

Coexistence of phycoerythrin and a chlorophyll *a/b* antenna in a marine prokaryote

(Prochlorophyta/cyanobacteria/phycoobilins/photosynthesis/endosymbiosis)

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ABSTRACT *Prochlorococcus marinus* CCMP 1375, a ubiquitous and ecologically important marine prochlorophyte, was found to possess functional genes coding for the α and β subunits of a phycobiliprotein. The latter is similar to phycoerythrins (PE) from marine *Synechococcus* cyanobacteria and bind a phycourobilin-like pigment as the major chromophore. However, differences in the sequences of the α and β chains compared with known PE subunits and the presence of a single bilin attachment site on the α subunit designate it as a novel PE type, which we propose naming PE-III. *P. marinus* is the sole prokaryotic organism known so far that contains chlorophylls *a* and *b* as well as phycobilins. These data strongly suggest that the common ancestor of prochlorophytes and the *Synechococcus* cyanobacteria contained phycobilins. Flow cytometric data from the tropical Pacific Ocean provide evidence that deep populations of *Prochlorococcus* possess low amounts of a PE-like pigment, which could serve either in light harvesting or nitrogen storage or both.

When the first prokaryote containing chlorophyll (Chl) *b* and lacking phycobilins, *Prochloron didemni*, was discovered, it was proposed to belong to a new Division: the Prochlorophyta (1). However, phylogenetic analyses of three such prochlorophytes, including *Prochlorococcus* (2) and *Prochlorothrix* (3), suggested that these organisms have a polyphyletic origin among cyanobacteria (4–6). Recently, the Chl *a/b* antenna proteins of prochlorophytes were found to be closely related to CP43', an iron stress-induced Chl *a* protein of cyanobacteria (7, 8). Prochlorophytes may have evolved from ancestral cyanobacteria by acquiring Chl *b* and losing the phycobilisomes (9). Alternatively, prochlorophytes and cyanobacteria might both derive from ancestors possessing Chl *a/b*-protein complexes and phycobiliproteins (10, 11). Up to now, no prokaryote combining Chl *a*, Chl *b*, and phycobiliproteins had been described. A preliminary search for genes encoding components of the phycobilisomal apparatus, which consists of 12–18 different structural proteins in cyanobacteria (12), was unsuccessful in *Prochlorothrix* (9). The marine genus *Prochlorococcus*, which has been detected in a large range of ecological conditions, appears to be a better candidate. Field populations as well as the different *Prochlorococcus* strains available in culture have strikingly distinct pigment ratios and are genetically very diverse (13–15).

We found that a phycoerythrin (PE)-like pigment is present in natural *Prochlorococcus* populations in the central Pacific Ocean at depths below 120 m. A molecular genetic analysis of the type strain *Prochlorococcus marinus* CCMP 1375 revealed the presence of functional genes coding for a novel type of phycoerythrin (PE-III), whereas functional genes were seemingly absent in four other *Prochlorococcus* isolates. The exci-

tation maximum of the major chromophore bound by PE-III corresponds to that of phycourobilin.

MATERIALS AND METHODS

Flow Cytometric Measurements. Sea water samples were collected at different depths during the France-Joint Global Ocean Flux Study OLIPAC cruise held in November 1994 aboard the N.O. l'Atalante. Samples were analyzed immediately using a FACScan (Becton Dickinson) flow cytometer and cell concentrations of *Prochlorococcus* and *Synechococcus* were determined as described (16). Orange fluorescence was collected through a 585/42 nm bandpass filter and normalized to 0.95 μ m fluorescent beads (16).

Culture. Several *Prochlorococcus* isolates and marine *Synechococcus* strains were grown as described (14, 15). The *P. marinus* clone CCMP 1375 and the *Prochlorococcus* sp. clone CCMP 1378 were obtained from the Center for the Culture of Marine Phytoplankton (Bigelow Laboratory, West Boothbay Harbor, ME). Four other *Prochlorococcus* isolates (NATL1, NATL2, TATL1, and TATL2) described in ref. 15 and the *Synechococcus* isolate ROSO4 came from the culture collection of the Station Biologique (Roscoff, France). Strain WH 8103 was kindly provided to us by D. Scanlan (University of Warwick, United Kingdom).

