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Photosynthetic Electron Flow Regulates Transcription of the *psaB* Gene in Pea (*Pisum sativum* L.) Chloroplasts Through the Redox State of the Plastoquinone Pool

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Plants respond to changing light conditions by altering the stoichiometry between components of the photosynthetic electron transport chain of chloroplast thylakoids. We measured specific run-on transcription of the chloroplast genes *psaB*, *psbA* and *rbcL* in pea (*Pisum sativum* L.) seedlings grown under three different conditions of illumination: light selective for photosystem I (PSI-light); light selective for photosystem II (PSII-light); and a combination of PSI- and PSII-light (mixed light, ML). The transcriptional rate of the *psaB* gene increased under PSII-light and decreased under PSI-light, while the transcriptional rates of the *psbA* and *rbcL* genes were affected only in a non-specific way. Similar effects also occurred in plants grown under ML and switched to either PSI- or PSII-light for 4 h. Addition of the inhibitors of photosynthetic electron transport 3-(3,4 dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) influenced *psaB* transcription in isolated, illuminated chloroplasts: DCMU addition resulted in oxidation of the plastoquinone pool and decreased transcription of *psaB*; DBMIB addition resulted in reduction of the plastoquinone pool and increased transcription of *psaB*. The experimental results obtained in vivo and in vitro provide evidence for coupling between the redox state of plastoquinone and the rate of transcription of the *psaB* gene in pea.

Key words: Chloroplast — Photosynthesis — *Pisum sativum* L. — Plastoquinone — Redox — Transcription.

Abbreviations: CP47, chlorophyll-binding protein of 47 kDa relative moleular mass; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; D1, reaction centre protein of PSII (*psaB* gene product); F_o, chlorophyll fluorescence emission when all PSII traps for excitation energy are open; F_m, chlorophyll fluorescence emission when all PSII traps for excitation energy are closed; F_t, chlorophyll fluorescence emission at time *t*; LHCII, light-harvesting pigment-protein complex of PS II; PSI-light, light absorbed by PSI more than by PSII; PSII-light, light absorbed by PSII more than by PSI; PQ, plastoquinone; ML, mixed light (PSI-light and PSII-light together); Rubisco, ribulose-1,5-bisphosphate carboxylase-oxygenase; Q_A, primary plastoquinone electron acceptor of PSII; WL, white light.

Introduction

Plants and other photosynthetic organisms respond to changing light conditions in their natural habitats. At low light intensities, such responses tend to optimise photosynthetic efficiency, and they accomplish this by balancing the activities of components of their electron transport chains. Changes in light quality result in adjustment of photosystem stoichiometry (Chow et al. 1990, Kim et al. 1993, Murakami and Fujita 1993, Melis et al. 1996, Pfannschmidt et al. 1999a). Changes in light quality also affect the degree of oxidation-reduction of components in the electron transport chain, and changes of this nature are known to influence gene expression at various levels. Redox control of gene expression is thought to occur in eukaryotic green algae such as *Dunaliella* sp., where the expression of the nuclear *cab* genes coding for light harvesting complex II species (LHCIIs) is regulated by the redox state of an intersystem component (Maxwell et al. 1995), or more specifically by the redox state of the plastoquinone pool (PQ-pool) (Escoubas et al. 1995). Redox regulation of chloroplast gene expression has been demonstrated to occur at the post-transcriptional level. In isolated pea chloroplasts, the stability of *petB* mRNA, coding for the cyt b_6 protein, is controlled in vitro by redox poise (Alexciev and Tullberg 1997). Under oxidising conditions, the amounts of *petB*-containing message drastically decrease compared to control levels while reducing conditions leave the *petB* mRNA levels unchanged. Regulation of chloroplast gene expression by photosynthetic electron transport may be particularly important for *psbA*, the gene encoding the D1 reaction centre protein of photosystem II (PSII). In the unicellular green alga, *Chlamydomonas reinhardtii*, redox-dependent mechanisms affect both the splicing of *psbA* pre-mRNAs (Deshpande et al. 1997) and the translation of the mature *psbA* mRNA (Danon and Mayfield 1994, Kim and Mayfield 1997).

The expression of the majority of plant chloroplast-encoded genes has been thought to be controlled predominantly at the post-transcriptional and translational levels (Gruissem et al. 1988, Mullet 1988, Gruissem 1989, Link 1996, Stern et al. 1997), although transcriptional control is not excluded (Mullet and Klein 1987, Klein and Mullet 1990, Schrubar et al. 1990).

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One proposal for the function of chloroplast genomes is that they contain genes whose expression must be controlled by the redox state of electron carriers with which their gene products interact (Allen 1993a, Allen 1993b). Here we show that there is a direct link between photosynthetic electron flow and gene-specific control of transcription in pea chloroplasts. We have used both in vivo and in vitro experiments to show that photosynthetic electron flow regulates the transcription of the *psaB* gene in pea. This gene is part of the *psaA/B* operon of the chloroplast genome and codes for the photosystem I (PSI) reaction centre protein PsaB. Our results indicate that the redox state of the plastoquinone pool is the signal to which the *psaB* gene responds.

Materials and Methods

Plant material and growth conditions

Peas (*Pisum sativum* L. cv. Oregon sugar pod) were grown for 8 d at 20–22°C under different light conditions in a 16 h light/8 h dark regimen. Fluorescent strips (F 30W/GRO, Sylvania, Germany) covered with orange filter of half-maximal transmission at 560 nm (orange nr. 405, Strand Lighting, Isleworth, U.K.) supplied light stimulating mainly photosystem II (PSII-light). Light absorbed mainly by photosystem I (PSI-light) was produced by incandescent light bulbs (40 W) covered with red filter of half-maximal transmission at 650 nm (medium red 027, LEE Filters, Andover, U.K.). A combination of both light-sources provided "mixed light" (ML) illumination, used as a reference light (Deng et al. 1989). The spectral characteristics of the different light-sources were measured with a spectroradiometer (model 754-O-PMT, Optronic Laboratories inc., Orlando, FL, U.S.A.). Photon flux density of the PSII-light was maximally 11.02 μE m^{-2} s^{-1} as measured with a LI-COR LI-189 photometer (Lambda Instruments, Lincoln, NE, U.S.A.), and that of the PSI light source was 9.15 μE m^{-2} s^{-1}. A neutral density metal grid filter was used to reduce the photon flux density of the ML illumination to 9.40 μE m^{-2} s^{-1}.

Plants were grown under these three light conditions and used for chloroplast preparation. To prevent any variations in the data due to circadian periods (Millar et al. 1995, Oelmüller et al. 1995), leaves were harvested exactly 4 h after the dark period or at different timepoints thereafter in the case of the light-switch experiments.

Chloroplast isolation

Chloroplast isolation was based on the method of Walker (1971) using modifications previously described (Harrison and Allen 1992, Alexciev and Tullberg 1997). Chloroplast intactness was routinely monitored in the phase-contrast microscope, and chlorophyll content was measured according to Porra et al. (1989). Plastid number was determined by counting in a hemocytometer.

Fluorescence measurements

The response of ML-grown peas switched to either PSI- or PSII-light was monitored by fluorescence measurements using a PAM 101/103 Walz-apparatus (Heinz Walz GmbH, Effeltrich, Germany) (Schreiber et al. 1986). Four single leaves were exposed to saturating, actinic pulses of 1 s duration from a quartz-halogen light source. The response was recorded at a rate of 1 actinic pulse per min for 4 h. The magnitude, y, of the change in amplitude of the fluorescence signal upon addition of the actinic flash is given by: $y = 1 - (F_m - F_t) / (F_m - F_o)$.

