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Differential phosphorylation of individual LHC-II polypeptides during short-term and long-term acclimation to light regime in the green alga *Dunaliella salina*

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The green alga *Dunaliella salina* is able to adapt to long-term changes in irradiance by adjusting the complement of auxiliary antenna complexes associated with each photosystem. This organism is also able to respond to short-term changes in incident light regime by transient adjustments in the absorption cross-section of Photosystem II by the process of state 1–state 2 transitions, which are mediated via redox-dependent phosphorylation of polypeptides of the chlorophyll *a*/*b*-binding light-harvesting complex of PS II (LHC-II). We have observed protein phosphorylation-dependent state transitions in *Dunaliella salina* and demonstrate that redox-controlled phosphorylation of LHC-II polypeptides of molecular mass 27, 26 and 25 kDa occurs in vivo during such adaptations. Redox-controlled phosphorylation of the 25 kDa polypeptide also occurred in high irradiance-adapted cells which have a depleted Photosystem II antenna and which are incapable of state transitions. We conclude that this polypeptide may be phosphorylated constitutively and may represent a component of the non-mobile, inner antenna of Photosystem II. State transitions were also observed in high irradiance-grown cells reacclimating to low irradiance, and conclude that the absorption cross-section of only PS II_α centres changes as a result of phosphorylation of LHC-II polypeptides. PS II_β has an unchanged absorption cross-section. An important conclusion is reached that during incremental assembly the PS II antenna on recovery from high light conditions, phosphorylation of individual LHC-II polypeptides is accompanied by an increase in the effect of Light 1 on variable fluorescence. This suggests that the 26 kDa and 25 kDa LHC-II polypeptides are phosphorylated in a redox-regulated reaction, with a 27 kDa polypeptide becoming phosphorylated finally to decouple a proportion of the antenna of PS II_α.

Introduction

Photosynthetic organisms adapt to prolonged changes in light regime by adjusting photosystem stoichiometry and antenna size (reviewed in Ref. 1). They also adapt to relatively short-term changes in light regime by a process known as state 1–state 2 transitions [2]. Imbalance in photosynthetic electron transport induced by preferential excitation of Photosystem II (PS II) results in net reduction of the plastoquinone pool and subsequent activation of a redox-dependent protein kinase [3]. This kinase phosphorylates polypeptides of the chlorophyll *a*/*b*-binding light-harvesting antenna protein complex of PS II (LHC-II), inducing its dissociation from PS II. The resultant decrease in PS II absorption cross-section constitutes the transition to state 2 [4]. Transition to state 1 occurs as a consequence of plastoquinone oxidation by preferential excitation of PS I, resulting in inactivation of the LHC-II kinase and permitting a phosphatase activity to dephosphorylate LHC-II [5]. This permits the reassociation of LHC-II with PS II. State transitions therefore represent a compensatory mechanism whereby preferential excitation of either photosystem induces a com-

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Abbreviations: A_m the unnormalized area above the fluorescence induction curve; A_t, area above the fluorescence induction curve after time t; Chl, chlorophyll; F_m, maximal fluorescence occurring when all PS II centres are closed; F_o, minimal fluorescence occurring when all PS II centres are open; F_v, variable fluorescence, $F_v = F_m - F_0$ DCMU, 3-(3′, 4′-dichlorophenyl)-1,1-dimethylurea; LHC-II, chlorophyll *a*/*b*-binding light-harvesting protein of PS II; PS II, photosystem II; Light 1, light absorbed predominantly by PS I; Light 2, light absorbed predominantly by PS II; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

plementary change in PS II antenna size and in PS II electron transport at limiting light intensity. Molecular events governing state transitions are reviewed in Ref. 6.

The phenomenon of state transitions has long been known to occur in green algae [7]. Since the photosynthetic apparatus of green algae does not differ fundamentally from that of higher plants, it might be anticipated that state transitions in green algae are mediated by the same redox-dependent protein phosphorylation mechanism. Indeed, protein phosphorylation in isolated algal thylakoid membranes has been shown to occur in vitro in the presence of [γ-^{32}P]ATP under conditions which give rise to quenching of PS II fluorescence indicative of decreased PS II antenna size [8–10].

Several distinct differences do however occur between chloroplasts isolated from higher plants and green algal cells, in particular with respect to the state of the photosynthetic apparatus in the dark. Unlike higher plant chloroplasts, the dark state in green algae is state 2 [9,11,12]. This state is generated as a result of reduction of plastoquinone by a chlororespiratory pathway which oxidises NADH/NADPH and uses plastoquinone as an electron carrier common to the photosynthetic and respiratory electron transport chains [13]. A membrane-bound NADH-plastoquinone oxidoreductase may initiate chlororespiratory activity [14,15].