Polymerase Chain Reaction (PCR). The following primers, taking into account the specific codon usage in *Prochlorococcus* (6, 17), were designed: primer P1, 5'-GC(A/T)CGNCT-NGA(A/G)GC(A/T)GC(A/T)GA(A/G)AA-3' corresponding to the peptide motif ARLEAAEK (residues 36–43 in *Synechococcus* WH 7803 α -PE); primer P2, 5'-CNCCNGC(G/C/T)ATNCCCCA(C/T)TC(A/G)TC-3' equivalent to DEW-GIAG (residues 106–112, antisense orientation). The primers were used in PCR, applying 35 cycles of 94°C for 60 s, 62°C for 60 s, and 72°C for 75 s. Single amplification products were obtained for *P. marinus* (204 nt) and the marine *Synechococcus* (228 nt, control).

Southern Blot Hybridization, Analysis, and Identification of Genes. Experiments were performed according to standard methods (18). Nonstringent hybridizations were performed in 7% SDS and 250 mM Na[PO₄] (pH 7.2) at 52°C, and otherwise at 64°C. Positive clones were isolated from a genomic library in bacteriophage gt10 (6, 17) and from a library in cosmid vector SuperCos1 (Stratagene). DNA sequence analysis occurred from both strands using the chain termination method and an automated laser fluorescence sequencer (Applied Biosystems model 373A). The identity of *P. marinus* CCMP

Abbreviations: Chl, chlorophyll; PE, phycoerythrin.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. Z68890).

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1375 was verified by partial sequence analysis of the 16S ribosomal RNA gene (5).

Isolation and Analysis of RNA and Proteins. Total RNA was isolated and analyzed (10 μ g per lane) as described previously (6). For analysis of proteins, cells from a 10-liter culture of *P. marinus* grown at 4 μ E m⁻²s⁻¹ were centrifuged for 15 min at 9000 \times *g*. For Western blotting, the method for the isolation of phycobilisomes from *Synechococcus* was followed (19). PEG 6000 was added to a final concentration of 15% (wt/vol) to the supernatant of a Triton X-100 extract, and then the solution was stirred at room temperature for 1 h and spun at 27,000 \times *g* for 30 min. The lower (water) phase was collected, and the proteins were precipitated with 10% trichloroacetic acid. Western blotting was performed with an antibody against purified PE of *Synechocystis* PCC 6808 (courtesy of J. Houmard, Institut Pasteur, Paris). Phycobilisomes from *Calothrix* PCC 7601 grown in green light to increase PE production (courtesy of J.-C. Thomas, École Normale Supérieure, Paris) were used as a control.

Fluorescence Spectra. Cells were resuspended in TENS buffer (50 mM Tris-HCl/2 mM EDTA/10 mM NaCl/200 mM sorbitol/1 mM benzamidine/5 mM ϵ -amino-*n*-caproic acid/0.1 mM phenylmethylsulfonyl fluoride; pH 8.0). This treatment causes cell lysis and release of soluble proteins. After centrifugation for 30' at 27,000 \times *g* to remove cell debris and membranes, the supernatant was analyzed using a LS50 spectrofluorimeter (Perkin-Elmer) equipped with a red-sensitive

photomultiplier. The emission spectrum was corrected for band shape distortions using a correction file provided by the manufacturer.

RESULTS

On board flow cytometric measurements made in the tropical Pacific Ocean revealed that natural populations of *Prochlorococcus* from the bottom of the euphotic layer displayed a significant orange fluorescence, almost equivalent to that of near surface, light-saturated *Synechococcus* cyanobacteria (Fig. 1). This suggested the presence of low amounts of PE within *Prochlorococcus* cells at depth. However, DNA from cultured *Prochlorococcus* strains isolated from different locations and depths did not hybridize significantly to PE gene probes from various marine and freshwater cyanobacteria (data not shown). In contrast, primers directed to conserved residues in known cyanobacterial α -PEs allowed the specific amplification of a gene fragment from one of these strains (*P. marinus* CCMP 1375) by PCR. The PCR product was used to isolate several genomic clones (see *Materials and Methods*). Genomic DNA (Fig. 2A) as well as several of the genomic clones hybridized specifically to this PCR product. Single prominent hybridizing gene fragments were also found in the genomic DNA from the marine cyanobacterium *Synechococcus* ROS04 and from one additional *Prochlorococcus* strain,

