick, T.B., Mutwil, M., Fernie, A.R. and Obata, T. (2019) Appropriate thiamin pyrophosphate levels are required for acclimation to changes in photoperiod. *Plant Physiol.* **180**, 185–197.

Roux, S.J. and Steinebrunner, I. (2007) Extracellular ATP: an unexpected role as a signaler in plants. *Trends Plant Sci.* **12**, 522–527.

Schaber, J., Liebermeister, W. and Klipp, E. (2009) Nested uncertainties in biochemical models. *IET Syst. Biol.*, **3**, 1–9.

Scharfenberg, M., Mittermayr, L., Von Roepenack-Lahaye, E., Schlicke, H., Grimm, B., Leister, D. and Kleine, T. (2015) Functional characterization of the two ferrochelatases in *Arabidopsis thaliana*. *Plant Cell Environ.* **38**, 280–298.

Schierenbeck, L., Ries, D., Rogge, K., Grewe, S., Weisshaar, B. and Kruse, O. (2015) Fast forward genetics to identify mutations causing a high light tolerant phenotype in Chlamydomonas reinhardtii by whole-genome-sequencing. *BMC Genom.* **16**, 57.

Schmid, L.-M., Ohler, L., Möhlmann, T., Brachmann, A., Muiño, J.M., Leister, D., Meurer, J. and Manavski, N. (2019) PUMPKIN, the sole plastid UMP kinase, associates with group II introns and alters their metabolism. *Plant Physiol.* **179**, 248–264.

Schneider, K., Venn, B. and Mühlhaus, T. (2020) TMEA: a thermodynamically motivated framework for functional characterization of biological responses to system acclimation. *Entropy*, **22**, 1030.

Schuster, M., Gao, Y., Schottler, M.A., Bock, R. and Zoschke, R. (2020) Limited responsiveness of chloroplast gene expression during acclimation to high light in tobacco. *Plant Physiol.* **182**, 424–435.

Selinski, J. and Scheibe, R. (2019) Malate valves: old shuttles with new perspectives. *Plant Biol.* **21**, 21–30.

Shikanai, T. (2007) Cyclic electron transport around photosystem I: genetic aproaches. *Ann. Rev. Plant Biol.* **58**, 199–217.

Singh, D.P., Cornah, J.E., Hadingham, S. and Smith, A.G. (2002) Expression analysis of the two ferrochelatase genes in Arabidopsis in different tissues and under stress conditions reveals their different roles in haem biosynthesis. *Plant Mol. Biol.* **50**, 773–788.

Stitt, M. and Hurry, V. (2002) A plant for all seasons: alterations in photosynthetic carbon metabolism during cold acclimation in Arabidopsis. *Curr. Opin. Plant Biol.* **5**, 199–206.

Stoppel, R., Lezhneva, L., Schwenkert, S., Torabi, S., Felder, S., Meierhoff, K., Westhoff, P. and Meurer, J. (2011) Recruitment of a ribosomal release factor for light- and stress-dependent regulation of petB transcript stability in Arabidopsis chloroplasts. *Plant Cell*, **23**, 2680–2695.

Suorsa, M., Järvi, S., Grieco, M. *et al.* (2012) PROTON GRADIENT REGULATION5 is essential for proper acclimation of *Arabidopsis* photosystem I to naturally and artificially fluctuating light conditions. *Plant Cell*, **24**, 2934–2948.

Suorsa, M., Rossi, F., Tadini, L. *et al.* (2016) PGR5-PGRL1-dependent cyclic electron transport modulates linear electron transport rate in *Arabidopsis thaliana*. *Mol. Plant*, **9**, 271–288.

Susek, R.E., Ausubel, F.M. and Chory, J. (1993) Signal transduction mutants of Arabidopsis uncouple nuclear CAB and RBCS gene expression from chloroplast development. *Cell*, **74**, 787–799.

Szabo, E.X., Reichert, P., Lehniger, M.K., Ohmer, M., de Francisco Amorim, M., Gowik, U., Schmitz-Linneweber, C. and Laubinger, S. (2020) Metabolic labeling of RNAs uncovers hidden features and dynamics of the Arabidopsis transcriptome. *Plant Cell*, **32**, 871–887.

Tadini, L., Pesaresi, P., Kleine, T. *et al.* (2016) GUN1 controls accumulation of the plastid ribosomal protein S1 at the protein level and interacts with proteins involved in plastid protein homeostasis. *Plant Physiol.* **170**, 1817–1830.

Terry, M.J. and Smith, A.G. (2013) A model for tetrapyrrole synthesis as the primary mechanism for plastid-to-nucleus signaling during chloroplast biogenesis. *Front. Plant. Sci.* **4**, 14.

