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Energy distribution between Photosystems I and II in the photosynthetic prokaryote *Prochlorothrix hollandica* involves a chlorophyll *a* / *b* antenna which associates with Photosystem I

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Prochlorothrix hollandica is a photosynthetic prokaryote, whose main thylakoid intrinsic chlorophyll *a* / *b* antenna copurifies with PS I and is both structurally and functionally distinct from chloroplast LHC II. The 35 kDa apoprotein of the antenna forms the main target for light/redox controlled reversible phosphorylation (Post, A.F., Gal, A., Ohad, I., Milbauer, K.M. and Bullerjahn, G.S. (1992) Biochim. Biophys. Acta 1100, 75–82). The occurrence of state 1 → 2 transitions in cells illuminated with light 1 (710 nm) and light 2 (652 nm) was shown from differences in fluorescence properties using the chlorophyll fluorescence induction technique. The same technique showed that the redox state of the PQ pool responded to light conditions, being more oxidized in dark-incubated and light-1-illuminated cells. Following the transfer of light-1-treated cells to light 2 conditions, state 2 was reached in approx. 10 min. Addition of the phosphatase inhibitor NaF locked cells in state 2. These observations lend support to the hypothesis that the molecular mechanism driving the state 1 → 2 transitions involves the reversible phosphorylation of the main chlorophyll *a* / *b* antenna. 77 K fluorescence spectra of whole cells and of PS I complexes obtained from detergent-treated thylakoids showed strong energy coupling between the antenna and PS I preferentially. Fluorescence quenching measurements showed an increase in PS I activity during a state 1 → 2 transition. These observations suggest that during a state 1 → 2 transition an increasing fraction of the excitation energy arriving from the antenna is diverted to PS I. The antenna copurified with PS I complexes under all conditions examined. We have summarized the principal differences between eukaryotic and prokaryotic chlorophyll *a* / *b* antennae. A model for the regulation of photosynthetic activity in *P. hollandica* is proposed and it involves light controlled reversible phosphorylation of the chlorophyll *a* / *b* antenna. Our model claims that in state 1 (non-phosphorylating conditions) the bulk chlorophyll *a* / *b* antenna is shared by both photosystems. Under phosphorylating conditions (state 2) the antenna associates more tightly with PS I, effectively reducing energy transfer to PS II centers. It is noted that reversible phosphorylation of a chlorophyll *a* / *b* antenna may have evolved as a regulatory mechanism prior to the evolution of chloroplast LHC II.

Introduction

All photosynthetic organisms exhibit plasticity in thylakoid organization and composition while responding to variations in the ambient light. Of all intrinsic pigment-protein complexes the strongest adaptive response is in general observed in LHC II, the light harvesting chlorophyll (Chl) *a* / *b* antenna of Photosystem II (PS II) in the green chloroplast [1–4]. LHC II from different plant/algal species contain immunologically related sets of apoproteins in the range of 20–30 kDa [5–9]. The protein complex binds Chl *a* and *b* in a ratio of 1.5 [2,10]. The principal task of the complex is light harvesting [2,6,8,10]. It further functions in the regulation of energy distribution between PS I and PS II, as visualized from changes in the effective absorption cross-section of RC II [11–14]. The molecular mechanism behind this modulation is a reversible protein-kinase activity regulated by imbalance in PS II activity relative to that of PS I [11,15,16]. The target of kinase activity is the LHC II. Following phosphorylation of LHC II apoproteins a subpopulation of the LHC II decouples from PS II [5] and is thought to reassociate with PS I [13,14].

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Chl *a*/*b* antennae are not unique to eukaryotic 'higher plant' type photosynthesis, and they are observed among photosynthetic prokaryotes also [17–19]. Whereas uniformity prevails among higher plant and algal Chl *a*/*b* antennae [11], it appears that the prokaryotic Chl *a*/*b* antenna is distinctly different from its eukaryotic counterpart in pigment composition, structure, localization and function. In *Prochloron* sp. a 34-kDa protein is reported as the apoprotein in the Chl *a*/*b* antenna [20,21] and it copurifies with PS I [21]. The Chl *a*/*b* antenna of *Prochlorothrix hollandica* contains a group of extremely hydrophobic apoproteins of 30–35 kDa which in part copurify with PS I [22–24]. The Chl *a*/*b* ratio in the antenna of both organisms is 2.4–2.5 [21,24]. The *P. hollandica* antenna apoprotein is immunologically related to that of *Prochloron* and distinct from LHC-II-type antennae from various sources [23]. Conversely, antibodies raised against LHC II apoproteins fail to recognize thylakoid proteins in *Prochloron* [21] or *Prochlorothrix* [23]. It is still not known which thylakoid complex forms the accessory antenna to PS II in these photosynthetic prokaryotes, although a 33 kDa Chl *a*/*b* apoprotein has been shown to copurify with PS II [24]. The 34 kDa Chl *a*/*b* apoprotein of *Prochloron* is phosphorylated in vitro by a constantly active protein kinase and state $1 \rightarrow 2$ transitions could not be detected [20]. However, the obligate symbiotic relationship between *Prochloron* and its host may be cause to this apparent lack of plasticity. *P. hollandica* displayed state $1 \rightarrow 2$ transitions upon transfer from far red to red illumination of cells [25]. In a recent publication we reported on a light/redox controlled kinase activity in *P. hollandica* thylakoids responsible for phosphorylation of the 35 kDa antenna apoprotein both in vivo and in vitro [24]. The PQ pool was largely oxidized in vitro [24] opening the possibility of light/redox control over kinase activity as observed in chloroplasts [26–28]. Choosing the appropriate experimental conditions, the dark state in green chloroplasts is shown to be state 1 correlating with a fully-oxidized PQ pool [29]. In cyanobacteria the dark state is state 2 in the presence of a reduced PQ pool [30]. Here we report on a study of state $1 \rightarrow 2$ transitions and the regulation of photosynthetic activity in *P. hollandica*. A model is discussed involving the regulation of the PS II absorption cross-section through reversible phosphorylation of a Chl *a*/*b* binding antenna protein which interacts directly with PS I. The prokaryotic antenna-PS I interactions are very different from those encountered in the chloroplast.