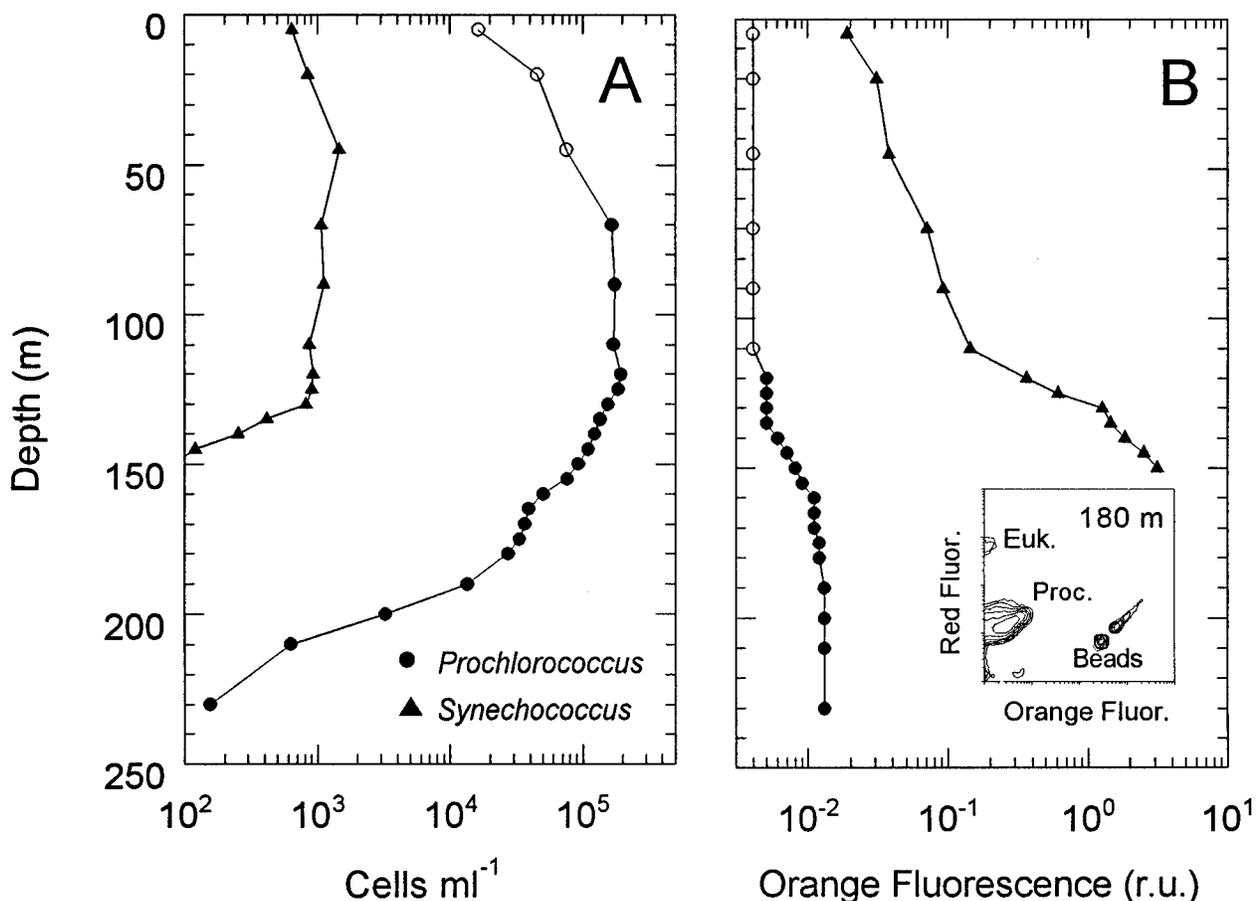


FIG. 1. Vertical profiles of flow cytometric cell concentrations per ml (A) and orange fluorescence per cell (B) of *Prochlorococcus* and *Synechococcus* in the tropical Pacific Ocean at 16°S 150°W (cast no. 127). *Prochlorococcus* populations were not completely resolved in the upper 50 m layer (open circles in A) and their orange fluorescence was too low to be discriminated from noise above 120 m (open circles in B). Nitrates were only detectable (i.e., >10 nM) below 145 m (P. Raimbault, personal communication). (Inset) A cytogram of orange versus red fluorescence for a natural population at 180 m, a depth at which only *Prochlorococcus* (Proc) and picoeukaryotes (Euk) were detectable. Note that despite their very small size, the orange fluorescence of *Prochlorococcus* is significantly higher than that of picoeukaryotes, the latter probably resulting from the "leaking" of the red fluorescence into the orange signal.

