

Prochlorococcus, a Marine Photosynthetic Prokaryote of Global Significance

F. PARTENSKY,^{1*} W. R. HESS,² AND D. VAULOT¹

Station Biologique, CNRS, INSU et Université Pierre et Marie Curie, F-29680 Roscoff, France,¹
and Humboldt-Universität Berlin, Institut für Biologie/Genetik, D-10115 Berlin, Germany²

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INTRODUCTION

The large body of work achieved since the discovery about 10 years ago of the minute and ubiquitous photosynthetic prokaryote *Prochlorococcus* (20, 21) has changed our view of the community structure of oceanic picoplankton. It also has implications in fields as different as the phylogeny of cyanobacteria and photosynthesis. *Prochlorococcus* has proven exceptional from several standpoints.

(i) The tiny size of *Prochlorococcus* (equivalent spherical diameter in culture, 0.5 to 0.7 μm [110]) makes it the smallest known photosynthetic organism, having the lowest predictable size for an O_2 evolver (136). The discovery and first field studies of this organism were made possible only by the use of sensitive flow cytometers onboard research vessels (21, 88, 116). Since then, the development of procedures to fix and preserve picoplanktonic cells has allowed cells to be transported to the laboratory for analysis (104, 173). As a result, the number of studies of picoplankton including *Prochlorococcus* has steadily increased. The ubiquity of this organism within the 40°S to 40°N latitudinal band, its high density, and its occupation of a

100- to 200-m-deep layer make it the most abundant photosynthetic organism in the ocean and presumably on Earth.

(ii) *Prochlorococcus* possesses a remarkable pigment complement, which includes divinyl derivatives of chlorophyll *a* (Chl *a*) and Chl *b*, the so-called Chl *a*₂ and Chl *b*₂ (41), that are unique to this genus. Recent developments of high-performance liquid chromatography (HPLC) (42) and spectrofluorimetric techniques (114) have made it possible to identify these pigments in natural assemblages and therefore to assess precisely the contribution of *Prochlorococcus* to the total planktonic photosynthetic biomass. Another peculiarity of its photosynthetic apparatus is the presence of light-harvesting complexes which are similar in function but not in structure to those of higher plants or green algae (77). In some strains, there are also small amounts of a particular type of phycoerythrin (55). The combination of Chl *a*, Chl *b*, and at least one phycobiliprotein is a unique trait among oxygen-evolving phototrophs.

(iii) In subtropical oligotrophic areas of the Atlantic and Pacific Oceans, the vertical distribution of *Prochlorococcus* often exceeds the boundaries of the euphotic layer (i.e., the part of the water column extending from the surface to the depth that receives 1% of the surface irradiance). Thus, cells of this genus seem to be able to sustain growth and photosynthesis over an irradiance range extending for more than 3 orders of magnitude. This raises the question whether natural *Prochlorococcus* populations exhibit an outstanding ability for photo-

* Corresponding author. Mailing address: Station Biologique, B.P. 74, 29682 Roscoff Cedex, France. Phone: (33) 2-9829-2314. Fax: (33) 2-9829-2324. E-mail: partensky@sb-roscoff.fr.

acclimation or whether other processes such as the occurrence of different *Prochlorococcus* species or pigment types along the vertical light gradient are implicated in this intriguing phenomenon. Recent biochemical studies (132) suggest that different *Prochlorococcus* strains may have different antenna systems specifically adapted to the light environment from which they have been isolated.

(iv) *Prochlorococcus* typically divides once a day in the sub-surface layer of oligotrophic areas, such as the central oceanic gyres (94), where it dominates the photosynthetic biomass (16). In these environments, where nutrients such as nitrogen are extremely scarce, *Prochlorococcus* has an obvious advantage with respect to uptake because of its high surface/volume ratio resulting from its very small cell size (19). However, it must also possess either unusually small nutrient requirements (at least compared to other phytoplankton) or an extremely efficient capacity to scavenge elements recycled by heterotrophic bacteria.

(v) The remarkable diversity of *Prochlorococcus* observed both among laboratory isolates (145, 166) and within field populations (124) raises questions about the role of genetic variability in the success of *Prochlorococcus* in the field. One may wonder how environmental constraints, such as the absence of iron in oligotrophic areas, conditioned the evolution of *Prochlorococcus* over geologic timescales from the ancestor it shares with the cyanobacteria.

This review critically assesses the basic knowledge acquired about *Prochlorococcus* both in the ocean and in the laboratory since its recent discovery. It clearly indicates that some research areas, such as the ecological distribution, photosynthetic capacities, and phylogeny of *Prochlorococcus*, are well ahead of others, in particular nutrient acquisition, which need to be developed.

CHARACTERIZATION AND CULTIVATION

Methods of Characterization

The first published records of *Prochlorococcus* in nature are the electron microscopy sections of “type II” cells from marine samples reported in the paper by Johnson and Sieburth (63). They revealed what is now known as the typical ultrastructure of *Prochlorococcus* (Fig. 1A). Although these authors clearly suggested that the cells they observed were probably devoid of phycoerythrin (63), Guillard et al. mistakenly identified them as *Synechococcus* (47), the other coccoid photosynthetic prokaryote discovered in abundance in marine waters around that time (184). The second published historical record came indirectly in 1983 from the discovery of an unknown “red-shifted” Chl *a* derivative in subtropical Atlantic waters (37) (Fig. 1B). In fact, years later, Gieskes (36) revealed that in 1977 he had found both red-shifted Chl *a* and Chl *b* in these waters by using thin layer chromatography. *Prochlorococcus* was clearly identified only once flow cytometry was used (21). During a cruise out of Barbados in 1985, Rob Olson and Ginger Armbrust were the first to visualize in a deep sample a new population of red-fluorescing particles, dimmer than *Synechococcus* (117). The finding that these cells possessed an unusual divinyl derivative of Chl *a* and the visualization of their ultrastructure led to the announcement in 1988 of the discovery of *Prochlorococcus* by Chisholm et al. (21). By then, other workers had begun to record *Prochlorococcus* either by flow cytometry or by epifluorescence microscopy (88, 116).

Since the late 1980s, many observations on *Prochlorococcus* in natural waters have been made. Most of these observations were obtained by flow cytometry on either live (Fig. 2) or

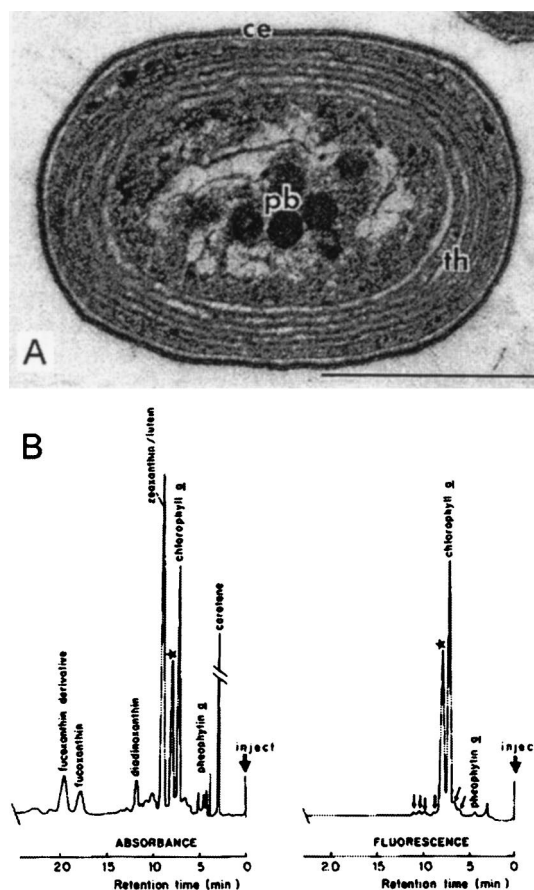


FIG. 1. First records of the occurrence of *Prochlorococcus*. (A) Electron microscope photograph of “type II cells” from deep samples of the North Atlantic ocean. Reprinted from reference 63 with permission of the publisher. ce, cell envelope; pb, polyhedral bodies; th, thylakoids. Scale bar, 0.5 μm . (B) Analysis of a pigment extract obtained by normal-phase HPLC showing the “unknown Chl *a* derivative,” indicated by a star. Reprinted from reference 37 with permission of the publisher.

preserved samples (99, 129, 173). One disadvantage of most commercially available flow cytometers is their inability to completely resolve surface populations from background noise in very oligotrophic surface waters, due to the very low Chl fluorescence of surface *Prochlorococcus*. This remains a serious problem for water column studies in areas where *Prochlorococcus* dominates the phytoplankton community. Two possible solutions to this problem have been put forward. The first requires the use of very high laser power (above 1 W) and modified optics on old instruments equipped with water-cooled lasers (119). Such bulky instruments are not very convenient for field work. The second possibility involves modifying the optics of the small instruments equipped with air-cooled lasers to focus laser beam more narrowly and increase the excitation power (28).

Alternative methods to quantify the *Prochlorococcus* cell population in the field include epifluorescence microscopy, electron microscopy, and pigment analysis. Although it has been claimed that *Prochlorococcus* abundance can be recorded by ordinary epifluorescence microscopy (60, 61, 88, 92), sophisticated image recording with, for example, a cooled charge-coupled device camera (158) is necessary to avoid severe underestimates of cell abundance, especially in surface waters, where the fluorescence is very low. For example, concentra-

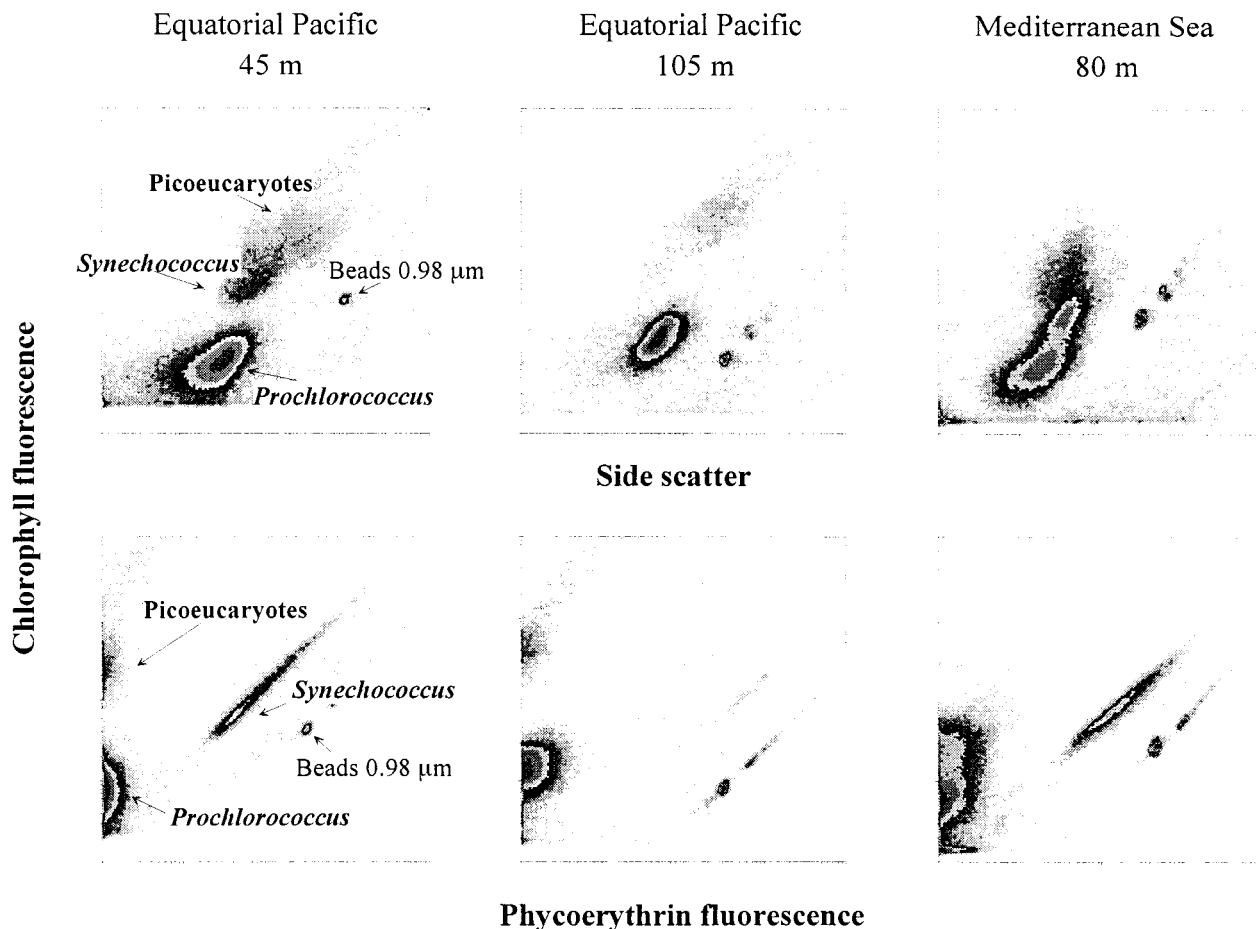


FIG. 2. Analysis of natural *Prochlorococcus* populations by flow cytometry. (Top) Side scatter (a function of cell size) plotted against red fluorescence of chlorophyll. (Bottom) Orange fluorescence of phycoerythrin plotted against red fluorescence of chlorophyll. All scales are 4-decades logarithmic from 1 to 10,000 (arbitrary units). (Left) Typical surface-layer sample (45 m, deep Equatorial Pacific, 7°S, 150°W, collected 10 November 94). *Synechococcus* is easily distinguished from *Prochlorococcus* by its orange phycoerythrin fluorescence. (Middle) Typical deep sample (105 m deep, Equatorial Pacific, 7°S, 150°W, collected 10 November 94). *Synechococcus* is virtually absent, and the chlorophyll fluorescence of *Prochlorococcus* is much higher than near the surface (compare the fluorescence of *Prochlorococcus* and that of the standard beads). Note also the weak orange fluorescence displayed by *Prochlorococcus* at this depth. (Right) Example of two *Prochlorococcus* populations coexisting at the same depth (80 m deep, Mediterranean Sea, 37°5'N, 16°52'E, collected 20 June 1996).

tions of *Prochlorococcus* in the Central Pacific estimated by epifluorescence microscopy (60) are twofold lower than those estimated by flow cytometry in the same region. Epifluorescence microscopy can, however, provide direct estimates of cell size, a key characteristic of oceanic biomass budgets (158). Because of its technical difficulty, electron microscopy has very seldom been used with marine samples (1, 21, 63). Finally, pigment analysis can be used to routinely detect *Prochlorococcus* in the field. Although it has been possible to discriminate Chl a_2 from its monovinyl counterpart by direct-phase HPLC analyses for quite a long time (37), a major step forward came with the ability to separate these pigments by reverse-phase HPLC, the most commonly used oceanographic HPLC technique, which also allows reliable separation of Chl b_1 and b_2 (see, e.g., references 3, 42, 170, and 182). Moreover, HPLC analysis offers the ability to measure the growth rate of *Prochlorococcus* by monitoring the incorporation of ^{14}C into Chl a_2 (see "Growth rates and loss processes in the ocean" below). Spectrofluorometry, a technique that is hardly ever used in oceanography, offers a very attractive alternative to detect divinyl Chls, especially because of its sensitivity, speed, and ease of implementation (114).