Some debate surrounds the significance of LHC-II phosphorylation in the mechanism of state transitions in green algae in vivo. Under some circumstances phosphorylation of LHC-II in vivo appears to be independent of wavelength of incident light and insensitive to the presence of the herbicide DCMU [8], reflecting the possibility that no significant variations in the redox state of plastoquinone occur under normal physiological conditions [16]. This has led to the suggestion that LHC-II phosphorylation in green algae is responsible for the maintenance or assembly of the PS II antenna, rather than for state transitions [8,17].

Green algae are certainly able to modify the composition of the photosynthetic apparatus in response to irradiance. This is exemplified by the response of the green alga *Dunaliella salina* to growth under high irradiance. Cells grown under high light exhibit a high chlorophyll *a*/*b* ratio (4.6 for low-light cells, 16.5 for high-light cells [19]), reflecting a greatly depleted auxiliary antenna system for both photosystems [18,19]. Variable fluorescence yield from PS II is also greatly reduced and arises with exceptionally slow kinetics [19]. In a previous study [20], we have described the molecular events which occur when high irradiance-adapted *Dunaliella* cells are returned to moderate growth irradiance. In the present study, we investigate the role of redox-dependent protein phosphorylation in this organism, and define the conditions under which phosphorylation occurs. In addition, we use the reacclimating photosynthetic apparatus of high irradiance-grown *Dunaliella* cells to demonstrate differential phosphorylation of LHC-II species. This may indicate specific physiological roles for LHC-II phosphorylation during differing stages of assembly of the PS II auxiliary antenna.

Material and Methods

Cell culture

Cells of *D. salina* were grown in a medium containing 2.0 M NaCl as described in Refs. 18 and 19. Control cells were grown under incandescent lights giving approximate photon flux density 150–200 μE m^{-2} s^{-1} High-light-grown (HL) cells and low-light-grown (LL) cells were grown under light intensities of 2150 and 100 μE m^{-2} s^{-1}, respectively, as previously described [20].

Radiolabelling in vivo was performed by harvesting cells by centrifugation at $6500 \times g$ for 10 min and resuspending in fresh, phosphate-free growth medium in the presence of [^{32}P]orthophosphate at an activity of 5 μCi ml^{-1}. Where light regime was altered during in vivo labelling, Light 1 was defined by 710 nm interference filter and Light 2 was defined by 670 nm short wavelength pass filter, with stabilised 250 W tungsten-halogen light source (CUEL, Kenilworth, UK).

Thylakoid membrane isolation

Thylakoid membranes were isolated by the method detailed in Ref. 20, and washed to remove ferredoxin from the membrane. Phosphorylation in vitro with [γ-^{32}P]ATP was performed on membranes resuspended to a chlorophyll concentration of 0.5 mg ml^{-1} in 100 mM sorbitol, 10 mM $MgCl_2$, 50 mM Tricine-NaOH (pH 7.8), with 400 μM ATP at a specific activity of 100 nCi (nmol ATP)$^{-1}$.

Electron transport measurements

Electron transport rates were measured in a Hansatech DW2 oxygen electrode at 20°C. Whole cells were resuspended in fresh growth medium to a chlorophyll concentration of 50 μg ml^{-1}.

Polyacrylamide gel electrophoresis

SDS-PAGE was performed on 12%–22% gradient gels as described previously [20]. Autoradiography of Coomassie brilliant blue-stained gels was performed with Amersham Hyperfilm MP with exposure at −80°C. Autoradiographs were scanned using an LKB-Pharmacia Ultrascan XL laser densitometer.

Room temperature fluorescence induction

Fluorescence induction in the presence of DCMU was performed as described in a previous study [20].

Kinetics of fluorescence rise were analysed by the procedure of Melis and Homann [21] and the proportion of PS II_β determined from the semilogarithmic plot of the rate of complementary area growth above the fluorescence induction curve. This first-order analysis reveals the biphasic nature of the fluorescence induction kinetics, with fast and slow components arising from PS II_α and PS II_β respectively. The relative proportion of β-centres is determined from the intercept on the y-axis of the linear extrapolation of the slow kinetic component. Cells were incubated under Light 1, Light 2 or in the dark for 15 min prior to addition of DCMU and recording the induction transient. The light regimes were defined by the filter systems described above. For fluorescence induction in the absence of DCMU, anaerobic conditions were generated by the action of glucose oxidase (2 mg ml^{-1}) with glucose (20 mM).