Since saturating light completely reduces the plastoquinone pool for the duration of the pulse, y is a measure of the state of oxidation of the PQ-pool before the flash. The amplitude of the fluorescence trace of the WL-grown plants before the light-switch was used as reference.

Oxygen electrode measurements

Oxygen electrode measurements were carried out using a Rank Bros. electrode (Rank Brothers Ltd., Cambridge, U.K.) with a 1 ml reaction vessel. Measurements were carried out using chloroplasts containing 50 μg chlorophyll in a total volume of 500 μl. Chloroplasts were made to perform CO_2-dependent oxygen evolution by the presence of 10 mM $NaHCO_3$, 5 mM $Na_4P_2O_7$ and 1 mM ATP (Allen and Bennett 1981) in 500 μl total volume. Titrations were performed with the inhibitors of electron transport 3-(3,4 dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB). Both compounds were purchased from Sigma (Sigma Chemical Company, St. Louis, MO, U.S.A.). 100 μl chloroplast suspension containing 50 μg chlorophyll were pre-incubated on ice for 10 min in the presence of increasing concentrations of DCMU (0.125, 0.6, 1.0, 1.3 or 2.0 μM) or DBMIB (0.1, 0.5, 1.0 or 5.0 μM) before being added to 400 μl of reaction medium (grinding medium supplemented with 10 mM $NaHCO_3$, 5 mM $Na_4P_2O_7$ and 1 mM ATP) in the electrode cuvette, thus giving a 1 : 4 dilution of chloroplasts and inhibitors. Final ethanol concentrations in the pre-incubations did not exceed 0.25%, except in the case of 5 μM DBMIB, where the final ethanol concentration was 0.5%. As a control for these measurements, we used chloroplasts pretreated with the respective final concentration of ethanol. Light was provided by a slide projector fitted with a Philips 5E light bulb placed 5 cm away from the cuvette. The resulting oxygen evolution curves were recorded both onto a Macintosh computer via a Mac-Lab unit (MacLab/4e, AD Instruments Ltd., Hastings, U.K.) with Chart 3.3.5 software and onto an analogue chart recorder.

Plasmids and slot-blot filters

The following pBluescript plasmids containing barley chloroplast genes were used in our experiments: pBB22 (containing *psaB*), pBHE319 (*psbA*), pBPH134 (*rbcL*), and pBSS24 (*16S rDNA*).

The equivalent of 1 pmol of insert was blotted onto Biodyne A transfer membranes (Biosupport Division Pall Process Filtration, Portsmouth, U.K.) in a BIO-DOT SF slot-blot apparatus (Bio-Rad Laboratories, Hercules, CA, U.S.A.); 1 pmol of pBluescript was used as a negative control and for background correction of the hybridisation signals; 3.5 μg wheat germ tRNA (Sigma) was included to serve as an additional negative control for the hybridisation.

Run-on transcription experiments

Run-on chloroplast transcription was measured essentially as described for barley (Mullet and Klein 1987) and for spinach (Deng and Gruissem 1987) with minor modifications. All reactions were performed in a total volume of 100 μl with chloroplasts containing 10 μg chlorophyll. For adaptation and light-switch experiments, 20 μl of chloroplast suspension were lysed under a dim green safe light of 480 nm wavelength (green acetate foil No. 17632, Oskar Vangerow, Munich, Germany) in 55 μl H_2O prewarmed to 25°C and containing 0.5 mg ml^{-1} heparin. A reaction mixture (25 μl) was added such that the final concentrations in 100 μl assays were 30 mM KCl, 10 mM $MgCl_2$, 50 mM K-HEPES (pH 8.0), 125 μM each of ATP, CTP and GTP, 10 μM UTP and 100 μCi of [α-^{32}P]UTP (specific activity 800 Ci $mmol^{-1}$: Amersham International plc, Little Chalfont, U.K.). Transcription was allowed to proceed for 5 min at 25°C. The reaction was stopped by addition of 35 μl "stop-buffer" containing 200 mM Tris-Cl pH 8.5, 300 mM NaCl, 2 mM EDTA and 1% SDS. Samples were extracted with 1 vol. Phenol : chloroform (1 : 1, v/v), the organic phase was washed with 0.5 vol. TE-buffer (10 mM Tris-Cl pH 7.0, 1 mM EDTA), and the pooled aqueous phases were extracted once

with 1 vol. pure chloroform. Samples were precipitated overnight at 4°C with 1 vol. 5 M ammonium acetate and 2.5 vol. isopropanol; total nucleic acids were then pelleted at 16,000×*g* in a bench-top Eppendorf centrifuge and washed with 70% ethanol. The dried pellets were resuspended in DNase buffer (40 mM Tris-Cl pH 7.9, 10 mM NaCl, 6 mM $MgCl_2$ and 10 mM KCl) and treated with RNase-free DNase (Promega, Madison, WI, U.S.A.) for 20 min at 37°C. Suspensions were stored at 4°C until needed for hybridisation.

For inhibitor experiments, 20 µl chloroplasts were preincubated on ice for 10 min with 10% ethanol (as a control), or with equal volumes of 10% ethanol containing DCMU (giving final concentrations of 0.6, 1.0 and 2.0 µM) or DBMIB (giving final concentrations of 0.1, 1.0 and 5.0 µM). The final ethanol concentration per sample did not exceed 0.25% (0.5% in the case of the highest DBMIB concentration). The same quantity of inhibitor per µg chlorophyll was used in the run-on experiments as in the oxygen evolution experiments. Thereafter, chloroplasts were made to perform CO_2-dependent oxygen evolution by addition of 10 mM $NaHCO_3$, 5 mM $Na_4P_2O_7$ and 1 mM ATP prior to illumination for 7 min as described previously. Chloroplasts were then lysed and further subjected to run-on assays as described above. As in the oxygen electrode experiments, the additions made to the chloroplast suspension produced a 1 : 4 dilution of the chloroplasts and the inhibitors.

TCA-precipitations were carried out to determine total transcriptional activity and transcript abundance. Equal amounts (c.p.m.) of labelled transcripts were hybridised to each filter. All solutions used for RNA-work were pretreated with 0.1% diethyl pyrocarbonate.

Nucleic acid hybridisations

Hybridisations were performed in 50 ml Sarstedt (Sarstedt, Nümbrecht, Germany) tubes in 5 ml hybridisation buffer (50% formamide, 3× SSC (1× SSC = NaCl 0.3 M, Na-citrate 0.03 M), 5× Denhardt's solution (1× Denhardt's = 0.02% polyvinylpyrrolidone, 0.02% Ficoll 400, 0.02% bovine serum albumin), 0.08% SDS, 10 µg ml^{-1} denatured salmon sperm DNA). Prehybridisation was carried out for a minimum of 2 h at 45°C before addition of the run-on generated transcripts. Filters were hybridised for 16–20 h at 45°C, and then washed twice in 2× SSC/0.1% SDS for 5 min each time, then twice in 0.2× SSC/0.1% SDS for 15 min each time. Whenever required, additional washes in 0.1× SSC/0.1% SDS were carried out to reduce the background. All post-hybridisation washes were performed at 45°C. Following washing, filters were briefly dried, wrapped in plastic foil, and exposed in PhosphorImage cassettes (Molecular Dynamics, Sunnyvale, CA, U.S.A.). The resulting signals were quantified using ImageQuant software. Additional exposures were made using X-ray film (Hyperfilm-MP, Amersham), after which the bands were excised and the hybridisation signals again quantified using a scintillation counter (type LS 6000IC, Beckman Instruments, Fullerton, CA, U.S.A.).