Cultivation

Available cultures and isolation methods. The first *Prochlorococcus* strain was isolated by Palenik in May 1988 from the bottom of the euphotic zone in the Sargasso Sea (depth, 120 m). It was initially dubbed LG (125), for "Little Greens," but was then renamed SARG for its geographical origin (131). Since then, several strains have been isolated both at the surface and at depth from various sites in oceans around the world (Table 1 and Fig. 3). Strains are now available from most areas where *Prochlorococcus* populations have been sampled.

For most of the strains reported in Table 1, samples were collected and processed by trace-metal clean techniques (30), although some other strains (e.g., MED) were obtained without special precautions. Isolation steps usually consist of a gentle filtration through two stacked 0.6- μm -pore-size filters followed by enrichment of the filtrate with sterile stock solutions of phosphate, nitrogen, and chelated trace metals (20, 105). Another elegant way of obtaining isolates is flow cytometric cell sorting, which can be directly applied to natural samples. For example, this technique has been used to obtain

TABLE 1. *Prochlorococcus* strains isolated in culture for which published data are available

| Strain | Derived clone | Other name | Origin | Date ^a | Latitude | Longitude | Depth (m) | Isolator | Remarks | Reference(s) |
|---------|---------------|-----------------|---------------------|-------------------|----------|-----------|-----------|------------------------|-----------------------------|--------------|
| SARG | SS120 | LG CCMP 1375 | Sargasso Sea | 30/5/88 | 28°59'N | 64°21'W | 120 | B. Palenik | First strain to be isolated | 131 |
| | PCC 9511 | | | | | | | L. R. Moore | Obtained by serial dilution | 20, 107 |
| MED | | DV | NW Mediterranean | 8/1/89 | 43°12'N | 6°52'E | 5 | R. Rippka | First axenic strain | 143 |
| | | | | | | | | D. Vault, F. Partensky | | 131 |
| | MED4 | CCMP 1378 | | | | | | L. R. Moore | Obtained by serial dilution | 20, 107 |
| NATL1 | | FP12 | N Atlantic | 1/4/90 | 37°39'N | 40°1'W | 30 | F. Partensky | | 131 |
| NATL2 | | FP5 | N Atlantic | 1/4/90 | 38°59'N | 40°33'W | 30 | F. Partensky | | 145, 166 |
| MIT9107 | | | S Pacific | 8/8/91 | 14°60'S | 134°60'W | 25 | J. A. Dusenberry | | 166 |
| TATL 1 | | EUM11 | E Tropical Atlantic | 19/10/91 | 20°57'N | 31°6'W | 20 | F. Partensky | | 145, 166 |
| TATL 2 | | EUM17 | E Tropical Atlantic | 21/10/91 | 20°25'N | 31°8'W | 30 | F. Partensky | | 145, 166 |
| GP2 | | | W Pacific | 10/9/92 | 8°32'N | 136°31'E | 150 | A. Shimada | | 155, 156 |
| SB | | | Sugura Bay, Japan | 21/10/92 | 35°0'N | 138°30'E | 40 | A. Shimada | | 156, 157 |
| PAC 1 | | | N Tropical Pacific | 1/4/92 | 22°45'N | 158°0'W | 100 | L. Campbell | | 145, 166 |
| MIT9201 | | | S Pacific | 26/9/92 | 11°60'S | 145°25'W | 0 | B. Binder | | 106 |
| MIT9202 | | | S Pacific | 26/9/92 | 11°60'S | 145°25'W | 79 | B. Binder | | 106 |
| MIT9211 | | | Equatorial Pacific | 10/4/92 | 0 | 140°W | 83 | R. J. Olson | | 106 |
| MIT9215 | | | Equatorial Pacific | 3/9/92 | 0 | 140°W | 0 | B. Binder | | 106 |
| MIT9302 | | | Sargasso Sea | 15/7/93 | 34°45'N | 66°11'W | 100 | L. Moore | Sorted by flow cytometry | 108 |
| MIT9303 | | | Sargasso Sea | 15/7/93 | 34°45'N | 66°11'W | 100 | L. Moore | Sorted by flow cytometry | 108, 166 |
| MIT9312 | | | N Atlantic | 17/7/93 | 37°30'N | 68°14'W | 135 | L. Moore | Sorted by flow cytometry | 108 |
| MIT9313 | | | N Atlantic | 17/7/93 | 37°30'N | 68°14'W | 135 | L. Moore | Sorted by flow cytometry | 108 |
| AS9601 | | | Arabian Sea | 1/11/95 | 19°12'N | 67°10'E | 50 | R. J. Olson | | 150 |

^a Day/month/year.

separate strains from subpopulations coexisting at the same depth in the Gulf Stream and the Sargasso Sea (108).

Optimal growth medium. Chisholm et al. (20) showed that among the various media initially tested, seawater enriched with urea, β-glycerophosphate, a minimum trace metal mix, and 100 μM CPTC (*cis,cis,cis,cis*-1,2,3,4-cyclopentanetetra-carboxylic acid), a chelator, led to sustained growth of *Prochlorococcus* (Table 2). However, *Prochlorococcus* can also be adapted to a seawater-based medium containing only inorganic additions (20). To date, the most widely used culture media (PC, PRO2, and modified K/10-Cu [Table 2]) are derived from the K medium used for marine microalgae (69), with EDTA as the chelator, a 10-fold-diluted trace metal stock solution, and no copper (Table 2). The PRO2 medium proved to be very efficient for isolation purposes (105). Other media, such as PC with trace metals as in f/20 (48) or PCR-S11 (142) (Ta-

ble 2), which use a modified “Gaffron” metal stock solution (141) have also been successful in tests for culturing. Maximum cell yields with these media are 2×10^8 to 3×10^8 cells ml⁻¹, corresponding to a Chl *a*₂ yield of ca. 0.2 to 0.4 mg liter⁻¹.

Growth on solid medium, despite repeated attempts, has not been successful to date, restraining the possible use of *Prochlorococcus* for genetic manipulations such as site-directed mutagenesis. This is one of the most critical bottlenecks for future research on *Prochlorococcus*, and it is the focus of much investigation. At present, cloning is possible only by using extinction serial dilutions, leading to “statistical” clones. Clones SS120 and MED4 (Table 2) have been obtained in this way. The same technique, combined with centrifugation to eliminate contaminant heterotrophic bacteria larger than *Prochlorococcus*, has been used to isolate the first axenic strain of *Prochlorococcus*, PCC 9511 (143).

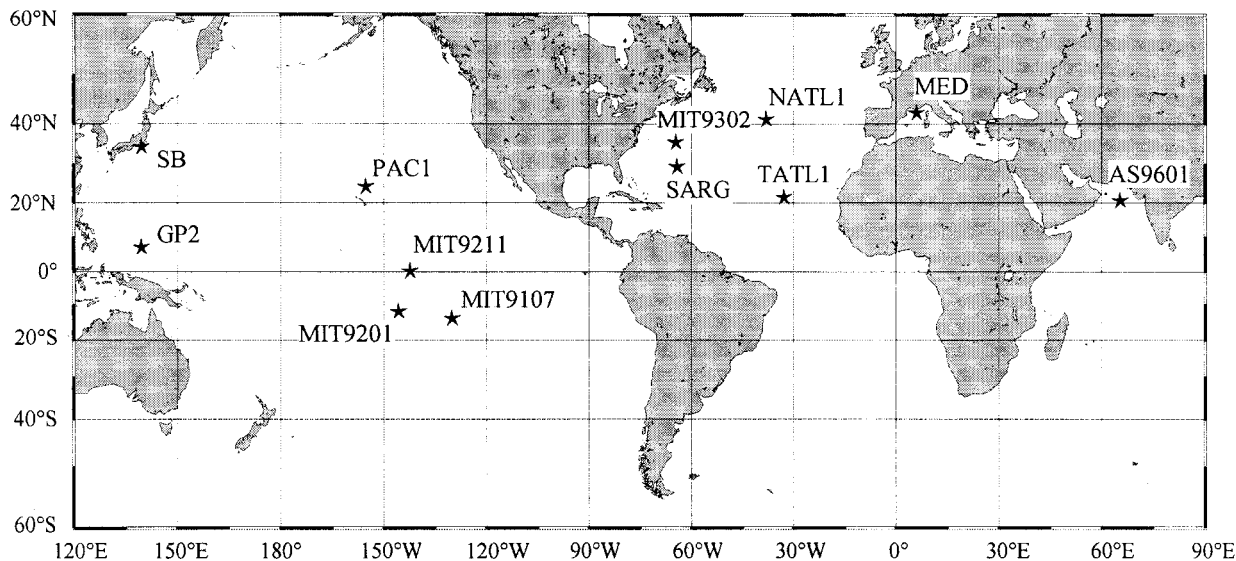


FIG. 3. Locations of some of the available *Prochlorococcus* strains (see Table 1).

TABLE 2. Composition of media used for growing *Prochlorococcus*^a

| Component | Concn of component in ^b : | | | | |
|---|--------------------------------------|-------------|---------|----------|-------------|
| | CPTC-based (A) | K/10-Cu (B) | PC (C) | PRO2 (D) | PCR-S11 (E) |
| Nutrients | | | | | |
| Urea | 20 μM | | 50 μM | 100 μM | |
| NH ₄ Cl ^{B,C,D} or (NH ₄) ₂ SO ₄ ^E | | 50 μM | 50 μM | 50 μM | 400 μM |
| β-glycerol-phosphate ^{A,C} (or NaH ₂ PO ₄) ^{B,D,E} | 10 μM | 10 μM | 10 μM | 10 μM | 50 μM |
| Buffer | | | | | |
| HEPES | | | | | 1 mM |
| Chelator/trace metals | | | | | |
| CPTC | 100 μM | | | | |
| EDTA-Na ₂ | | 11.7 μM | 11.7 μM | 1.2 μM | 8 μM |
| FeSO ₄ ^A or FeCl ₃ ^{B,C,D,E} | 0.1 μM | 1.2 μM | 1.2 μM | 1.2 μM | 8 μM |
| MnCl ₂ ^{A,B,C,D} or MnSO ₄ ^E | 10 nM | 90 nM | 90 nM | 90 nM | 30 nM |
| ZnCl ₂ ^{B,C,D} or ZnSO ₄ ^E | | 8 nM | 8 nM | 8 nM | 3 nM |
| CoCl ₂ ^{B,C,D} [or Co(NO ₃) ₂] ^E | | 5 nM | 5 nM | 5 nM | 1.5 nM |
| Na ₂ MoO ₄ ^{A,B,C,D} or (NH ₄) ₆ Mo ₇ O ₂₄ ^E | 10 nM | 3 nM | 3 nM | 3 nM | 1.5 nM Mo |
| Na ₂ SeO ₃ ^{B,C,D} or SeO ₂ ^E | | 10 nM | 10 nM | 10 nM | 1.5 nM |
| NiSO ₄ or NiCl ₂ ^{D,E} | | 10 nM | 10 nM | | 1.5 nM |
| Na ₂ WO ₄ | | | | | 0.3 nM |
| KBr | | | | | 3 nM |
| KI | | | | | 1.5 nM |
| Cd(NO ₃) ₂ | | | | | 1.5 nM |
| CuSO ₄ | | | | | 1.5 nM |
| Cr(NO ₃) ₃ | | | | | 0.3 nM |
| VOSO ₄ | | | | | 0.3 nM |
| KaI(SO ₄) ₂ | | | | | 3 nM |
| H ₃ BO ₃ | | | | | 150 nM |
| Vitamins | | | | | |
| Thiamine-HCl | | 10 μM | | | |
| Biotin | | 50 nM | | | |
| B ₁₂ | | 50 nM | | | 7 nM |

^a When different forms of a chemical are used in different media, a letter code (A through E) is used to indicate which form is used in which medium.