Materials and Methods

Growth conditions

Batch cultures of *Prochlorothrix hollandica* were grown in BG 11 mineral medium (300 ml in 1 litre Erlenmeyer flasks) at 22°C on an orbital shaker. Continuous illumination was provided by warm white fluorescence lamps at a flux of 40 μmol quanta/m^2 per s. Cells adapted to light preferentially driving PS II activity (light 2) and PS I activity (light 1) were obtained by shifting white light-grown cells for the duration of 24 h into flasks masked with broad band red (max. transmission at 660 nm) and far red (max. transmission at 695 nm) filters at a light intensity of 15 μmol quanta/m^2 per s.

Fluorescence measurements

Excitation light for chlorophyll fluorescence induction was obtained by passing a collimated light beam through a short-pass filter transmitting wavelengths < 550 nm. PS II fluorescence emission was defined as the photon flux reaching the pin diode detector after passing a long-pass filter transmitting wavelengths of > 675 nm. Before measurement cells were either dark-adapted for 30 s or adapted to 652 nm red (light 2) and 712 nm far red illumination (light 1) for 10 min at 20 μmol quanta/m^2 per s. Fluorescence induction parameters were estimated after digitized data acquisition as follows: initial fluorescence F_0 was determined at 3 ms, directly after complete opening of the electronic shutter. Steady-state fluorescence, F_s, and maximal fluorescence in the presence of 10^{-6} M DCMU, F_{max}, were determined after 3 s of excitation. Fluorescence quenching was measured using a Walz pulse amplitude modulated (PAM) fluorimeter described by Schreiber et al. [31]. Low-temperature fluorescence excitation and emission spectra of whole cells frozen at 77 K were obtained with a SLM 8000 fluorimeter using an excitation slit width of 5 nm and an emission slit width of 2 nm. Fluorescence yields at each wavelength are averaged values over 10 s sample intervals.

In vivo phosphorylation

Phosphorylation of thylakoid proteins in vivo was performed as described previously [24], except that cells were resuspended in BG-11 medium containing 0.5 × the original phosphate concentration. Carrier-free $^{32}PH_3^{35}PO_4$ (ICN, 9 Ci/mmol PO_4) was added to the medium to yield a final activity of 2 μCi/ml. Cultures were then exposed to the PS II and PS I illumination regimes described above.

PS I isolations and non-denaturing protein electrophoresis

Photochemically active preparations of PS I were prepared by resuspending *P. hollandica* cells to 300 μg chlorophyll *a* in TMN buffer (50 mM Tris-HCl (pH 8.0), 10 mM NaCl, 10 mM NaF, 5 mM $MgCl_2$). The NaF added to the buffer acts as inhibitor of any phosphatase activity present in broken cells [24]. Henceforth, all procedures were performed at 4°C.

Cells were lysed by the dropwise addition of 30% aqueous Deriphat 160 (Serva), followed by gentle stirring for 1 min. Following centrifugation for 20 min at 15000 × *g* to pellet cell wall and chromosomal material, the optically clear green supernatant was loaded onto a 5–30% sucrose gradient made up in TMN buffer containing 1% Deriphat 160. Centrifugation for 16 h at 60000 × *g* yielded a major non-fluorescent green band which contained high levels of PS I activity, as judged by methylviologen photoreduction (320 μmol O_2 consumed/mg chlorophyll per h [32]). The preparation contained only trace amounts of PS II activity as determined by electron transport from diphenylcarbazide to dichlorophenolindophenol (40 μmol DCPIP reduced/mg chlorophyll per h.

Deriphat extracts of whole cells were prepared as described previously [24]. Non-denaturing electrophoresis of Deriphat extracts and PS I preparations were performed on 6% acrylamide slab gels. [22–24]. Green bands were excised and analyzed by 77 K fluorescence excitation spectroscopy and denaturing electrophoresis.

Denaturing protein electrophoresis

Denaturing LDS polyacrylamide gel electrophoresis was performed at 4°C according to Guikema and Sherman [33]. Prior to loading, the samples were solubilized in 3% LDS sample buffer for 10 min at room temperature. Immunoblotting of gels employed the protocols of Towbin et al. [34]. The antibody recognizing the chlorophyll *a*/*b* binding proteins in Prochlorophytes has been described previously [23]. The antibody to the PS I-P700 protein raised against the *Synechococcus* PCC7942 *Psa*A/*Psa*B polypeptides were a kind gift from Dr. J. Guikema, Kansas State University. Antibody-antigen complexes were visualized by immunodecoration of blots with alkaline-phosphatase-conjugated goat-anti-rabbit IgG (Promega). Protein determinations were carried out according to Lowry et al. [35] using a bovine serum albumin standard.

Results

Fluorescence induction

A common property of all O_2-evolving photosynthetic organisms is the transient increase in PS II fluorescence yield in response to exposure of the cells to a continuous light flux (fluorescence induction). Such transients provide valuable information on PS II and its antenna, since fluorescence emission originates from different sources: (1) an immediate emission following the onset of illumination (F_0) is attributed to decoupled chlorophylls in the PS II antenna, antennae decoupled from photosystems and 'missing hits' at PS II reaction centers; (2) on a millisecond time-scale one sees a progressive contribution to fluorescence emission by the back-reaction from the primary electron acceptor I^- to $P680^+$ to form P680-I in PS II reaction centers which contain the quinone Q_A in the reduced state (F_v). F_v may reach any value between F_0 and maximal fluorescence (F_{max}) and may follow a so-called Kautsky transient, thought to reflect activation of carbon fixation, before reaching steady-state fluorescence (F_s); (3) F_{max} is routinely determined in the presence of the herbicide DCMU, which ensures the presence of a fully-reduced Q_A pool following the first charge separation in each RC II. Assuming that F_0 has a constant contribution to the fluorescence signal during measurement, it is reasonable to take F_{max} as a relative measure of the amount of excitation energy arriving at closed PS II reaction centers and, hence, reflecting the effective absorption cross-section of PS II. Fig. 1 shows typical fluorescence induction curves for the green alga *Chlamydomonas reinhardtii*, the cyanobacterium *Synechococcus* PCC 7941 and *Prochlorothrix hollandica*. *Synechococcus* was characterized by a high F_0, the absence of a Kautsky transient and a low variable fluorescence. The excitation wavelengths were not favorable for absorption by the accessory pigment C-phycocyanin and, therefore, we consider the contribution of phycobilisome emission negligible under our

