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Chloroplast-mediated regulation of nuclear genes in *Arabidopsis thaliana* in the absence of light stress

Mirva Piippo, Yagut Allahverdiyeva, Virpi Paakkarinen,
Ulla-Maija Suoranta, Natalia Battchikova, and Eva-Mari Aro

Department of Biology, University of Turku, Finland

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Piippo, Mirva, Yagut Allahverdiyeva, Virpi Paakkarinen, Ulla-Maija Suoranta, Natalia Battchikova, and Eva-Mari Aro. Chloroplast-mediated regulation of nuclear genes in *Arabidopsis thaliana* in the absence of light stress. *Physiol Genomics* 25: 142–152, 2006. First published January 10, 2006; doi:10.1152/physiolgenomics.00256.2005.—Chloroplast signaling involves mechanisms to relay information from chloroplasts to the nucleus, to change nuclear gene expression in response to environmental cues. Aside from reactive oxygen species (ROS) produced under stress conditions, changes in the reduction/oxidation state of photosynthetic electron transfer components or coupled compounds in the stroma and the accumulation of photosynthesis-derived metabolites are likely origins of chloroplast signals. We attempted to investigate the origin of the signals from chloroplasts in mature *Arabidopsis* leaves by differentially modulating the redox states of the plastoquinone pool and components on the reducing side of photosystem I, as well as the rate of CO₂ fixation, while avoiding the production of ROS by excess light. Differential expression of several nuclear photosynthesis genes, including a set of Calvin cycle enzymes, was recorded. These responded to the stromal redox conditions under prevailing light conditions but were independent of the redox state of the plastoquinone pool. The steady-state CO₂ fixation rate was reflected in the orchestration of the expression of a number of genes encoding cytoplasmic proteins, including several glycolysis genes and the trehalose-6-phosphate synthase gene, and also the chloroplast-targeted chaperone DnaJ. Clearly, in mature leaves, the redox state of the compounds on the reducing side of photosystem I is of greater importance in light-dependent modulation of nuclear gene expression than the redox state of the plastoquinone pool, particularly at early signaling phases. It also became apparent that photosynthesis-mediated generation of metabolites or signaling molecules is involved in the relay of information from chloroplast to nucleus.

light acclimation; redox regulation; plastoquinone pool; carbohydrate metabolism; photosynthesis

THE EFFICIENCY OF PHOTOSYNTHESIS at low light intensity is highly dependent on the rate of linear electron transfer, driven by two distinct photosystems in chloroplasts. Saturated electron transfer under high light conditions, on the other hand, produces reducing equivalents in the stroma in excess of that required to drive the Calvin cycle at maximum rate, to produce triose phosphates for energy conservation. Sudden changes in both the quality and quantity of light tend to cause the redox poise of the photosynthetic electron carriers to become imbalanced. Changes in light quality might lead to preferential excitation of either photosystem (PS)II or PSI, thus leading to redox changes in intersystem electron carriers, particularly the PQ pool and

the cytochrome b₆f complex, whereas high light reduces the whole electron transfer chain and causes accumulation of reducing equivalents in the stroma as well as the production of reactive oxygen species (ROS). These various species are likely to form an important source of signals from chloroplasts to the nucleus, to change nuclear gene expression according to environmental cues (13). For example, the PQ pool, cytochrome b₆f, ferredoxin, thioredoxin, and ROS have been implicated as being sources of plastid signals (14, 28, 38). In addition, the intermediates of chlorophyll biosynthesis have been shown to play an important role in chloroplast-to-nucleus signaling under stress conditions (17, 46). In addition, the photosynthesis-derived metabolites have recently been attributed a possible role in regulation of nuclear genes (29, 50). Multiple mechanisms and cross-signaling with light are thus likely to coordinate the expression of nuclear and chloroplast genes to rapidly respond to changes in environmental conditions.

Redox signals from photosynthetic electron transfer components have been shown to control gene expression in chloroplasts at both the transcriptional and translational levels and the expression of nuclear genes mainly at the transcriptional level (32). The redox state of the PQ pool in particular has been strongly suggested as a prominent candidate for the origin of chloroplast redox signals (1, 33). This has been demonstrated by using the photosynthetic electron transfer inhibitors 2,5-dibromo-3-methyl-6-isopropyl-benzoquinone (DBMIB) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and by modulating the redox state of the PQ pool by light that predominantly excites either PSII or PSI and thus reduces or oxidizes the PQ pool, respectively. Several genes, and photosynthesis-related genes in particular, have been reported to respond to signals originating from the PQ pool, e.g., the chloroplast-encoded *psaAB*, *psbA*, and *psbB* genes, and nuclear-encoded *petE*, *lhcb*, and *apx* genes (12, 18, 34, 44, 51). Genes regulated by some component in the electron transfer chain, distinct from the PQ pool, include *psaD*, *psaF*, *ferredoxin* and *nitrate reductase* (31, 34, 44).

Photosynthesis-mediated production of ROS has also been studied extensively as a source of chloroplast signals. High light, well in excess of that required for light-saturated fixation of CO₂, generally induces a strong expression of various antioxidant genes, cellular chaperones and heat shock proteins, which also respond to externally applied ROS species such as H₂O₂ (10, 20, 38, 48). On the contrary, several thylakoid proteins are strongly downregulated under such excessive light conditions.

Most studies addressing the role of chloroplast-mediated signals in nuclear gene expression have ignored the possible effects of carbon metabolism, e.g., by using inhibitors of the

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Address for reprint requests and other correspondence: E.-M. Aro, Plant Physiology and Molecular Biology, Dept. of Biology, Univ. of Turku, FI-20014 Turku, Finland (e-mail: evaaro@utu.fi).

electron transfer chain, thus blocking the production of reducing equivalents and ATP for CO₂ fixation. Here, we have modulated the redox state of the photosynthetic electron transfer chain by short-term illumination with different qualities and quantities of light, namely, PSII light that preferentially excites photosystem II, PSI light that excites predominantly photosystem I, high but not excessive light, low light, and darkness. All of these treatments were compared with the standard growth light (white light) conditions. Light conditions inducing ROS production were particularly avoided, and emphasis was placed on the role of the redox state of the PQ pool, the stromal redox compounds, and the CO₂ fixation products as sources of chloroplast signals during the 3-h illumination period. Many of the genes responding to different light treatments in this study are among the genes involved in carbohydrate metabolism and protein synthesis. Their expression is most likely regulated from the reducing side of PSI and via carbon and energy metabolism, whereas the role of the redox state of the PQ pool in nuclear gene expression appears to be only marginal, or negligible.

MATERIALS AND METHODS

PSII- and PSI-specific light. The PSII light source was a fluorescent tube (Gro-Lux F58W/GRO-T8; Sylvania, Germany) covered with an orange filter (Lee 105 filter; Lee Filters, Hampshire, UK), whereas PSI light was derived from halogen lamps (500 W) covered with an orange filter (Lee 105) and a "Median blue" filter (Roscolux no. 83; Rosco Europe, London, UK). The spectral characteristics of the normal growth light (OSRAM PowerStar HQI-T 400/D metal halide lamp) and the PSI and PSII light sources (Fig. 1) were tested with a diode array spectrophotometer (S2000; Ocean Optics, Dunedin, FL).

