Phosphorylation of the D1 Photosystem II Reaction Center Protein Is Controlled by an Endogenous Circadian Rhythm

Isabelle S. Booij-James, W. Mark Swegle, Marvin Edelman, and Autar K. Mattoo*

Vegetable Laboratory, The Henry A. Wallace Beltsville Agricultural Research Center-West, United States Department of Agriculture-Agricultural Research Service, Beltsville, Maryland 20705–2350 (I.S.B.-J., M.S., A.K.M.); and Department of Plant Sciences, The Weizmann Institute of Science, Rehovot 76100, Israel (M.E.)

The light dependence of D1 phosphorylation is unique to higher plants, being constitutive in cyanobacteria and algae. In a photoautotrophic higher plant, *Spirodela oligorrhiza*, grown in greenhouse conditions under natural diurnal cycles of solar irradiation, the ratio of phosphorylated versus total D1 protein (D1-P index: \([\text{D1-P}] / [\text{D1}]\)) of photosystem II is shown to undergo reproducible diurnal oscillation. These oscillations were clearly out of phase with the period of maximum in light intensity. The timing of the D1-P index maximum was not affected by changes in temperature, the amount of D1 kinase activity present in the thylakoid membranes, the rate of D1 protein synthesis, or photoinhibition. However, when the dark period in a normal diurnal cycle was cut short artificially by transferring plants to continuous light conditions, the D1-P index timing shifted and reached a maximum within 4 to 5 h of light illumination. The resultant diurnal oscillation persisted for at least two cycles in continuous light, suggesting that the rhythm is endogenous (circadian) and is entrained by an external signal.

Photosynthetic oxygen evolution involves a supramolecular protein-pigment complex, PSII (Ort and Yocum, 1996; Mattoo et al., 1999). The PSII reaction center, which includes the D1 and D2 protein heterodimer, binds most of the nonprotein components of the PSII electron transport chain (Nanba and Satoh, 1987; Michel and Deisenhofer, 1988; Mattoo et al., 1989; Hankamer et al., 1997). Light is central to the metabolism of the D1 protein, regulating its synthesis (Mattoo et al., 1984), intramembrane translocation (Mattoo and Edelman, 1987; Callahan et al., 1990), posttranslational phosphorylation (Michel et al., 1988; Elich et al., 1992) and acylation (Mattoo and Edelman, 1987; Mattoo et al., 1993), and its rate of degradation (Mattoo et al., 1984; Greenberg et al., 1987; Aro et al., 1993). The posttranslational phosphorylation of D1 occurs at its N-terminal Thr residue, catalyzed by a light-dependent redox-regulated kinase (Michel et al., 1988; Elich et al., 1992).

Protein phosphorylation is a mechanism used by eukaryotes to regulate cellular activity (Stone and Walker, 1995). In plants, protein phosphorylation is a key response to environmental signals such as wounding (Usami et al., 1995) and light (Allen, 1992). The greatest concentration of phosphoproteins in plants is found in the chloroplast membranes (Bennett, 1991). Phosphorylation and dephosphorylation of the D1 protein are strictly light dependent (Elich et al., 1993, 1997). Reversible, redox-sensitive phosphorylation of the light-harvesting chlorophyll apoprotein is thought to be a mechanism maximizing quantum yield by equalizing electron flow through PSII and PSI (Allen, 1992); however, the role of phosphorylation of D1 or other PSII proteins is largely unknown. It has variously been suggested that phosphorylation regulates D1 degradation, maintaining it as a storage form prior to its replacement (Rintamäki et al., 1995a), or that it regulates dimerization of the reaction center (Santini et al., 1994).

The effect of light intensity on D1 metabolism has, without exception, been studied under unnatural conditions by illuminating plants or thylakoid membranes with radiance of constant intensity. There is usually a pretreatment of complete darkness or exposure to a light intensity different from that ultimately to be used. The intensity of the constant radiation not only influences the rate of D1 degradation, but also the ratio of phosphorylated to unphosphorylated D1 (Elich et al., 1992; Rintamäki et al., 1995a). To gain insight into the physiological function of phosphorylation of D1, we characterized this process under a natural diurnal cycle, i.e. with naturally fluctuating solar intensity. Antibodies were raised against synthetic peptides, which selectively detect each form of D1, phosphorylated and unphosphorylated, and allow measurement of the proportion of D1 that is phosphorylated under specific conditions.