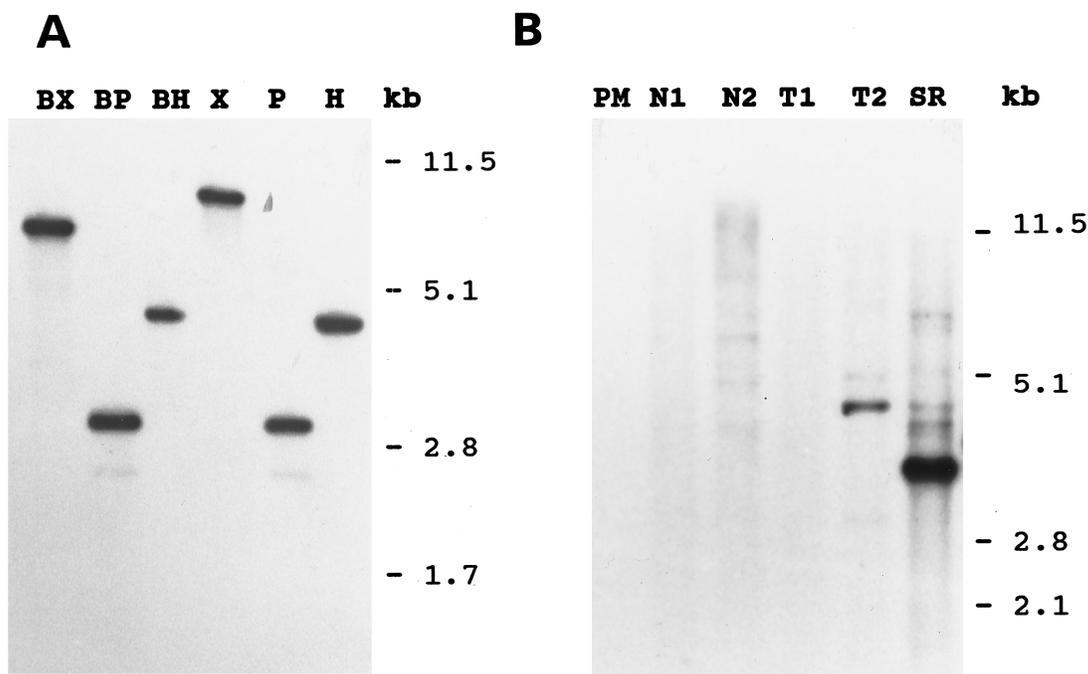


FIG. 2. Identification of phycoerythrin genes in the genome of *P. marinus* CCMP 1375. (A) Primers (P1 and P2) based on the cyanobacterial phycoerythrin were used for amplifying *Prochlorococcus* DNA (see Fig. 3). The resulting 204 nucleotide PCR product hybridized strongly to genomic DNA cleaved by the restriction enzymes *Bam*HI (B), *Hind*III (H), *Pst*I (P), and *Xho*I (X), individually or in double digests. (B) Southern blot hybridization using the same probe as in A but under less stringent conditions revealed a single hybridizing band also in the *Hind*III-digested DNA of *Prochlorococcus* strain TATL2 (T2), but not in the four other strains CCMP 1378 (PM), NATL1(N1), NATL2 (N2), and TATL1 (T1). DNA from marine *Synechococcus* strain ROS04 (SR) was included for comparison.

TATL2, whereas there was no signal for four other *Prochlorococcus* isolates, including clone CCMP 1378 (Fig. 2B).