Results and Discussion

State transitions and phosphorylation of LHC-II JR in vivo

Changes in PS II absorption cross-section indicative of state transitions are reflected by differences in the yield of variable fluorescence induced in the presence of DCMU. Fluorescence induction curves obtained in the presence of DCMU for whole cells of *D. salina* are shown in Fig. 1. Control cells, grown under light intensity 150–200 μ E m^{-2} s^{-1}, were resuspended in the presence of [^{32}P]orthophosphate prior to adaptation to Light 1 (L1), Light 2 (L2), or to darkness (D) for 15 min. Fluorescence yield from PS II was greatest from cells stably adapted to Light 1, indicating a largest absorption cross-section in state 1. This yield was reduced by 27% and 29% when cells were adapted to Light 2 or darkness, respectively. Note that the ratio F_m/F_o showed no variation under differing illumination conditions, indicating an absence of any 'spillover' type effects [22].

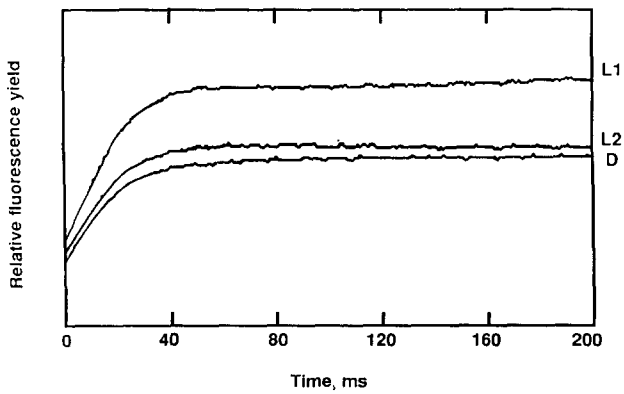

Fig. 1. Fluorescence induction transients in the presence of DCMU for cells of *Dunaliella salina* incubated for 15 min under Light 1(L1), Light 2 (L2) or in darkness (D) prior to addition of DCMU. Cells were grown under light intensity 150–200 μE m^{-2} s^{-1}.

Adaptation to state 2 in the dark has been shown to result from the action of the chlororespiratory pathway in reducing plastoquinone [10,13]. The influence of chlororespiration on light state adaptation is discussed in detail below. It is clear from Fig. 1 that cells of *D. salina* are capable of reversible changes in room-temperature fluorescence yield which should at least in part be due to phosphorylation-induced changes in the PS II auxiliary antenna. The observed changes are quantitatively similar to those observed for other species of green algae [23,24].

A previous study on the green alga *Chlamydomonas reinhardtii* showed phosphorylation of LHC-II to occur in vivo, but to be independent of illumination wavelength and to be unaffected by the presence of DCMU [8]. Phosphorylation of LHC-II was proposed therefore to have a role in maintaining the structural integrity of the PS II unit, rather than in light-state adaptations alone. The variations in PS II fluorescence yield, observed under differing illumination conditions [8,12] could conceivably be explained by a quenching mechanism related to energy state of the thylakoid membrane, rather than to phosphorylation-induced changes in PS II antenna size. Supporting this notion is the observation that redox state of plastoquinone appears to show little variation under different illumination conditions [16]. Phosphorylation of *D. salina* thylakoid membrane proteins in vivo resulting from incubation under the light regimes described above is shown in Fig. 2: differential degrees of phosphorylation of LHC-II polypeptides in the molecular mass range 28–25 kDa occurred under the three light regimes. Incubation under Light 1 resulted in phosphorylation principally of a 25 kDa polypeptide, whereas incubation under Light 2 gave enhanced phosphorylation of this polypeptide and of species with somewhat higher mass (26–28 kDa). Maximum phosphorylation of these LHC-II species, and of two polypeptides of mass 32 kDa and 9 kDa, was achieved under dark conditions. These latter polypeptides may correspond to the D1 *psbA* polypeptide of the PS II reaction centre, and the *psbH* gene product, respectively, [6]. Although it is difficult to distinguish individual LHC-II phosphoprotein species from the mini-gels shown in Fig. 2, there is a clear correlation between the overall degree of phosphorylation and the changes in PS II fluorescence characteristic of altered PS II absorption cross-section during state transitions. Differential phosphorylation of LHC-II subunits was further investigated using a more highly resolving gel system, and is discussed below.

Phosphorylation of LHC-II occurred to some extent in cells adapted to state 1 (Fig. 2). Also note that incubation in the dark resulted in enhanced LHC-II

