Planta (1995) 197:553–562

Planta

Dynamic responses of photosystem II and phycobilisomes to changing light in the cyanobacterium *Synechococcus* sp. PCC 7942

Adrian K. Clarke, Douglas Campbell, Petter Gustafsson, Gunnar Öquist

Department of Plant Physiology, University of Umeå, S-901 87 Umeå, Sweden

Received: 9 May 1995 / Accepted: 13 July 1995

Abstract. We have examined the molecular and photosynthetic responses of a planktonic cyanobacterium to shifts in light intensity over periods up to one generation (7 h). *Synechococcus* sp. PCC 7942 possesses two functionally distinct forms of the D1 protein, D1:1 and D1:2. Photosystem II (PSII) centers containing D1:1 are less efficient and more susceptible to photoinhibition than are centers containing D1:2. Under 50 μmol photons · m^{-2} · s^{-1}, PSII centers contain D1:1, but upon shifts to higher light (200 to 1000 μmol photons · m^{-2} · s^{-1}), D1:1 is rapidly replaced by D1:2, with the rate of interchange dependent on the magnitude of the light shift. This interchange is readily reversed when cells are returned to 50 μmol photons · m^{-2} · s^{-1}. If, however, incubation under 200 μmol photons · m^{-2} · s^{-1} is extended, D1:1 content recovers and by 3 h after the light shift D1:1 once again predominates. Oxygen evolution and chlorophyll (Chl) fluorescence measurements spanning the light shift and D1 interchanges showed an initial inhibition of photosynthesis at 200 μmol photons · m^{-2} · s^{-1}, which correlates with a proportional loss of total D1 protein and a cessation of growth. This was followed by recovery in photosynthesis and growth as the maximum level of D1:2 is reached after 2 h at 200 μmol photons · m^{-2} · s^{-1}. Thereafter, photosynthesis steadily declines with the loss of D1:2 and the return of the less-efficient D1:1. During the D1:1/D1:2 interchanges, no significant change occurs in the level of phycocyanin (PC) and Chl *a*, nor of the phycobilisome rod linkers. Nevertheless, the initial PC/Chl *a* ratio strongly influences the magnitude of photoinhibition and recovery during the light shifts. In *Synechococcus sp.* PCC 7942, the PC/Chl *a* ratio responds only slowly to light intensity or quality, while the rapid but transient interchange between D1:1 and D1:2 modulates PSII activity to limit damage upon exposure to excess light.

Key words: Acclimation–D1 turnover–Photosystem II–Phycobilisome–*Synechococcus* sp. PCC 7942–Variable light

Introduction

Cyanobacteria are prokaryotes that share with plants the capacity for oxygenic photosynthesis. These photobionts contain similar thylakoid membranes with multi-protein complexes that use light energy to drive electron transfer, with concomitant production of NADPH and ATP. Photosystem II (PSII) houses the key contituents necessary for the light-driven oxidation of water and primary charge separation. The primary redox components are bound to two structurally similar proteins, D1 and D2, which form a heterodimer. One important distinction between cyanobacteria and chloroplasts, however, is the light-harvesting antennae associated with PSII. In cyanobacteria, these consist of several different pigmented phycobiliproteins arranged in macro-molecular complexes, the phycobilisomes, which are attached to the surface of the membrane. In contrast, plants have an array of integral membrane proteins that bind both chlorophyll (Chl) *a* and *b*. In both plants and cyanobacteria, the cellular content of light-harvesting complexes is under light regulation, although the cyanobacterial phycobilisome content also responds strongly to other factors such as nitrogen and carbon dioxide supply (Grossman et al. 1993; Reuter and Müller 1993; Tandeau de Marsac and Houmard 1993).

Loss of photosynthetic activity after absorption of excessive light energy, or photoinhibition, is common to all plants and cyanobacteria (Samuelsson et al. 1985, 1987; Lönneborg et al. 1988; Krupa et al. 1990). Photoinhibition results under conditions where light capture exceeds energy dissipation, as in shifts to increased light intensi-

Abbreviations: Chl=chlorophyll; F'_M=maximum Chl fluorescence in light-adapted state; F'_v-variable Chl fluorescence in light-adapted state; PC-phycocyanin; PPFD=photosynthetic photon flux density; q_p=photochemical quenching

Correspondence to: A.K. Clarke; Fax: 46 (90) 166676;
E-mail: adrian.clarke@plantphys.umu.se

ty, but also under moderate light if excess light-harvesting antennae are present or if low temperature limits energy dissipation (Huner et al. 1993). The site most susceptible to photoinhibition is the reaction center of PSII, although which of the key reactions become primarily inactivated *in vivo* has yet to be resolved. Photoinhibition *in vitro*, however, can occur after primary charge separation if electron donation to the oxidized reaction-center Chl P_{680}^+ is blocked, or alternatively by over-reduction of the primary quinone acceptor of PSII, Q_A, on the acceptor side of PSII (De Las Rivas et al. 1992). Both forms of inhibition result in the eventual degradation of the D1 protein (Shipton and Barber 1991; De Las Rivas et al. 1992; Vass et al. 1992).

The D1 protein of all plants is encoded by a single active *psbA* gene situated on the chloroplast genome. In contrast, cyanobacteria possess small *psbA* multi-gene families (Curtis and Haselkorn 1984; Mulligan et al. 1984; Golden et al. 1986; Jansson et al. 1987; Gingrich et al. 1988; Bouyoub et al. 1993). The unicellular cyanobacterium *Synechococcus* sp. PCC 7942 possesses three active *psbA* genes (Golden et al.1986); the *psbAI* gene codes for the D1 form 1 (D1:1) protein while both *psbAII* and *psbAIII* code for D1 form 2 (D1:2). D1:1 and D1:2 differ in only 25 of the total 360 amino acids, 12 of which are in the first 16 amino acids and 7 within the putative trans-membrane helices II and III (Golden et al. 1986).

The *psbA* genes in *Synechococcus* sp. PCC 7942 are differentially expressed over a range of photosynthetic photon flux densities (PPFDs). The *psbAI* gene is active primarily under low light, comprising around 94% of the total *psbA* mRNA (Golden et al. 1986). With increasing light, steady-state *psbAI* expression significantly decreases, while the combined expression of the *psbAII/III* genes rises proportionally (Schaefer and Golden 1989). Similarly, when cells grown at 125 μmol photons · m^{-2} · s^{-1} are shifted to 500 μmol photons · m^{-2} · m^{-1}, *psbAI* expression decreases by around 70%, and the degradation rate of *psbAI* mRNA increases while the combined expression of *psbAII/III* increases 500% (Bustos et al. 1990). Prolonged exposure to 500 μmol photons · m^{-2} · s^{-1} leads to the reappearance of *psbAI* message after 3 h, along with a recovery in *psbAI* mRNA stability, but during this period the level of *psbAII/III* mRNA also remains high (Kulkarni and Golden 1994).