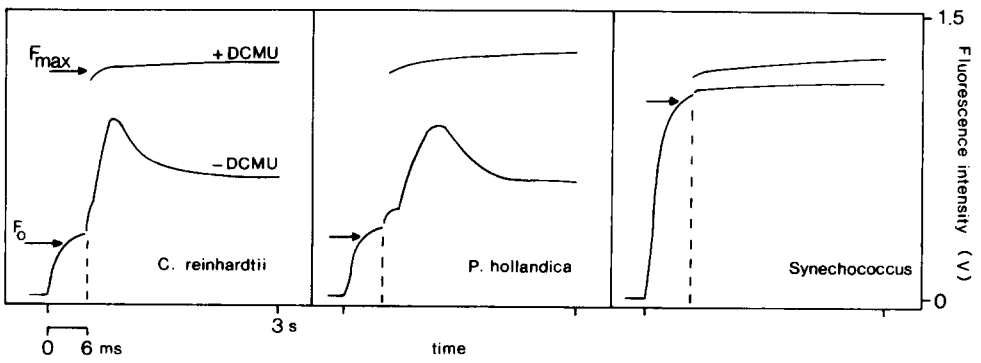

Fig. 1. Fluorescence induction characteristics of the green alga *Chlamydomonas reinhardtii*, the chlorophyll *a*/*b* prokaryote *Prochlorothrix hollandica* and the cyanobacterium *Synechococcus*. Chlorophyll concentrations were adjusted to yield equal F_{max} values. F_{max} was measured as the maximal fluorescence yield in the presence of 10^{-6} M DCMU. The fluorescence rise in the presence of DCMU has been omitted.

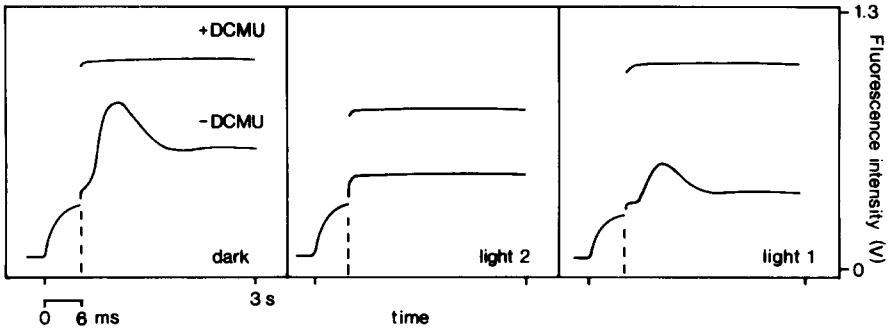

Fig. 2. Fluorescence induction curves of *P. hollandica* adapted to dark, light 2 (652 nm) and light 1 (712 nm), respectively, for 10 min. The fluorescence rise in the presence of DCMU has been omitted.

experimental conditions. Hence, these features are indicative for a PQ pool which is substantially reduced in the dark (see, e.g., Refs. 30,36). Both *P. hollandica* and *C. reinhardtii* showed a low F_0, a distinct Kautsky transient and a substantial variable fluorescence. This suggests that the PQ pool in the prokaryotic *P. hollandica* is more oxidized than that in most dark-adapted cyanobacteria. As is the case for green chloroplasts, its redox state may be subject to modulation through light 1 and light 2 treatments.

State 1 → 2 transitions

Fig. 2 shows the fluorescence induction curves for *P. hollandica* after adaptation to dark, light 1 and light 2. Adaptation to dark and to light 1 yielded similar fluorescence transients and apparently the dark state in *P. hollandica* represented state 1. Adaptation to light 2 was expressed in a lower F_{max} and a higher F_0 value as compared to dark-adapted *P. hollandica* and they are indicative of a state 2 situation. Table I summarizes the values of the important parameters together with the F_0 values in the presence of DCMU. Since cells that were submitted to light 1 and light 2 treatment had an equal number of PS II per cell, we interpret the decrease in F_{max} in state 2 cels as reflecting a decrease in the PS II absorption cross-section. A decrease in F_{max} was expected to be accompanied by a proportional decrease in F_0, should the latter be a function of antenna size only. However, the backreaction in RC IIs which are in the Q_A^- state inevitably contributes to F_0 when measured in the millisecond time-scale. Thus the higher F_0 values (both with and without DCMU) for cells adapted in state 2 reflect a faster accumulation of Q_A^- in state 2 cells. Since the halftime of Q_A^- is less than 50 μs [37], this phenomenon most probably reflects the inability to transfer electrons from the Q_A^- pool to secondary acceptors as a consequence of the redox state of the PQ pool in state 2 cells. State 1 → 2 transitions were completed after 10 min. of light 2 illumination (Fig. 3). Addition of 10 mM of the phosphatase inhibitor NaF during illumination with light 2 caused an even stronger decrease in F_{max} (Fig. 4). Whereas 10 min illumination with light 1 was sufficient to return to a state 1 situation in untreated cells, NaF treated cells were prevented from returning to the high-fluorescent state 1 and they were locked into state 2 (data not shown). In a related paper [24] we reported, that NaF effects an enhanced phosphorylation of the 23 and 25 kDa thylakoid proteins in vitro. These observations indicate that light/redox controlled reversible phosphorylation in *P. hollandica* may form the basis for state 1 → 2 transitions.

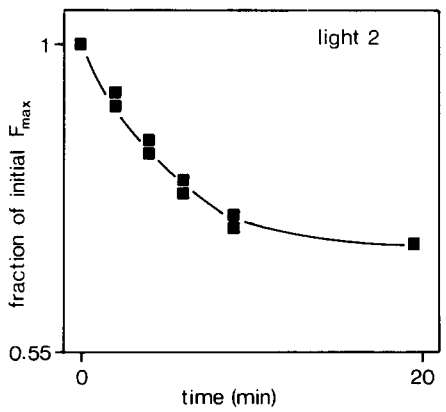

Fig. 3. Kinetics of the state 1 → 2 transition in dark-adapted cells of *P. hollandica* shifted to 20 μmol quanta/m^2 per s of red (652 nm) light. The F_{max} readings were normalized to the F_{max} value of dark-adapted cells.

TABLE I

Initial fluorescence rise (± DCMU) and maximal fluorescence by P. hollandica after adaptation to dark, light 1 (712 nm) and light 2 (652 nm)

Fluorescence intensity is given in mV.