DNA sequence analysis of several independent genomic clones revealed the presence of two tightly linked open reading frames of 465 and 546 nucleotides (Fig. 3A). Their derived amino acid sequences are strikingly similar to known PE α - and β -chains, respectively. Both *P. marinus* PE genes are somewhat smaller than the corresponding *cpeA* and *cpeB* genes from marine *Synechococcus* (Fig. 3 B and C), but the possible deletions are restricted to less-conserved regions and the reading frames are preserved. The α - and β - chains consist of 155 and 182 aa and have estimated molecular weights of 17.3 and 19.3 kDa, respectively. Several residues known for their functional importance are present: chromophore attachment sites, Cys-73 in the α subunit and Cys-50, -61, -82, and -163 in the β subunit; chromophore interaction, Arg-75, Asp-76 in the α subunit and Arg-84, Asp-85, Arg-77 and -78, Ala-81 in the β subunit (11, 20–25). The known cyanobacterial α -PEs contain either two or three chromophore attachment sites per molecule and are designated as types I and II, respectively (11, 21, 24). In contrast, the presence of only one attachment site for a chromophoric group as well as the unusual sequence aa 51–70 in the α chain and aa 147–162 in the β chain characterize *Prochlorococcus* PE as a new type that we propose to call PE-III. As in other cyanobacteria, the *cpeA* and *cpeB* genes of *P. marinus* are arranged in tandem and are cotranscribed (Fig. 3A), as indicated by a transcript size of 1.3 kb (Fig. 4A). Maintenance of continuous reading frames and of functionally important residues as well as the occurrence of a transcript demonstrate that these genes are functional in *Prochlorococcus*.

The presence of a phycobiliprotein in *P. marinus* CCMP 1375 was demonstrated by immunoblotting. A polyclonal antiserum raised against the PE of *Synechocystis* PCC 6808 recognized a protein with an apparent molecular weight of 21 kDa in water soluble extracts from *P. marinus* (Fig. 4B). Spectrofluorimetric analyses demonstrated that this protein

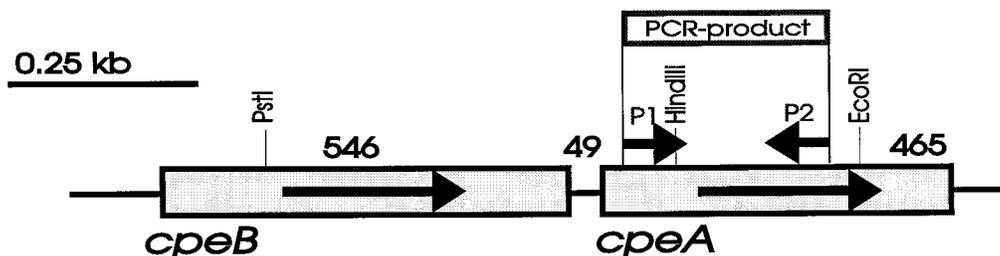
binds phycobilins (Fig. 4 C and D). Fluorescence excitation spectra exhibited a major peak at 496 nm (Fig. 4C), suggesting that phycourobilin is the dominant prosthetic group in *P. marinus* PE (25). The occurrence of a second peak at ca. 550 nm suggests that some phycoerythrobilin is present as well. Both these chromophores have a main emission peak at 572 nm (Fig. 4D), slightly red-shifted (ca. 6 nm) compared with that of a suspension of phycobilisomes from *Synechococcus* WH 8103 (data not shown). In contrast, no PE fluorescence was observed in *Prochlorococcus* sp. CCMP 1378, in agreement with the absence of hybridizing DNA fragments (Fig. 2B).

DISCUSSION

This is the first report of the presence of phycobiliproteins in a prokaryote, *P. marinus*, also possessing an intrinsic Chl *a/b* antenna. The unique pigment composition of *P. marinus*, which includes (divinyl-) Chl *a*, (divinyl- and monovinyl-) Chl *b*, low amounts of a Chl *c* derivative (14, 26) and at least one phycobiliprotein (PE), resembles a situation suggested previously for an hypothetical ancestor of prokaryotes performing oxygenic photosynthesis (10, 11). Thus, *P. marinus* could derive from such an ancestral form without major pigmentary changes, whereas most other cyanobacterial descendants would have lost the ability to synthesize or use Chl *b* and Chl *c*.