In recent studies, we found that *Synechococcus* sp. PCC 7942 cells grown at 50 μmol photons · m^{-2} · s^{-1} respond to a sudden shift to 500 μmol photons · m^{-2} · s^{-1} by rapidly replacing the normally predominant D1:1 polypeptide with D1:2. This interchange between D1 forms is essential for limiting the extent of photoinhibition of photosynthesis resulting from the high light exposure, since inactivation mutants lacking D1:2 are very susceptible to photoinhibition (Krupa et al. 1990), even if the D1:1 form is overexpressed from an artificial DNA construct (data not shown). The D1:1/D1:2 interchange reverses fully once cells are returned to 50 μmol photons · m^{-2} · s^{-1}, allowing for a rapid return to normal photosynthetic activity (Clarke et al. 1993a). Using specific *psbA* inactivation mutants (Golden et al. 1986), we found that PSII reaction centers containing D1:2 were somewhat less susceptible to photoinhibition (Krupa et al. 1990) and more photochemically efficient than those containing D1:1 (Clarke et al. 1993b).

To date, nothing is known regarding the biological role of D1 interchange in cyanobacteria. To place this intriguing regulatory phenomenon in a functional context, we directly compared changes in D1 content to shifts in photosynthetic activity and pigment composition during acclimation to increased light over one generation. We show that in *Synechococcus* sp. PCC 7942 coordinated changes in D1 protein composition generate a rapid modulation of PSII properties in response to excess light, while the light-harvesting antennae are more stable in the face of short-term fluctuations in light.

Materials and methods

Cell material and growth conditions. Synechococcus sp. PCC 7942 was grown in BG-11 inorganic medium (Rippka et al. 1979) supplementally buffered with 10 mM 3-(N-morpholino)propanesulfonic acid, pH 7.5, at 37° C under a continuous light of 50 μmol photons · m^{-2} · s^{-1} in batch cultures as detailed previously (Clarke et al. 1993a). Cultures were bubbled with 5% CO_2 in air, except for the 'ambient' treatment of Fig. 7, which was bubbled with water-saturated air. For cells grown under blue light, a cellulose acetate filter blocked transmission from 550 to 650 nm. Cells in linear growth were used for all experiments. For construction of growth curves and calculation of pigment content, whole cell spectra were measured from 400 to 750 nm and phycocyanin (PC) and Chl contents calculated according to Myers et al. (1980).

High light and CO_2 treatments. For the short-term high light treatments, cells were harvested by centrifugation at 4000 · *g* for 5 min and resuspended in fresh growth medium containing 10 mM $NaHCO_3$ at a Chl concentration of 5 μg · mL^{-1}. Treatments were carried out in 50-mL transparent tubes bubbled with air in a water bath at 37° C. The light source was a halogen lamp power star HQI-IS 400 W; Osram, Berlin, Germany. The incident light was measured inside the tube using a GaAsP diode (Model G1125-02; Hamamatsu, Herrsching, Germany) sensitive to white light only and recorded on a voltameter (Model 192; Keithley, München, Germany), calibrated against a quantum sensor (Li-Cor 185A; Li-Cor, Inc., Lincoln, Neb., USA) with the desired light obtained using neutral density filters. Cells were incubated for 30 min at 50 μmol photons · m^{-2} · s^{-1}. Mild and severe photoinhibitory treatments were carried out for 2 h at PPFDs of 200 and 1000 μmol · m^{-2} · s^{-1}, respectively. The subsequent recovery period was at a PPFD of 50 μmol · m^{-2} · s^{-1} for a further 2 h. Controls were kept at a PPFD of 50 μmol · m^{-2} · s^{-1} for the complete 4 h. Samples for protein isolation were taken at the beginning of the treatments and every 30 min thereafter.

For the prolonged high light treatment, cells grown to a Chl concentration of about 2 μg · mL^{-1} in flat flasks with a culture depth of 1 cm were shifted directly from 50 to 200 μmol photons · m^{-2} · s^{-1} in the same growth chamber and maintained under identical CO_2 and temperature conditions. The PPFD was measured on a quantum sensor (Li-Cor 185A). Samples for spectroscopy, photosynthetic measurements and protein isolation were taken just prior to (0 h) and during (0.5, 1–7 h) the high light treatment.

Photosynthetic measurements. Chl *a* fluorescence induction was measured using a pulse amplitude modulated fluorometer (PAM; Walz, Effeltrich, Germany) as described in detail elsewhere (Clarke et al. 1993b; Campbell et al. 1995). A PAM-compatible system of cuvette, magnetic stirrer, oxygen electrode and Bjork-

man-type actinic lamp were used for the simultaneous measurement of fluorescence and oxygen evolution (Hansatech, King's Lynn, U.K.). Culture samples were transferred directly from the growth flask to the cuvette and dark-adapted for 5–7 min, allowing measurement of dark respiration. During this time' whole-cell spectra were taken for the estimation of pigment content and cell number. Minimal fluorescence (F_o) was measured using the modulated measuring beam at 0.14 µmol photons · m^{-2} · s^{-1}, this PPFD did not alter the rate of dark respiration. The actinic light provided 50 or 200 µmol photons · m^{-2} · s^{-1} to the sample and light-stimulated oxygen evolution was measured. Maximum fluorescence in the dark-adapted ($F_M$dark) and light-adapted ($F_{M'}$) states were measured before or after addition of the actinic light using 1-s pulses of saturating white light (8000 µmol photons · m^{-2} · s^{-1}) to close all reaction centers and drive photochemical quenching to zero. True maximum fluorescence was measured by adding 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) to the sample which eliminates photochemical quenching and allows non-photochemical quenching to collapse over a period of minutes. The cells did not become CO_2-limited during the measurement, as addition of 10 mM NaH_2CO_3 had no effect on the oxygen evolution nor the fluorescence signal (data not shown). The parameters F_o (initial Chl fluorescence), F_o (initial Chl fluorescence in light-adapted state), F_M (maximal Chl fluorescence) F'_M (maximal Chl fluorescence in light-adapted state) and F_s (steady/state Chl fluorescence under actinic light PPFD) were used to calculate photochemical (q_p) quenching of fluorescence, which measures the proportion of open PSII centers (van Kooten and Snel 1990); and the efficiency of excitation energy capture by open PSII reaction centers (F'_v/F'_M; Genty et al. 1989). See Miller et al. (1991), and Clarke et al. (1993b) for detailed discussions and empirical justifications of the application of these parameters to cyanobacteria.

Immuno-blot analysis. Total cellular proteins were extracted by centrifuging samples (1 mL) at 14 000 · *g* for 30 s and then resuspending the resulting cell pellet in 120 µL of 100 mM Tris-HCl (pH 8.6), 150 mM sucrose, 30 mM dithiothreitol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride and 2% lithium dodecyl sulphate (LiDS). Cell were disrupted by 15–30 s of continuous sonication (Model B15 Sonifer Branson, Danbury, Conn., USA) and proteins solubilized by heating at 75° C for 5 min. Insoluble material was removed by centrifugation at 14 000 · *g* for 5 min. Supernatants were removed and stored at –20° C to await Chl determination and electrophoresis.

Protein samples containing equivalent amounts of Chl (0.5–1.5 µg) were separated on linear 15% LiDS-polyacrylamide gels, transferred electrophoretically to nitrocellulose followed by immuno-blot analysis according to Clarke and Critchley (1992) with detection using the ECL chemiluminescent kit (Amersham Braunschweig, Germany). The polyclonal antibodies against D1:1 and D1:2 are completely form-specific and show no detectable cross-reactivity, as shown by Clarke et al. (1993a) and Soitamo et al. (1994) using inactivation mutant strains which express only D1:1 or D1:2. The specificities of the polyclonal antibodies against total D1 and phycobilisome have been characterized by Tyystjärvi et al. (1994) and Bhalerao et al. (1994), respectively. Density scanning of films from the immuno-blot exposures was carried out using the ImageMaster Desktop Scanner and software (Pharmacia LKB, Uppsala, Sweden) following the manufacturer's recommendations.

