to my father

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Mediation of Photosynthetic Redox Signals in the Regulation of Plant Gene Expression

Thesis

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Referees:

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Abbreviations

AA	ascorbic acid
AO	ascorbate oxidase
AOX	alternative oxidase
APX	ascorbate peroxidase
ATP	adenosine tri-phosphate
bp	base pairs
bzw.	beziehungsweise
CAB	chlorophyll a/b-binding
cDNA	complementary DNA
cGMP	cyclic guanosine monophosphate
cop	constitutive photomorphogenetic
Cry	cryptochrome
cue	CAB underexpressed
Cys	cysteine
Cyt	cytochrome
DBMIB	2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone
DCMU	3-(3',4'-dichlorophenyl)-1,1'-dimethylurea
DHLA	dehydro lipoic acid
DNA	desoxyribonucleic acid
e.g.	for example *(example gratia)*
et al.	and others *(et alii)*
ETC	electron transfer chain
Fig.	figure
FNR	ferredoxin:NADP:oxidoreductase
FR	far red
FTR	ferredoxin-thioredoxin reductase
F_m	maximum fluorescence
F_s	steady state fluorescence
Grx	glutaredoxin
GSH/GSSG	glutathione
gun	genome uncoupled

H_2O_2	hydrogen peroxide
HIR	high irradiance response
hy	hypocotyl
i.e.	that is *(id est)*
K	Kelvin
kDa	kilo-Dalton
KO	knock out
LA	lipoic acid
LHC	light harvesting complex
LTR	long-term response
MAP	mitogen activated protein
MAPK	MAP kinase
µM	micromolar
mM	millimolar
mRNA	messenger RNA
NAD	nicotine amide adenine dinucleotide
NADP	nicotine amide adenine dinucleotide phosphate
NEP	nuclear encoded polymerase
NO	nitric oxide
NO_2	nitric dioxide
NR	nitrate reductase
NTR	NADPH-thioredoxin reductase
O_2	oxygen
oe	overexpressed
ORF	open reading frame
PAGE	poly-acrylamide gel electrophoresis
PC	plastocyanine
PEF	photosynthetic electron flow
PEP	plastid encoded polymerase
ph	potential (of) hydrogen *(pondus hydrogenii)*
phy	phytochrome
PKA	protein kinase A
PQ	plastoquinone
Prx	peroxiredoxin

PS	photosystem
PTK	plastid transcription kinase
Q_O (site)	quinol oxidation (site)
R	red
redox	reduction/oxidation
RNA	ribonucleic acid
RNPase	RNA polymerase
ROS	reactive oxygen species
s.	siehe
Ser	serine
SLF	sigma-like factor
SO_2	sulfur dioxide
TAK	thylakoid associated kinase
Thr	threonine
Trx	tyroxine
Tyr	tyrosine
UV	ultraviolet
UTR	untranslated region
WT	wild type

Contents

1 Introduction

One of the most developing topics of the last few years in the field of plant molecular biology is the regulation of plant internal processes via 'Redox control'. 'Redox' reactions are chemical reactions that involve the transfer of either electrons or hydrogen atoms as charge carrier between molecules. 'Reduction' means the reception of at least one electron or hydrogen atom by an electron acceptor, while oxidation is the release of at least one electron or hydrogen atom by an electron donor. The availability or the lack of charges defines a molecule as to be 'reduced' or 'oxidized', respectively. The charge of the molecule or the charge state in a system consisting of many molecules with different charges at a certain point of time defines the 'Redox state' of the molecule or the system. By changing the charge state the physical properties of the molecule are changed. In a biological system thereby it may be able to perform regulatory activities resulting in a molecular response, directly or indirectly via a chain of signalling components. This regulation is referred to as 'Redox control', the control of a biological phenomenon which is subject to the redox state of one or more components of a signalling cascade.

1.1 Main components of the cellular redox network

Redox control in plant cells appears in a manifold manner and a large number of molecular responses were reported, including regulation at the level of transcription, post-transcriptional events as well as translation and post-translational modification. Especially for the latter many processes are known to be redox controlled. Main components of the cellular redox network are pyridine nucleotides, ascorbate, glutathione, lipoic acid, oxylipins, tocopherol, thioredoxins, glutaredoxins, peroxiredoxins, and other thiol proteins.

Hydrogen peroxide (H_2O_2) and *nitric oxide* (NO) should also be mentioned in this context since they belong beside the hydroxyl radical (HO^-), singlet oxygen ($^1O_2$), or the superoxide anion (O_2^-) to the so called reactive oxygen species (ROS) which are increased in their concentration under biotic or abiotic stress conditions. ROS traditionally were considered as unavoidable by-products of aerobic metabolism with respect to their capability of unrestricted oxidation what can lead to oxidative destruction of the cell. This view resulted from the ob-

servation that they are produced in reactions involved in normal cell metabolism, such as photosynthesis or respiration, but also deriving from sources belonging to pathways enhanced under stress conditions. In recent years, however, new sources of ROS have been identified in plants, including NADPH oxidases, amine oxidases and cell-wall-bound peroxidases. (reviewed by Mittler, 2002). Special emphasis was placed on H_2O_2 what is produced in a genetically controlled manner by cytosolic membrane bound NADPH oxidases which serve as a source beside the mentioned processes localised in chloroplasts, mitochondrions, peroxisomes, and the apoplast. H_2O_2 has been implicated to mediate a wide range of stress responses including defence reactions against pathogens and herbivores, stomata closure, and the regulation of cell expansion and plant development (for review see e.g. Neill *et al.*, 2002; Laloi *et al.*, 2004). The synthesis and action of H_2O_2 apparently are linked to those of nitric oxide in that production of NO has been detected under conditions enhancing H_2O_2 generation. NO also plays a role in a broad spectrum of pathophysiological and developmental processes in plants but no biosynthetic origin is known so far. Nitrate reductase (NR) was reported to be a metabolic source for NO and nitrite/ascorbate interaction or the light-mediated conversion of nitrogen dioxide to NO via carotenoids were implicated as routes to NO generation. A role for NO in programmed cell death, a process where H_2O_2 also was reported to be involved, was implicated for *Arabidopsis* via control of cGMP synthesis (for review see e.g. Neill *et al.*, 2002; Lamattina *et al.*, 2003). Both hydrogen peroxide and nitric oxide were shown to activate mitogen activated protein (MAP) kinases which are known to be modulators of gene expression via phosphorylation of transcription factors (Hirt, 1997). Some examples of ROS mediated responses in a specific context with emphasis on regulation of gene expression will be discussed.

Because *nicotinic acid* is a pyridine derivate, the coenzymes of oxidoreductases [nicotine amide adenine dinucleotide (NAD) and the respective phosphate (NADP)] are called pyridine nucleotides. They are acting as so called reduction equivalents in many dissimilatory and assimilatory processes. In photosynthesis they play an important role as final electron acceptor of the photosynthetic electron transport chain. The concentration in the cell usually is determined to be around 100 µM. Cytoplasmic pyridine nucleotides are mostly in a reduced state, while the redox state in the chloroplast is shifted from very oxidised to reduced upon illumination after dark treatment and back to a value slightly above the dark value in the steady state of photosynthesis (reviewed by Dietz, 2003). The involvement of pyridine nucleotides in metabolic and signalling processes in the plant cell has many facettes and will be discussed in the respective context below.

Ascorbate (AA) is the most abundant antioxidant in plants and especially in photosynthetic tissues. It has important functions in oxidative stress response and may be involved in cell division and growth (Smirnoff, 2000; Pignocchi *et al.*, 2003). Approximately 90% of the AA content of the leaf is localised in the cytoplasm (reviewed by Noctor and Foyer, 1998). The remaining 10% are exported to the apoplast where they reach millimolar concentrations. Apoplastic AA is considered to be the first line of defence against potentially damaging external oxidants such as ozone, SO_2, and NO_2 (Pignocchi *et al.*, 2003). This redox buffering function is facilitated by enzymes that are involved in AA metabolism, such as ascorbate oxidase (AO) and monodehydroascorbate reductase (MDHAR) which are found either on the plasmalemma or in the apoplast (reviewed by Pignocchi and Foyer, 2003). In the chloroplast the ascorbate concentration can reach up to 300 mM (Smirnoff, 2000). A well known pathway related to photosynthesis is the glutathione-ascorbate cycle. It is used to detoxify H_2O_2 which is generated in consequence of the reduction of molecular oxygen (O_2) to O_2^- at photosystem I (PS I) in the so called Mehler reaction (reviewed by Noctor and Foyer, 1998).

In plants, *gluthathion* has three major functions: sulfur metabolism, antioxidant defence, and redox-signal transduction as a so called redox agent (reviewed by Noctor and Foyer, 1998; Pfannschmidt *et al.*, 2001). Gluthathion is available in two interchangeable forms, the reduced GSH and the oxidised GSSG. GSH is a tripeptide and the most widespread form consists of γ-glutamine, cysteine, and glycine (γ-glu-cys-gly). GSH regulates sulfur uptake at root level, is used by the GSH-S transferases in the detoxification of xenobiotics and is a precursor of the phytochelatins. In both plants and animals, glutathion is important as antioxidant and redox buffer (reviewed by Noctor and Foyer, 1998). High light experiments with *Arabidopsis* indicated a correlation of the redox state of glutathione and gene expression in both the nuclear and chloroplast (Karpinski *et al.*, 1997).

Another redox agent which was studied intensively in recent years is *thioredoxin* or the ferredoxin-thioredoxin reductase system of the chloroplast, respectively (reviewed by Buchanan and Balmer, 2004). Thioredoxins (Trx) are small proteins with a redox active disulfide bridge and found in plant, bacterial and animal cells. In *Arabidopsis* at least 20 thioredoxin genes have been reported by analyzing the whole sequenced genome (Meyer *et al.*, 2002; reviewed by Gelhaye *et al.*, 2005). Plant Trxs can be classified in two families. Family I proteins contain one distinct thioredoxin domain, whereas family II proteins are fusion proteins with one or more such domains. In the cytosol of plant cells, one of so far six known family I thioredoxin forms is present, namely thioredoxin *h*. It forms together with the NADPH-thioredoxin reductase (NTR) a cytosolic thioredoxin system, in which electrons from NADPH are trans-

ferred to thioredoxin by NTR (reviewed by Schürmann and Jacquot, 2000; Gelhaye *et al.*, 2004). In chloroplasts four groups of family I proteins and one family II Trx are found, the nuclear encoded Trxs *f*, *m*, *x*, and *y*, and CDSP32 (chloroplast drought-induced stress protein of 32 kDa), an also nuclear encoded protein consisting of two thioredoxin modules, what was characterised due to its thioredoxin properties by Broin *et al.* (2002). Chloroplast Trxs *f* and *m* are reduced by ferredoxin via a ferredoxin-thioredoxin reductase (FTR) (Dai *et al.*, 2000) and are able to activate specifically selected target enzymes (reviewed by Gelhaye *et al.*, 2005). Trx *x* is probably specifically involved in defence against oxidative stress via high efficient Prx reduction, particularly of 2-Cys Prx (Collin *et al.*, 2003). Trx *y* was found to reduce various Prxs in *Chlamydomonas reinhardtii* but with less efficiency (Lemaire *et al.*, 2003). Former work already showed an involvement of thioredoxins in regulation of Calvin cycle enzymes by covalent redox modification (reviewed by Scheibe, 1991).

Laloi *et al.* (2001) identified a Trx/NTR system in plant mitochondria. The localisation of both the Trx AtTRX-o1 and the NTR AtNTRA in mitochondria of *Arabidopsis* and their activity was shown *in vitro*. Furthermore, a Trx *h* from poplar (PtTRX*h*2) recently was demonstrated to be targeted to mitochondria and to be reduced by AtNTRA (Gelhaye *et al.*, 2004). Evidence for a mitochondrial Trx system was obtained already from experiments studying the interaction of thioredoxins with 2-oxoacid dehydrogenase complexes (Bunik *et al.*, 1999). The results showed that the plant mitochondrial thioredoxins can be reduced *in vitro* by lipoic acid generated via the 2-oxoacid dehydrogenase complex, as in animal mitochondria (Bunik *et al.*, 1995).

Lipoic acid (LA) is an eight carbon acid with two sulfhydryl groups and a low molecular weight (reviewed by Dietz, 2003). Beside the mentioned activity in the mitochondria it functions as a cofactor in the multienzyme complexes that catalyse the oxidative decarboxylation of α-keto acids such as pyruvate, α-ketoglutarate, and branched-chain α-keto acids, in which it is covalently bound as lipoamide (Sgherri *et al.*, 2002). Both the reduced [dihydrolipoic acid (DHLA)] and the oxidised form (LA) may act as antioxidants, although DHLA is the more effective one; they have metal-chelating ability and quench activated oxygen species. Due to the hydrophobic as well as the hydrophilic properties lipoic acid is able to connect the activity of antioxidants in the cell membrane (α-tocopherol) with antioxidants in the cytosol (ascorbic acid and GSH), possibly via non-enzymatic reduction of dehydroascorbate, GSSG, and tocopheroxyl radicals (Navari-Izzo *et al.*, 2002; D'Amico *et al.*, 2004). In human, rat, yeast, and *Escherichia coli* the reduction of GSSG by reduced lipoamide catalyzed by glutaredoxins has been demonstrated *in vitro* (Porras *et al.*, 2002).

Glutaredoxins (Grx) are ubiquitous oxidoreductases of the thioredoxin family. They are about 10-15 kDa in size and exhibit an active site sequence CxxC or CxxS required for their redox properties. Grx are regenerated via reduction by NADPH, glutathione reductase (GR), and glutathione (GSH) (reviewed by Rouhier *et al.*, 2004). Indeed, the most results were obtained in *E. coli*, mammal cells and yeast, but most recent studies more and more put Grxs together with Trxs ahead concerning their role in cellular redox control in plants, apart from ascorbic acid and glutathione. So far, about 30 Grx genes could be identified in *Arabidopsis*, a number similar to this of Trxs what amounts to around 20 (reviewed by Lemaire, 2004; Gelhaye *et al.*, 2005). However, about the function of plant Grx very little is known until now, indeed only some target proteins could be identified or predicted, respectively, and real evidence is available for a Grx from poplar phloem what is able to reduce a Prx II (Rouhier *et al.*, 2001).

Peroxiredoxins represent an own family of peroxidases that are found in all organisms. They can be grouped into four subfamilies based on their structural an biochemical properties: 2-Cys Prx, Prx Q, Prx II, all containing two catalytic Cys residues, and 1-Cys Prx what contains only one conserved Cys residue. Members of all subfamilies are present in *Arabidopsis*, as indicated by sequence analyses. 10 open reading frames (ORFs) could be identified, of which eight were shown to be expressed. Higher plant Prxs are nuclear encoded (reviewed by Dietz, 2003). The one identified 1-Cys Prx is targeted to the nucleus, the 2-Cys Prxs and the Prx Q are targeted to the chloroplast, whereas the fourth group includes proteins targeted to mitochondria and chloroplasts as well as cytosolic Prxs (Horling *et al.*, 2002; Horling *et al.*, 2003). Expression of Prx genes depending on light, ascorbate, or oxidative stress were investigated and found to be regulated specifically for different Prxs, but, Prx II C was found to be induced by all applied treatments (Horling *et al.*, 2003). Shortly before type II Prx C was already shown to be highly efficient for H_2O_2 detoxification (Horling *et al.*, 2002). Activity of various Prxs towards peroxides was described in many cases (reviewed by Dietz *et al.*, 2002; Rouhier *et al.*, 2004) as well as their function as redox sensors (reviewed by Dietz, 2003).

Tocopherol is a lipid-soluble redox compound considered to have antioxidant activity in lipid membranes where it quenches lipid radicals and singlet oxygen, thus preserving membrane integrity. Both tocopherols and the closely related tocotrienols belong to the vitamin E group and are synthesised exclusively in plastids of photosynthetic organisms whereby the major form is α-tocopherol. This is present in green tissues while the latter is found predominantly in seeds (reviewed by Munné-Bosch and Falk, 2004). To prevent membranes from destruction by consequences of a radical chain reaction initiated by generation of an alkyl radical from a (poly)unsaturated fatty acid and O_2, a lipid hydroperoxide and a tocopheroxyl radical are

formed. The lipid hydroperoxide, an oxidized polyenoic fatty acid, may be oxidised to jasmonic acid, while the tocopheroxyl radical is regenerated by electron donors such as glutathione and ascorbic acid, as shown for the latter in the *vtc-1 Arabidopsis* mutant under drought (Munné-Bosch and Alegre, 2002; reviewed by Dietz, 2003). Jasmonic acid and other oxidation products deriving from lipid hydroperoxides belong to the large group of so called oxylipins which are involved in many processes constituting stress tolerance for pathogens and herbivores (Feussner and Wasternack, 2002). Jasmonates are known to regulate gene expression in the nucleus, affecting photosynthesis, anthocyanin and antioxidant metabolism (reviewed by Creelman and Mullet, 1997). While photosynthesis related genes are inhibited upon regulation by jasmonic acid, genes involved in tocopherol synthesis are induced. This impact could be interpreted as self-regulation of the tocopherol level and, thereby and besides down-regulation of photosynthetic activity, serve as a further system for plant protection under stress conditions (reviewed by Munné-Bosch and Falk, 2004).

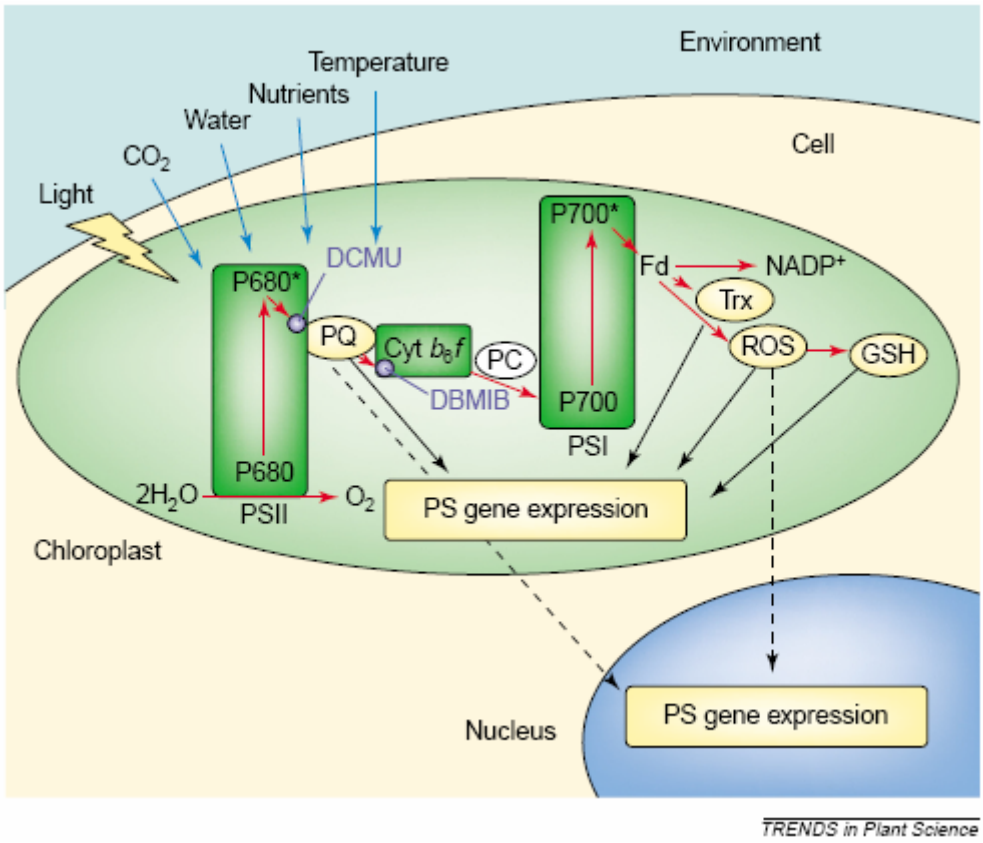

Fig. 1 - Factors in photosynthetic redox chemistry that influence photosynthesis gene expression. The electron transport chain of a chloroplast is drawn schematically according to the Hill–Bendall Z scheme and the electron flow is represented by red arrows.

From Pfannschmidt (2003).

1.2 Redox regulation of gene expression

Due to their inability to avoid environmental changes concerning mainly light intensity, water supply, nutrient availability, and temperature by moving to a more comfortable place, plants have developed sophisticated intracellular respond mechanisms. The mentioned parameters affect the necessary balance generated between absorbed light energy synonymous with the induced electron flow, and all molecules serving as electron acceptors. Already small interferences can cause a disintegration of this redox equilibrium and such trigger a complex, redox signal mediated response. This response may occur within seconds or in a time scale of hours or even days. A rapid reaction, a so called short term response, was described, for example, for the regulation of light energy distribution between the photosystems, named "state transition". The shift of the moveable part of the LHCII complex was first described in the alga *Chlorella pyrenoidosa* as 'rapid adaptive response to the immediate incident light conditions' or 'State I-State II transition' (Williams and Salamon, 1976). The process is triggered by the redox state of the photosynthetic electron transport chain, particularly by the interaction of reduced PQ with the Q_O site of the cytochrome b_6f (Cyt b_6f) complex and the state of thiol compounds reduced via ferredoxin at PSI (see Fig. 5). It ensures a sufficient supply of PSI with excitation energy under light conditions preferring PSII (for review see e.g. Allen and Pfannschmidt, 2000; Aro and Ohad, 2003).

Another aspect of the regulation of photosynthetic efficiency is the adjustment of photosystem stoichiometry, a well described example for a long term response (LTR) to changing light conditions. In plant populations with a high density or in the ground vegetation of a forest, for instance, changes in quality or quantity of incident light occur constantly, leading to an imbalance in excitation energy distribution between both photosystems. To maintain an optimal photosynthetic efficiency, the amount of the disfavoured PS is increased whereas the preferentially excited PS is reduced (reviewed by Allen and Pfannschmidt, 2000). In cyanobacteria, an acclimation to changes in light quality is achieved by an increase of PSI particles, whereas the amount of PSII remains constant. Changes in the redox state of cytochrome b_6 in the Cyt b_6f complex are suggested to trigger a signalling cascade which regulates expression of the *psaAB* genes at the translation or posttranslational level (reviewed by Fujita, 1997).

Experimentally, photosystem stoichiometry adjustment in higher plant chloroplasts can be induced by growing plants in light sources favouring excitation of either PSI or PSII (Melis, 1991), consequently termed PSI- and PSII-light, respectively. Application of PSII-light to non-acclimated plants causes a reduction of the PQ pool, leading in conclusion to state transi-

tion and expression of PSI genes. PSI-light results in an oxidation of the PQ pool and thus, by eliminating the triggering event in a loss of the short-term response. At the same time a signalling cascade is initiated what finally leads to down-regulation of *psaAB* gene transcription and enhanced expression of PSII genes (see Fig. 2). The adjustment of photosystem stoichiometry resulting from both reduction and oxidation of the PQ pool contributes essentially to the long-term-acclimation via these processes. In contrary to other systems using differences in light quantity (e.g. high light and low light, respectively) variations in light quality do not include the destructing side effects of light stress conditions, such as photooxidation via ROS and subsequent photoinhibition. Furthermore variations in light quantity always affect both photosystems whereas a certain light quality only stimulates the activity of the preferentially excited photosystem.

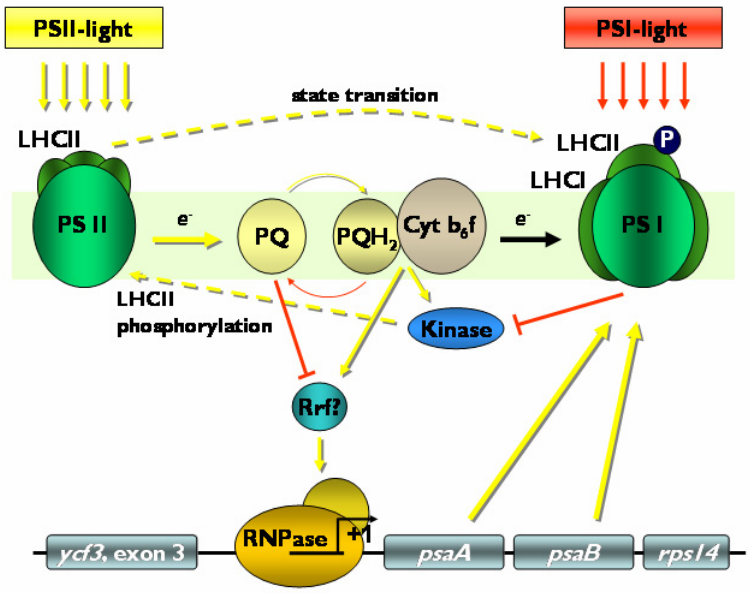

Fig. 2 – Schematic view of the light system. Application of PSII-light causes an oxidation of the PQ pool. Upon interaction of PQH_2 with the Cyt b_6f complex the LHCII kinase is activated what leads to LHCII phosphorylation and state transition (dotted yellow arrows). Furthermore reduction of the PQ pool promotes transcription of *psaAB* via a proposed redox-responsive factor (Rrf) (yellow arrows).
PSI-light causes an oxidation of the PQ-pool abolishing the activation of the LHCII kinase and the positive signal targeting *psaAB* transcription (red arrows and lines).

For prokaryotes redox-sensitive transcription factors have been shown earlier, such as OxyR what mediates the response to H_2O_2 in *E. coli*, or SoxR what is activated under oxidative stress from O_2^- and consequently activates SoxS. SoxS activity leads in conclusion to an increased expression of genes encoding antioxidant enzymes (reviewed by Carmel-Harel and Storz, 2000). Analogous to OxyR, the functional homologous transcription factor Yap1 from *Saccharomyces cerevisiae* is activated by conformational changes resulting from oxidation of protein thiols (reviewed by Georgiou, 2002). Anoxygenic photosynthetic bacteria, such as *Rhodobacter sphaeroides*, exhibit a well characterised redox regulation of photosynthesis

gene expression, co-regulated by a two-component signal transduction system consisting of RegB and RegA. RegB was predicted to be a membrane bound histidine protein kinase with an autophosphorylation site, whereas RegA is a DNA-binding protein with a putative phosphorylation site, strongly implicating a two-component regulatory system constituted of both factors. Photosystem as well as several non-photosynthesis genes of *R. sphaeroides* and *R. capsulatus* are expressed in anaerobic, but not aerobic conditions. Aerobic suppression of photosynthesis gene expression is mediated by another conserved factor known as CrtJ in *R. capsulatus* and PpsR in *R. sphaeroides*. CrtJ effectively binds DNA under oxidising, but not reducing, conditions which cause a conformational change initiated by formation of an intramolecular disulphide bond (reviewed by Bauer *et al.*, 2003).

In plants, several *cis*-elements and *trans*-factors have been described to be involved in responses to stress factors, such as freezing, drought, or high salt conditions; however, so far no redox-regulated transcription factor could be identified (reviewed by Pastori and Foyer, 2002). Despite of this lack of information a large number of redox signals that modulate gene expression have been reported. Exposure to abiotic or biotic stress leads to an accumulation of H_2O_2 and oxidative stress. A cDNA microarray approach identified 175 genes which are regulated by H_2O_2, a molecule implicated as stress factor in plants (Desikan *et al.*, 2001). Activation of a mitogen-activated protein kinase (MAPK) by H_2O_2 in *Arabidopsis* has been demonstrated earlier. In suspension cultures, treatment with the bacterial protein harpin resulted in a rapid generation of H_2O_2 and activation of two MAPK-like enzymes of 39 and 44 kDa (Desikan *et al.*, 1999). By direct treatment with H_2O_2 the 44 kDa protein could be identified as MAPK what is phosphorylated at tyrosine and threonine residues upon activation (Desikan *et al.*, 1999). In further experiments this protein turned out to be the *Arabidopsis* MAPK AtMPK6 with an apparent molecular mass of 47 kDa (Desikan *et al.*, 2001). MAPK cascades are universal signal transduction modules in eukaryotes. In *Arabidopsis* 20 MAPKs, 10 MAPK kinases and 60 MAPK kinase kinases could be identified (Ichimura, 2002).

Photosystem stoichiometry adjustment necessitates regulation on the level of gene expression in both nucleus and chloroplasts (see Fig. 1). Photosynthesis proteins are encoded mainly in the nucleus and must be imported into the chloroplast after their translation. In photosynthetic organisms, redox signals from the chloroplast have been shown to target almost all levels of gene expression in both compartments (reviewed by Rodermel, 2001; Pfannschmidt, 2003) (see Fig. 3).

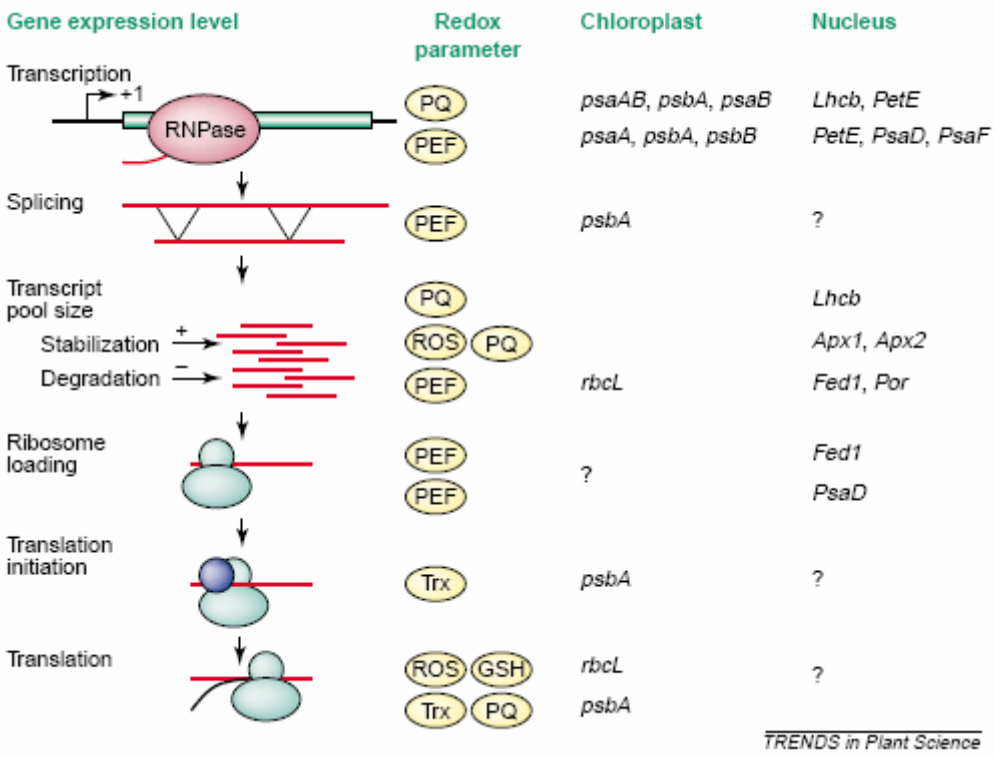

Fig. 3 - Summary of redox-regulated expression levels of nuclear and chloroplast photosynthesis genes in higher plants and algae. The left-hand column represents the various steps of general gene expression, beginning with transcription and ending with translation. The green bar represents a gene that is transcribed by the RNA polymerase, with +1 as the transcription start site. The resulting transcript is represented by a red line including introns (black). The small and large subunits of a ribosome are shown as light-blue circles and ovals, respectively. Additional factors for translational initiation are represented by a dark-blue circle. The generated polypeptide chain is shown as a black line. The second column summarizes the known redox parameters that affect the gene expression level (compare with Fig. 1). Columns 3 and 4 list the affected photosynthesis genes that are known to date, listed by chloroplast and nuclear location, respectively. Affected genes and regulatory redox factors are drawn at the same height. Abbreviations: GSH, glutathione; PEF, photosynthetic electron flow; PQ, plastoquinone; RNPase, RNA polymerase; ROS, reactive oxygen species; Trx, thioredoxin.

From Pfannschmidt (2003).

Impact of plastidic redox state on nuclear gene expression was shown for *Lhcb* genes in various organisms (reviewed by Pfannschmidt *et al.*, 2003). In algae shifted from high-light intensities to low-light *Lhcb* gene transcription were increased. By applying site-specific electron transport inhibitors 3-(3',4'-dichlorophenyl)-1,1'-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) (Trebst, 1980) the redox state of the PQ pool could be determined as controlling parameter of a signal transduction cascade (Escoubas *et al.*, 1995). In similar experiments using high or low temperature as stress factor a comparable excitation pressure was produced. At constant light intensities high temperature caused a low-

light response whereas low temperature led to high-light stress (Maxwell *et al.*, 1995). Pursiheimo *et al.* (2001) reported a co-regulation of the phosphorylation state of thylakoid light harvesting proteins LHCII and CP29 and the mRNA level of the nuclear encoded photosynthesis genes *Lhcb* and *RbcS*, and the plastome encoded gene *psbA* in winter rye. Plants were grown under contrasting environmental conditions or transiently shifted to different light intensity or temperature conditions. Short-term shifts revealed that LHCII phosphorylation and accumulation of *Lhcb* mRNA occurs under similar conditions implicating a regulatory role of the LHCII kinase also in *Lhcb* gene expression.

In low-light acclimated *Arabidopsis* plants which were exposed to light stress conditions an increase of mRNA levels of two cytosolic ascorbate peroxidases (*Apx1* and *Apx2*) was observed (Karpinski *et al.*, 1997). Inhibitor experiments again pointed to the PQ pool as origin of the responsible signalling cascade. Addition of GSH abolished the signal underlining the important role of glutathione or the redox state of the glutathione pool in this context and implicating ROS as mediating factors for the signal transduction from chloroplasts to the nucleus. In further experiments a transport of the high-light induced signal to untreated tissues was observed, called "systemic acquired acclimation" (Karpinski *et al.*, 1999). H_2O_2 is suggested to be one responsible component for such a creation of an acclimatory response in unstressed regions of the plant. Beside excess-light also other stress factors were determined to induce ROS formation, such as chilling, wounding, or pathogen attack. Those stresses have been shown to initiate transcription of components of the cytosolic ROS-scavenging system (reviewed by Mullineaux *et al.*, 2000).