^b CPTC-based medium (20), K/10-Cu (20), PC (2), PRO2 (105), and PCR-S11 (142) are described elsewhere.

PHYSIOLOGY

Basic Cellular Features

Ultrastructure. Because of their tiny size, *Prochlorococcus* cells are very difficult to identify by optical microscopy. They are hardly distinguishable from small heterotrophic bacteria, except for their very weak Chl fluorescence. Transmission elec-

tron microscopy reveals typical cyanobacterial architecture (Fig. 4), which is best compared to that of the other abundant oceanic photosynthetic prokaryote, *Synechococcus*. However, the *Prochlorococcus* cell is distinctly elongated (Fig. 4A) whereas the *Synechococcus* cell is much more spherical (see 21, 63, 158). In some cases, the membrane appears fairly electron dense, but it is not known whether this is a consequence of environmental conditions or if it is strain specific. The cytoplasm contains DNA fibrils, carboxysomes that can be labeled with an antibody against ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and glycogen granules, located near or between thylakoids (90). In cross-sections, there are generally between two and four thylakoids, and there are sometimes up to six (157). They run parallel to the cell membrane (Fig. 4) and are much more appressed than in *Synechococcus*. In *Prochlorococcus marinus* type strain (SARG) and in most cells from natural populations, they are closed. In contrast, in the surface isolate *Prochlorococcus* sp. strain MED (90) and in the deep isolate MIT 9313 (Fig. 4A), they are horseshoe shaped. Interestingly, Johnson and Sieburth (63) described a "type III" cell with noncircular thylakoids that looks somewhat like MED. No phycobilisomes are visible, but since phycoerythrin has been found in strains such as CCMP 1375 (55), immunocytochemistry would be useful to investigate its intracellular localization.

Size and carbon content. Most methods used for cell sizing, such as electronic (Coulter) sizing and optical and electronic microscopy, exhibit a number of biases for such tiny objects as *Prochlorococcus* cells. The best estimates made on cultures give a range of 0.5 to 0.8 μm for length and 0.4 to 0.6 μm for width (90, 110). The cell size appears to vary with environmental conditions. For example, it was shown to increase from 0.45 to 0.75 μm between the surface and a depth of 150 m in the Sargasso Sea (158). Moreover, forward scatter measured by flow cytometry, a function of both size and refractive index, increases from dusk to dawn at the equator, a corollary of synchronized cell division (7, 174).

Determination of the carbon content of *Prochlorococcus* is important to assess its relative contribution to the oceanic biomass (16, 87). A commonly used method is to assume a given cell size and to rely on some universal carbon-to-volume ratio derived from the literature. Depending on the hypotheses made, this yields values ranging between 50 and 60 fg of C

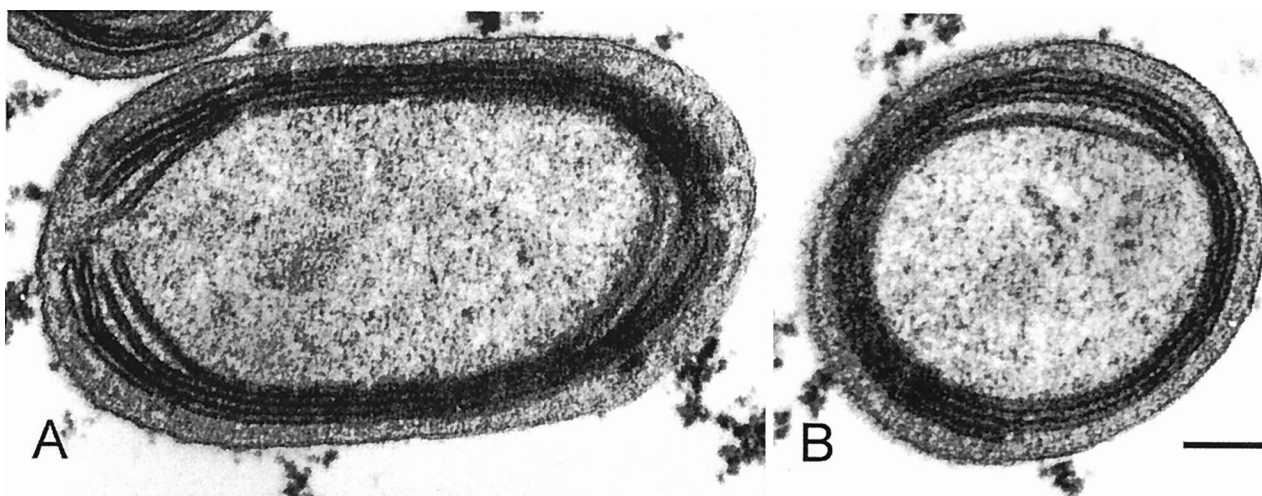


FIG. 4. Electron micrographs of longitudinal and cross sections of *Prochlorococcus* strain MIT9313 showing tightly appressed thylakoids at the periphery of the cell. Scale bar, 0.1 μm. Unpublished photographs courtesy of C. Ting, J. King, and S. W. Chisholm.

TABLE 3. Genes sequenced in *Prochlorococcus*

| Category | Gene name | Molecule encoded | Localization or function | EMBL/GenBank accession no. | Reference(s) | |
|-----------------|--------------------|---|---|----------------------------|--------------|-----|
| Photosynthesis | <i>cpeY</i> | Phycocerythrobilin, phycocourobilin lyase? | Light harvesting? | AJ001230 | 52 | |
| | <i>cpeZ</i> | Phycocerythrobilin, phycocourobilin lyase? | Light harvesting? | AJ001230 | 52 | |
| | <i>mpeX</i> | Bile pigment biosynthesis, coupling? | Light harvesting? | AJ001230 | 52 | |
| | <i>orf463</i> | Assembly of light-harvesting structures? | Light harvesting? | AJ001230 | 52 | |
| | <i>pcb</i> | Chl <i>a</i> ₂ and Chl <i>b</i> ₂ antenna | PS II antenna | U57660, U57661 | 77 | |
| | <i>petB</i> | <i>b</i> -type cytochrome | Cytochrome <i>b₆-f</i> complex | AF001487–AF001489 | 166 | |
| | <i>petD</i> | Subunit IV | Cytochrome <i>b₆-f</i> complex | AF001487–AF001489 | 166 | |
| | <i>cpeA</i> | Phycocerythrin III α subunit | Light harvesting? | Z68890, AJ001230 | 55 | |
| | <i>cpeB</i> | Phycocerythrin III β subunit | Light harvesting? | Z68890, AJ001230 | 55 | |
| | <i>ppeC</i> | Linker polypeptide of phycocerythrin | Light harvesting? | Z92525, AJ001230 | 54 | |
| | <i>psaA</i> | PsaA | PS I core | | 167 | |
| | <i>psaB</i> | PsaB | PS I core | | 167 | |
| | <i>psaF</i> | Subunit PsaF of PS I | Binding of plastocyanin? | | 167 | |
| | <i>psaI</i> | Subunit PsaI of PS I | Trimerization of PS I | Z98595, AJ002427 | 168 | |
| | <i>psaJ</i> | Subunit PsaF of PS I | Binding of plastocyanin? | | 167 | |
| | <i>psaL</i> | Subunit PsaL of PS I | Trimerization of PS I | Z98595, AJ002427 | 168 | |
| | <i>psbA</i> | D1 | PS II core | Z49201 | 57 | |
| | <i>psbB</i> | CP47 | Internal antenna of PS II | AF001481–AF001485 | 166 | |
| | <i>rbcL</i> | Large subunit of Rubisco | Carbon assimilation | U93857, U93858 | 52, 133, 155 | |
| | General metabolism | <i>aroC</i> | Chorismate synthase | Amino acid metabolism | Z49201 | 51 |
| <i>aspA</i> | | Aspartoacylase | Amino acid metabolism | Z80110 | 51 | |
| <i>cpn60</i> | | Chaperonin-60 | Protein folding | Z37730 | 52 | |
| <i>dapA</i> | | Dihydrodipicolinate synthase | Amino acid metabolism | Z37733, Z68126 | 95 | |
| <i>rpoC1</i> | | DNA-dependent RNA polymerase | Biosynthesis of RNA | Z11160 | 125 | |
| <i>mnpB</i> | | RNase P ribozyme | RNA processing | Y12789, AJ001135 | 53 | |
| <i>mc</i> | | RNase III | RNA processing | AJ001135 | 52 | |
| <i>rrn</i> | | 16S rRNA | Protein synthesis | X63140, AF001466–AF001476 | 165, 166 | |
| <i>trnR</i> | | Transfer RNA arginine (CCU) | Translation | Y12789, AJ001135 | 53 | |
| Nutrient uptake | | <i>pstS</i> | Phosphate-binding protein | Phosphorus assimilation | U75514 | 146 |
| Cell cycle | | <i>dnaA</i> | DnaA | DNA replication | U44977 | 139 |
| | <i>ftsZ</i> | FtsZ | Cell division | AJ011025 | 58 | |
| | <i>uvrD</i> | DNA helicase | DNA replication and DNA repair processes | AJ001230 | 52 | |

cell⁻¹ (8, 16, 87), although higher values have been proposed (124 fg of C cell⁻¹ [181]). The only direct measurements made on cultures (13) provided an average value of 49 fg of C cell⁻¹, surprisingly close to those indirect estimates. The recent availability of an axenic strain (143) should allow us to further refine this value.

Genome size and base composition. Existing data obtained on the axenic strain PCC 9511 either by DNA renaturation kinetics (143) or by flow cytometry (97) suggest a genome size of 1.9 to 2.0 Mbp. This value is within the size range of genomes of free-living eubacteria, which extends from 1.55 Mbp for *Aquifex aeolicus* (25) to 4.21 Mbp for *Bacillus subtilis* (71). Genome sizes have also been estimated for a variety of cyanobacteria (mainly freshwater) by renaturation kinetics (49). These range from 2.55 to 13.2 Mbp (1.66×10^9 to 8.58×10^9 Da (49; Catalogue of the Pasteur Culture Collection at <http://www.pasteur.fr>). For instance, the only sequenced cyanobacterial genome, *Synechocystis* strain PCC 6803 (67), is about twice as large (3.6 Mbp) as that of *Prochlorococcus* whereas *Prochlorothrix hollandica* has a genome size of 5.5 Mbp (149). Although more genome size data are required for marine cyanobacteria, it seems that *Prochlorococcus* possesses the smallest genome of all prokaryotes evolving oxygen (or oxyphotobacteria; see "Phylogeny" below). Some of the genetic characteristics of *P. marinus* SS120, such as the presence of only one copy of *psbA* (57), overlapping genes in the dihydrodipicolinate synthase operon (95), or overlapping promoter

regions (53), are consistent with the finding of a relatively compact genome in this microorganism. The genes sequenced to date in *Prochlorococcus* are listed in Table 3.

In contrast to marine *Synechococcus* cyanobacteria, characterized by a high G+C content (47.4 to 69.5%; [185]), most *Prochlorococcus* strains have a low G+C content. This was suggested first from the relatively high cell DNA fluorescence when cells were stained with DAPI (4',6-diamino-2-phenylindole), which is AT specific (9), and then from the analysis of *rpoC1* gene sequences of cultivated strains (125). When the whole set of sequences available for *P. marinus* SS120, the strain which at present has the best-known genome, is examined (Table 4), the global G+C content is 36.82% over an accumulated length of 25,083 nucleotides. This G+C content is comparable to the ones estimated for a few freshwater *Synechocystis* strains (34.7 to 36.7%), which have the lowest G+C scores among 176 cyanobacteria strains examined by Herdman et al. (50). Since the G+C content of *P. marinus* is an absolute number based on sequences of both genes and noncoding intergenic regions, it can be compared to the notably higher absolute G+C value of 43% obtained for the total genome of *Synechocystis* strain PCC 6803 (68). In accordance with its low G+C content, the codon usage of *P. marinus* SS120 (Table 4) is shifted towards A or T at the third base position (T > A > C > G), suggesting mutational biases as the most likely cause (65, 153, 161). From a practical point of view, it is important to take these factors into account, e.g., when oligonucleotide

TABLE 4. Codon usage in *P. marinus* CCMP 1375 (6,365 codons)^a

| Amino acid | First and second positions | No. of occurrences at third position | | | |
|------------|----------------------------|--------------------------------------|-----|-----|-------|
| | | A | C | G | T |
| Ala | GC | 247 | 70 | 34 | 255 |
| Arg | CG | 27 | 16 | 5 | 52 |
| Arg | AG | 121 | | 41 | |
| Asn | AA | | 89 | | 222 |
| Asp | GA | | 86 | | 259 |
| Cys | TG | | 21 | | 43 |
| Gln | CA | 158 | | 77 | |
| Glu | GA | 244 | | 101 | |
| Gly | GG | 152 | 107 | 66 | 167 |
| His | CA | | 44 | | 131 |
| Ile | AT | 145 | 72 | | 294 |
| Leu | TT | 236 | | 106 | |
| Leu | CT | 119 | 36 | 29 | 199 |
| Lys | AA | 239 | | 100 | |
| Phe | TT | | 123 | | 190 |
| Pro | CC | 103 | 22 | 10 | 123 |
| Ser | TC | 124 | 27 | 20 | 150 |
| Ser | AG | | 65 | | 103 |
| Thr | AC | 111 | 65 | 11 | 137 |
| Tyr | TA | | 50 | | 111 |
| Val | GT | 115 | 40 | 36 | 203 |
| Stop | TA | 6 | | 4 | |
| Stop | TG | 6 | | | |
| Total | | 2,153 | 933 | 640 | 2,639 |

^a The absolute number of occurrences of each codon was computed from 17 genes (see Table 3 for references): *aspA*, *cpeY*, *cpeZ*, *cpn60*, *dapA*, *dnaA*, *mpeX*, *pcb*, *ppeA*, *ppeB*, *psaA*, *psaB*, *psbA*, *ppeC*, *mc*, *uvrD*, and *orf463*. The noninformative Trp and Met codons are omitted. For *uvrD*, only the first 518 codons are known.

primers or gene probes are constructed. Noncoding regions are frequently even more enriched in A and T. Lorenz et al. (96) found G+C scores as low as 29% for one noncoding DNA fragment in *P. marinus* SS120. When individual genes are considered, *ppeC* has the lowest G+C content (33.7%) whereas the highly expressed *psbA* gene is less biased, with 44.6% G+C. Similar differences in G+C content in strongly versus weakly expressed genes are known for other bacteria. In *Escherichia coli* genes, for instance, the codon usage is biased towards higher G+C contents with increasing gene expression (59, 152). The evolutionary forces causing the compositional bias toward a low G+C content in most investigated *Prochlorococcus* strains are not known. It might be related to higher mutation rates relative to other prokaryotes, including *Synechococcus* (124), perhaps as a result of the higher sensitivity of AT-rich DNA sequences to UV light-induced oxidative DNA damage (187).