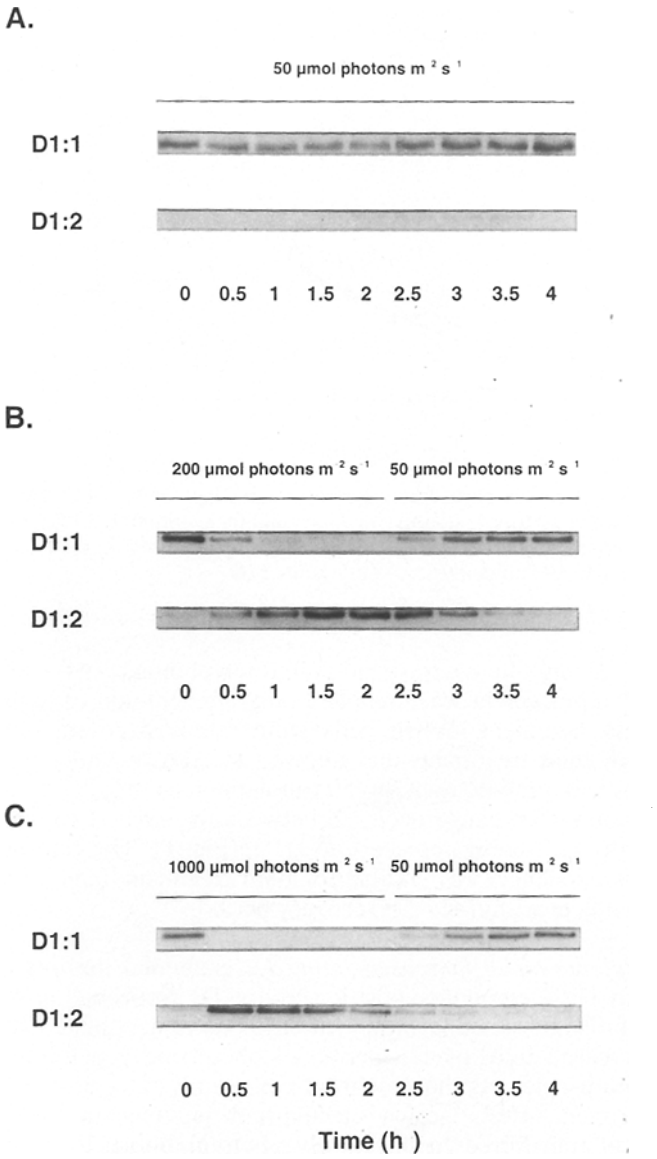

Fig. 1A–C Immuno-blot detection of D1:1 and D1:2 during different light treatments in *Synechococcus* sp. PCC 7942. Cellular proteins were extracted every 30 min from cells exposed to the following light regimes: **A** 50 µmol photons · m^{-2} · s^{-1} for 4 h (control); **B** 200 µmol photons · m^{-2} · s^{-1} for 2 h (mild photoinhibition) followed by 50 µmol photons · m^{-2} · s^{-1} for 2 h (recovery); **C** 1000 µmol photons · m^{-2} · s^{-1} for 2 h (severe photoinhibition) followed by 50 µmol photons · m^{-2} · s^{-1} for 2 h (recovery). Proteins were separated by LiDS-polyacrylamide gel electrophoresis on the basis of equal Chl content (1.5 µg per lane). D1:1 and D1:2 proteins were detected by immuno-blot analysis using specific polyclonal antibodies. All treatments were repeated in three separate experiments, of which a representative is shown

Results

Rate of D1 exchange. The D1:1 and D1:2 protein contents of *Synechococcus* sp. PCC 7942 cells exposed to three different light treatments are shown in Fig 1. Under the moderate growth light of 50 µmol photons · m^{-2} · s^{-1}, D1:1 was the predominant D1 form making up over 95% of the total D1 protein (Fig. 1A). When cells were exposed to mild (200 µmol photons · m^{-2} · s^{-1}, Fig 1B) or severe (1000 µmol photons · m^{-2} · s^{-1}, Fig. 1C) photoinhibitory light, the D1:1 protein was rapidly replaced with the alternate D1:2, with the rate of exchange dependent upon the PPFD. At 1000 µmol photons · m^{-2} · s^{-1}, complete replacement of D1:1 with D1:2 occurred with-

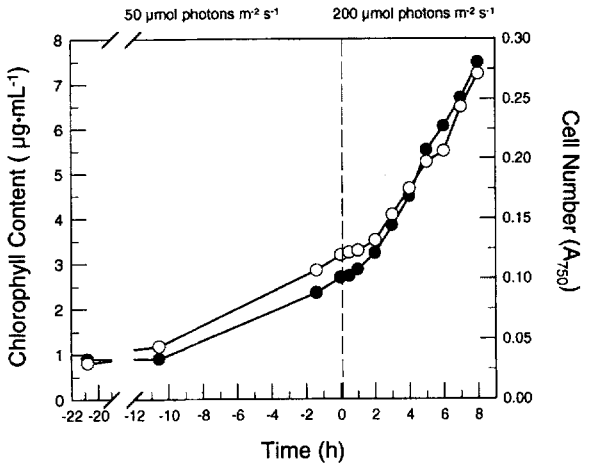

Fig. 2 Growth curves for *Synechococcus* sp. PCC 7942 cells grown at 50 µmol photons · m^{-2} · s^{-1} and then shifted to 200 µmol photons · m^{-2} · s^{-1} for 8 h. Cell growth was measured on the basis of either Chl content (○) or cell number (●)

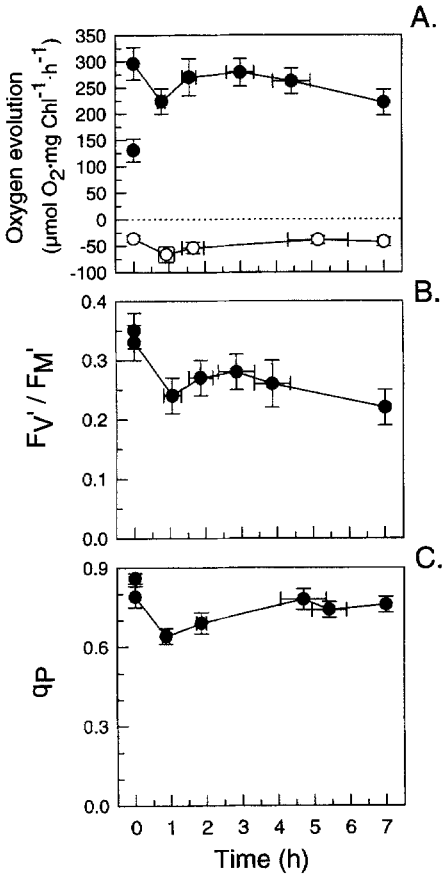

Fig. 3A–C Photosynthetic activities in *Synechococcus* sp. PCC 7942 cells following shifts to increased light. Cells grown at 50 µmol photons · m^{-2} · s^{-1} were shifted to 200 µmol photons · m^{-2} · s^{-1} for 8 h. **A** Oxygen evolution (●) and dark respiration (○) as mesured by µmol O_2 · (mg Chl)$^{-1}$ · h^{-1}. **B** Efficiency of excitation-energy capture by open PSII reaction centers (F'_V/F'_M); **C** Photochemical quenching (q_P). Results were obtained from seven replicates. The initial values for each individual replicate, measured at 50 and 200 µmol photons · m^{-2} · s^{-1} are plotted. Subsequent values are averages of measures at 200 µmol photons · m^{-2} · s^{-1}. To fully represent the data, we have presented error bars (±SE) in the *y* dimension to indicate the variation in the extent of changes, and error bars (±SE) in the *x* dimension to indicate variability in the timing of the changes

in 30 min. In contrast, at 200 µmol photons · m^{-2} · s^{-1} D1 replacement was complete only after 90 min of high light treatment. When cells were removed from both high light treatments and allowed to recover under the original growth light of 50 µmol photons · m^{-2} · s^{-1}, a second interchange occurred between the two D1 forms, with D1:2 being replaced by D1:1 (Fig. 1). The rate of this exchange was similar for both treatments and was complete within the 2-h recovery period.