Prominent examples for nuclear encoded enzymes functional in chloroplasts are peroxiredoxins. Reporter gene plants harbouring an *Arabidopsis* 2-Cys peroxiredoxin-A (2CPA) promoter fused to the glucuronidase *(gus)* gene revealed a correlation between promoter activity and availability of electron acceptors at PSI (Baier *et al.*, 2004). Furthermore inhibitor studies showed an involvement of a MAPK kinase and serine/threonine kinase are involved in the signal transduction process in response to an oxidative or antioxidant signal, respectively.

Redox control of *psbA* translation initiation in *Chlamydomonas* is a well researched example for transduction of signals inside the chloroplast. To counteract photoinactivation of PSII by unavoidable by-products of photosynthesis, photodamaged D1 protein is constantly replaced. This maintaining mechanism requires a continuous protein turnover ensured by *de novo* synthesis of D1, regulated by light dependent binding of a multiprotein complex to specific elements in the 5' untranslated region (UTR) of the *psbA* mRNA (Danon and Mayfield, 1994;

Mayfield *et al.*, 1994). In chloroplasts of dark adapted cells, RNA binding is inhibited *in vitro* through ADP-dependent phosphorylation by a serine/threonine phosphotransferase associated with the binding complex (Danon and Mayfield, 1994). The redox state of another member of this complex, termed RB60, was found to be crucial for translation initiation (Trebitsh *et al.*, 2000). The responsible signal leading to a reduction of RB60 at a dithiol site and subsequent translation initiation is generated at PSI and mediated by the ferredoxin-thioredoxin system. A yet unidentified prerequisite pathway probably involving specific oxidation of RB60 originates from the redox state of the PQ pool (Trebitsh and Danon, 2001) whereby reduction of PQ seems to initiate the signal. A similar protein complex was found to bind the 5'UTR of the psbA mRNA in *Arabidopsis* (Shen *et al.*, 2001). Association was prevented under oxidising conditions, while incubation with a reductant could recover it, suggesting a redox control of *psbA* translation also in higher plants.

In mustard a photosystem stoichiometry adjustment could also be assigned to the redox state of the PQ pool (Pfannschmidt *et al.*, 1999). Transcription of chloroplast genes *psaAB* and *psbA* was shown to be regulated in plants grown under PSI- or PSII-light, respectively. In studies which are part of this work the same light conditions were used. The results confirm the redox-control of the reaction centre proteins of PSI and PSII, respectively, for *Arabidopsis* (Fey, Wagner et al. 2005).

In a study using the same light quality conditions for plant growth, transgenic tobacco plants harbouring promoters of nuclear-encoded PSI genes *PetE*, *PetH*, *PsaD*, and *PsaF* [encoding plastocyanin (PC), the ferredoxin:NADP:oxidoreductase (FNR), and the PSI subunits *PsaD* and *PsaF*] fused to the *uidA* gene, it could be demonstrated that redox signals generated in the photosynthetic electron transport chain also affect nuclear gene expression (Pfannschmidt *et al.*, 2001). In *Arabidopsis* the nitrate reductase *(Nia2)* promoter as well as the activity of the expressed enzyme was shown to respond also to varying light conditions as used in the experiments mentioned before (Sherameti *et al.*, 2002). However, the transcription was activated by an oxidation of electron transport components either by PSI light or by DCMU or DBMIB treatment.

1.3 Photoreceptors in plants

Via the mechanisms discussed above the chloroplast biochemical machinery contributes significantly to the control of nuclear gene expression and acts beside cytosolic photoreceptors as an independent and important sensor for changes in light conditions.

The red and blue light absorbing photoreceptors are well known to regulate nuclear gene expression. Studies using *Arabidopsis* mutants exhibiting a pale phenotype [*cue4* and *cue9* (cue=chlorophyll a/b-binding [CAB] protein-underexpressed)], delayed greening (*cue3*, *cue6*, and *cue8*), or phytochrome deficiency [*hy1* (long hypocotyl] and *phyB* (phytochrome)], respectively, revealed a close interaction of plastid- and phytochrome-derived signalling pathways targeting nuclear expression of plastid proteins (Lopez-Juez *et al.*, 1998). The authors present two models concerning the order of signal transduction. According to this either the plastid signal controls the amplitude of the phytochrome signal, or the phytochrome signal targets primarily the organelle modulating its activity and is subsequently relayed to the nucleus.

Plants use three major classes of photoreceptors to monitor variations in direction, duration, quantity, and wavelength of light. The cryptochromes and phototropins sense the blue/UV-A region of the spectrum, whereas the phytochromes perceive primarily the red (R) and far-red (FR) wavelengths. UV-B receptors are still unknown, but several developmental responses have been reported to be regulated by UV-B light, like inhibition of hypocotyl elongation and transcriptional regulation. An optimisation of photosynthetic yields is achieved through several light responses mediated by phototropins, while cryptochromes are very important during de-etiolation (reviewed by Chen *et al.*, 2004). Phytochromes which can interact with the cryptochromes are the best characterized photoreceptors. In *Arabidopsis* phytochromes are encoded by a small gene family consisting of five genes (*PHYA-PHYE*). PhyA is mainly responsible for very low fluence responses and for the far red (FR) light-dependent high-irradiance responses (HIRs). PhyB to PhyE have similar functions in responses to continuous red and white light. Phytochrome molecules are able to undergo light-induced, reversible switching between two conformers, the inactive P_r (red-light-absorbing) form and the active P_{fr} (far-red-light-absorbing) form (reviewed by Quail, 2002).

A large number of mutants with defects in function of photoreceptors or in transduction of signals triggered by photoreceptors have been created (for review see e.g. Chory *et al.*, 1995; Briggs and Liscum, 1997; Whitelam *et al.*, 1998; Kuno and Furuya, 2000; Strand, 2004). Among these are knock-out-mutants which were used to investigate the signal transduction

pathways targeting nuclear gene expression (see above). However, yet little is known about the correlation between responses deriving from another important light receptor for the respective wavelength', the photosynthetic apparatus, and the cytosolic photoreceptors.

1.4 Redox control and phosphorylation

Protein phosphorylation has been shown to be one of the most important modification processes in plant signalling, confirmed by many reports describing signalling cascades involving one or more phosphorylation events (see above). Further support came from analyses of the *Arabidopsis* genome which revealed the existence of nearly 1000 genes encoding serine/threonine kinases (The-Arabidopsis-Genome-Initiative, 2000).

The most prominent member of so far described redox-controlled kinases is the LHCII kinase responsible for phosphorylation of the LHCII complex proteins Lhcb1 and Lhcb2 (reviewed by Aro and Ohad, 2003). The function of the kinase is controlled by interaction of reduced PQ (PQH_2) with the quinol oxidation site (Q_O site) of the Cyt b_6f complex. Activation of the kinase occurs if the Q_O site is occupied by PQH_2 (Vener *et al.*, 1997; Zito *et al.*, 1999) but the quinol cannot be oxidised due to unavailability of downstream electron acceptors, as e.g. under light conditions favouring PSII. In its inactivated form, while bound to the Cyt b_6f complex, the kinase can be reversibly inhibited by reducing dithiol agents as thioredoxin and reactivated by thiol oxidants (Rintamäki *et al.*, 1997; Hazra and DasGupta, 2003).

The identity of the kinase still remains unknown; however, several candidates have been introduced (Allen and Race, 2002). The most interesting group comprises the so called thylakoid associated kinases (TAKs) identified in *Arabidopsis* (Snyders and Kohorn, 1999, 2001). Probably three TAKs form a complex, and the enzymes themselves are subject to phosphorylation. In *Chlamydomonas* a thylakoid associated serine/threonine protein kinase (Stt7) was described recently that shows sequence homologies with *Arabidopsis* proteins (Depege *et al.*, 2003). Stt7 was shown to be required for LHCII phosphorylation and for state transition. Together these data suggest an involvement of more than one protein kinase in phosphorylation of the light harvesting complex of which not all are redox regulated, and a model of a kinase cascade with one initially redox-activated kinase has been proposed (Snyders and Kohorn, 1999).

Transcription of *psaAB* and *psbA* is regulated by light and an integral factor in photosystem stoichiometry adjustment (Pfannschmidt, 2003). It requires a multi-subunit bacteria-like plas-

tid encoded polymerase (PEP) (Link, 1996; for review see Hess and Börner, 1999; Liere and Maliga, 2001) what is suggested to be a main target for redox regulation. In mustard chloroplasts, subunits of PEP including sigma-factors are targets for phosphorylation what is also thought to be a key event for its activity (Baginsky *et al.*, 1997). The responsible kinase was named plastid transcription kinase (PTK). It exhibits serine/threonine protein kinase activity and is a heterotrimeric associated factor of the RNA polymerase (Baginsky *et al.*, 1999). PTK activity *in vitro* depends on its phosphorylation state and the redox state of glutathione. Further investigations classified the enzyme as a casein kinase II family member (Ogrzewalla *et al.*, 2002). Also in mustard, processing and RNA binding activity of an endoribonuclease of 54 kDa (p54) was found to be stimulated by phosphorylation (Liere and Link, 1997). Addition of oxidised glutathione also led to an activation, whereas incubation with the reduced form as well as dephosphorylation caused inhibition. Earlier experiments revealed an involvement of p54 in 3' end processing of *trnk* and *rps16* precurser transcripts *in vitro* (Nickelsen and Link, 1993).

A long known phosphoprotein associated with the thylakoid membrane was characterised recently by Carlberg *et al.* (2003) in spinach *(Spinacea oleracea)*. The protein is named thylakoid soluble phosphoprotein (TSP9) due to its partial release from the photosynthetic membrane on illumination and subsequent phosphorylation, and a molecular mass of 9 kDa. TSP9 was demonstrated to be a plant protein, with no homologs in cyanobacteria and algae. This attribute may the protein make a unique factor evolved only in oxygenic photosynthetic organisms.

On the basis of the obtained data mentioned above a model was proposed which includes the action of phosphorylated TSP9 and its possible role as interlinking factor connecting redox-controlled thylakoid protein phosphorylation to chloroplast gene expression (Zer and Ohad, 2003).

1.5 Aim of this thesis

The regulation of transcription of the genes *psaAB* and *psbA* encoding the P700 apoprotein of PSI and the D1 protein of PSII, in a redox signal-dependent manner has been demonstrated (Pfannschmidt *et al.*, 1999; Pfannschmidt *et al.*, 1999). The existence of components involved in the photosynthetic electron transport chain capable of generation of such redox signals as well as a number of mediating factors was manifold reported (for review see e.g. Allen and

Pfannschmidt, 2000; Dietz, 2003). These components are either able to perform redox reaction themselves or only activated by a redox reaction and thus part of a subsequent signal transduction cascade, whereas downstream-compounds operating at the target point of the signalling pathway may act as so called "redox-responsive factor".

Because the phytochrome signalling pathways may be affected by our light sources, I tested several *Arabidopsis* mutants with defects in cytosolic photoreceptors for their capability to perform a LTR. Seedlings with defects in phytochrome A *(phyA)*, phytochrome B *(phyB)*, a phytochrome double mutant (*phyA/phyB*), and a phytochrome B overexpressing mutant *(phyB-oe)* were exposed to either reducing or oxidising light signals and subsequently compared to a white-light control using chlorophyll fluorescence as measuring parameter. Additionally two cryptochrome (cry) mutants lacking cryptochrome 1 *(hy4)* or cryptochrome 2 *(cry2-1)*, respectively, were tested. Phytochrome and cryptochrome signalling plays a major role in photomorphogenesis and is associated with proteolytic active components which belong to the so called *COP/DET/FUS* class and are involved in transcriptional control of nuclear genes (for review see e.g. Quail, 2002; Schäfer and Bowle, 2002; Chen *et al.*, 2004). To investigate the interaction of redox-controlled and photomorphogenesis pathways I included *cop1-5* and *cop9-1* KO mutants in my investigation.

Because the postulated redox-responsive factor(s) are most probably nuclear encoded as it was shown for the great majority of chloroplast proteins (for review see Pfannschmidt *et al.*, 2003), and their expression is addressed by a likely unidentified pathway being part of the so called "plastid factor" (Oelmüller, 1989), I tested also signal transduction mutants for their acclimation capacity. The CAB gene underexpressed 1 *(cue1)* mutant underexpresses light-regulated nuclear genes encoding chloroplast localised proteins (Streatfield *et al.*, 1999). *cue1* is defect in the phosphoenolpyruvate/phosphate translocator of the chloroplast inner envelope. In genomes uncoupled (*gun*) mutants *Lhcb1* expression occurs in the absence of chloroplast development (Susek *et al.*, 1993). I investigated *gun1-1*, *gun4-1*, and *gun5-1*, all of them defective in the respective gene.

In parallel *psaAB* transcript accumulation was determined using primer extension analysis with a gene specific primer. The intention was to compare the results obtained from the chlorophyll fluorescence measurements on the transcriptional level to prove whether the light-dependent regulation can also be found for transcript accumulation.

The second approach aimed at the phosphorylation state of enriched protein fractions purified from isolated chloroplasts. Chloroplasts were isolated from mustard cotyledons from seed-

lings grown under the described yellow or red light sources, respectively. Subsequently nucleic acid binding proteins were enriched using Heparin-Sepharose (HS) chromatography columns. Such HS-fractions were shown earlier to have RNA polymerase activity (Pfannschmidt and Link, 1994). Furthermore results obtained from mass spectrometry (MS) confirmed the existence of proteins with predicted DNA-binding motifs and chloroplast target sequences in HS-fractions (Pfannschmidt *et al.*, 2000; Ogrzewalla *et al.*, 2002; Suzuki *et al.*, 2004). An important role of phosphorylation events in triggering light-dependent responses in the thylakoid membrane was suggested and proven manifold (for review see e.g. Gal *et al.*, 1997; Rintamäki and Aro, 2001). Especially state transition was shown to be functional linked to activity of a kinase what is activated by the redox state of PQ in conjunction with the interaction of PQH_2 and the Q_o-site of the Cyt b_6f complex (Vener *et al.*, 1997; reviewed by Aro and Ohad, 2003). Because transcription of *psaAB* and *psbA* is linked to the redox state of the PQ pool (Pfannschmidt *et al.*, 1999), too, it stood to reason to test HS-fractions for their phosphorylation properties. Thereby I concentrated on the following questions: Do the fractions have endogenous kinase activity? What changes in the phosphorylation pattern result from a treatment with an exogenous kinase before or after phosphatase treatment, respectively? What is the basic phosphorylation pattern of HS-fractions?

The presented work shows that redox signals generated in the photosynthetic electron transport chain have a deeper impact on the cellular signalling network as supposed so far. It was proved that the photosynthetic apparatus acts as an important sensor for changes in the light environment of land plants and that signals originating from the redox state of electron transport components are an own class of plastid signals targeted to the nucleus.

2 Compendium of the manuscripts

I) Pfannschmidt, T., Schütze, K., Fey, V., Sherameti, I., and Oelmüller, R. (2003). Chloroplast redox control of nuclear gene expression-a new class of plastid signals in interorganellar communication. Antioxid Redox Signal **5,** 95-101.

The review focuses on the role of chloroplast redox signals in nuclear gene expression. It summarizes the present knowledge about such pathways and describes the interaction with other signalling cascades, as well as specific problems in the transduction of these signals. It refers to data from two recent publications [**Pfannschmidt, T., Schütze, K., Brost, M., and Oelmüller, R.** (2001). A novel mechanism of nuclear photosynthesis gene regulation by redox signals from the chloroplast during photosystem stoichiometry adjustment. J Biol Chem **276,** 36125-36130.; **Sherameti, I., Sopory, S.K., Trebicka, A., Pfannschmidt, T., and Oelmüller, R.** (2002). Photosynthetic electron transport determines nitrate reductase gene expression and activity in higher plants. J Biol Chem **277,** 46594-46600.] and especially to unpublished work of Vidal Fey, which was later published in detail (see the following manuscript).

II) Fey, V., Wagner, R., Bräutigam, K., Wirtz, M., Hell, R., Dietzmann, A., Leister, D., Oelmüller, R., and Pfannschmidt, T. (2004). Retrograde plastid redox signals in the expression of nuclear genes for chloroplast proteins of Arabidopsis thaliana. J Biol Chem **Epub ahead of print**.

In this study the role of plastid redox signals in the regulation of plastid and nuclear genes during photosystem stoichiometry adjustment is characterised in *Arabidopsis thaliana*. For the first time the molecular response to PSI- or PSII-light in chloroplasts of *A. thaliana* is described. Determinations of glutathione content and redox state were performed to check possible interactions of different redox signals in this event. Via a macroarray approach the impact of plastid redox signals on the nuclear transcriptome of chloroplasts was determined.

Vidal Fey performed molecular and physiological characterisation of various Arabidopsis mutants using chlorophyll fluorescence measurements and determination of *psbA* and *psaAB* transcript amounts of *Arabidopsis* mutants. He established the primer extension analysis using a Li-Cor DNA sequencer and its use as RNA quantification method. Raik Wagner performed the western analyses. RNA used in the macroarray study was prepared by Thomas Pfannschmidt. Thomas Pfannschmidt, Raik Wagner and Katharina Bräutigam analysed and evaluated the macroarray data. All work described above was performed at the Department of Plant Physiology of the University of Jena of Prof. Ralf Oelmüller. Glutathione measurements were performed by Markus Wirtz and Rüdiger Hell at the Heidelberg Institute of Plant Sciences (HIP) of the University of Heidelberg. Hybridisation experiments for the macroarray study, signal quantification, and statistical analysis were performed by Angela Dietzmann and Dario Leister at the Max Planck Institute for Plant Breeding Research in Köln.

III) Wagner, R., Fey, V., Borgstädt, R., Kruse, O., and Pfannschmidt, T. (2004). Screening for Arabidopsis thaliana mutants deficient in acclimatory long-term response to varying light qualities using chlorophyll fluorescence imaging. In 13th International Congress on Photosynthesis, A.v.d. Est and D. Bruce, eds (Montréal: Allen Press) **in press**

In this work a screen for *Arabidopsis* mutants with defects in their acclimatory long-term response is introduced. Using chlorophyll fluorescence video imaging it is demonstrated that differences in chlorophyll fluorescence between PSI- and PSII-light acclimated Arabidopsis seedlings can be used as screening parameter to identify individuals with a changed or missing LTR.

Raik Wagner established the screening method using a video imaging system. He was responsible for plant growth, measurements, and data evaluation. Vidal Fey performed control measurements to determine chlorophyll fluorescence of WT plants, provided initial data during the establishment of the Fs/Fm values and constructed an improved PAM101 device (using PDA100) together with Raik Wagner, which provided the experimental base for the screening method. Rüdiger Borgstädt and Olaf Kruse at the Department of Cell Physiology of the University of Bielefeld helped Raik Wagner with the growth of mutant populations and the transfer of the measurement technique from the PAM101 to the FluorCam device.

IV) Fey, V., Allahverdiyeva, Y., Aro, E.M., and Pfannschmidt, T. (to be submitted to JBC). Photosynthetic redox control during light-quality acclimation in Sinapis alba has specific effects on phosphorylation state and composition of proteins involved in plastid gene expression.

In this work the relationship between light-quality-induced redox signals from photosynthetic electron transport and the phosphorylation state of thylakoid proteins and chloroplast protein fractions with DNA-binding and RNA polymerase activities was investigated. The results show distinct differences in accumulation as well as phosphorylation state for a small number of proteins suggesting an important role of phosphorylation events in the mediation of photosynthetic redox signals to the level of gene expression.

The project was elaborated by Vidal Fey and Thomas Pfannschmidt. Vidal Fey was responsible for plant growth, coordination as well as realisation of all molecular and biochemical work. A major part of the experiments was performed in collaboration with and in the Laboratory of Plant Physiology and Molecular Biology of Prof. Eva-Mari Aro at the Department of Biology of the University of Turku. Yagut Allahverdiyeva determined the 77K fluorescence spectra and participated in the respective method and results description.

3 Publications/Manuscripts I – IV

I

Pfannschmidt T, Schütze K, Fey V, Sherameti I, Oelmüller R (2003) Chloroplast redox control of nuclear gene expression-a new class of plastid signals in interorganellar communication. Antioxid Redox Signal **5:** 95-101

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Forum Review

Chloroplast Redox Control of Nuclear Gene Expression—A New Class of Plastid Signals in Interorganellar Communication

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IRENA SHERAMETI, and RALF OELMÜLLER

ABSTRACT

Chloroplasts are genetically semiautonomous organelles that contain their own subset of 100–120 genes coding for chloroplast proteins, tRNAs, and rRNAs. However, the great majority of the chloroplast proteins are encoded in the nucleus and must be imported into the organelle after their translation in the cytosol. This arrangement requires a high degree of coordination between the gene expression machineries in chloroplasts and nucleus, which is achieved by a permanent exchange of information between both compartments. The existence of such coordinating signals has long been known; however, the underlying molecular mechanisms and signaling routes are not understood. The present data indicate that the expression of nuclear-encoded chloroplast proteins is coupled to the functional state of the chloroplasts. Photosynthesis, which is the major function of chloroplasts, plays a crucial role in this context. Changes in the reduction/oxidation (redox) state of components of the photosynthetic machinery act as signals, which regulate the expression of chloroplast proteins in both chloroplasts and nucleus and help to coordinate the expression both in compartments. Recent advances in understanding chloroplast redox regulation of nuclear gene expression are summarized, and the importance for intracellular signaling is discussed. *Antioxid. Redox Signal.* 5, 95–101.

INTRODUCTION

CHLOROPLASTS, THE TYPICAL ORGANELLES of higher plants and green algae, provide all structural and functional properties necessary for photosynthesis, a process that converts light energy into chemical energy. Changes in environmental parameters such as light intensity, light quality, or temperature affect the photosynthetic electron transport and subsequently change the efficiency of light energy fixation. Photosynthetic organisms therefore developed several mechanisms to acclimate to a wide range of environmental conditions and to maintain the photosynthetic efficiency as high as possible (2, 4, 20).

The light-driven chemistry of photosynthesis involves a series of redox steps in structural components or functionally coupled pools of redox-active compounds, such as thioredoxin or glutathione. An increasing number of reports show that environmentally induced changes in the redox state of these electron transport components act as signals that regulate the expression of proteins of the photosynthetic machinery (for reviews, see 3, 9, 26, 42). This feedback mechanism couples the present function of photosynthesis to the expression of its structural constituents and thus acclimates photosynthesis to changing environmental cues. As the photosynthetic machinery consists of both plastid and nuclear encoded products, this requires a high coordination in the expression of these genes. Chloroplast redox signals play an important role in this intracellular coordination; their impact on different levels of plastid gene expression is also reviewed in this issue. This review focuses on the role of chloroplast redox signals in nuclear gene expression. It summarizes the present knowledge about such pathways and describes the interaction with other signaling cascades, as well as specific problems in the transduction of these signals.

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NUCLEAR GENE EXPRESSION—A TARGET FOR CHLOROPLAST REDOX CONTROL

Light regulation of nuclear gene expression is an extensively investigated field of research in plant biology, and the impact of photoreceptor-controlled signaling cascades is well documented (for reviews, see in 1, 5, 14, 16, 25, 33, 47). Chloroplast redox control, however, is a new concept of how light can influence the expression of nuclear genes. By this way, photosynthesis contributes important information to the regulation of nuclear gene expression that is not sensed by cytosolic photoreceptors. Through this, the chloroplast serves as a sensor for environmental changes and can induce physiological acclimation reactions (Fig. 1). The activation of transcription of nuclear encoded photosynthesis genes by illumination is a long-known phenomenon, but the first clear evidence that such regulation can be coupled to photosynthetic electron transport came from studies with the unicellular algae *Dunaliella tertiolecta* and *Dunaliella salina* (17, 28).

Escoubas *et al.* (17) demonstrated that transcription of the *Lhcb* genes [encoding chlorophyll-binding proteins of light-harvesting complex II (LHCII)] is stimulated when high light-acclimated cells are exposed to low-light intensities. By performing the same experiments in the presence of the site-specific electron transport inhibitors 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) (51), they could show that this increase is coupled to photosynthetic electron transport. In addition, they identified the redox state of the plastoquinone pool (PQ) as the controlling parameter: an oxidized PQ pool (generated by low light or DCMU treatment) activates *Lhcb* transcription, whereas a reduced one (generated by high light or DBMIB treatment) represses it.

In an independent study, Maxwell *et al.* (28) came to similar conclusions. They analyzed *Lhcb* transcription and LHCII apoprotein content in response to varying light intensities under controlled temperature environments. This approach is based on the observation that light-intensity effects also depend on ambient temperature. For instance, the same light intensity that represents low-light condition at a high temperature can also represent high-light condition under low temperature. The enzymatic steps in the photosynthetic dark reaction become rate-limiting under low temperature, thus generating a higher excitation pressure. In their experiments, Maxwell *et al.* (28) found that relaxation of high excitation pressure both thermodynamically (shifting cells from 13°C to 30°C under constant light) and photodynamically (shifting cells from light to dark growth regimes under constant temperature) results in an increase of *Lhcb* transcription and LHCII protein accumulation. They concluded that the redox poise of intersystem electron transport represents a common sensing/signaling pathway for *Lhcb* transcription under various stress conditions.

A recent study using *Lemna perpusilla* as model organism demonstrated that *Lhcb* transcript abundance and LHCII protein content are increased under low-light intensities also in a higher plant (52). Using a cytochrome $b_6 f$ complex *(cyt* $b_6 f$*)* deficient mutant, Yang *et al.* (52) showed that this regulation is coupled to photosynthetic electron transport. The mutant failed to respond to changing light intensities and exhibited always a high light-acclimated phenotype. Further investigations with DCMU in combination with results from the photosynthetic mutant led to the conclusion that the redox state of the PQ pool represents the controlling redox parameter in this regulation, which is fully consistent with the observations described above.

High-light treatment was also used to demonstrate the existence of another type of chloroplast redox signals in the higher plant *Arabidopsis thaliana* (22). An increase of incident light intensity from 200 to 2,000 μE increased the transcript level of two genes for cytosolic ascorbate peroxidases (*Apx1*, *Apx2*) within 15 min. DCMU and DBMIB treatments

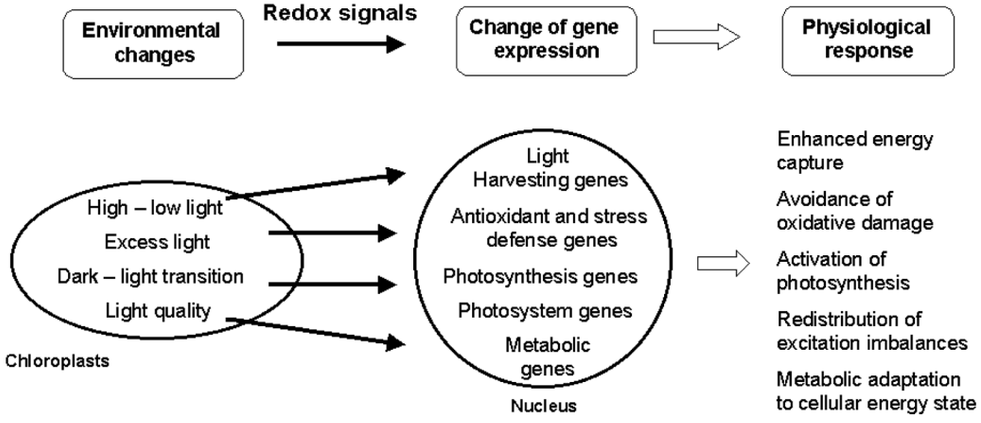

FIG. 1. Physiological role of chloroplast redox signals. Fluctuating environmental conditions, especially changes in illumination, result in changes of photosynthetic efficiency. Concomitantly, the redox state of chloroplast components is affected. Such redox changes represent a sensor for the environmental fluctuation and serve as signals that influence the expression of nuclear genes whose products, in turn, are involved in an appropriate physiological response to counteract the limitation of photosynthesis by the environment. A general scheme for this is given at the top of the figure; several concrete examples are summarized below. For more details, see text.

of leaf discs indicated that this signaling pathway originates from the PQ pool; however addition of reduced glutathione abolished this signal, suggesting that the redox state of the glutathione pool may play an interacting regulatory role in this context. Both ascorbate peroxidases and glutathione are scavengers of reactive oxygen species (ROS) that are generated preferentially under stress conditions, such as high light. Further experiments with transgenic *Arabidopsis* plants containing *Apx2* promoter:luciferase fusions demonstrated that the high light-induced signal can be transported from a high light-treated tissue to an untreated tissue plant probably via high light-generated hydrogen peroxide (H_2O_2) (23). This phenomenon, called "systemic acquired acclimation," suggests that redox signals in higher plants not only are transduced from chloroplasts to the nucleus of the same cell, but also function in a tissue overriding manner. Further investigations demonstrated that also transcripts for a peroxisomal catalase (*Cat2*), the chloroplast glutathione peroxidase (*Gpx2*), and glutathione *S*-transferase (*Gst*), as well as the pathogenesis-related type 2 protein (*Pr2*), were induced by light stress, but interestingly only in systemic, nonilluminated leaves (31).

In transgenic tobacco plants harboring a pea ferredoxin transcribed region (*Fed1*) under the control of the cauliflower 35S promoter, the abundance of this *Fed1* message was four- to fivefold increased in reilluminated plants compared with dark-adapted plants (38). The light-induced increase was shown to be coupled to photosynthetic electron transport because DCMU treatment could abolish the light effect. This response is gene-specific because *Lhcb* transcript accumulation was not affected by the drug. In addition, it could be demonstrated that light-dependent polyribosome loading of *Fed1* mRNA, an important prerequisite for efficient translation, was also diminished by the DCMU treatment. The same was observed for the *Lhcb* message. Further experiments indicated that interruption of photosynthetic electron transport by either dark or DCMU treatment led to a rapid destabilization of the *Fed1* mRNA (39). Taken together, these observations demonstrate that chloroplast redox signals affect also post-transcriptional events besides transcription.

In mustard photosystem stoichiometry adjustment in response to varying light-quality conditions was shown to be regulated by PQ redox control of transcription of the chloroplast genes *psbA* and *psaAB* [encoding reaction center proteins of photosystem II (PSII) and photosystem I (PSI)] (41). In a recent study using transgenic tobacco plants harboring promoters of nuclear-encoded PSI genes *petE*, *petH*, *psaD*, and *psaF* [encoding plastocyanin (PC), the ferredoxin:NADP:oxidoreductase (FNR), and the PSI subunits *PsaD* and *PsaF*] fused to the *uidA* gene, it could be demonstrated that the same light-quality variations also affect nuclear gene expression (43). In combination with DCMU and DBMIB treatments, these experiments showed that the PC promoter activity is controlled by the redox state of the PQ pool, whereas the *psaD* and *psaF* promoters are under the control of a yet unidentified downstream component of the photosynthetic electron transport chain. All three promoters have in common that they are activated by the reduction of photosynthetic electron transport components. The FNR promoter revealed no regulation under any condition. A further study using the same experimental approach showed that the *Arabidopsis* nitrate reductase (*Nia2*) promoter also responds to the light-induced redox signals. However, it is activated by a predominant oxidation of the electron transport components either by PSI light or by DCMU or DBMIB treatment (45). Beside transcription, the nitrate reductase enzyme activity was regulated in exactly the same way. The use of a *cyt* b_6 *f*-deficient *Lemna aequinoctialis* mutant (8) confirmed that the photosynthetic electron transport regulates nitrate reductase activity. The mutant showed constitutive high activity under all conditions similar to PSI light-acclimated wild type. These results show that redox signals that are shown to act within the organelle (41) can also extend to the nucleus and that the same signal can have different gene-specific effects.

These examples show that chloroplast redox signals affect nuclear gene expression under various physiological conditions and at different levels of expression. The increasing number of reports describing such regulation pathways suggests that a complex network of redox signals rather than a single signaling pathway couples chloroplast function to the nuclear gene expression machinery.

INTERACTION OF REDOX SIGNALS WITH OTHER SIGNALING PATHWAYS

All chloroplast redox signals described in the previous section are connected to illumination and photosynthesis. As photosynthesis is the central point of plant energy metabolism that is connected with almost all processes in the cell, it should be expected that there exist interactions with other regulating signals (Fig. 2). An example for this can be found in a recent study with an *Arabidopsis* cell culture (36). It is well known that high external sugar concentrations repress nuclear photosystem gene expression (46). Oswald *et al.* (36) found that the increase in transcription of *Lhcb* and *RbcS* genes, which is normally observed when the external sugar is removed, can be blocked by addition of DCMU to the culture medium. The same experiments performed with transgenic *Arabidopsis* lines carrying *Lhcb2* and PC promoters fused to

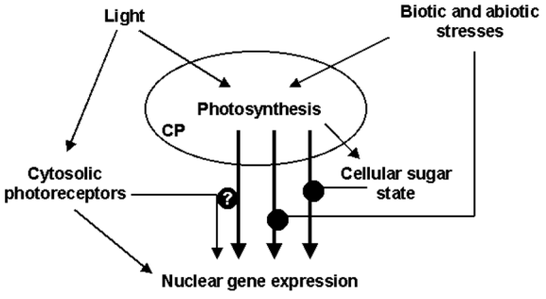

FIG. 2. Known and putative interactions of plastid redox signals with other signaling pathways during control of nuclear gene expression. Thick arrows indicate redox signals; thin arrows are influences of various parameters on cellular components or processes. Black circles indicate interactions of signaling pathways. CP, chloroplast. For more details, see text.

the luciferase reporter gene confirmed these results *in planta*. Further investigations using the sugar-insensitive *Arabidopsis* mutant *sun6* also carrying the PC-LUC construct revealed that the PC promoter activity is increased by sugars and that DCMU treatment has no effect on this response. Oswald *et al.* (36) postulated that under weak light a redox signal from photosynthetic electron transport activates the derepression of nuclear photosynthetic genes when the strong antagonistic suppression by external sugars is removed. This signal is not active once derepression has occurred. This suggests that photosynthetic gene expression is balanced by cellular sugar status and photosynthetic activity to fulfil the demands of energy metabolism. If this model also accounts for high-light conditions has to be tested.