Although it is a salient feature for most available *Prochlorococcus* strains, a low G+C content is not a general characteristic within this genus. One strain, MIT9303, has a significantly higher G+C content than all other *Prochlorococcus* strains available in culture (55% G+C at third codon positions [164]). Interestingly, molecular studies with the 16S rRNA gene (166) place MIT9303 as the closest known relative of the high-G+C marine *Synechococcus* strains, whereas strain MED4, which has a very low G+C content of 35.7% (when considering concatenated sequences for three regions: *pcb*, *rpoC1*, and the *psbB-petB/D* intergenic region) belongs to the most recently evolved clade within *Prochlorococcus* (see "Genetic diversity" below). Therefore, there may be a correlation between the global G+C content of a particular genotype and its phylogenetic position. A systematic analysis of the G+C

content in all isolates should allow us to verify this interesting hypothesis.

Photosynthesis

Pigment composition. The presence of Chl *a*₂ and Chl *b*₂ is a trait common to all *Prochlorococcus* strains characterized to date (41, 107, 131, 166). Besides *Prochlorococcus*, Chl *a*₂ (but not Chl *b*₂) has been observed only in a mutant of corn (5), although divinyl derivatives of chlorophyll(ide) are probable intermediates in the biosynthesis of Chl in higher plants (126). Both Chl *a*₂ and Chl *b*₂ have absorption and fluorescence excitation maxima in the blue part of the visible spectrum red-shifted by 8 to 10 nm compared to their monovinyl counterparts (107, 110). The other pigments of *Prochlorococcus*, which include zeaxanthin, α -carotene, and small amounts of a Chl *c*-like pigment (Mg,3-8 divinyl phaeoporphyrin *a*_s), are shared with a limited number of other phytoplanktonic groups, including chlorophytes, cryptomonads, and cyanobacteria (41). It is noteworthy that natural populations of *Prochlorococcus* from suboxic waters of the Arabian Sea also possess a novel 7,8-dihydro derivative of zeaxanthin, probably parasiloxanthin, which is absent in cultured strains (40). One notable peculiarity of *Prochlorococcus* is the dramatic difference in pigment ratios among different isolates. For example, several isolates, notably SARG, have a Chl *b*₂/Chl *a*₂ ratio equal to or higher than 1 whereas other isolates display much lower ratios, with the MED strain exhibiting the lowest (0.13 [105–107, 131]). At least three isolates (MIT9302, MIT9312, and SARG and its clonal derivative SS120 [Table 1]) also synthesize normal (monovinyl) Chl *b* (or Chl *b*₁) when grown under high light conditions (105, 107, 131), suggesting that this light condition may trigger the expression of enzymes which can transform, probably in a single step, Chl *b*₂ into Chl *b*₁ (5, 131, 144). Surprisingly, although these enzymes should also be able to allow the transformation of Chl *a*₂ into Chl *a*₁, no Chl *a*₁ has been detected in *Prochlorococcus*. Another difference between these strains is the presence in SS120 but not in MED4 of a novel type of phycoerythrin (55). Because of its low concentration and the fact that classical pigment HPLC analyses do not detect phycobiliproteins, this pigment remained undetected for a long time. It also was overlooked by researchers making absorption measurements, because the major phycobilin associated with this phycoerythrin is phycourobilin. Its absorption maximum at 495 nm is very close to that of the very abundant Chl *b*₂ (480 nm), and thus they cannot be discriminated by their absorption spectra. It was only after the discovery of the phycoerythrin gene that Hess et al. (55) examined water-soluble fractions by spectrofluorimetry and found evidence of phycobilins.

Photosynthetic performances. In a study with the MED and SARG isolates (131), the observed ranges of assimilation rates, expressed per Chl unit, at various growth irradiances were similar between these strains (1.5 to 4.8 and 1.4 to 5.6 fg of C fg of Chl⁻¹ h⁻¹ for MED and SARG, respectively), but expressed per cell, they were almost constant for MED (4.9 to 5.8 fg of C cell⁻¹ h⁻¹) and more variable for SARG (2.8 to 6.2 fg of C cell⁻¹ h⁻¹). However, light-saturated carbon fixation rates (P_m^{Chl} or P_m^{cell} [*m* stands for maximum]) were found to vary significantly between strains grown under similar irradiances (131). A more recent study confirmed that differences in pigmentation among isolates correlate with differing photosynthetic efficiencies: when grown at 9 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, two *Prochlorococcus* isolates (MIT9303 and MIT9313) with high ratios of Chl *b*₂ to Chl *a*₂ (>1.1) had a significantly higher P_m^{Chl} than did two other isolates (MIT9302 and MIT9312) with Chl *b*₂/Chl *a*₂ ratios lower by a factor of 2 (2.4 and 1.8 fg

of C fg of Chl⁻¹ h⁻¹) (105). Laboratory values also compare fairly well with data on natural *Prochlorococcus* populations from the Moroccan upwelling (0.6 to 4 fg of C cell⁻¹ h⁻¹), obtained by labeling a natural seawater sample with ¹⁴C and sorting individual *Prochlorococcus* cells by flow cytometry (82). However, photosynthetic rates measured by the same method for *Prochlorococcus* cells at the base of the euphotic zone in the open ocean were significantly lower (0.03 to 0.3 fg of C cell⁻¹ h⁻¹). This method also allowed us to estimate the fraction of the total phytoplanktonic production attributable to *Prochlorococcus*, which varied from 11 to 57% (82).

Some of its photosynthetic properties may give *Prochlorococcus* a definite selective advantage for growth at depth in oligotrophic areas, particularly compared to *Synechococcus*. These properties include (i) its absorption characteristics with maxima (for the SARG-like pigment type) in the range from 430 to 490 nm, which are more suitable than those of oceanic *Synechococcus* for collecting photons in the blue part of the spectrum, the wavelengths which penetrate the deepest in the ocean (107, 131); (ii) its higher photosynthetic yield than *Synechococcus* at all wavelengths (156); and (iii) its higher probability of absorbing rather than scattering incident photons (in contrast to *Synechococcus*), especially in the blue part of the spectrum (110).

In contrast, all *Prochlorococcus* isolates studied to date appear fairly sensitive to high irradiances, such as those available in near surface waters. In particular, curves of photosynthesis versus irradiance (108, 131, 156) saturate at relatively low irradiances (typically around 200 μmol quanta m⁻² s⁻¹) and display a strong inhibition at high irradiances, in a way reminiscent of that observed for deep natural populations (82). This is true even for *Prochlorococcus* strains belonging to the so-called high light clade, such as MED (see "Genetic diversity" below). One may wonder, therefore, whether researchers have been successful at obtaining isolates that are truly representative of populations thriving in the uppermost layers of the ocean.

Photoacclimation versus photoadaptation. One remarkable characteristic of the distribution of *Prochlorococcus* is that viable cells can be found over a very thick (150 to over 200 m) layer when the hydrologic conditions are stable enough to allow the water column to stratify, either quasi-permanently, such as in the tropical Atlantic and Pacific Oceans, or seasonally, such as in the Sargasso Sea or the Mediterranean Sea in summer. Over this natural light gradient, ranging from ca. 1,500 μmol quanta m⁻² s⁻¹ near the surface to less than 1 μmol quanta m⁻² s⁻¹ below 150 m, cells display a variety of differences. The most obvious ones are concomitant increases in cell size (see "Size and carbon content" above) and pigment content, as well as variations of the ratios of accessory pigments to Chl *a*₂, which generally occur below the depth of the mixed layer.

Goericke and Repeta (42) were among the first to measure Chl *a*₂ and Chl *b*₂ simultaneously in field populations and thus to provide direct information on the response of wild *Prochlorococcus* cells to changes in light intensity. The ratio of Chl *b*₂ to Chl *a*₂ they measured ranged from 0.15 in the surface layer to 2.9 below the deep Chl maximum. Similar ranges were observed in the Red Sea by using normal phase HPLC (180) and in the tropical Atlantic by using spectrofluorometry (129). In these two studies, *Prochlorococcus* cell concentrations were also enumerated by flow cytometry, which allowed variations of the content of *Prochlorococcus* cells in divinyl Chls to be computed. The Chl *b*₂ content varied 45-fold from 0.1 to 4.5 fg cell⁻¹, and the Chl *a*₂ content varied only 12-fold, from 0.23 to 2.7 fg cell⁻¹. In samples dominated by *Prochlorococcus*, such

large variations in *Prochlorococcus* divinyl Chl content and in the ratio of Chl *b*₂ to Chl *a*₂ are also reflected by conspicuous changes in the absorption and fluorescence excitation spectra of total phytoplankton pigments, which at depth display a marked peak at 480 nm, specific of Chl *b*₂ (10, 78).

All these variations concern the whole *Prochlorococcus* population, which is considered homogeneous. In fact, the ranges of size, Chl *a*₂ or Chl *b*₂ content per cell, ratio of Chl *b*₂ to Chl *a*₂, or red Chl fluorescence determined by flow cytometry measured in the field largely exceed those measured for individual *Prochlorococcus* isolates (43, 107, 131). A close look at flow cytometric red fluorescence histograms obtained from field samples reveals that several populations may be present over the vertical light gradient (Fig. 5). Bimodal red-fluorescence distributions of *Prochlorococcus* have very often been observed around the deep chlorophyll maximum layer in oligotrophic areas (7, 8, 17, 102, 121, 129). In such bimodal distributions, the fluorescence of the dim population is ca. 2 to 3 times lower than that of the bright population. Only the dim population seems to occur in the upper layer, while the bright population is the sole representative below the deep chlorophyll maximum. Each population, considered separately, does exhibit photoacclimation, in the physiological sense, as suggested by the increase of its modal red fluorescence (and scatter) with depth (Fig. 5). However, the bright and dim populations seem to be genetically distinct and have significantly different irradiance optima for growth. This hypothesis is supported by the fact that these populations also have genome sizes that differ by 14%, as determined by flow cytometry (17). Recently, Reckermann and Veldhuis (137) showed that if a sample with two populations is incubated at 20 m deep, only the dim population, i.e., the one that is acclimated to the prevailing light at 20 m, can grow, while the bright one rapidly disappears, probably consumed by grazers. Finally, using flow cytometric cell sorting, Moore et al. (108) were able to sort cooccurring bright and dim populations from natural samples at two sites and grow coisolates separately. Even after 2 years of culture, these coisolates maintained their physiological distinctness. Isolates with high red fluorescence had a higher ratio of Chl *b*₂ to Chl *a*₂ and were adapted to growth and photosynthesis at lower light levels relative to the dim populations. In addition, the high- and low-red-fluorescence coisolates were more than 2% different in their 16S rRNA sequence (see "Genetic diversity" below), confirming that genetically and physiologically different populations of *Prochlorococcus* can coexist. The relative differences in the light-dependent physiological parameters between the high- and low-red-fluorescence coisolates are similar to the differences observed between SS120 and MED4, leading to the hypothesis that *Prochlorococcus* isolates can be distinguished as low- or high-light-adapted ecotypes (108). The low-light-adapted isolates (SS120-like) have ratios of Chl *b*₂ to Chl *a*₂ that are 2- to 10-fold higher than those of the high-light-adapted isolates (MED4-like) over all irradiances (105). Furthermore, these two ecotypes display shifted optimal growth irradiances. Culture studies have also shown that no single isolate of *Prochlorococcus* is able to thrive over a range greater than 2.5 orders of magnitude of light irradiance (107). Thus, the ability of wild *Prochlorococcus* strains to stand a very wide range of light conditions in the field apparently results from both physiological and genetic diversity among species or groups within this genus.

