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Additions and Corrections

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Page 5321, Fig. 2: The positions of the *psaA* transcripts given in Fig. 2 are incorrect. The correct figure is shown below. As a result, a sentence appearing in the left column, lines 29–31 of the same page, should read as follows: "For *psaA* we found two prominent 5′-ends in a distance of 189 and 76 bases upstream of the translation initiation codon."

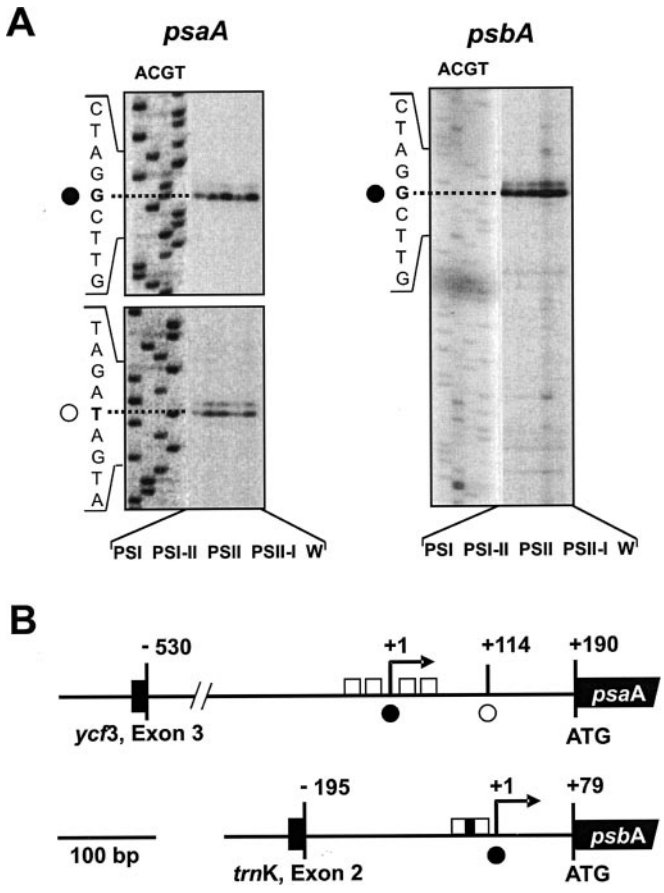

FIG. 2

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.

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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

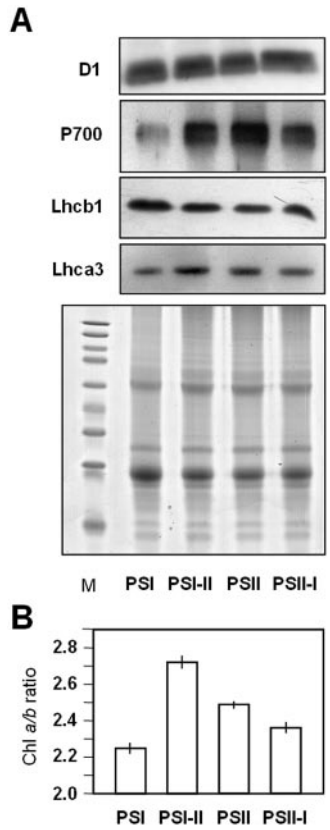

FIG. 1. **Changes in photosynthesis protein and chlorophyll amounts during long term acclimation.** *A*, Western immunological analysis of photosystem core and antenna protein content. Chloroplasts of the differentially grown plants were isolated, and proteins of ~2 × 10^5 organelles were separated by 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

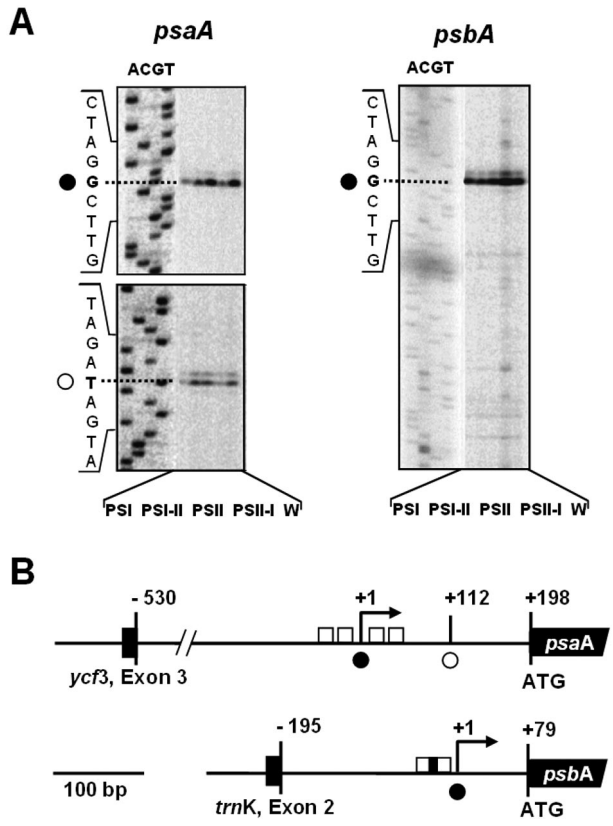

FIG. 2. **Primer extension analysis of position and accumulation of 5′- ends of *psaA* and *psbA* transcripts.** Plants were grown under the respective light sources, and total RNA was isolated. Fluorescence dye-labeled primers were designed to anneal within the first 50 bp of the coding region of the *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.