	F_0-DCMU	F_0 + DCMU	F_m
Dark	227	462	1032
Light 1	255	473	1042
Light 2	300	531	805

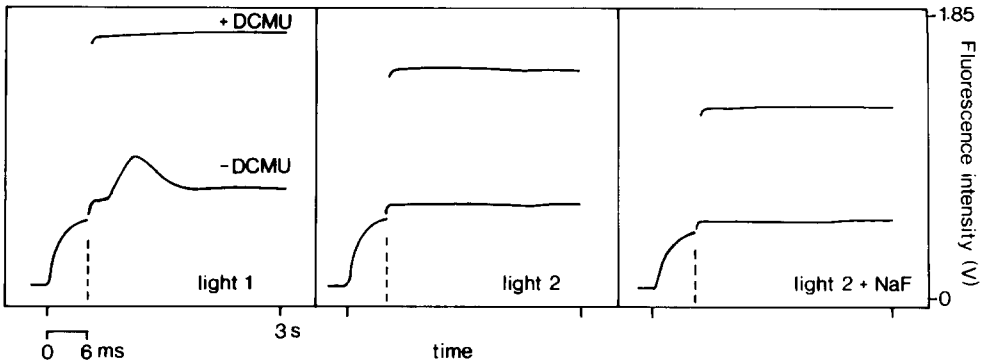

Fig. 4. Fluorescence induction curves of *P. hollandica* adapted to light 1 (712 nm) and light 2 (652 nm) without and with 10 mM Na-fluoride (NaF) The fluorescence rise in the presence of DCMU has been omitted.

Lower PS II fluorescence yield accompanied increased PS I activity

Basic to state 1 → 2 transitions is the ability of the cell to discriminate between wavelengths promoting either PS I or PS II activity. PS II activity can be defined as photochemical quenching (Q_P) of PS II fluorescence, since light energy is trapped and converted into chemical energy with a high redox potential. Non-photochemical quenching (Q_{NP}) of PS II fluorescence is attributed to the formation of a ΔpH over the thylakoid membrane, potentially driven by PS I activity only. These parameters can be simultaneously determined in the modulated fluorimeter setup described in Ref. 31. The measurement of modulated PS II fluorescence is a well-known tool in distinguishing between photosynthetic properties in light 1 or light 2 [13,25,30,31]. Dark-adapted cells built up a minor Q_{NP} due to the probing flashes (650 nm), whereas Q_P was unaffected (data not shown). Exposing dark-adapted cells to light 2 (20 μmol quanta/m^2 per s) revealed that the formation of Q_{NP} slowed down drastically (Fig. 5). Illumination with 20 μmol quanta/m^2 per s light 1, which is much less absorbed, invoked a pronounced formation of Q_{NP} with a clear fast phase (< 1 min) followed by a slow phase. This suggests that the absorption cross-section for Q_{NP} formation was limited to the long-wavelength-absorbing components of PS I only and served to perpetuate the state 1 situation resulting from dark incubation. When cells were adapted for 30 min to either light 1 or light 2 Q_{NP} had reached identical steady-state levels. This phenomenon may indicate a movement of the antenna away from PS II towards PS I where it contributes to the absorption cross-section of PS I and hence Q_{NP} formation. Therefore, the observed decrease in PS II fluorescence yield in state 2 (Fig. 2) may represent a gain in energy conservation by PS I in the form of enhanced photophosphorylation due to cyclic electron flow.

The chlorophyll a / b antenna is energy-coupled to PS I

Equal concentrations of total chlorophyll in samples of the green alga *Chlamydomonas reinhardtii* and *P. hollandica* yielded distinctly different fluorescence rise patterns (Fig. 6). Not only did the fluorescence emission from *P. hollandica* reach much lower maximal levels, also the rise to F_{max} was much slower. These

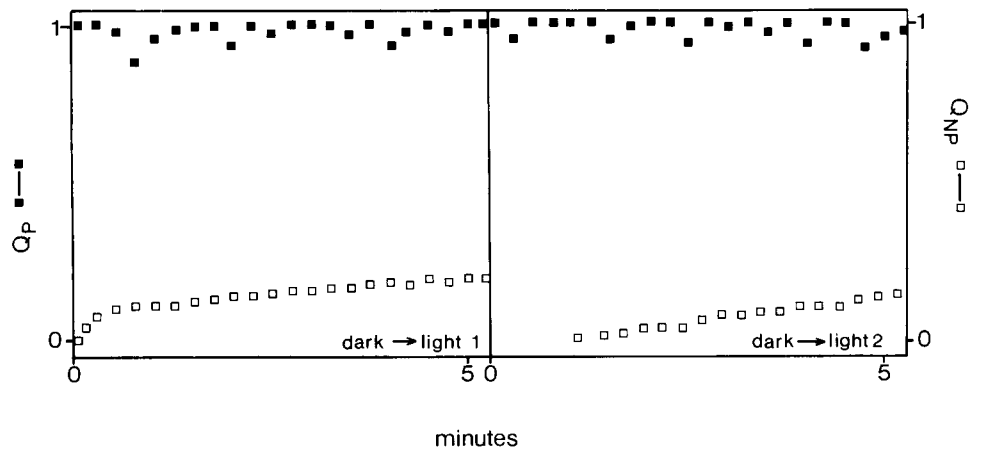

Fig. 5. Kinetics of photochemical (Q_P) and non-photochemical (Q_{NP}) fluorescence quenching for *P. hollandica* shifted from dark to 20 μmol quanta/m^2 per s of light 1 (712 nm) and light 2 (652 nm)

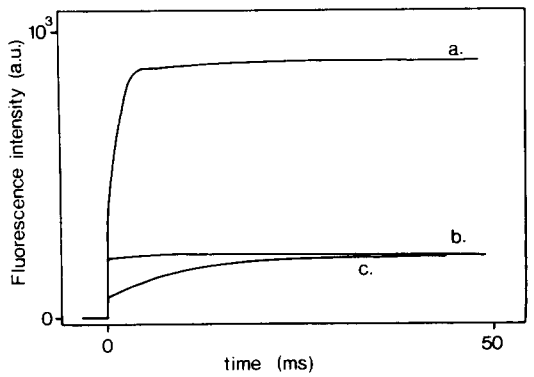

Fig. 6. Initial rise to maximal fluorescence in the presence of 10^{-6} M DCMU for fully dark-adapted samples (P680-Q_A) of *Chlamydomonas reinhardtii* (a) and *P. hollandica* (c). The actinic light (90 μmol quanta/m^2 per s broad band blue light) was sufficient to induce light-saturated photosynthesis in both species. Trace b represents the fluorescence rise after 15 s illumination with the actinic light effectively closing all RC IIs.

findings suggest, that (1) in *P. hollandica* a larger fraction of the chlorophylls is associated with PS I, and (2) that the antenna serving PS II in *P. hollandica* is much smaller than its counterpart in *Chlamydomonas* sp. Low-temperature fluorescence spectra of whole *P. hollandica* cells excited at 435 nm showed emission peaks at 686, 696 and 713 nm attributable to antenna + PS II, PS II and PS I, respectively (Fig. 7). When cells were excited at 470 nm (preferentially absorbed by Chl *b*) a pronounced increase in PS I emission was observed. This further suggests profound differences between the *P. hollandica* light harvesting antenna and LHC II.