Acclimation to increased light. We examined the interplay between photosynthetic activity, D1 forms and phycobilisome antenna organization as cells acclimated to increased light over a period of about one generation. Figure 2 depicts the growth of cells during a typical experiment. Cells grown at 50 µmol photons · m^{-2} · s^{-1} were transferred to the mildly photoinhibitory light of 200 µmol photons · m^{-2} · s^{-1} and their growth, photosynthetic performance and cellular content of pigments, D1 proteins and phycobiliproteins were followed for 8 h. Prior to the light shift, cells were growing with a generation time of around 6 h. The shift to the higher light caused a cessation in growth during the first hour, as measured by both cell number (A_{750}) and Chl content. Growth then resumed at a rate nearly twice that observed in cells growing at 50 µmol photons · m^{-2} · s^{-1} (Fig. 2). Cells harvested from this culture were used for the representative examples presented in Fig. 4 and 5.

Photosynthetic activity. Figure 3 shows average oxygen evolution and Chl *a* fluorescence parameters from cultures measured over 7 h following the shift from 50 to 200 µmol photons · m^{-2} · s^{-1}. Each of seven replicate cultures was measured at 0 h at both 50 and 200 µmol photons · m^{-2} · s^{-1}, to show the instantaneous effect of the light shift. Subsequent measures were under 200 µmol photons · m^{-2} · s^{-1} at 0.5, 1, 2, 3, 4, 5, 6 and 7 h after the shift (along with sampling for pigment, growth and protein analysis). Each parameter showed a characteristic pattern, with moderate variability between replicates in both the extent and timing of changes. For each replicate, each parameter was plotted over time, and the value and timing were determined for: the time-zero measurements at 50 and 200 µmol photons · m^{-2} · s^{-1}; the maximum initial drop after the shift; the beginning of recovery; maximum recovery; start of final decline (if any); and the final value at 7 h. Figure 3 presents the average value and average timing of each of these key points.

Oxygen evolution at 50 µmol photons · m^{-2} · s^{-1} was 130 µmol O_2 · mg Chl^{-1} · h^{-1} (SE±22), and increased over two fold upon the shift to 200 µmol photons · m^{-2} · s^{-1}. Within 1 h after the light shift, oxygen evolution declined by around 25% but then partially recovered (Fig. 3A). From 3 h onwards, the oxygen evolution rate again

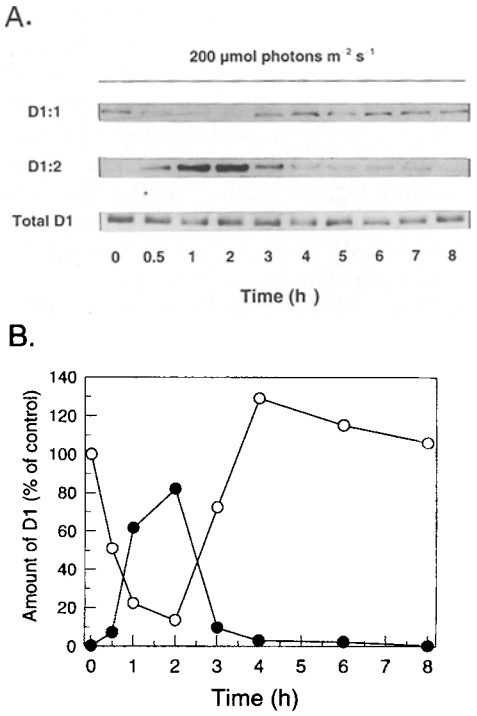

Fig. 4A, B D1 proteins in *Synechococcus* sp. PCC 7942 cells following a prolonged shift to increased light. Cells grown at 50 µmol photons · m^{-2} · s^{-1} were shifted to 200 µmol photons · m^{-2} · s^{-1} for 8 h. **A** Immuno-blot detection of D1 proteins. Cellular proteins were extracted before (0 h) and during the high light treatment (0.5–8 h). Proteins were separated by LiDS-PAGE on the basic of equal Chl content (0.5 µg per lane), with D1:1, D1:2 and total D1 detected on replicate blots using specific polyclonal antibodies. **B** Quantification of D1:1 and D1:2 during the high light treatment. D1:1 (○) is presented as a percentage of the control, where D1:1 contributed 100% of total D1 at 50 µmol photons · m^{-2} · s^{-1}. The D1:2 (●) content was normalized to the maximum level found 2 h after the light shift, when D1:2 contributed 92% of the total D1 protein, which had reached 106% of the initial control level

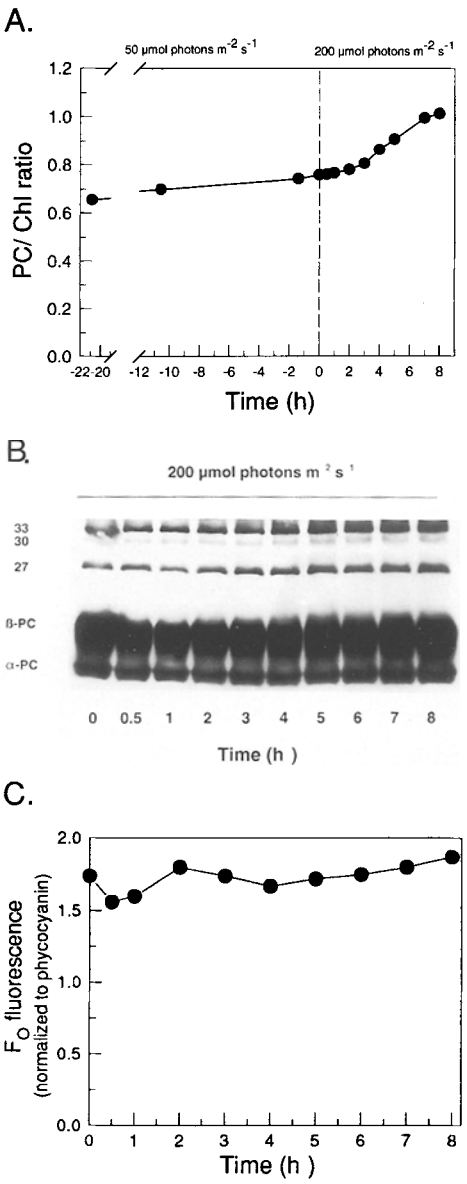

Fig. 5A–C Phycocyanin to Chl ratio and organization of phycobilisomes in *Synechococcus* sp. PCC 7942 cells following a prolonged shift to increased light. **A** Phycocyanin to Chl ratio calculated from whole-cell spectra. **B** Detection of phycobilisome proteins by immuno-blotting using a polyclonal antibody directed against purified whole phycobilisomes. **C** Minimal fluorescence (F_o) normalized to PC content

declined, but remained higher than the initial rate at 50 µmol photons · m^{-2} · s^{-1}. Respiration initially increased following the light shift, as previously observed in *Synechococcus* sp. PCC 7942 (Shyam et al. 1993). Afterwards, the respiratory rate declined slightly and then remained steady throughout the remaining high light treatment, at a rate that was significantly higher than the control. This pattern of oxygen evolution accurately reflects the activity in the culture vessel since the light in the measuring cuvette was equivalent in intensity and quality to the high growth light. As the cells grew during the experiment, around one-third of the drop in oxygen evolution from 5 to 8 h was caused by increased self-shading, effectively decreasing the light.