As described above, excess-light-induced ROS formation activates nuclear antioxidant genes (22); however, the generation of ROS can be also observed under other abiotic or biotic stresses, such as chilling, wounding, or pathogen attack. It has been shown that transcripts for several components of the cytosolic ROS-scavenging system are induced by such stresses (for review, see 31). ROS formation also affects the degree of reduction of major antioxidant pools, such as glutathione, which is regarded as a key component of antioxidant defense in plants (18). In general, there is increasing evidence that ROS and the redox states of antioxidant pools regulate the expression of nuclear antioxidant genes. In this instance, redox signals via ROS serve as integrating components of several signaling pathways that help the plant to adapt to various types of stresses. As the cell is not able to identify the respective type of stress only by recognizing the ROS levels in the stroma of the plastid or in the cytoplasm, further signals are required. For instance, in the case of systemic acquired resistance, ROS provide the common signal for stress, but the response appears to be elicited only in co-action with nitric oxide (13). Therefore, ROS-mediated redox signals may interact with many other signals to define changes in environmental conditions. The importance for a sensitive sensing of cellular ROS levels has been demonstrated also in transgenic tobacco plants overexpressing a plastid γ-glutamylcysteine synthetase. Despite the potentially higher ROS scavenging capacity, these plants suffered from strong oxidative stress that is caused most probably by their inability to sense changes in ROS formation (12).

Redox signals induced by the photosynthetic process are strictly light-dependent. From the present data, it appears that different types of signals can be generated depending on the incident-light quantity, and a model has been proposed in which such different signals operate in a hierarchical order (42). It appears likely that under certain conditions, different redox-controlled signaling pathways may interact with each other.

Light-regulated nuclear gene expression is largely affected by the red and blue light-absorbing cytosolic photoreceptors (see Introduction). Furthermore, there exists increasing evidence that plastid signals interact with phytochrome signaling pathways that also influence expression of nuclear genes for plastid proteins (27). At present, it is unclear if chloroplast redox signals interfere or interact with cytosolic photoreceptor cascades. The availability of photoreceptor mutants provides a useful tool to solve this problem. First results show that phytochrome A- and phytochrome B-deficient *Arabidopsis* mutants respond in the same way to light quality-induced redox signals as the wild type, which indicates that the redox signaling cascade is functional in phytochrome A- and phytochrome B-deficient *Arabidopsis* mutants (Fey and Pfannschmidt, unpublished observations).

CHLOROPLAST REDOX SIGNALS AND THEIR ROLE AS "PLASTID FACTOR"

Early investigations in the 1980s especially with norflurazon, an inhibitor of phytoene desaturase, gave the first hints that chloroplast function or development influences nuclear gene expression. This led to the postulation of a so-called "plastid factor" that couples the functional state of the plastids to the expression of nuclear encoded plastid proteins (for reviews, see 34, 50). Norflurazon-treated plants exhibit a white phenotype that is caused by an arrest in chloroplast biogenesis through photobleaching because the inhibitor blocks chlorophyll biosynthesis. As a concomitant effect, an inhibition of the expression of some nuclear genes (*i.e., RbcS*, *Lhcb*) encoding chloroplast proteins was observed. The same results were obtained when chloroplast gene expression was blocked by inhibitors such as tagetitoxin, rifampicin, or cycloheximide (for review, see 19), suggesting the involvement of (a) chloroplast gene product(s) in this retrograde signaling. Further studies point to intermediates or enzymatic components of the chlorophyll biosynthesis pathway as putative plastid regulators of nuclear gene expression (for reviews, see 6, 21, 37, 44).

The molecular nature of the "plastid factor(s)," as well as the way this signal is transduced across the chloroplast envelope into the cytosol, is still unknown. Recent studies with *Arabidopsis cue1*, *gun5*, and *laf6* mutants, however, have shed more light on this complex signaling network. Their defects are located in the phosphoenolpyruvate/phosphate translocator (*cue1*), the *H* subunit of the magnesium chelatase (*gun5*), and a new ABC transporter protein (*laf6*). All three components are located in the chloroplast envelope, suggesting that the defect interrupts the transport of one or several plastid factor(s), and models of respective signaling pathways have been proposed (29, 30, 48). Earlier studies with mustard (35, 40) and a recent study on the pea *lip1* mutant (49), which shows photomorphogenic development in the dark, demonstrated that nuclear photosynthesis gene expression depends on plastid translation or transcription in a light-independent manner. This suggests that the plastid factor(s) is not necessarily coupled to light, thylakoid formation, or photosynthesis. At present, it is not clear how this light-independent signal relates to the signaling pathways identified in the *Arabidopsis* mutants; however, as redox signals from photosynthesis are strictly light-dependent, they represent a different class of plastid signals.

Mutants with defects in chloroplast-to-nucleus signaling often show strong developmental defects (19, 44). Especially in the beginning of cell or tissue differentiation, the developmental state of plastids may be a very important parameter for further development of its host cell. Early plastid signals

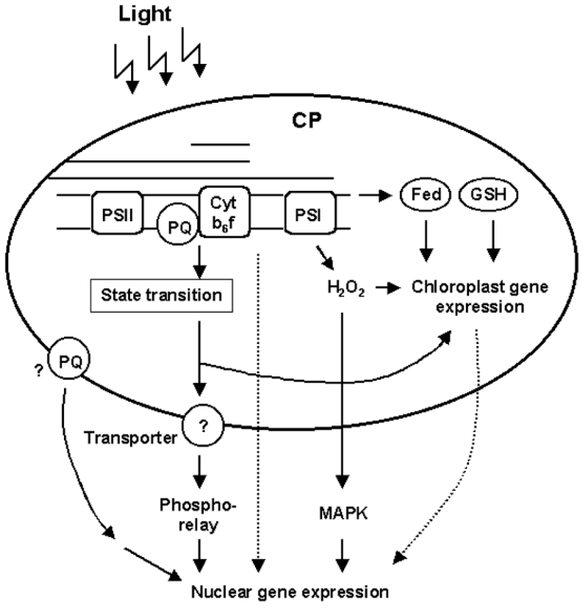

FIG. 3. Putative transduction mechanisms of chloroplast redox signals to the nucleus. Photosynthetic electron transport components are sketched within a chloroplast (CP). Horizontal lines above them represent the thylakoid membrane system and indicate its connection with the envelope of the chloroplast. Arrows represent redox signals; dotted arrows represent putative redox signaling pathways. MAPK, mitogen-activated protein kinase. For details, see text.

therefore may represent light-independent developmental signals, whereas plastid signals in fully developed tissue report the actual physiological state of the organelle. From the present data, it is apparent that photosynthetic redox signals play an important role in this second class of signals (compare Fig. 1). In addition, it can be assumed that cytosolic photoreceptors may play an important role in gene expression control of plastid proteins during greening of seedlings and that its role is overtaken by other factors such as chloroplast redox signals in fully green plants.

Although the function of chloroplast redox signals appears to be relatively clear, the molecular mechanisms of its transduction toward the nucleus are only poorly understood (Fig. 3). Best models for signal transduction exist for H_2O_2–mediated redox signals. H_2O_2 is known to be membrane-permeable and may pass the chloroplast envelope without directed transport. In the cytosol it induces the respective change in gene expression by activating a mitogen-activated protein kinase cascade (24). For redox signals starting from the PQ pool, the transduction is less clear. It is likely that the same signaling pathway that regulates the state transition also regulates chloroplast gene expression (3), and it is conceivable that an additional branch of this pathway extends to the nucleus. How the signal passes the envelope, for instance via a transporter, is unknown to date. However, there exists experimental evidence that the PQ redox signal is transformed into a phosphorylation signal in the cytosol (10, 17). As a second possibility, PQ redox signals may be sent directly to the nucleus via PQ molecules located in the envelope membrane. As the inner-chloroplast membrane system is in close contact to the envelope and envelope-located electron transport has been found (32), such PQ molecules could report the redox state of the PQ pool to a putative cytosolic receptor. Chloroplast redox signals may be also reported to the nucleus indirectly by their effects on chloroplast gene expression or by affecting the biochemistry of the organelle. As outlined above, there exist plastid signals that tightly couple nuclear gene expression to the function of chloroplast gene expression and therefore to inner-chloroplast redox signals. Besides these possible transduction pathways, several redox-regulated gene expression events are described from which the transduction of the redox signals is completely unknown.

TABLE 1. SUMMARY OF REDOX-CONTROLLED NUCLEAR GENES SORTED BY TYPE OF REGULATING REDOX SIGNAL

*Gene and gene class**	*Activating redox control parameter* *Oxidation signals*	*Organism and reference*
Lhcb (LH)	Oxidized PQ pool	*Dunaliella tertiolecta* (17)
Lhcb (LH)	Oxidized intersystem electron carrier	*Dunaliella salina* (28)
Lhcb (LH)	Oxidized PQ pool	*Lemna perpusilla* (52)
Apx1, Apx2 (AOS)	Reduced PQ pool, high H_2O_2 concentration	*Arabidopsis, Arabidopsis* (transgenic) (22, 23)
Gpx2, Cat2, Gst, Pr2 (AOS)	High H_2O_2 concentration	*Arabidopsis* (31)
Lhcb2, RbcS (PSY)	Photosynthetic electron transport (oxidized)	*Arabidopsis* (cell culture) (36)
Nia2 (M)	Photosynthetic electron transport (oxidized)	Tobacco (transgenic) (45)
	Reduction signals	
Fed1 (PSY)	Photosynthetic electron transport (reduced)	Tobacco (transgenic) (38)
PetE (PSY)	Photosynthetic electron transport (reduced)	*Arabidopsis* (transgenic) (36)
PetE (PSY)	Reduced PQ pool	Tobacco (transgenic) (43)
PsaD, PsaF (PS)	Photosynthetic electron transport (reduced)	Tobacco (transgenic) (43)

*For physiological details and encoded proteins, see text. LH, light-harvesting genes; AOS, antioxidant and stress genes; M, metabolic genes; PSY, photosynthesis genes; PS, photosystem genes.

PERSPECTIVES

The increasing number of reports describing chloroplast redox control of nuclear gene expression suggests that there exist more signals than previously anticipated and underlines the importance of such signals for the development and metabolism of the cell. Currently, we have more questions than answers; however, the present data already show that redox signals are involved in many signaling pathways, such as light, stress, energy, and metabolic signaling, and more are expected. In addition, redox signals from other cell components, *i.e.,* mitochondria and peroxisomes (for review, see 11), may interact with those from the chloroplast. Furthermore, the identity and number of all redox-controlled genes are unknown to date. Genomic approaches using microarray techniques will give us a more complete picture within the next few years. A first study using a H_2O_2-treated *Arabidopsis* cell culture showed that at least 175 different open reading frames responded to oxidative stress (15). As the number and identities of redox-responsive genes will vary depending on the physiological test systems, the presently known genes (Table 1) may represent only the tip of the iceberg. It will be fascinating work to unravel all the roles that redox signals play in the intracellular signaling network.

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ABBREVIATIONS

cyt b_6 *f,* cytochrome b_6 *f* complex; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea; FNR, ferredoxin:NADP: oxidoreductase; H_2O_2, hydrogen peroxide; LHCII, light-harvesting complex of photosystem II; PC, plastocyanin; PQ, plastoquinone; PSI, photosystem I; PSII, photosystem II; ROS, reactive oxygen species.

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Retrograde Plastid Redox Signals in the Expression of Nuclear Genes for Chloroplast Proteins of *Arabidopsis thaliana**[S]

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Excitation imbalances between photosystem I and II generate redox signals in the thylakoid membrane of higher plants which induce acclimatory changes in the structure of the photosynthetic apparatus. They affect the accumulation of reaction center and light-harvesting proteins as well as chlorophylls *a* and *b*. In *Arabidopsis thaliana* the re-adjustment of photosystem stoichiometry is mainly mediated by changes in the number of photosystem I complexes, which are accompanied by corresponding changes in transcripts for plastid reaction center genes. Because chloroplast protein complexes contain also many nuclear encoded components we analyzed the impact of such photosynthetic redox signals on nuclear genes. Light shift experiments combined with application of the electron transport inhibitor 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea have been performed to induce defined redox signals in the thylakoid membrane. Using DNA macroarrays we assessed the impact of such redox signals on the expression of nuclear genes for chloroplast proteins. In addition, studies on mutants with lesions in cytosolic photoreceptors or in chloroplast-to-nucleus communication indicate that the defective components in the mutants are not essential for the perception and/or transduction of light-induced redox signals. A stable redox state of glutathione suggest that neither glutathione itself nor reactive oxygen species are involved in the observed regulation events pointing to the thylakoid membrane as the main origin of the regulatory pathways. Our data indicate a distinct role of photosynthetic redox signals in the cellular network regulating plant gene expression. These redox signals appear to act independently and/or above of cytosolic photoreceptor or known chloroplast-to-nucleus communication avenues.

The light environment of plants is highly variable. This is of particular importance for photosynthesis, because changes in incident light intensity or quality can reduce the efficiency of photosynthetic electron transport and therefore the net energy fixation. Plants have developed many acclimatory mechanisms at the molecular level that enable them to cope with such changes. Most prominent responses are dynamic changes in the structure and composition of the photosynthetic apparatus (1–3).

Light quality and quantity gradients that occur *e.g.* in dense plant populations induce an imbalance in excitation energy distribution between the two photosystems (which work electrochemically in series) and therefore reduce photosynthetic efficiency. To counteract such imbalances plants re-distribute light energy in a short term by state transitions (4, 5) and in a long term by a re-adjustment of photosystem stoichiometry. This results in a supply of more light quanta to the less active side of the electron transport chain (6–8). Both processes are regulated by light-induced changes in the redox state of photosynthetic components (9–11). While the short term response acts via post-translational phosphorylation of existing antenna proteins, the long term response (LTR)[1] requires the synthesis of new components and hence has to affect gene expression. This implies signaling routes that connect photosynthetic electron transport/efficiency with the expression machinery. Studies in the last decade show that such functional connections exist at multiple levels and in virtually all classes of photosynthetic organisms. In higher plants photosynthetic redox control has been found at the levels of transcription (12–19), transcript stability (20–23), ribosome loading (24–26), translation initiation (27), and protein accumulation (28).

The origin of the respective signal transduction pathways can be very different. To date three classes of redox signals can be distinguished: the first one is generated directly within the electron transport chain, the second is represented by photosynthesis-coupled redox-active compounds such as thioredoxin or glutathione, and the third is constituted by reactive oxygen species, which are unavoidable by-products of photosynthesis (29–31). Such signals operate within the chloroplast, but have also been shown to affect the expression of some nuclear genes for plastid proteins. Therefore, they may represent a new class of the so-called "plastid signals" (32–35). Retrograde signaling represents an important feedback control that couples the expression of nuclear encoded plastid proteins to the functional state of the chloroplast. Underlying signaling mechanisms in this communication still represent a great field of open questions in plant cell biology. To date neither the impact of retro-

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[S] The on-line version of this article (available at http://www.jbc.org) contains Supplemental Table SI.

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[1] The abbreviations used are: LTR, long term response; Chl, chlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea; PAM, pulse-amplitude modulation; *psaAB*, chloroplast genes for PsaA and PsaB reaction center proteins of photosystem I; *psbA*, chloroplast gene for reaction center D1 protein of photosystem II; PSI, photosystem I; PSII, photosystem II; PIPES, 1,4-piperazinediethanesulfonic acid; GST, glutathione *S*-transferase; PEP, plastid-encoded RNA polymerase; μE, μmol photons per m^2 and s.

grade redox signals on the nuclear transcriptome of chloroplasts nor possible interaction with other retrograde signals or with photoreceptor-mediated light signals are known while an interaction with sugar signals has been reported (19).

In this study we characterize the role of plastid redox signals in the regulation of plastid and nuclear genes during photosystem stoichiometry adjustment in *Arabidopsis thaliana*. By the use of this model organism we take advantage from the mutant and array resources available for this organism offering experimental strategies, which are not possible with tobacco and mustard used in earlier studies (9, 14). We describe for the first time the molecular response to PSI or PSII light in chloroplasts of *A. thaliana.* Determinations of glutathione content and redox state were performed to check possible interactions of different redox signals in this event. Cross-talk of the LTR with other signaling routes has been tested in mutants lacking either photoreceptors or components of plastid-to-nucleus signal pathways. By using a macroarray approach we determined the impact of plastid redox signals on the nuclear transcriptome of chloroplasts. Our study indicates that chloroplast redox signals from the thylakoid membrane represent a novel and separate class of plastid signals.

EXPERIMENTAL PROCEDURES

Plant Growth—Plants were grown in temperature-controlled growth chambers at 22 °C under continuous light. *Arabidopsis* seeds (var. Col 0 or *Landsberg erecta* and mutant lines in the respective backgrounds) were sown either sterile on half-strength Murashige and Skoog (MS) medium containing 1.35% sucrose or on earth substituted with vermiculite. Density of seeds was adjusted in such a way that 16-day-old plants did not shadow each other. After 2 days at 4 °C plants were grown for 10 days under white light provided by 30-watt white stripe lamps (OSRAM, München, Germany) with a photosynthetic-active radiation of ~35 μE. This white light pre-treatment was found to be necessary for the plants to develop a normal leaf anatomy and hence a true acclimatory response. Direct germination and growth under the PSI or PSII light sources resulted in aberrant leaf anatomy due to the lack of blue radiation of these light sources. After growth in white light, plants were acclimated to PSI (photosynthetic active radiation, ~20 μE) or PSII (photosynthetic active radiation, ~30 μE) light for 6 days or they were first acclimated to one light source for 2 days followed by 4 days under the respective other light source. PSI and PSII light sources have been described earlier (9, 12); however, the incandescent bulbs of the PSI light source were replaced by 18-watt fluorescent stripe lamps "Red" (OSRAM, München, Germany) of the same photon flux density to reduce thermal radiation. The photosynthetic active radiation was determined by using the lightmeter LI-250 (Heinz Walz GmbH, Effeltrich, Germany). It must be noted that the far-red spectrum of the PSI light is outside of the detection range of the LI-250. White light control plants were grown for 16 days under the white light source alone.

Chlorophyll Fluorescence Measurements—In vivo Chl *a* fluorescence parameters were determined at room temperature with a pulse amplitude-modulated (PAM) fluorometer (PAM101/103, Heinz Walz). 10–15 seedlings grown on MS medium were measured simultaneously as described previously (14). After dark acclimation (8–10 min) the measuring beam was turned on, and minimal fluorescence (F_o) was determined. Then leaves were exposed to a 500-ms flash of saturating white light (6000 μE) to determine maximal fluorescence (F_m) and the optimum quantum yield F_v/F_m value was calculated as $F_m - F_o/F_m$ (36). Subsequently, leaves were illuminated with 100 μmol of photons m^{-2} s^{-1} of actinic red light of 600 nm (Walz 102-R). Fluorescence was recorded in the saturation pulse mode by application of saturating flashes every 30 s to determine maximal fluorescence of illuminated leaves (F_m') until a stable fluorescence level (F_t) was reached. Actinic light was switched off, and far-red light (Walz 102-FR) was turned on to oxidize the electron transport chain and to determine minimal fluorescence (F_o') in the light-acclimated state. The steady-state fluorescence F_s was then calculated as $F_t - F_o' = F_s$. The optimum quantum yield describes the maximal photosynthetic capacity of a plant and was taken as a measure for photosynthetic efficiency of the mutant lines analyzed in this study in comparison to wild type. For wild type we found F_v/F_m values of 0.8–0.83, which typically indicate that the plant analyzed has no decreased photosynthetic efficiency. Only plants with a wild type like behavior were tested for their response to the two light sources. A proper acclimation response to PSI or PSII light is characterized by a significant change in the F_s/F_m value as shown earlier (14) and reflects the structural differences in the photosynthetic apparatus of these plants. The difference of 10 μE in photosynthetic active radiation between PSI and PSII light has no detectable impact on this acclimation, because in control experiments PSI plants showed the same decrease in F_s/F_m after acclimation to either 20 or 30 μE PSII light (data not shown). One-way analysis of variance was used to reveal significant differences in F_s/F_m values of plants grown under the defined conditions. Light treatment was used as a factor, and the F_s/F_m value as a dependent variable. If a significant influence of light treatment was determined, post-hoc tests (pairwise multiple comparison test for lowest significance difference) was performed to find out which groups differ from each other. $p < 0.5$ determines significant differences between various samples (see Supplementary Table SI). All tests were performed using SPSS 11.5.

Chlorophyll Content Determination—Total chlorophyll was determined spectroscopically after grinding of leaves in liquid nitrogen and extracting chlorophylls with 80% (v/v) buffered acetone. Concentrations of chlorophylls *a* and *b* were calculated by using the extinction coefficients from previous studies (37).

Western Analyses of Chloroplast Proteins—20 g of leaf material of plants grown on soil were harvested under the respective light source and directly homogenized in ice-cold buffer containing 0.05 M Hepes/KOH, pH 8.0, 0.33 M sorbitol, 0.001 M $MgCl_2$, and 0.002 M EDTA. The material was filtered through four layers of muslin and one layer of Miracloth, followed by a centrifugation (10 s at 6000 rpm). The pellet was washed twice in homogenization buffer and resuspended in 1 ml of the same buffer. Concentrations of chloroplasts were determined microscopically by counting diluted aliquots in a Fuchs-Rosenthal chamber. 2×10^5 plastids of each preparation were lysed and denatured in 5× SDS sample buffer (final concentrations: 0.4% SDS, 0.1% β-mercaptoethanol, 2% glycerol, 0.02% bromphenol blue) by incubation for 5 min at 95 °C. Insoluble particles were removed by centrifugation, and samples were loaded on denaturing 10% SDS-polyacrylamide gels (38) and separated overnight at 45 V. Proteins were transferred to a nylon membrane (Roti-Nylon Plus, Roth, Karlsruhe, Germany) at 400 mA for 1 h using a semi-dry blotting apparatus, and the membrane was saturated in Tris-buffered saline containing 2% fat-free milk powder. Incubation with polyclonal antisera followed standard protocols (39). Antibodies for D1, Lhca3, and Lhcb1 were purchased from AgriSera (Vannas, Sweden). Detection of the first antibody was performed with a goat-anti-rabbit-IgG-peroxidase conjugate (Sigma, München, Germany) and the enhanced chemiluminescent (ECL) detection system. For visualization of marker proteins and to prove blotting efficiency, membranes were stained with Amido Black (39) after the ECL reaction.

RNA Preparations—RNA for primer extension analyses was isolated from plants grown on MS medium. RNA for array analyses was isolated from wild-type (Col-0) plants grown on soil. Leaf material was harvested and frozen in liquid N_2 under the respective light source. Total RNA was isolated using the TRIzol reagent (Invitrogen) following a protocol described earlier (40). Concentration and purity of RNA samples were determined spectroscopically in a Biophotometer (Eppendorf, Hamburg, Germany). Intactness was proven by ethidium bromide staining of rRNA species after electrophoretic separation of aliquots on denaturing 1.2% agarose gels containing formaldehyde (39). Isolated RNA was stored at −80 °C until further use.

Primer Extension Analyses—Primer extension analyses (41) were carried out according to a protocol from Li-Cor (Bad Homburg, Germany). 5 μg of total RNA was resolved in 20 μl of hybridization mixture containing 1.25 μM infrared dye 700-labeled *psaA*-specific and infrared dye 800-labeled *psbA*-specific primers and 18 μl hybridization buffer (50% formamide, 1 mM EDTA, 400 mM NaCl, 40 mM PIPES, pH 6.4). After denaturation at 80 °C for 15 min RNA/primer hybrids were allowed to form at room temperature for 1 h. Hybrids were precipitated with 2.5 volumes of 96% EtOH at −80 °C for 30 min and washed with 100 μl of 70% EtOH. Precipitates were dried and resolved in 2 μl of 5× buffer for Moloney murine leukemia virus reverse transcriptase, 4 μl of 5 mM dNTPs, 3 μl of H_2O, and 1 μl of Moloney murine leukemia virus reverse transcriptase (MBI Fermentas, 200 units/μl)), and incubated 1 h at 42 °C. 1 μl of the samples was mixed 1:1 with formamide loading dye (Amersham Biosciences), applied onto a sequencing gel (4% acrylamide, 1× TBE (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, ph 8.0), 7 M urea, 66 cm × 0.25 mm) and separated according to Li-Cor (Bad Homburg, Germany) recommendations. Gene-specific primer sequences: *psaA*, 5′ infrared dye 700, 5′-CCC ATT CCT CGA AAG-3′ (sequence position +65 to +79 relative to ATG); *psbA*, 5′ infrared dye 800, 5′-AGA CGG TTT TCA GTG-3′ (sequence position +69 to +83

relative to ATG). The same primers were used to sequence the respective region of *Arabidopsis* chloroplast DNA using a cycle sequencing kit (MBI Fermentas, St. Leon-Roth, Germany).

Determination of Thiol Group Content and Redox State of Glutathione—For isolation of total glutathione and cysteine 25 mg of leaf material was ground in liquid N_2 and extracted with 0.5 ml of buffer E (100 mM phosphate, pH 7.1, 50% methanol, 5 mM dithiothreitol) for 10 min at 60 °C while shaking. Homogenates were centrifuged twice at 15,400 × *g* for 5 min at room temperature, and supernatants were used for further analysis. Determination of oxidized glutathione was based on the same extraction, but dithiothreitol in buffer E was replaced by 5 mM *N*-ethylmaleimide to block reduced glutathione (42). Reduction of oxidized thiols in the extracts (0.02 ml) was carried out at room temperature for 60 min in a total volume of 0.27 ml containing 134 mM Tris, pH 8.3, 1 mM dithiothreitol. Then thiols were derivatized for 15 min by adding 0.03 ml of monobromobimane (Calbiochem, La Jolla) to a final concentration of 3 mM (2.5-fold excess above total thiol concentration). Resulting monobromobimane derivatives were stabilized by addition of 0.7 ml of 5% acetic acid and detected fluorometrically (Fluorometer RF 551, Shimadzu) at 480 nm by excitation at 380 nm after separation by reverse-phase HPLC using a Waters HPLC-system (Waters 600E Multisolvent Delivery system, Autosampler 717plus) connected to a Nova-Pak C18 4.6 × 250-mm column (pore size, 4 μm). Glutathione and other thiols were separated by applying an isocratic flow (1.3 ml/min) of buffer A (100 mM potassium acetate, pH 5.5, 9% methanol) for 12.5 min. The column matrix was washed with 100% methanol for 3 min and re-equilibrated for 8.5 min in buffer A. Data acquisition and processing was performed with Millenium^{32} software (Waters). Reduced glutathione concentrations were calculated from the difference between total and oxidized glutathione. Recovery rates were higher than 95% for reduced and oxidized glutathione and higher than 90% for cysteine, respectively, as determined by spiking of samples with internal standards. Samples were analyzed in quadruplicate.

Expression Profiling—The 3292-GST nylon array, including 2661 nuclear chloroplast genes and 631 genes coding for non-chloroplast proteins, has been described previously (43). Experiments were performed with plant material corresponding to pools of at least 250–500 individuals. To obtain larger amounts of tissue of healthy and unstressed plants, seedlings were initially grown 22 days under white light (short day periods, 8-h light/16-h dark) on soil. Plants were then acclimated to: (i) PSI light (5 days), (ii) PSI light (3 days) followed by PSII light (2 days), (iii) PSI light (3 days) followed by PSII light plus 5 μM DCMU (2 days), or (iv) PSI light plus 5 μM DCMU. DCMU (Sigma) has been applied to plants directly before performing the light shifts using a fine sprayer as described before (14). DCMU stock solution was 10 mM in 50% ethanol, and the applied concentration was prepared by dilution in sterile water directly prior use. The drug was found to be completely stable during the 2-day period of experiment as determined by the effect on chlorophyll *a* fluorescence using a PAM101 fluorometer. Effects of DCMU on photosynthetic electron flow have been proven by determination of ΦPSII (44) at the end of the treatments (PSI: 0.72 ± 0.02; PSI–II: 0.8 ± 0.02; PSI–II plus DCMU: 0.49 ± 0.05; PSI plus DCMU: 0.53 ± 0.05). Three independent experiments with different filters and independent cDNA probes were performed thus minimizing variation between individual plants, filters, or probes. cDNA synthesis was primed by using a mixture of oligonucleotides matching the 3292 genes in antisense orientation and hybridized to the GST array as described (43, 45). Images were read using a Storm PhosphorImager (Amersham Biosciences). Hybridization images were imported into the ArrayVision program (version 6, Imaging Research Inc., Ontario, Canada), where artifacts were removed, background correction was performed, and resulting values were normalized with reference to intensity of all spots on the array (45). In the next step, those data were imported into the ArrayStat program (version 1.0 Rev. 2.0, Imaging Research Inc.), and a *z*-test (nominal α set to 0.05) was performed employing false discovery rate (46) correction to identify statistically significant differential expression values. Only differential expression values fulfilling the criteria of this statistical procedure were used for the expression profiling.

RESULTS

Changes in Photosystem Structure of Arabidopsis during Acclimation to PSI and PSII light and Transcriptional Regulation of Plastid Reaction Center Genes psaA and psbA—Imbalances in excitation energy distribution between the photosystems can be induced by illumination with light sources that differentially excite PSII or PSI (PSII or PSI light, respectively) resulting either in a more reduced or more oxidized state of the electron transport components (data not shown). To study how plants deal with and acclimate to such imbalances, *Arabidopsis* seedlings were grown first under white light until the four- to six-leaf stage before they were subjected to PSI or PSII light (PSI or PSII plants). Responses of such plants were compared with responses of plants acclimated to PSI or PSII light followed by an additional acclimation to the respective other light source (PSI–II plants or PSII–I plants). The analysis of PSI and PSII plants show the acclimation to the two light sources in general, whereas the analysis of the plants shifted between the light sources proves the reversibility of the observed responses (an indicator for true acclimatory effects). To test photosystem stoichiometry adjustment in response to light quality in *Arabidopsis* we analyzed photosystem protein abundance and chlorophyll contents. The overall protein pattern of whole tissue protein extracts did not reveal any major differences between the four growth conditions when analyzed by SDS gel electrophoresis. In Western analyses with antisera raised against the D1 protein and the P700 apoproteins (representing the core proteins of PSII and PSI) (Fig. 1*A*) the D1 protein exhibited more or less constant amounts under all conditions, whereas the amounts of P700 apoproteins increased in PSI–II plants in

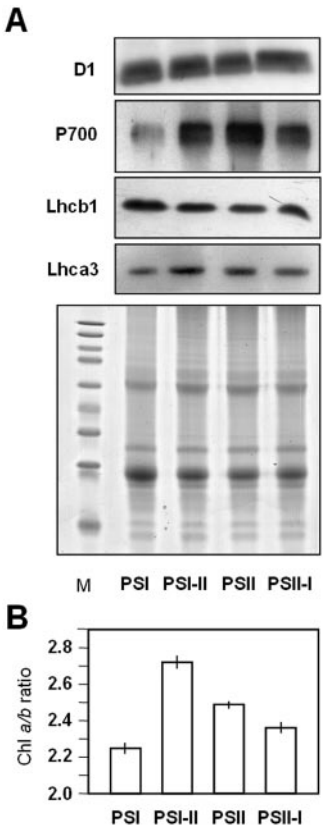

FIG. 1. **Changes in photosynthesis protein and chlorophyll amounts during long term acclimation.** *A*, Western immunological analysis of photosystem core and antenna protein content. Chloroplasts of the differentially grown plants were isolated, and proteins of ~2 × 10^5 organelles were separated by SDS-PAGE per lane and transferred to nylon membranes. Respective growth conditions are given at the *bottom*. D1 protein, P700 apoproteins, Lhcb1, and Lhca3 were detected with polyclonal antisera and a peroxidase-coupled secondary antibody using enhanced chemiluminescence. Representative results from three independent experiments are shown. A Coomassie Blue-stained SDS gel is shown below as loading control. Marker proteins (*lane M*) range from 116 to 14 kDa. Growth conditions are given at the *bottom*. *B*, Chl *a/b* ratio. Chlorophylls of acclimated plants were extracted, spectrophotometrically determined, and calculated as described under "Experimental Procedures." Growth conditions are given at the *bottom*. All experiments were repeated three times.

comparison to PSI plants and decreased in PSII–I plants in comparison to PSII plants. Taking the amount of the reaction center proteins as an indicator for the relative number of the photosystems, the PSII/PSI ratio is high under PSI light and decreases after a shift to PSII light, whereas the opposite can be observed under PSII light and a shift to PSI light. Furthermore, we tested the abundance of antenna proteins Lhcb1 and Lhca3, two important components of the PSII and PSI antennae, respectively. Lhca3 showed a similar accumulation under the light sources as the P700 apoproteins, whereas the opposite effect was observed for the Lhcb1 protein suggesting a concomitant increase in antenna size of the respective rate-limiting photosystem. Such changes in the antennae are also indicated by characteristic changes in the Chl *a/b* ratio. After acclimation to PSI light the Chl *a/b* ratio is low and increases significantly after a shift to PSII light (Fig. 1*B*, PSI–II). Under PSII light the Chl *a/b* ratio is high and decreases after a shift to PSI light. Because Chl *b* is mainly associated with the PSII antenna, these observations are consistent with the observed changes in the amounts of antenna proteins.

In mustard the adjustment of photosystem stoichiometry is controlled by changes in the transcription of the reaction center genes *psaA* and *psbA* (9, 12). To test if this is also true for *Arabidopsis* we performed primer extension analyses (Fig. 2) for these genes that allowed us to check for changes in transcript initiation sites and amounts of the respective RNAs in the same experiment. Both *psaA* and *psbA* transcripts exhibited the same 5′-ends under all conditions investigated, although in varying amounts. For *psaA* we found two prominent 5′-ends in a distance of 197 and 111 bases upstream of the translation initiation codon. The first (more prominent) end corresponds to the transcription start sites for *psaA* in mustard (47), the second one has not been reported in any other organism and might represent a species-specific start or processing site. The regulation of *psaA* transcript accumulation is comparable to the situation observed for the respective proteins with an increase in transcripts after a PSI–II light shift (in comparison to PSI light) and a decrease after a PSII–I light shift (in comparison to PSII light). For *psbA* we found a single prominent 5′-end 78 bases in front of the translation start site, consistent with earlier reports (27, 47, 48). The accumulation of this transcript showed only a slight decrease after a PSI–II light shift and a slight increase after a PSII–I light shift. The observed changes in RNA amounts are in agreement with the observations at protein level suggesting that redox-regulated transcription plays an important role also in *Arabidopsis*.