Theis, J., Lang, J., Spaniol, B. *et al.* (2019) The Chlamydomonas *deg1c* mutant accumulates proteins involved in high light acclimation. *Plant Physiol.* **181**, 1480–1497.

Theis, J., Niemeyer, J., Schmollinger, S. *et al.* (2020) VIPP2 interacts with VIPP1 and HSP22E/F at chloroplast membranes and modulates a retrograde signal for HSP22E/F gene expression. *Plant Cell Environ.* **43**, 1212–1229.

Thormählen, I., Meitzel, T., Groysman, J., Öchsner, A.B., von Roepenack-Lahaye, E., Naranjo, B., Cejudo, F.J. and Geigenberger, P. (2015) Thioredoxin1 and NADPH-dependent thioredoxin reductase C have overlapping functions in regulating photosynthetic metabolism and plant growth in response to varying light conditions. *Plant Physiol.* **169**, 1766–1786.

Thormählen, I., Zupok, A., Rescher, J. *et al.* (2017) Thioredoxins play a crucial role in dynamic acclimation of photosynthesis in fluctuating light. *Mol. Plant*, **10**, 168–182.

Tikkanen, M., Grieco, M., Kangasjärvi, S. and Aro, E.-M. (2010) Thylakoid protein phosphorylation in hgher plant chloroplasts optimizes electron transfer under fluctuating light. *Plant Physiol.* **152**, 723–735.

Tillich, U.M., Lehmann, S., Schulze, K., Duhring, U. and Frohme, M. (2012) The optimal mutagen dosage to induce point-mutations in Synechocystis sp. PCC6803 and its application to promote temperature tolerance. *PLoS One*, **7**, e49467.

Tillich, U.M., Wolter, N., Franke, P., Duhring, U. and Frohme, M. (2014) Screening and genetic characterization of thermo-tolerant Synechocystis sp. PCC6803 strains created by adaptive evolution. *BMC Biotechnol.* **14**, 66.

Tiwari, B., Habermann, K., Arif, M.A., Weil, H.L., Garcia-Molina, A., Kleine, T., Muhlhaus, T. and Frank, W. (2020) Identification of small RNAs during cold acclimation in *Arabidopsis thaliana*. *BMC Plant Biol.* **20**, 298.

Töpfer, N., Caldana, C., Grimbs, S., Willmitzer, L., Fernie, A.R. and Nikoloski, Z. (2013) Integration of genome-scale modeling and transcript profiling reveals metabolic pathways underlying light and temperature acclimation in Arabidopsis. *Plant Cell*, **25**, 1197–1211.

Trentmann, O., Mühlhaus, T., Zimmer, D., Sommer, F., Schroda, M., Haferkamp, I., Keller, I., Pommerrenig, B. and Neuhaus, H.E. (2020) Identification of chloroplast envelope proteins with critical importance for cold acclimation. *Plant Physiol.* **182**, 1239–1255.

Trosch, R., Barahimipour, R., Gao, Y., Badillo-Corona, J.A., Gotsmann, V.L., Zimmer, D., Muhlhaus, T., Zoschke, R. and Willmund, F. (2018) Commonalities and differences of chloroplast translation in a green alga and land plants. *Nat. Plants*, **4**, 564–575.

Udy, D.B., Belcher, S., Williams-Carrier, R., Gualberto, J.M. and Barkan, A. (2012) Effects of reduced chloroplast gene copy number on chloroplast gene expression in maize. *Plant Physiol.* **160**, 1420–1431.

Vogel, M.O., Moore, M., Konig, K., Pecher, P., Alsharafa, K., Lee, J. and Dietz, K.J. (2014) Fast retrograde signaling in response to high light involves metabolite export, MITOGEN-ACTIVATED PROTEIN KINASE6, and AP2/ERF transcription factors in Arabidopsis. *Plant Cell*, **26**, 1151–1165.

Vu, D.P., Martins Rodrigues, C., Jung, B. *et al.* (2020) Vacuolar sucrose homeostasis is critical for plant development, seed properties, and nighttime survival in Arabidopsis. *J. Exp. Bot.* **71**, 4930–4943.

Walley, J., Xiao, Y., Wang, J.Z., Baidoo, E.E., Keasling, J.D., Shen, Z., Briggs, S.P. and Dehesh, K. (2015) Plastid-produced interorgannellar stress signal MEcPP potentiates induction of the unfolded protein response in endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA*, **112**, 6212–6217.

Wang, L., Leister, D., Guan, L., Zheng, Y., Schneider, K., Lehmann, M., Apel, K. and Kleine, T. (2020) The Arabidopsis SAFEGUARD1 suppresses singlet oxygen-induced stress responses by protecting grana margins. *Proc. Natl. Acad. Sci. USA*, **117**, 6918–6927.