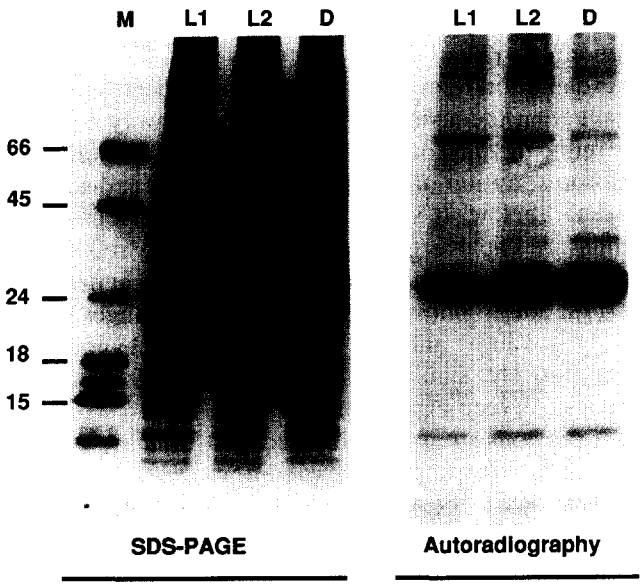

Fig. 2. SDS-PAGE analysis (left) and subsequent autoradiography (right) of *Dunaliella salina* thylakoid membrane polypeptides phosphorylated in vivo with [^{32}P]orthophosphate. Cell growth and light incubation conditions were as described for Fig. 1. Positions and molecular masses of marker proteins (M) are indicated.

phosphorylation with respect to the levels achieved under Light 2, but gave only a very minor decrease in PS II variable fluorescence compared to the yield from cells adapted to Light 2 (Fig. 1). Thus, a non-linear relationship between LHC-II phosphorylation and fluorescence decrease is suggested. This reflects the possibility that distinct physiological roles are fulfilled by differential phosphorylation of individual LHC-II subspecies.

Chlororespiratory activity in Dunaliella salina

Adaptation to functional state 2 in the dark results from reduction of plastoquinone by chlororespiration. Net reduction of plastoquinone – by inhibition of the terminal oxidase by generation of anaerobic conditions – strongly promotes LHC-II phosphorylation, even in PS-II-deficient mutants [25]. The generation of state 2 in the dark has been suggested to be advantageous [26,27] in the respect that it could represent a mechanism whereby cyclic electron transport, and therefore ATP production, is favoured over NADPH production during the initial onset of illumination. This would tend to promote assimilatory reactions within the chloroplast [28]. Indeed, it has been suggested that regulation of the NADPH/ATP ratio is a principal function of the state transition in green algae [28,29].

Since photosynthetic and chlororespiratory electron transport chains use plastoquinone as a common electron transport intermediate, stimulation of chlororespiratory activity in vitro in the dark by NADPH would be expected to give redox-dependent kinase activity and LHC-II phosphorylation. Fig. 3 shows phosphorylation in vitro of isolated washed *Dunaliella* thylakoid membranes incubated in the dark for 15 min in the presence of [γ-^{32}P]ATP, with or without the inclusion of electron donors NADPH or NADH. Since efficient reduction of plastoquinone in the dark can be achieved with ferredoxin and NADPH [30], washing was required to ensure removal of any ferredoxin which may have been present. The presence of NADH (lane 2) or NADPH (lane 5) in the presence of oxygen gave increased LHC-II phosphorylation with respect to the levels achieved without electron donors (lane 1), suggesting that reoxidation of plastoquinone by oxygen or by any terminal oxidase is rate-limiting. Removal of oxygen gave enhanced phosphorylation in accordance with the idea of increased plastoquinone reduction by chlororespiration (lanes 3 and 6). NADPH appeared to be a somewhat better electron donor than was NADH, as judged by higher levels of phosphorylation in the presence of this donor. Note that preincubation with rotenone did not appear to inhibit phosphorylation to any extent, suggesting a failure to inhibit electron transport from NADH to plastoquinone in this system (lane 4).

The direct effect of chlororespiratory electron transport on plastoquinone redox state was confirmed by measurement of fluorescence induction from isolated

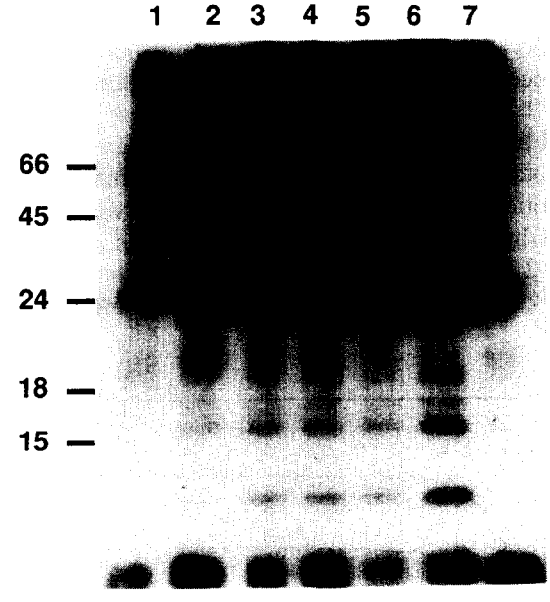

Fig. 3. In vitro phosphorylation of *Dunaliella salina* thylakoid membrane proteins. Isolated thylakoid membranes were incubated in the presence of [γ-^{32}P]ATP in the dark for 15 min under various conditions prior to SDS-PAGE analysis (not shown) and subsequent autoradiography (above). Lanes were loaded with thylakoid membrane material equivalent to 3 μg chlorophyll. Positions and molecular masses of SDS-PAGE marker proteins are indicated. (1) no additions; (2) 2 mM NADH/aerobic; (3) NADH/anaerobic; (4) preincubated with 30 μm rotenone, then as (3); (5) 2 mM NADPH/aerobic; (6) NADPH/anaerobic; (7) anaerobic, no electron donor.