Light Quality Acclimation in Photoreceptor and Chloroplast-to-Nucleus Signaling Mutants—Adjustment of photosystem stoichiometry in higher plants requires coordinated changes in the expression of plastid- and nuclear-encoded photosynthesis genes. To test whether cytosolic photoreceptors or components of plastid retrograde signaling pathways are involved in the detection and/or transduction of PSI or PSII light-induced redox signals, we analyzed the LTR in various *Arabidopsis* mutants (Fig. 3). We used the Chl fluorescence parameter F_s/F_m, which in wild type typically increases after acclimation to PSI light and decreases after acclimation to PSII light (13, 14)[2] and, therefore, can be used as a non-invasive indicator for a LTR. In the photosynthesis mutant *hcf109* (49), which exhibits partial impairment of PSII and PSI activities, no significant changes in the F_s/F_m values could be observed (data not shown) indicating that perturbations of photosynthetic electron transport lead to a loss of the LTR and/or its detectability. Therefore, before assessing the F_s/F_m value each mutant line was tested

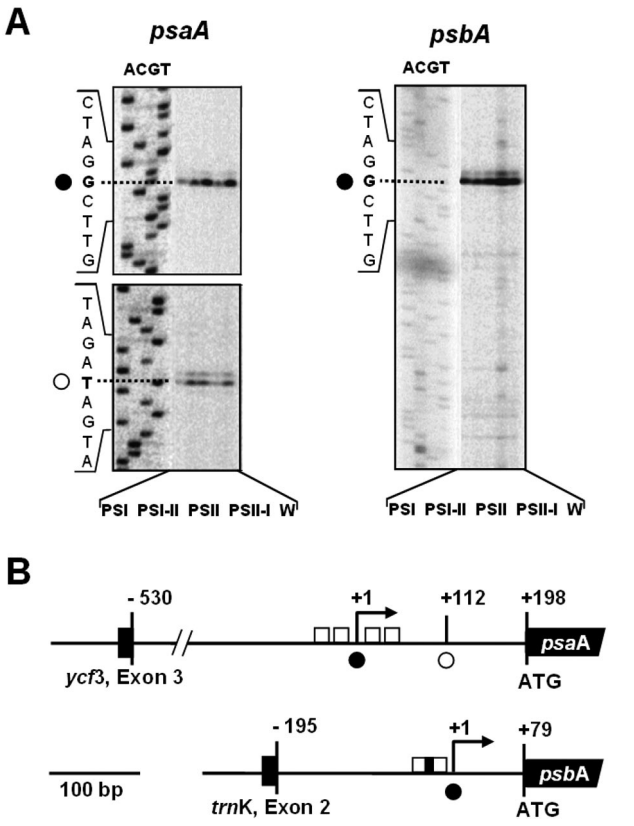

FIG. 2. **Primer extension analysis of position and accumulation of 5′- ends of *psaA* and *psbA* transcripts.** Plants were grown under the respective light sources, and total RNA was isolated. Fluorescence dye-labeled primers were designed to anneal within the first 50 bp of the coding region of the *psaA* and *psbA* genes and were used both in a reverse transcription reaction with isolated total RNA and a sequencing reaction of chloroplast DNA fragments covering the *psaA* and *psbA* 5′-gene and promoter regions. Products were separated in parallel on a denaturing 4% acrylamide gel containing 7 M urea and detected by laser excitation in a Licor 4200 sequencer. *A*, sequencer images of the primer extension analyses. The DNA sequences within the *psaA* (*left part*) and *psbA* (*right part*) promoters are shown each on the *left*, primer extension products on the *right*. Detected 5′-ends are marked by *dots* (*black* for transcript start; *white* for unknown end), and respective transcription start nucleotides are given in *bold letters*. Growth conditions are given at the *bottom*. *B*, structure of the *Arabidopsis psaA* and *psbA* promoter regions. Positions of 5′-ends are marked by the same *dots* as in Fig. 5*A*. Transcription start sites are indicated by +1, and all other positions are given relative to it. Pairs of *white boxes* indicate −10/−35 regions; a *black box* indicates a TATA-like *cis*-element.

for its Chl fluorescence parameter F_v/F_m as indicator for the general photosynthetic function. All mutants revealed wild type-like F_v/F_m values of >0.8 (data not shown) indicating that they can perform normal photosynthesis. We then tested the LTR in mutants lacking functional phytochrome A (*phyA*), phytochrome B (*phyB*), or both (*phyA*/*phyB*) (50) as well as for a transgenic line overexpressing phytochrome B (*phyB oe*) (51). In addition, we tested mutants lacking cryptochrome 1 (*hy4*) or 2 (*cry2-1*) (52, 53). A significant decrease or increase of F_s/F_m after the respective light switch was observed for all photoreceptor mutants indicating their ability to perform an appropriate LTR (Fig. 3*A*). Only the *phyA*/*phyB* double mutant revealed no significant decrease of the F_s/F_m value after a shift from PSI to PSII light, whereas the *cry2–1* mutant exhibited a significant LTR, however, with a less strong increase in F_s/F_m than usually observed after a shift from PSII to PSI light (compare Supplementary Table SI).

We also analyzed the response of *genome-uncoupled* (*gun*) (54) and *cab underexpressed* (*cue*) (55) mutants (Fig. 3*B*). Both types of mutants exhibit defects in chloroplast signaling routes

[2] R. Wagner and T. Pfannschmidt, unpublished observations.

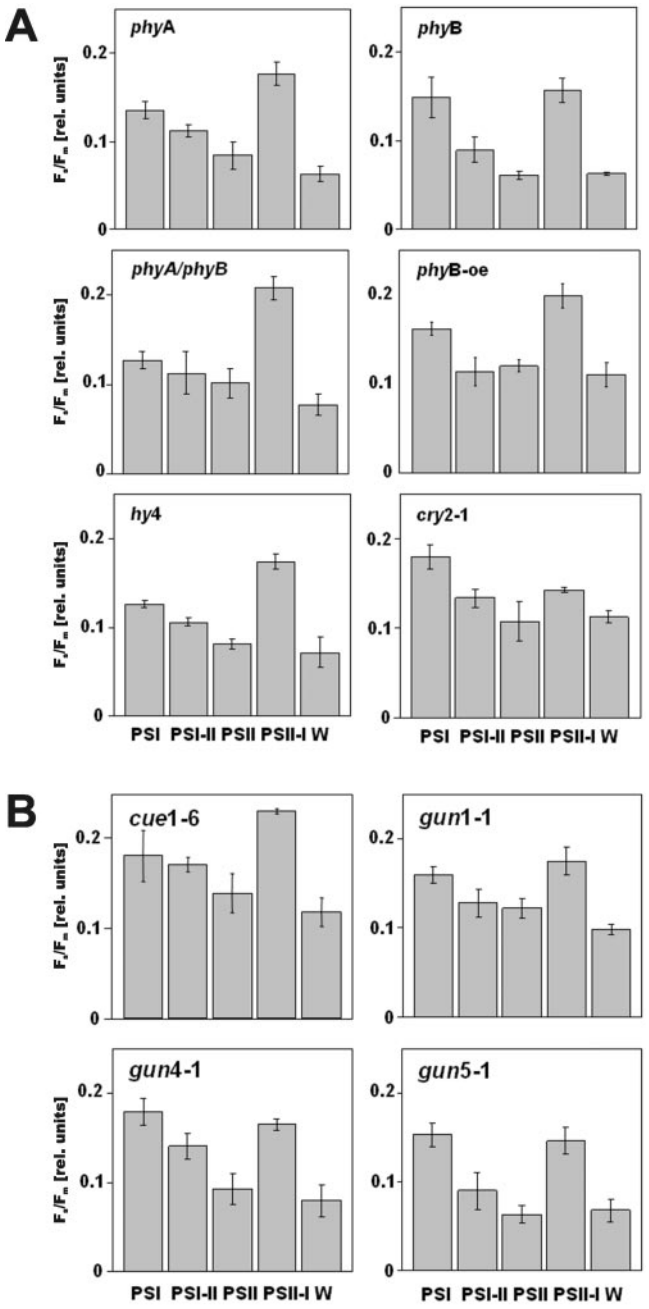

FIG. 3. **LTR in *Arabidopsis* mutants.** Mutant lines were acclimated to PSI or PSII light, and F_s/F_m values were determined using a PAM fluorometer. All values were determined in at least three independent experiments with 15–20 plants each, and the statistical significance of differences was proven using the SPSS statistic program (for details see Supplementary Table SI). The indication of the respective lines is given in the *upper left corner* of each graph (for designation see text). *A*, photoreceptor mutants; *B*, chloroplast-to-nucleus signaling mutants.

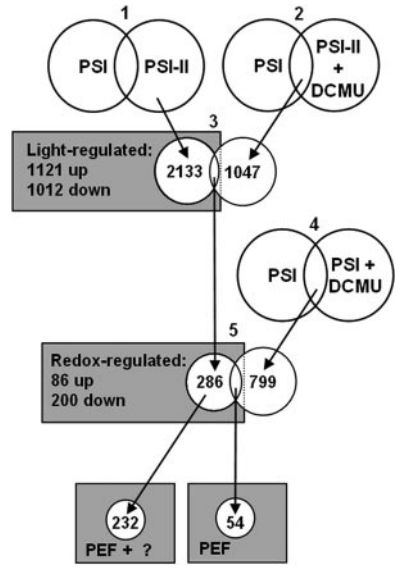

FIG. 4. **Macroarray strategy to define redox-regulated genes encoding chloroplast proteins.** White light-grown plants were acclimated to PSI light for 6 days (*PSI*) or 2 days followed by an acclimation to PSII light for additional 4 days (*PSI–II*). Parallel samples were treated with 5 μM DCMU after 2 days in PSI light and then shifted to PSII light or left under the PSI light. *Large circles* represent the respective expression profiles (test condition is given inside). Profiles of these conditions (*large circles*) were compared (for details see text). Intersections represent genes that do not differ significantly in their expression under the conditions compared. *Small circles* represent gene groups resulting from these comparisons (number is given inside), and their respective origin is indicated by *arrows*. *Gray boxes* list category and number of up- or down-regulated genes in the *small circles* that originate from comparisons between expression profiles represented by *large circles* (for details, see text). PEF, genes that are regulated by photosynthetic electron transport. PEF + ?, genes that are regulated by photosynthetic electron transport and an unknown additional redox signal from the thylakoid membrane.

toward the nucleus. *gun1-1* has still unknown defects, *gun5* encodes the H subunit of the magnesium chelatase in the chloroplast envelope, and *gun4* encodes a product that binds the substrate of the magnesium chelatase (56, 57). The *cue1* gene encodes the phosphoenol pyruvate/phosphate translocator of the chloroplast envelope (58). In our test system all *gun* mutants exhibited a wild type-like behavior with significant LTRs. The *cue1-6* mutant, however, showed no significant decrease of F_s/F_m after a shift from PSI to PSII light, whereas the expected increase after a shift from PSII to PSI light is present to a full extent (compare Supplementary Table I).

These data indicate that the defective components in the photoreceptor and retrograde signaling mutants are not essential for the LTR, otherwise we would have observed a complete loss of it. Thus, chloroplast redox signals represent a unique class of retrograde signals. The less pronounced effects in *phyA*/*phyB* and *cue1-6* mutants might be caused by general developmental effects (see "Discussion") suggesting that redox signals are an integral component of the intracellular signaling network.

Photosynthetic Control of the Nuclear Transcriptome of the Chloroplast—To analyze the global effects of light quality and redox signals on the expression of genes for chloroplast proteins, we performed a macroarray analysis using a GST array with probes covering respective nuclear genes (45). This preselection of genes guarantees that a high proportion of light-regulated genes are investigated. Light regulation is a prerequisite for the study of redox regulation under our conditions. Furthermore, this array has been shown in earlier studies to produce statistically reliable and reproducible expression profiles (43, 59). To assess the impact of redox signals we followed a three-step strategy. 1) First we compared gene expression profiles of PSI and PSI–II plants (Fig. 4, *comparison 1*). This showed the overall impact of a reduction signal induced by the shift from PSI to PSII light. Non-light-regulated genes could be identified and omitted from further analysis. 2) Next we compared gene expression profiles of PSI plants with PSI–II plants pre-treated with DCMU (Fig. 4, *comparison 2*). Genes with the same expression under both conditions represent either non-light-regulated genes or light-regulated genes whose expression change is abolished by the electron transport inhibitor.

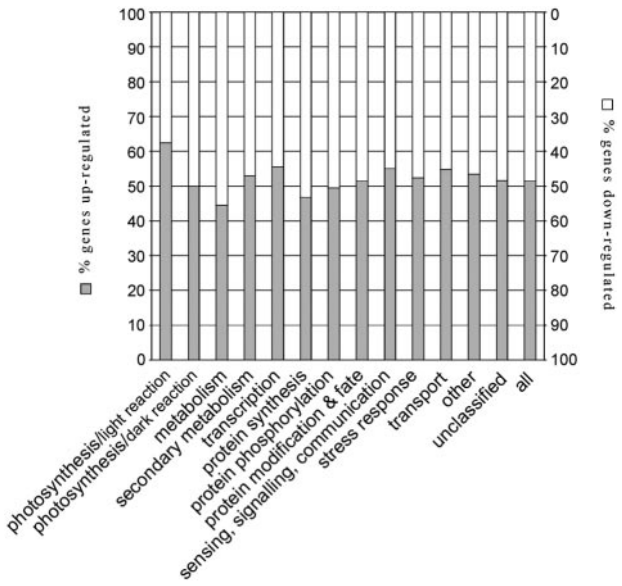

FIG. 5. **Relative distribution of light quality-induced expression changes of genes sorted by function of gene product.** Only genes with significant expression changes were included. Genes were grouped according to the known or predicted function of the encoded product (given at the *bottom*). Numbers of genes with increased (*gray part of bar*) or decreased (*white part of bar*) expression are given in percentages.

The latter are defined as redox-regulated genes and could be identified by comparing this group of non-regulated genes with those responsive to the light signal from step 1 (Fig. 4, *comparison 3*). 3) Finally, we compared gene expression profiles of PSI plants and PSI plants treated with the same amounts of DCMU as in step 2 (Fig. 4, *comparison 4*). Redox-regulated genes whose expression change is completely abolished by the DCMU treatment were controlled by the photosynthetic electron flow when the same DCMU treatment as in step 2 had no effect in step 3 indicating that the DCMU treatment has only neutralized the PSII light effect. Such "ideal" redox-regulated genes are defined by a comparison of non-regulated genes from comparison 4 with the group of redox-regulated genes of comparison 3 (Fig. 4, *comparison 5*).

Comparison 1 indicates that a light quality shift has a massive impact on the expression of genes encoding chloroplast proteins. A set of 2133 genes significantly responded to the shift from PSI to PSII light; 1121 genes were up-regulated while 1012 were down-regulated. Among these we found genes for all major functional classes of proteins (Fig. 5), including genes for photosynthesis, gene expression, metabolism, and transport. We found no gene class exhibiting unidirectional expression changes. As a general tendency it emerged that all gene classes responded in a balanced way with around 50% up- and 50% down-regulated genes. Out of the 2133 light-regulated genes, we identified 286 that are directly regulated by redox signals from the photosynthetic electron transport chain. 86 genes were up-regulated by a reduction signal while 200 genes were down-regulated by it. From these 286 redox-regulated genes 54 matched the theoretical constraints for an "ideal" expression profile to be expected for a gene regulated by redox signals from photosynthetic electron flow. The remaining 232 genes still represented redox-regulated genes but seemed to be regulated by more than one redox parameter (see "Discussion").

Only 76 of the 286 redox-regulated genes encode products with known functions, including all major gene groups such as photosynthesis, gene expression, metabolism, or signal transduction (Table I). The great majority of genes, however, codes for putative, hypothetical, or even unknown proteins (not shown). Nevertheless, several groups of functionally related genes can be identified that exhibit similar expression patterns pointing to concerted regulatory events. The largest groups among the down-regulated genes include: (*a*) a large group of metabolic genes mainly encoding enzymes (or enzyme subunits) involved in amino acid or nucleotide metabolism; (*b*) several chaperones and signal recognition particle components partially involved in photosystem assembly; (*c*) genes for transcription and its regulation in the nucleus and chloroplast; and (*d*) genes for components involved in sulfur and glutathione metabolism. The largest groups among the up-regulated genes include (*a*) metabolic genes for amino acid and nucleotide metabolism as well as energy metabolism and (*b*) photosynthesis genes. Beside these major groups many individual genes encoding products with functions necessary for the establishment of the LTR are identified (see "Discussion"). This result demonstrates that redox signals from the thylakoid membrane have an extensive influence on the expression of nuclear genes reflecting the multiple functional involvements of chloroplasts within the metabolic pathways of the cell.

Effects of PSI and PSII Light on Thiol Group Content and Glutathione Redox State—Glutathione is an important cellular redox buffer and functions also as a potent regulator of gene expression especially in chloroplasts (16, 17, 22, 60). The array analysis exhibited several regulated genes involved in glutathione metabolism. To test if changes in glutathione redox state are involved in the LTR, we determined the content of cysteine and glutathione as well as the redox state of glutathione in plants (Table II) grown under the four different conditions. We found comparable thiol contents under all growth conditions, and only PSII plants exhibited slightly increased amounts in glutathione and cysteine. In addition, glutathione appeared to be mainly reduced (around 90%) under each light regime, as it is described for *Arabidopsis* grown under standard white light sources. Therefore it is unlikely that changes in the glutathione redox state are responsible for the observed changes in plastid gene expression. Aside from this, the highly reduced state indicates that the plants do not suffer from strong reactive oxygen species-mediated stresses, which is typically indicated by an increase in oxidized glutathione concentrations. Thus, superimpositions from reactive oxygen species-induced redox signaling cascades under the different light qualities are unlikely, and we conclude that in *Arabidopsis* light quality changes are reported mainly via redox signals from intersystem electron transport components.

DISCUSSION

Light Quality Effects on Photosystem Stoichiometry—We found significant acclimatory changes in the structure of the photosynthetic apparatus in an extent comparable to those reported for other higher plants (9, 14, 61, 62). Our Western analyses, however, suggest that photosystem stoichiometry adjustment in *Arabidopsis* is mainly regulated by changes in PSI complexes and PSII antenna size. This differs from observations in pea and mustard were antiparallel changes in both PSI and PSII were observed (12, 61), whereas it is in accordance with observations in spinach and cyanobacteria for which mainly changes in PSI were reported (7, 62). Changes in PSII content in *Arabidopsis* have been reported to occur only under higher light intensities (63). Spectroscopic analyses might help to determine more precisely the absolute changes in photosystems in *Arabidopsis* under our conditions. The immunologically detected changes in D1 and P700 apoprotein levels are accompanied by corresponding changes in respective transcript pool sizes as observed earlier (9, 12). At both promoters redox regulation occurs at the major transcription start site (Fig. 2), which is located directly behind typical promoter elements for

TABLE I
Redox-regulated genes encoding known products

ATG[a]	Ratio[b] PSII/PSI	Ratio[b] PSII_DCMU/PSI	Ratio[b] PSI_DCMU/PSI	Description[c]
Down-regulated				
Metabolism				
At5g38530	0.63	0.81	0.49	Tryptophan synthase, β chain
At4g16700	0.64	0.85	0.72	Decarboxylase-like protein
At2g43090	0.69	0.82	0.71	3-Isopropylmalate dehydratase, small subunit
At5g13280	0.69	0.88	0.45	Aspartate kinase
At4g27070	0.70	0.96	0.54	Tryptophan synthase, β-subunit (TSB2)
At4g16800	0.71	0.85	0.50	Enoyl-CoA hydratase
At4g19710	0.72	0.81	0.51	Aspartate kinase-homoserine dehydrogenase-like protein
At5g03650	0.72	0.88	0.48	1,4-α-glucan branching enzyme isoform SBE2.2
At3g10050	0.73	0.81	0.57	Threonine dehydratase/deaminase (OMR1)
At4g18440	0.74	0.82	0.57	Adenylosuccinate lyase-like protein
At4g09740	0.74	1.03	0.51	Cellulase-like protein
At5g08300	0.76	0.81	0.65	Succinyl-CoA-ligase, α subunit
At4g11010	0.76	0.82	0.47	Nucleoside diphosphate kinase 3 (ndpk3)
At2g03220	0.79	0.85	0.50	Xyloglucan fucosyltransferase AtFT1
At4g31180	0.76	0.89	0.40	Aspartate-tRNA ligase-like protein
Other				
At4g35770	0.68	1.15	2.01	Senescence-associated protein sen1
At5g18810	**0.77**	**1.05**	**0.99**	**Serine/arginine-rich protein-like**
At5g25380	0.77	0.92	0.62	Cyclin 3a
At5g24020	0.80	0.95	0.76	Septum site-determining MinD
Photosynthesis				
At4g15530	0.69	0.80	0.54	Pyruvate, orthophosphate dikinase
At1g76450	0.78	0.86	0.27	Unknown thylakoid lumen protein, PsbP domain
Protein modification and fate				
At2g39990	0.59	0.82	0.50	26 S proteasome regulatory subunit
At5g15450	0.73	0.81	0.70	ClpB heat shock protein-like
At4g36040	**0.75**	**0.90**	**1.25**	**DnaJ-like protein**
At2g28800	**0.76**	**0.86**	**0.98**	**Chloroplast membrane protein ALBINO3 (ALB3)**
At5g03940	0.79	0.96	0.51	Signal recognition particle 54CP (SRP54) protein
At4g37910	0.79	0.85	0.51	Hsp70.3
Protein phosphorylation				
At4g23650	**0.64**	**0.83**	**1.19**	**Calcium-dependent protein kinase (CDPK6)**
Stress response				
At4g29890	**0.62**	**1.16**	**0.89**	**Choline monooxygenase-like protein**
At3g45140	**0.76**	**0.82**	**0.81**	**Lipoxygenase AtLOX2**
Transcription				
At1g59940	**0.75**	**0.87**	**0.90**	**Response regulator ARR 12**
At3g57040	0.79	0.96	0.54	Response regulator ARR 9
RpoB	**0.62**	**1.05**	**1.05**	**Plastid gene; RNA polymerase catalytic chain**
At5g24120	0.63	0.81	0.67	Sigma-like factor (emb CAA77213.1)
At3g56710	0.73	0.88	0.38	SigA-binding protein
At3g60490	0.73	0.92	0.39	Transcription factor-like protein
At1g68990	0.75	0.99	0.51	DNA-directed RNA polymerase (mitochondrial)
At1g03970	0.79	0.81	0.69	G-box binding factor, GBF4
Transport				
At4g33650	0.68	1.03	0.35	*Arabidopsis* dynamin-like protein ADL2
At4g18290	0.68	0.94	0.51	Potassium channel protein KAT2
At4g36580	**0.75**	**1.04**	**0.92**	**ATPase-like protein**
At5g59030	0.76	0.90	0.53	Copper transport protein
Unclassified				
At2g13870	0.79	1.06	0.65	En/Spm-like transposon protein
S-metabolism				
At4g02520	**0.57**	**0.85**	**0.91**	**Atpm24.1 glutathione *S*-transferase**
At5g56760	0.66	0.81	0.47	Serine *O*-acetyltransferase (EC 2.3.1.30) Sat-52
At4g39940	**0.67**	**1.23**	**0.95**	**Adenosine-5-phosphosulfate kinase**
At5g43780	0.75	0.97	0.72	ATP sulfurylase precursor (gb AAD26634.1)
Up-regulated				
Metabolism				
At1g29900	1.26	1.18	0.60	Carbamoyl phosphate synthetase, large chain (carB)
At4g24620	1.27	1.17	0.70	Glucose-6-phosphate isomerase
At3g11670	1.28	0.98	0.67	Digalactosyldiacylglycerol synthase
At2g43100	**1.34**	**0.96**	**0.81**	**3-Isopropylmalate dehydratase, small subunit**
At1g01090	1.41	1.08	0.77	Pyruvate dehydrogenase E1, alpha subunit
At1g24280	1.53	1.03	0.75	Glucose-6-phosphate 1-dehydrogenase
At5g16290	1.74	1.15	0.72	Acetolactate synthase-like protein
At1g76490	1.81	0.86	1.77	Hydroxymethylglutaryl-CoA reductase (AA 1–592)
Photosynthesis				
At3g16140	1.26	0.92	0.67	PsaH1
At5g66190	1.27	1.25	0.62	PetH2; FNR; ferredoxin-NADP+ reductase
At5g66570	1.30	1.17	1.60	PsbO1
At3g08940	**1.49**	**0.92**	**0.84**	**Lhcb4.2 (CP29)**
At1g79040	1.53	0.91	1.66	PsbR
At1g15820	1.61	0.80	0.70	Lhcb6 (CP24)

TABLE I—*continued*

ATG[a]	Ratio[b] PSII/PSI	Ratio[b] PSII_DCMU/PSI	Ratio[b] PSI_DCMU/PSI	Description[c]
At1g31330	**1.71**	**0.91**	**1.17**	**PsaF**
At4g29670	1.89	0.82	1.58	Thioredoxin-like protein
Protein modification and fate				
At4g20740	1.30	1.11	0.78	Similarity to CRP1
At5g42390	**1.31**	**1.14**	**0.81**	**SPP/CPE**
Protein phosphorylation				
At5g25930	1.47	1.24	1.78	Receptor-like protein kinase-like
Protein synthesis				
At4g17300	**1.28**	**1.14**	**0.83**	**Asparagine-tRNA ligase**
Secondary metabolism				
At4g20230	1.46	1.18	1.36	Terpene cyclase-like protein
At5g38120	1.49	1.22	1.49	4-Coumarate-CoA ligase-like protein
At4g32540	**1.56**	**1.22**	**0.93**	**Dimethylaniline monooxygenase-like protein**
Stress response				
At4g11230	**1.36**	**1.12**	**0.89**	**Respiratory burst oxidase homolog F-like protein**
Transport				
At4g36520	1.30	1.15	0.77	Trichohyalin-like protein
At1g80830	**1.38**	**1.23**	**0.93**	**Metal ion transporter**
S-metabolism				
At5g27380	**1.26**	**1.20**	**0.96**	**Glutathione synthetase gsh2**

[a] Accession number.
[b] Expression data under the respective test condition relative to the expression data under PSI-light.
[c] Those genes among the classified 286 genes that have a clear functional assignment have been listed according to their down- or up-regulation under PSII light in comparison to PSI light. Genes are grouped into functional categories and listed according to their degree of regulation. Genes matching conditions of "ideal" redox regulation are given in bold letters.

TABLE II
Thiol group content and redox state of glutathione in differentially acclimated Arabidopsis seedlings

Growth light regime	Cysteine[a]	Glutathione	Reduced glutathione
	pmol/mg		*%*[b]
PSI	10.7 ± 0.7	319.5 ± 33.9	90.0 ± 2.4
PSI–II	10.8 ± 2.1	344.6 ± 65.0	87.3 ± 3.6
PSII	12.4 ± 1.2	390.2 ± 18.8	92.0 ± 1.5
PSII–I	10.0 ± 2.2	322.2 ± 73.1	88.7 ± 1.7

[a] Each value represents the average of four independent samples based on fresh weight, and S.D. is given.
[b] "%" refers to the proportion of reduced glutathione of total glutathione content.

the plastid-encoded RNA polymerase (PEP) (64). This suggests the existence of specific regulatory protein factors that might mediate the redox signal to the RNA polymerase. It is interesting to note that components for the PEP complex are found in the group of redox-regulated genes (see below). Furthermore, our primer extension studies identified a not yet described *psaA* 5′-end; however, this does not provide hints on putative redox-responsive *cis*-elements because the technique does not distinguish between transcript initiation and processing. *In vitro* DNA-protein interaction studies in spinach suggest that the *psaA* promoter may contain additional important regulatory elements, *i.e.* a so-called region D (65). Transcript initiation at this promoter therefore may play a key role during light quality acclimation in *Arabidopsis*. Experiments are in progress to characterize this regulation in more detail.

LTR in Photoreceptor and Chloroplast-to-Nucleus Signaling Mutants—Our PSI light source contains wavelengths over 700 nm, whereas the PSII light does not, resulting in different red/far red ratios that might affect the intracellular ratio of the phytochrome P_r and P_{fr} forms. However, because the LTR is present in all photoreceptor mutants tested, we conclude that the acclimatory response operates either independently from or above the photoreceptor signaling network. The observation that the LTR is only partially functional in the *phyA*/*phyB* mutant is most probably caused by pleiotropic side effects, because the double mutant exhibits severe developmental effects that may interfere with the LTR even if the general photosynthetic performance does not seem to be disturbed. The reversibility of the LTR within the single mutants provides a strong argument that the LTR is regulated without the signaling avenues of *phyA* or *phyB*. Both the PSI and the PSII light do not contain blue or UV-light, which is consistent with the observation that the LTR is not mediated by cryptochromes. The observed weaker response in the *cry2-1* mutant after a PSII–I light shift (Fig. 3*A*) must therefore be caused by a developmental side effect in this mutant. These data do not exclude interactions between redox and photoreceptor signaling networks, especially because many more genes are light- than redox-regulated, however, for the LTR, this appears to be meaningless. *Arabidopsis* photoreceptor mutants have also been used to test the involvement of photoreceptors in photosynthetic acclimation responses to high light (66, 67). In these studies the photoreceptor mutants acclimated to shifts in light intensity in a wild type-like manner. Although acclimations to light quality or light quantity involve different responses (1, 63), they all function in the absence of photoreceptors underlining the importance of photosynthetic acclimation in the response to environmental changes.

In the chloroplast-to-nucleus signaling mutants we also detected clear responses to the PSI and PSII light, indicating that the LTR operates independently of the lesions in these mutants. Only *cue1-6* lacks a significant LTR after a PSI–II light shift (as *phyA*/*phyB)*. The lack of the phosphoenol pyruvate carrier in *cue1-6*, however, has a strong impact on the energy metabolism of the mutant, and adult plants exhibit a reticular phenotype (58). Similar to the *phyA*/*phyB* double mutant, these developmental lesions might affect the LTR. None of the mutant lines investigated here lack the LTR completely except *hcf109*, which is the only mutant with defects in photosynthesis. The observation that in *phyA*/*phyB*, *cue1-6*, and *cry2-1* only one response is affected while the other is not could be a hint that reduction and oxidation signals can be separated and may operate via different pathways. It is interesting to note that in *cue* mutants a connection between phytochrome and plastid regulation of nuclear gene expression has been observed (68), although a connection between photosynthetic redox signals and other plastid retrograde signals or photoreceptors was not found here.

Impact of Light Quality on the Nuclear Chloroplast Transcriptome—The major goal of our array study was to determine the global impact of light quality and photosynthetic redox signals on the expression of nuclear genes for chloroplast proteins to assess the importance of such signals for higher plants. Light quality affects over 2000 genes encoding not only photosynthesis but also many other structural and functional components. Around 15% of these genes appear to be regulated by redox signals suggesting that many genes among the 2000 may be secondary or tertiary targets that are affected through the long term impact of redox signals on the overall cellular signaling network and/or the action of other light perceiving systems. Many genes exhibit relatively small changes in their expression. This can be best explained by the fact that the expression profiles were determined at the end of the acclimatory response when a new expression equilibrium has been established. Genes transiently affected only for a short time after a light switch or an inhibitor application might be not detected by this approach and will be identified by further, more detailed, studies.

The expression profile after acclimation to a reduction signal exhibits similar numbers of up- and down-regulated genes (Fig. 5). In a hierarchical cluster analysis of expression profiles in 35 different physiological situations or mutants with this macroarray, our profile was found to be the most prominent representative of the so-called class 2 profiles, which are characterized by balanced expression changes (43). Class 1 profiles showed mainly up-regulated and class 3 profiles mainly down-regulated genes. Among the latter two classes the profiles of the *gun* (class 1) and *cue* mutants (class 3) were found. The different profile clustering is an independent confirmation that in these mutants gene expression regulation appears to be totally different from that observed under our conditions. This again argues for the independence of light quality-induced redox signals from the plastid signaling pathways, which are defective in the *gun* and *cue* mutants.

It is difficult to discuss complex results such as transcript profiles on the level of individual genes, however, the study uncovered many interesting genes responding to redox signals. Some of them that are of special interest for the LTR and its regulation are highlighted in the following. We found several groups of redox-regulated genes encoding products with related functions, including those for photosynthesis (Table I, up-regulated). All affected genes encoding components of the photosynthetic machinery were found to be up-regulated by a reduction signal. A prominent representative is the *PsaF* gene, which exhibits essentially the same expression profile as obtained earlier with transgenic tobacco lines containing a *PsaF*-promoter::*uidA* construct (14), demonstrating the reproducibility of the expression data. We also found a thioredoxin-like protein that is of interest because thioredoxins regulate many processes in chloroplasts such as light induction of Calvin cycle enzymes or translation initiation of *psbA* (69). In general, up-regulation occurred for both PSII and PSI genes suggesting that the stoichiometric adjustment of the nuclear encoded components includes additional regulatory steps at other levels of expression and/or complex assembly (see below). This might also be the reason why we did not find all nuclear photosynthesis genes to be regulated in this array.

Metabolic genes represent the most prominent group among the redox-regulated genes identified here. Most encode components involved in amino acid and nucleotide metabolism and are regulated in the opposite way to photosynthesis genes. Amino acids and nucleotides are central molecules in many biosynthetic pathways demonstrating that the acclimation response is not restricted to photosynthesis but has also a deep impact on the metabolism of a plant. A metabolic gene of special interest here is the succinyl-CoA-ligase, which produces the precursor molecule for aminolevulinic acid, the entry substance for chlorophyll biosynthesis, a process that is clearly affected during the LTR (Fig. 1*C*). In addition, we found the digalactosyldiacylglycerol synthase, which produces the major lipid of thylakoid membranes (70). The LTR involves major re-arrangements of the thylakoid membrane system in chloroplasts.[2] Because of these results we have started further studies to investigate the LTR effects on plant metabolism in more detail.