Alternatively, *P. marinus* could have evolved more recently from marine *Synechococcus*-like cyanobacteria. The latter hypothesis is consistent with the phylogenetic analysis of *Prochlorococcus* 16S rRNA gene, which places this organism close to marine *Synechococcus* and other unidentified marine cyanobacteria (5). Tentative phylogenetic trees obtained using all known cyanobacterial α - or β -PE sequences (data not shown) also suggested a closer relatedness of *P. marinus* PE to marine *Synechococcus* PE, although the low number of available sequences did not allow to draw any other meaningful phylogenetic conclusions. Further, the

A



B

P. MAR. **MKSTVTTVIASADAAGRFP**TISDIESVKGSFDRAKDRLEAAEKLSIHIDR 50
 WH 7803 **MKS**VVTTVVSGADAAGRFP**SQ**NDLEAVQGNIQRRARLEAAEKLAAGLDA 50

P. MAR. **FTSQALD**-----**YVY**-----**G**TENYE**Q**ANKDK**C***SRDIHHYLRLIN**Y**CLVTG 91
 WH 7803 **VTKEAGDACFNKYPYLKQ**PGEAGEN**Q**TKV**D**K**CY**RD**LGH**YLRLIN**Y**CLV**V**G 100

P. MAR. **GTGPLDEWGIAGMREVI**RIQL**LPT**AA**YIEA**FTYIRD**NLDIP**ND**M**G**Q**Q**A**GA 141
 WH 7803 **GTGPLDEWGIAGAREVY**RTL**GLPT**GPY**EA**LT**Y**TRDRA**C**AP**RD**MSP**Q**ALN 150

P. MAR. **EFKNLLDY**LINALA 155
 WH 7803 **EFKSYLDY**VINALS 164

C

P. MAR. **MLDAFSRAVVSADSKG**ATIG**S**AE**LSS**LRKYVADANKRIDATL**AITQ**NV**S***C 50
 WH 7803 **MLDAFSRSVVSADAKT**APVGGSD**L**AGLRSYVRDGN**KRLDA**VNAIT**S**NA**S**C 50

P. MAR. **IAADAISGMV***CENT**GLTQ**PGGH**CYP**TR**MAA**CLRDGEI**ILR**YV**S**YAL**F**AG 100
 WH 7803 **IVSDAVTGMIC**ENT**GLI**QAGG**NCYP**NR**MAA**CLRDGEI**ILR**Y**S**YAL**L**AG 100

P. MAR. **DPSVLDDRCINGL**K**E**TYIAL**GV**PL**SNA**IRAI**EIM**KIAT**V**AIM**TET**NSGRK 150
 WH 7803 **DASVLDDRCIN**GL**K**E**T**YIAL**GV**PA**Q**SAARAVAIM**K**ASATAHIG**ET**NT**Q**AN 150

P. MAR. **MFEGIN**--SGSGAE**C**KDIASE**EA**SY**FDR**VIDALN 182
 WH 7803 **GGAKFRKMET**T**Q**GDC**S**ALVA**EA**SY**FDR**VISAIA 184

FIG. 3. (A) Organization of *P. marinus* CCMP 1375 *cpe* genes and the location of the PCR-generated probe used in Fig. 2. Direction of transcription is shown by arrows, numbers indicate length of genes, and spacer in base pairs. (B and C) Predicted protein sequences of *P. marinus* α -PE (B) and β -PE chains (C) aligned with C-PE type I α and β genes of marine *Synechococcus* WH7803 (27). Residues identical to the *P. marinus* sequences are boldface type, functionally important amino acids mentioned in the text are in italics, conserved cysteine residues that serve as bilin attachment sites are labeled by an asterisk. The second chromophore attachment site of cyanobacterial C-PE type I, not conserved in *Prochlorococcus*, is double underlined.

organization of the genes coding for the two subunits (β then α) in the PE operon, as well as the presence of a phycourobilin-like chromophore, are considered as more modern characteristics (20). Consequently, it can be reasonably speculated that in prochlorophytes the development of a constitutive Chl *a/b* antenna, which derived from a cyanobacterial Chl *a* protein (7, 8), allowed the disappearance of phycobilisomes in some lines (such as *Prochlorococcus* sp. CCMP 1378 and possibly the other two prochlorophyte genera), whereas in others lines (such as *P. marinus* CCMP 1375) both light harvesting systems were kept.