Antenna–PS I reaction center interactions

In an attempt to establish the association of the antenna with the PS I reaction center, we analyzed PS I complexes obtained from non-denaturing green gels, as well as purified PS I particles. These preparations depend on the use of the detergent Deriphat 160, which we have previously shown to stabilize PS I complexes on green gels [24].

Deriphat solubilization of *P. hollandica* membranes yields a number of green bands upon non-denaturing electrophoresis (Fig. 8A). The major complexes CP1–3 were non-fluorescent upon UV transillumination, indicating the presence of actively quenching PS I centers [22–24]. The major fluorescent complex, CP4, migrates more rapidly than the PS I complexes. In this gel system, there is virtually no detectable free Chl running at the gel front. Denaturing electrophoresis of CP1–4 revealed the presence of the *Psa*A and *Psa*B PS I reaction center polypeptides in CP1–3 (Fig. 8B), while

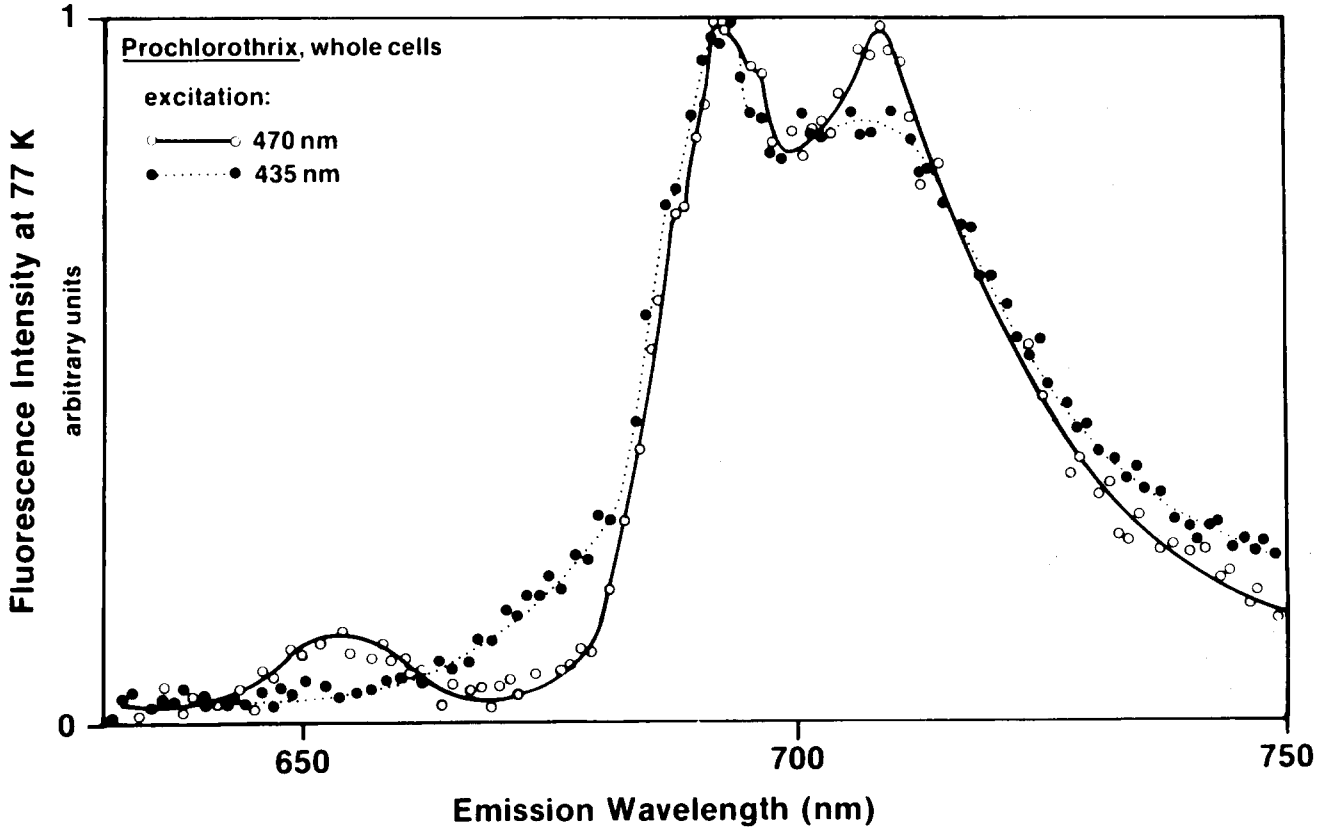

Fig. 7. Low-temperature (77 K) fluorescence emission spectra of whole cells of *P. hollandica* at wavelengths exciting chlorophyll *a* (435 nm) and chlorophyll *b* (470 nm)