A further striking observation is the regulatory impact on components of the chloroplast PEP enzyme (*rpoB*, sigma-like factor, *SigA* binding factor; Table I, transcription, down-regulated), which is responsible for the redox regulation at the *psbA* and *psaAB* promoters (Fig. 2). The *rpoB* gene is plastid-localized, encodes the catalytic β-subunit of PEP, and is transcribed by the nuclear encoded RNA polymerase (64). This suggests a redox regulation of nuclear encoded RNA polymerase activity. Interestingly the paralogue nuclear encoded RNA polymerase gene, which encodes the mitochondrial nuclear encoded RNA polymerase, appears to be redox-regulated in its expression like ARR9, ARR12, and GBF4, transcriptional regulators of nuclear transcription, as well as the sigma-like-factor and *SigA* binding factor, transcriptional regulators of chloroplast transcription. This suggests a complex signaling network controlling in parallel the expression of the different components of the plastid gene transcription machinery in the nucleus and in the organelle. Furthermore, regulation of the PEP enzyme has been shown *in vitro* to be under phosphorylation control via the plastid transcription kinase, which itself is under control of glutathione redox state (16). Our results do not indicate major differences in the glutathione redox state under the various light conditions thus supporting the idea of several different redox control pathways in chloroplast transcription (60, 71), depending on environmental conditions as to be expected for different acclimation responses under low or high light (see above).

The photosystems are multiprotein complexes, which have to be assembled in a highly coordinated manner. Several chaperones and assembly proteins were identified as being redox-regulated (Table I, up-regulated, protein modification, and fate). Important in this context are ALB3 and SRP54, two proteins of the SRP complex in thylakoid membranes that are responsible for the import of light-harvesting proteins into the thylakoid membrane (72, 73).

Of special interest is the observation that several genes for enzymes involved in sulfur and glutathione metabolism (Table I, *S*-metabolism) together with genes for products involved in various stress responses (choline monooxygenase, lypoxygenase, and respiratory burst oxygenase) are found to be redox-regulated. It is possible that these changes in glutathione genes are responsible for the relatively stable glutathione redox state found here. It is also possible that these genes are regulated together with stress genes (see above) in a kind of overlap reaction between photosynthetic redox signals and other environmentally induced stress signals such as cold (indicated by choline monooxygenase (74)) or pathogen attack (indicated by lypoxygenase, respiratory burst oxygenase (75)), which are also mediated by redox signals. It is well known that interactions between photosynthesis, temperature, or pathogen attack exist and that redox signals of various origin play a central role in this scenario. Antioxidant molecules such as glutathione are involved in all these processes indicating that multiple connections between the responses to the different environmental stress situations exist (76). The dominant regulatory signals

controlling the LTR appear to come from the electron transport chain, because the glutathione redox state remained relatively stable under all conditions. Because the redox state of ascorbate is tightly coupled to that of glutathione (31), we expect that the antioxidant network remains in homeostasis during the LTR, which makes it very unlikely that in our light quality system reactive oxygen species play a significant regulatory role.

To our knowledge this is the first report describing the effects of DCMU on gene expression in a higher plant using an array approach. A similar study has been performed so far only with a whole genome array of *Synechocystis* (77). By the use of DCMU and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, 140 genes have been reported to be affected by redox signals from the electron transport chain, which is in the same order of magnitude as in our experiment. However, Hihara *et al.* (65) concluded that the redox regulation of photosynthesis genes in *Synechocystis* might be totally different from that in algae and plants. A gene-by-gene comparison between both studies does not provide much useful information, even if we consider that in our array the eukaryotic complement of the cyanobacterial genome is present, because the physiological conditions used in both studies are very different.

DCMU also affected photosynthetic electron flow in plants grown continuously under PSI light indicating that these plants perform linear electron transport. The expression profile of these plants, however, is different from that of PSI–II plants treated with DCMU suggesting that possibly more redox-regulated genes exist than described here. The combined action of DCMU and PSI light on photosynthetic electron flow is difficult to understand to date and requires further detailed analyses; therefore, we described only those genes as redox-regulated that allow us to conclude unambiguously on such a regulation. Data from different studies suggest the existence of several yet unknown redox signals originating from the electron transport chain, including PSII (30, 78, 79). Furthermore, any change in linear electron flow will affect the redox state of components downstream of PSI such as thioredoxin, which in turn will affect the efficiency of the Calvin cycle (69). Whether such signals influence gene expression events in our experimental system is currently under investigation.

Our study indicates that photosynthetic redox signals play an important role in the intracellular signaling network. The photosynthetic redox signals contribute essential information about the light environment in addition to cytosolic photoreceptors thus significantly expanding the ability of plants to sense environmental cues. It appears that this information is transferred from the organelle to the nucleus by mechanisms that differ from other chloroplast-to-nucleus signaling avenues and without the help of photoreceptor-mediated signaling.

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III

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SCREENING FOR *ARABIDOPSIS THALIANA* MUTANTS DEFICIENT IN ACCLIMATORY LONG-TERM RESPONSE TO VARYING LIGHT QUALITIES USING CHLOROPHYLL FLUORESCENCE IMAGING

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Keywords: photosynthetic acclimation, light quality gradients, chlorophyll fluorescence, video imaging, mutant screen

INTRODUCTION

Photosystem stoichiometry adjustment is an important acclimatory response to excitation imbalances between photosystem I and II which often result from natural occuring light quality gradients. Typically, the photosystem ratio is changed in favour of the rate-limiting photosystem (Melis 1991, Fujita 1997, Allen & Pfannschmidt 2000) which improves excitation energy distribution and photosynthetic efficiency (Chow et al 1990, Allen 1992). Changes in photosystem stoichiometry require co-ordinated changes in the expression of photosynthesis genes that are encoded both in the nucleus and the chloroplast genome (Race et al 1999, Rodermel 2001, Pfannschmidt 2003). Photosystem stoichiometry adjustment can be experimentally induced by illumination with artificial light sources that differentially excite PSII (PSII-light) or PSI (PSI-light). In higher plants grown under PSII-light the PSII/PSI ratio decreases while it increases under PSI-light (Glick et al 1986, Chow et al 1990, Kim et al 1993, Pfannschmidt et al 1999). This response is fully reversible (Kim et al 1993) and is based on changes in the expression of plastid (Glick et al 1986, Deng et al 1989, Pfannschmidt et al 1999a, b, Tullberg et al 2000) and nuclear genes (Pfannschmidt et al 2001, Sherameti et al 2002) that are controlled by the changes in the redox state of electron transport components (e.g. the plastoquinone pool). The underlying signal transduction pathways and their components are mainly unknown. The target of our approach was to establish a screen for *Arabidopsis* mutants that have defects in their acclimatory long-term response (LTR) to the PSI- and PSII-light sources. Such a forward genetic approach would allow to identify components of regulatory pathways in chloroplasts and the nucleo-cytosolic compartment. Using chlorophyll fluorescence video imaging we demonstrate that differences in chlorophyll fluorescence between PSI- and PSII-light acclimated *Arabidopsis* seedlings can be used as screening parameter to identify individuals with a changed or missing LTR.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Wild-type and mutant seeds (M_2 population after fast neutron bombardment (55Gy) (Lehle Seeds, USA)) of *Arabidopsis thaliana* (Columbia) were surface-sterilised, germinated (after 2 days at 4 °C) and grown on full MS medium containing 2% (w/v) sucrose. All plants were initially grown for 10 days under white light (~30 μE). The LTR was induced by continuous illumination of seedlings with PSI- or PSII-light as indicated. The light sources were described earlier in detail (Pfannschmidt et al 2001b). Pre-selected LTR-mutants from the first screening step were grown to full rosette stage on soil at a temperature of 20–22 °C and 60–80% humidity under a short-day light regime (8 hours light/ 16 hours dark) until the second LTR screening step was performed.

Determination of F_s/F_m Values. F_s/F_m values of PSI- and PSII-light acclimated seedlings were recorded by chlorophyll fluorescence video imaging using a Fluorcam 700 MF (Photon System Instruments, Brno, Czech Republic) and the following programme: 10 min dark-adaptation, 3 s measuring F_0, strong light pulse of 1.6 s to determine F_m, actinic light for 10 min to determine F_t, dark relaxation of 2 min for F_0'. The $(F_t - F_0')/F_m = F_s/F_m$ value was calculated for each individual. The distribution of F_s/F_m-values over all plants was analysed and the standard deviation calculated to define the thresholds for lacking LTRs. Plants with a F_s/F_m-value beyond these thresholds were regarded as putative mutants. In the second screening step the F_s/F_m values of the selected putative mutant were determined in three parallels with a PAM101/PDA100 fluorometer (Walz, Effeltrich, Germany) as described earlier (Pfannschmidt et al 2001).

Chlorophyll Determination. Spectroscopic determination of Chl *a* and *b* was performed after extraction of N_2–ground material with 80% buffered acetone according to Porra et al (1989).

RESULTS AND DISCUSSION

White light-grown *Arabidopsis* wild-type seedlings were acclimated to PSI- and PSII-light (PSI- and PSII-plants, respectively) and chlorophyll fluorescence was determined with a PAM101 fluorometer. PSI-plants exhibited a higher F_s/F_m value than PSII-plants. If PSI-plants were acclimated to PSII-light the F_s/F_m value declined while it increased when PSII-plants were acclimated to PSI-light (Fig. 1A). This coincides with earlier observations and demonstrates the reversibility of this acclimatory response (Pfannschmidt et al 2001, Sherameti et al 2002). The kinetic of the LTR was followed by determination of Chl *a/b* and F_s/F_m at different time points after LTR induction. In *Arabidopsis* (Col) the LTR took place to 70–80% within 2 days and was completed after 5–7 days (data not shown). The establishment of a screening protocol for acclimation mutants basing on these changes in F_s/F_m was inspired from screens for state transition mutants in which changes in chlorophyll fluorescence of similar extent were used (Borgstädt and Kruse 2001). To screen chlorophyll fluorescence from many seedlings we used a video imaging camera working with pulse-amplitude modulation allowing a simultaneous F_s/F_m-determination of several individuals. Mutagenised *Arabidopsis* seeds were grown for 10 days under white light followed by 2 days in PSI-light and further 2 days in PSII-light. After each acclimation step chlorophyll fluorescence of the seedlings was determined. A maximum of 25 seedlings per plate could be analysed. The small seedling number prohibited an overlay of leaves due to seedling growth which would interfere with exact F_s/F_m determinations of the individuals. Plants acclimated to PSI-light showed an average F_s/F_m-value of 0.163 (standard deviation (SD): 0.046) while they exhibited an average F_s/F_m-value of 0.123 (SD:

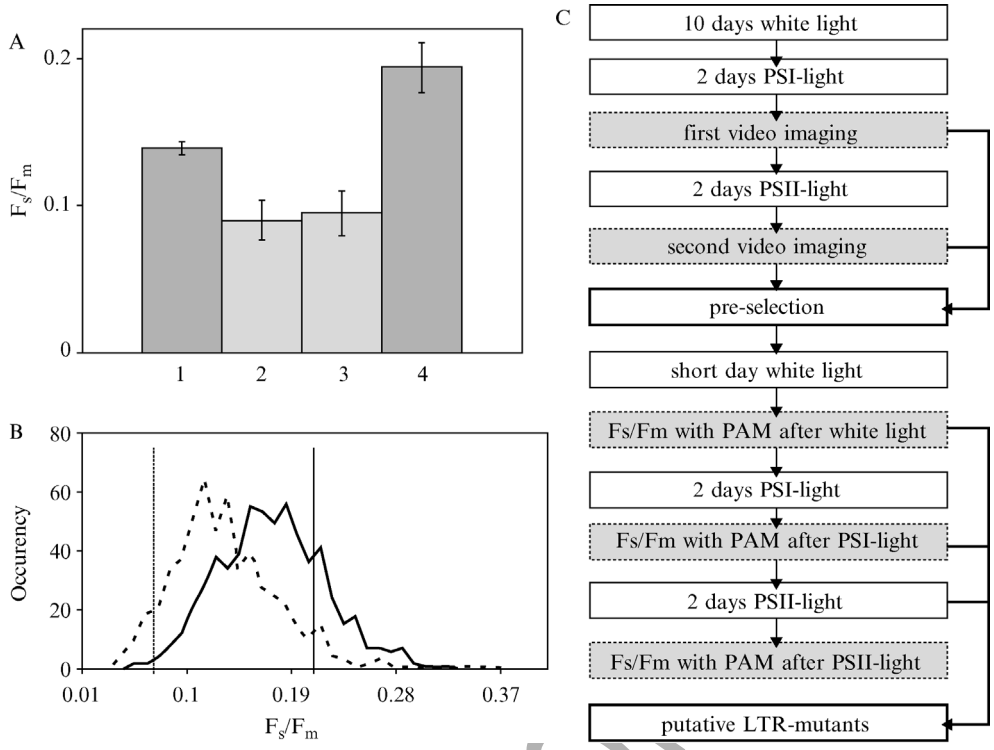

Figure 1: F_s/F_m values in PSI- or PSII-light acclimated *Arabidopsis* seedlings and its use as screening parameter. (A) PAM determination of F_s/F_m from 5 plants each in 3 independent experiments. All plants were grown first for 10 days in white light before the acclimation reactions were induced. Lane 1: 2 days in PSI-light, lane 2: 2 days in PSI-light followed by 4 days in PSII-light, lane 3: 2 days in PSII-light, lane 4: 2 days in PSII-light followed by 4 days in PSI-light. (B) Difference in F_s/F_m of PSI- and PSII-light acclimated mutagenised *Arabidopsis* seedlings. Plants were grown as described followed by F_s/F_m determination with video imaging. Graphs show the distribution of F_s/F_m values of around 600 seedlings (solid line: PSI-light acclimation; broken line: PSII-light acclimation). Thresholds defining putative mutants are indicated by perpendicular lines. (C) Screening strategy for LTR mutants.

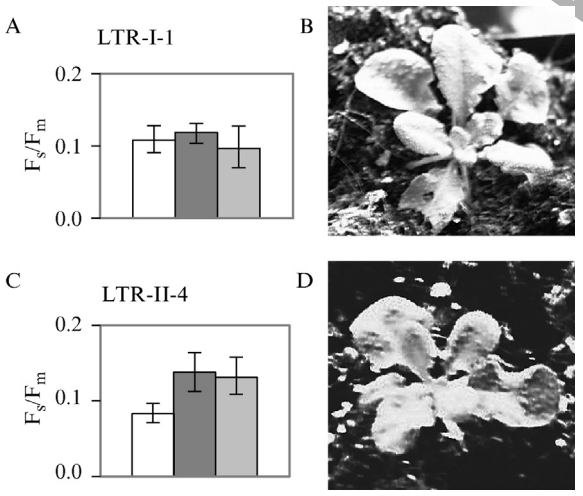

Figure 2: Classes of putative LTR mutants. (A, C) F_s/F_m values of representative plants after several weeks white light (white bar), after 2 days PSI-light (dark-grey bar) and after further 2 days PSII-light (grey bar). (B, D) Phenotypes of LTR-I-1 and LTR-II-4 mutants after 3 weeks in white light.

0.047) after the following acclimation to PSII-light. Both averages were significant different from each other as determined by a student t-test of associated samples. The upper border of SD of PSI-plants was set as threshold for a lacking LTR to PSII-light, i.e. PSII-plants exhibiting a F_s/F_m value above the threshold were regarded as individuals lacking the typical response. The lower border of SD of PSII-plants was defined as threshold for plants lacking a LTR to PSI-light i.e. PSI-plants exhibiting a F_s/F_m-value below this threshold were regarded as individuals lacking a proper response (Fig. 1B). The complete screening strategy is shown in Fig. 1C.

Plants meeting these conditions were selected, grown up to full-rosette stage and F_s/F_m values were determined again after a second acclimation to PSI- and PSII-light with a PAM101/PDA100. This two-step strategy was succesful in identifying two different groups of mutants (Fig. 2). One group showed no reaction to the oxidation signal by PSI-light, i.e. it exhibited no increase in the F_s/F_m value while a second group revealed such an increase, however did not show a reaction to the reduction signal by PSII-light since no decrease in the F_s/F_m value could be observed. We regard the groups as putative LTR mutants named LTRI (for the first group lacking the response to PSI-light) and LTRII (for the second group lacking the response to PSII-light). The existence of two groups suggest that the LTR involves more than one regulatory pathway for acclimation. All isolated plants grow autotrophically on soil and none of them shows a phenotype that differs from wild-type under standard white light (Fig. 2) as expected for acclimation mutants.

The screening method presented here therefore is a useful tool to identify mutants with defects in regulatory components of photosynthetic acclimation to varying light qualities and might be of importance also for identifying new regulatory components of photosynthesis in general.

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IV

Fey V, Allahverdiyeva Y, Aro EM, Pfannschmidt T (to be submitted) Photosynthetic redox control during light-quality acclimation in *Sinapis alba* has specific effects on phosphorylation state and composition of proteins involved in plastid gene expression.

Title: Photosynthetic redox control during light-quality acclimation in *Sinapis alba* has specific effects on phosphorylation state and composition of proteins involved in plastid gene expression

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Summary

Long-term exposition of mustard seedlings to light sources favouring either photosystem I or photosystem II leads to an acclimation response in which the stoichiometry of the photosystems is readjusted. This can be detected in chlorophyll fluorescence spectra at 77 K. In addition, the structural differences in the photosynthetic apparatus of such plants are accompanied by differential phosphorylation states of photosystem II proteins. The photosystem stoichiometry adjustment involves a transcriptional regulation of the *psaAB* genes encoding the P700 apoproteins of photosystem I. In order to analyse the molecular basis of this regulation we purified chloroplast protein fractions containing RNA polymerase and DNA-binding activity from mustard seedlings acclimated to PSI or PSII light. We compared the protein composition and the biochemical properties of such fractions with special emphasis on kinase activities and phosphorylation state. Our results demonstrate that long-term acclimation to varying light qualities not only affects structure and phosphorylation state of the photosynthetic apparatus but also involves very specific changes in the accumulation and phosphorylation of proteins performing or affecting chloroplast gene expression. The data suggest a possible pathway by which redox signals from the thylakoid membrane may be mediated to the level of gene expression.

Introduction

Photosynthetic organisms possess a great number of molecular mechanisms which enable them to acclimate the photosynthetic process to a fluctuating environment (1,2). Especially changes in incident light directly affect the photosynthetic electron transport and can dramatically reduce its efficiency. Acclimation responses maintain or restore the photosynthetic elec-

tron flux under such adverse conditions and, by such means, help to keep the net energy fixation as high as possible.

Naturally occurring light quality gradients often result in a favoured excitation of one photosystem over the other. Since the two photosystems work electrochemically in series this induces an imbalance in excitation energy distribution between them. In the short-term this is counteracted by a process called state transition which occurs in the order of minutes. In this acclimation response the mobile part of the light harvesting complex of photosystem II (PSII) (LHCII) is phosphorylated through the action of a redox-sensitive kinase and subsequently migrates and attaches to photosystem I (PSI). The variation of the antenna cross section redirects a part of the incident light between the photosystems and restores the excitation imbalance between them. The activity of this LHCII kinase is controlled by the redox state of the plastoquinone (PQ) pool where its reduction results in activation and its oxidation in inactivation of the kinase (3-5). The transduction of the signal is still not understood; however, mutation analyses showed that it involves the Qo site of the cytb_6f complex which serves as a sensor for the redox state of the PQ pool (6-8). In the long-term, excitation imbalances are counteracted by a readjustment of photosystem stoichiometry which occurs within hours to days dependent on the species. This long-term response (LTR) results in the same but longer lasting effect as the state transition and can be observed over a wide range of species ranging from cyanobacteria over unicellular algae up to higher plants (9-12). In mustard and pea it could be shown that this response is also regulated by the redox state of the PQ pool *via* a mechanism in which the expression of the core proteins of the two photosystems is oppositely regulated (13-15). Under conditions resulting in a reduced PQ pool transcription of *psaAB* (encoding the P700 apoproteins A and B of PSI) is enhanced while this occurs for *psbA* (encoding the D1 protein of PSII) under conditions leading to an oxidized PQ pool. How the redox signal from the PQ pool is transduced to the level of transcription is still unknown.

Chloroplast transcription involves the activity of two different RNA polymerases, a single-subunit phage-type nuclear encoded polymerase (NEP) which appears in two different copies (rpoT1 and rpoT2) and a multi-subunit bacteria-like plastid encoded polymerase (PEP) (16-18). The genes *psbA* and *psaAB* are predominantly transcribed by PEP suggesting that it is the main target for redox regulation. Phosphorylation was also shown to be a key event in the regulation of chloroplast transcription initiation. Both phosphorylation of PEP subunits and of the so-called sigma factors have been shown to be important (19,20). It is catalysed by a serine-specific kinase termed plastid transcription kinase (21). The kinase belongs to the family of casein kinases II (22). Its *in vitro* phosphorylation activity was shown to be regulated by the redox state of glutathione (21) which correlates to *in planta* observations showing that its activity is enhanced under high light stress when the glutathione redox state in chloroplasts turns to be more oxidised (23).

In the present study we investigate the relationship between light quality induced redox signals from photosynthetic electron transport and the phosphorylation state of thylakoid proteins and chloroplast protein fractions with DNA-binding and RNA polymerase activities. Chloroplasts of mustard seedlings acclimated to light qualities which induce short-term and long-term acclimations were isolated and DNA-binding proteins and RNA polymerases were enriched by subsequent heparin-Sepharose chromatography. Biochemical properties of these fractions with respect to phosphorylation state and kinase activity were analysed and compared. Our results show distinct differences in accumulation as well as phosphorylation state for a small number of proteins suggesting an important role of phosphorylation events in the mediation of photosynthetic redox signals to the level of gene expression.

Material and Methods

Plant material and growth conditions

Mustard seedlings (*Sinapis alba*, L., var. Albatros) were grown on soil for seven days under continuous illumination at 20°C and 60% humidity. Developing seedlings were subjected to a growth light regime of 5 days under PSI-light followed by two days under PSII-light or *vice versa* as described earlier (13). For primer extension analyses additionnal control plants were grown for 7 days under PSI- or PSII-lights. Lamps and filters for the light sources favouring PSI or PSII were as described (24). White light control plants were grown under comparable conditions; however, under white light fluorescent stripe lamps (Osram). After 7 days cotyledons were harvested under the respective growth light, placed on ice and immediately used for preparation of chloroplasts or thylakoids.

Thylakoid isolation and 77 K chlorophyll fluorescence measurements

Thylakoids were isolated from cotyledons in a cold room under dim green safelight in the presence of 10 mM NaF to inhibit phosphatase activity as described (25). Chlorophyll content of thylakoid preparations was determined by extraction in 80% (v/v) buffered acetone (2.5 mM Hepes/NaOH pH 7.5) and quantified according to Porra et al. (26). Fluorescence emission spectra were measured at 77 K using a diode array spectrophotometer (S2000, Ocean Optics) equipped with a reflectance probe. Fluorescence was excited with light below 500 nm and the emission was recorded between 600 and 780 nm. For measurements the thylakoid preparations were diluted to a final Chl concentration of 8 µg/ml in storage buffer (50 mM Hepes/KOH, pH 7.5, 100 mM sorbitol, 10 mM $MgCl_2$ and 10 mM NaF). Three parallels of each sample were measured and averaged before plotting.

Protein phosphorylation in isolated thylakoids

Thylakoids were diluted on ice to a final Chl concentration of 0.5 µg/µl in storage buffer (see above) and ATP was added to a final concentration of 0.4 mM. Samples were illuminated with a photon flux density of 100 µE for 20 min at room temperature using Osram Dulux S 11W/41 lamps (Osram, München, Germany). Subsequently fractions were solubilized in SDS-sample buffer (1% SDS, 5% glycerol, 3.67 mM 2-mercaptoethanol, 10.7 mM Tris-HCl pH 6.8, 0.2% bromo phenol blue) and an amount corresponding to 2.5 µg Chl each was subjected to SDS-PAGE using a gel containing 15% acrylamide and 6% urea. After electrophoresis proteins were transferred to a PVDF membrane (Immobilon-P, Millipore, USA) by electroblotting and the membrane was blocked with 1% fatty acid-free bovine serum albumin. Phosphoproteins were immuno-detected using a polyclonal rabbit anti-phospho-threonine serum (Zymed Laboratories, San Francisco, USA) according to Rintamäki et al. (27). Visualisation was performed using an alkaline phosphatase-goat anti-rabbit IgG conjugate and the Phototope-Star Chemiluminescent Detection Kit (New England Biolabs, Beverly, USA).

Primer extension analyses

For RNA isolation 2 g of leaf material was harvested and frozen in liquid N_2 under the respective light source. Total RNA was isolated using the TRIzol reagent (Invitrogen Life Technologies, Karlsruhe, Germany) following a protocol described earlier (28). Concentration and purity of RNA samples were determined spectroscopically in a Biophotometer (Eppendorf, Hamburg, Germany). Intactness was proven by ethidium bromide staining of rRNA species after electrophoretic separation of aliquots on denaturing 1.2% agarose gels containing formaldehyde (29). Isolated RNA was stored at -80°C until further use. Primer extension analyses (30) were then carried out essentially as described earlier (24). The gene-specific primer for the *psaAB* operon was: 5' IRD 700, 5' CCC ATT CCT CGA AAG 3' (sequence position +65 - +79 relative to ATG). The same primer was used to sequence the respective region of

mustard chloroplast DNA using a cycle sequencing kit (MBI Fermentas, St. Leon-Roth, Germany).

Preparation of protein fractions with RNA polymerase and DNA-binding activity

Chloroplasts were isolated by differential centrifugation followed by sucrose gradient centrifugation as described (31,32). The isolated plastids were lysed and subjected to a heparin-Sepharose CL-6B (HS) chromatography according to (19). After extensive washing the bound proteins were eluted with a single step of 2 M $(NH_4)_2SO_4$. Collected fractions containing the elution peak were identified visually by separating aliquots of all samples *via* SDS-PAGE on 7.5 – 15% gradient gels followed by silver staining and enzymatically by determination of the *in vitro* transcription activity of the fractions using a standard protocol described earlier (33). Identified peak fractions were dialysed against storage buffer (50 mM Tris/HCl pH 7.6, 0.1 mM EDTA, 5 mM 2-mercaptoethanol, 0.1% Triton X-100 and 50% glycerol) and stored at -20°C until further use.

Phosphorylation and dephosphorylation of heparin-Sepharose fractions

Phosphorylation/dephosphorylation reactions were performed according to Tiller and Link (19) and Baginsky et al. (21) with slight modifications. 100 µg protein of the heparin-Sepharose fractions were phosphorylated either by endogenous kinase activity or by adding 30 U of the catalytic subunit of the cAMP-dependent protein kinase of bovine heart (PKA) in a final volume of 60 µl. The reaction mixture contained final concentrations of 130 mM Tris-HCl pH 8.0, 40 mM KCl, 4.5 mM $MgCl_2$, 2 mM $CaCl_2$, 3.5 mM 2-mercaptoethanol, 0.5 mM DTE, 0.4 mM EDTA, 0.1 mM ATP and 0.1 mM $MgCl_2$ and were incubated for 30 min at 30°C. For radioactive labelling of proteins 0.74 MBq γ-^{32}P-ATP in 5 µl containing 3.3 µM ATP and 3.3 µM $MgCl_2$ were added instead of the unlabelled 0.1 mM ATP and 0.1 mM $MgCl_2$. Radioactive PKA phosphorylation of pre-treated heparin-Sepharose fractions was

performed after heat-deactivation of endogenous proteins (5 min at 90°C). Pre-treaments were either non-radioactive phosphorylation (see above) or dephosphorylation as indicated. For dephosphorylation 100 µg protein of the heparin-Sepharose fractions were incubated in the same reaction mixture as for phosphorylation but in presence of 1.2 U shrimp alkaline phosphatase (SAP) (Roche). After phosphorylation/dephosphorylation reactions all proteins were heat-inactivated for 5 min at 90°C in the presence of SDS-sample buffer (see above) and subjected to SDS-PAGE on a 7.5 - 15% acrylamide gradient gel. After electrophoresis the gel was silver-stained and dried on a vacuum gel dryer. Radioactive signals were detected and visualized using a Storm phosphorimager and the *ImageQuant* software (Amersham Biosciences AB, Uppsala, Sweden).

Detection of phospho-aminoacids in heparin-Sepharose fractions

To examine the *in vivo* phosphorylation state of the heparin-Sepharose fractions, 25 µg of each fraction were solubilised and subjected to SDS-PAGE using 7.5 – 15% gradient gels as before. In parallels 25 µg of each HS-fraction were dephosphorylated using 1.5 U calf intestinal alkaline phosphatase (CIAP) (Roche). Subsequently NaF was added to a final concentration of 10 mM to inhibit the phosphatase and an autophosphorylation assay in presence of 0.1 mM ATP was performed as described above. Western-immuno-detection of the phosphorylation state of proteins was performed using polyclonal rabbit anti-phosphoserine, polyclonal rabbit anti-phosphothreonine, and monoclonal mouse anti-phosphotyrosine antibodies (Zymed Laboratories, San Francisco, USA) and enhanced chemiluminescence (see above).

Results

State transitions in mustard seedlings acclimated to PSI- or PSII-light

In earlier experiments we grew mustard seedlings 7 days under PSI- or PSII-light (PSI or PSII plants, respectively) or they were grown first for 5 days under PSI-light followed by 2 days in PSII-light or *vice versa* (PSI-II or PSII-I plants, respectively) (13,14). Monitoring the room-temperature chlorophyll fluorescence of such seedlings uncovered that plants after they have acclimated to PSI- or PSII-light exhibit differences in light-quality-induced state transition experiments which are most pronounced in PSI-II and PSII-I plants (14). This effect can be best explained by the phenomenon of photosystem stoichiometry adjustment which occurs under the long-term exposition to the different light qualities and thus also affects the short-term response. Here we tested the 77 K Chl fluorescence of thylakoids isolated from PSI-II and PSII-I plants (Fig. 1). The scans revealed no differences in the position of the PSII [CP43 (684 nm) and CP47 (694 nm)] or PSI (732 nm) emission peaks. After normalization of the data to the PSII emission peak, however, the PSI-II plants exhibit a higher PSI-fluorescence than PSII-I (Fig. 1A). This resembles measurements typically obtained with plants which are in state 2 or state 1. State transitions are also known to modulate in the short-term the phosphorylation state of thylakoid proteins preferably at N-terminal threonine residues (34). To test if phosphorylation differences are also present after long-term acclimation we proved the appearance of phospho-threonine residues in the major PSII thylakoid proteins by western-immuno-detection (Fig. 1B). PSI-II and PSII-I thylakoid preparations after white light-induced *in vitro* phosphorylation exhibit comparable phosphorylation patterns (Fig. 1B, lanes 1, 2) which show slightly stronger signals as in white light-grown control plants and a non-illuminated phosphorylation control (Fig. 1B, lanes 3, 4). Thylakoid samples without any pre-treatment, however, exhibit differences in the phosphorylation pattern. With the exception of D2 all proteins are stronger phosphorylated in the PSII-I than in the PSI-II sample (Fig. 1B,

lanes 5, 6). Since in all assays phosphatase activities are inhibited by NaF and the illuminated controls (lanes 1, 2) show equal phosphorylation patterns we conclude that these observed differences are due to differential kinase activities in the PSI-II and PSII-I plants. This indicates that long-term exposition to the different light sources affect the phosphorylation state of PSII proteins in a specific way, suggesting that these differences are important for the stoichiometry adjustment.

Transcriptional activity at the *psaAB* promoter

Earlier experiments in mustard indicated a redox-controlled transcriptional regulation of the *psaAB* operon under the different light qualities (13,14). We performed primer extension analyses with isolated RNA from PSI, PSI-II, PSII and PSII-I plants to elucidate transcript initiation points in the *psaAB* promoter region under these conditions (Fig. 2). Under all conditions we found the same 5'- transcript end occurring at position – 187 relative to the translation start. No further ends could be detected. The signal increases after a shift from PSI to PSII-light while it decreases in the reverse experiment. This observation is consistent with the earlier reports with respect to changes in the RNA accumulation. In addition, it indicates that the redox regulation occurs at a single transcript initiation site located directly behind typical PEP promoter elements suggesting that the PEP is the target for the redox signal from the thylakoid membrane.

Purification of chloroplast protein fractions with RNA polymerase and DNA-binding activity

We assumed that the mediation of the PQ redox signal involves the action of unknown trans-factors and/or post-translational modifications of the transcriptional machinery. In order to get deeper insight into the *psaAB* expression regulation we grew mustard seedlings for 5 days under PSI-light followed by 2 days under PSII-light and *vice versa*, harvested the cotyledons,

isolated chloroplasts by sucrose-gradient centrifugation and lysed the respective isolates in a buffer containing the non-ionic detergent Triton X-100. The resulting lysates were subjected to heparin-Sepharose chromatography and bound proteins were eluted with a high-salt step (Fig. 3A). Such fractions have been shown earlier to contain chloroplast RNA polymerase and proteins with DNA-binding activity (19,33). The collected fractions were analysed for unspecific RNA polymerase activity (Table 1) and aliquots of active peak fractions were subjected to SDS-PAGE followed by silver staining (Fig. 3B). The fractions from both plant sources exhibit comparable transcriptional activities and reveal an almost identical protein composition. One weak band at around 27 kDa appears in the PSI-II but not in the PSII-I sample while a second weaker band at 30 kDa appears in the PSII-I but not the PSI-II sample. All other visible proteins can be identified in both samples and in most cases in even identical amounts suggesting only minor structural differences between both samples. In the next steps we therefore focussed on differences in biochemical properties of the proteins in the two samples.