---

1 This work was supported in part by the Avron-Wilsttter Minerva Center for Research in Photosynthesis (to M.E.).
* Corresponding author; e-mail mattooa@ba.ars.usda.gov; fax 301–504–5555.
Article, publication date, and citation information can be found at www.plantphysiol.org/cgi/doi/10.1104/pp.013441.
We show here that *Spirodea oligorrhiza* plants, entrained to the natural light/dark cycle in a greenhouse, exhibit diurnal oscillation of the ratio of phosphorylated to total D1 protein ([D1-P]/[D1] + [D1-P]), which is paralleled by de novo D1 phosphorylation in vivo. When plants were shifted from a light/dark cycle to continuous light conditions, the D1-P index rhythm was maintained for several cycles. These results show circadian regulation of the phosphorylation of D1, a key PSII reaction center protein of the chloroplast membranes.

**RESULTS**

**Immunodifferentiation of Phosphorylated and Non-Phosphorylated D1**

In vivo light-dependent phosphorylation of D1 protein is transient and increases with increasing light intensity (Elich et al., 1992; Rintamaki et al., 1995a). The light (redox) dependence can be reproduced in vitro by incubating isolated thylakoids in the dark under redox-generating conditions using ferredoxin and reducing power (Elich et al., 1992, 1993). Thylakoid membranes, extracted from *S. oligorrhiza* plants that had been held in the dark for 3 d to allow for protein dephosphorylation, were phosphorylated in vitro. Affinity-purified antibodies (anti-SP1 and anti-SP2) were tested for their abilities to detect phosphorylated and unphosphorylated D1 (Fig. 1). Dark incubation of thylakoids with ATP, NADPH, and ferredoxin resulted in the progressive phosphorylation of D1 as the time of incubation increased. A distinct separation into two D1 forms, identified as phosphorylated (D1-P) and unphosphorylated D1 (Elich et al., 1992), was obtained. Anti-SP2 recognizes both forms of D1, whereas anti-SP1 recognizes only the unphosphorylated form (Fig. 1B). Nonrecognition of D1-P by anti-SP1 suggests that the phosphorylated form of the N-terminal TAILERR... region assumes a more structured, protected conformation than the unphosphorylated form. Such conformational changes upon phosphorylation are well documented (Barford et al., 1991) and have been evoked for chlorophyll proteins sp29 (Croce et al., 1996) and light-harvesting chlorophyll apoprotein (Nilsson et al., 1997).

Plants rapidly dephosphorylate D1-P in the light in the presence of DCMU, which inhibits D1 kinase activity (Elich et al., 1993, 1997). Under this condition, the ratio of unphosphorylated to phosphorylated D1 should increase. Such was the case when immunoblots of the DCMU-treated samples were probed with anti-SP2 and anti-SP1 (Fig. 1C, +DCMU). In a converse manner, under conditions where phosphorylation is inhibited by DCMU and D1-P dephosphorylation is inhibited by NaF (an inhibitor of phosphatase), D1 and D1-P levels should remain unchanged. This was the case, as shown in a separate experiment with anti-SP2 (Fig. 1C, +DCMU+NaF). These observations confirm the specificity of the antibodies and the identification of the upper and lower immunoreactive bands in Figure 1, B and C, as D1-P and unphosphorylated D1, respectively. These results are consistent with previous conclusions (Elich et al., 1992, 1993).

**Diurnal Oscillations of the D1-P Index**

Thylakoid samples isolated from *S. oligorrhiza* plants grown in the greenhouse under natural diurnal cycles of solar irradiation were immunoblotted and analyzed for D1 and D1-P. The data in Figure 2 are plotted as the D1-P index, which is the apparent percentage of D1 in the phosphorylated form, versus time over three light/dark cycles. Reproducible oscillations were obtained in the D1-P index, which were clearly out of phase with the period of maximum radiation (Figs. 2A, 3, and 4). Thus, light intensity per se does not directly correlate with the ratio of phosphorylated versus total D1.

The D1-P index maximum consistently preceded the peak of maximum light intensity (Fig. 2A). This 24-h oscillation was observed on three consecutive days and on all the additional occasions when the experiment was repeated (Figs. 3 and 4). During these experiments, the temperature in the greenhouse was kept constant (at 13°C, 20°C, or 26°C; Fig. 3B presents data with plants maintained at 26°C) or...
was allowed to rise and fall with changing sunlight intensity (Fig. 3A). The oscillations and D1-P index pattern seen in Figure 2A were indifferent to temperature within the range tested (Fig. 3, A versus B). These data show that the phase of the rhythm as entrained to the light/dark cycle is not much affected by different temperatures.