Photosynthetic apparatus. One basic question raised by the observation of different *Prochlorococcus* pigment types both in the field (17, 129) and in culture (107, 110, 131) is whether strains or populations representative of those found near the surface and those found at the bottom of the euphotic zone

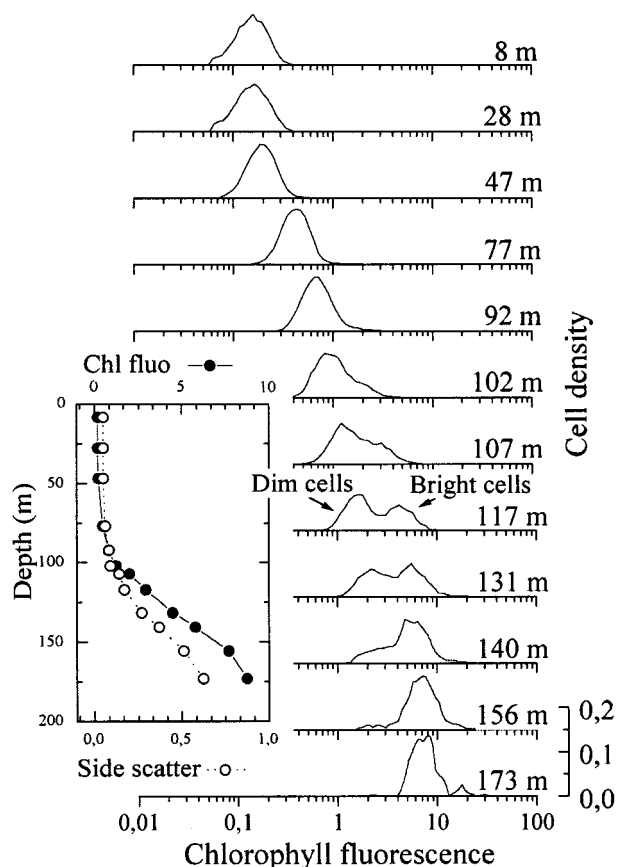


FIG. 5. Vertical distributions from bright and dim *Prochlorococcus* populations in the subtropical Pacific Ocean off Hawaii. The insert represents the vertical profile of side scatter and red chlorophyll fluorescence of the total *Prochlorococcus* population measured by flow cytometry. Adapted from reference 17 with permission of the publisher.

have not only different pigmentation and ecophysiological features but also structurally different photosynthetic apparatuses (Fig. 6). This question was addressed through the biochemical characterization of the pigment complexes of clones MED4 and SS120 (132). Since these strains differed mainly in their ratios of Chl b_2 to Chl a_2 (even at a given growth irradiance; see "Pigment composition" above) and since the majority of Chl b is located in antenna systems (46), the most striking structural difference between strains was expected to be found at the level of their major light-harvesting complexes. Proteins constituting these antenna complexes have apparent molecular masses on denaturing electrophoresis gels of 32.5 and 34 to 38 kDa in MED4 and SS120, respectively. Moreover, they are more abundant (at low light intensities), their relative amount varies more with irradiance, and they bind ca. 7 times as much Chl b_2 in SS120 as in MED4 (132). Sequencing of the *pcb* genes, which encode these antenna proteins, confirmed the large differences between strains, since polypeptides deduced from gene sequences have only 76% identity at the amino acid level (77). These polypeptides are, however, similar between MED4 and SS120 in length (352 and 351 amino acids, respectively), mass (44.9 and 44.6 kDa, respectively), and the presence of six putative transmembrane helices (insert in Fig. 6), suggesting that the discrepancy noticed between their apparent molecular masses resulted from posttranslational modifications, e.g., different levels of phosphorylation, affecting their

migration properties in denaturing gels (132). The antenna proteins of *Prochlorococcus* (Pcb), as well as those of the two other "prochlorophytes" (*Prochlorothrix* and *Prochloron*; see "Phylogeny" below), are closely related to the IsiA proteins found in iron-stressed freshwater cyanobacteria, such as *Synechococcus* strain PCC 7942, and are probably derived from them (77). All these proteins belong to the same family of Chl-binding proteins as the *psbC* and the *psbB* gene products (CP43 and CP47), which are major constituents of the photosystem II (PS II) internal antenna in all oxygenic photosynthesizing organisms (77). Despite the progress made recently in the identification of "prochlorophyte" antenna proteins, major questions still remain to be answered. The presence in both *Prochlorothrix* and *Prochloron* of several *pcb* genes (77, 169) raises the question whether some or all *Prochlorococcus* strains might not also possess multiple *pcb* genes, since these genes could play an important role in the photoacclimation capacity of these organisms. Other important topics are the exact localization of Chl-binding residues within the Pcb amino acid sequences and their total Chl-binding capacity per molecule. Furthermore, state transitions, a characteristic of photosynthesis in many cyanobacteria (see reference 147 and references therein), and the possible role of Pcb-antenna complexes in this phenomenon, if it takes place in *Prochlorococcus*, might be specifically targeted in future studies.

Although in both *Prochlorococcus* strains, most Chl b_2 is found associated with these major antenna complexes, a significant quantity of this pigment is also found in PS I fractions (35). This suggests that Chl b_2 may be associated either with some minor PS I-specific Chl a_2 -Chl b_2 -protein complexes or with the PS I core itself. Both hypotheses imply an unusual organization of the *Prochlorococcus* PS I with regard to other phototrophs since (i) cyanobacteria do not have any PS I-specific antenna and (ii) the PS I core of all photosynthetic organisms studied to date does not include any Chl b . Additionally, two proteins found in purified PS I fractions have unusual apparent molecular masses of 21 and 25 kDa, whereas no PS I proteins with such masses are known in cyanobacteria (35). Sequence data show that these proteins are related to PsaF and PsaL, respectively. Their anomalous sizes, compared to the sizes of the equivalent molecules known in other phototrophs, are caused by an insertion in the central part of the molecule in PsaF (167) and an N-terminal extension in PsaL (168). As in most cyanobacteria, the *psaL*-like gene is found in an operon with the *psaI* gene, which is located upstream of *psaL*, and the two genes are cotranscribed (168). In cyanobacteria, PsaL-PsaI complexes are known to be implicated in the formation or stabilization of PS I trimers (44), and the presence of such trimers in *Prochlorococcus* (35) suggests that despite its anomalous length, the PsaL-like protein of *Prochlorococcus* and its PsaI-like companion must have maintained similar functions. Knockout mutants of *Synechococcus* strain PCC 7002 with *psaL* mutation revealed that transitions from state 2 to state 1 (resulting from excess excitation of PS II and PS I, respectively) proceeded approximately three times more rapidly than in the wild type, possibly linked to the missing ability to form PS I trimers and the consequently enhanced mobility of PS I particles in the mutant (147). An interference of the N-terminal extension of *Prochlorococcus* PsaL with similar processes would be intriguing. The function of the PsaF-like protein, which in cyanobacteria also makes complexes with another protein, PsaJ, is even less clear so far. It has been suggested that the PsaF-PsaJ complex is active in docking negatively charged donors such as plastocyanin (44). Further genetic studies are required to know whether these modifica-

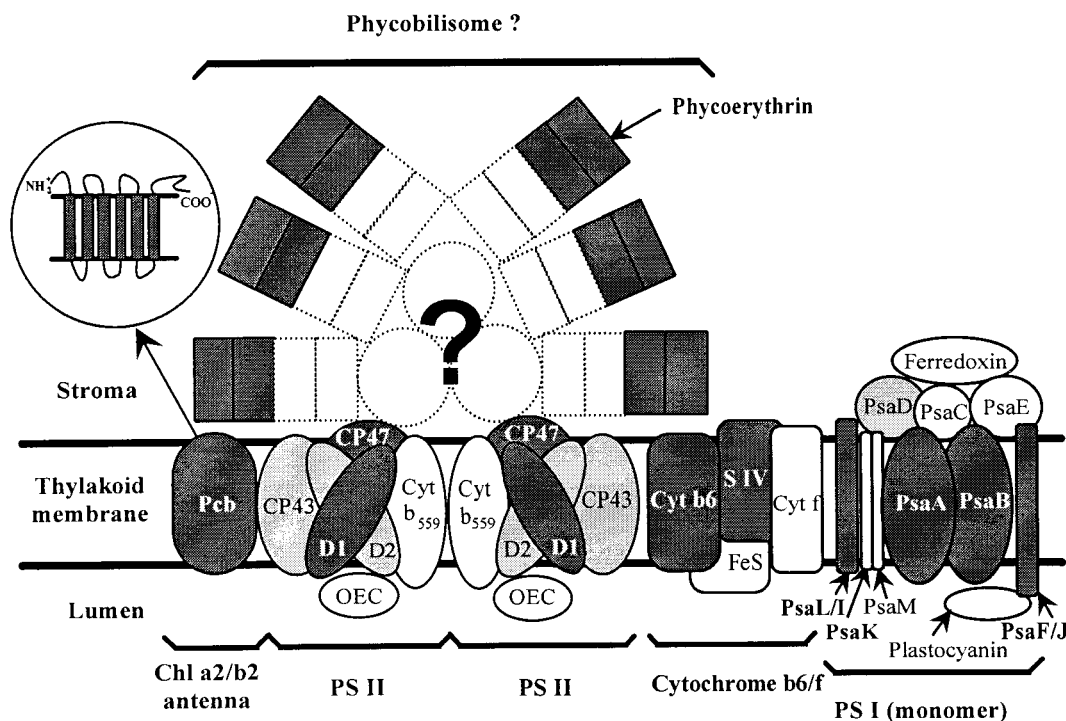


FIG. 6. Diagram showing the thylakoid proteins in *Prochlorococcus* and their putative organization by homology to the photosynthetic apparatus of cyanobacteria. The proteins whose genes have been sequenced partially or totally are shown in dark gray (see Table 3), and those which have been characterized only by immunoblotting (35, 90, 132) are shown in light gray. Although phycocerythrin is present in some strains such as SS120, it is not clear whether it is integrated in phycobilisomes, which, if present, would be very scarce. PS I is probably organized in trimers (35), but only one monomer is shown. The insert shows a detail of the Pcb protein which includes six transmembrane hydrophobic domains. For the electron transport chain, only the cytochrome b_6/f complex is shown because the existence of other components (such as NADH dehydrogenase and plastoquinone) has not been demonstrated yet. Other probable components of the photosynthetic apparatus such as cytochrome oxidase or ATP synthase are not shown either, since they are still uncharacterized in *Prochlorococcus*. Abbreviations: CP, chlorophyll-protein complex; Cyt, cytochrome; OEC, oxygen evolving complex.

tions in PS I proteins have some consequences in the structural organization and function of this photosystem.

Despite their originality, the PS I components appear fairly similar between SS120 and MED4. However, there is one striking difference between the two *Prochlorococcus* pigment types (beside their antenna proteins). Hess et al. (55) found a functional operon encoding the two subunits (α and β) of a phycocerythrin in SS120. Attempts to detect phycocerythrin genes in MED4 by hybridization and PCR did not provide any evidence for their presence in this strain (55). With a homologous antiserum to the SS120 phycocerythrin β subunit, it was not possible to detect even traces of this protein in MED4 or PCC 9511, whereas the same serum showed a strong reaction with total proteins from SS120 (56). The phycocerythrin found in SS120 was characterized as "type III" (55), based on major differences with regard to type I, which is widespread in cyanobacteria, and type II, which is present only in some marine *Synechococcus* (122). Characteristics of type III phycocerythrin include the presence of a single chromophore-binding site in the α subunit (as opposed to two or three in other phycocerythrin α subunits) and the absence of a few amino acids in the central part of the molecule (55). Future comparative and functional studies are required to elucidate whether these modifications play a functional role. Preliminary immunocytochemical data suggest an association of phycocerythrin with thylakoid membranes in SS120 (56). However, whether this phycocerythrin is included in structures comparable to the phycobilisomes of cyanobacteria (Fig. 6) or is present in the thylakoid lumen as in cryptophytes (32) is not known. In this context, it is noteworthy that the newly discovered Chl *d*-con-

taining prokaryote *Acaryochloris marina*, a species similar in many aspects to *Prochloron* sp. (see "Phylogeny" below), contains phycobiliprotein aggregates that are not organized in the form of phycobilisomes (100, 103). The phycocerythrin genes in SS120 are part of a larger gene cluster. The latter includes *ppeC*, a gene encoding a putative gamma phycocerythrin-like protein which may serve as linker polypeptide (54), and two genes homologous to *cpeY* and *cpeZ* (56). These two genes are thought to encode lyases involved in chromophore attachment to the α or β phycocerythrin subunits in *Fremyella diplosiphon* (66), and a similar role may be attributed to their gene products in *Prochlorococcus*. The presence of at least one linker polypeptide and two putative lyases in SS120 suggests that its phycocerythrin is chromophorylated and may participate in light harvesting. In contrast, this finding, together with the relatively low concentration of phycocerythrin in the cell and its probable intracellular localization within the thylakoids, constitute evidence against a role for *Prochlorococcus* phycocerythrins as nitrogen storage molecules, a function which was proposed for marine nitrogen-replete *Synechococcus* (190). Future studies are clearly needed to clarify the functional relevance of *Prochlorococcus* phycocerythrin.