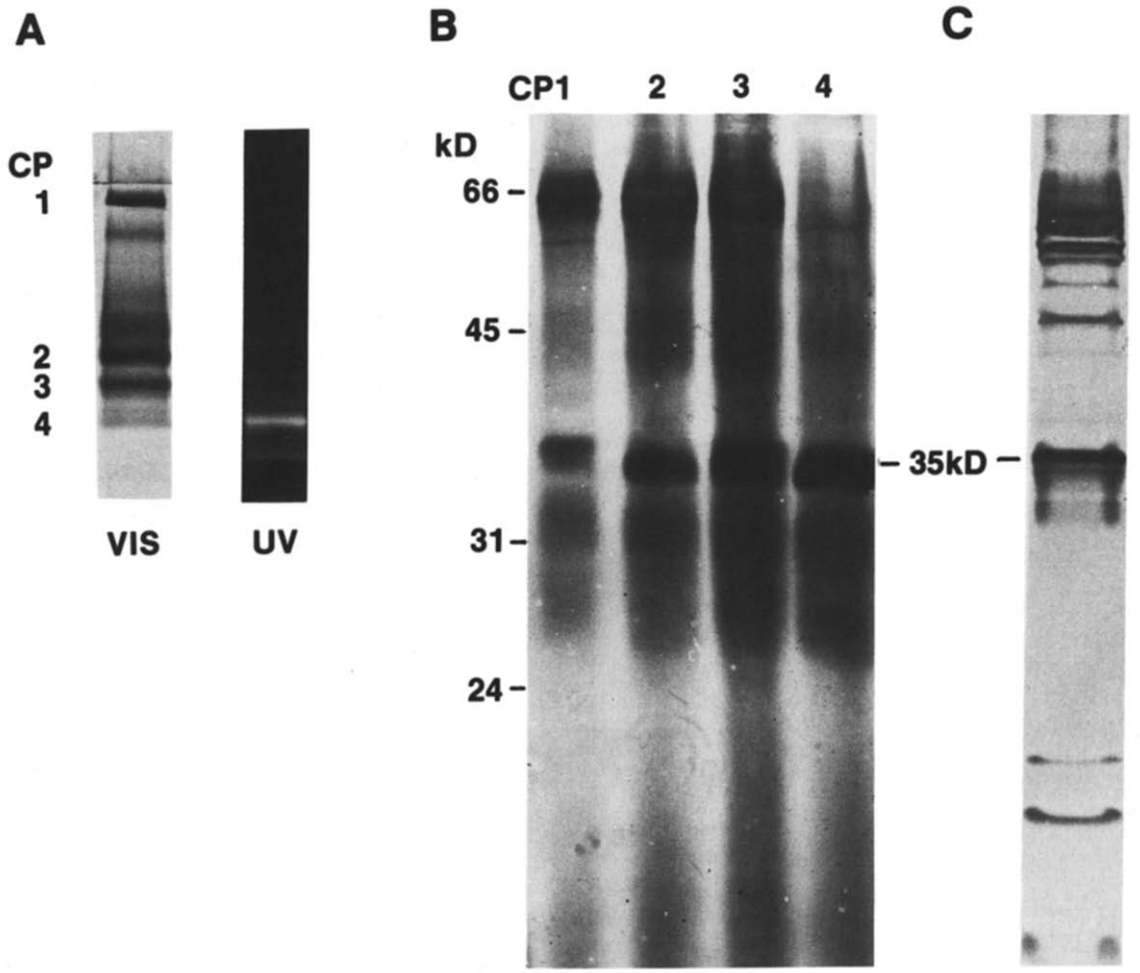

Fig. 8. Analysis of Chl-protein complexes protein gel electrophoresis. Panel A: Deriphat-solubilized green complexes resolved by non-denaturing electrophoresis and photographed during transillumination by visible (VIS) and long-wavelength ultraviolet (UV) light. Panel B: polypeptide composition of CP1–4 obtained following denaturing electrophoresis. Panel C: polypeptide composition of PS I particles obtained following sucrose gradient centrifugation; note the presence of the 35 kDa antenna protein in both the green gel bands and the PS I preparation.

antibodies to the major Chl *a*/*b* antenna complex identified the antenna apoproteins in CP1–4 (Fig. 9). These data indicate that CP1–3 represent oligomeric forms of a PS I/antenna complex, while CP4 is composed of free antenna Chl-proteins. As the majority of the Chl is found in CP1–4 and the most abundant Chl-protein complexes are CP1–3, it is clear that much of the antenna comigrates with PS I on non-denaturing gels. To confirm further that the major 35 kDa antenna protein was associated with PS I, denaturing electrophoresis of PS I particles purified by sucrose gradient centrifugation revealed the presence of this protein (Fig. 8C).

A previous study has suggested that isolated PS I complexes obtained by dodecyl β-D-maltoside solubilization retain an antenna complex which facilitates energy transfer to PS I as shown form the 77 K emission at 717 nm [22]. The antenna shows 77 K emission at 689 nm, whereas free antenna complexes, i.e., antenna complexes in which no PS I or PS II components could be detected, emit at 687 nm [22]. Hence, we assign the 77 K emission at 690 nm mainly to fluorescence by the abundant antenna both in whole cells, isolated thylakoids and thylakoid subfractions. This emission is always pronounced irrespective of freezing procedures and it may be due to a loss in energy transfer to photosystems following freezing. The data of Fig. 10 confirm that the Chl *a*/*b* antenna was functionally bound to PS I both in the green gel complexes and in PS I particles obtained by Deriphat solubilization. Fluorescence emission spectra show that such PS I preparations yield fluorescence from both the antenna (690 nm emission) and chlorophyll of the PS I core (approx. 717 nm). Setting the emission monochromator at the long-wavelength peak yields an excitation peak at 468 nm, diagnostic for Chl *b*. These data

along with the in vivo fluorescence studies (Figs. 6 and 7) lend further evidence for a stable functional association of the major antenna complex to PS I centers.

Wavelength dependence of phosphorylation

Analysis of the phosphorylation state of the 35 kDa Chl *a*/*b* protein as a consequence of light condition shows that the protein was maximally phosphorylated in light 2 (Fig. 11). This enhancement of phosphorylation was seen both in the polypeptides resolved from green gel bands of PS I samples and in whole cell extracts (Fig. 11). These data show that the 35 kDa Chl *a*/*b* antenna contained the major phosphoprotein in whole thylakoid membranes and that it was associated with PS I irrespective of phosphorylation state. Indeed, the phosphorylation state in whole membranes reflected closely the phosphorylation state of the fraction which was bound to PS I. All these data together suggest that the phosphorylation state of the antenna is modulated similarly to that in chloroplasts, but the overall antenna organization does not resemble that of LHC II.