Experimental material. *Arabidopsis thaliana* (ecotype RLD) plants were grown in white light (100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, where 1 $\mu\text{mol} = 6 \times 10^{17}$ photons) under an 8:16-h light-dark cycle at a temperature of 23°C. Six different light treatments were applied: light that predominantly excites PSII (50 and 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, denoted by PSII and PSII* light, respectively), light that predominantly excites PSI (30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), high-intensity white light resulting in 80% saturation of CO₂ fixation (450 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), low-intensity white light (20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and darkness. Five-week-old plants were transferred to the respective light treatments for 3 h at the end of the diurnal

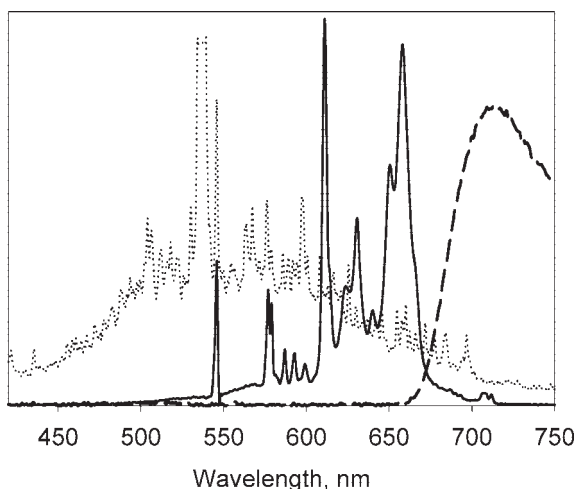


Fig. 1. Emission spectra of the normal growth light (dotted line), photosystem (PS)II light (continuous line), and PSI light (dashed line) used for plant treatments.

dark period. Control plants were kept in the growth light for the same time as the respective treatments, to avoid the effects of circadian rhythm.

Thylakoid isolation and phosphoprotein analysis. Isolation of thylakoids was done as described in Ref. 5. All buffers contained 10 mM NaF, and the chlorophyll content was determined as previously described (35). SDS gel electrophoresis was carried out in 15% polyacrylamide gels with 6 M urea (23). For immunoblot analysis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Belford, MA). The phosphoproteins were immunodetected by polyclonal phospho-threonine antibody (New England BioLabs, Beverly, MA), using a Phototope-Star Chemiluminescent Detection Kit (New England Biolabs).

Chlorophyll fluorescence measurements. Steady-state fluorescence quenching parameters were determined in intact leaves directly in the growth chamber using a PAM 101/103 Chl fluorometer (Walz, Effeltrich, Germany). After a specific light treatment of 3 h, the leaf was detached and immediately transferred to the PAM fluorometer; steady-state fluorescence (F_s) was maintained with the same quality and quantity of light as prevailed during the previous 3-h light treatment, and the maximal fluorescence (F_m') was induced by a 0.8-s saturating flash of white light (3,000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). To determine the minimal level of fluorescence (F_0'), far-red light was provided using the light-emitting diode (LED) light source (PAM-102-FR, Walz). The PSII excitation pressure ($1 - qP$) was calculated according to the equation, $1 - qP = (F_s - F_0') / (F_m' - F_0')$.

Seventy-seven-Kelvin (77K) fluorescence emission spectra of thylakoid membranes were recorded with a diode array spectrophotometer (S2000; Ocean Optics) equipped with a reflectance probe as previously described (19). Fluorescence excitation was obtained with light below 500 nm, defined using LS500S and LS700S filters (Corion, Holliston, MA) placed in front of a slide projector, and the emission was recorded between 600 and 800 nm.

NADP-malate dehydrogenase activity and CO₂ fixation assays. NADP-malate dehydrogenase (NADP-MDH) activity was measured as described previously (42). CO₂ assimilation rates under different light conditions were measured from the whole plants with the Ciras-1 photosynthesis system (PP Systems, Hertfordshire, UK) in atmospheric CO₂ concentration (360 ppm) using a special *Arabidopsis* chamber (PP Systems).

cDNA microarrays. *Arabidopsis* cDNA microarray chips are based on the GEM1 clone set from IncyteGenomics (Palo Alto, CA). The GEM1 set contains 7,942 elements, which corresponds to ~6,500 unique genes. To prepare DNA samples ready for spotting, the original collection was amplified with the TECAN robotic station. Plasmids were isolated with a Wizard SV 96 Plasmid DNA Purification System (Promega, Madison, WI), and the quality of plasmids was assessed by agarose gel electrophoresis. Expressed sequence tags (ESTs) in plasmids were amplified by PCR, and their quality was checked with agarose gel electrophoresis. PCR products were purified with the Montage PCR₉₆ Cleanup Kit (Millipore, Belford, MA). Each clone was spotted three times to an array to provide technical replicates, and the whole set was divided between two slides.

RNA preparation, labeling, and hybridization for microarrays and Northern blot analysis. Three independent leaf samples were homogenized with a mortar and pestle in liquid nitrogen. Total RNA was extracted with the Trizol reagent (Invitrogen, Carlsbad, CA), and poly(A)⁺ RNA was isolated with magnetic beads (Dynabeads; Dynal Biotech, Oslo, Norway). One microgram of poly(A)⁺ RNA was labeled with d(T)₁₂₋₁₈ primers (Amersham Biosciences, Piscataway, NJ) with direct incorporation of either Cy3- or Cy5-dUTP (Amersham Biosciences, Little Chalfont, UK) using SuperScript II RT (Invitrogen).

The microarray slides were UV cross-linked (90 mJ/cm²) and prehybridized with 1% BSA (Sigma, St. Louis, MA). The labeled samples were combined and hybridized in a total volume of 80 μl at 65°C overnight under a LifterSlip (Eric Scientific, Portsmouth, NH).

For Northern blot analysis, 5 μg of total RNA prepared for microarray analysis were run in a 1.2% denaturing agarose gel using glyoxal as denaturing agent and blotted to a Hybond-N membrane (Amersham) as described (40). The PCR products of EST clones from the microarray clone collection were used as probes for the respective genes in hybridization. The DNA probe was labeled with Prime-a-Gene labeling kit (Promega) using [α - ^{32}P]dCTP (Amersham) and hybridized to membranes at 65°C overnight. Washed membranes were exposed to X-ray films, and signal density was quantified with Fluor Chem version 3.04A (Alpha Innotech, San Leandro, CA).

Sequencing of the clones. The clones used for Northern blot analysis were picked from the spotting plates, and their sequences were verified by sequencing with the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) at the Centre for Biotechnology, Turku, Finland.