The photochemical efficiency of PSII (F'_V/F'_M) after the shift to 200 µmol photons · m^{-2} · s^{-1} followed a pattern similar to that for oxygen evolution (Fig. 3B). In the first hour, F'_V/F'_M dropped but recovered partially from 1 to 3 h; F'_V/F'_M then declined from about 3 h onwards. The redox state of the quinone acceptor Q_A (q_P), which reflects the proportion of open PSII reaction centers, also dropped following the light shift but then recovered almost completely, although more slowly than oxygen evolution or F'_V/F'_M (Fig. 3C). Interestingly, q_P remained relatively constant after the initial drop and recovery despite decreasing photosynthesis as shown by both oxygen evolution and Chl fluorescence.

Composition of D1. Upon the shift to 200 µmol photons · m^{-2} · s^{-1}, D1:1 was rapidly replaced by D1:2 (Fig. 4A), with the total amount of D1 protein decreasing by around 30% during this period when growth stopped. The rate of D1:1 loss over the first hour was identical to

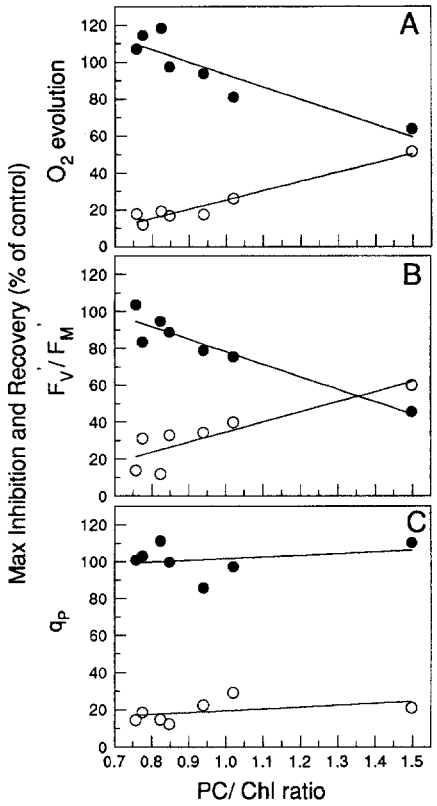

Fig. 6A–C Phycocyanin to Chl ratios predict photoinhibition and recovery of *Synechococcus* sp. PCC 7942 cells after shifts to increased light. For seven independent treatments, plots of oxygen evolution, F_V'/F_M', and q_P were constructed similar to those of Fig. 3. The maximum initial drop and then the maximum subsequent recovery for each parameter was calculated as the percentage of the time 0 h control value. These percentage maximum declines and recoveries are plotted versus the initial PC/Chl ratio of the corresponding cultures, and fitted with a linear regression line. **A** Inhibition (○, R=0.97, slope=50.4) and recovery (●, R=0.91, slope=–67.6) of oxygen evolution *vs.* PC content. **B** Inhibition (○, R=0.87, slope=54.8) and recovery (●, R=0.95, slope=–67.9) of photochemical efficiency of open PSII centers (F_V'/F_M') vs. PC. **C** Decline (○, R=0.44, slope=10.0) and recovery (●, R=0.27, slope=9.0) of photochemical quenching (q_P) vs. PC content

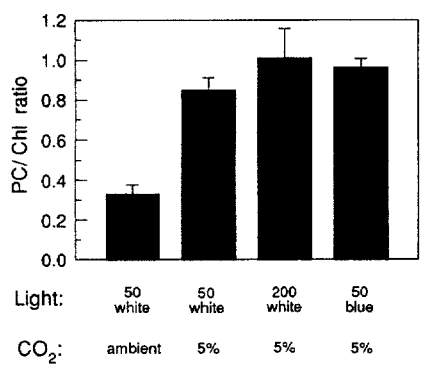

Fig. 7 Light, CO_2 and PC to Chl *a* ratio. *Synechococcus* sp. PCC 7942 cells were grown under the following conditions: 50 µmol photons · m^{-2} · s^{-1} white light, ambient CO_2; 50 µmol photons · m^{-2} · s^{-1} white light, 5% CO_2; shift from 50 to 200 µmol photons · m^{-2} · s^{-1} white light for 8–14 h, 5% CO_2; 50 µmol photons · m^{-2} · s^{-1} blue light, 5% CO_2. Values represent averages ±SE (n=3–5)

the degradation rate of D1:1 previously determined at 200 µmol photons · m^{-2} · s^{-1} using an inhibitor of protein synthesis (Clarke et al. 1993b), indicating an almost immediate cessation in D1:1 synthesis upon the shift to high light. After 2 h, the total level of D1 protein had returned to its control value and about 90% of the D1 protein was D1:2 (Fig. 4B). Between 2 to 3 h after the shift to 200 µmol photons · m^{-2} · s^{-1}, however, the two D1 forms once again exchanged so that D1:1 was again the predominant D1 form and remained so for the final 5 h. Concomitantly, the level of D1:2 dropped to less than 10% of total D1 protein and was almost completely absent by the end of the high light treatment. Similar to the initial rapid loss of D1:1, the rate of D1:2 loss between 2 and 3 h was identical to the measured rate of D1:2 degradation at 200 µmol photons · m^{-2} · s^{-1} (Clarke et al. 1993b). This indicates an abrupt cessation of D1:2 synthesis after about 2 h at high light.

Phycobilisome composition. During the two D1:1/D1:2 interchanges (0–3 h), there was no significant change in either the proportion of PC to Chl (Fig. 5A), or in the level of PC apoproteins (α- and β-PC) and rod-linker polypeptides of the phycobilisome (27, 30 and 33 kDa; Fig. 5B). This also indicates that the PSI to PSII ratio remained unchanged during the D1:1/D1:2 exchanges, a finding supported by electron paramagnetic resonance (EPR) measurements (data not shown). Afterwards, however, the PC/Chl *a* (PC/Chl) ratio rose, with a corresponding rise in the level of the phycobilisome proteins after 3 h at 200 µmol photons · m^{-2} · s^{-1}. This increase in cellular phycobilisome content occurred even though the light intensity remained at or near photoinhibitory levels throughout this period. These new phycobilisomes were not uncoupled from the reaction centers since minimal fluorescence (F_o) per PC did not increase over the course of the experiment (Fig. 5C), whereas uncoupling of phycobilisomes would have been detected by an increased fluorescence yield (Murata and Fork 1975; data not shown).