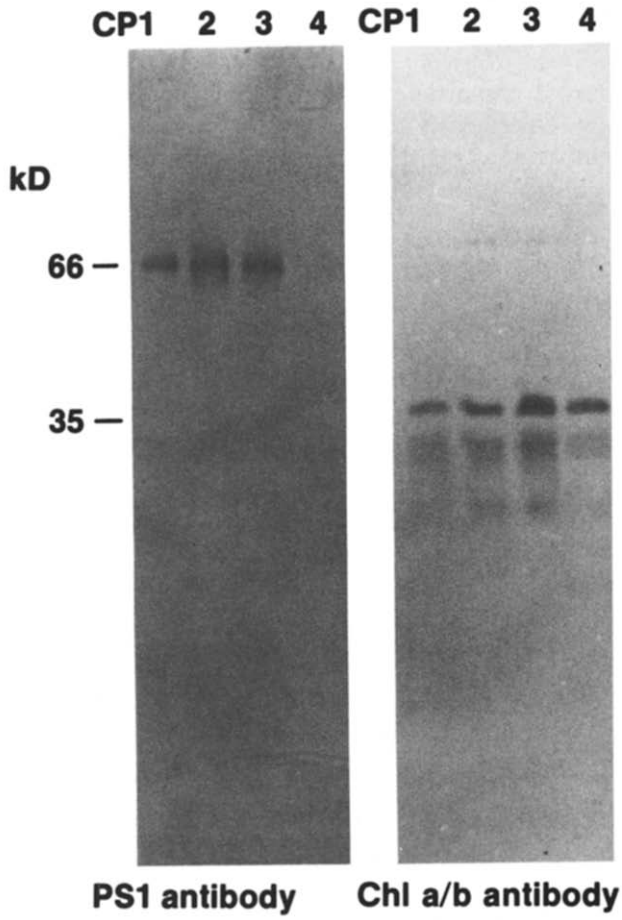

Fig. 9. Immunoblot analysis of CP1–4. Polypeptides of CP1–4 were separated by denaturing gel electrophoresis and immunoblots probed with antibodies raised against the PS I reaction center (left-hand panel) and the major Chl *a*/*b* antenna apoprotein (right-hand panel).

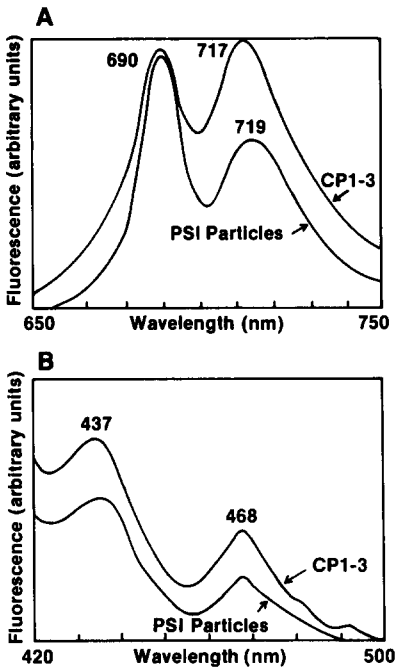

Fig. 10. Fluorescence emission and excitation studies of CP1–3 and isolated PS I particles. Panel A: 77 K emission profiles with the excitation monochromator set to 435 nm. Panel B: excitation spectra of the same samples with the emission monochromator tuned to the long-wavelength emission peak at 717 nm.

Discussion

Prokaryotic Chl *a*/*b* antennae share few common properties with the LHC II of green chloroplasts. Their highly hydrophobic apoproteins are of 30–35 kDa molecular mass [20–24,40,41]. No immunological cross-reactivity could be detected between prokaryotic and eukaryotic antenna structures, whereas the antenna apoproteins of *Prochlorothrix* and *Prochloron* are immunologically related [23]. The Chl *a*/*b* ratio of the antenna is 2.5 [21,24] as opposed to 1.5 in LHC II [10]. The orientation of the antenna in the thylakoid membrane differs from that of LHC II [42]. The organization of chlorophyll *b* in the antenna, as judged from circular dichroism spectroscopy, differs from that of LHC II [42]. Low-temperature fluorescence spectra of whole cells and thylakoid subfractions indicate a strong energy coupling between the Chl *a*/*b* antenna and PS I (see Refs. 21,22 and this article). Additionally, the 35 kDa Chl *a*/*b* apoprotein copurifies preferentially with PS I (Refs. 21–24; this article). While changes in light intensity and wavelength modulate the phosphorylation state of the 35 kDa protein (see also Ref. 24), a physical association with PS I is retained (this article). The non-phosphorylated form of the antenna allows for efficient energy transfer not only to PS

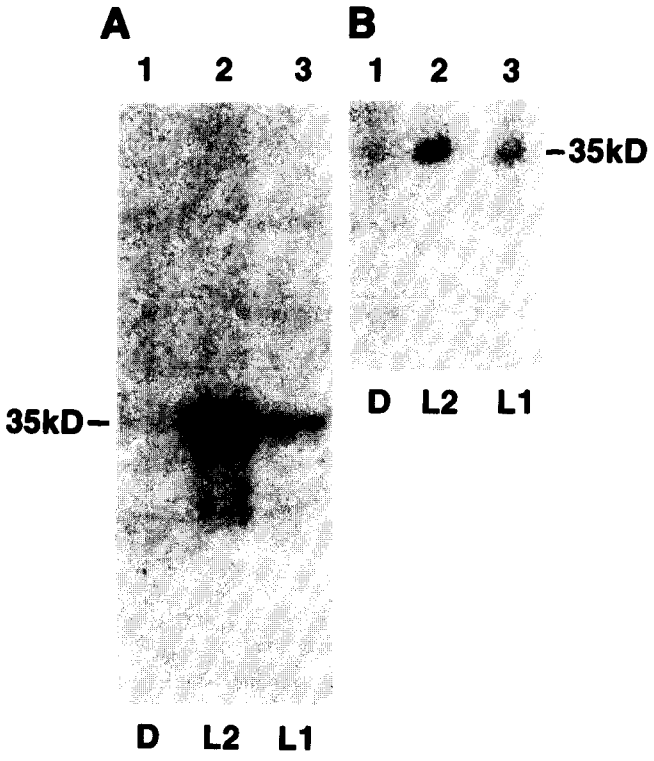

Fig. 11. Wavelength dependence on phosphorylation. Panel A: pattern of phosphoproteins obtained in whole cell extracts following in vivo phosphorylation in the dark (D), light 2 (L2) and light 1 (L1) Note the major phosphorylation of the 35 kDa antenna apoprotein; the minor phosphorylation at approx. 30 kDa does not correspond to a Chl *a*/*b* apoprotein on immunoblots (data not shown) Panel B: pattern of phosphoproteins obtained following electrophoresis of PS I particles prepared from in vivo ^{32}P-labeled cells in the Dark (D), light 2 (L2) and light 1 (L1)

I but also to PS II. Non-denaturing detergent solubilization of the thylakoid membranes routinely shows the Chl *a*/*b* antenna to be associated with PS I [24]. On the basis of these findings we conclude that prokaryotic Chl *a*/*b* antennae involving the 35 kDa apoprotein form a structural and functional antenna of PS I, capable of energy transfer to PS II.