thylakoid membranes incubated under the conditions described for Fig. 3. Changes in the rate of area growth above the fluorescence induction curve recorded in the absence of DCMU reflect changes in the photochemical quenching capacity of the plastoquinone pool, and therefore permit indirect measurement of the redox state of plastoquinone. Using this methodology, fluorescence induction from *Dunaliella salina* thylakoid membranes was used to confirm increased plastoquinone reduction by chlororespiration under anaerobic conditions, and relatively more efficient oxidation of NADPH by the chlororespiratory pathway (data not shown).

Redox-dependent protein phosphorylation in high light- and low light-acclimated Dunaliella salina

The light harvesting system of *Dunaliella salina* shows great flexibility in its response to intensity of incident illumination [18–20], exhibiting greatly reduced antenna size for both photosystems when cells are grown under high irradiance. High-light-grown cells have a very low variable fluorescence yield, arising with slow kinetics which are characteristic of the predominant form of PS II in this high irradiance acclimated system, termed PS II_γ. In a previous report [20], we have shown by immunoblotting that the thylakoid membranes of HL-grown *Dunaliella salina* contain residual amounts of four LHC-II species in the mass range 28–25 kDa. Subsequently, we described the events which occur during the reassembly of the PS II antenna during the course of reacclimation of HL-grown cells to physiological light intensity. This reacclimation process took the form of discrete, stepwise incremental additions to the functional PS II antenna. In this study, we have used the flexibility in response of the photosynthetic apparatus of this organism to probe further the roles of LHC-II phosphorylation in this system. Having established conditions under which plastoquinone reduction and therefore kinase activation takes place, we have investigated further the phosphorylation events which occur not only in the intact photosynthetic apparatus but also in the HL-grown system with its truncated PS II antenna. By investigating phosphorylation phenomena in the structurally and functionally simplified antenna present in HL-grown cells, and by observing changes in phosphorylation