The precise structural organization and function of *Prochlorococcus* PE as well as the possible presence of other phycobilisome components remain to be investigated. In cyanobac-

teria, PE is situated at the periphery of phycobilisomes and transfers the energy it collects from photons to other types of phycobiliproteins. In marine *Synechococcus*, PE has functions both in light harvesting and in nitrogen-storage (28) and PE-III may play similar roles in *Prochlorococcus*. We show that in the field only populations from the bottom of the euphotic zone that are exposed to very low levels of blue light and detectable levels of nitrates contain some PE. This is consistent with the dominance of phycourobilin, a chromophore that presents a blue-shifted absorption maximum compared with phycoerythrobilin (21, 22, 25), and the fact that the biosynthesis of phycobiliproteins requires more nitrogen than the biosynthesis of a Chl *a/b* antenna [110 aa per chromophore instead of 15–16 aa per Chl for a Chl *a/b* antenna (10)]. The discrepancies

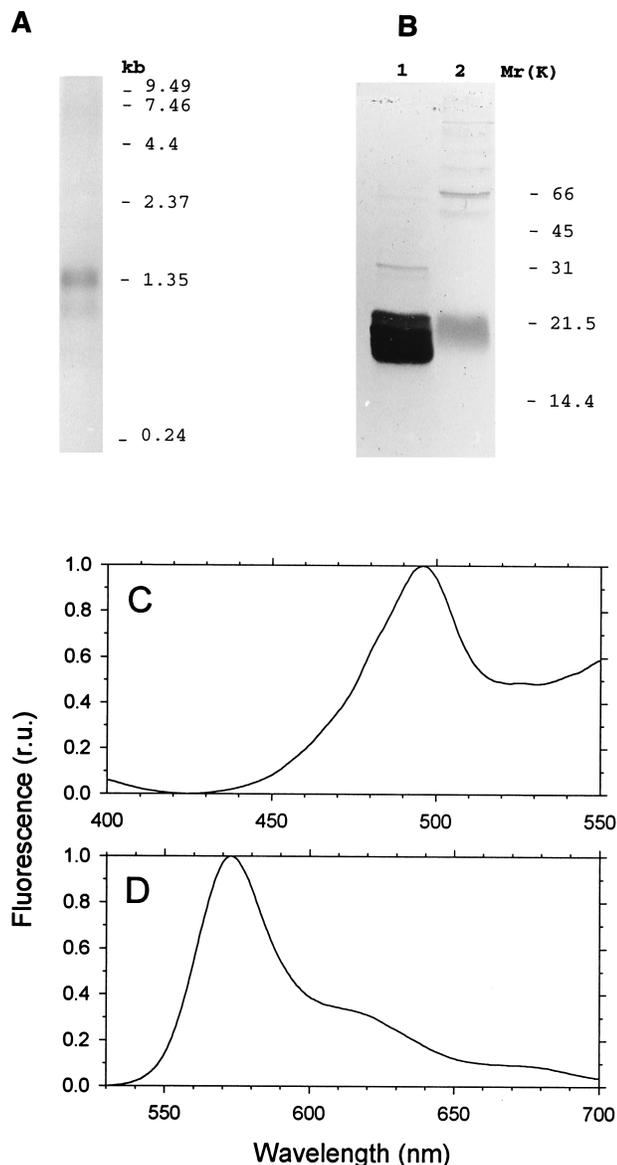


FIG. 4. Characterization of phycoerythrin expression in *Prochlorococcus*. (A) A 1.3-kb mRNA is detected using a labeled *cpeA* fragment from *Prochlorococcus* as a probe. (B) Western blot of *Prochlorococcus* water soluble extract (lane 2) and phycobilisome preparations from *Calothrix* PCC 7601 (control, lane 1) using antibody raised to PE of *Synechocystis* PCC6808. (C and D) Spectrofluorimetric analyses of a *Prochlorococcus* soluble extract. (C) Fluorescence excitation spectrum with emission at 572 nm. (D) Fluorescence emission spectrum with excitation at 496 nm.

noticed between surface and deep populations of *Prochlorococcus* may furthermore reflect a genetic differentiation, as suggested by the genotypic differences between *P. marinus* CCMP 1375, a strain isolated from 120 m depth and other *Prochlorococcus* strains originating from shallower layers, including *Prochlorococcus* sp. CCMP 1378 (Fig. 2; see also refs. 14, 15, and 26).