Microarray data analysis. The arrays were scanned with ScanArray 5000 (GSI Lumonics, Billerica, MA), and the spot intensities were quantified with the ScanArray Express Microarray Analysis System 2.0 (Perkin-Elmer Life Sciences, Wellesley, MA) using the adaptive circle method. Raw signal intensities were normalized with BioConductor software (<http://www.bioconductor.org/>) using print-tip-group Lowess scale normalization function. Data filtering was performed with Kensington Discovery Edition version 1.8 (InforSense, London, UK). From the normalized data, the poor data points were discarded if the spot was flagged as bad, either manually or by the software during spot intensity quantification. The three technical replicates from each slide were averaged, followed by averaging of the dye swaps for samples they were made of. The preprocessed data were imported into GeneSpring 7.2 (Silicon Genetics, Redwood City, CA) from the three independent biological experiments under each illumination condition, and the averages, standard deviations, *P* values, and clustering were obtained. Any given *P* values are derived from Student's *t*-test. For each treatment, the *q*-values (45) were calculated using BioConductor's QVALUE package, and the *P* value threshold that allowed a maximum false discovery rate of 0.10 (*q*-value ≤ 0.10) was selected as significant. The *P* value thresholds for different treatments were as follows: dark, 0.03362; low light, 0.01562; PSI light, 0.01003; PSII* light, 0.00452; PSII light, 0.00083; and high light, 0.00052. The gene annotation used was derived from The Arabidopsis Information Resource (TAIR; <http://www.arabidopsis.org>).

RESULTS

The emission spectra of PSI and PSII light used for illumination of *Arabidopsis* plants can be seen in Fig. 1. Throughout the text, the higher-intensity PSII light ($150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) is denoted by an asterisk (PSII* light) to distinguish it from lower-intensity PSII light ($50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The PQ pool redox state of treated plants was monitored by analyzing the

Table 1. Effects of different light conditions on peak ratios of 77K fluorescence emission spectrum and PSII excitation pressure

Treatment	F733/F685	1-qP
Growth light	1.76 \pm 0.01	0.099 \pm 0.004
PSII* light	1.94 \pm 0.02	0.422 \pm 0.050
PSI light	1.44 \pm 0.02	0.069 \pm 0.014
PSII light	ND	0.117 \pm 0.030
Low light	1.67 \pm 0.08	0.028 \pm 0.008
High light	1.57 \pm 0.05	0.686 \pm 0.155
Darkness	1.54 \pm 0.04	ND

Values are means of 3 independent illumination treatments. PS, photosystem; F733, fluorescence emission peak at 733 nm; F685, fluorescence emission peak at 685 nm; 1-qP, the PSII excitation pressure; ND, not determined.

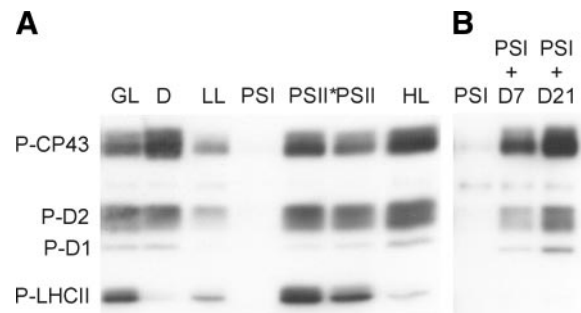


Fig. 2. Phosphorylation (P) of *Arabidopsis* PSII core and LHCII proteins. A: phosphorylation of thylakoid PSII core and LHCII proteins in growth light (GL; $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, where $1 \mu\text{mol} = 6 \times 10^{17}$ photons), darkness (D), low light (LL; $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), PSI light (PSI; $30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), PSII light (PSII; $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), PSII* light (PSII*; $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and high light (HL; $450 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) after 3 h of treatment. B: phosphorylation of PSII core proteins in the dark was examined by transferring the plants after 3 h of treatment in PSI light (PSI) to darkness for 7 (PSI + D7) and 21 h (PSI + D21). Proteins equivalent to 1 μg of Chl were loaded in each lane.

PSII excitation pressure ($1 - qP$) and the phosphorylation state of PSII core proteins (Table 1 and Fig. 2).

The PSII excitation pressure is a measure of the redox state of primary quinone electron acceptor (Q_A). It thus reflects the balance between light energy absorbed by PSII and that utilized by the intersystem chain and/or PSI, and can be modulated by different light conditions. Plants treated with PSI light and low light showed lower PSII excitation pressures (0.069 and 0.028, respectively) than plants in growth light (0.099), which indicates a more oxidized redox status of the PQ pool under PSI and low light conditions compared with plants kept under growth light.

Low phosphorylation levels of PSII proteins D1, D2, CP43, and LHCII in *Arabidopsis* plants treated with PSI light and low light are in accordance with oxidation of the PQ pool under those conditions (3). On the other hand, a strong phosphorylation level of PSII core proteins and an approximately four- and sevenfold increase in the PSII excitation pressure after exposure of plants to PSII* light and high light, respectively, indicate a more reduced PQ pool under these conditions compared with growth light. Thus the PSII excitation pressure and steady-state phosphorylation levels of the PSII core proteins suggest that the PSII* and PSI illumination conditions modulate the redox state of the PQ pool in opposite directions. However, plants treated with low PSII light ($50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) showed only a slight increase in PSII excitation pressure (0.117), and the phosphorylation level of the PSII core proteins was similar to that in growth light. Clearly, the low-intensity PSII light was not strong enough to keep the PQ pool highly reduced but enabled the monitoring of the effects of light quality.

To find out whether PSII core protein phosphorylation in darkness (Fig. 2A) was due to an inactivation of the protein phosphatase or to a persistent kinase activity in the dark, the plants were treated with PSI light for 3 h to dephosphorylate all thylakoid proteins and then transferred to darkness for 7 and 21 h. The phosphorylation level of PSII core proteins gradually increased in dark-treated plants, indicating that the kinase responsible for phosphorylation of PSII core proteins is active in *Arabidopsis* leaves in darkness (Fig. 2B), due to reduction of the PQ pool by alternative electron transfer routes. On the other

hand, the kinase responsible for LHCII protein phosphorylation remained inactive in the dark.

Changes in the peak ratios of the 77K fluorescence emission spectrum at 733 nm (emission from PSI) relative to 685 and 695 nm (Chl fluorescence emission from PSII) are typical of state transitions *in vivo* (2). As shown in Fig. 2 and Table 1, the phosphorylation level of LHCII and the F733/F685 fluorescence emission ratio increased under PSII* light (state 2) and decreased in dark-treated, low light-treated, and PSI light-treated (state 1) plants compared with plants in growth light. It is interesting to note that, under high light conditions, when the PQ pool was highly reduced, the 77K fluorescence peaks revealed a lower F733/F685 fluorescence emission ratio than under growth light, which is in line with dephosphorylation of LHCII proteins under high light (Fig. 2). Inhibition of LHCII protein phosphorylation under high light, on the other hand, implies an accumulation of reducing equivalents in chloroplast stroma in addition to strong reduction of the PQ pool (37).