At the end of the light period and 2 h into darkness, a set of plants was exposed for 5 min to 300 μmol m⁻² s⁻¹ fluorescent light (arrow) and thereafter returned to darkness (■). Error bars indicate ss based on a sample size of four. B. D1-P index maintains oscillations in free-running conditions in continuous light. Plants were grown in the greenhouse under natural light/dark cycles for a week in medium lacking Suc. Then, at the end of the light cycle and 2 h into darkness, a set of plants was left in the greenhouse until the end of the experiment (arrow), whereas another was brought into the laboratory and incubated in continuous light at 200 μmol m⁻² s⁻¹ until the end of the experiment. Error bars indicate ss based on a sample size of three.

In Vivo D1 Phosphorylation Mirrors D1-P Index Oscillations

The preceding experiments were based on immunological separation of D1 and D1-P to measure the D1-P index. To determine if these steady-state patterns reflected de novo phosphorylation, the in vivo patterns of D1 synthesis and phosphorylation were determined through the diurnal cycle using [³⁵S]Met and [²⁵P]orthophosphate, respectively. The pattern for D1 phosphorylation mirrored that for the D1-P index, both peaking 4 h into the light phase (at 10 AM). In contrast to its phosphorylation, D1 continued to be synthesized as the day progressed, peaking after about 10 h of the light phase (at about 4 PM), and well beyond the maximum in light intensity (Fig. 4, high light). The steady-state level of D1 as quantified by immunoblot analysis of these samples did not show any major alterations.

Similar patterns and identical peak times were obtained on a cloudy day, with maximum greenhouse illumination at 200 μmol m⁻² s⁻¹ and peaks for D1 phosphorylation, D1-P index and D1 synthesis all...
circadian oscillations in D1 phosphorylation are part of a regulatory system controlling the operation of the photosynthetic apparatus, as well as a signal to alter the metabolism of the D1 protein. Rieselmann and Piechulla (1992) suggested that proteins damaged during the light cycle might be substituted at the very beginning of the light period of the following day. If this mechanism were under the control of an endogenous, circadian rhythm, it would enable restoration of thylakoid membrane protein complexes early in the morning to allow optimal photosynthetic reactions during the day.

The protein kinase that phosphorylates D1 is localized in the chloroplast membranes (Elich et al., 1992), but is likely encoded by a nuclear gene because no open reading frame for a kinase-like gene has been identified in the chloroplast genome (Sugiura, 1992). The endogenous rhythm in D1 protein phosphorylation does not reflect the quantity of the kinase activity measured in thylakoids. It is possible that activation (plastoquinone redox) state of the D1 kinase (Elich et al., 1992) or the phosphatase (Elich et al., 1993) may be involved. However, whether it is a consequence of redox versus direct regulation of the kinase, the outcome still is clock regulation of the kinase, which continued to rise till late in the afternoon, or photoinhibition. Thus, the oscillations are indicative of an endogenous regulator controlling D1 metabolism and function.

Circadian control of D1 phosphorylation adds another dimension to the as yet unsolved role of light-dependent D1 protein turnover or D1 phosphorylation in chloroplast function. D1 phosphorylation has been suggested to be a means of turning off PSII, or rerouting electron transport, to protect the photosystem from damage caused by high light intensity (Rintamäki et al., 1995a). However, we show here that the greatest amount of phosphorylation occurs hours before maximal light intensity, and at light intensities well below those saturating for photosynthesis (Jansen et al., 1996) or initiation of photoinhibition (Jansen et al., 1999). We note here that the rate of D1 degradation is least in the dark but increases during the day with increase in light intensity (Mattoo et al., 1984; Jansen et al., 1999). It is possible that circadian oscillations in D1 phosphorylation are part of a regulatory system controlling the operation of the photosynthetic apparatus, as well as a signal to alter the metabolism of the D1 protein.