Most of the other photosynthetic genes investigated so far in one or several *Prochlorococcus* strains (Table 1) show few unique characteristics compared to known cyanobacterial, algal, or plant models. However, the *psbA* gene raises some interesting questions. It is present as a single copy in both the *Prochlorococcus* strains SS120 (57) and MED4 genomes (145). This contrasts with other cyanobacteria, such as *Synechococcus* strain PCC 7942, which generally possess two isoforms of the

D1 protein, D1:1 and D1:2, that are differentially regulated by light (45, 123). D1:1 (encoded by *psbAI*) is the only type detectable in the thylakoid membrane at low light intensities. Upon a shift to high light intensities, the *psbAII/III* genes encoding the D1:2 form are induced, the *psbAI* mRNA is actively degraded, and D1:2 is substituted for D1:1 in the PS II reaction center (70). However, following this transient photoinhibition phase, the process reverses and D1:1 dominates again. Thus, D1:1 corresponds to long-term photoacclimation whereas D1:2 is expressed only transiently. The latter form provides a higher photochemical efficiency to PS II, thus dissipating excess energy via photochemical quenching and preventing photodamage to the photosynthetic machinery (123, 160). The two *Synechococcus* strain PCC 7942 D1 isoforms differ by only 25 amino acids. Among the few internal differences, residue 130 is a prime candidate for being responsible for the respective photochemical properties of each D1 type (38): D1:1 has a glutamine at this site, whereas D1:2 has a glutamate. Because of its single *psbA* gene copy, *Prochlorococcus* cannot use a similar regulation mechanism. The D1 protein of *Prochlorococcus* strain SS120 is phylogenetically closer to D1:1 of *Synechococcus* strain PCC 7942 than to D1:2 (57), and a glutamine is present at position 130 as in D1:1. In steady-state cultures of strains SS120 and MED4 acclimated to different irradiances, *psbA* transcript levels are proportional to light irradiances (34). They increase when cultures are shifted from low to high light intensities, more quickly in MED4 than in SS120, and decrease during the opposite shift. Thus, the D1 protein from both strains seems to have a mode of regulation closer (but not identical) to that of D1:1. Although the highly developed antenna system of *Prochlorococcus* strain SS120 is particularly efficient at harvesting low photon fluxes (132), it may become detrimental in the setting of abrupt light changes, because it conveys excess energy to the reaction center that cannot be completely dissipated by the turnover of D1 because its transcription is too slow.

Nutrient Assimilation

One of the most intriguing ecological characteristics of *Prochlorococcus*, besides its capacity to grow over a very wide range of irradiances in nature, is its ability to colonize extremely oligotrophic areas. For example, *Prochlorococcus* represents on average 73% of the photosynthetic carbon in the surface mixed layer off Hawaii (16). Under these conditions, its very small cell size and the resulting high surface-to-volume ratio are obvious adaptative advantages for nutrient uptake (19). However, very little is known about its basic physiological characteristics with respect to nutrient assimilation, partly because of the unavailability of axenic cultures, a gap only recently filled (143).

Most oceanic areas are assumed to be limited by nitrogen. In tropical areas, *Prochlorococcus* is present both in the surface layer, where presumably only reduced nitrogen forms are available, and in the deep chlorophyll maximum, where nitrates are present. However, in the Mediterranean Sea in winter, NO_3^- addition was able to stimulate *Prochlorococcus* cell cycling (177), suggesting that some populations might be able to take up oxidized forms of nitrogen. These data raise the possibility that there are different physiological types of *Prochlorococcus* adapted to grow on different nutrient sources, a hypothesis consistent with the available information for marine *Synechococcus* (185) and with the existence of genetically different *Prochlorococcus* (see "Genetic diversity" below). Nutrient uptake experiments must be performed with the axenic strain now available (143) and other forthcoming axenic strains more rep-

resentative of deep ecotypes. It will also be very interesting to investigate whether all *Prochlorococcus* strains possess the components necessary for NO_3^- uptake, such as the *ntcA* gene, whose product is involved in the activation of transcription of a set of genes required for the use of oxidized nitrogen sources (91).

Although nitrogen is the primary limiting nutrient in many oceanic areas, recent evidence points to phosphorus as limiting, either permanently, in areas such as the Mediterranean Sea, or transiently, in areas such as the Sargasso Sea (23, 162). *Prochlorococcus* is well represented in both areas, suggesting that it also has the ability to thrive under very low P concentrations. Parpais et al. (127) showed that *Prochlorococcus* becomes limited only at P-PO_4^{3-} concentrations of the order of 30 nM. Although the initial medium used to isolate *Prochlorococcus* contained organic instead of mineral phosphorus (see "Optimal growth medium" above), *Prochlorococcus* can grow very well on the latter form. In fact, organic phosphorus is rapidly mineralized by the heterotrophic bacteria present in the culture (127). It is possible that a similar mechanism occurs in situ and provides natural *Prochlorococcus* populations with mineral P, which is consumed so quickly that it remains undetectable. It is worth noting that *Prochlorococcus* possesses a *pstS* gene encoding a phosphate-binding protein, that is expressed only under P depletion and may play a role in its adaptation to oligotrophy; however, this gene is not unique to this organism (146).

Iron is the third major nutrient recently implicated in the limitation of primary productivity in remote areas of the ocean not subjected to eolian inputs, such as the equatorial Pacific (101), as well as in more coastal areas (76). The low iron requirement of *Prochlorococcus* could be a key factor in its success in central oceanic areas. This is suggested in particular by the fact that the *Prochlorococcus* PS II antenna is similar to IsiA, a protein induced under iron stress in *Synechococcus* sp. strain PCC 7942 (77). However, field experiments suggest that natural populations of *Prochlorococcus* are somewhat iron limited (although much less so than larger cells such as diatoms), since the addition of iron induces increases in cell size and chlorophyll fluorescence (192). Clearly, future laboratory experiments should aim at a better understanding of the regulation of cellular processes by iron in *Prochlorococcus* and should search for the eventual presence of siderophores, as evidenced previously in a variety of marine and freshwater cyanobacteria (189).

Cell Cycle

Although little studied in photosynthetic prokaryotes, cell cycling, a key cellular process that coordinates growth, DNA replication, and cell division, has received some attention in *Prochlorococcus* because of its application to assessment of growth rate and population status with respect to nutrient limitation in the field (see "Growth rates and loss processes in the ocean" below). The *Prochlorococcus* cell cycle can be easily studied by flow cytometry after staining with a DNA-binding dye (98, 104). The *Prochlorococcus* cell cycle resembles that of eukaryotes, with a discrete DNA synthesis phase (S phase) and two well-defined G_1 and G_2 phases, in contrast to some strains of marine *Synechococcus*, which may have more than two genome copies (6). As in most phytoplankton species (171), light or nutrient deprivation arrests cells in the G_1 phase of the cell cycle. A notable exception occurs when phosphorus is depleted. In this case, cells are blocked in all phases of the cell cycle, including the DNA synthesis S phase. Cells arrested in S are not able to recover upon addition of fresh phosphorus, in con-

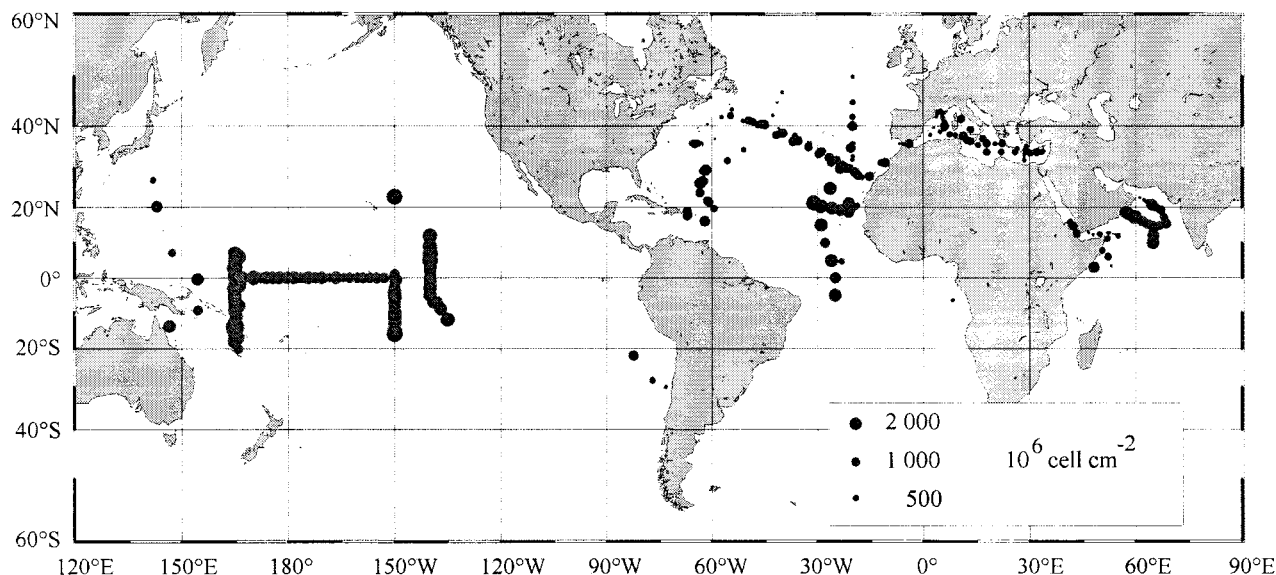


FIG. 7. Concentrations *Prochlorococcus* integrated over the water column throughout the world oceans. Based on data from Table 5.

trast to cells arrested in G_1 and G_2 (127). Light-dark entrainment induces a very strong synchronization of the cell cycle both in cultures (150) and in nature (94, 129, 175). The S phase usually takes place in late afternoon, and division occurs during the early part of the night. Natural *Prochlorococcus* populations from the Arabian Sea, as well as two cultured strains, can divide more than once per photocycle while remaining highly synchronized (150). In such cases, two cohorts of dividing cells occur in rapid succession, the second with a G_1 duration as short as 1 h. It would be interesting to investigate whether *Prochlorococcus* can display behaviors typical of circadian clock control (e.g., free running in continuous light), as demonstrated for *Synechococcus* strain PCC 7942 (62). Indeed, S-phase initiation appears to be linked to a light-triggered timer (150).

The only gene related to the cell cycle that has fully been characterized in *Prochlorococcus* so far is *dnaA*. In most bacteria, the encoded protein, DnaA, is essential for initiating chromosomal replication recognizing short, asymmetric sequence elements, the DnaA boxes, near the origin of replication, *oriC* (159). In *P. marinus* CCMP 1375 (139), DnaA consists of 461 amino acids and has 63% identity in a 315-residue overlap to *Synechocystis* strain PCC 6803 DnaA (140). Among the more than 35 bacterial DnaA sequences available in data banks, those of *Prochlorococcus* and *Synechocystis* form a distinguishable subfamily. The C-terminal DNA-binding domain of the *Prochlorococcus* recombinant DnaA protein expressed in vitro recognizes and binds specifically the heterologous *oriC* from *Bacillus subtilis* and *E. coli*, suggesting that the primary structure of the DnaA boxes might be evolutionarily conserved in *Prochlorococcus*. In many bacteria, *oriC* is physically near the *dnaA* gene and DnaA boxes are frequently present in its promoter region, providing a basis for binding and autoregulation (159). Surprisingly, no putative DnaA box could be identified in the analyzed genomic region of 5,304 bp containing *Prochlorococcus dnaA* (139). However, this is consistent with a unique gene organization in *Prochlorococcus*, which is very different from the highly conserved gene order, *rnpA-rpmH-dnaA-recF-gyrB*, found in a wide variety of bacteria. It is worth noting that the cyanobacterium *Synechocystis* strain PCC 6803

also lacks a DnaA-binding box near *dnaA* and displays an unusual gene arrangement in this region (140). Surprisingly, knockout mutants of *Synechocystis* strain PCC 6803 grow as well as the wild type, suggesting that DnaA is not essential for this strain, in contrast to all known eubacteria (138).

ECOLOGY

Oceanic Distribution

Prochlorococcus appears to have a very wide oceanic distribution. This is shown in Fig. 7, which is based on the analysis of more than 8,400 field measurements of *Prochlorococcus* made throughout the world oceans by flow cytometry (Table 5). *Prochlorococcus* is virtually ubiquitous in the latitudinal band extending from 40°N to 40°S (Fig. 7). It can still be found beyond 40°, but its concentrations decline fairly rapidly. The highest latitude where it has been recorded is 60°N off Iceland in the North Atlantic (11). This oceanic distribution suggests that low temperatures are lethal to *Prochlorococcus*, and this is confirmed by culture data (107). In the field, the lowest surface temperature at which *Prochlorococcus* is recorded is about 10°C (Fig. 8A). This contrasts strongly with that for *Synechococcus*, the other major marine unicellular cyanobacterium, which can be encountered, albeit at low concentrations, at temperatures as low as 2°C (151). There does not appear to be an upper temperature limit for *Prochlorococcus* distribution, since it is found in warm equatorial waters that reach 30°C at the surface. Still, the maximum integrated concentrations occur between 26 and 29°C and decrease above that temperature (Fig. 8A).

Although *Prochlorococcus* is most abundant in oligotrophic waters, both in absolute terms and relative to the other photosynthetic populations, it is by no means restricted to nutrient-depleted waters (Fig. 8B). In particular, it can be found in relatively coastal areas, such as the plume of the Rhone river in the Mediterranean Sea (178) or the Japanese waters of Suruga Bay (157). It has also been observed in the inner lagoons of Pacific atolls (18). Whether *Prochlorococcus* grows actively in such environments or is simply advected is an open question.