Kinase activities in PSI-II and PSII-I fractions

Phosphorylation events are crucial determinants in the regulation of both state transitions and chloroplast transcription initiation (35,36). Heparin-Sepharose fractions of mustard chloroplast lysates have been shown to contain endogenous kinase activities (19,20). To test a possible light quality effect on such kinase activities in our heparin-Sepharose fractions an autophosphorylation assay with γ-^{32}P-ATP was performed (Fig. 4). Radioactively labelled proteins were visualised after electrophoretic separation and exposition to a phosphorimager. Both samples revealed endogenous kinase activity; however, the radioactive signals in the PSII-I sample are much stronger than in the PSI-II sample (Fig. 4, lanes 1, 2). To elucidate if this is due to different kinase activities or general protein phosphorylation state endogenous kinase activities were heat-inactivated and the proteins were phosphorylated by exogenously added bovine heart kinase (PKA) (Fig. 4, lanes 3 and 4). The PKA treatment resulted in simi-

lar phosphorylation patterns of both samples but revealed a few bands at around 140, 28, 24 and 16 to 12 kDa which differ in signal strength (black arrows) suggesting that they could vary in their phosphorylation state. To prove this parallel samples were pre-treated with a phosphatase before radioactive phosphorylation through PKA to display the maximum of phosphorylatable proteins (Fig. 4, lanes 5 and 6). The resulting patterns are almost identical as with PKA treatment alone indicating that it is not a differing phosphorylation state which causes the variations in the signal strength. This result therefore points more to varying kinase activities and/or varying accessibilities of phosphorylation site(s) in the PSI-II and PSII-I samples as reason for the pattern differences observed in lanes 1 and 2.

Protein phosphorylation state of heparin-Sepharose fractions

To analyse the phosphorylation state of the proteins in the PSI-II and PSII-I fractions in more detail we visualised the original phosphorylation state after purification by western-immuno-detection using antibodies directed against phospho-serine (P-Ser), phospho-threonine (P-Thr) and phospho-tyrosine (P-Tyr) (Fig. 5). The overall phosphorylation patterns for all three amino acids is very similar in both fractions, but a few proteins exhibit exclusive or preferential phosphorylation under one specific condition when the signals were compared over all three eluted peak fractions (indicated by arrows in Fig. 5). Most prominent examples are: i) one phosphorylation signal at around 54 kDa which occurs exclusively in the P-Thr test and which is higher phosphorylated in the PSI-II sample; ii) one prominent signal at around 31 kDa in all three phospho-amino acid tests where it is always higher phosphorylated in the PSI-II fraction iii) one signal at around 30 kDa that occurs also in all three phospho-aminoacid tests, but here exclusively in the PSII-I sample. Other examples can be found in the lower molecular range (compare Fig. 5). This indicates that specific differences in the protein phosphorylation state in the PSI-II and PSII-I samples exist and that DNA-binding proteins

like thylakoid proteins (compare Fig. 1) are targets for selective or preferential phosphorylation under the different light qualities.

Phosphorylation level of heparin-Sepharose fractions after dephosphorylation and subsequent *in vitro* phosphorylation

In order to characterise the endogenous kinase activities in more detail we dephosphorylated the heparin-Sepharose fractions, inactivated the phosphatase by addition of NaF and subjected such pre-treated samples to an autophosphorylation experiment using the endogenous kinase activities. The resulting phosphorylation patterns were again visualised using western-immuno-detection (Fig. 6). In this experiment we also found comparable phosphorylation patterns in the PSI-II and PSII-I samples; however, with all three phospho-amino acid antibodies we detected for many proteins higher phosphorylation degrees in the PSI-II than in the PSII-I sample. Only two signals detected with the P-Tyr antibody exhibit a higher phosphorylation state in the PSII-I sample (Fig. 6, lanes 5 and 6). This suggests the action of several kinases and/or a selective activation/deactivation of kinases by the phosphatase pre-treatment. Interestingly most of the phosphorylated proteins above the 32.5 kDa marker (compare Fig. 5) are not phosphorylated by the endogenous kinases neither in PSI-II nor in PSII-I samples.

Discussion

Long-term acclimation of mustard seedlings to light conditions favouring either PSII or PSI results in a photosystem stoichiometry adjustment which has also an impact on the ability to perform short-term responses (14). This is reflected by the different Chl fluorescence of PSI-II and PSII-I seedlings in the 77 K spectra (Fig.1A). The extent to which each lateral antenna movement and photosystem stoichiometry adjustment contributed to this difference cannot be

defined yet and requires further investigation. However, the experiment clearly demonstrates the functional consequences of the acclimation response. We also observed differences at the level of thylakoid PSII protein phosphorylation (Fig. 1B). Phosphorylation of photosystem proteins is known to be redox-controlled and to be important for assembly and reconstruction of these membrane multiprotein complexes (37,38). In general, the PSII-I plants exhibit stronger phosphorylation signals of PSII subunits than PSI-II plants with the exception of the D2 protein which appears higher phosphorylated in PSI-II plants. This opposite phosphorylation of the D1 and D2 proteins is a surprising result which cannot be explained by current models and suggests a specific regulation event which is not addressed in this study. Nevertheless, the results show that prolonged exposition to the different light qualities has a defined impact on the phosphorylation state of PSII proteins even when the LTR is finished.

The main topic of this study was to investigate the molecular basics of the LTR at the level of gene expression. The primer extension analyses confirm earlier studies which show that the regulation of transcription initiation at the *psaAB* promoter operon is a crucial point in the LTR. We detected specific light-quality-dependent changes in the accumulation of a single transcript end starting directly behind the PEP promoter. The detected initiation point and the observed changes in accumulation correspond to earlier results obtained with *Arabidopsis* grown under a comparable light regime (24). A second transcript end as detected in *Arabidopsis* could not be identified pointing to species-specific differences. The present experiment confirms that a redox control of *psaAB* transcription initiation must be mediated at the standard PEP promoter. To prove if PEP or other DNA-binding proteins are affected we enriched chloroplast protein fractions with transcriptional (Table I) and nucleotide-binding activity (19,39). The protein compositions of the PSI-II and PSII-I samples after heparin-Sepharose chromatography appear almost identical (Fig. 3B); however, because of the limited resolution of one-dimensional gel electrophoresis possible light–quality effects on the accumulation of proteins masked by others of the same size cannot be excluded. This question is currently

under investigation using two-dimensional SDS-PAGE combined with mass spectroscopy. However, because no major differences in protein composition could be identified we focussed on post-translational modifications, i.e. protein phosphorylation events as possible regulatory processes responsible for the varying *psaAB* transcription in PSI-II and PSII-I samples. Phosphorylation has been shown earlier to be very important for promoter recognition in mustard (19) suggesting it as a likely regulation level involved in the mediation of the PQ redox signal especially since the light sources are able to induce differential phosphorylation states of photosystem proteins (Fig. 1B). The autophosphorylation experiment indeed revealed endogenous kinase activity in both fractions but resulted in a stronger phosphorylation pattern with the PSII-I than with the PSI-II samples. To test if such differences reflect variations in kinase activity or protein phosphorylation state we performed a phosphorylation experiment with an exogenously added kinase (PKA). This resulted in very similar phosphorylation patterns in general but also revealed a few specific differences between the samples. This and the observation that the substrate specificities of PKA and the endogenous kinase(s) appear different (compare Fig. 4) led to an unclear result concerning the problem in question. A further phosphorylation experiment after a pre-dephosphorylation resulted in identical phosphorylation patterns as with untreated samples suggesting a very similar protein phosphorylation state of both samples. However, this experimental approach represents a very crude method to detect the phosphorylation state of a sample. To get more detailed information we analysed the original phosphorylation state of the proteins after the purification using phospho-aminoacid specific antibodies. These experiments clearly demonstrate that the general phosphorylation pattern for all three amino acids tested is indeed very similar in both samples but a few differences also appeared (Fig. 5, black arrows). One protein at around 30 kDa seems to be phosphorylated at all three amino acids only in the PSII-I sample (Fig. 6, pictures 4 and 6). The size of this signal coincides with a weak protein band which appeared exclusively in this sample after SDS-PAGE (compare Fig. 3). A second protein at 31 kDa appears to be higher phos-

phorylated in the PSI-II sample at all three P-aminoacid sites (Fig. 6). The corresponding protein band after SDS-PAGE is more prominent in the PSI-II plants (Fig. 3). Therefore, in both cases a stronger phosphorylation signal corresponds to a higher protein amount suggesting a differential accumulation of the respective protein as the reason for the differing phosphorylation signals. The signal at around 54 kDa, however, seems to be unique in that a differential phosphorylation signal occurs only for P-Thr site(s) and in that the corresponding protein band after SDS-PAGE does not exhibit major differences in accumulation (Fig. 3) suggesting in this case a differential phosphorylation state. A few differentially phosphorylated bands occur in the range below 25 kDa, however, because of the high amounts of proteins in this area identification of corresponding proteins is difficult.

Apparently, both preferential accumulation and phosphorylation occur for a limited number of proteins under the different light quality conditions. Such proteins may account for the observed regulation of the *psaAB* promoter utilisation and possibly other regulatory gene expression events in the LTR. These observations imply both different kinase activities as well as different accumulation of putative regulatory proteins during acclimation to PSI- or PSII-light. The identification of such proteins is currently addressed by the above mentioned mass spectrometry approach.

Of special interest in this context is the differentially phosphorylated protein at 54 kDa. Earlier studies in mustard identified a kinase activity of the same size in heparin-Sepharose fractions which was able to phosphorylate sigma-like-factors *in vitro* (20). This plastid transcription kinase (PTK) was found to be less active in a phosphorylated state and to be activated antagonistically by the redox state of glutathione *in vitro* (20,21). The redox state of glutathione under PSI- or PSII-light appears to be stable (24) excluding this level of regulation of the kinase activity. This suggests that only the phosphorylation state under our conditions is of importance. This is consisting with our observation that the 54 kDa band is higher phosphorylated at (a) P-Thr site(s) in PSI-II plants (Fig. 5, panel 3 and 4) and that the en-

dogenous kinase activity in these fractions is low (Fig. 3, lane 1). Under PSII light the electron transport chain, i.e. the PQ pool, is more reduced than under PSI-light (40,41) leading to an activation of the LHCII kinase which is known to be a threonine kinase. It is tempting to speculate that the 54 kDa protein is a substrate of the LHCII kinase which phosphorylates this protein in response to the light quality induced redox changes of the thylakoid membrane i.e. of the PQ pool. This is consistent with the observation that after dephosphorylation of the heparin-Sepharose fractions endogenous kinase activities were not able to phosphorylate the 54 kDa protein again suggesting that the responsible kinase (i.e. the LHCII kinase) is lost during the chromatography (Fig. 6). If the 54 kDa protein indeed represents the PTK which phosphorylates sigma-like-factors (or other unknown transcription initiation factor(s)) in its low phosphorylated state this would provide a mechanistic link between the redox state of PQ and PTK activity which regulate the transcription initiation at PEP promoters by differential phosphorylation of proteins involved in transcription initiation. This idea is supported by studies on *Arabidopsis* mutants lacking LHCII kinase activity. A regulation of PTK *via* phosphorylation through LHCII kinase activity cannot occur in these mutants. Under such conditions we would expect that no differences in the Chl fluorescence parameter Fs/Fm, an indicator for the occurrence of the LTR (40-42), are detectable between PSI- and PSII-light acclimated plants. Exactly this can be observed in these mutants (R. Wagner, T. Pfannschmidt, D. Leister, unpublished data). In summary, this provides an attractive working hypothesis which can be tested by detailed analyses of photosystem stoichiometry adjustment in these mutants.

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Figure legends

Fig. 1: *State transitions in mustard seedlings acclimated to different light qualities.* (A) 77 K chlorophyll fluorescence spectra. Thylakoids isolated from PSI-II (I-II) and PSII-I (II-I) seedlings as well as from white-light grown control plants (W) were analysed. Resulting Chl fluorescence spectra were normalised to the PSII emission peak (F684). Graphs represent the mean of three measurements. (B) Thylakoid protein phosphorylation. Phosphorylation state after (lanes 1-3) and before (lanes 5-7) light-induced *in vitro* phosphorylation of the same thylakoid preparations as in (A). Lane 4: Dark control. Equal thylakoid amounts (2.5 µg Chl) were tested for the phosphorylation state of PSII proteins (CP43, D2, D1, LHCII and a putative CP29 as indicated) using a P-Thr antibody.

Fig. 2: *Primer extension assays at the psaAB promoter*

Plants were grown under PSI, PSI-II, PSII and PSII-I growth light regimes and total RNA was isolated from each condition. A fluorescence dye labelled primer was designed to anneal within the first 50 bp of the coding region of the *psaAB* operon and was used both in a reverse transcription reaction with the isolated RNA and a sequencing reaction of chloroplast DNA fragments covering the *psaAB* promoter region. Products were separated in parallel on a denaturing 4 % acrylamide gel containing 7 M urea and detected by laser excitation in a Licor

4200 sequencer. A sequencer image of the primer extension analysis is shown. The DNA sequence within the *psaAB* promoter is shown on the left, primer extension products on the right flanked by a magnification of the primer extension products. The detected 5´-end is marked by a dotted line, the respective transcription start nucleotide is given in bold letters. Growth conditions are given on bottom.

Fig. 3: *Purification and electrophoretic separation of putative DNA-binding proteins with RNA polymerase activity*. (A) Scheme of isolation procedure for chloroplast protein fractions with RNA-polymerase and DNA-binding activity. (B) Peak fractions with RNA-polymerase activity after step elution (compare table I). Equal volumes (10 µl) of collected fractions were separated on a 7.5 - 15% SDS gel and stained with silver. Sizes of marker proteins separated in parallel are given in the left margin. Number of transcriptional active fractions (compare Table I) are given on the bottom.

Fig. 4: *Characterisation of endogenous kinase activity in the heparin-Sepharose fractions*. Kinase activity from heparin-Sepharose purified proteins from PSI-II and PSII-I chloroplasts was assayed in *in vitro* phosphorylation reactions using γ-^{32}P-ATP. Radioactively labelled proteins were separated by SDS PAGE and detected by using a phosphorimager. Radioactive bands are shown. Sizes of marker proteins separated in parallel and stained with silver are given in the left margin. Lanes 1 and 2: Autophosphorylation of pooled peak fractions after heparin-Sepharose chromatography. Lanes 3 and 4: Peak fractions were heat-inactivated and subjected to phosphorylation by an exogenously added PKA. Lanes 5 and 6: Peak fractions were dephosphorylated using shrimp alkaline phosphatase, heat-inactivated and finally radioactively labelled by PKA. The strong additional band at 58 kDa represents the deactivated phosphatase. Lane 7: PKA autophosphorylation control.

Fig. 5: *Protein phosphorylation state of heparin-Sepharose fractions*. The phosphorylation states of the purified PSI-II and PSII-I peak fractions (compare Fig. 3) were characterised by western-immuno-detection after SDS-PAGE using anti-phospho-amino acid antibodies directed against phospho-serine (P-Ser, lane 1 and 2), phospho-threonine (P-Thr, lane 3 and 4), and phospho-tyrosine (P-Tyr, lane 5 and 6). Black arrows mark proteins which exhibit exclusive or clearly higher phosphorylation (with respect to all three peak fractions) in that sample to which the arrow is placed. No phosphorylated amino acids were detectable in proteins above appr. 70 kDa even after extended detection. This region is not shown to increase resolution of the lower parts of the gel.

Fig. 6: *Phosphorylation level of heparin-Sepharose fractions after dephosphorylation and subsequent in-vitro phosphorylation*. Fractions were dephosphorylated by phosphatase treatment. After inactivation of phosphatase by adding NaF the fraction were subsequently phosphorylated by the endogenous kinase activities. As in Fig. 5 arrows mark proteins which are phosphorylated exclusively or preferentially (with respect to all three peak fractions) in that sample to which the arrow is placed. As in Fig. 5 no phosphorylated amino acids were detectable in the molecular weight region above 70 kDa (therefore not shown).

Tables

Table I: General transcriptional activiy in fractions eluted from the heparin-Sepharose column.

	fraction	transcription activity [cpm]	standard error [%]
PSI-PSII			
	6	567,8	7,54
	7	2035,82	4,97
	8	1302,16	5,1
	9	188,16	12,39
	10	420,5	8,75
PSII-PSI			
	6	2082	4,98
	7	2239,1	4,94
	8	2929,13	4,98
	9	621,44	7,2
	10	425,5	8,73

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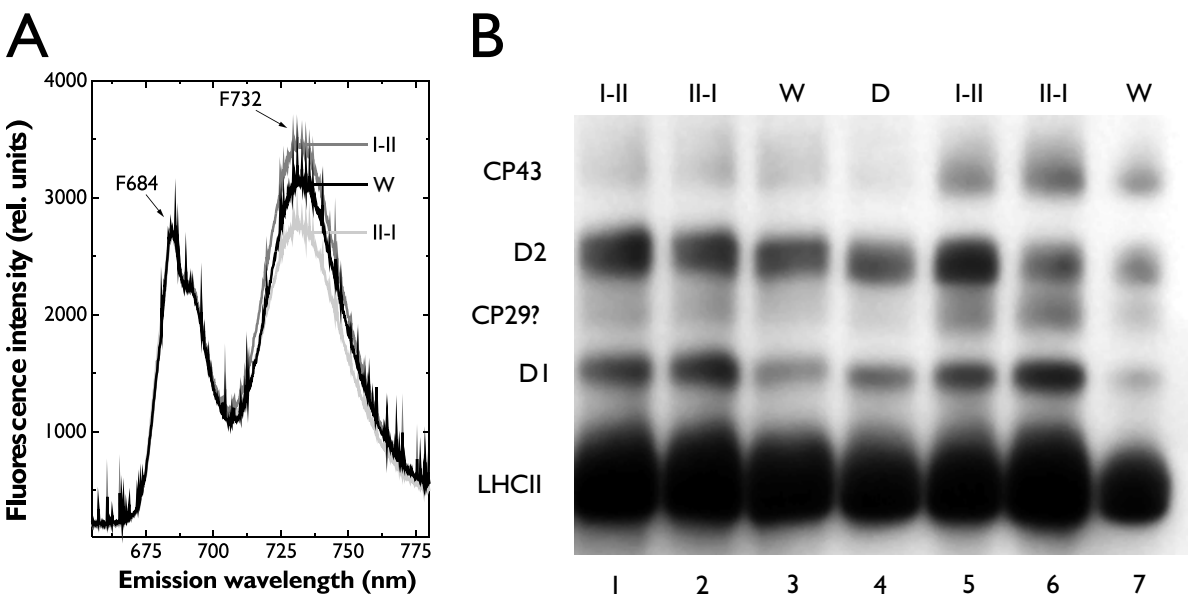
Fig 1


A
B
4000
3000
2000
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F732
F684
I-II
W
II-I
Fluorescence intensity (rel. units)
675
700
725
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Emission wavelength (nm)
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CP43
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CP29?
D1
LHCII
1
2
3
4
5
6
7

Fig 2

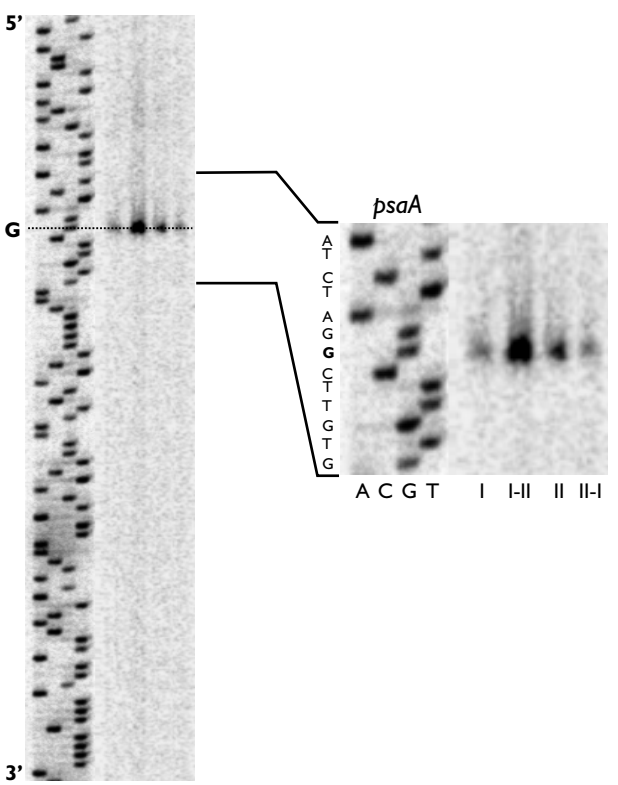


Fig 3

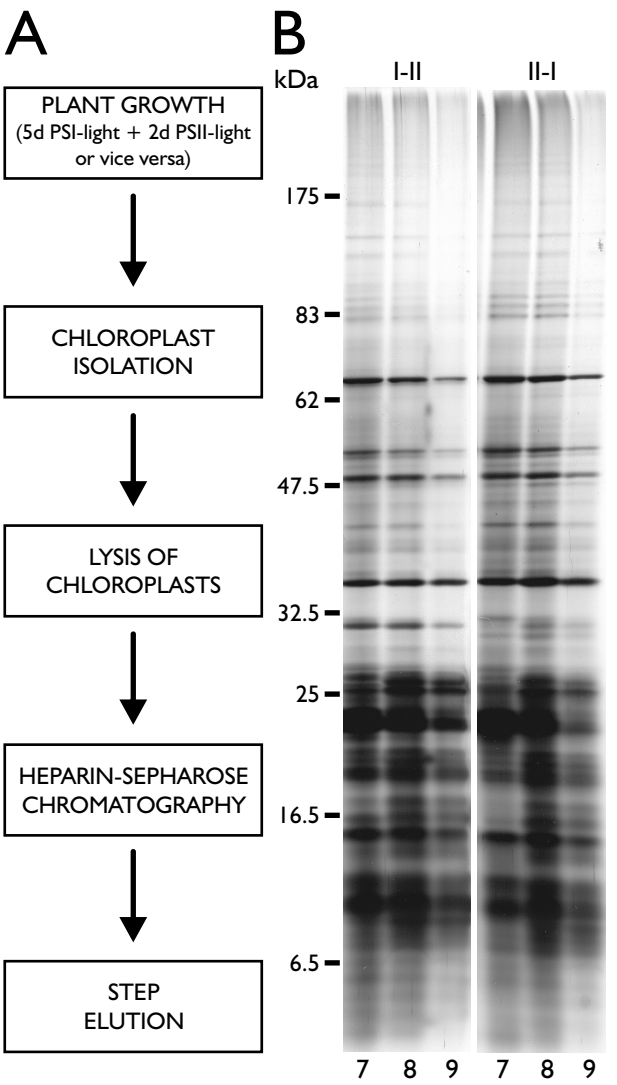

A
PLANT GROWTH
(5d PSI-light + 2d PSII-light
or vice versa)
CHLOROPLAST
ISOLATION
LYSIS OF
CHLOROPLASTS
HEPARIN-SEPHAROSE
CHROMATOGRAPHY
STEP
ELUTION
B
kDa
I-II
II-I
175
83
62
47.5
32.5
25
16.5
6.5
7 8 9 7 8 9

Fig 4

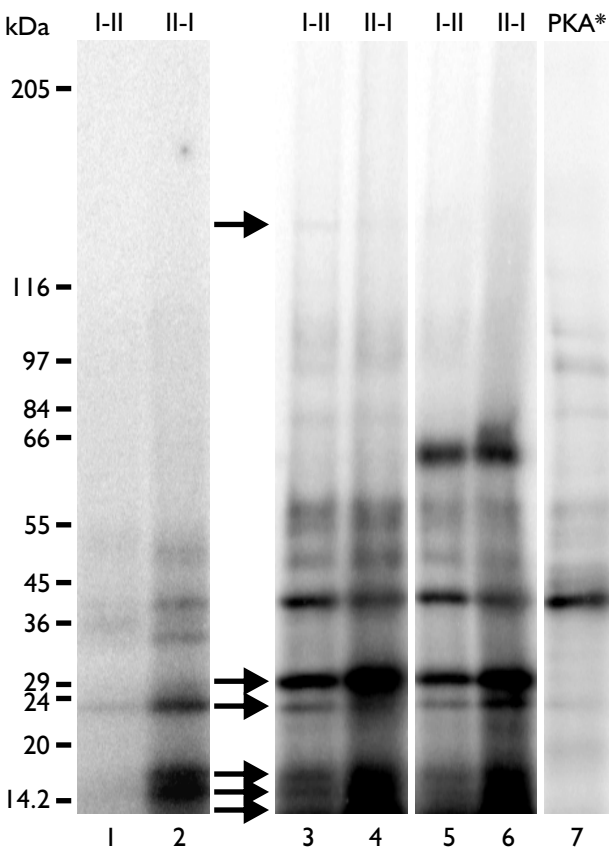

kDa
I-II
II-I
I-II
II-I
I-II
II-I
PKA*
205
116
97
84
66
55
45
36
29
24
20
14.2
1
2
3
4
5
6
7

Fig 5

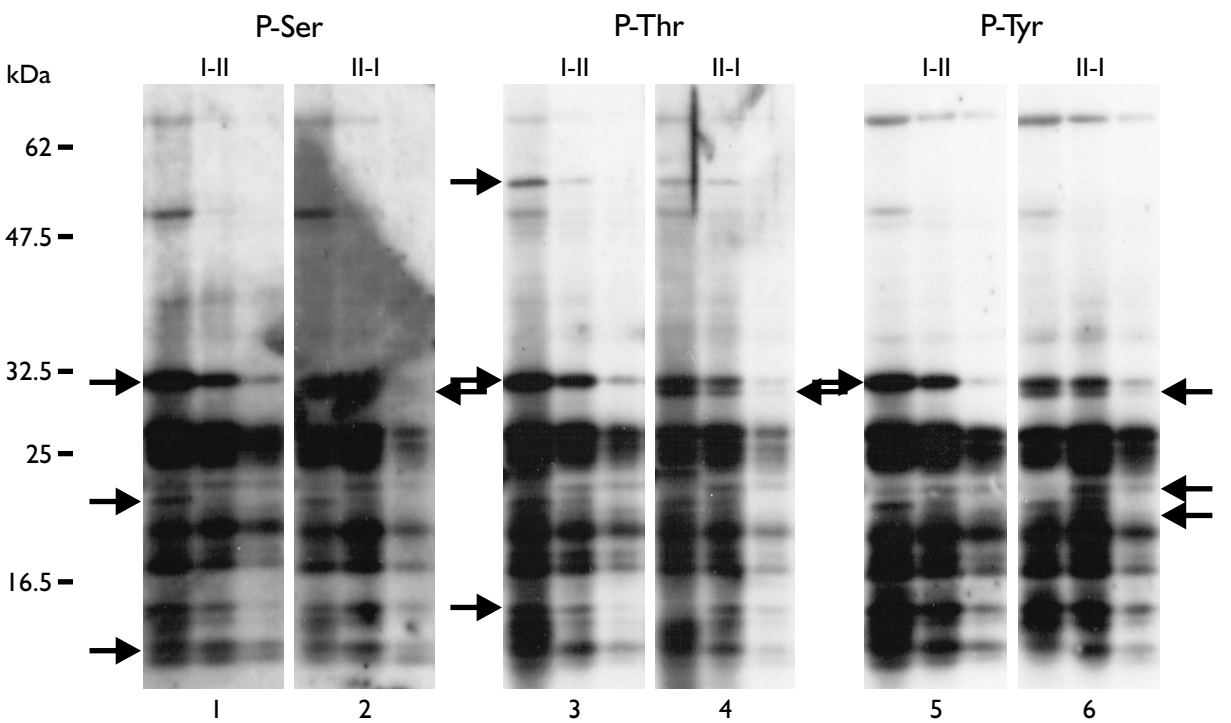
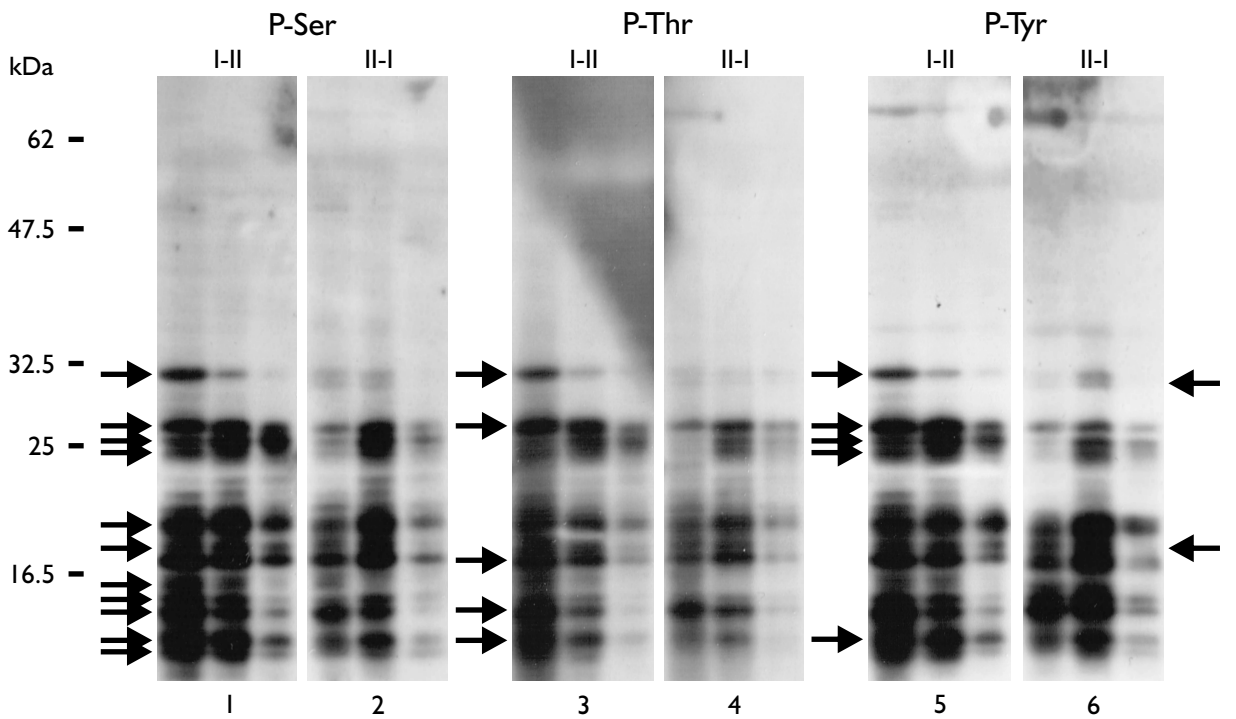


Fig 6



4 Discussion

Acclimation to varying light conditions requires a close and highly complex interaction of gene expression in both nucleus and chloroplasts. The nuclear gene expression of proteins active in the plastid is controlled to a considerable degree by chloroplast derived signals (reviewed by Pfannschmidt *et al.*, 2003). Such retrograde signals are generated in the light energy accepting machinery of photosynthesis and by pathways of photosynthetic metabolism, and include porphyrins, reactive oxygen intermediates, and carotenoids (reviewed by Rodermel, 2001). Among the factors sensing environmental variations, such as different stresses, nutrient availability, or light conditions are molecules which are changed in their reduction/oxidation (redox) state. Signals immediately generated by the light driven process of photosynthesis are known to originate from imbalances in the redox state of components of the photosynthetic electron transport chain (ETC). For our experiments we used special light sources which are able to set up a distinct redox signal either at photosystem II or at photosystem I. A combination of lamps emitting a certain spectra of white light and special yellow or red filters, respectively, generates so called PSII- or PSI-light. Application of PSII-light leads to reduction of ETC components whereas PSI-light causes an oxidation, both resulting in respective acclimatory responses. Shifts of acclimated plants to the respective other light source result in the opposite acclimation, confirming the reversibility of the process and thus, that the observed effects are in fact results of acclimatory reactions. These reactions can be distinguished into fast short-term responses occurring within minutes and long-term responses (LTR) which take place in hours or days, respectively. In mustard (*Sinapis alba* L.) long-term acclimation to PSII- or PSI-light, respectively, results in a photosystem stoichiometry adjustment which affects also the ability to perform short-term responses (Pfannschmidt *et al.*, 1999). The origin of the responsible signal transduction cascade is the redox state of the plastoquinone (PQ) pool. Mustard has several very important properties making it a suitable model plant for our experiments: first, it grows very fast, and thereby produces a sufficient amount of biomass to perform biochemical investigations. Second, it is well characterised with respect to the chloroplast transcription machinery (see e.g. Pfannschmidt *et al.*, 2000; Loschelder *et al.*, 2004) and moreover, it is a close relative of *Arabidopsis thaliana* (L.), which facilitates database searches because of high homologies between mustard and *Arabidopsis* genes. To reveal a reduced or oxidised PQ pool, respectively, seedlings were grown five days under PSI-light followed by two days under PSII-light, or *vice versa*. Since shifted

plants show the same, and even stronger, response than non-shifted plants, as it is known from earlier experiments (Pfannschmidt *et al.*, 1999), only these growth variants were used for biochemical studies with mustard. After chloroplast isolation and subsequent purification of nucleotide binding proteins via heparin-Sepharose chromatography and high-salt elution the obtained protein fractions were tested for RNA-polymerase activity and protein content to be used in further experiments. RNA-polymerase activity in such fraction was demonstrated earlier by Pfannschmidt and Link (1997). Photosystem stoichiometry adjustment is closely connected to transcription of the plastid encoded genes for photosystem core proteins *psaAB* and *psbA* which are transcribed by an also plastid encoded multi-subunit bacteria-like RNA polymerase (PEP) making this enzyme a likely target for redox control derived from the photosynthetic electron flow (PEF). The main topic of this thesis was to investigate the impact of light quality induced redox signals on gene transcription with respect to involved signalling components and whose putative interaction with other signalling networks acting in both chloroplasts and cytosol.