DISCUSSION

We demonstrate that beyond the known redox regulation of phosphorylation of the D1 photosystem II reaction center protein (Elich et al., 1992; Silverstein et al., 1993), the overriding control is exerted by an internal, circadian clock. Diurnal rhythm of D1 phosphorylation follows parameters that are fundamental to circadian rhythms (Golden et al., 1997; Dunlap, 1998; McClung, 2001), i.e. it has a 24-h periodicity and can be entrained. In most circadian systems, light is a primary signal for entraining rhythmic activity to the daily light/dark cycle by resetting the phase of the clock without altering the cycle length (Feldman, 1982; Wilkins, 1992; Anderson and Kay, 1996). This appears true for the D1-P index as well. The clock was reset by interrupting the dark period with continuous light of ≥200 μmol m⁻² s⁻¹. Therefore, it appears that light resets the phase and then acts in concert with the clock to regulate D1 phosphorylation in vivo. The nonpersistence of rhythmic behavior in the D1-P index in total darkness is likely due to the fact that light is an absolute requirement for most chloroplast activities (Oort and Yocum, 1996), in particular D1 dynamics (Mattoo et al., 1999).

Figure 4. D1-P index (□) parallels in vivo labeling of D1 with [³²P]orthophosphate (●), but is independent of de novo D1 synthesis (○)—quantified by isotope incorporation into D1 (percentage of maximum)—and the daylight maximum (broken lines). Light intensity at the indicated times is shown in micromoles per meter per second. Data points were averaged from four independent experiments. High light (on the left) indicates experiments done on a sunny day. Low light (on the right) indicates experiments done on a cloudy day.

occurring at 100 ± 25 μmol m⁻² s⁻¹ (Fig. 4, low light). These intensities are well below the initiation point for photoinhibition in S. oligorrhiza (>500 μmol m⁻² s⁻¹; Jansen et al., 1996).
D1 protein of the moss plants and algae also bears further investigation. The organism. The state of D1 phosphorylation in lower to determine if D1 can be phosphorylated in this

a single psbA and c

maki et al., 1995b). However, information on gene diance in vivo or under in vitro conditions (Rintanenuus, 1967) containing 0.5% (w/v) Suc under 25 µmol m⁻² s⁻¹ cool-white fluorescent light. Plants were transferred to medium lacking Suc for at least 48 h before each experiment. Thylakoids were prepared according to published methods (Elich et al., 1992). The final thylakoid pellet was suspended in a small volume of buffer A (10 mM Tricine-NaOH, pH 7.8, 100 mM sorbitol, 10 mM MgCl₂, and 10 mM NaCl) such that the chlorophyll concentration was greater than 250 µg mL⁻¹. Chlorophyll concentrations were determined in 80% (w/v) acetone (Arnon, 1949).

In Vitro Phosphorylation

In vitro phosphorylation of S. oligorrhiza thylakoids was carried out in the dark essentially as described (Elich et al., 1992). Thylakoids were diluted to a chlorophyll concentration of 200 µg mL⁻¹ in buffer A containing 10 mM NaF, 0.5 mM NADPH, 3.5 µM ferredoxin, and 0.2 mM ATP. Reactions were stopped at the indicated times by addition of 0.5 volumes of 3X SDS sample buffer (Mattoo et al., 1981).

Diurnal Oscillations Experiments

S. oligorrhiza plants were maintained on medium lacking Suc under fluorescent lighting as above and were then shifted to the greenhouse at Beltsville, MD for at least 3 d on the same medium before experiments were started. Temperature and light intensity were recorded at 1- or 2-h intervals. Samples (10–20 plants) were harvested in quadruplicate, immediately frozen on dry ice, and then stored at −80°C until thylakoids were isolated. Three sets of samples were used for analysis of phosphorylated and unphosphorylated D1 by SDS-PAGE/immunoblotting, and one set for in vitro kinase experiments. For constant temperature experiments, plants were held in temperature-controlled water baths at 15°C or 26°C. For cycle shift experiments, 1-week-old greenhouse-grown plants were divided into five sets and, at nightfall, were transferred in darkness to an indoor controlled growth chamber where they were maintained in darkness for 1.5 h. One set of plants was left in darkness for the duration of the experiment, and the other four were exposed to 200 or 300 µmol m⁻² s⁻¹ fluorescent light in

D1 Phosphorylation Is Controlled by a Circadian Rhythm

**Materials and Methods**

**Plant Material and Thylakoid Membrane Preparation**

*Spirodela oligorrhiza* plants were maintained as described (Elich et al., 1992) at 25°C in one-half strength Hunter’s medium (Posner, 1967) containing 0.5% (w/v) Suc under 25 µmol m⁻² s⁻¹ cool-white fluorescent light. Plants were transferred to medium lacking Suc for at least 48 h before each experiment. Thylakoids were prepared according to published methods (Elich et al., 1992). The final thylakoid pellet was suspended in a small volume of buffer A (10 mM Tricine-NaOH, pH 7.8, 100 mM sorbitol, 10 mM MgCl₂, and 10 mM NaCl) such that the chlorophyll concentration was greater than 250 µg mL⁻¹. Chlorophyll concentrations were determined in 80% (w/v) acetone (Arnon, 1949).