The redox state of the stroma under different light treatments was indirectly assayed by measuring the NADP-MDH activity (Fig. 3), which is directly related to the reduction state of stromal thioredoxins (26, 41). The NADP-MDH activity measurements indicated that PSI light marginally activates NADP-MDH, and slight activation occurred during 3 h of illumination in low light and in both types of PSII light. On the other hand, in growth light and high light, the NADP-MDH activity was

2.4 and 4.7 times higher, respectively, than in PSII* light, implying a drastically more reduced stroma under the two former light conditions. Indeed, high light-treated plants had ~80% of their NADP-MDH activated. The effects of differential light treatments on CO₂ assimilation rates did not directly follow the activation state of NADP-MDH. Low light induced only marginal CO₂ fixation in leaves. Low-intensity PSII light induced a CO₂ fixation rate of ~1.1 $\mu\text{mol m}^{-2}\text{s}^{-1}$, PSII* light ~2.4 $\mu\text{mol m}^{-2}\text{s}^{-1}$, growth light 3.0 $\mu\text{mol m}^{-2}\text{s}^{-1}$, and high light 7.5 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Thus growth light and PSII* light gave very similar CO₂ assimilation rates, and the different light conditions clearly induced a gradient of CO₂ fixation rates in differentially illuminated leaves.

Changes in gene expression in plants treated with different light conditions were recorded using microarrays. Control plants harvested 3 h after the onset of the illumination period were compared with plants transferred to different light conditions (or kept in darkness) for the same 3-h period, immediately after the dark period. This experimental design also allowed consideration of the effects of the products of photosynthesis on nuclear gene expression. To find an optimal time point, plants were illuminated for 1, 3, and 6 h and even 27 and 51 h (data not shown). Major changes in gene expression could be seen already after 1 and 3 h of treatment. Because we wanted to study early acclimation responses to chloroplast-mediated signals, the 3-h time point was chosen for more extensive investigation. To simplify the terminology, a gene will be described as being upregulated if the expression level recorded was higher in treated plants than in plants in growth light, and downregulated if the expression level recorded was lower in the treated plants than in plants in growth light.

A total of 2,027 genes (~25% of the genes on the array) changed their expression significantly as a result of at least one light treatment. According to the TargetP software (11), 370 (18%) of them were targeted to the chloroplast and 150 (7%) to mitochondria. The general expression profile (Fig. 4) in different treatments vs. growth light showed that, although the gene expression changes were strongest in the dark-treated, PSI light-treated, and low light-treated plants and weakest in PSII* light- and PSII light-treated plants, the overall pattern of gene regulation in *Arabidopsis* leaves was rather similar under all of these conditions and generally opposite to that occurring in high light. The dark treatment alone caused >1,500 genes to change their level of expression significantly; both low light and PSI light caused >500 to change their expression, and PSII, PSII*, and high light each caused <300 to change. The three illumination conditions that caused the most changes in gene expression, namely, dark, low light and PSI light, had 270 genes in common that changed their expression under all three conditions.

The genes that underwent differential expression were related to metabolic pathways (mainly C-compound and carbon metabolism and amino acid metabolism), protein synthesis (ribosome biogenesis and translation), energy production (glycolysis and gluconeogenesis, photosynthesis, the TCA cycle), and stress responses, as deduced from the Munich Information Center for Protein Sequences Functional Catalogue of *Arabidopsis* genes (39). The MapMan software (49) was used to obtain further functional grouping of transcriptional changes. Genes categorized as being especially associated with glycolysis, the Calvin cycle, photorespiration, the oxidative pentose

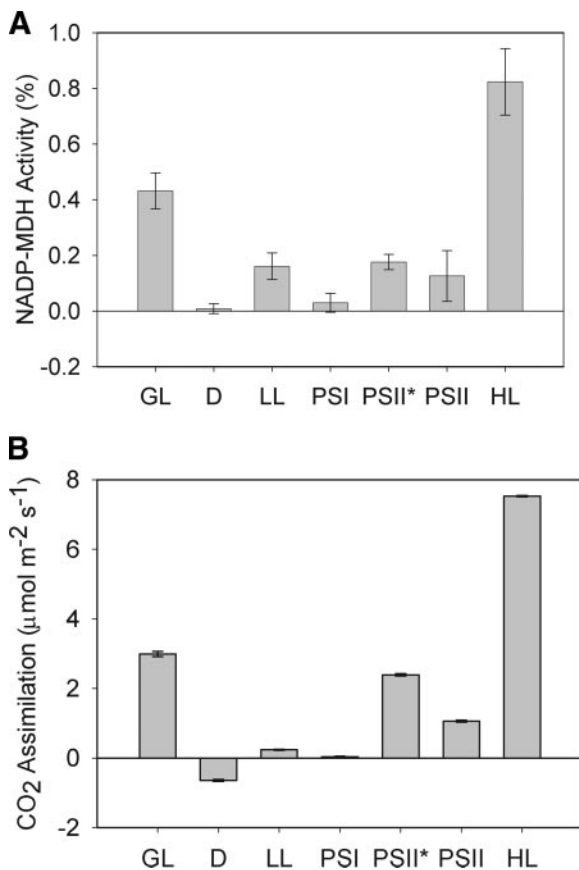


Fig. 3. NADP-malate dehydrogenase (NADP-MDH) activity and CO₂ assimilation rate of *Arabidopsis* leaves under different light treatments. A: fraction of active NADP-MDH enzyme for different light treatments. B: CO₂ steady-state assimilation rate for different treatments. Light conditions are as in Fig. 2.

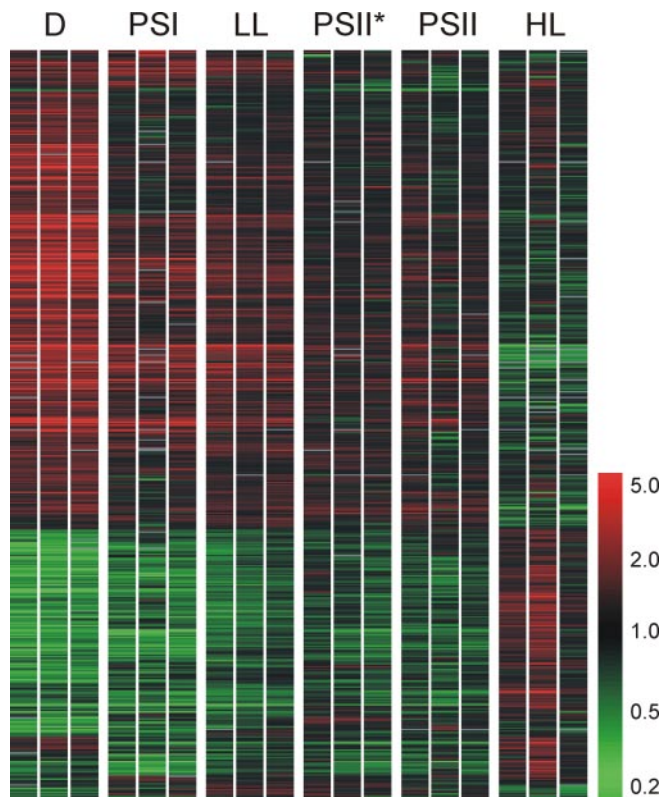


Fig. 4. Clustering of all 2,027 genes that showed significant changes in expression in at least 1 of the light treatments. Control sample treated with growth light served as a baseline reference for each treatment. Three independent biological replicates, starting from the growth of a new set of plants, are shown for each light treatment for comparison of the repeatability of the biological replicates. Each biological replicate measurement is an average of technical replicates that were obtained by spotting each DNA fragment 3 times on a single slide, producing 3 technical replicates for each measurement. For detailed data analysis procedure, see MATERIALS AND METHODS. Light conditions are as in Fig. 2 (red, upregulated; green, downregulated; gray, missing value).