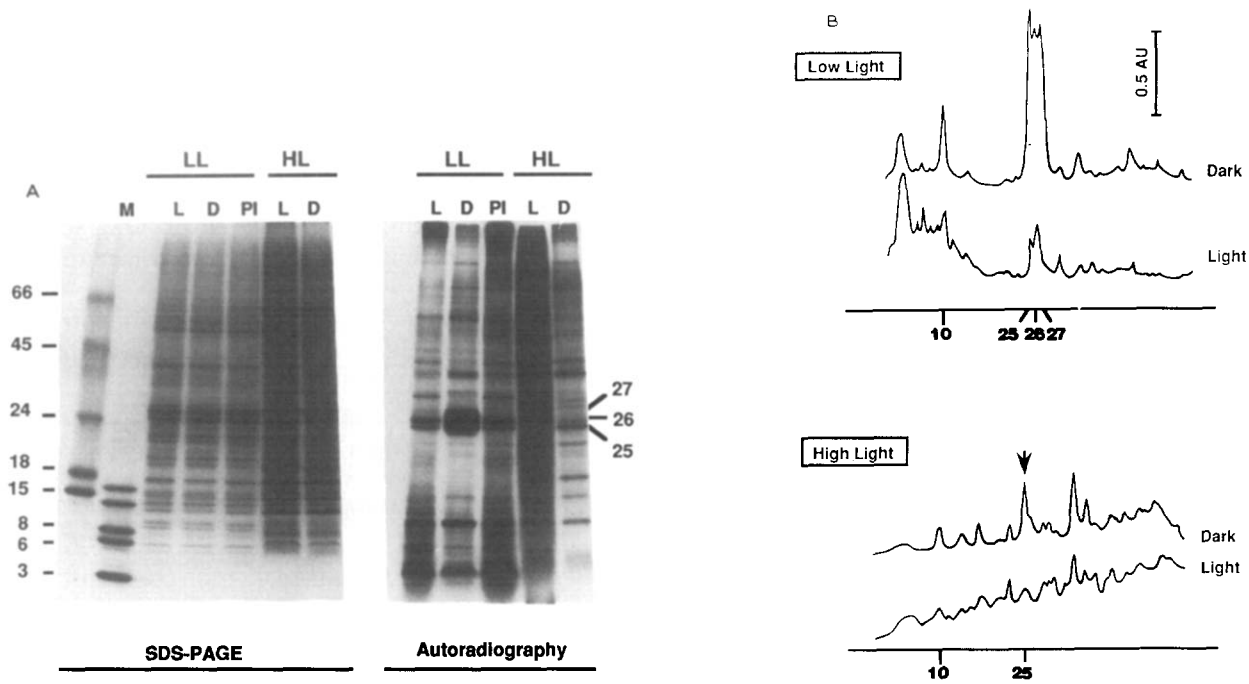

Fig. 4. In vivo phosphorylation of thylakoid membrane polypeptides from LL-grown and HL-grown *Dunaliella salina*. Cells were grown under LL or HL, resuspended in fresh growth medium supplemented with [^{32}P]orthophosphate and returned to their respective light regimes for 30 min. Cells were subsequently removed to Light 1 or dark conditions for 15 min prior to isolation of thylakoid membranes. SDS-PAGE analysis (left panel) and subsequent autoradiography (right panel) are shown for thylakoid membrane polypeptides isolated from LL-grown cells (LL) or HL-grown cells (HL) incubated under Light 1 (L) or dark (D) conditions. In an additional experiment, LL-grown cells were instead removed to the HL regime after equilibration with [^{32}P]orthophosphate, resulting in photoinhibition of PS II activity. SDS-PAGE and autoradiography of thylakoid membrane polypeptides from these cells are indicated PI. Positions and approximate molecular masses of major LHC-II polypeptides are indicated, as are positions of molecular mass markers (M). (b) Densitometric scans of the autoradiographs in (a). The upper panel shows scans of the autoradiograph obtained for light and dark incubations of LL-grown cells (LL, lanes L and D in Fig. 4a). Scans of the autoradiographs from the corresponding incubations for HL-grown cells (HL, lanes L and D in Fig. 4a) are shown below. The position of the LHC-II polypeptide undergoing dark-dependent phosphorylation in the thylakoid membrane of HL-grown cells is indicated by the arrow.

which occur during reassembly of the PS II auxiliary antenna as HL-grown cells reacclimate to low light, it may be possible to differentiate between diverse roles for protein phosphorylation in distinct components of the PS II antenna system.

Cells of *D. salina* grown under high irradiance (HL) or low irradiance (LL) in the presence of [^{32}P]orthophosphate, and subsequently removed to light or darkness, exhibit variations in phosphorylation of thylakoid membrane proteins, as shown in Fig. 4. Cells grown under LL or HL were harvested, resuspended in phosphate-free growth medium supplemented with [^{32}P]orthophosphate and returned to the original light regime for 30 minutes to ensure phosphate uptake. Cells were subsequently incubated for 15 min under Light 1 or under dark conditions. Thylakoid membranes were then isolated from the radiolabelled cells. Fig. 4a shows SDS-PAGE analysis and subsequent autoradiography of thylakoid membrane proteins undergoing redox-dependent phosphorylation in vivo in both LL-grown and HL-grown *D. salina* cells. There is apparent phosphorylation of many polypeptides, with radiolabelling of LHC-II species in the mass range 25–27 kDa prominent in the sample from LL-grown cells incubated in the dark. Incubation under Light 1 resulted in greatly decreased phosphorylation of these polypeptides. Significantly, a polypeptide of 25 kDa present in the thylakoid membrane of HL-grown cells was also phosphorylated specifically in the dark (Fig. 4a)

Polypeptides of LHC-II are apparent in the SDS-PAGE tracks showing analysis of thylakoid membranes from LL-grown cells (Fig. 4a, left panel). Interpretation of the LHC-II content of thylakoid membranes from HL-grown cells is more difficult, but our previous immunoblotting study [20] has indicated relatively low levels of all LHC-II polypeptides in the membrane of HL-grown cells, consistent with the decreased functional chlorophyll antenna size for PS II under HL conditions.

Densitometric scans of the autoradiographs from Fig. 4a are shown in Fig. 4b, and indicate high levels of phosphorylation of LHC-II polypeptides of masses 25, 26 and 27 kDa specifically under plastoquinone-reducing conditions defined earlier in this study (that is, in the dark). These three polypeptide species were phosphorylated equally under these dark conditions. However, densitometry of the SDS-PAGE lanes (not shown) indicates that the 27 kDa polypeptide is somewhat less abundant than are the other two, suggesting greater specific phosphorylation for the 27 kDa polypeptide under reducing conditions. Incubation under plastoquinone-oxidising conditions gave no detectable phosphorylation of the 27 kDa polypeptide, and greatly reduced phosphorylation of the 25 and 26 kDa species (Fig. 4b). Phosphorylation of a polypeptide with a mass of approx. 10 kDa, which probably corresponds to the *psbH* gene product of *Dunaliella salina* [31], was also decreased under oxidising conditions.