**In Vitro Phosphorylation**

In vitro phosphorylation of S. oligorrhiza thylakoids was carried out in the dark essentially as described (Elich et al., 1992). Thylakoids were diluted to a chlorophyll concentration of 200 µg mL⁻¹ in buffer A containing 10 mM NaF, 0.5 mM NADPH, 3.5 µM ferredoxin, and 0.2 mM ATP. Reactions were stopped at the indicated times by addition of 0.5 volumes of 3X SDS sample buffer (Mattoo et al., 1981).

**Diurnal Oscillations Experiments**

*S. oligorrhiza* plants were maintained on medium lacking Suc under fluorescent lighting as above and were then shifted to the greenhouse at Beltsville, MD for at least 3 d on the same medium before experiments were started. Temperature and light intensity were recorded at 1- or 2-h intervals. Samples (10–20 plants) were harvested in quadruplicate, immediately frozen on dry ice, and then stored at −80°C until thylakoids were isolated. Three sets of samples were used for analysis of phosphorylated and unphosphorylated D1 by SDS-PAGE/immunoblotting, and one set for in vitro kinase assays. For constant temperature experiments, plants were held in temperature-controlled water baths at 15°C or 26°C. For cycle shift experiments, 1-week-old greenhouse-grown plants were divided into five sets and, at nightfall, were transferred in darkness to an indoor controlled growth chamber where they were maintained in darkness for 1.5 h. One set of plants was left in darkness for the duration of the experiment, and the other four were exposed to 200 or 300 µmol m⁻² s⁻¹ fluorescent light in

regulate chloroplast function. This is, perhaps, a means for the chloroplast to anticipate environmental changes. Light intensities supersaturating for photosynthesis cause deleterious effects in the chloroplast; therefore, there is a need for a sensing mechanism to down-regulate PSII. It is not known how the chloroplast achieves this. Does it use the D1-P index as a sensor to anticipate the onset of higher light intensities (Mattoo and Edelman, 1985)? Is the phosphorylation state of PSII reaction core proteins a consequence or a determinant of the relative energy distribution between the two photosystems in oxygenic photosynthesis? Because irradiance-dependent D1 turnover is a fact of life for PSII and because phototrophs are normally subjected to a daily light/dark cycle, circadian regulation of D1 metabolism is not unexpected. However, how regulation is achieved may differ for different photosynthetic forms. In higher plants, where D1 is reversibly phosphorylated, circadian regulation of metabolism can be at the phosphorylation level, as is suggested here for *S. oligorrhiza*. In cyanobacteria, redox-regulated phosphorylation of D1 does not appear to occur, but these oxygenic bacterial phototrophs often possess multiple copies of the psbA gene coding for D1 (Golden, 1995; Chen et al., 1999), with different D1 isoforms adapted in vivo to varying photon irradiation, one dominant at lower and another at higher light intensities (Bustos et al., 1990; Clarke et al., 1993; Kulkarni and Golden, 1994). Several studies have shown that light-induced transcription of cyanobacterial psbA occurs within the larger framework of circadian control (Liu et al., 1995; Chen et al., 1999). Thus, a generalized hypothesis can be forwarded that reversible phosphorylation of D1 (and maybe, other phosphorylated PSII proteins) in higher plants evolutionarily replaced multiple DNA copies in cyanobacteria as a more energy-efficient substrate for circadian clock regulation of PSII core metabolism.