Wang, S., Bai, G., Wang, S., Yang, L., Yang, F., Wang, Y., Zhu, J.K. and Hua, J. (2016) Chloroplast RNA-Binding protein RBD1 Promotes chilling tolerance through 23S rRNA processing in Arabidopsis. *PLoS Genet.* **12**, e1006027.

Wanner, L.A. and Junttila, O. (1999) Cold-induced freezing tolerance in Arabidopsis. *Plant Physiol.* **120**, 391–400.

Weber, A., Servaites, J.C., Geiger, D.E., Koffler, H., Hille, D., Groner, F., Hebbeker, U. and Flügge, U.I. (2000) Identification, purification, and molecular cloning of a putative plastidic glucose translocator. *Plant Cell*, **12**, 787–802.

Weiszmann, J., Fürtauer, L., Weckwerth, W. and Nägele, T. (2018) Vacuolar sucrose cleavage prevents limitation of cytosolic carbohydrate metabolism and stabilizes photosynthesis under abiotic stress. *FEBS J.* **285**, 4082–4098.

Westrich, L.D., Gotsmann V.L., Herkt C. *et al.* (2020) The versatile interactome of chloroplast ribosomes revealed by affinity purification mass spectrometry. *Nucleic Acids Research*, http://dx.doi.org/10.1093/nar/gkaa 1192.

Woodson, J.D., Perez-Ruiz, J.M. and Chory, J. (2011) Heme synthesis by plastid ferrochelatase I regulates nuclear gene expression in plants. *Curr. Biol.* **21**, 897–903.

Wu, W., Liu, S., Ruwe, H. *et al.* (2016) SOT1, a pentatricopeptide repeat protein with a small MutS-related domain, is required for correct processing of plastid 23S–4.5S rRNA precursors in *Arabidopsis thaliana*. *Plant J.* **85**, 607–621.

Xu, D., Dhiman, R., Garibay, A., Mock, H.P., Leister, D. and Kleine, T. (2020a) Cellulose defects in the Arabidopsis secondary cell wall promote early chloroplast development. *Plant J.* **101**, 156–170.

Xu, D., Leister, D. and Kleine, T. (2020b) VENOSA4, a human dNTPase SAMHD1 Homolog, contributes to chloroplast development and abiotic stress tolerance. *Plant Physiol.* **182**, 721–729.

Xu, D., Marino, G., Klingl, A., Enderle, B., Monte, E., Kurth, J., Hiltbrunner, A., Leister, D. and Kleine, T. (2019) Extrachloroplastic PP7L functions in chloroplast development and abiotic stress tolerance. *Plant Physiol.* **180**, 323–341.

Yang, X., Li, Y., Qi, M., Liu, Y. and Li, T. (2019) Targeted control of chloroplast quality to improve plant acclimation: from protein import to degradation. *Front. Plant Sci.* **10**, 958.

Yoshida, Y., Sarmiento-Manus, R., Yamori, W., Ponce, M.R., Micol, J.L. and Tsukaya, H. (2018) The Arabidopsis phyB-9 mutant has a second-site mutation in the VENOSA4 gene that alters chloroplast size, photosynthetic traits, and leaf growth. *Plant Physiol.* **178**, 3–6.

Zampieri, G., Vijayakumar, S., Yaneske, E. and Angione, C. (2019) Machine and deep learning meet genome-scale metabolic modeling. *PLoS Comput. Biol.* **15**, e1007084.

Zerges, W. and Hauser, C. (2009) Protein synthesis in the chloroplast. In *The Chlamydomonas sourcebook: organellar and metabolic processes*, vol **2**, (Stern, D.B., ed). Oxford: Elsevier, pp. 967–1025.

Zhao, C., Wang, Y., Chan, K.X. *et al.* (2019) Evolution of chloroplast retrograde signaling facilitates green plant adaptation to land. *Proc Natl Acad Sci USA*, **116**, 5015–5020.

Zhao, W.T., Feng, S.J., Li, H., Faust, F., Kleine, T., Li, L.N. and Yang, Z.M. (2017) Salt stress-induced FERROCHELATASE 1 improves resistance to salt stress by limiting sodium accumulation in *Arabidopsis thaliana*. *Sci Rep*, **7**, 14737.

Zoschke, R. and Bock, R. (2018) Chloroplast translation: structural and functional organization, operational control, and regulation. *Plant Cell*, **30**, 745–770.

Zoschke, R., Watkins, K.P. and Barkan, A. (2013) A rapid ribosome profiling method elucidates chloroplast ribosome behavior in vivo. *Plant Cell*, **25**, 2265–2275.