Southern blot analysis of total DNA isolated from pea plants grown under the different light regimens and from plants subjected to a 4 h light-switch was carried out as described earlier (Deng and Gruissem 1987).

Results

Effects of light quality on the transcription of chloroplast-encoded photosynthesis genes

The quality and intensity of growth lights were chosen such that they favoured excitation of predominantly either photosystem I (PSI-light; Fig. 1a), or photosystem II (PSII-light; Fig. 1b). The light sources used produced functional light-1 and light-2 as shown by PQ-reduction-oxidation studies (Pfannschmidt et al. 1999b). Control plants received mixed light (ML) from both light sources (Fig. 1c), which is absorbed by both photosystems (Deng et al. 1989). Plants grown under ML or PSII-light show essentially the same leaf chlorophyll *a*/*b* ratio, while plants subjected to PSI-light illumination exhibit a decreased chlorophyll *a*/*b* ratio (Table 1).

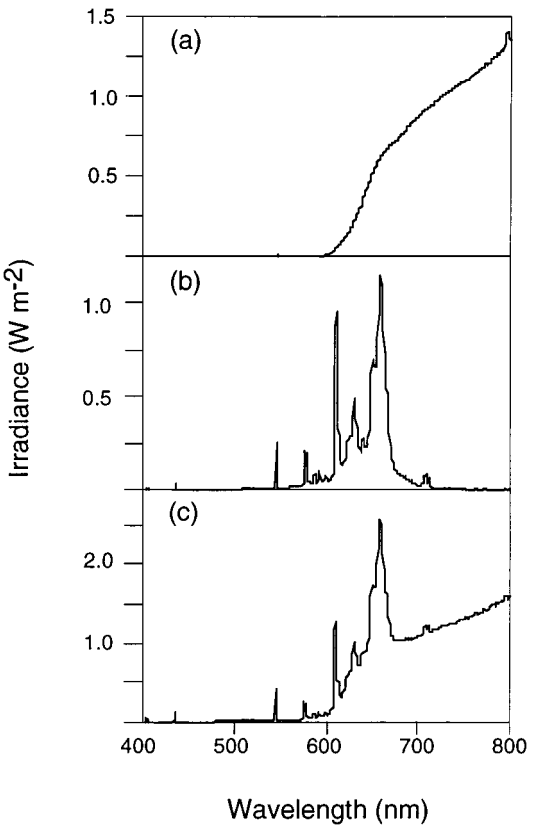

Fig. 1 Emission spectra of the light sources used. (a) PSI-light; (b) PSII-light and (c) mixed light (ML), control.

Pea plants were grown under lights of different spectral qualities for 8 d. We compared the transcriptional rates of several chloroplast-encoded photosynthesis genes after adaptation of the plants to the different light regimens. In order to measure the transcriptional activity of pea plastids, we adapted the chloroplast run-on assays developed for barley (Mullet and Klein 1987), and for spinach (Deng and Gruissem 1987). Transcriptional run-on experiments were carried out on the basis of equal amounts of chlorophyll. The results for the gene-specific transcription are shown in Fig. 2. The gene probes used in the slot-blot hybridisation were directed towards psaB, coding for the PSI reaction centre protein PsaB; psbA, coding for the D1 reaction centre protein of PSII; and rbcL, coding for the large subunit of ribulose-1,5-bisphosphate carboxylase oxygenase

Table 1 Changes in chlorophyll and transcriptional activity in pea plants grown in different light qualities.

	Chl *a*/*b* [a]	Chlorophyll content per plastid (ng)	Total transcriptional activity [b]
PSI-light	2.91 (±0.06)	2.32	0.75
Mixed light	3.02 (±0.03)	2.32	1.0
PSII-light	3.04 (±0.05)	2.22	0.53

[a] Calculated according to Porra et al. (1989). The values are the means (± SE) of 4–7 independent experiments.

[b] Measured as incorporation of [α-^{32}P]UTP into RNA, pmol/ 5×10^6 plastids/5 min. The values, in relative units to ML, represent the mean of two independent measurements.

(Rubisco). We also monitored the transcriptional rate of the gene for 16S rRNA as a representative of constitutively expressed genes of the chloroplast genetic machinery (Glick et al. 1986), and used it as an internal standard for quantification. Within the linear range of the hybridisation signal, psaB was the only gene analysed which exhibited opposing trends in transcriptional rates depending on light quality (Fig. 2b). In PSI-light grown plants, the transcriptional rate of the psaB gene was decreased by 45% of the value of the control, the corresponding transcriptional rate for ML-grown plants. When plants were grown under PSII-light, the relative transcriptional rate of psaB was increased by 119% of the value of the same control (Fig. 2b). In contrast with these results for psaB, the transcriptional rates for psbA were increased under both light regimens: by 12% under PSI-light, and 25% under PSII-light. Similar results were obtained for the transcriptional rates of the rbcL gene, which also increased under both light sources relative to ML values—by 16% under PSI-light, and 36% under PSII-light.

During the short incubation time of 5 min in our run-on assays, the steady-state levels of mRNA do not change significantly as a result of changed stability of the messages. In higher plant chloroplasts, the half-life of most mRNA species is in the range of h (Klaff and Gruissem 1991). We therefore had to make sure that the differences observed in the transcriptional rates of specific genes did not result from changed gene dosage of the template and/or changed overall transcriptional rates. Total DNA was isolated from pea plants grown under the different light qualities (PSI-light, PSII-light and ML) and also from plants whose light regimen had been changed (ML to PSI-light; ML to PSII-light) 4 h previously. Total DNA digested with *Eco*RI was analysed by Southern blotting, and no discernible differences in DNA quantities were detected (data not shown). This finding eliminates the possibility that the variations in the transcriptional rate of the psaB gene result from changed amounts of the DNA template. Alternatively, the alterations in transcriptional rates might result from non-specific changes in overall transcriptional activity. We analysed the incorporation of radioactive precursors into RNA by measuring the TCA-precipitable radioactivity of the run-on transcripts from isolated pea chloroplasts. The results presented in Table 1 show a decrease in total transcriptional activity in seedlings grown under PSI- or PSII-light, compared to the ML-control. This indicates that the variations in psaB transcription detected during adaptation to varying light regimens are specific and do not reflect a general trend of altered gene expression. This conclusion is also consistent with the relatively constant quantity of 16S rRNA seen in Fig. 2a.

Modulation of electron transport by changing light quality

Variations in light quality are accompanied by changes in the relative rates of electron transfer through the two photosystems. A key mediator in this process is the PQ-pool which transfers electrons from PSII to the cyt b_6/f complex. PSI oxidises PQ while PSII reduces it. We followed relative changes in oxidation-reduction of the PQ-pool by measuring the change in the amplitude of chlorophyll fluorescence emission by the sample leaf during a period of 4 h after light-switch from ML to either PSI- or PSII-light (Fig. 3). Transition from ML to PSI-light produced a 7% oxidation of the PQ-pool in a 40 min period compared to ML control plants. This degree of oxidation was maintained for an hour, then declined to 5% after 4 h. The opposite effect, with more rapid kinetics, was observed when plants grown under ML were switched to PSII-light: a maximum of 10% reduction of the PQ-pool was reached after 30 min. The pool is re-oxidised over time, but is still 5% more reduced than in the control plants after 4 h.