phosphate pathway, and photosynthetic light reactions were downregulated in dark-, low light-, PSI-, PSII-, and PSII*-treated plants, and the response was strongest in dark-treated and PSI light-treated plants. In addition, the expression of some genes involved in tetrapyrrole, amino acid, and starch metabolism had changed in different treatments. Some interesting genes and their relative changes in expression are listed in Fig. 5. A categorized list of genes can be found in the Supplemental Material (available at the *Physiological Genomics* web site).¹

The PSII excitation pressure, thylakoid protein phosphorylation, and 77K fluorescence emission data showed that the PQ pool is strongly oxidized in PSI light and strongly reduced in PSII* light. None of the genes on the array had significant changes in gene expression in PSI light accompanied by a significant change in PSII* light in the opposite direction. Moreover, only a few genes responded selectively and similarly to both PSII* and high light, for example the genes encoding calcium exchanger (At2g38170), calreticulin (At1g56340), and jacalin lectin (At3g16470) (although no

significant change in gene expression could be detected in PSI light). Thus a possible role for the reduced PQ pool acting as a transcriptional activator of these genes cannot be excluded. Interestingly, only the calcium exchanger protein is targeted to chloroplasts according to the TargetP software.

Surprisingly, the expression of genes encoding proteins associated with the thylakoid membrane was not dramatically affected by the light treatments (Fig. 5 and Supplemental Material). The expression of early light-induced protein gene *elip1* (At3g22840) was elevated 5.4-fold under high light and repressed on average 1.7-fold under all other conditions. Regarding the relatively extensively studied *lhcb* genes, the results from microarrays did not reveal statistically significant changes in expression for leaves treated with PSI or PSII* light. However, clear changes were recorded in the Northern blot hybridizations from the same leaf samples (see Fig. 7). *lhcb* genes were clearly downregulated under PSI light, which causes oxidation of the PQ pool, but also in high light, which leads to a more reduced PQ pool than in growth light. No significant change was recorded under PSII* light. Genes encoding the oxygen-evolving complex proteins changed their expression more distinctly than those encoding the other subunits of the photosynthetic complex. A clear downregulation under high light was recorded for the oxygen-evolving complex protein genes *psbO* and *psbQ*. On the other hand, two genes (At1g14150, At1g77090) that have a domain similar to that in the PsbQ and PsbP subunits of PSII oxygen-evolving complex were downregulated under all conditions except high light.

Genes of two distinct pathways, the Calvin cycle and glycolysis, responded strongly to different light treatments. Calvin cycle enzymes were downregulated with light treatments other than high light, where they were upregulated. The expression patterns of several enzymes of glycolysis, however, differed from those of Calvin cycle enzymes in both PSII and PSII* light, being very similar to their expression in growth light (Fig. 5). In addition, expression of trehalose phosphate synthase (At1g70290), which is involved in mobilization of energy reserves such as trehalose and glycogen, was upregulated under all conditions except high light, where it was downregulated (Table S1 of Supplemental Material). It is noteworthy that trehalose phosphate synthase was among nine genes that changed their expression markedly under different treatments. Interestingly, most of these genes showed changes in expression that were either directly or inversely related to the CO₂ assimilation rate under a given illumination condition (Fig. 6), suggesting a sensitive “metabolic” regulation of these genes.

Excess light, as well as other environmental stresses, can cause the formation of harmful ROS, which are removed by the antioxidant defense system. Mittler et al. (27) collected an extensive list of antioxidant-scavenging enzyme genes, of which 57 were found on our microarray. None of the antioxidant genes were statistically significantly upregulated in our high light-treated plant samples, indicating limited formation of ROS in the high light treatment used, which was not excessive with respect to saturation of CO₂ fixation (Supplemental Table S1). Only *cat2* and *dhar5* were induced more than twofold on average in our high light treatment, but the change was not statistically significant. In addition, of other well-known H₂O₂-induced transcripts gathered from the liter-

¹ The Supplemental Material for this article is available online at <http://physiolgenomics.physiology.org/cgi/content/full/00256.2005/DC1>.