Incubation of thylakoid membrane proteins from HL-grown cells with [^{32}P]orthophosphate invariably resulted in high background radiolabelling. However, densitometry was able to resolve individual phosphorylated species (the extent of autoradiographic development was well within the dynamic range of the instrument). The resulting scans are shown in Fig. 4b (lower). A polypeptide of mass 25 kDa was phosphorylated specifically under the reducing conditions defined earlier. Under these dark conditions, HL-grown cells exhibited chlororespiratory activity of 850 μmol O_2 (mg Chl)$^{-1}$ h^{-1}, a value apparently high because of the low cellular chlorophyll in HL-grown cells. Incubation of HL cells under plastoquinone oxidising conditions resulted in loss of phosphorylation of the 25 kDa polypeptide (Fig. 4b, lower), indicating redox control of the phosphorylation of this species and therefore implying that the polypeptide represents a residual population of the 25 kDa LHC-II polypeptide within the depleted PS II antenna of the HL-grown cell.

In an additional experiment, LL-grown cells were resuspended in [^{32}P]orthophosphate-supplemented medium (as described), maintained under LL conditions for 30 min and then removed to the HL regime (2150 μE m^{-2} s^{-1}) for 15 min. This resulted in complete but reversible photoinhibition of light-dependent oxygen evolution (a net oxygen uptake by chlororespiration of 90 μmol O_2 (mg Chl)$^{-1}$ h^{-1} was observed). However, photoinhibition did not give rise to increased specific phosphorylation of any polypeptide species (Fig. 4a, right panel, lane PI), contrary to other reports [32].

The data in Fig. 4 suggest that redox-controlled LHC-II phosphorylation may take place even in the highly depleted auxiliary antenna of HL-grown cells. This observation may reflect a capacity to perform redox-dependent changes in PS II absorption cross-section even in a system in which the peripheral PS II antenna is minimal or absent.

State transitions during reacclimation of HL-grown cells to physiological light intensity

We have previously reported changes which occur in the photosynthetic apparatus of *Dunaliella salina* during reacclimation of HL-grown cells to LL [20]. In the course of this reacclimation, a sequential reconstruction of the PS II antenna occurred over a time period of 24 h. This increase in PS II antenna size was characterised by higher PS II variable fluorescence yield, the kinetics of which reflected a shift in the population of PS II centres from exclusively small antenna β-centres to larger antenna α-centres after 12 h reacclimation. By using the redox conditions defined earlier, we have investigated the capacity of reacclimat-

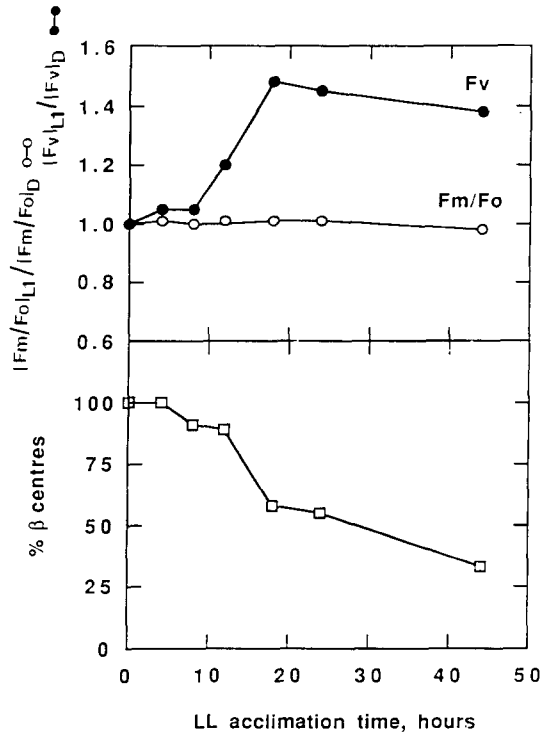

Fig. 5. State transitions during reacclimation of HL-grown *Dunaliella salina* to LL. Fluorescence induction curves were recorded with HL-grown cells reacclimated to LL for various times and incubated for 15 min under Light 1 or in the dark prior to the addition of DCMU. Light 1-induced changes in variable fluorescence yield (F_v) and in the ratio F_m/F_o are plotted as a function of LL reacclimation time, and are expressed relative to the corresponding dark value, giving the ratios $(F_v)_{L1}/(F_v)_D$ and $(F_m/F_o)_{L1}/(F_m/F_o)_D$ (upper panel). Changes only in F_v are diagnostic for altered PS II absorption cross-section, characteristic of state 1-state 2 transitions. Changes in the proportion of PS II centres which are β-centres occurring during LL reacclimation are also plotted as a function of reacclimation time (lower panel). %β is determined as described in the text.

ing *Dunaliella salina* cells to perform state transitions, as defined by an ability to make rapid adjustments to PS II absorption cross-section in response to altered redox conditions. The capacity of reacclimating HL-grown cells to alter PS II variable fluorescence yield in response to variations in redox state is shown in Fig. 5.