Having established that the redox state of the PQ-pool was affected by the light-switches, we examined whether these events influenced chloroplast transcription by performing run-on experiments on chloroplasts isolated from pea plants subjected to both kinds of light-switch. The plants switched to PSI-light were analysed after 40 min (corresponding to maximal oxidation of the PQ-pool as shown in Fig. 3) and 4 h. Measurement of the relative transcriptional rates (Fig. 4a) revealed an increase in transcription of the three genes 40 min after the plants were moved from ML to PSI-light (*psaB*: +16%, *psbA*: +39% and *rbcL*: +7%). After 4 h under the new light regimen, only the transcriptional rate of the *psaB* gene had decreased, by 20%, compared to the level of control plants (Fig. 4b). Although the *psbA* gene also exhibits a decreased tran-

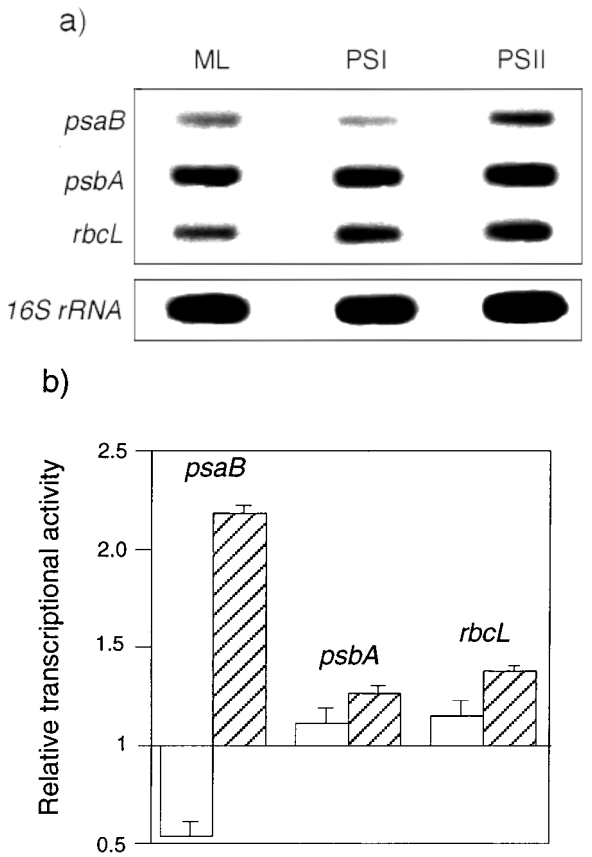

Fig. 2 Transcriptional activity of chloroplast genes in pea seedlings grown under different light regimens. Plants were grown for 8 d in ML, PSI- or PSII-light; chloroplasts were isolated and transcriptional rates analysed by run-on assays. (a) Run-on transcripts hybridised to membrane-bound gene-specific probes. (b) Histogram of the results from (a). Incorporation of [α-^{32}P]UTP into the run-on transcripts examined, as measured by the intensity of the signals, is expressed relative to the control incorporation in plastids isolated from ML-grown plants (arbitrarily set to 1). All values were normalised to the internal standard *16S rRNA*. Empty bars: plants grown under PSI-light; striped bars: plants grown under PSII-light. The results are the mean ± SE of two independent experiments.

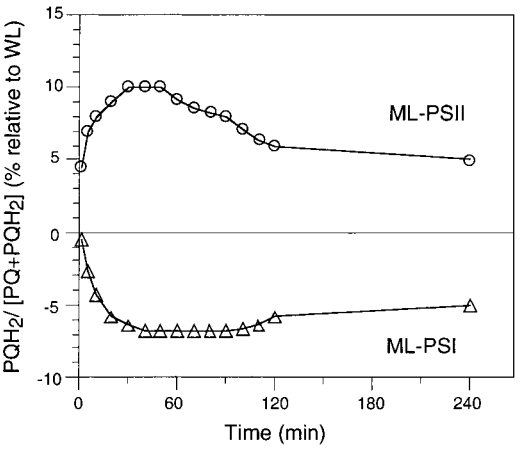

Fig. 3 Chlorophyll fluorescence emission following changes in light regimen. Plants grown for 8 d under ML were shifted for 4 h to either PSI- or PSII-light. Fluorescence was excited at 645 nm. The relative changes in fluorescence amplitude are compared to the traces of leaves kept in ML (control, 0). Triangles represent data points in the ML-PSI light-switch, and circles–data points in the ML-PSII light-switch. Results are representative of 3–4 independent measurements.

scriptional rate after 4 h, this is still 25% higher than measured in the control. Under these conditions, the transcriptional rate of the *rbcL* gene remained virtually unchanged even after 4 h of illumination with PSI-light.

For plants switched to PSII-light, chloroplasts were isolated after 30 min of illumination (corresponding to the maximal reduction of the PQ-pool under our protocol, as seen in Fig. 3) and after 4 h (Fig. 4c, d). Here again, on a short time-scale (30 min after the light-switch) the plastids respond non-specifically by decreasing the total transcriptional activity, as compared with ML-plants, thus the transcriptional rates of all three genes decrease (*psaB*: –20%, *psbA*: –13% and *rbcL*: –43%) (Fig. 4c). The run-on assays after 4 h show that the transcriptional rate of *psaB* increases by 41% when compared to the peas remaining under ML (Fig. 4d). The transcriptional rate of *psbA* also increases after 4 h of PSII-light illumination, by 34% compared to the ML-level. The transcriptional rate of *rbcL* remains low (–33% relative to the peas remaining in ML) even after illumination with PSII-light for 4 h (Fig. 4d).

In summary, the light-induced changes in the redox-state of the PQ-pool, as measured in vivo (Fig. 3), promote two opposing effects: changing the pool to a more oxidised state results in a general increase in transcriptional rates; conversely, a reduction of the pool causes a rapid decrease in transcriptional activity. When the plants have started adapting to the new light-conditions after 4 h, the gene-specific effects become discernible. The transcriptional rates of *psaB* and *psbA* in plants subjected to 4 h light quality switch (Fig. 4b, d) change in the same direction as in plants grown under a single, photosystem-specific light (Fig. 2). The results for *rbcL* do not show such an obvious correlation with the direction of change of transcriptional rate (Fig. 4d vs. Fig. 2b).