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- Lewin, R. A. (1976) *Nature (London)* **261**, 697–698.
- Chisholm, S. W., Frankel, S. L., Goericke, R., Olson, R. J., Palenik, B., Waterbury, J. B., West-Johnsrud, L. & Zettler, E. R. (1992) *Arch. Microbiol.* **157**, 297–300.
- Burger-Wiersma, T., Veenhuis, M., Korthals, H. J., van der Wiel, C. C. M. & Mur, L. R. (1986) *Nature (London)* **320**, 262–264.
- Palenik, B. & Haselkorn, R. (1992) *Nature (London)* **355**, 265–267.
- Urbach, E., Robertson, D. L. & Chisholm, S. W. (1992) *Nature (London)* **355**, 267–270.
- Hess, W. R., Weihe, A., Loiseaux-de Goër, S., Partensky, F. & Vault, D. (1995) *Plant Mol. Biol.* **27**, 1189–1196.
- La Roche, J., Partensky, F. & Falkowski, P. G. (1995) in *Photosynthesis: From Light to Biosphere*, ed. Mathis, P. (Kluwer, Dordrecht, The Netherlands), Vol. 1, pp. 171–174.
- van der Staay, G. W. M., Ducret, A., Aebersold, R., Li, R., Golden, S. S., Hiller, R. G., Wrench, P. M., Larkum, A. W. D. & Green, B. R. (1995) in *Photosynthesis: From Light to Biosphere*, ed. Mathis, P. (Kluwer, Dordrecht, The Netherlands), Vol. 1, pp. 175–178.
- Matthijs, H. C. P., van der Staay, G. W. M. & Muur, L. R. (1994) in *The Molecular Biology of Cyanobacteria*, ed. Bryant, D. A. (Kluwer, Dordrecht, The Netherlands), pp. 49–64.
- Bryant, D. A. (1992) *Curr. Biol.* **2**, 240–242.
- Glazer, A. N. & Wedemayer, G. J. (1995) *Photosynth. Res.* **46**, 93–97.
- Sidler, W. A. (1994) in *The Molecular Biology of Cyanobacteria*, ed. Bryant, D. A. (Kluwer, Dordrecht, The Netherlands), pp. 139–216.
- Palenik, B. (1994) *Appl. Environ. Microbiol.* **60**, 3212–3219.
- Partensky, F., Hoepffner, N., Li, W. K. W., Ulloa, O. & Vault, D. (1993) *Plant Physiol.* **101**, 285–296.
- Scanlan, D. J., Hess, W. R., Partensky, F., Newman, J. & Vault, D. (1996) *Eur. J. Phycol.* **31**, 1–9.
- Partensky, F., Blanchot, J., Lantoiné, F., Neveux, J. & Marie, D. (1996) *Deep-Sea Res.*, in press.
- Lorenz, M., Börner, T. & Hess, W. R. (1995) *Endocyt. Cell Res.* **11**, 59–68.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
- Glazer, A. N. (1988) *Methods Enzymol.* **167**, 304–312.
- Apt, K. E., Collier, J. L. & Grossman, R. (1995) *J. Mol. Biol.* **248**, 79–96.
- Ong, L. J. & Glazer, A. N. (1991) *J. Biol. Chem.* **266**, 9515–9527.
- Swanson, R. V., Ong, L. J., Wilbanks, S. M. & Glazer, A. N. (1991) *J. Biol. Chem.* **266**, 9528–9534.
- Wilbanks, S. M., de Lorimier, R. & Glazer, A. N. (1991) *J. Biol. Chem.* **266**, 9535–9539.
- Wilbanks, S. M. & Glazer, A. N. (1993) *J. Biol. Chem.* **268**, 1226–1235.
- Ong, L. J., Glazer, A. N. & Waterbury, J. B. (1984) *Science* **224**, 80–82.
- Moore, L. R., Goericke, R. & Chisholm, S. W. (1995) *Mar. Ecol. Prog. Ser.* **116**, 259–275.
- Newman, J., Mann, N. H. & Carr, N. G. (1994) *Plant Mol. Biol.* **24**, 679–683.
- Wyman, M., Gregory, R. P. R. & Carr, N. G. (1985) *Science* **230**, 818–820.