Plasticity of high-light response. Seven replicates of this high-light-shift experiment and several other related experiments all showed the same pattern of responses of D1 composition, photosynthetic activity and pigment composition. However, the magnitude of the initial inhibition and subsequent recovery of photosynthesis (Fig. 3) and the final ratio of D1:1/D1:2 (Fig. 4) varied between replicates. This variability was partly explained by the influence of the initial PC/Chl ratio on the responses to increased light. In Fig. 6, we have plotted the maximum initial photoinhibition and subsequent recovery observed in cells with different initial PC/Chl ratios. A direct correlation between the PC/Chl ratio and the extent of photoinhibition was observed as measured by both oxygen evolution and F_V'/F_M' (Fig. 6A and B, respectively). Conversely, the greatest recovery from photoinhibition was observed in cells with the lowest PC/Chl ratio. Since maximum photoinhibition and recovery are expressed as percentages of the initial control values, the slopes are directly comparable and are identi-

cal for the plots of photoinhibition of oxygen and F'_V/F'_M versus PC content, as are the slopes of the recovery of these parameters versus PC. In contrast, changes in the redox state of PSII (q_P) were unaffected by the variations in PC/Chl ratios (Fig. 6C), and recovery of q_P after the light shift generally reached initial control levels over the full range of PC/Chl.

Effectors of pigment composition. Figure 6 demonstrates the importance of the pre-shift PC/Chl ratio in determining the susceptibility of *Synechococcus* sp. PCC 7942 cells to photoinhibition. However, over one generation of growth at increased light the cells did not reduce their cellular PC content, which in fact tended to increase over the treatment period (Fig. 5). Thus, we investigated the relative influences of growth factors on the proportion of PC to Chl (reviewed in Reuter and Muller 1993). The results in Figure 7 show that when cultures were grown at 50 μmol · m^{-2} · s^{-1} PPFD, bubbling with 5% CO_2 more than doubled the PC/Chl ratio (>0.9) when compared to cells grown under ambient CO_2, with a PC/Chl ratio of 0.4. The shift from 50 to 200 μmol photons · m^{-2} · s^{-1} for 8–14 h, with continuous bubbling of 5% CO_2 gave a further slight increase (PC/Chl of 1.0). Preferential excitation of Chl failed to significantly change the PC/Chl ratio in cultures bubbled with CO_2, despite using a filter which specifically blocks transmission of the light absorbed by PC (550–650 nm). Thus, in this strain, CO_2 supply has a stronger influence on phycobilisome content than does light intensity or light quality (Muller et al. 1993).

Discussion

Light dependence of D1 interchange

The normally predominant form of D1 (D1:1) in *Synechococcus* sp. PCC 7942 is completely replaced with the alternate D1 form (D1:2) when cells are shifted to increased light, with the rate of interchange dependent on the light intensity. We showed previously that the rate of D1:1 loss during the exchange with D1:2 at 500 μmol photons · m^{-2} · s^{-1} was identical to the degradation rate for D1:1 (Clarke et al. 1993a). Similarly, the degradation rate of D1:1 at 200 and 1000 μmol photons · m^{-2} · s^{-1} (Clarke et al. 1993b; and data not shown) corresponds to the rate of D1:1 loss at the two PPFDs used in this study (Fig. 1). These results indicate that the synthesis of D1:1 ceases almost immediately when cells are exposed to light of 200 μmol photons · m^{-2} · s^{-1} and above.

When cells are re-exposed to the original growth light (50 μmol photons · m^{-2} · s^{-1}), the interchange reverses so that D1:1 again becomes the predominant D1 form. Interestingly, the rate at which D1:2 is lost when cells are shifted back to 50 μmol photons · m^{-2} · s^{-1} is significantly faster than expected from the degradation rate of D1:2 previously measured at 50 μmol photons · m^{-2} · s^{-1} (Clarke et al. 1993b). During recovery, however, it is likely that the faster rate of D1:2 degradation at PPFDs above 200 μmol · m^{-2} · s^{-1} does not immediately slow down to the steady-state level measured at 50 μmol photons · m^{-2} · s^{-1}. This possible continued high rate of D1:2 degradation during the initial stages of the recovery period may help explain the rapid exchange of D1:2 for D1:1 that occurs in association with a rapid induction and repression of *psbAI* and *psbAII/III* expression, respectively.

Phases of high-light acclimation. To place D1 exchange in the overall context of acclimation to changing light, we examined the changes in growth, PSII composition and function and phycobilisome content during a shift to increased light, prolonged over one generation of growth. A relatively mild shift from 50 to 200 μmol photons · m^{-2} · s^{-1} resulted in definite but limited loss of photosynthetic activity, and was chosen as an ecologically relevant treatment that avoided senescent changes resulting from extreme high-light stress. We observed at least three distinct phases as *Synechococcus* sp. PCC 7942 acclimated to the higher light.

The first two phases can be designated the photoinhibitory and regeneration phases which occur within 2 to 3 h after the shift from low to high light. In the first hour at 200 μmol photons · m^{-2} · s^{-1}, cell growth stopped, there was loss in total D1 protein and in photosynthetic activity. This correlation between nett loss of D1 protein and photoinhibition of photosynthesis has been previously observed in cyanobacteria (Clarke et al. 1993a), where the increased rate of D1 degradation presumably exceeds the rate of D1 synthesis. Such a clear correlation between D1 loss and photoinhibition does not always hold for the chloroplasts of higher plants (Chow et al. 1989; Ottander et al. 1993). During the loss in D1 and photosynthesis, there was a rapid removal of D1:1 and synthesis of D1:2, resulting in an almost complete interchange between the two D1 forms. Throughout this dramatic change in D1 composition and photosynthetic activity, there was little change in the abundance or organization of the phycobilisomes, as shown by constant rod/linker composition and PC/Chl ratio. Furthermore, there was no significant accumulation of uncoupled phycobilisomes, or changes in the ratio of PSI to PSII.

After 2 h of high light, the exchange between the two D1 forms was almost complete. Concomitantly, growth was restored at a rate significantly faster than before the shift, and PSII activity largely recovered. This shows that the rapid induction of *psbAII/III* expression and D1:2 synthesis enables additional synthesis of D1 protein to compensate for increased D1 degradation at high light (Clarke et al. 1993b). A parallel process also occurs for the other PSII reaction-center protein, D2, where expression of the constitutive *psbDI* gene is supplemented under high light with induction of the *psbDII* gene which codes an identical D2 protein (Golden et al. 1989; Bustos and Golden 1992), presumably to compensate for an increased rate of D2 degradation. Overall, by supplementing the synthesis of D1 and D2 at high light, the severity of damage to PSII may be minimized enabling a more rapid recovery.