Effects of inhibitors of photosynthetic electron transport on the expression of the psaB, psbA and rbcL genes in isolated chloroplasts

By illuminating pea plants with light of different qualities, we were able to create imbalances in electron flow in a non-invasive manner inside the pea chloroplasts, as measured by changes in chlorophyll fluorescence emission (Fig. 3). To complement our studies, we simulated the effects of different light qualities by reversible manipulation of the redox status of the PQ-pool in vitro. To this end, we used the site-specific electron transport inhibitors DCMU, which binds to the Q_B-site of

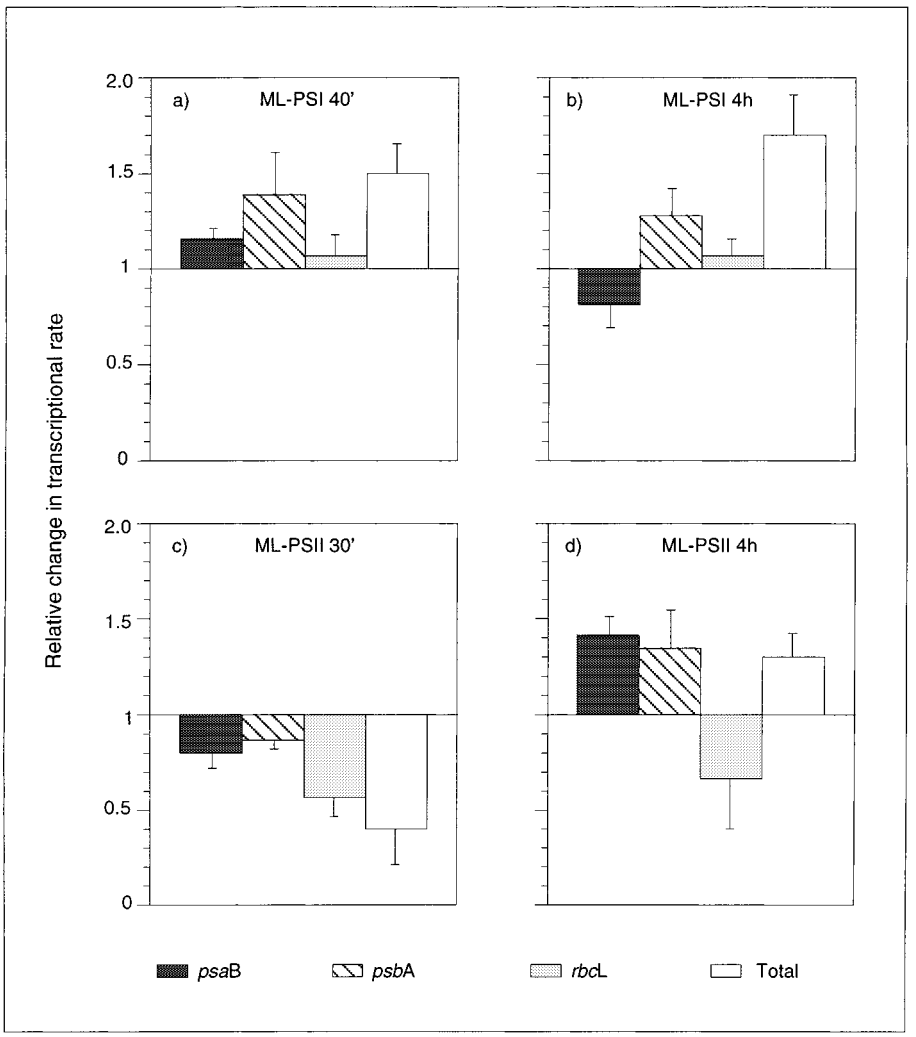

Fig. 4 Changes in relative transcriptional rates of chloroplast photosynthesis genes in response to altered light-regimen. Plants were grown as for Fig. 3 and chloroplasts were isolated after 40 min (a) and 4 h (b) following the switch ML-PSI, and after 30 min (c) and 4 h (d) following the switch ML-PSII. The gene-specific transcriptional rate was quantified as described for Fig. 2, and normalised to the values obtained from plants remaining in ML for each time-point. The initial transcriptional rate (at time-point 0) in ML-grown plants is arbitrarily set to 1 for each gene (*psaB*, shaded bars; *psbA*, diagonally striped bars and *rbcL*, dotted bars). For comparison, the total transcriptional activity in isolated chloroplasts from the different light-regimes (calculated as in Table 1) is given (empty bars). The results are the mean ± SE (n=3–4).

PSII (Trebst 1980), and DBMIB, which binds to the Q_O-site of the cytochrome b_6/f complex (Trebst 1980). DCMU inhibits electron transport from the Q_A-site of PSII into the PQ-pool, and addition of DCMU therefore results in oxidation of the PQ-pool, simulating the effect of PSI-light. DBMIB inhibits electron transport from the PQ-pool through the cyt b_6/f-complex. DBMIB addition therefore leads to reduction of the PQ-pool, thus simulating the effect of PSII-light. We chose to work with concentrations of inhibitors that give partial inhibition of oxygen evolution. In this way, we aimed to produce effects on electron transport of the same magnitude as those induced in vivo by changing light quality and also minimise side-effects on chloroplast metabolism.

Chloroplasts were prepared from ML grown peas, and CO_2-dependent oxygen evolution was measured using an oxygen electrode. Young pea chloroplasts (6–10 d old seedlings) have special requirements for CO_2-dependent oxygen evolution, because of the existence in the chloroplast envelope of an active adenine nucleotide transporter (Robinson 1977). Consequently, chloroplasts were supplemented with $NaHCO_3$, $Na_4P_2O_7$ and ATP (Allen and Bennett 1981). In a series of control experiments, the maximum rate of oxygen evolution measured was 37±2 μmol O_2 (mg Chl)$^{-1}$ h^{-1} (mean ± SE, n=9). To quantify the degree of inhibition of O_2-evolution, we used in-

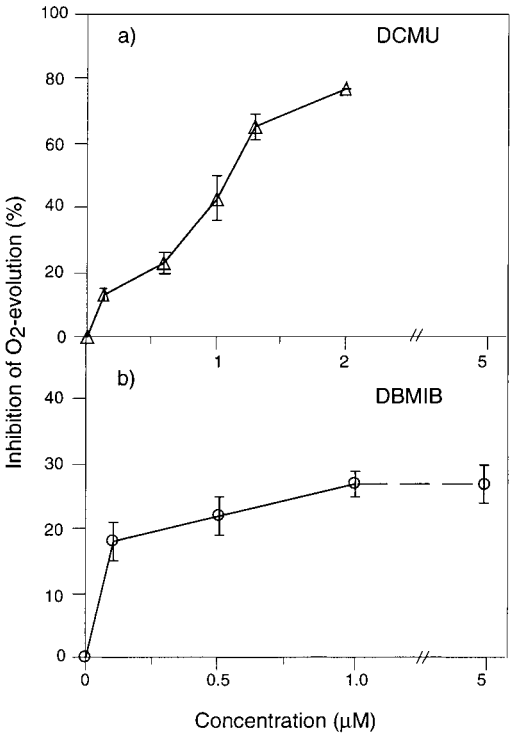

Fig. 5 Inhibition of CO_2-dependent O_2-evolution in intact pea chloroplasts. O_2-evolution was titrated with increasing concentrations of the photosynthetic electron transport inhibitors DCMU (a) and DBMIB (b). Each titration point is the mean ± SE (n=2–6), except 2 μM DCMU, which represents one measurement.