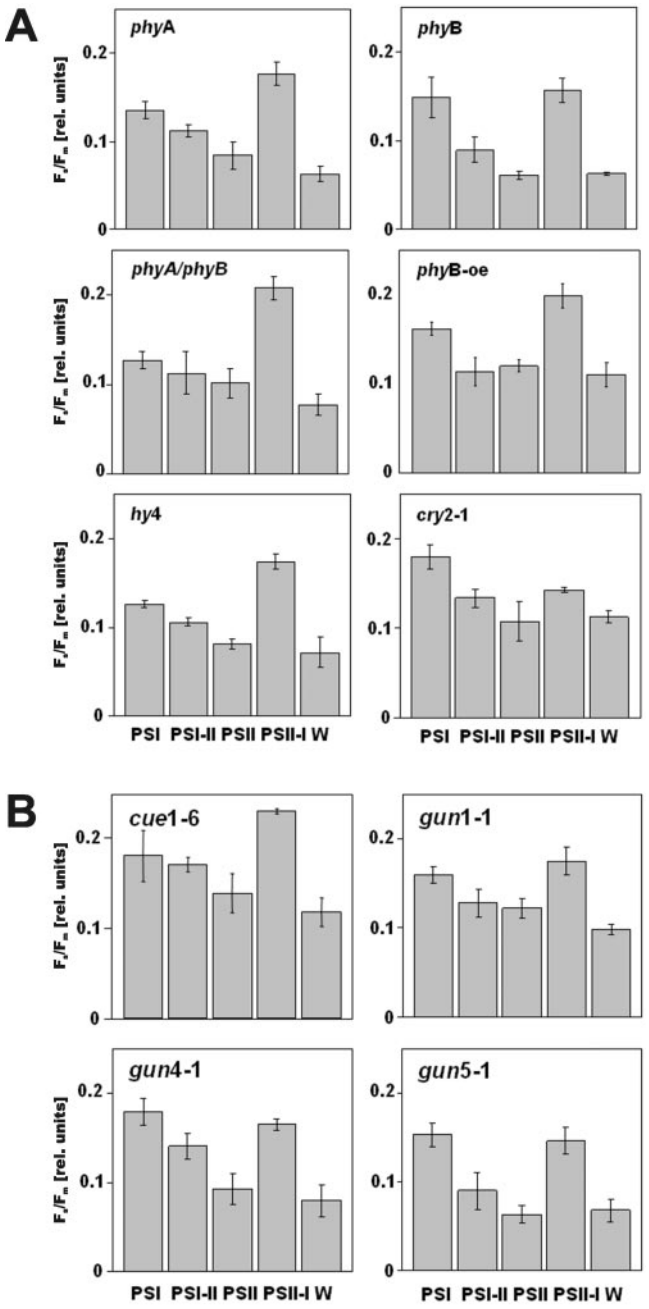

FIG. 3. **LTR in *Arabidopsis* mutants.** Mutant lines were acclimated to PSI or PSII light, and F_s/F_m values were determined using a PAM fluorometer. All values were determined in at least three independent experiments with 15–20 plants each, and the statistical significance of differences was proven 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.

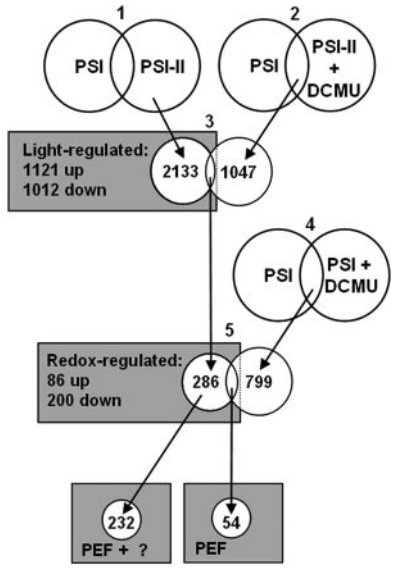

FIG. 4. **Macroarray strategy to define redox-regulated genes encoding chloroplast proteins.** White light-grown plants were acclimated to PSI light for 6 days (*PSI*) or 2 days followed by an acclimation to PSII light for additional 4 days (*PSI–II*). Parallel samples were treated with 5 μM DCMU after 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.

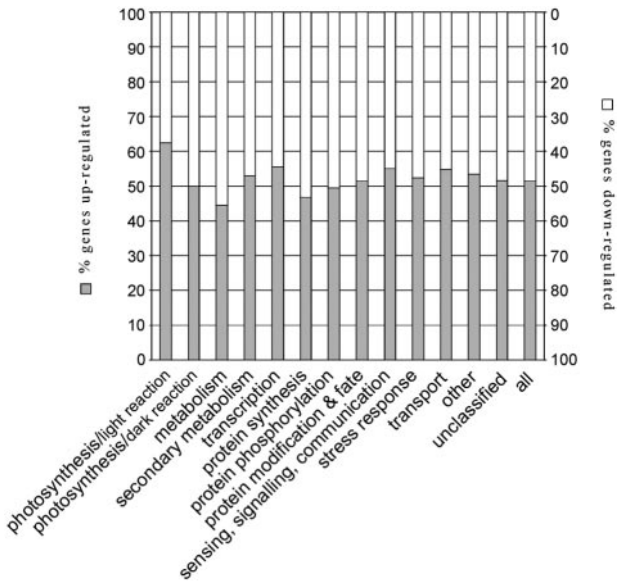

FIG. 5. **Relative distribution of light quality-induced expression changes of genes sorted by function of gene product.** Only genes with significant expression changes were included. Genes were grouped according to the known or predicted function of the encoded product (given at the *bottom*). Numbers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