In contrast to the supplemental expression of identical D2 proteins under high light, the D1:1 protein is actively

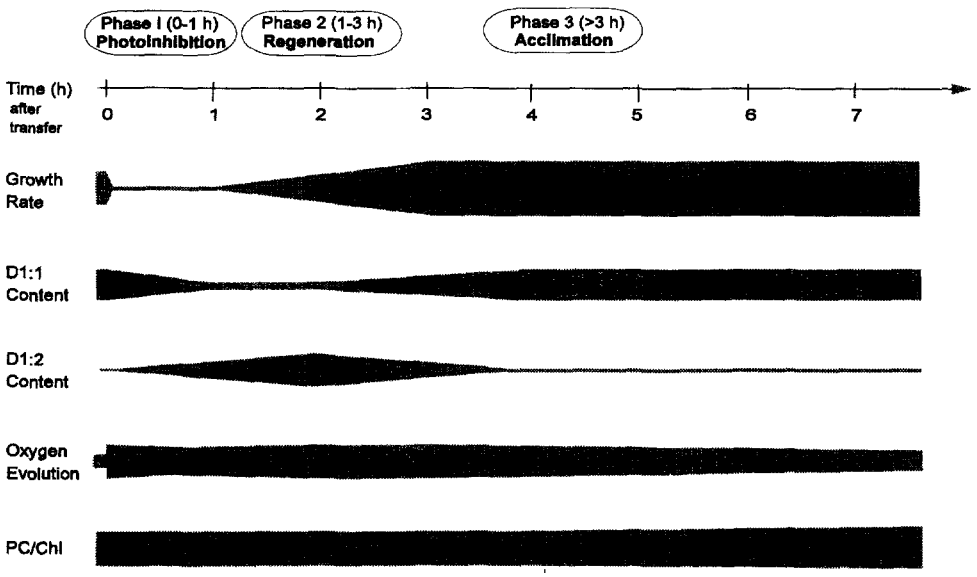

Fig. 8 Photoinhibition and acclimative changes of *Synechococcus* sp. PCC 7942 following a light shift from 50 to 200 μmol photons · m^{-2} · s^{-1}. Thickness of *horizontal bars* represents changes relative to the values of parameters at time zero

replaced by D1:2 upon exposure of cells to high light. We have previously shown that PSII reaction centers containing D1:2 have a higher photochemical efficiency (25%) than PSII reaction centers containing D1:1, and also have somewhat higher intrinsic resistance to photoinhibition (Krupa et al. 1991; Clarke et al. 1993b; Campbell unpublished). Replacement of D1:1 with D1:2 allows cells to maintain overall PSII activity in the face of photoinhibitory loss of centers, since the rate of oxygen evolution per D1 protein is significantly higher when D1:2 is the predominant form (2 h) compared to D1:1 (4-8 h). Events within the PSII core may well limit the dissipation of energy via photochemistry, resulting in reaction centers which are susceptible to photoinhibition. The enhanced photochemical efficiency of PSII containing D1:2 apparently allows increased transfer of electrons into the transport chain leading away from PSII, which remains non-saturated, as shown by maintenance of high photochemical quenching. This strategy stands in marked contrast to the situation in higher plants and green algae, where downstream electron transport is frequently thought to limit energy dissipation by PSII (Öquist et al. 1992; Maxwell et al. 1994).

The third phase during prolonged exposure to high light is the second interchange between the two D1 forms that occurs after 2 h. Despite continued exposure to high light, the synthesis of D1:2 ceases soon after it has become the predominant D1 form, and the cells then revert to the less efficient D1:1 protein. The resynthesis of D1:1 protein after 2 h is consistent with the known reactivation of *psbAI* expression under continuous high light (Kulkarni and Golden 1994). In contrast, the *psbAII/III* mRNA remained at high levels over the period of rapid loss of D1:2 protein, suggesting that strong post-transcriptional regulation plays an important role in determining the form of D1 protein present in the PSII reaction center. Despite little change in the nett amount of D1 protein, the reversion is clearly correlated with decreasing photosynthesis, although the growth rate does not decline in parallel (Fig. 2). These cooordinated changes are summarized in Fig. 8. D1:2 protein is apparently required only transiently after a shift to increased light, while the D1:1 protein is used for steady-state growth. These D1:1 centers may be more competent in the distribution of excitation energy between the photosystems (Campbell unpublished), especially under low or fluctuating light regimes, outweighing the penalty of lower efficiency under most growth situations in nature. Furthermore, natural cells experience high light for only short daily periods of 4–5 h or less, depending on position in the water column and mixing. In this context, the rapid but transient induction of the D1:2 protein at high light may also be viewed as an excitation stress response, analogous to stress responses such as those induced by heat shock.

Phycocyanin and the plasticity of acclimation responses. The changes within PSII upon shifts to high light are variable in extent, a finding largely explained by a strong direct correlation between the initial PC/Chl ratio and the severity of the inhibition of oxygen evolution and F'_V/F'_M after the shift. Differing PC/Chl ratios between cultures directly reflect the relative light-capture capacity of PSII versus PSI and thus the level of excitation pressure on PSII at a given light (Reuter and Müller 1993). The initial PC/Chl ratio is also strongly negatively correlated with recovery after inhibition, with cultures of high PC content unable to recover to the same degree as those with less PC. The validity of these correlations is supported by the identical relations between PC/Chl and inhibition or recovery as measured by oxygen evolution or by F'_V/F'_M.

Although the initial PC/Chl ratio influences photoinhibition and acclimation processes in this strain, PC content responds slowly to light intensity or quality and appears more strongly influenced by other factors such as CO_2 supply (Eley 1971; Muller et al. 1993) which change the relative cellular requirements for NADPH

and ATP (Manodori and Melis 1984; Reuter and Müller 1993). More generally, phycobilisome content and photosystem ratios in cyanobacteria are regulated by many factors other than light intensity or quality, including nitrogen and sulphur metabolism (Grossman et al. 1993; Tandeau de Marsac and Houmard 1993), consistent with the multiple roles of the phycobilisomes in the flexible metabolism of cyanobacteria. In our shift experiments, there was no detectable change in the cellular phycobilisome content nor in the arrangement of the phycobilisome rods, until after the interchanges between D1:1 and D1:2, when the PC/Chl ratio actually increased even though the cells remained under near saturating light. Therefore, PC content is a strong but relatively static influence on susceptibility to photoinhibition. In contrast, rapid turnover and exchange of the D1 protein is exploited by *Synechococcus* sp. PCC 7942 to modulate the properties of PSII to accommodate short-term changes in excitation pressure, which depends not only on light intensity but on the existing phycobilisome content.

The authors would like to thank Professor Eva-Mari Aro (Department of Biology, University of Turku, Finland) for her gift of the total D1 antibody, and Dr Vaughan M. Hurry (RSBS, Australian National University, Canberra, Australia) for his critical reading of the manuscript. This research was supported by the Swedish Natural Science Research Council.

References

Bhalerao RP, Lind LK, Gustafsson P (1994) Cloning of the *cpcE* and *cpcF* genes from *Synechococcus* sp. PCC 6301 and their inactivation in *Synechococcus* sp. PCC 7942. Plant Mol Biol 26:313–326

Bouyoub A, Vernotte C, Astier C (1993) Functional analysis of the homologous *psbA* gene copies in Synechocystis PCC 6714 and PCC 6803. Plant Mol Biol 21:249–258

Bustos SA, Golden SS (1992) Light-regulated expression of the *psbD* gene family in *Synechococcus* sp. strain PCC 7942: evidence for the role of duplicated *psbD* genes in cyanobacteria. Mol Gen Genet 232:221–230

Bustos SA, Schaefer MR, Golden SS (1990) Different and rapid responses of four cyanobacterial *psbA* transcripts to changes in light intensity. J Bacteriol 172:1998–2004

Campbell D, Zhou G, Gustafsson P, Öquist G, Clarke AK (in press) Electron transport regulates exchange of two forms of photosystem II DI protein in the cyanobacterium *Synechococcus*. EMBO J

Chow WS, Osmond CB, Huang LK (1989) Photosystem function and herbicide binding sites during photoinhibition of spinach chloroplasts in vivo and in vitro. Photosynth Res 21:17–26