The reacclimation procedure was performed as described previously [20]. Briefly, HL-grown cells were transplanted from a single stock culture into identical subcultures and maintained under HL for a further 48 h. Cells were then removed to the LL regime for the various specified times, after which they were harvested, resuspended in fresh growth medium and incubated under Light 1 or dark conditions for 15 min to induce state transitions. The capacity of reacclimating cells to perform state transitions is expressed in Fig. 5 (upper) in terms of the capacity of plastoquinone-oxidising Light 1 conditions to induce an increase in PS II variable fluorescence yield (F_v) relative to the fluorescence yield under dark conditions (see Fig. 1). The ratio $(F_v)_{L1}/(F_v)_D$ therefore provides a direct measure of redox-dependent state transitions during LL acclimation.

Alterations in the relative proportion of PS II_β centres during LL reacclimation were also measured as changes in the slow kinetic component of the area growth above the fluorescence induction curve. Since this slow component has been proposed to arise from the small antenna size β-centres, any change in the proportion of the biphasic fluorescence rise attributable to this component is indicative of altered PS II_β abundance.

During the first 12 h reacclimation, cells contained only PS II_β centres (Fig. 5, lower), as defined by the rate constant for photon uptake [18,20], and showed no capacity for increasing PS II fluorescence yield (F_v) in response to incubation under plastoquinone-oxidising Light 1 conditions (Fig. 5, upper). Between 12–18 h after the onset of reacclimation, cells demonstrated a greatly increased capacity to perform state transitions, as judged by a Light-1-dependent increase in variable fluorescence yield (Fig. 5, upper). No further variations in F_v were observed subsequent to this dramatic step, which correlated with the shift in the PS II population from PS II_β centres to PS II_α centres (Fig. 5, lower). No redox-dependent variations in the ratio F_m/F_o, which might indicate phenomena other than state transitions, were observed during the reacclimation time course (Fig. 5, upper. See also Fig. 1).

Since the capacity to adjust PS II antenna size in response to plastoquinone-reducing conditions correlates with increased PS II_α abundance, it is strongly suggested that only PS II_α centres are capable of reducing PS II absorption cross-section by a redox-controlled protein phosphorylation mechanism, and therefore only these centres are involved in state transitions. This contrasts with an earlier study in which it was suggested that both α- and β-centres underwent complementary changes in absorption cross-section during state transitions [33].

It should be emphasised that with the methodology applied in this study it is not possible to discriminate between PS II centres which are capable or incapable of plastoquinone reduction, referred to as Q_B-reducing and Q_B-non-reducing centres, respectively, (for discussion, see [1]). An underlying phenomenon involving changes in the relative proportion of these centres during LL reacclimation cannot therefore be discounted. Although there are significant overlaps in the functional characteristics of β-centres and Q_B-non-reducing centres, it seems unlikely that the PS II_β population of reacclimating cells is exclusively or even largely Q_B-non-reducing. The situation is more likely to resemble that which occurs in chlorophyll *b*-deficient

mutants, in which PS II centres exhibit a uniformly small antenna size but are competent for plastoquinone reduction.

The data in this report indicate that the green alga *Dunaliella salina* is capable of performing state transitions by a protein phosphorylation mechanism in response to variations in plastoquinone redox state resulting from fluctuations in light regime. Since it is the 27 kDa LHC-II polypeptide which undergoes the greatest enhancement in phosphorylation under dark and plastoquinone-reducing conditions in LL-grown cells, it is most likely the phosphorylation of this polypeptide which would be responsible for inducing the transition to state 2. The 'mobile' or 'peripheral' antenna of PS II [1] which becomes detached from PS II upon transition to state 2 [34,35], would therefore be enriched in this polypeptide. The 25 kDa and 26 kDa LHC-II polypeptides would be relatively more abundant in the 'inner' antenna of PS II [1,35], which would not dissociate from PS II during the state 2 transition. It is important to note, however, that phosphorylation of these latter species can occur to a limited extent in vivo even under state 1-inducing oxidising conditions, and even in the truncated photosynthetic apparatus of HL-grown cells incapable of performing state transitions. Although this observation would tend to suggest some constitutive phosphorylation of LHC-II, this idea may be refuted by the observation that oxidising conditions completely abolish phosphorylation of residual LHC-II polypeptides in the PS II antenna of HL-grown cells. If phosphorylation were constitutive, it should be expected to occur to an invariable degree in a system which has no peripheral antenna and is incapable of performing state transitions. Instead, the data suggest that phosphorylation of the more abundant 25 and 26 kDa LHC-II polypeptides is under redox control and may represent an intermediate state preceding phosphorylation of the 27 kDa polypeptide, which triggers dissociation of the peripheral antenna from PS II and induces the transition to state 2.

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