4.1 Redox-controlled gene expression in mutants of *Arabidopsis thaliana*

Chlorophyll fluorescence measurements: F_s/F_m as a parameter for the long-term acclimation in photoreceptor and chloroplast-to-nucleus signalling mutants

To gain more knowledge about the interaction of signals derived from PEF on the one hand and cytosolic photoreceptors on the other hand, a set of *Arabidopsis* mutants with defects in phytochromes and cryptochromes was tested for their ability to perform the LTR. Phytochromes may be affected by our light system since the PSI light contains wavelengths over 700 nm while the PSII light does not suggest different P_r/P_{fr} ratios in plants grown under them. To exclude their impact on long-term acclimation we performed chlorophyll fluorescence measurements with phytochrome knock-out (KO) mutants and one overexpression mutant which were grown under white light and subsequently subjected to either PSII- or PSI-light. The white light pre-treatment was necessary for a normal leaf development. Plants either were grown only under one light source or they were shifted to the respective other light after acclimation to the first light [(Fey, Wagner et al. 2005), Fig. 3A]. All mutants revealed a wild-type like LTR as observed earlier (Pfannschmidt *et al.*, 1999; Pfannschmidt *et al.*, 2001) suggesting that the acclimatory response is acting either independently or above the phytochrome signalling network. The variations observed in the double mutant *phyA/phyB* for plants shifted from PSI- to PSII-light most probably reflect developmental effects which are interacting with

the LTR. For the tested blue-light receptors also a WT-comparable acclimation was observed as expected since the light sources do not contain any blue or UV-light wavelengths. However, the response of *cry2-1* after a shift from PSII- to PSI-light was weaker than in all other mutants what might be explained by a side effect of this mutation. Cryptochromes are involved in de-etiolation and act in coordination with the phytochromes (reviewed by Chen *et al.*, 2004). In *Arabidopsis* three cryptochromes could be identified: cry1 and cry2 with known functions, and cry3, for which no function was described so far. Phytochromes regulate nuclear gene expression mainly during skoto- and photomorphogenesis. While phyA is highly abundant in etiolated seedlings, it is degraded and expression of the *PHYA* gene is repressed upon exposure to light, and phyB becomes the most abundant form (reviewed by Schäfer and Bowle, 2002). All *Arabidopsis* phytochrome forms have been reported to undergo light-induced nuclear import, and the active P_{fr} forms of phyA and phyB were shown to bind nuclear transcription factors *in vitro* suggesting a direct light regulation of specific promoters (reviewed by Quail, 2002). phyA is thought to target primarily a master set of rapidly responding genes encoding transcription factors which in turn are responsible for the regulation of the transcriptional network that drives photomorphogenesis affecting photosynthesis only very indirect. Both phyA and phyB have been demonstrated to bind to the phytochrome-interacting factor 3 (PIF3) and the regulation of CAB (former name of Lhcb) gene expression by phyB is suggested to occur through this mechanism (see Fig. 4). However, photosynthetic acclimation to changing light conditions, such as varying light qualities as used here, requires a simultaneous expression of specific genes encoded in both the nucleus and the chloroplast. A direct signal from the affected physiological process, i.e. photosynthesis, to such genes would provide the most effective regulation. Since phytochromes are not able to detect the excitation imbalances in the photosynthetic electron transport, it is unlikely that they play an important role during LTR even if regulatory effects on other genes cannot be excluded. This view is supported by work from Walters and Horton who found a retained regulation of photosystem stoichiometry in phy mutants grown under red light with varying blue light irradiance (Walters and Horton, 1995). Furthermore, the response to higher levels of blue light was attenuated by changing growth light intensities what led the authors to the assumption that photosynthetic processes are part of the regulatory system responsible for photosystem stoichiometry adjustment. This fits into the model of the „grand design of photosynthesis", a concept which describes photosynthesis as sensor for environmental information, that was originally proposed by Arnon (1982) and further extended by Anderson (1995; for review see Pfannschmidt *et al.*, 2001).

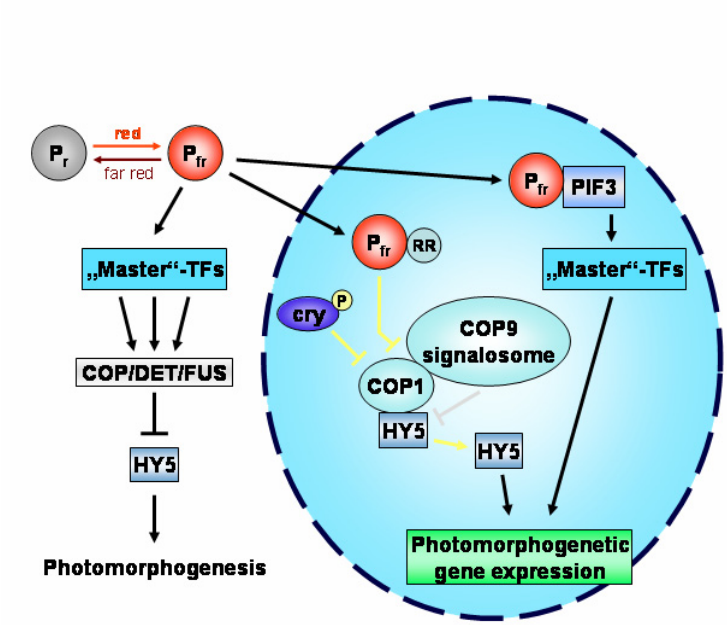


Fig. 4 - Simplified schematic of phy and cry signalling pathways. The active phy form P_{fr} is translocated to the nucleus, where it binds transcription factors (in particular PIF3) and thereby induces activation of a set of "Master" transcription factors required for transcription of several light-regulated genes. Additionally phy is thought to be stabilised in its activated form upon binding to response regulators (RR). The stabilised phy as well as activated cry are proposed to inhibit COP1-dependent proteolysis via action of the COP9 signalosome (gray blocked line) of the HY5 transcription factor facilitating release of HY5 (yellow lines and arrow) and subsequent photomorphogenetic gene expression.
With respect to the available data the translocation of activated phy causes the activation of key regulatory TFs and the different signalling pathways are suggested to merge at, or upstream of, the COP/DET/FUS mediated proteolysis of the HY5 transcription factor and other downstream components regulating photomorgenetic gene expression.

According to (Quail, 2002; Schäfer and Bowle, 2002).

A further fact supporting the hypothesis that the processes leading to the LTR are acting independently or above of phytochrome and cryptochrome signalling is the observation, that cry1, cry2, and phyB interact with COP1 (constitutively photomorphogenetic 1), an E3 ubiquitin-protein ligase targeting proteins for degradation (reviewed by Quail, 2002; Chen *et al.*, 2004). COP1 belongs to a group of components that were termed *COP/DET/FUS* class. These components are thought to be members of a signalling cascade that acts negatively in darkness to suppress photomorphogenesis via proteolytic degradation of positively acting factors, whereby the other class members are subunits of the oligomeric COP9 signalosome complex (see Fig. 4). Chlorophyll fluorescence measurements of the mutants *cop1-5*, and *cop9-1* revealed a functional LTR in *cop1-5*, whereas *cop9-1* is impaired in its ability to perform a wild-type like LTR (Fey and Pfannschmidt, unpublished data, see addendum, Fig. 7). If COP1 would be involved in signal transduction from PEF to nuclear gene expression, then in case of a lack of this factor the acclimation would fail. This is not the case. Thus, since COP1 is thought to act late in the signalling process downstream of phy and cry pathways, respectively, a merging of cytosolic photoreceptor signalling cascades and those deriving from redox imbalances of ETC components appears not likely. However, plants with lesions disabling the COP9 signalosome do not per-

form a proper LTR, suggesting an involvement of proteolytic active compounds in the regulation of genes encoding factors required for chloroplast gene expression.

Also the chloroplast-to-nucleus signalling mutants revealed a clear response to the changing light environment [(Fey, Wagner et al. 2005), Fig. 3B] indicating the independence of the LTR from the affected components, what is further confirmed by macroarray data (see below). However, the *cue* mutant showed no significant LTR after a PSI-II shift. Similar to the *phyA/phyB* double mutant the *cue1-6* mutation, namely the lack of the phosphoenolpyruvate carrier, might affect the LTR due its strong impact on energy metabolism. This impact may be responsible for the reticular phenotype observed in *cue1-6* (Streatfield *et al.*, 1999). Additionally, both the gene underexpression and the leaf cell morphology phenotypes were dependent on light intensity. The affected locus of *gun1-1* is still unknown; however, defects in signalling pathways coupling nuclear and plastidic gene expression, and in establishing photoautotrophic growth, indicating a role during chloroplast biogenesis (Mochizuki *et al.*, 1996). *GUN5* encodes the ChlH subunit of Mg-chelatase (Mochizuki *et al.*, 2001), while the product of *GUN4* is required for a signalling pathway triggered by accumulation of magnesium-protoporphyrin IX, an intermediate in chlorophyll biosynthesis, what is produced by Mg-chelatase. Mg-chelatase is activated by *GUN4* through binding a substrate of the enzyme (Larkin *et al.*, 2003). In the mutants which showed a partially impaired acclimation (*phyA/phyB, cue1-6*, and *cry2-1*), only one response is affected while the respective opposite is not. In consistence with the data obtained from the *cop1-5* mutant that also lacks a significant acclimation after a shift from PSI- to PSII-light, this specific interruption of one part of the LTR might suggest a separate mediation of oxidative and reductive signals. Lopez-Juez *et al.* (1998) found a connection between plastid- and phytochrome derived signals targeting nuclear gene expression in *cue* mutants and light intensity was reported to affect *CAB* transcript accumulation during de-etiolation of *cue1* seedlings (Streatfield *et al.*, 1999). However, a correlation between photosynthetic redox signals and other plastid retrograde signals or photoreceptors was not found here. This illustrates the difference between signals generated by variations in light quantity and those deriving from light-quality changes on the one hand, and points on the other hand rather to a specific signal as required for the LTR than to a more general impact on nuclear gene expression as necessary for photomorphogenesis. Furthermore, our findings of a still intact LTR in *cue1* and the *gun* mutants which have nuclear *Lhcb1* expression in the absence of chloroplast development (Susek *et al.*, 1993) might be a hint to a signalling network acting above of phy and cry pathways, maybe on the post-transcriptional level. This assumption is in turn supported by the results from the *cop1-5* mutant that showed

also an intact LTR despite the lack of COP1, an important factor in *CAB* gene expression. However, this model is purely theoretical and requires further investigation.

Primer extension analyses: transcriptional regulation of psaAB and psbA during the long-term acclimation occurs at the standard transcription initiation site

The primer extension analysis confirmed earlier studies which demonstrated the importance of transcription initiation at the *psaAB* promoter. We found the same transcription start site as it was reported for mustard by Summer *et al.* (2000) for both mustard [(Fey *et al.*, to be submitted), Fig. 2] and *Arabidopsis* [(Fey, Wagner et al. 2005), Fig. 2B] positioned directly behind the PEP promoter. Furthermore, a yet unidentified *psaA* 5'-end was found for *Arabidopsis* what may represent a start or processing site. However, it cannot be ascertained if the observed band indeed reflects a new species-specific redox-responsive *cis*-element or just a processing product since the primer extension technique does not distinguish between both phenomenons. No different transcription initiation sites for different light treatments were detected for mustard. However, each light quality revealed a different transcript accumulation confirming chlorophyll fluorescence measurements with respect to the ability to perform a LTR, what highlights the impact of redox regulation of this acclimation process on the *psaAB* transcription start site. For *psbA* a single prominent 5'-end 78 bases in front of the translation start site was found in *Arabidopsis*, consistent with earlier reports (Liere *et al.*, 1995; Shen *et al.*, 2001). *psbA* transcript accumulation also is consistent with results obtained from fluorescence measurements; however, to a smaller degree than it was observed for *psaAB*. Nevertheless the data indicate that redox regulation may also play an important role for *psbA* transcription. These results are confirmed by our observations on the protein level. Western analyses suggest that PS stoichiometry adjustment in *Arabidopsis* is mainly regulated by changes in PSI complexes and PSII antenna size [(Fey, Wagner et al. 2005), Fig. 1A]. This differs from observations in pea and mustard were antiparallel changes in both PSI and PSII were observed (Glick *et al.*, 1986; Pfannschmidt *et al.*, 1999) while it is in accordance with observations in spinach and cyanobacteria for which mainly changes in PSI were reported (Deng *et al.*, 1989; Fujita, 1997). Changes in PSII content in *Arabidopsis* have been reported to occur only under higher light intensities (Bailey *et al.*, 2001). The available data implicate that the acclimatory response itself was conserved during evolution while the molecular details vary between different organisms. Apparently there exist two prominent mechanisms, both leading to an adjustment of PS stoichiometry: An alteration of both PS or an alteration of only one PS,

respectively, whereby the other remains unchanged. The idea of a conservation of the acclimation process throughout evolution fits in the theory of a retained genome in chloroplasts (Allen, 1993; Race *et al.*, 1999) since the regulation of gene expression within the organelle and hence, of the LTR, seems to be strictly associated with the redox state of photosynthetic components.

As described above, the primer extension technique provides an experimental alternative to compare mRNA amounts. This feature was used in further experiments to determine transcript accumulation levels in long-term acclimated *Arabidopsis* seedlings with the same KO mutations for cytosolic photoreceptors and chloroplast envelope components involved in plastid-to-nucleus signalling as used for the chlorophyll fluorescence measurements (Fey and Pfannschmidt, unpublished data, see addendum, Fig 8). The results reflect the fluorescence data with respect to the main transcription start site, with exception of the *gun*-mutants. Surprisingly no or only very weak signals were detected at this transcription initiation point, instead the primer extension products accumulated almost exclusive on the same level with the second in the WT observed start site. Data obtained from genetic analysis of the *gun1*, *gun4*, and *gun5* mutations implicated two different signalling pathways for *gun1* and for *gun4* and *gun5*, respectively (Vinti *et al.*, 2000; Mochizuki *et al.*, 2001). GUN4 and GUN5 are involved in tetrapyrrole biosynthesis and thus in a suggested tetrapyrrole mediated plastid signal (Mochizuki *et al.*, 2001; Larkin *et al.*, 2003) while GUN1 seems to be affected by inhibition of plastid protein synthesis (Gray *et al.*, 2003). Despite these observations our data suggest that the regulation of chloroplast transcription of photosystem genes is affected by all *gun* mutations in the same way leading in its consequence most likely to an increase in processing activity at the *psaAB* promoter. This increase would explain the very strong reduction of the signal for the standard transcription site and the strong signal found downstream of it instead. In contrary to the results obtained from WT plants where the transcript accumulation at this site always followed the accumulation pattern at the standard transcription start site, the light-switch-induced regulation of *psaAB* gene expression seems to be impaired whereas the final LTR is not affected. This implicates the activation of an alternative regulatory mechanism in these mutants leading to the acclimatory response, a proposal consistent with the findings from the macroarray study which revealed a profile clustering for *gun* and *cue* mutants different from that observed for a reduction signal (Richly *et al.*, 2003). A hierarchical cluster analysis of expression profiles in 35 environmental and genetic conditions, including plastid-to-nucleus signalling mutations, revealed three main classes of nuclear chloroplast transcriptome responses: Two classes involved predominantly either up- or down-regulated genes,

whereas the third class included approximately equal numbers of up- and down-regulated genes. The *gun* profiles belong to class 1 which showed mainly up-regulated genes, whereas the *cue* profiles are assigned to class 3 involving mainly down-regulated genes.

The expression profiles in the physiological situations generated with respect to redox control deriving from PEF however belong to class 2 responses associated with balanced expression changes. Over 2000 genes were affected by light quality changes, whereby around 15% seem to be redox-regulated either directly by PEF or by an interaction of PEF derived signals with other chloroplast signals generated by variations in the redox equilibrium. This indicates an impact of redox-generated signals also on expression of a large number of genes what acts through second or third pathways of the cellular signalling network during the long-term acclimation. Overall, 3292 genes were analysed (Richly *et al.*, 2003); thus, about two-thirds exhibited expression changes in response to varying light quality, including genes for photosynthesis, gene expression, metabolism and transport. This finding underlines the massive impact of a light quality shift on nuclear genes encoding chloroplast proteins. Interestingly, all genes encoding components of the photosynthetic apparatus were up-regulated by a reducing signal, among them genes for both photosystems. Additionally, the plastid-localised gene *rpoB*, encoding the catalytic β-subunit of PEP, was found to be down-regulated. The *psaAB* and *psbA* genes are transcribed by PEP which itself is a target for redox regulation (Baginsky *et al.*, 1999; Pfannschmidt *et al.*, 2000; Ogrzewalla *et al.*, 2002; reviewed by Link, 2003). The *rpob* gene in turn is transcribed by NEP, the nuclear encoded RNA polymerase (Liere and Maliga, 2001), suggesting a redox regulation of NEP activity. Since PEP activity is responsible for transcription of genes for both PSI and PSII, the different regulation provides further evidence for a transduction of reducing and oxidising signals, respectively, via different pathways. These results indicate the existence of a complex regulatory network, in which light generated redox signals act on various levels of gene expression in both the nucleus and the chloroplast.

4.2 Effects of redox control on phosphorylation state and composition of chloroplast proteins

Biochemical analyses of mustard chloroplast proteins: heparin-Sepharose fractions with RNA polymerase and kinase activity

Phosphorylation was shown to be a crucial regulating event in both short-term and long-term acclimatory responses. In heparin-Sepharose fractions a kinase activity could be detected what was able to phosphorylate sigma-like transcription factors associated with the PEP *in*

vitro (Baginsky *et al.*, 1997). An impact of membrane-bound redox-regulated protein kinase activity on the phosphorylation state of thylakoid proteins was first described by Bennett (1977) and has been an area of intensive investigation (for review see e.g. Bennett, 1991; Gal *et al.*, 1997; Ohad *et al.*, 2001; Rintamäki and Aro, 2001). Phosphorylation of these proteins is known to be important for assembly and reconstruction of the PSII protein complex as well as for state transition (Andersson and Aro, 2001; Aro and Ohad, 2003). We observed differences in the phosphorylation level of thylakoid PSII proteins [(Fey *et al.*, to be submitted), Fig. 1B]. With exception of the D2 protein all detected PSII subunits are stronger phosphorylated in PSII-I plants than in PSI-II plants. The D2 protein appears higher phosphorylated in PSI-II plants and thus reveals the opposite result to the D1 protein suggesting a specific regulation. Chlorophyll fluorescence measurements at 77K also confirmed an acclimatory response to extended treatment with different light qualities [(Fey *et al.*, to be submitted), Fig. 1A]. To what extend each lateral antenna movement and photosystem stoichiometry adjustment contributed to the observed differences in the PSI emission peak cannot be defined yet and requires further investigation.

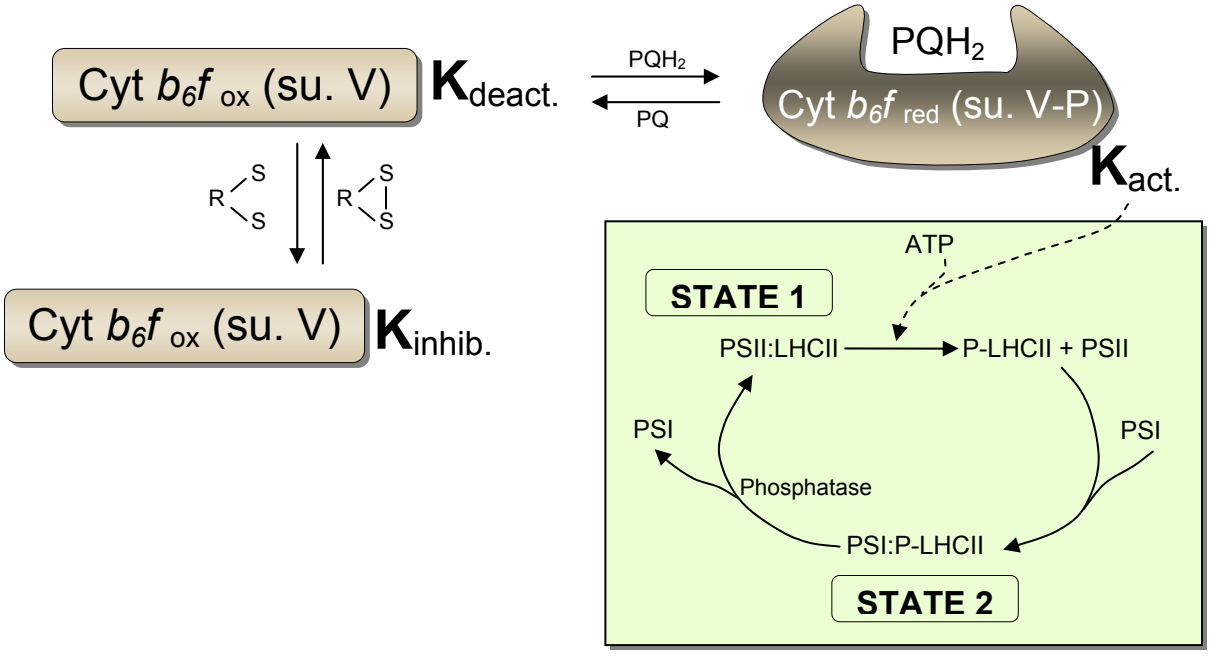


Fig. 5 - Hypothetical scheme showing the activation of the LHCII kinase while interacting with the Cyt *b*$_6$*f* complex and the dithiol-mediated reversible inhibition of the kinase. State1 – state 2 transitions promoted by an active LHCII kinase are diagrammed in the box.

According to Aro and Ohad (Aro and Ohad, 2003).

The primer extension results confirmed that a redox control of *psaAB* transcription must occur at the standard PEP promoter. To prove the impact on the chloroplast pool of DNA-binding proteins including PEP we enriched chloroplast protein fractions with transcriptional and nucleotide-binding activity (Tiller and Link, 1993; Pfannschmidt and Link, 1997). One- and two-dimensional gel electrophoresis revealed a very similar protein composition with only little differences between PSI-II and PSII-I samples after heparin-Sepharose chromatography [(Fey *et al.*, to be submitted), Fig. 3B; Fey and Pfannschmidt, unpublished data, see addendum, Fig. 9]. However, these differences are reproducible in several purifications demonstrating a high accuracy of the purification procedure. The identity of the concerned proteins is currently under investigation using mass spectrometry. Because no major differences in protein composition were found we focussed on post-translational modifications as protein phosphorylation to gain further insight into the regulation mechanisms affecting *psaAB* transcription. Phosphorylation has been shown earlier to be very important for promoter recognition in mustard (Tiller and Link, 1993). Endogenous kinase activity indeed could be detected in an autophosphorylation experiment exhibiting a stronger phosphorylation pattern in the PSII-I than in the PSI-II samples [(Fey *et al.*, to be submitted), Fig. 4]. Further experiments using an exogenous kinase (PKA) with and without prior dephosphorylation revealed some specific differences between the samples and displayed different substrate specificities for PKA and endogenous kinases; however, no further differences appeared after pre-dephosphorylation suggesting a similar phosphorylation state of both fractions. Furthermore, the observed phosphorylation patterns rather reflect differences in the protein amount and/or in the ability to be phosphorylated than in the phosphorylation state. Addressing this question concerning the original phosphorylation state after purification we used specific anti-phospho-amino acid antibodies. The experiments clearly demonstrate that the phosphorylation pattern indeed is very similar in the PSI-II and the PSII-I fractions for all three amino acids tested but a few differences are visible [(Fey *et al.*, to be submitted), Fig. 5]. These differences partially reflect different protein amounts in comparison with the silver stain [(Fey *et al.*, to be submitted), Fig. 3B]; however, the signal arising at around 54 kDa seems to represent a higher phosphorylation of the respective protein due to the fact that it appears only for the P-Thr antibody and that there are no major differences visible in the silver stain. The differential phosphorylated proteins in the range below 25 kDa are difficult to address since the protein density in this area is very high.

Apparently, only a limited number of chloroplast DNA-binding proteins is differentially expressed or phosphorylated, respectively, when grown in different light qualities. The differ-

ences in the phosphorylation pattern indicate different kinase activities and underlining the impact of protein phosphorylation as regulatory mechanism of chloroplast gene expression. The data further suggest that the endogenous kinase activity may be regulated by phosphorylation of kinases themselves and that light quality has an impact on protein structure while leading to conformational changes and thus, a differential exposition of phosphorylation sites what might be another regulating factor.

Kinase activity regulation via phosphorylation is demonstrated impressively by the mentioned protein of 54 kDa. Earlier studies in mustard identified a kinase of the same size in heparin-Sepharose fractions which was able to phosphorylate sigma-like-factors *in vitro*, termed plastid transcription kinase (PTK) (Baginsky *et al.*, 1997). It was down-regulated via phosphorylation and activated antagonistically by the redox state of glutathione *in vitro* (Baginsky *et al.*, 1997; Baginsky *et al.*, 1999). The 54 kDa band only appears in PSI-II samples [(Fey *et al.*, to be submitted), Fig. 5, panel 3 and 4] and the endogenous kinase activity in these fractions is low (Fig. 3, lane 1). However, the redox state of glutathione under PSI- or PSII-light was shown to be stable (Fey, Wagner et al. 2005) excluding it as regulating factor and highlights the role of the phosphorylation state. The fact that the 54 kDa protein obviously is phosphorylated exclusively at a threonine residue lead to the speculation that it is a substrate of the LHCII kinase which is known to be a threonine kinase and to be activated by a reduced PQ pool (reviewed by Aro and Ohad, 2003) (see Fig. 5). The LHCII kinase is not expected to be still present in heparin-Sepharose purified fractions. This is consistent with our observations that after pre-dephosphorylation the endogenous kinase activity was not able to re-phosphorylate the 54 kDa protein [(Fey *et al.*, to be submitted), Fig.6]. Together these data suggest that this protein indeed might be the PTK what would provide a mechanistic link between the redox state of the PQ pool and phosphorylation of PEP associated components. Thereby, activation of the LHCII kinase would be a crucial regulating factor in *psaAB* gene expression via triggering the PTK phosphorylation state and thus, its activity. A striking new result in this context is the absence of a LTR in a stt7 KO mutant (Wagner and Pfannschmidt in cooperation with D. Leister, unpublished data). stt7 was presented by Depege *et al.* (2003) as a candidate for the LHCII kinase. If this kinase is responsible for PTK phosphorylation, then a lack of stt7 would lead to a permanent PTK activity and thus, to a failure of a crucial regulating event in gene transcription at the PEP promoter and in conclusion, to a loss of the LTR. Although the direct link remains to be elucidated, the data indicate a mechanistic interface between signal cascades mediating state transition and long-term acclimatory response, respectively, as proposed earlier (Allen and Pfannschmidt, 2000).

Pursiheimo *et al.* (2001) suggested a role of the LHCII kinase in regulation of *Lhcb* transcript levels with respect to its activity state. LHCII phosphorylation and accumulation of *Lhcb* mRNA occurred under similar conditions, whereby conditions promoting an inactivation of the kinase led to dampening of the circadian oscillation in the amount of *Lhcb* mRNA while maximal accumulation of *Lhcb* mRNA during the diurnal light phase seems to require an active LHCII kinase. Short term shifts to higher light intensities and low temperature were used to generate a PSII excitation pressure, i.e. a reduced PQ pool. This led to the conclusion that the redox state of the PQ pool is not directly involved in the regulation of *Lhcb* mRNA accumulation. Instead stromal electron carriers of PSI and the correlated activation state of the LHCII kinase are suggested to be crucial for *Lhcb* gene expression. With respect to the results from Pfannschmidt *et al.* (1999) and our data obtained from western analysis and 77K fluorescence measurements combined with the findings for stt7 and the PTK however, this view may be modified. The chlorophyll fluorescence data show an acclimation to PSI-light as increase in the F_s/F_m value, i.e. the PSII fluorescence. These results are confirmed by the observed increase of Lhcb1 under the respective conditions [(Fey, Wagner et al. 2005), Fig. 1A]. The opposite results are obtained from plants treated *vice versa*. Thus, *Lhcb* gene expression occurs under conditions inhibiting LHCII kinase activity, such as treatment with PSI-light, and is suppressed while the kinase is active as under PSII-light. These findings support the theory of a negative regulator of *Lhcb* gene expression (Escoubas *et al.*, 1995). Under low light the LHCII kinase would be active (Pursiheimo *et al.*, 2001) since only the occupation of the Q_O site of the Cyt b_6f complex is enough for its fully activation (Vener *et al.*, 1997) and *Lhcb* mRNA is accumulated because the reductive pressure from PSII is rather low. However, a more reduced PQ pool, such as under high light intensities and PSII-light, causes down-regulation of *CAB* gene expression implicating the activation of a second signal acting independently of the kinase. In contrary, the LHCII kinase seems to play a crucial role in regulating PS stoichiometry adjustment via phosphorylation of the PTK upon activation, either directly or downstream of a phosphorylation cascade, and thereby control of *psaAB* gene expression in the chloroplast (see above). This proposed model provides a likely regulatory mechanism which connects the redox state of PQ to *psaAB* transcription.

Despite the knowledge about the origins of redox signals and the genes regulated in consequence of these signals, the molecular basis of the transduction from the chloroplast to the nucleus remains unknown. Several models have been suggested involving participation of photosynthesis related compounds, such as H_2O_2 or PQ. H_2O_2, generated or accumulated in the plastid as a result of biotic or abiotic stress, was shown to activate a MAPK cascade in the

cytosol of *Arabidopsis* leaf cells (Kovtun *et al.*, 2000). Desikan *et al.* (2001) identified an *Arabidopsis* MAPK what is activated in response to H_2O_2 generated upon treatment with the bacterial elicitor harpin. Evidence for phosphorylation-dependent signalling in the cytosol related to the redox state of the PQ pool comes from studies with the green alga *Dunaliella tertiolecta*, where a regulation of CAB gene expression on the transcriptional level was shown in response to varying irradiances (Escoubas *et al.*, 1995). Another model suggests a transduction of the PQ signal via PQ molecules themselves, which are located in the envelope membrane (for review see Pfannschmidt *et al.*, 2003). Alternatively, the redox signals targeting the chloroplast expression machinery may be relayed to the nucleus via gene products encoded in the organelle.

Interaction of signalling pathways derived from chloroplasts and the cytosolic light receptors has been suggested to be involved in plastid-to-nucleus signalling (Lopez-Juez *et al.*, 1998; Mullineaux *et al.*, 2000; Vinti *et al.*, 2000; Moller *et al.*, 2001). However, a role of phytochromes or cryptochromes in mediation of redox imbalance promoting differences in the incident light could not be excluded. For the first time, the molecular response to PSI- or PSII-light in chloroplasts of *Arabidopsis* was described here. It was shown that phytochrome and cryptochrome derived pathways targeting nuclear gene expression, are not interconnected with redox signalling routes from the chloroplast. The results obtained from the tested *cop* mutants indicate an independent transduction of chloroplast redox signals and cytosolic light-generated signals; however, the LTR of the *cop9-1* mutant to a reducing light signal implies an involvement of nuclear proteolytic components in regulating transcriptional events. Despite this observation, the data obtained from the chloroplast-to-nucleus signalling mutants suggest a regulation of the LTR above the cytosolic photoreceptor pathways, since the proteolytic activity of the COP9 signalosome affects several genes, including those for transcription factors, what may cause the observed effect.

The crucial factor for adjusting PS stoichiometry in *Arabidopsis* seems to be an up- or down-regulation of nuclear *Lhcb* gene expression affecting PSII and regulation of plastid *psaAB* gene expression affecting PSI, respectively [(Fey, Wagner et al. 2005), Figs. 1A and 2A}]. Our data from the phosphorylation experiments confirm the assumption of a direct signal transduction from PEF to the gene transcription level inside the chloroplast. Especially the observations concerning the 54 kDa protein which might represent the PTK (Baginsky *et al.*, 1999; Ogrzewalla *et al.*, 2002) support the idea that this enzyme is the last component of a

phosphorylation cascade originating at the same kinase which is responsible for LHCII phosphorylation.

5 Summary

This work contributes novel aspects to the understanding of function and co-action of cellular signalling networks in the course of acclimation to a changing light environment.

Application of PSI- and PSII-light as changeable environmental factor has been introduced by Melis *et al.* (Melis, 1991) for higher plants and was used for the first time by Pfannschmidt *et al.* to generate redox imbalances in mustard (Pfannschmidt *et al.*, 1999). For our experiments we improved this system and established a growth installation what enables us to perform large scale plant growth under the respective light quality and in constant light intensity, temperature, and humidity conditions. An important advance was the replacement of incandescent bulbs by fluorescent tubes to avoid temperature stress. In the presented work I took advantage of this possibility and performed physiological, molecular, and biochemical studies with *Arabidopsis* and mustard. The availability of mutant lines of the model plant *Arabidopsis* allowed for the investigation of signalling integration with respect to the plastid light energy absorbing machinery and the cytosolic photoreceptors phytochrome and cryptochrome (*phyA*, *phyB*, *phyA/phyB*, *phyB-oe*, *hy4*, and *cry2-1*). Furthermore chloroplast-to-nucleus signalling mutants (*cue1-6*, *gun1-1*, *gun4-1*, and *gun5-1*) were tested to investigate a possible interaction of the affected components with redox-signalling pathways. The data, obtained from chlorophyll fluorescence measurements and determination of transcript accumulation of *psaAB* using a primer extension approach, showed a functional LTR in all mutants. In the *gun* mutants, however, differences at the transcriptional level were observed, although these mutants also exhibited a WT-like fluorescence pattern. These results confirm the assumption that the photosynthetic redox signals act independent or above the cytosolic photoreceptor pathways. This is supported by fluorescence data obtained from photomorphogenesis mutants (*cop1-5* and *cop9*), which indicate separate signalling routes targeting nuclear transcription of genes encoding PSII light harvesting complex proteins. Furthermore, chloroplast redox signals are reported independently of the affected components in the signalling mutants. Such, although experimental evidence is missing, our data suggest a regulation on the post-transcriptional level in the course of *Lhcb* gene expression (see discussion, page 118). Western analyses showed that regulation of these genes is most likely an integral step in PS stoichiometry adjustment in *Arabidopsis* concerning PSII. In the same experiments, however, adaptation of PSI to changing light qualities was shown to depend mainly on the regulation of plastid *psaAB* gene expression.

The primer extension approach further revealed that the transcriptional regulation of both *psaAB* and *psbA* occurs at the standard transcription initiation site; however, a much more intensive regulation was observed for *psaAB* than for *psbA*. A second, weaker signal appeared for the *psaAB* promoter with a regulation pattern similar to that what was observed for the standard start site in WT plants; however, this signal became dominant in the *gun* mutants, suggesting an increased processing activity in *Arabidopsis* which occurs in these mutants as a consequence of their defects.