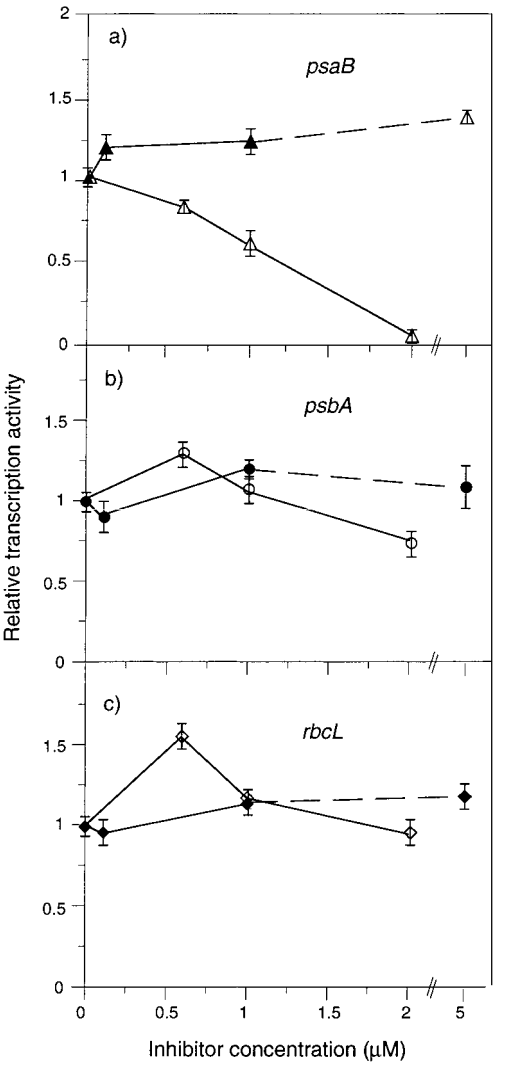

Fig. 6 Transcription of chloroplast photosynthesis genes in the presence of inhibitors of photosynthetic electron transport. Chloroplasts were isolated from ML-grown 8 day-old peas and pretreated with increasing concentrations of the electron transport inhibitors DCMU (open symbols) or DBMIB (filled symbols). After induction of CO_2-dependent O_2-evolution for 7 min, chloroplasts were diluted fourfold and the rate of run-on transcription was measured. The hybridisation signals of *psaB* (a), *psbA* (b) and *rbcL* (c) were normalised to *16S rRNA* for each titration point. The controls (with no inhibitors added) were arbitrarily set to 1. Each data point is the mean ± SE of two or three individual assays.

creasing concentrations of the two inhibitors. A 10 min preincubation of the intact chloroplasts with DBMIB on ice was necessary to obtain maximal inhibition with this inhibitor (data not shown). For consistency, a 10 min preincubation was also performed in the presence of DCMU (although the inhibitory effect of DCMU was found to be complete within s of mixing). Analysis of the two titration curves shown in Fig. 5 revealed that 0.6 μM DCMU or 1 μM DBMIB caused approximately 30% inhibition of oxygen evolution. Increasing concentrations of DBMIB up to 5 μM did not extend the inhibition beyond 30%. At higher concentrations, this compound binds not only to its primary site but also to the Q_B-site of PSII (Trebst 1980), thus mimicking the effect of DCMU and rendering interpretation of results difficult.

On the basis of this titration data from the electron transport inhibitors, we performed a titration of run-on transcription in intact chloroplasts. It is assumed that gene transcription is altered in response to changes in electron flow. The subsequent run-on transcription experiments therefore permit the assessment of inhibitor-induced changes in transcriptional rates of the genes of interest. As shown in Fig. 6a, DCMU decreased the transcriptional rate of *psaB*, which approached zero at 2 μM DCMU. DBMIB increased the transcriptional rate of the same gene up to a maximum of 30%. As can be seen in Fig. 6b, c, DCMU at 0.6 μM increases the transcriptional rate of both the *psb*A (by 25%) and the *rbcL* (by 60%) genes; higher concentrations caused a decrease in transcriptional rates. This in vitro effect resembles the behaviour of these two genes in vivo during the light-quality switch (Fig. 4). In the presence of increasing DBMIB concentrations, the transcriptional rates of *psbA* and *rbcL* remain largely unaltered compared to the control sample

of non-treated chloroplasts (Fig. 6b, c).

Discussion

The chloroplast is an organelle containing its own genome (reviewed by Sugiura 1992) coding for components of its genetic machinery, as well as some photosynthesis-related proteins. The chloroplast is thought to have originated as an endosymbiotic prokaryote, and in the course of evolution, most of the genes surviving from the ancestral endosymbiont have been transferred to the nucleus. Retention of some genes inside the chloroplast could be a consequence of the need for these genes to respond to the specialised redox chemistry being performed inside this organelle (Allen 1993a, Allen 1993b). In this context, it has been suggested that chloroplast gene expression is tightly coupled to photosynthetic electron transport, in a process involving the interplay of redox sensors and response regulators (Allen 1992, Allen 1993a, Allen 1993b, Allen et al. 1995, Race et al. 1999)

Light is an essential environmental factor in plant growth and in leaf and chloroplast development. In natural habitats, changes occur in both light quantity and quality and photosynthesis must be tuned to these changes if high efficiency is to be maintained. At the molecular level, this tuning involves genetic responses ensuring optimal photosystem stoichiometry according to the prevailing light conditions (Kim et al. 1993, Pfannschmidt et al. 1999a). Earlier studies on photosynthesis genes have shown that transcription of the PSII genes *psbA* and *psbB* (the latter coding for the CP47 protein) are largely unaffected by light quality or intensity (Glick et al. 1986, Deng et al. 1989, Tonkyn et al. 1992). Our results support these observations but demonstrate a redox effect on *psaB* transcription that provides further insight into the molecular mechanisms of light-dependent chloroplast gene regulation.

The rate-limiting step of the light-reactions of photosynthesis under normal light-conditions is electron transfer from the PQ-pool to plastocyanin via the cyt b_6/f complex (Junge 1977). The redox state of the PQ-pool is thought to regulate photosystem composition in cyanobacterial cells (Fujita et al. 1987) and plants (Allen 1995, Pfannschmidt et al. 1999a), and the transcription of *cab* genes, coding for light-harvesting complex species associated with PSII (LHCIIs), in the green alga *Dunaliella* (Escoubas et al. 1995, Maxwell et al. 1995). PQ redox state is also involved in the transcriptional regulation of the nuclear-encoded cytosolic ascorbate peroxidase genes in *Arabidopsis* (Karpinski et al. 1997). The redox state of the PQ-pool is known to govern the activity of a redox-dependent protein kinase (Allen et al. 1981) whose action, through phosphorylation of specific LHCII proteins under reducing conditions, results in redistribution of light energy between the two photosystems (Allen 1992). The PQ-pool is now also implicated in signalling changes in electron flow to the chloroplast genetic machinery: our results with pea plants subjected to changed excitation pressure by modulating light quality show that the chloroplast-encoded *psaB* gene is transcriptionally regulated by changes in the redox state of the PQ-pool, which in turn are caused by alterations in light quality. When the redox state of the PQ-pool is more oxidised as a result of the light-switch from ML to PSI, this is signalled to the *psaA/B* operon and the response is a decrease in its transcriptional rate. The opposite effect, of enhanced transcription, is observed when the PQ-pool becomes more reduced as a result of the light switch from ML to PSII. The specific genetic response requires about 4 h of changed illumination. The physiological relevance of such specific, slow responses has been shown for *Chlamydomonas* (Murakami et al. 1997) and higher plants (Kim et al. 1993). The rapid change in the redox status of the PQ-pool, maximal oxidation within 40 min of PSI light and maximal reduction within 30 min of PSII light under our experimental conditions, however, seems to have a non-specific effect on the general transcription in the chloroplast. This behaviour of the transcriptional apparatus towards instant light-quality changes might be a stress response with no immediate impact on the photosystem quantity and stoichiometry.