Clarke AK, Critchley C (1992) The identification of a heat shock protein complex in chloroplasts of barley leaves. Plant Physiol 100:2081–2089

Clarke, AK, Soitamo A, Gustafsson P, Öquist G (1993a) Rapid interchange between two distinct forms of cyanobacterial D1 photosystem II reaction center protein in response to photoinhibition. Proc Natl Acad Sci USA 90:9973–9977

Clarke AK, Hurry VM, Gustafsson P, Öquist G (1993b) Two functionally distinct forms of the photosystem II reaction center D1 protein in the cyanobacterium *Synechococcus* sp. PCC 7942. Proc Natl Acad Sci USA 90:11985–11989

Curtis SE, Haselkorn R (1984) Isolation, sequence and expression of two members of the 32-kd thylakoid membrane protein gene family from the cyanobacterium *Anabaena* 7120. Plant Mol Biol 3:249–255

De Las Rivas J, Andersson B, Barber J (1992) Two sites of primary degradation of the D1-protein induced by acceptor or donor side photo-inhibition in photosystem II core complexes. FEBS Lett 301:246–252

Eley JH (1971) Effect of carbon dioxide concentration of pigmentation in the blue-green algae *Anacystis nidulans*. Plant Cell Physiol 12:311–316

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92

Gingrich JC, Buzby JS, Stirewalt VL, Bryant DA (1988) Genetic analysis of two new mutations resulting in herbicide resistance in the cyanobacterium *Synechococcus* sp. PCC 7002. Photosynth Res 16:83–99

Golden SS, Brusslan J, Haselkorn R (1986) Expression of a family of *psbA* genes encoding a photosystem II polypeptide in the cyanobacterium *Anacystis nidulans*. R2. EMBO J 5:2789–2798

Golden SS, Cho D-SC, Nalty MS (1989) Two functional *psbD* genes in the cyanobacterium *Synechococcus* sp. strain PCC 7942. J Bacteriol 171:4707–4713

Grossman AR, Schaefer MR, Chiang GG, Collier JL (1993) Environmental effects on the light-harvesting complex of cyanobacteria. J Bacteriol 175:575–582

Huner NPA, Öquist G, Hurry VM, Krol M, Falk S, Griffith M (1993) Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. Photosynth Res 37:19–39

Jansson C, Debus RJ, Osiewacz HD, Gurevitz M, McIntosh L (1987) Construction of an obligate photoheterotrophic mutant of the cyanobacterium *Synechocystis* 6803 Plant Physiol 85:1021–1025

Krupa Z, Öquist G, Gustafsson P (1990) Photoinhibition and recovery of photosynthesis in *psbA* gene-inactivated strains of cyanobacterium *Anacystis nidulans*. Plant Physiol 93:1–6

Krupa Z, Öquist G, Gustafsson P (1991) Photoinhibition of photosynthesis and growth responses at different light levels in *psbA* gene mutants of the cyanobacterium *Synechococcus*. Physiol Plant 82:1–8

Kulkarni RD, Golden SS (1994) Adaptation to high light intensity in *Synechococcus* sp. strain PCC 7942: Regulation of three *psbA* genes and two forms of the D1 protein. J Bacteriol 176:959-965

Lönneborg A, Kalla SR, Samuelsson G, Gustafsson P, Öquist G (1988) Light-regulated expression of the *psbA* transcript in the cyanobacterium *Anacystis nidulans*. FEBS Lett 240:110–114

Maxwell DP, Falk S, Trick CG, Huner NPA (1994) Growth at low temperature mimics high-light acclimation in *Chlorella vulgaris*. Plant Physiol 105:535–543

Manodori A, Melis A (1984) Photochemical apparatus organization in *Anacystis nidulans* (cyanobacteria). Effect of CO_2 concentration during cell growth. Plant Physiol 74:67–71

Miller AG, Espie GS, Canvin DT (1991) The effects of inorganic carbon and oxygen on fluorescence in the cyanobacterium *Synechococcus* UTEX 625. Can J Bot 69:1151–1160

Muller C, Reuter W, Wehrmeyer W, Dau H, Senger H (1993) Adaptation of the photosynthetic apparatus of *Anacystis nidulans* to irradiance and CO_2-concentration. Bot Acta 106:480–487

Mulligan B, Schultes N, Chen L, Bogorad L (1984) Nucleotide sequence of a multiple-copy gene for the B protein of photosystem II of a cyanobacterium. Proc Natl Acad Sci USA 81:2693–2697

Murata N, Fork D (1975) Temperature dependence of chlorophyll a fluorescence in relation to the physical phase of membrane lipids in algae and higher plants Plant Phys 56:791–796

Myers J, Graham JR, Wang RT (1980) Light harvesting in *Anacystis nidulans* studied in pigment mutants. Plant Physiol 66:1144–1149

Öquist G, Chow WS, Anderson JM (1992) Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. Planta 186:450–460

Ottander C, Hundal T, Andersson B, Huner NPA, Öquist G (1993) Low temperature photoinhibition in barley results in structurally stable photosystem II reaction centers. Photosynth Res 35:191–200

Reuter W, Müller C (1993) Adaptation of the photosynthetic apparatus of cyanobacteria to light and CO_2. J Photochem Photobiol B: Biol 21:3–27

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stainer RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Samuelsson G, Lönnesborg A, Rosenqvist E, Gustafsson P, Öquist G (1985) Photoinhibition and reactivation of photosynthesis in the cyanobacterium *Anacystis nidulans* Plant Physiol 79:992–995

Samuelsson G, Lönneborg A, Gustafsson P, Öquist G (1987) The susceptibility of photosynthesis to photoinhibition and the capacity of recovery in high and low light grown cyanobacteria, *Anacystis nidulans.* Plant Physiol 83:438–441

Schaefer MR, Golden SS (1989) Differential expression of members of a cyanobacterial *psbA* gene family in response to light. J Bacteriol 171:3973–3981

Shipton CA, Barber J (1991) Photoinduced degradation of the D1 polypeptide in isolated reaction centers of photosystem II: Evidence for an autoproteolytic process triggered by the oxidizing side of the photosystem. Proc Natl Acad Sci USA 88:6691–6695

Shyam R, Raghavendra AS, Sane PV (1993) Role of dark respiration in photoinhibition of photosynthesis and its reactivation in the cyanobacterium *Anacystis nidulans.* Physiol Plant 88:446–452

Soitamo AJ, Zhou G, Clarke AK, Öquist G, Aro E-M, Gustafsson P (1994) Over-production of the D1 protein of photosystem II reaction centre in the cyanobacterium *Synechococcus* sp. PCC 7942. Plant Mol Biol 26:709–721

Tandeau de Marsac N, Houmard J (1993) Adaptation of cyanobacteria to environmental stimuli: New steps towards molecular mechanisms. FEMS Microbiol. Rev 104:119–190

Tyystjärvi E, Kettunen R, Aro E-M. (1994) The rate constant of photoinhibition in vitro is independent of the antenna size of photosystem II but depends on temperature. Biochim Biophys Acta 1186:177–185

van Kooten O, Snel JFH (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosyn Res 25:147–150

Vass I, Styring S, Hundal T, Koivuniemi A, Aro E-M, Andersson B (1992) Reversible and irreversible intermediates during photoinhibition of photosystem II: Stable reduced QA species promote chlorophyll triplet formation. Proc Natl Acad Sci USA 89:1408–1412