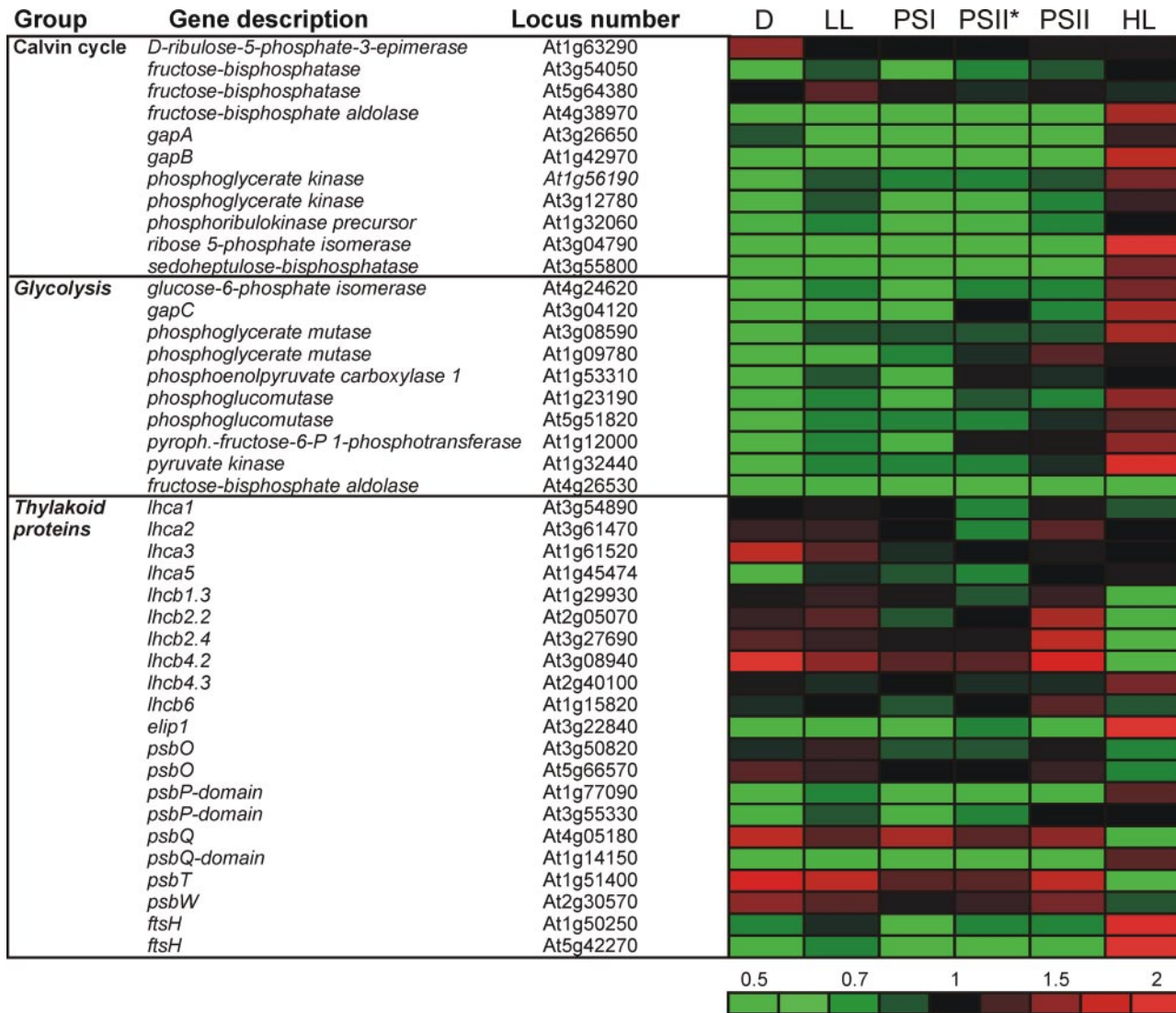


Fig. 5. Relative changes in expression of distinctive groups of genes. Complete list can be found in the Supplemental Material. *Arabidopsis* plants were exposed to different light conditions for 3 h and compared with growth light-treated plants. Light conditions are as in Fig. 2.

ature, only glutathione *S*-transferase (*gstI*) was upregulated 2.7-fold in high light.

One might ask whether the phytochromes, the red light receptors of green plants, play a role in the results presented here, since the plants were treated with red light (PSII light) and far-red light (PSI light). Some genes, such as those encoding chalcone synthase (*chs*), the Rubisco small subunit (*rbcS*), NADP:protochlorophyllide oxidoreductase (*por*), and the *lhcb* genes, are known to respond strongly to the phytochrome photoreceptor (22, 24). It is known that phytochromes have a significant role in regulating seed germination, deetiolation, shade avoidance, and the circadian clock (47). Most of these experiments, however, have been done with young seedlings, and there is not much information available on the role of phytochromes in the acclimation of mature green leaves to an altered environment.

We examined the expression of some well-known phytochrome-regulated genes on our microarrays to find out whether the response to red light (PSII light) and far-red light (PSI light) is as apparent also in developed green leaves. Taking a

simple approach, it would be expected that, if a gene is regulated by phytochrome in our light conditions, it should be regulated in a similar way both in the dark and in PSI light but in the opposite direction under both PSII and PSII* light. None of the known phytochrome-regulated genes on the array fulfilled these criteria, indicating that phytochromes do not play a major role under the light conditions used (Supplemental Table S1). It is worth noting, however, that *porB* (At4g27440) was specifically upregulated in both PSII and PSII* light and downregulated only in high light. The gene for PhyA photoreceptor (At1g09570), which autoregulates itself at the transcriptional level, had increased expression levels in dark-, low light-, and PSI light-treated leaves and (although not statistically significant) also in both PSII and PSII* light, relative to its expression in growth light. This result is also affected, at least partially, by posttranscriptional degradation of *phyA* mRNA (9) in growth light and probably also in PSII and PSII* light but not in darkness, low light, or PSI light. It is, however, important to note that *Arabidopsis* seedlings lacking four major photoreceptors, namely phytochromes A and B and crypto-

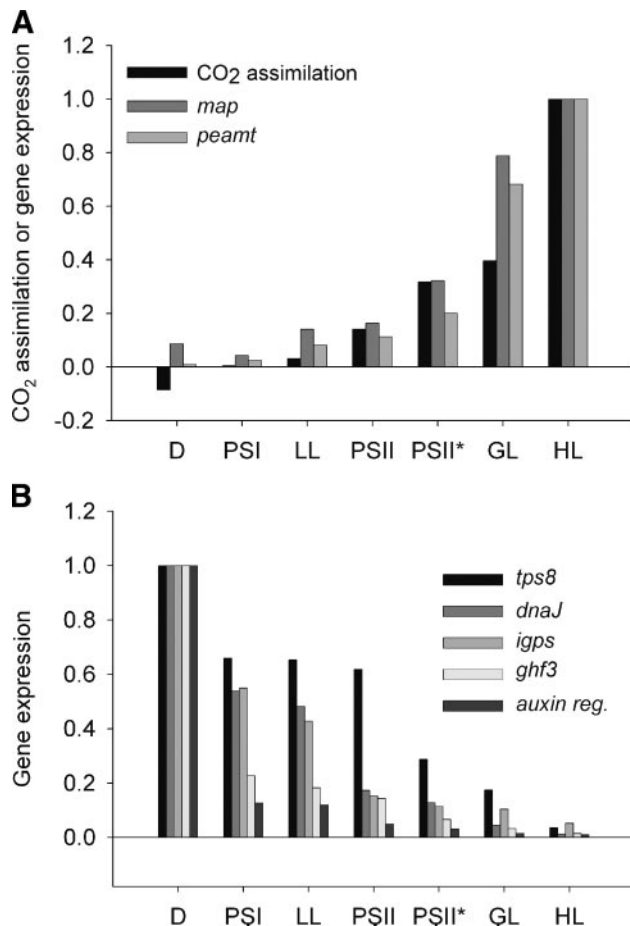


Fig. 6. Genes that showed drastic changes in gene expression with different light treatments in relation to the rate of CO₂ fixation. Both rates of gene expression and CO₂ assimilation have been normalized to the highest observed gene expression ratio or assimilation rate, respectively. Growth light value is indicated for gene expression data as well and was set to be 1 before normalizations of all treatments to the highest value. For absolute values, see Supplemental Table S1. *A*: genes that show increasing expression as CO₂ assimilation increases. *B*: genes that have reduced expression as CO₂ assimilation increases. Light conditions are as in Fig. 2. *map*, Microtubule-associated protein (At5g55230); *peamt*, phosphoethanolamine *N*-methyltransferase (At1g73600); *dnaJ*, chloroplast-targeted chaperone protein (At1g80920); *auxin regulated*, auxin-regulated protein (At2g33830); *ghf3*, glycosyl hydrolase family 3 (At5g49360); *tps8*, trehalose phosphate synthase (At1g70290); *igps*, indole-3-glycerol phosphate synthase (At2g04400).

chromes 1 and 2, are capable of showing light-induced changes in gene expression resembling those of the wild type, even though the quadruplet mutant does not respond morphologically to light (30).

The reliability of the microarray results was assessed with Northern blot analysis of the expression profiles of 13 genes on the array (Fig. 7). The Northern analysis also demonstrated that genes expressed at relatively low levels, such as *psbQ*, *eli3-1*, and *fba*, had relatively consistent fold changes in both the microarray and Northern blot results. This shows our results to be reliable also for low-expression genes, since the data-filtering procedure used did not explicitly remove spots with low values of signal intensity. Thus the results from microarrays were reproducible with Northern blot analysis, demonstrating that the reliability of our assays was good.