The hypothesis can be investigated phylogenetically by viewing atypical species. *Prochlorococcus marinus*, a ubiquitous, free-living marine cyanobacterium, is unusual in that it contains chlorophyll a, b, and c, but no phycobilisomes. In addition, it has only a single psbA gene and one type of D1 protein (Garcia-Fernandez et al., 1998). It will be interesting to determine if D1 can be phosphorylated in this organism. The state of D1 phosphorylation in lower plants and algae also bears further investigation. The D1 protein of the moss *Ceratodon purpureus* is reported as not being phosphorylated under high irradiance in vivo or under in vitro conditions (Rintamaki et al., 1995b). However, information on gene copy number and regulation of D1 metabolism in this model lower plant organism is currently lacking. The literature for *Chlamydomonas reinhardtii*, although extensive, is surprisingly ambiguous concerning phosphorylation of the D1 and D2 proteins. The most recent investigation suggests that neither of these PSII core proteins is phosphorylated in this green alga (Andronis et al., 1998). The psbA gene of *C. reinhardtii* maps to the edge of the inverted repeat region; thus, there are two identical copies of the gene and a single type of D1 protein. Transcription of chloroplast-encoded genes, including psbA, is controlled by a nuclear-regulated circadian clock in *C. reinhardtii* (Hwang et al., 1996; Kawazoe et al., 2000), which would be predicted by our hypothesis above.

The isolation of a D1 kinase gene and elucidation of its role could be a step toward understanding the role of D1 phosphorylation and its regulatory mechanisms in higher plants. More than one kinase is clearly involved with phosphorylation of PSII proteins (Elich et al., 1997). We have purified and cloned a *S. oligorrhiza* kinase that phosphorylates a synthetic peptide mimicking the D1 protein in a calcium-dependent manner (A. Raskind, M. Swegle, I. Booij-James, V. Kumar, M. Edelman, and A. Mattoo, unpublished data). However, it is yet to be ascertained if this is the protein kinase that phosphorylates D1 in vivo. One approach being followed is the antisense RNA technology to silence this protein and check if the knockout transformants phosphorylate D1.

**Plant Physiol. Vol. 130, 2002 2073**

Copyright © 2002 American Society of Plant Biologists. All rights reserved.
controlled growth chambers. Two of the four sets were returned to darkness following a 5-min light pulse, whereas the remaining two were kept in continuous light for the duration of the experiment.

**In Vivo Pulse-Labeling Experiments**

S. elodea plants were transferred for at least 2 d to medium lacking 
Suc and phosphate and were maintained under fluorescent lighting as above. Plants were then shifted to the greenhouse and were maintained at an constant temperature on the same medium for 2 d prior to the experiment. Plants were pulse-labeled for 1 h with 0.5 mCi mL$^{-1}$ of [35S]orthophosphate (Elich et al., 1992) or 0.1 mCi mL$^{-1}$ of [35S]Met (Mattoo et al., 1981). The plants were subsequently washed three times with ice-cold water, collected on dry ice, and stored at $-80^\circ$C until thylakoids were isolated.

**Preparation of Antiserum**

Based on the archetype D1 sequence of spinach (Spinacia oleracea; Zuraws-ki et al., 1982), two peptides corresponding to amino acids 1 through 17 and 57 through 85 of the mature protein were synthesized and designated SP1 and SP2, respectively (Fig. 1A). The SP2 peptide contains at its N terminus a Cys added for conjugation purposes. These two synthetic peptides were conjugated to bovine serum albumin through Cys residues, and the conju-gates were used to immunize rabbits. The resulting antisera were purified by immunoaffinity using Sulfolink columns (Pierce, Rockford, IL) to which gates were used to immunize rabbits. The resulting antisera were purified.

**Electrophoresis and Immunoblots**

Thylakoid proteins were solubilized in 1× SDS sample buffer for 1 h at room temperature and were separated by SDS-PAGE on 10% to 20% (w/v) acrylamide gradient gels (Elich et al., 1992). The samples were loaded on an equal chlorophyll basis (1 μg of chlorophyll$^{-1}$ lane). The gels were stained with Coomassie Blue R-250 or were electrotransferred to nitrocellulose membranes for at least 5 h at 0.2 mA. The blots were immunodecorated with immunopurifiication of the psbA gene in the marine oxyphotobacteria Prochlorococcus spp. Arch Biochem Biophys 359: 17–23.

**ACKNOWLEDGMENTS**

We thank Dr. Todd Elich for preparing and testing the antibodies, his input into Figure 1, and many discussions. We also thank Prof. Susan Golden for constructive comments.

Received August 21, 2002; returned for revision September 13, 2002; ac-cepted September 19, 2002.

**LITERATURE CITED**