Heparin-Sepharose chromatography with chloroplast lysates from mustard was performed to obtain protein fraction with nucleic acid binding and transcriptional activity. These fractions were used in one- and two-dimensional gel electrophoresis experiments which showed a reproducible protein composition in the silver stain what provides good evidence for the reproducibility and accuracy of the purification procedure. Comparing seedlings acclimated to either PSI- or PSII-light in shift experiments only a few proteins were found to be expressed differently. Phosphorylation experiments revealed a stronger endogenous kinase activity in the PSII-I fraction and differentially phosphorylated proteins upon autophosphorylation or treatment with an exogenous kinase, respectively. Further experiments using an exogenous applied phosphatase suggested a similar phosphorylation state of both PSI-II and PSII-I samples, what was confirmed by immuno-blot analyses using anti-phospho-amino acid antibodies raised against P-Ser, P-Thr, and P-Tyr, respectively. In these experiments, a few differences between the light treatments were observed reflecting most probably different amounts of the respective proteins; however, one protein arising at around 54 kDa apparently is regulated in its phosphorylation state in a redox-dependent manner. This protein might represent the plastid transcription kinase, an RNA polymerase associated enzyme that mediates redox control of plastid gene transcription (Baginsky *et al.*, 1999).

From these data, a model is proposed which shows a possible signal transduction derived from the photosynthetic electron transport targeting nuclear gene expression independently of cytosolic photoreceptors. Furthermore a regulatory mechanism for chloroplast gene expression via a phosphorylation cascade starting with the kinase responsible for LHCII phosphorylation is suggested (Fig. 6).

6 Zusammenfassung

Die Ergebnisse dieser Arbeit leisten einen wesentlichen Beitrag zum Verständnis der intrazellulären Signalübermittlung von den Chloroplasten zum Nukleus. Dabei werden im Speziellen die vom photosynthetischen Elektronentransport (PEF, *photosynthetic electron flow*) als Antwort auf Veränderungen der umgebenden Lichtverhältnisse generierten Signale betrachtet. Diese so genannten Redox-Signale nehmen einen besonderen Platz in den zellulären Signal-Netzwerken ein, da sie einem Ungleichgewicht im Reduktions- bzw. Oxidationzustand von Komponenten des Photosyntheseapparates entspringen, welches direkt Unterschiede im eingestrahlten Licht widerspiegelt. Im Gegensatz zu den klassischen Photorezeptoren handelt es sich also um die Interaktion von licht-sensitiven Systemen und die an sie gekoppelten Elektronentransportkomponenten, welche das Signal erzeugt.

Um solch ein beschriebenes Ungleichgewicht zu erzeugen, verwenden wir für unsere Experimente spezielle Lichtquellen, so genanntes Photosystem (PS) I- und PSII-Licht. PSII-Licht führt zu einem Überschuss an reduziertem Plastochinon (PQ, *plastoquinone*), dem nachfolgenden Elektronentransporter, und somit zu einer Reduktion des PQ *pools*. PSI-Licht erzeugt einen starken Elektronensog, der eine Oxidation des PQ *pools* zur Folge hat. Pflanzen haben im Laufe der Evolution eine Reihe verschiedener Mechanismen entwickelt, um solchen Redox-Ungleichgewichten entgegen zu wirken. Ein sehr gut untersuchter Mechanismus ist als so genannte *state transition* beschrieben worden (Haldrup *et al.*, 2001), eine Verlagerung eines Teils des Antennenkomplexes von Photosystem II (LHCII, *light harvesting complex* II) von diesem zum Photosystem I. Diese Antwort erfolgt innerhalb weniger Minuten, weshalb sie auch als Kurzzeitantwort bezeichnet wird. Eine so genannte Langzeitantwort (LTR, *long-term response*) hingegen zeigt sich als Stöchiometrieänderung der Photosystemkomplexe, welche durch die Veränderung der Transkriptmenge der beteiligten Gene induziert wird. Dies konnte am Beispiel von *psaAB* und *psbA*, welche die Reaktionszentrumsproteine von Photosystem I respektive II kodieren, gezeigt werden (Pfannschmidt *et al.*, 1999). Der Vorteil unterschiedlicher Lichtqualitäten liegt vor allem darin, dass jeweils nur ein PS angeregt wird, während bei Veränderungen in der Lichtintensität immer beide PS betroffen sind. Weiterhin werden destruktive Nebeneffekte von Lichtstress wie Photooxidation mittels reaktiver Sauerstoffspezies (ROS) ausgeschlossen. Dieses Lichtsystem wurde erstmals von Pfannschmidt und Mitarbeitern verwendet, um Redox-Ungleichgewichte im Gelbsenf (*Sinapis alba*, L.) zu generieren (Pfannschmidt *et al.*, 1999). In unserer Arbeitsgruppe in Jena wurde es weiterentwickelt, um Pflanzenanzucht in den verschiedenen Lichtqualitäten bei konstanten Umweltbedingungen im großen Maßstab zu ermöglichen. Ein wichtiger Fortschritt war dabei der Austausch von Glühbirnen gegen Leuchtstoffröhren, um Temperaturstress zu

Glühbirnen gegen Leuchtstoffröhren, um Temperaturstress zu vermeiden. Für meine Experimente nutzte ich das Lichtsystem für physiologische, molekulare und biochemische Studien an *Arabidopsis thaliana* und Senf. Die Verfügbarkeit von *Arabidopsis*-Mutanten bietet die Möglichkeit, gezielt die Funktion bestimmter Faktoren zu untersuchen, die Aufgaben in einem größeren Kontext in der Zelle ausüben. Um mögliche Einflüsse der Lichtquellen auf cytosolische Photorezeptoren auszuschließen und mehr Details der Transduktion Redox-generierter Signale im Chloroplasten und vom Chloroplasten zum Nukleus in Erfahrung zu bringen, untersuchte ich mehrere Mutanten mit Defekten in den cytolsolischen Photorezeptoren Cryptochrom und Phytochrom (*phyA*, *phyB*, *phyA/phyB*, *phyB-oe*, *hy4* und *cry2-1*) und Plastiden-Signal-Mutanten (*cue1-6*, *gun1-1*, *gun4-1* und *gun5-1*). Die Pflanzen wurden zunächst im Weißlicht und anschließend unter beiden Lichtqualitäten angezogen (PSI- bzw. PSII-Licht) bzw. nach der Akklimation an eine Bedingung einem Lichtwechsel ausgesetzt (PSI-II bzw. PSII-I), um eine umgekehrte Reaktion zu erzeugen. Diese Umkehrbarkeit der Akklimationsreaktion ist gleichzeitig eine Bestätigung für eine echte Akklimation auf die verwendete Lichtqualität, im Gegensatz zu Entwicklungsprozessen, welche nicht umkehrbar sind. Die Anzucht im Weißlicht erwies sich als notwendig, da die unmittelbare Keimung unter den PSI- bzw. PSII-Lichtquellen zu abnormalem Blattwachstum führte, was auf den Mangel an Blaulicht in diesen Lichtquellen zurückzuführen ist. Anschließende Chlorophyllfluoreszenz-Messungen und Transkriptmengenbestimmungen von *psaAB* ergaben eine funktionierende LTR in allen Mutanten. Nur die *gun*-Mutanten zeigten Unterschiede auf der Transkriptionsebene, obwohl auch hier eine Wildtyp-ähnliche Fluoreszenz beobachtet wurde. Diese Ergebnisse bestätigen die Vermutung, dass die Redox-Signale unabhängig von oder übergeordnet zu den Signalwegen der cytosolischen Photorezeptoren übertragen werden. Ein weiteres Indiz hierbei sind die Fluoreszenzdaten von Photomorphogenese-Mutanten (*cop1-5* and *cop9*), die auf eine Photorezeptor-unabhängige Regulation der nukleären Transkription von LHCII-Genen durch plastidäre Redox-Signale hinweisen. Insgesamt deuten diese Ergebnisse auf eine Regulation der nukleären *Lhcb*-Expression auf post-transkriptioneller Ebene hin, was allerdings nicht experimentell bestätigt wurde.

In *Western-blot*-Experimenten zeigte sich, dass die LTR in *Arabidopsis* vorwiegend durch Veränderungen der LHCII-Menge für PSII bzw. durch die Regulation der *psaAB*-Expression für PSI erreicht wird, was durch die Ergebnisse der *primer extension* – Analyse bestätigt wurde. Diese offenbarte eine Regulation der Transkription von *psaAB* und *psbA* in *Arabidopsis* und Senf am Standard-Transkriptions-Startpunkt in Abhängigkeit von der Lichtqualität. Für *Arabidopsis* wurde ein weiteres Signal stromabwärts des ersten gefunden, was auch eine Regulation zeigte, aber schwächer ausgeprägt war. Dieses Signal wurde allerdings in den *gun*-Mutanten dominant, während das Signal am Standard-Transkriptionsstart fast vollständig veschwand. Eine mögliche Erklärung ist, dass es sich bei dem zweiten Signal um eine Prozes-

sierungsstelle handelt und in diesen Mutanten infolge ihrer Defekte eine verstärkte Prozessierungsaktivität vorliegt, welche zu einer starken Abnahme des RNA-Vorläufers und stattdessen zu einer Anreicherung des Prozessierungsproduktes führt.

Um Nukleinsäuren bindende Proteine mit RNA-Polymerase-Aktivität anzureichern, wurde eine Heparin-Sepharose-Chromatografie mit Chloroplastenlyaten aus Senfkeimlingen mit anschließender Fraktoinierung durchgeführt. Die Keimlinge wurden in Lichtwechselbedingungen angezogen (PSI-II und PSII-I), welche erfahrungsgemäß die gleiche, aber stärker ausgeprägte LTR hervorrufen wie die Belichtung mit nur einer Lichtqualität. In ein- und zweidimensionalen Gel-Elektrophorese-Experimenten zeigte sich, dass die HS-Fraktionen eine reproduzierbare Proteinzusammensetzung mit nur wenigen Unterschieden zwischen beiden Lichtbedingungen aufweisen, was für die Reproduzierbarkeit und Genauigkeit der gesamten Aufreinigungsprozedur spricht. In Phosphorylierungs-Experimenten konnte zudem endogene Kinase-Aktivität in den Fraktionen ermittelt werden, welche in den PSII-I – Proben stärker war als in den PSI-II – Proben. Behandlung mit einer exogenen Kinase zeigte eine unterschiedliche Substratspezifität zwischen dieser und den endogenen Kinasen und offenbarte nur wenige Unterschiede zwischen beiden Lichtbedingungen. Behandlung mit einer exogenen Phosphatase und anschließend mit der exogenen Kinase unterstützte ebenfalls die Vermutung, dass beide Proben eine ähnliche Grundphosphorylierung aufweisen. Dies wurde in Experimenten mit anti-Phospho-Aminosäure-Antikörpern (P-Ser, P-Thr und P-Tyr) bestätigt, in welchen ebenfalls nur wenige Unterschiede zwischen PSI-II – und PSII-I – Proben gefunden wurden. Diese Unterschiede konnten zudem teilweise auf unterschiedliche Proteinmengen zurückgeführt werden. Ein Protein von ungefähr 54 kDa jedoch zeigte im Vergleich mit der Silberfärbung keine Unterschiede in der Proteinmenge und schien in Abhängigkeit vom Redox-Zustand differentiell phosphoryliert zu werden. Dieses Protein könnte die Plastiden-Transkriptions-Kinase (PTK) repräsentieren (Baginsky *et al.*, 1999), welche die Redox-Regulation der *psaAB*- und *psbA*-Transkription ausgehend vom PEF vermittelt.

Auf Grundlage dieser Daten wird ein Modell konstruiert, welches die Regulation der nukleären Genexpression unabhängig von den cytosolischen Photorezeptoren darstellt und eine mögliche Kontrolle der plastidären Genexpression mittels einer Phosphorylierungskaskade aufzeigt, ausgehend von derselben Kinase, welche für die LHCII-Phosphorylierung verantwortlich ist (Fig. 6).

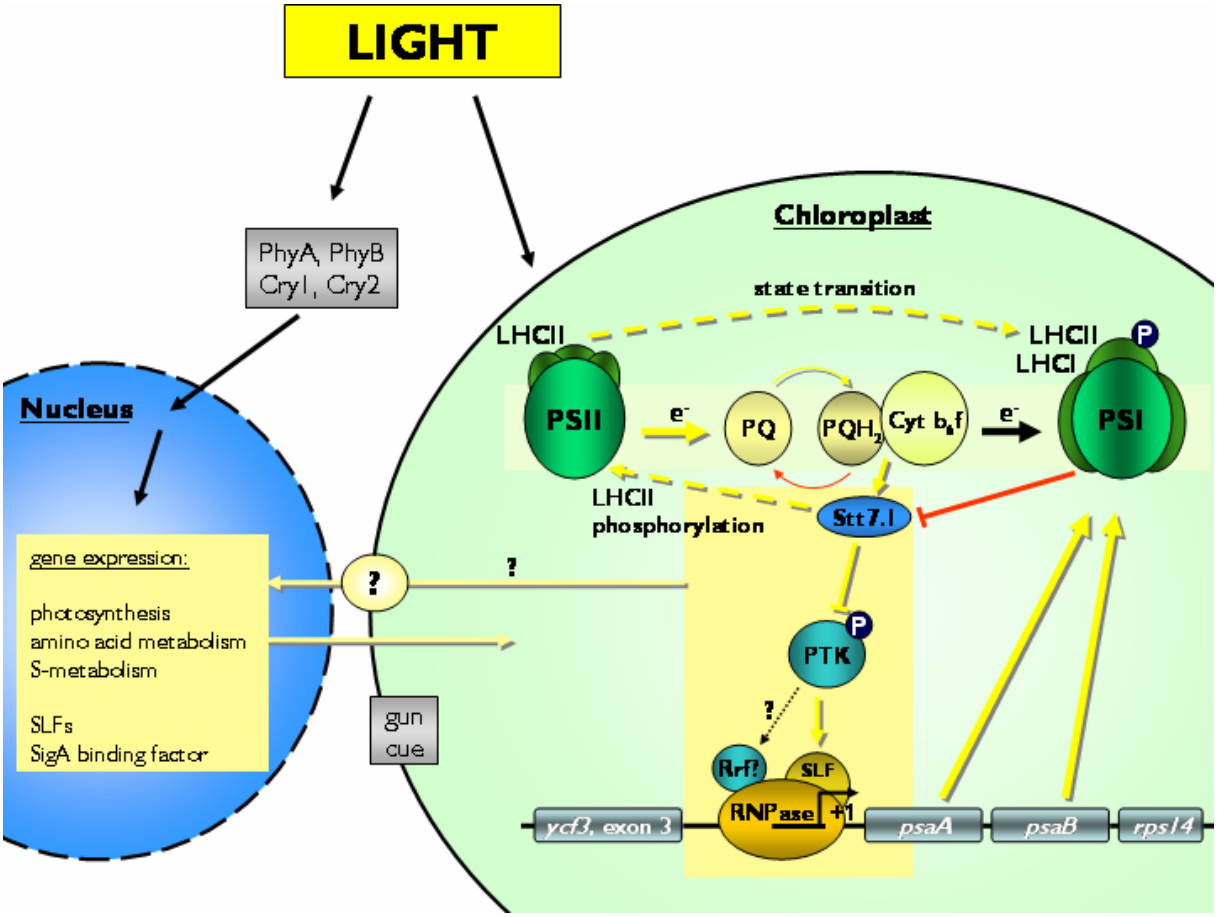


Fig. 6 – Model of redox signalling pathways affecting gene expression in the nucleus and the chloroplast. Incident light is perceived by both the photosynthetic machinery in the chloroplast and cytosolic photoreceptors. Excitation of PSII causes a reduction of the PQ pool leading to activation of the LHCII kinase Stt7.1 and in consequence to state transition (dotted yellow arrows). The same kinase is presumably responsible for deactivation of PTK by phosphorylation. As a result of the inactive PTK, sigma-like factors (SLFs) are not phosphorylated which results in transcription initiation at the promoter of *psaAB*. In this regulatory complex (yellow field covering the phosphorylation cascade) one or more signals are generated targeting the expression of several classes of nuclear genes (yellow field in the nucleus) which in turn have impact on chloroplast function. The signal(s) are reported to the nucleus by a novel pathway which acts independently of cytosolic photoreceptors phytochrome (Phy) and cryptochrome (Cry) and of the affected compounds in *cue* and *gun* mutants.

7 Thesen

I) Das Hauptthema dieser Arbeit war eine Untersuchung der Einflüsse von Ungleichgewichten im Redox-Zustand der photosynthetischen Elektronentransportkette auf die Genexpression in Bezug auf die beteiligten Komponenten und deren mögliche Interaktion mit anderen Signal-Netzwerken im Chloroplasten und im Cytosol.
Redox-Ungleichgewichte im photosynthetischen Elektronentransport und die damit verbundenen Kurzzeit- und Langzeitantworten (LTR) in höheren Pflanzen können durch Bestrahlung mit unterschiedlichen Lichtqualitäten erzeugt werden, welche bevorzugt Photosystem (PS) I oder PSII anregen.

II) Ein entsprechendes Lichtsystem, welches so genanntes PSI-Licht und PSII-Licht verwendet, wurde weiterentwickelt und für die Pflanzenanzucht im großen Maßstab bei konstanten Umweltbedingungen etabliert.

III) Chlorophyll-Fluoreszenz-Messungen und Untersuchungen der Transkript-Akkumulation von *psaAB* an Photorezeptor-Mutanten, Plastiden-Signal-Mutanten und Photomorphogenese-Mutanten von *Arabidopsis* zeigten eine Wildtyp-ähnliche LTR, was zu folgenden Schlussfolgerungen führt:

   a) Die Transduktion lichtgenerierter plastidärer Redox-Signale erfolgt unabhängig von oder übergeordnet zu den cytosolischen Photorezeptor-Signal-Netzwerken.
   b) Die Redox-Regulation der nukleären Expression der Lichtsammelkomplex-Proteine von PSII erfolgt möglicherweise auf post-transkriptioneller Ebene.

IV) In *Arabidopsis* wird eine Stöchiometrieänderung der PS vorwiegend durch eine Regulation der Lichtsammelkomplex-Proteine im Falle von PSII bzw. durch eine Änderung der P700-Proteinmenge, verantwortlich ist für die Anpassung von PSI, erreicht.

V) Die *primer extension* – Technik zur Ermittlung von Transkriptionsstartpunkten mithilfe von genspezifischen *primern* wurde für die Benutzung eines Li-Cor DNA *sequencers* optimiert und zur Bestimmung der mRNA-Akkumulation von *psaAB* und *psbA* genutzt. Dies führte zu folgenden Resultaten:

a) Die Transkription dieser beiden Gene wird am Standard-Transkriptionsstartpunkt redox-reguliert, wie Untersuchungen an WT-Pflanzen zeigten.
b) Da sich diese Regulation in *Western*-Analysen widerspiegelt, was eine Korrelation der Protein- bzw. Transkript-Akkumulation aufzeigt, wurde die Bestimmung der Transkript-Akkumulation auch für die Mutanten als Parameter für die Stöchiometrieänderung herangezogen.
c) In *Arabidopsis* gibt es ein zweites reguliertes 5'-Ende im *psaAB*-Promotor stromabwärts vom Standard-Startpunkt.
d) Dieses zweite Ende dominiert in *gun*-Mutanten, was auf eine verstärkte Prozessierungsaktivität an diesem Promotor hindeutet, die in diesen Mutanten infolge ihrer Defekte auftritt.

VI) Heparin-Sepharose-Chromatografie mit Chloroplastenlysaten und anschließende Fraktionierung führt zur Anreicherung von Proteinen mit Nukleinsäurebinde-, RNA-Polymerase-, und Kinase-Aktivität.

VII) Mit diesem Chromatographie-Verfahren können hochreproduzierbare Protein-Aufreinigungen erzielt werden.

VIII) Es gibt nur wenige Unterschiede im Phosphorylierungsstatus und der spezifischen Phosphorylierung zwischen beiden Lichtbedingungen.

IX) Die Fluoreszenzwerte beider Photosysteme zeigen eine deutliche Regulation im 77K-Spektrum isolierter Senf-Thylakoide von unterschiedlich akklimatisierten Pflanzen.

X) Der Phosphorylierungszustand von PSII-Proteinen wird von Änderungen der Lichtqualität beeinflusst.

XI) Die Transkription von *psaAB* wird wahrscheinlich durch eine Phosphorylierungskaskade reguliert, welche von der LHCII-Kinase und damit vom Redox-Zustand des PQ *pools* ausgeht und die plastidäre Transkriptionskinase PTK einschließt.

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10 List of publications and presentations

Journal articles

Pfannschmidt T, Schütze K, Fey V, Sherameti I, Oelmüller R (2003) Chloroplast redox control of nuclear gene expression-a new class of plastid signals in interorganellar communication. Antioxid Redox Signal **5:** 95-101

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Fey V, Allahverdiyeva Y, Aro EM, Pfannschmidt T (to be submitted) Photosynthetic redox control during light-quality acclimation in Sinapis alba has specific effects on phosphorylation state and composition of proteins involved in plastid gene expression.

Conference proceedings

Wagner R, Fey V, Borgstädt R, Kruse O, Pfannschmidt T (2004) Screening for Arabidopsis thaliana mutants deficient in acclimatory long-term response to varying light qualities using chlorophyll fluorescence imaging. *In* Avd Est, D Bruce, eds, 13th International Congress of Photosynthesis. Allen Press, Montréal, in press

Talks

Fey V, Sherameti I, Schütze K, Wagner R, Oelmüller R, Pfannschmidt T (2002) Redoxregulation der pflanzlichen Genexpression. *In* 15. Tagung Molekularbiologie der Pflanzen, Dabringhausen

Fey V, Wagner R, Oelmüller R, Pfannschmidt T (2002) Redoxregulation der pflanzlichen Genexpression. *In* VIII. Jahrestagung der Deutschen Sektion der Internationalen Gesellschaft für Endocytobiologie, Blaubeuren

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Fey V, Schütze K, Wagner R, Oelmüller R, Pfannschmidt T (2002) Photosynthetic control of chloroplast and nuclear gene expression. *In* International Workshop of the Special Research Focus (Forschergruppe) FOR 387 of the DFG - Redox Regulation: From Molecular Responses to Environmental Adaptation, Bielefeld

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Fey V, Allahverdiyeva Y, Paakkarinen V, Aro EM, Pfannschmidt T (2004) Phosphorylation of DNA binding proteins in chloroplasts of Sinapis alba after acclimation to different light qualities. *In* 13th International Congress of Photosynthesis, Montréal

11 Ehrenwörtliche Erklärung zur Anfertigung der Dissertation

Entsprechend der Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität erkläre ich hiermit, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Hilfsmittel und Literatur angefertigt habe.
Personen, die bei der Auswahl und Auswertung des Materials und der Erstellung der Manuskripte behilflich waren sind am Beginn eines jeden Manuskripts angegeben.
Die Hilfe eines Promotionsberaters wurde nicht in Anspruch genommen.
Die vorgelegte Arbeit wurde bislang weder an der FSU, noch an einer anderen Hochschule als Dissertation eingereicht.

Jena, 24. Februar 2005

12 Addendum

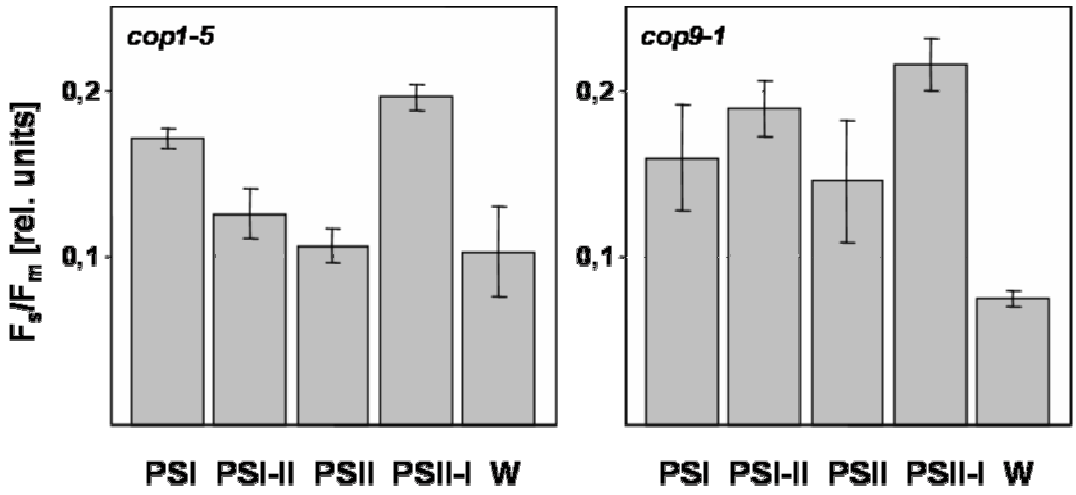

Fig. 7 – LTR in Arabidopsis mutants. Seedlings were acclimated to either PSI- or PSII-light as indicated. Fs/Fm values were determined using a PAM fluorometer in at least three independent experiments with 15 to 20 plants each.

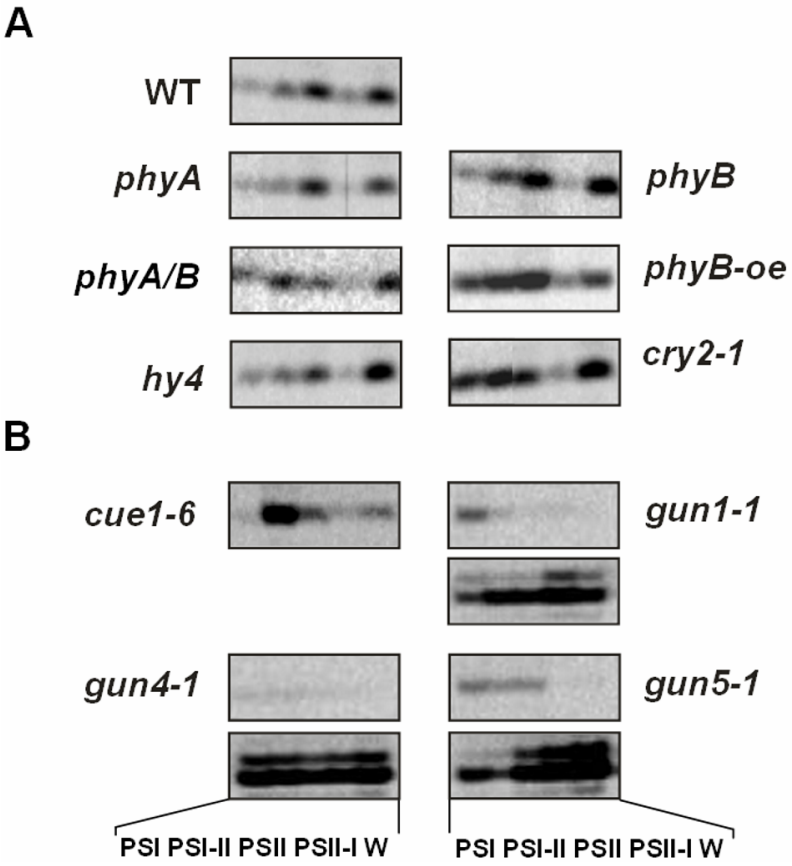

Fig. 8 – LTR in *Arabidopsis* mutants. Plants were grown under the respective light sources given on bottom. Transcript accumulation level was determined using primer extension technique. Total RNA was subjected to reverse transcription reaction with infrared dye labelled *psaA* specific primer and resulting cDNAs were analysed using a Li-Cor 4200 sequencer. (A) Photoreceptor mutants. (B) Chloroplast-to-nucleus signalling mutants

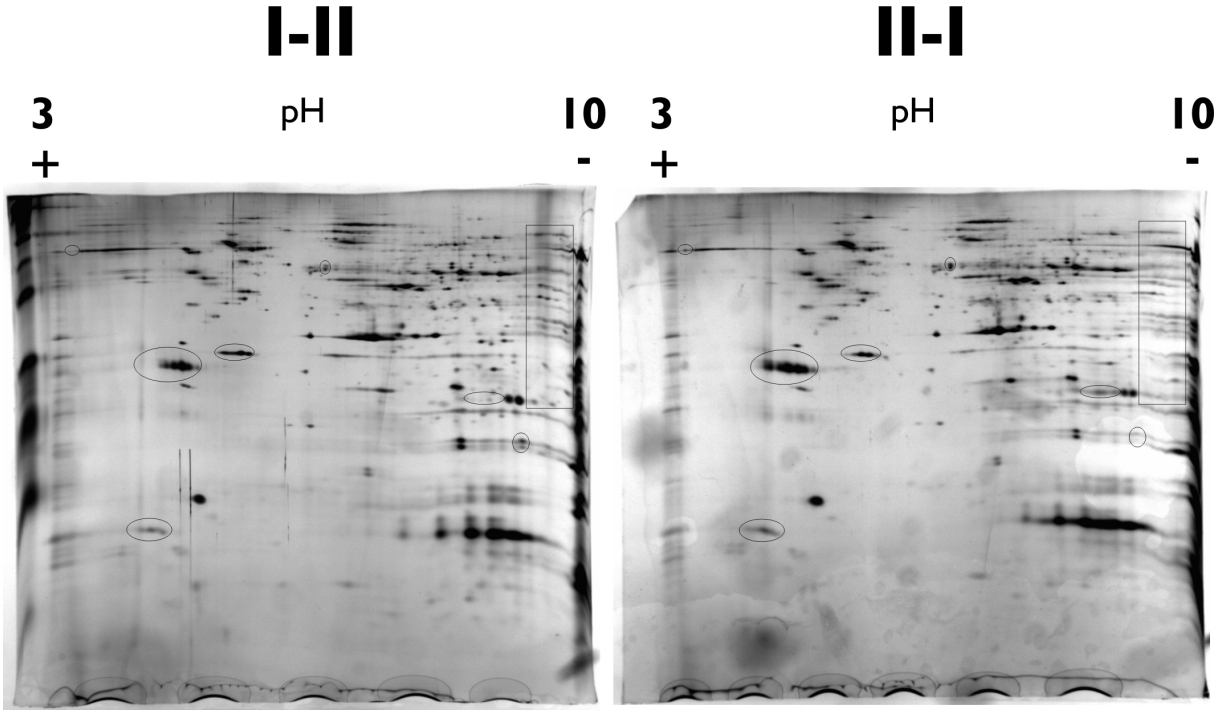

Fig. 9 – 2D SDS-PAGE with heparin-Sepharose fractions. Proteins were purified from mustard seedlings as described (Fey *et al.*, to be submitted). Following acetone/methanol precipitation proteins were subjected to isoelectric focussing and subsequently SDS-PAGE using 14% of acrylamide according to Hippler *et al.* (Hippler *et al.*, 2001) with slight modifications. The left image shows the protein composition on a silver stained gel after a switch from PSI- to PSII-light and the right image from plants treated *vice versa*. Regions with reproducible differences in the protein pattern are marked.

Tabellarischer Lebenslauf

Name: Vidal Fey

Geburtsdatum und -ort: 29.11.1974 in Eisenach

Schule:

09/1981-08/1990	Besuch der Polytechnischen Oberschule (POS)
09/1990-08/1991	Besuch der Erweiterten Oberschule (EOS)
09/1991-06/1993	Besuch des Gymnasiums mit Abschluss Abitur

Wehrdienst:

10/1993-09/1994	Ableistung des Grundwehrdienstes in Rothenburg/Fulda und Frankenberg/Eder

Wissenschaftlicher Werdegang:

Studium

10/1994-12/1998	Studium in der Fachrichtung Biologie an der Friedrich-Schiller-Universität Jena Hauptfach Botanik, Nebenfächer Ökologie, Biochemie und Mikrobiologie, Diplomprüfung in diesen Fächern
01/1999-06/2000	Diplomarbeit am Lehrstuhl für Pflanzenphysiologie des Instituts für Allgemeine Botanik und Pflanzenphysiologie unter Anleitung von Prof. Dr. R. Oelmüller; Thema: „Etablierung einer Methode zur Detektion von kernkodierten, plastidär aktiven Nukleinsäuren bindenden Proteinen in *Arabidopsis thaliana*“

Abschluss des Studiums als Diplom-Biologe am 30. Juni 2000 mit der Note 1,7

seit Juli 2000 Wissenschaftlicher Mitarbeiter am Lehrstuhl für Pflanzenphysiologie des Instituts für Allgemeine Botanik und Pflanzenphysiologie in der Arbeitsgruppe von Dr. Thomas Pfannschmidt

Freiwillige Exkursionen und Praktika während des Studiums

1997	6 Wochen molekularbiol. Praktikum am IPK Gatersleben
1998	Botanische Exkursion nach Tunesien
1999	Botanische Exkursion nach Teneriffa
	Botanische Exkursion in die Schweiz
2001	Botanische Exkursion nach Cuba

Auslandsaufenthalte

2003	von September bis Dezember Durchführung eines Forschungsprojektes im Rahmen der Dissertation in der Arbeitsgruppe von Prof. Eva-Mari Aro in Turku/Finnland

Preise und Stipendien

2002	Peter-Sitte-Preis für die mündliche Präsentation auf der ISE-G – Tagung in Blaubeuren
2003	DAAD-Kurzzeit-Doktorandenstipendium

Sonstige Aktivitäten

2000	Mitarbeit bei der Vorbereitung und Durchführung der Botanikertagung, im Speziellen Erstellung des Tagungsbandes

Betreuung von Grund-, Ober- und Forschungspraktika im Bereich Pflanzenphysiologie

Besondere Kenntnisse und Fertigkeiten:

Sprachkenntnisse

Englisch	(gut in Wort und Schrift)
Finnisch, Russisch, Tschechisch, Albanisch	(Grundkenntnisse in Wort und Schrift)

sonstige Fertigkeiten

November 1992 Erwerb des Führerscheins der Klasse 3

sehr gute PC-Kenntnisse, umfassende Erfahrungen im Umgang mit MS Office, verschiedenen Grafikprogrammen, HTML-Editoren, Sequenzanalyseprogrammen, Datenbankrecherchierungssoftware, Literaturdatenbanken, Netzwerksoftware auf Basis verschiedener MS-Betriebssysteme