As the light-sources used in our experiments contain wavelengths absorbed by phytochrome, we considered the possibility that the observed effects could be mediated by phytochrome activation. However, this is unlikely because we obtained the same response by the *psaB* gene in the in vivo light-switch experiments (where phytochrome could conceivably play a role after 4 h of illumination) as in the in vitro inhibitor studies which take place under a green safe-light. Therefore, we conclude that the observed light-quality effects are mediated by changes in the PQ-pool rather than by activation of the phytochrome system.

A direct link between the PQ-pool of the thylakoid membranes and gene expression was established by the use of specific electron transport inhibitors. This in vitro approach enabled us to modulate the redox state of the PQ-pool by titration with DCMU or DBMIB. As anticipated, when photosynthetic electron transport was partially inhibited by DCMU, which promotes oxidation of the PQ-pool, just as PSI-light does, the transcription of the *psaB* gene was decreased by approximately the same degree as the inhibition of oxygen evolution in the system. Conversely, the partial reduction of the PQ-pool by DBMIB, mimicking PSII-light, led to enhanced transcriptional rates of the *psaB* gene, again by the same degree as the inhibition of oxygen evolution. In addition to these correlations within the inhibitor experiments, the close agreement between the in vivo (after 4 h light treatment) and in vitro results provides strong evidence for coupling of the transcriptional rate of *psaB* and photosynthetic electron flow through plastoquinone. These effects are achieved to a similar degree in both experimental systems despite differences in the time-scale. The apparenly slower effects measured in the in vivo system might be readily explained if one considers the lack of cytosolic components in the in vitro system capable of maintaining redox homeostasis upon fluctuations in light quality.

We also investigated transcription of the *psbA* gene, which did not respond here to the alteration in electron flow induced by the light-quality switch (Fig. 4). This result is in contrast with experiments with mustard seedlings that suggest that *psbA* transcription is also under plastoquinone redox regulatory control (Pfannschmidt et al. 1999a, Pfannschmidt et al. 1999b). The differences might reflect differences in the kinetics of short-term response as both plants grown under different light regimes adapt the transcription of their *psb*A gene in the same way. It has also been shown previously that *psbA* is regulated in a redox-dependent manner at the translational level in *C. reinhardtii* (Danon and Mayfield 1994). An RNA-activator complex interacts with the 5′-untranslated region of the message in a light-dependent manner, suggesting that the light signal is transduced by photosynthetic electron transport. The *psbA* gene has a relatively stable message, with a half-life of 40 h in barley (Mullet and Klein 1987), so new D1 protein (its translation product) can be produced from existing mRNA when required. Such translational control may be particularly appropriate for the D1 protein, as its location in the PSII reaction centre renders it highly susceptible to radical-induced damage and it therefore requires frequent replacement (Mattoo et al. 1984, Aro et al. 1993). The *psaB* gene is part of the *psaA-psaB-rps14* operon. The *psaB* message has a relatively low stability (6 h), while the stability of the PsaB protein in the PSI complex is much higher than that of D1, one possible reason being that the PSI complex is surrounded by oxygen-radical scavenging enzymes such as superoxide dismutase and ascorbate peroxidase. Furthermore, studies in *C. reinhardtii* have revealed a possible role for the PsaB protein as an anchor for the assembly of PSI (Stampacchia et al. 1997). All these considerations make the transcriptional regulation of *psaB* plausible: PsaB needs to be replaced seldom, therefore its message has a relatively low stability. However, as PsaB is an anchor of PSI, the rate of synthesis of its message needs to be altered quickly in response to persisting changes in the environment. The third gene studied, *rbcL*, shows no major alterations in transcriptional rates in response to the changes in electron flow. This expected result is in accordance with earlier studies from spinach (Deng et al. 1989). An explanation for the seeming discrepancy in the transcriptional rate of the *rbcL* gene under PSII-light acclimation and under the 4 h light-switch ML-PSII is that the *rbcL* gene needs a longer adaptation time to reach the transcriptional rates of the PSII-light grown plants.

A DNA-binding protein that interacts specifically with the *psaA-psaB-rps14* promoter has been isolated from spinach chloroplasts (Cheng et al. 1997). However, the respective promoter region in pea does not contain similar binding sites (results not presented) which makes the involvement of this particular protein in the light-quality regulation of pea *psaB* rather unlikely. Thus the details of the redox signalling mechanism of transcriptional regulation of *psaB* remain to be clarified. We predict that the affinity of a DNA-binding protein(s) for the *psaB* promoter will vary in response to changes in the redox state of the PQ-pool, and this interaction in turn switches the transcription of *psaB* on or off by allowing an RNA polymerase to bind or not to the promoter. The regulatory process could also involve thioredoxin, and possibly follow a mechanism related to that described earlier for the redox regulation of *psbA* translation in *C. reinhardtii* (Kim and Mayfield 1997) suggesting a whole cascade of redox and phosphorylation events. This could explain the lag-phase in the gene-specific responses upon light switch. The redox-regulated thylakoid protein kinase(s) (Allen 1992) might also be involved in signalling changes in the redox state of the PQ-pool to the chloroplast genetic machinery, perhaps via a protein kinase acting on a component of the chloroplast transcriptional apparatus (Baginsky et al. 1999).

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References

Alexciev, K. and Tullberg, A. (1997) Regulation of *petB* mRNA stability in pea chloroplasts by redox poise. *Physiol. Plant.* 99: 477–485.

Allen, J.F. (1992) Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta* 1098: 275–335.

Allen, J.F. (1993a) Control of gene expression by redox potential and the requirement for chloroplast and mitochondrial genomes. *J. Theor. Biol.* 165: 609–631.

Allen, J.F. (1993b) Redox control of gene expression and the function of chloroplast genomes—an hypothesis. *Photosynth. Res.* 36: 95–102.

Allen, J.F. (1995) Thylakoid protein phosphorylation, state 1-state 2 transitions, and photosystem stoichiometry adjustment: redox control at multiple levels of gene expression. *Physiol. Plant.* 93: 196–205.

Allen, J.F., Alexciev, K. and Håkansson, G. (1995) Photosynthesis. Regulation by redox signalling. *Curr. Biol.* 5: 869–862.

Allen, J.F. and Bennett, J. (1981) Photosynthetic protein phosphorylation in intact chloroplasts. *FEBS Lett.* 123: 67–70.

Allen, J.F., Bennett, J., Steinback, K.E. and Arntzen, C.J. (1981) Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. *Nature* 291: 25–29.

Aro, E.-M., Virgin, I. and Andersson, B. (1993) Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. *Biochim. Biophys. Acta* 1143: 113–134.

Baginsky, S., Tiller, K., Pfannschmidt, T. and Link, G. (1999). PTK, the chloroplast RNA polymerase associated protein kinase from mustard (*Sinapis alba*), mediates redox control of plastid in vitro transcription. *Plant Mol. Biol.* 39: 1013–1023.

Cheng, M.C., Wu, S.P., Chen, L.F.O. and Chen, S.C.G. (1997) Identification and purification of a spinach chloroplast DNA-binding protein that interacts specifically with the plastid *psa*A-*psa*B-*rps*14 promoter region. *Planta* 203: 373–380.

Chow, W.S., Melis, A. and Anderson, J.M. (1990) Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. *Proc. Natl Acad. Sci. USA* 87: 7502–7506.

Danon, A. and Mayfield, S.P. (1994) Light-regulated translation of chloroplast messenger RNAs through redox potential. *Science* 266: 1717–1719.