DISCUSSION

Differentially illuminated *Arabidopsis* plants were subjected to cDNA microarray analysis to gain a better understanding of the chloroplast-nuclear signaling. It is noteworthy that more than one-quarter of the genes in the array responded to differential illumination conditions and are thus likely to be regulated by chloroplast-mediated signals. Even though our array contained only one-third of the whole *Arabidopsis* genome, it is a solid random representation of the whole genome, since the genes were not preselected on the basis of their function or localization. Particular emphasis was placed on the PQ pool, the stromal redox state, and the products of CO₂ fixation as signal sources for nuclear gene expression. Thus our high light treatment of plants was chosen to be strong enough to enhance the reduction of the whole photosynthetic electron transfer chain and stroma compared with growth light, but at the same time to be only moderately high to avoid the harmful effects of excess light, such as strong formation of ROS. This could indeed be prevented, as seen from the low number of upregulated antioxidant- and H₂O₂-induced genes in our high light-treated plants (Supplemental Table S1).

Although the redox state of the PQ pool has been attributed a significant role in chloroplast-to-nucleus signaling (1, 33), we present here results showing that it is not a major source of chloroplast signaling in mature higher plants. First, a noticeable similarity in the changes in gene expression profiles after light treatments other than the high light was evident from the clustering of all the genes that changed their expression under at least one condition, despite very different redox states of the PQ pool (Fig. 4). The similarities in the changes in gene expression seen with the treatments used implies that there is a similar mechanism or mechanisms regulating genes under these conditions, and the redox state of the PQ pool is apparently not one of them.

Second, the absence of genes that are consistently regulated according to reduction or oxidation of the PQ pool in PSI light and PSII* light, respectively, strongly suggests that the changes in nuclear gene expression are not dependent on the redox state of the PQ pool. This is in accordance with the results of a microarray experiment carried out using the whole genome of *Synechocystis* sp. 6803, in which only a few genes were found to follow the oxidation and reduction of the PQ pool (16).

Third, the expression of plant-specific *lhcb1* and -2 genes has frequently been suggested to be under regulation of the redox state of the PQ pool (12, 25, 53). According to this model, the *lhcb1* and -2 genes should be downregulated in PSII* light and upregulated in PSI light, which (under our experimental conditions) induced reduction and oxidation of the PQ pool, respectively. The microarray results however, showed that neither PSI light nor PSII* light noticeably affect the transcription of *lhcb* genes. Moreover, confirmation of three *lhcb1* and -2 genes with Northern blot analysis showed that these genes are downregulated in both PSI light- and high light-treated plants, where the PQ pool is oxidized and reduced (Fig. 7B), respectively. These results are in line with recent investigations on the regulation of *lhcb1* gene transcription in *Dunaliella tertiolecta* (8). Our results strongly support the notion that a mechanism(s) other than the PQ pool must be involved in the early regulation of the expression of *lhcb1* and

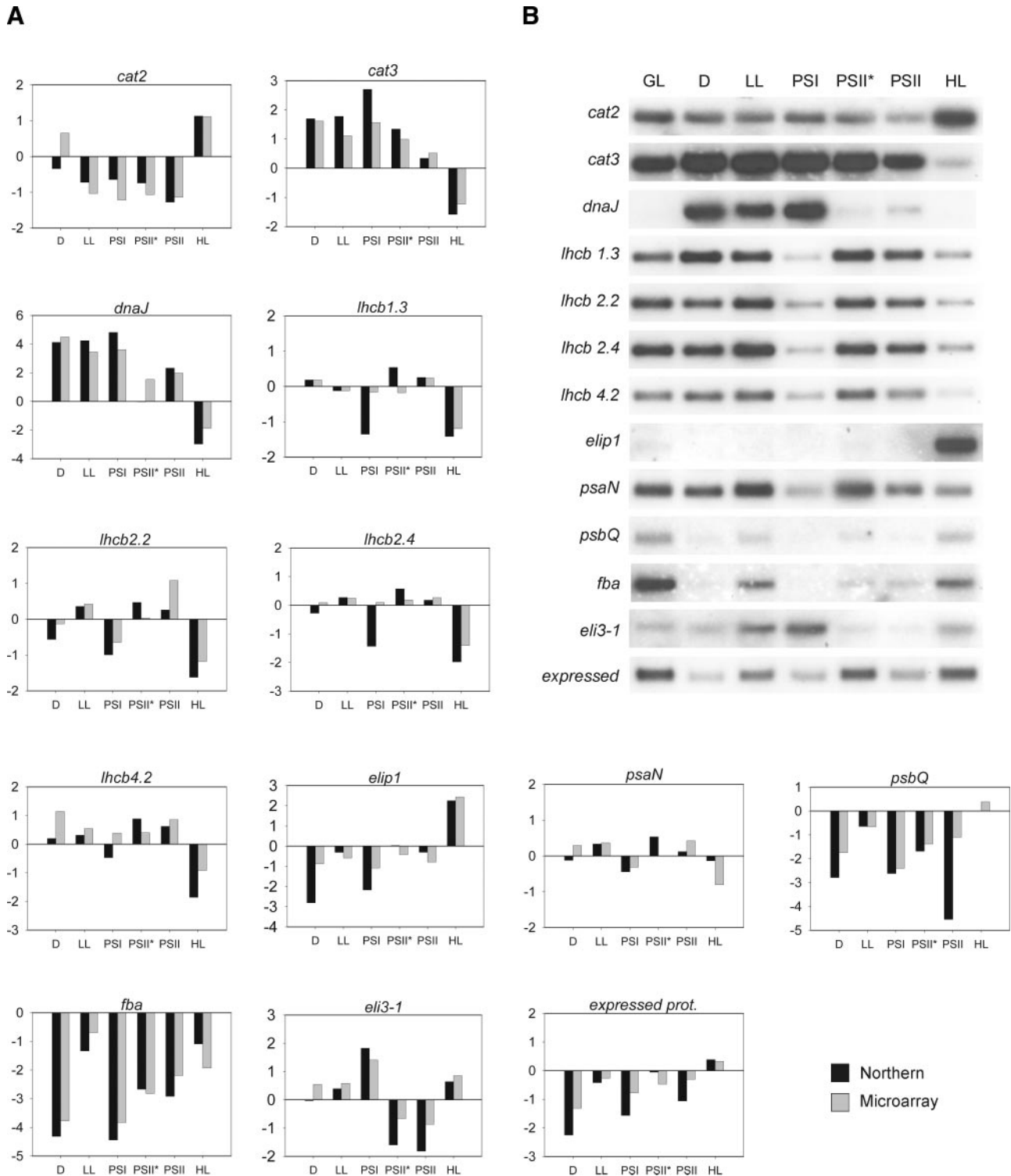


Fig. 7. Correlation of Northern blot analysis and microarray results. *A*: changes in gene expression analyzed from microarray and Northern results are expressed as log₂ values for the following selected genes, *cat2* (catalase 2, At4g35090), *cat3* (catalase 3, At1g20620), *dnaJ* (chloroplast-targeted chaperone protein, At1g80920), *lhcb1.3* (PSII light-harvesting complex gene, At1g29930), *lhcb2.2* (PSII light-harvesting complex gene, At2g05070), *lhcb2.4* (PSII light-harvesting complex gene, At3g27690), *lhcb4.2* (PSII light-harvesting complex gene, At3g08949), *elip1* (early light-induced protein, At3g22840), *psaN* (N subunit of photosystem I, At5g64040), *psbQ* (*psbQ* domain-containing gene, At1g14150), *fba* (fructose biphosphate aldolase, At4g26530), *eli3-1* (cinnamyl alcohol dehydrogenase, At4g37980), and *expressed* (expressed protein, At2g34860). *B*: Northern blot analysis of the same genes. Light conditions are as in Fig. 2.