TABLE 5. Oceanographic data used to construct Fig. 7 to 10

| Ocean or sea | Area | Cruise | Ship | Yr(s) | Months | Reference |
|--------------------------|-----------------------|-----------------------------|------------------------------|-------------------|----------|-----------|
| Atlantic Ocean | North Atlantic | 87022 | CSS <i>Hudson</i> | 1987 | June | 88 |
| | | 88026 | CSS <i>Hudson</i> | 1988 | Sept | 87 |
| | | 89003 | CSS <i>Baffin</i> | 1989 | Apr | 86 |
| | | NIOZ Natl 89 | RV <i>Tyro</i> | 1989 | Aug–Sept | 179 |
| | | 91001 | CSS <i>Hudson</i> | 1991 | Apr | 81 |
| | | 92037 | CSS <i>Hudson</i> | 1992 | Sept | 83 |
| | | 93002 | CSS <i>Hudson</i> | 1993 | May | 83 |
| | | NOAA 93 | NOAA <i>Malcolm Baldrige</i> | 1993 | July–Aug | 11 |
| | | Delaware 95 | RV <i>Cape Henlopen</i> | 1995 | Apr | 84 |
| | | 95016 | CSS <i>Hudson</i> | 1995 | July | 84 |
| | Sargasso Sea | CHLOMAX | NO <i>Suroit</i> | 1987 | Sept–Oct | 116 |
| | | Lopez 96 | Launch | 1995 | June | 85 |
| | Tropical Atlantic | EUMELI 3 | NO <i>Atalante</i> | 1991 | Oct | 129 |
| | | EUMELI 4 | NO <i>Atalante</i> | 1992 | June | 129 |
| | | EUMELI 5 | NO <i>Atalante</i> | 1992 | Dec | 129 |
| | Indian Ocean | Arabian Sea | Arabian TTN043 | RV <i>Thomson</i> | 1995 | Jan |
| Arabian TTN045 | | | RV <i>Thomson</i> | 1995 | Mar–Apr | 14 |
| Arabian TTN049 | | | RV <i>Thomson</i> | 1995 | July–Aug | 117 |
| Indian Ocean and Red Sea | | NIOZ Indian | | 1992–1993 | May–Feb | 181 |
| Pacific Ocean | Equatorial Pacific | EQPAC TT007 | RV <i>Thomson</i> | 1992 | Feb–Mar | 74 |
| | | EQPAC TT008 | RV <i>Thomson</i> | 1992 | Mar–Apr | 7 |
| | | EQPAC TT008D | RV <i>Thomson</i> | 1992 | Mar–Apr | 27 |
| | | EQPAC TT011 | RV <i>Thomson</i> | 1992 | Aug–Sept | 74 |
| | | EQPAC TT012 | RV <i>Thomson</i> | 1992 | Sept–Oct | 27 |
| | | SURTROPAC 17 | NO <i>Noroit</i> | 1992 | Aug | 8 |
| | | OLIPAC | NO <i>Atalante</i> | 1994 | Nov | 115 |
| | | FLUPAC | NO <i>Suroit</i> | 1994 | Sept–Oct | 115 |
| | Tropical Pacific | HOT | <i>Moana Wave</i> | 1990–1994 | | 15 |
| | Coast of Japan | Suruga Bay | Boat | 1992–1993 | May–Oct | 157 |
| | South Pacific | Chile 95 | RV <i>Sonne</i> | 1995 | June | 85 |
| | West Tropical Pacific | Australia | RV <i>Sohgen-Maru</i> | 1990 | Nov–Dec | 154 |
| Mediterranean Sea | EROS Discovery 89 | HMS <i>Discovery</i> | 1989 | Jan | 178 | |
| | EROS Bannock 89 | <i>Bannock</i> | 1989 | July | 128 | |
| | POEM 91 | RV <i>Bilim/RV Shikmona</i> | 1991 | Oct | 89 | |
| | Eddy 92 | RV <i>Shikmona</i> | 1992 | Mar | 193 | |
| | EROS Valdivia 92 | <i>Valdivia</i> | 1992 | Mar | 97 | |
| | EROS Discovery 93 | HMS <i>Discovery</i> | 1993 | July | 4 | |
| | Malaga 93 | Launch | 1993 | Jan | 33 | |
| | MINOS | NO <i>Suroit</i> | 1995 | Jun | 176 | |

In contrast, although it is often present in offshore temperate waters, it has never been observed in some temperate, permanently mixed shallow seas such as the English Channel (175). Very recently, it has been discovered in yet another niche, in secondary deep chlorophyll maxima situated below the oxycline in the Arabian Sea (40, 64). Still, vast areas of the world oceans remain uncharted (Fig. 7), especially in the southern hemisphere, although more data are now available from regions such as the Indian Ocean (Table 5), where large oceanographic surveys have been recently completed as part of the Joint Global Ocean Flux Study.

Vertical plots of the available measurements of *Prochlorococcus* and *Synechococcus* concentration by flow cytometry (Fig. 9) reveal the broad features that distinguish these two photosynthetic prokaryotes. On average *Synechococcus* concentrations are about 1 order of magnitude lower than *Prochlorococcus* concentrations, although their abundance maxima are very comparable. Maximum *Prochlorococcus* concentrations on the order of 700,000 cells ml⁻¹ have been recorded in the Arabian Sea (14). Clearly, *Prochlorococcus* extends much deeper than *Synechococcus*, since the latter disappears virtually beyond 100 m deep. Three major types of vertical distributions are

observed (Fig. 10). The first type is encountered mostly in nearshore waters (Fig. 10A and B). *Prochlorococcus* is restricted to the surface mixed layer, and its abundance drops abruptly below the thermocline (Fig. 10A), where a sharp and narrow maximum can be localized (Fig. 10B). In such cases, *Synechococcus* has a vertical distribution that parallels that of *Prochlorococcus* and its abundance is similar or slightly higher. In the second type of distribution (Fig. 10C and D), *Prochlorococcus* presents a very sharp maximum concentration on the order of 10⁵ cells ml⁻¹ near the bottom of the euphotic zone, which decreases by at least 1 order of magnitude at the surface. The third type of vertical distribution (Fig. 10E and F), with *Prochlorococcus* extending from the surface to the bottom of the euphotic zone at nearly constant concentrations on the order of 1 × 10⁵ to 3 × 10⁵ cells ml⁻¹, is the most widespread in oceanic waters. Under these circumstances, the *Synechococcus* concentration is 1 to 2 orders of magnitude lower. Very often, a slight *Prochlorococcus* maximum occurs just above the depth at which concentrations begin to drop off (e.g., at 70 m in Fig. 10E). Generally, two different types of populations are found in the surface mixed layer and near the bottom of the euphotic zone (Fig. 5). This type of vertical profile is typical of

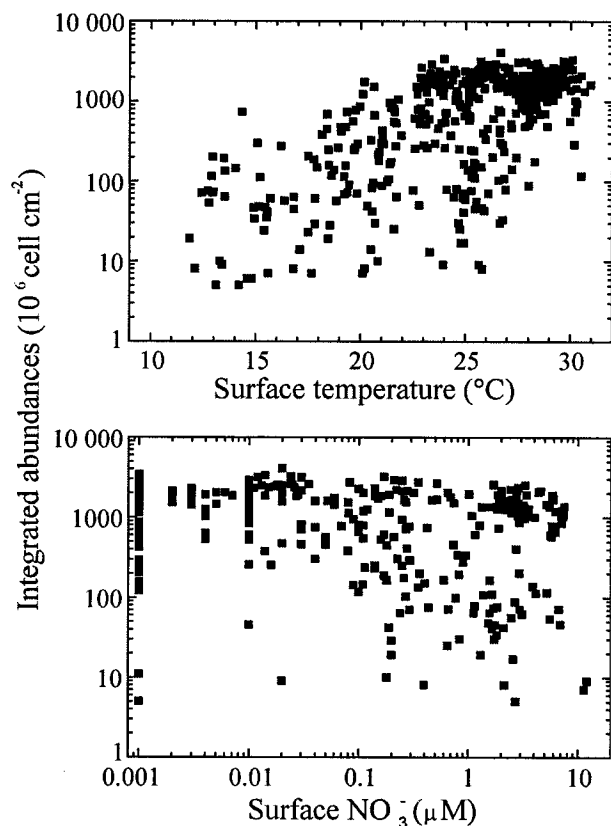


FIG. 8. Relationship between *Prochlorococcus* integrated concentrations and surface temperature (A) and surface nitrate concentrations (B). Based on data from Table 5.

oligotrophic waters. As oligotrophy increases (e.g., in the Pacific, going from the equator to the south gyre [Fig. 10E and F]), the *Prochlorococcus* layer deepens and *Synechococcus* concentrations decrease. Under these circumstances, the chlorophyll fluorescence of *Prochlorococcus* is very weak near the surface and cells are difficult to detect. This may lead to underestimates of cell concentrations at the surface and to creation of artifactual abundance maxima at depth.

In oceanic areas, where the water column is never mixed beyond 100 to 150 m deep (roughly between 30°S and 30°N), *Prochlorococcus* depth profiles vary only slightly throughout the year and are of the third type, as observed, for example, off Hawaii (15). In contrast, when the water column is mixed seasonally to depths below the euphotic zone, which usually happens in winter in more temperate waters, *Prochlorococcus* completely disappears during that period of the year (92). In spring, when the water column begins to re-stratify and abundant nutrients are found at the surface, *Synechococcus* may start to bloom in the mixed layer, followed by *Prochlorococcus*, yielding vertical profiles of the first type (119). As the bloom develops and nutrients become depleted, a well-defined *Prochlorococcus* layer deepens, resulting in a vertical profile of the second type (119). At these sites, profiles of the third type are not observed, at least to our knowledge, suggesting that there is not enough time between the end of the spring bloom and the next winter mixing event for a *Prochlorococcus* population to recolonize fully the oligotrophic surface layer. However, such recolonization may happen at locations where deep mixing does not occur every winter.

Growth Rates and Loss Processes in the Ocean

In culture, maximal steady-state division rates reported for *Prochlorococcus* are in general slightly below (or, more rarely, above [150]) one division per day, which is equivalent to a population growth rate of 0.5 to 0.6 day^{-1} (107, 131, 156). Besides being under the control of parameters such as light and nutrients, the division rate is under the strict control of temperature. For example, the MED4 and SS120 strains both have optimal growth rates at 24°C and cannot grow at or above 28°C , while their minimum growth temperatures are, respectively, 15 and 12.5°C (107).

In the field, division rates are very difficult to estimate directly from changes in cell concentrations because loss and growth processes balance each other and maintain populations at near constant levels. Initially, average estimates of *Prochlorococcus* growth rates of 0.2 to 0.3 day^{-1} in the top, euphotic layer of the Sargasso Sea off Bermuda were provided by the labeling of Chl a_2 with ^{14}C (43). However, this method yields strong over- or underestimates for populations subjected to photoacclimation (13), a common occurrence in the field. Use of the dilution method (72) to measure division rates in Equatorial Pacific waters gave variable and in some cases unrealistically low estimates for the *Prochlorococcus* division rate (0 to 0.5 day^{-1} at 10 m deep in reference 73), while its use to measure rates in the western Arabian Sea off Somalia suggested that *Prochlorococcus* can undergo more than two divisions per day in the field (137), i.e., higher than ever observed in culture.

Using cell cycle analysis, a method avoiding all artifacts linked to sample incubation, several groups demonstrated that the *Prochlorococcus* cell cycle is highly synchronized in the field, with DNA synthesis taking place in the afternoon and

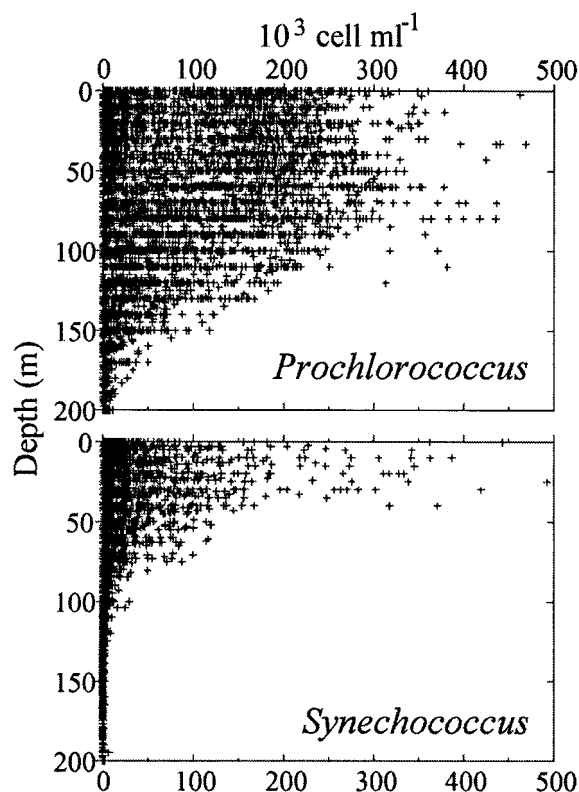


FIG. 9. Vertical distributions of *Prochlorococcus* (A) and *Synechococcus* (B). Based on data from Table 5.