Deng, X.-W. and Gruissem, W. (1987) Control of plastid gene expression during development: the limited role of transcriptional regulation. *Cell* 49: 379–387.

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

Deshpande, N.N., Bao, Y.J. and Herrin, D.L. (1997) Evidence for light/redox-regulated splicing of *psb*A pre-RNAs in *Chlamydomonas* chloroplasts. *RNA – A Publication of the RNA Society* 3: 37–48.

Escoubas, J.M., Lomas, M., Laroche, J. and Falkowski, P.G. (1995) Light intensity regulation of *cab* gene transcription is signaled by the redox state of the plastoquinone pool. *Proc. Natl Acad. Sci. USA* 92: 10237–10241.

Fujita, Y., Murakami, A. and Ohki, K. (1987) Regulation of photosystem composition in the cyanobacterial photosynthetic system: the regulation occurs in response to the redox state of the electron pool located between the two photosystems. *Plant Cell Physiol.* 28: 283–292.

Glick, R.E., McCauley, S.W., Gruissem, W. and Melis, A. (1986) Light quality regulates expression of chloroplast genes and assembly of photosynthetic membrane complexes. *Proc. Natl Acad. Sci. USA* 83: 4287–4291.

Gruissem, W. (1989) Chloroplast gene expression: how plants turn their plastids on. *Cell* 56: 161–170.

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

Harrison, M.A. and Allen, J.F. (1992) Protein phosphorylation and Mg^{2+} influence light harvesting and electron transport in chloroplast thylakoid membrane material containing only the chlorophyll-*a*/*b*-binding light-harvesting complex of photosystem I and photosystem II. *Eur. J. Biochem.* 204: 1107–1114.

Junge, W. (1977) Physical aspects of light harvesting, electron transport and electrochemical potential generation in photosynthesis of green plants. *In* Encyclopaedia of Plant Physiology, New series 6. Edited by Trebst, A. and Avron, M. pp. 59–93. Springer-Verlag, Heidelberg.

Karpinski, S., Escobar, C., Karpinska, B., Creissen, G. and Mullineaux, P.M. (1997) Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in Arabidopsis during excess light stress. *Plant Cell* 9: 627–640.

Kim, J.H., Glick, R.E. and Melis, A. (1993) Dynamics of photosystem stoichiometry adjustment by light quality in chloroplasts. *Plant Physiol.* 102: 181–190.

Kim, J.M. and Mayfield, S.P. (1997) Protein disulfide isomerase as a regulator of chloroplast translational activation. *Science* 278: 1954–1957.

Klaff, P. and Gruissem, W. (1991) Changes in chloroplast messenger RNA stability during leaf development. *Plant Cell* 3: 517–530.

Klein, R.R. and Mullet, J.E. (1990) Light-induced transcription of chloroplast genes. *J. Biol. Chem.* 265: 1895–1902.

Link, G. (1996) Green life: control of chloroplast gene transcription. *Bioessays* 18: 465–471.

Mattoo, A.K., Hoffman-Falk, H., Marder, J.B. and Edelman, M. (1984) Regulation of protein metabolism: Coupling of photosynthetic electron transport to in vivo degradation of the rapidly metabolized 32-kilodalton protein of the chloroplast membranes. *Proc. Natl Acad. Sci. USA* 81: 1380–1384.

Maxwell, D.P., Laudenbach, D.E. and Huner, N.P.A. (1995) Redox regulation of light-harvesting complex II and *cab* mRNA abundance in *Dunaliella salina*. *Plant Physiol.* 109: 787–795.

Melis, A., Murakami, A., Nemson, J.A., Aizawa, K., Ohki, K. and Fujita, Y. (1996) Chromatic regulation in *Chlamydomonas reinhardtii* alters photosystem stoichiometry and improves the quantum efficiency of photosynthesis. *Photosynth. Res.* 47: 253–265.

Millar, A.J., Straume, M., Chory, J., Chua, N.H. and Kay, S.A. (1995) The regulation of circadian period by phototransduction pathways in *Arabidopsis*. *Science* 267: 1163–1166.

Mullet, J.E. (1988) Chloroplast development and gene expression. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 39: 475–502.

Mullet, J.E. (1993) Dynamic regulation of chloroplast transcription. *Plant Physiol.* 103: 309–313.

Mullet, J.E. and Klein, R.R. (1987) Transcription and RNA stability are important determinants of higher plant chloroplast RNA levels. *EMBO J.* 6: 1571–1580.

Murakami, A. and Fujita, Y. (1993) Regulation of stoichiometry between PSI and PSII in response to light regime for photosynthesis observed with *Synechocystis* PCC 6714: relationship between redox state of Cyt b_6/f complex and regulation of PSI formation. *Plant Cell Physiol.* 34: 1175–1180.

Murakami, A., Fujita, Y., Nemson, J.A. and Melis, A. (1997) Chromatic regulation in *Chlamydomonas reinhardtii*: time course of photosystem stoichiometry adjustment following a shift in growth light quality. *Plant Cell Physiol.* 38: 188–193.

Oelmüller, R., Schneiderbauer, A., Herrmann, R.G. and Kloppstech, K. (1995) The steady-state mRNA levels for thylakoid proteins exhibit coordinate diurnal regulation. *Mol. Gen. Genet.* 246: 478–484.

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

Pfannschmidt, T., Nilsson, A., Tullberg, A., Link, G. and Allen, J.F. (1999b) Direct transcriptional control of the chloroplast genes *psbA* and *psaAB* adjust photosynthesis to light energy distribution in plants. *IUBMB Life* 48: 271–276.

Porra, R.J., Thompson, W.A. and Kriedemann, P.E. (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta* 975: 384–394.

Race, H.L., Herrmann, R.G. and Martin, W. (1999) Why have organelles retained genomes? *Trends Genet.* 15: 364–370.

Robinson, S.P. (1977) Inhibition of CO_2 fixation by adenosine 5′-diphosphate and the role of phosphate transport in isolated pea chloroplasts. *Arch. Biochem. Biophys.* 184: 546–554.

Schreiber, U., Schliwa, U. and Bilger, W. (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.* 10: 51–62.

Schrubar, H., Wanner, G. and Westhoff, P. (1990) Transcriptional control of plastid gene expression in greening *Sorghum* seedlings. *Planta* 183: 101–111.

Stampacchia, O., Girard-Bascou, J., Zanasco, J.L., Zerges, W., Bennoun, P. and Rochaix, J.-D. (1997) A nuclear-encoded function essential for translation of the chloroplast *psa*B mRNA in *Chlamydomonas*. *Plant Cell* 9: 773–782.

Stern, D.B., Higgs, D.C. and Yang, J.J. (1997) Transcription and translation in chloroplasts. *Trends Plant Sci.* 2: 308–315.

Sugiura, M. (1992) The chloroplast genome. *Plant Mol. Biol.* 19: 149–168.

Tonkyn, J.C., Deng, X.-W. and Gruissem, W. (1992) Regulation of plastid gene expression during photooxidative stress. *Plant Physiol.* 99: 1406–1415.

Trebst, A. (1980) Inhibitors in electron flow: tools for the functional and structural localization of carriers and energy conservation sites. *Methods Enzymol.* 69: 675–715.

Walker, D.A. (1971) Chloroplast (and grana): aqueous (including high carbon fixation ability). *Methods Enzymol.* 23A: 211–220.

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