-2 genes after changes in environmental conditions (8, 36). In fact, recent experiments have suggested that the plastoquinone regulation becomes operational only after long exposure of plants to contrasting environmental conditions (8, 13).

From the discussion above, it is clear that the changes in gene expression observed are largely independent of the redox state of the PQ pool. It is therefore highly unlikely that the redox state of the PQ pool as such would have a primary role in chloroplast-to-nucleus signaling in short-term experiments, as described here. On the other hand, it is becoming evident that other mechanisms such as the reduction state of stromal redox components, derivatives of CO₂ fixation, or the ATP/ADP ratio may be involved in chloroplast signaling (52). With the exception of high light, a common factor in all light treatments was a clearly reduced activity of NADP-MDH compared with growth light conditions, implying an oxidized stromal environment, particularly that of thioredoxins (Fig. 3A). We recognized a distinct set of nuclear genes, the expression of which responded to changes in light conditions similarly to that of NADP-MDH activity. Many of these gene products are associated with thylakoid membranes (e.g., Elip1, FtsH, PsbP domain, PsbQ, PsbQ domain-containing protein, PsbW, PsbT) or function in the Calvin cycle (e.g., fructose biphosphate aldolase, GapB, ribose-5-phosphate isomerase) or secondary metabolism (chalcone synthase). In addition to the ferredoxin-thioredoxin system, another important stromal redox pool is formed by reduced and oxidized glutathione. Reduced glutathione is capable of activating plastid transcription kinase and therefore acts as a significant regulator of plastid gene expression (4, 6). However, it has not been considered to regulate nuclear genes encoding chloroplast proteins (13). Indeed, the glutathione redox-mediated control of nuclear genes is probably limited to only stress defense genes (7), and these genes did not respond to our differential light treatments (Supplemental Table S1).

Although the general pattern of CO₂ fixation resembled that of the NADP-MDH activity, it is important to note that the PSII* light maintained nearly as high a steady-state CO₂ fixation rate as growth light. Comparison of overall gene expression profiles, both in terms of the number of genes changing their expression and in the magnitude of the changes (Fig. 4), with the CO₂ assimilation rates in different treatments revealed remarkable interrelationships. Indeed, the least deviation from growth light conditions was observed in PSII* light, followed by PSII light, low light, PSI light, and darkness, in order of decreasing CO₂ fixation by leaves under given conditions. High light that clearly enhanced CO₂ fixation resulted in opposite changes in the expression of most of the genes that responded to light treatments. Our results thus suggest that the initiation of CO₂ fixation and subsequent accumulation of metabolites when plants are exposed to light have an important role in orchestration of changes in nuclear gene expression.

The metabolic shift from utilization of the end products of photosynthesis to other energy reserves and metabolic pathways was observed as downregulation of the expression of Calvin cycle genes and glycolysis genes. As discussed above, the genes of several Calvin cycle enzymes seemed to respond to the thiol redox state of the stroma, whereas glycolysis genes more clearly followed the steady-state rates of leaf CO₂ fixation (Fig. 5). This difference was particularly evident when comparing the CO₂ fixation rates (Fig. 3), NADP-MDH acti-

vation (Fig. 3), and gene expression profiles (Fig. 5) between growth light-treated plants and PSII* light-treated plants. Several Calvin cycle genes and the NADP-MDH activity were downregulated to similar extents in PSII* light relative to growth light conditions, whereas the transcription levels of several glycolysis enzymes more closely followed the rates of CO₂ fixation, which did not differ much between these two light conditions. In addition, the trehalose-6-phosphate synthase (*tps8*) gene was strongly upregulated in darkness, low light, PSI light, and both PSII and PSII* light and was downregulated in high light. Trehalose is a low-abundance disaccharide molecule that can act as a metabolic or stress indicator in many organisms (15). The end product of trehalose phosphate synthase, trehalose-6-phosphate, has been shown to gate the carbon flux into glycolysis in *Arabidopsis* (43) and report cytosolic sucrose levels to plastids (21). The idea of metabolite-mediated regulation of this gene is strengthened by the negative correlation between its expression and CO₂ assimilation rates (Fig. 6). Such a signal(s) from chloroplasts or cytoplasm to nucleus cannot act simply by an on/off mechanism, since a comparison of the changes in gene expression and CO₂ assimilation rate inevitably shows a “dose effect” at transcript levels (Figs. 4–6). Changes in photosynthesis-derived metabolite levels acting as signals could be ideal in triggering the quantitative changes in gene expression observed. It is reasonable to deduce that the composition of sugar signaling molecules as well as of other metabolites was affected as a consequence of altered photosynthetic rates in different light treatments, implying that sugar signaling may be part of the signaling cascade from chloroplast to nucleus. This interpretation is supported by a recent report demonstrating similar regulation of a number of genes when the plants were exposed to light or fed with sugar in darkness (50).

Comparison of nuclear gene expression data with the functional and biochemical characteristics of photosynthesis in leaves treated under different light regimes has enabled us to make a rough distinction between genes that respond to signals originating from the stromal redox components on the reducing side of PSI, such as reduced ferredoxin and thioredoxins, and genes that respond to signals involving some photosynthesis-derived metabolites, or which may actually use the sugar signaling pathways at an early stage. This distinction will help in the search and identification of the various signaling components and signal transduction pathways that are apparently involved in the relay of information from chloroplasts to the nucleus. It should be kept in mind, however, that some photosynthetic genes have been shown to respond to both cellular sugar levels and plastid-derived redox signals (28), which suggests that the sugar and redox signaling pathways are likely to have significant cross talk with each other.

In conclusion, the redox poise of the photosynthetic electron transfer chain was found to be an important factor in controlling the expression of a number of nuclear genes. According to our data, the redox state of electron carriers on the reducing side of PSI is probably crucial in the early stages of chloroplast signaling, whereas the redox state of the PQ pool is only of minimal or no importance. We also hypothesize that the energy and metabolic status of the cell, depending on the steady-state CO₂ fixation rate, is a significant source of plastid signals for differential light regulation of nuclear genes in mature *Arabidopsis* leaves.

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