Here we have confirmed an earlier report [25] showing that state 1 → 2 transitions do occur in photosynthetic prokaryotes carrying Chl *a*/*b*, despite earlier claims for *Prochloron* sp. [20]. State 1 is obtained after prolonged dark incubation or after preillumination with far red light. State 2 is obtained by illumination with wavelengths absorbed by both Chl *a* and *b*. Lower fluorescence yield in state 2 coincided with increased energy coupling of antenna with PS I as judged from fluorescence quenching. This feature bears close resemblance to the situation engaged in green chloroplasts [13,14]. A number of observations support the possibility of reversible phosphorylation being the molecular mechanism underlying the state 1 → 2 transitions:

(1) The redox state of the PQ pool is subject to change depending on illumination conditions, a situation unlike that in cyanobacteria [28,30,36]. It may allow for light/redox control over the kinase activity involved in the phosphorylation of antenna apoproteins [15,16].

(2) A 35 kDa apoprotein of the main Chl *a*/*b* antenna is the main target for kinase activity in *P. hollandica* [24].

(3) The kinetics of the state 1 → 2 transitions are on a time-scale of minutes, enough for considerable phosphorylation of the antenna apoproteins in vitro [24] and in vivo [38]. 4. Addition of 10 mM NaF, an effective phosphatase inhibitor in *P. hollandica* [24], locks the photosynthetic system irreversibly in state 2.

The involvement of reversible phosphorylation in state 1 → 2 transitions in cyanobacteria is a subject of continued discussion [16,39]. However, it is less controversial in photosynthetic prokaryotes such as *P. hollandica*, since it involves a thylakoid intrinsic antenna complex, known to be a major kinase substrate [20,24,40].

The nature of the prokaryotic Chl *a*/*b* antenna and the apparent mode of regulation of photosynthetic activity in *P. hollandica* suggest a different type of thylakoid organization than the ones encountered in green chloroplasts or in phycobilisome containing cyanobacteria (Fig. 12). In dark or far red light illumi-

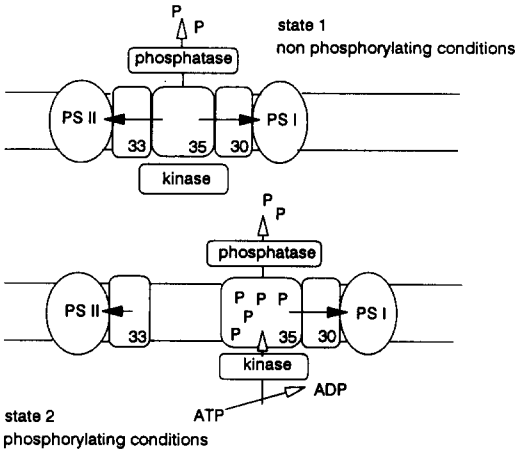

Fig. 12. Model for the molecular mechanism of state 1 → 2 transitions in photosynthetic prokaryotes carrying chlorophyll *a*/*b* antennae. The chlorophyll *a*/*b* antenna consists of a bulk antenna which is located on 30 and 35 kDa apoproteins and associates preferably with PS I. A minor chlorophyll *a*/*b* antenna is carried by a 33 kDa apoprotein and is found to copurify with PS II. The 35 kDa antenna protein forms the major target protein of light/redox-controlled kinase activity. Upon phosphorylation, the bulk antenna excludes PS II centers and enters a more tight association with PS I. Under such conditions, the energy transfer to PS I is enhanced. This process reverses to a state of balanced energy transfer by the bulk antenna following dephosphorylation of the antenna in either darkness or far red illumination.

nated *P. hollandica* one finds a non-phosphorylated antenna shared by PS II and PS I and capable of efficient energy transfer to both reaction centers. Following sufficient donation of electrons by PS II to the electron-transport chain, the PQ pool becomes more reduced. This or a related event is responsible for the activation of a thylakoid-based protein kinase activity. The phosphorylation of the antenna apoprotein is instrumental in breaking the energy coupling between PS II and the antenna. Consequently, a stronger association of the antenna with PS I is enforced, visualized from an increased potential to utilize quanta harvested in this antenna for the establishment of a PS-I-driven ΔpH over the thylakoid membrane. This is the first account of state $1 \rightarrow 2$ transitions involving an antenna structurally and functionally associated with PS I, a feature unique for Chl *a/b* prokaryotes. Our model for the prokaryotic Chl *a/b* antenna/photosystem interactions demands that PS I and PS II centers should not be laterally separated. As electron micrographs of *P. hollandica* have revealed that stacking is not extensive [18,23], it is certainly likely that lateral heterogeneity of reaction centers is limited.

Van der Staay et al. [41] recently presented a study examining the antenna organization in *P. hollandica* and proposed that the Chl *a/b* antenna associates exclusively with PS II. We believe that these authors have reached a conclusion conflicting the one presented here for the following reasons. (1) Their study did not address the question of energy transfer from antenna to photosystems, an essential part of our study. (2) The authors could not unequivocally identify the antenna apoproteins in their preparations, whereas our laboratories have characterized the polypeptide composition of Chl-protein complexes by employing antibodies to the major antenna apoproteins [22–24]. (3) Van der Staay et al. [41] obtained their Chl-protein complexes by Zwittergent solubilization of thylakoids. Recent work has indicated that this harsh detergent disrupts antenna/reaction center interactions [46].

From an evolutionary point of view Chl *a/b* prokaryotes are now considered unlikely ancestors of the green chloroplast and they were reassigned to the cyanobacterial radiation with no apparent relationship to each other [43–45]. Interestingly, our results seem to indicate a common molecular mechanism for regulation of energy transfer in *Prochloron* and *P. hollandica* [24], including an immunologically related antenna apoprotein [23] that serves as the major target for phosphorylation. This suggests that: (1) Prochlorophytes may have evolved from a common ancestor, and (2) light/redox-controlled reversible phosphorylation of thylakoid intrinsic Chl-protein complexes as a regulatory mechanism may have preceded the evolution of the LCH II, the main phosphorylated pigment-protein complex in green chloroplasts.

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