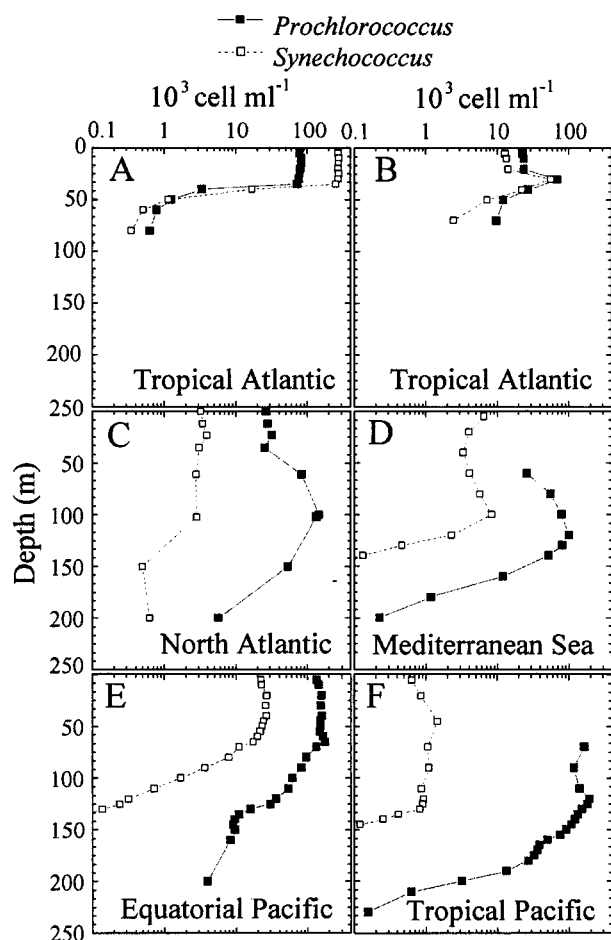


FIG. 10. Typical vertical distributions of *Prochlorococcus* and *Synechococcus*. (A and B) Surface layer maximum. (C and D) Deep maximum. (E and F) Uniform distribution over the euphotic zone. (A and B) North tropical Atlantic, EUMELI3 cruise (129). (A) EU site, off the coast of Mauritania (20°N, 18°W). (B) MESO site (18°N, 21°W). (C) North Atlantic 30°N, 23°W (11). (D) Eastern Mediterranean Sea, 34°N 18°E, MINOS cruise (176). (E) Equatorial Pacific 150°W, 5°S (174). (F) Tropical Pacific 150°W, 16°S (174). (D and F) *Prochlorococcus* chlorophyll fluorescence was too weak to be detected at the surface.

division taking place after dusk (94, 174, 175). Until recently, maximal growth rates recorded in areas such as the equatorial and subtropical Pacific Ocean were around 0.8 day^{-1} , i.e., slightly above 1 division per day. However, populations from the Arabian Sea seem to be able to sustain growth rates of almost 1 day^{-1} , i.e., about 1.4 divisions per day (150). Surprisingly, maximal rates were observed not at the surface but between 30 and 70 m deep. Above this depth, DNA synthesis is retarded by up to 4 h (a possible deleterious consequence of UV radiation that affects the top 20 to 30 m of the ocean) and, concomitantly, division rate decreases by up to 30% at the surface (174, 175).

Since *Prochlorococcus* cells reach division rates of the order of 1 division per day in marine waters and population abundance remain very stable over large timescales and spatial scales (15), loss processes must precisely balance division. This has been established in field experiments (93). However, the exact nature of the major loss processes has not been elucidated yet. Grazing by microzooplankton is probably important, as it is for heterotrophic bacteria (188). The single experimental study devoted to this question (22) revealed that ciliates

exhibit a marked preference for *Synechococcus* over *Prochlorococcus* when given both together, leading to the speculation that the micrograzer community could be very different in *Prochlorococcus*- and *Synechococcus*-dominated marine ecosystems. Large filtering organisms, such as sponges in coastal systems (134) or filter feeders in reefs (191), could also have a major impact on *Prochlorococcus*. Viral lysis of *Prochlorococcus* has not been studied, but it has been shown not to be a significant cause of mortality of *Synechococcus* natural populations (183). Finally, near the surface, UV-induced lysis probably affects *Prochlorococcus* as strongly as it does heterotrophic bacteria (111).

DIVERSITY AND PHYLOGENY

Genetic Diversity

Although in the previous sections we mainly discussed data obtained for two strains, MED (or its clonal derivative MED4) and SARG (or its clonal derivative SS120), which are representative of surface and deep oceanic layers, respectively, many more strains have been isolated (Table 1). Molecular studies of this set of isolates suggest that there is no correlation between their genetic distance, as assessed by using either restriction fragment length polymorphism patterns (145) or 16S rRNA gene sequence comparisons (166), and the geographic distance between sites from which these strains were isolated. For example, the genetic distance between strains isolated from the mixed layer of distant sites such as the Mediterranean Sea and the northwestern Atlantic Ocean seems to be much shorter than that between the latter isolates and others from deep waters ($\geq 100 \text{ m}$) of the Sargasso Sea. Thus, the natural vertical gradients of light and nutrients may provide more stringent conditions for speciation within the *Prochlorococcus* genus than does geographic distance. For isolates whose pigmentation has been determined, genetic differentiation seems to be related to differences in the ratios of Chl b_2 to Chl a_2 . The “high-light-adapted clade” (Fig. 11), as defined by Urbach et al. (166), groups strains from distant sites, all having low ratios of Chl b_2 to Chl a_2 (from 0.1 to 0.6). The well-studied clone MED4 is typical of this clade. Strains with low ratios of Chl b_2 to Chl a_2 , such as strain GP2, a_2 have sometimes been isolated from deeper layers (156), and the converse is also true. This is probably the main cause of exceptions in strain clustering, when the isolation depth is the only factor considered, as in the work by Scanlan et al. (145). It is most likely that strains with such deviant pigment signatures did not constitute the dominant genotype in the original seawater sample but were selected from a genetically mixed population by the conditions of culture following isolation (29, 108). A much larger genetic variation seems to exist among strains with high ratios of Chl b_2 to Chl a_2 than among those with low ratios (166), which is consistent with the more intense physical mixing rates experienced by surface oceanic populations. Moreover, their position in the phylogenetic tree suggests that members of the high-light clade are the most recently evolved of the *Prochlorococcus* strains.

As mentioned above, MED4 and SS120 differ by the presence in the latter strain but not in the former of a large gene cluster encoding phycoerythrin and related proteins (see “Photosynthetic apparatus” above). It would be interesting to know if this distinctive peculiarity extends to all strains, with those from the high-light-adapted clade being deprived of this gene cluster and others possessing it. This cluster is an example of the role that environmental parameters (in that case mainly light) may play in driving genetic diversity within the genus

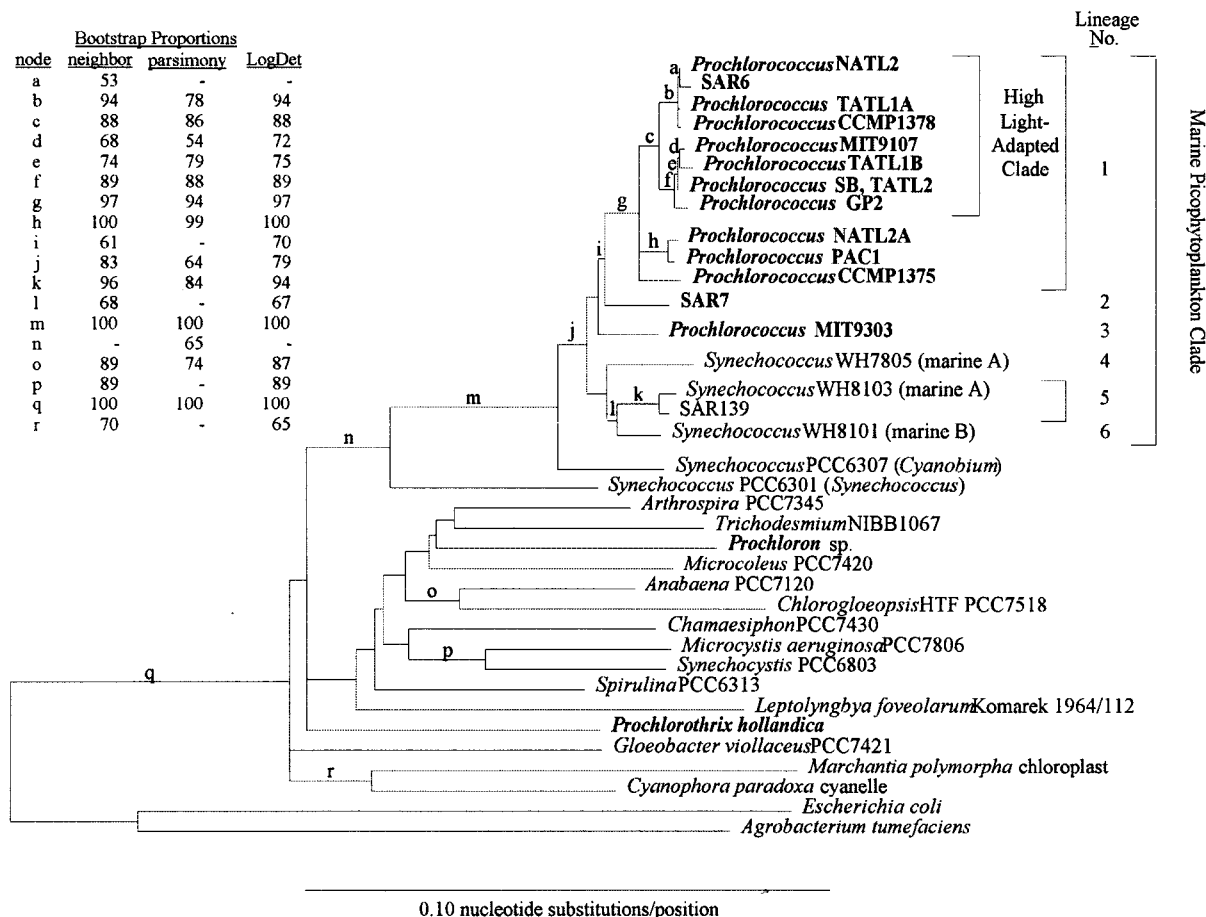


FIG. 11. Phylogenetic relationship between *Prochlorococcus* isolates and cyanobacteria inferred from 16S rRNA gene sequences. Reprinted from reference 166 with permission of the publisher.

Prochlorococcus, and it is expected that other genes or operons exclusive to one of the groups might soon be discovered. Furthermore, it would be very informative to compare whole genome regions between different *Prochlorococcus* genotypes to determine whether there are hot spots for recombination that change more rapidly than do other regions.

Most of the conclusions drawn from culture studies are confirmed by molecular studies on the genetic diversity of *Prochlorococcus* in the field (124, 163). In particular, they also suggest a very high genetic variability among individuals of *Prochlorococcus*-like populations. The first sequences from natural *Prochlorococcus* populations were determined in studies investigating the diversity of the whole prokaryotic community in oceanic waters by using the 16S rRNA gene as a marker (31, 39, 148). Since these sequences were obtained prior to the sequencing of this gene in cultured *Prochlorococcus* cells (165), they were first misinterpreted as belonging to organisms from the *Synechococcus* marine A cluster. However, a later reanalysis of a number of sequences of shotgun clones obtained from the field, i.e., SAR6 (39), ALO7, ALO23, and ALO37 (148), and NH16-15 (31), showed their closer relatedness to *Prochlorococcus*, with a sequence similarity of around 98% (112).

The *rpoC1* gene (encoding the RNA polymerase γ subunit) has also been used as a marker for more specific studies of natural populations of photosynthetic prokaryotes from the Sargasso Sea and the California Current (29, 124). In neither of these field studies was any gene sequence retrieved by am-

plifying *rpoC1* absolutely identical to that in cultured isolates. Environmental clones could be separated into two genetically distant clades, A and B, with the former being genetically close to the MED4 clone. In contrast, the SS120 clone did not fall into either clade. For samples obtained under well-established stratified conditions (e.g., in the California Current), surface and deep environmental clones fell neatly into the A and B clades, respectively. However, clones from recently or weakly stratified water columns fell into both clades, suggesting that surface and deep populations probably occur together in the water column following mixing and become separated only once stratification is sufficiently (or permanently) established. The A clade is probably equivalent to the "high-light clade" identified by using 16S rRNA (166). The variability of the *rpoC1* gene allows us to further subdivide this A clade into two deep branches (A1 and A2), which may differ in their temperature or nutrient preferences (29).

Another important outcome of the *rpoC1* studies (29, 124) is that the genetic diversity among clones of a cluster is significantly higher for *Prochlorococcus* than for the phylogenetically related cyanobacterial genus *Synechococcus*, which generally occurs together with *Prochlorococcus* in the field. In contrast, *Synechococcus* seems to display a larger number of deeply branching clades (29), which may correspond to the wider oceanic distribution of *Synechococcus* compared to *Prochlorococcus*. It must be stressed that, as for *Prochlorococcus*, several pigment types of *Synechococcus* may be identified by flow cy-

tometry or spectrofluorometry in the ocean, owing to their different ratios of phycourobilin to phycoerythrobilin. High-phycoerythrobilin *Synechococcus* strains dominate in mesotrophic environments, whereas only high-phycoerubilin populations are found in oligotrophic waters (75, 118, 120). Thus, in an oligotrophic environment, the genetic variability of *Synechococcus* is expected to be lower than that of *Prochlorococcus*. Moreover, because of the lower population density of *Synechococcus* than *Prochlorococcus* in oligotrophic environments, extinction of lineages due to selective pressures and random genetic drift may occur more frequently for the former (124). More work is needed to check whether the reverse is true in mesotrophic environments, where *Synechococcus* may outnumber *Prochlorococcus*.

In another study, Urbach and Chisholm (164) gathered an extended data set, consisting of sequences from *Prochlorococcus* cells that had been sorted by flow cytometry from different depths at two stratified sites in the North Atlantic ocean. They used the variable intergenic region separating *petB* and *petD* genes and part of these genes as a marker. Of 68 *Prochlorococcus* alleles analyzed, only 16 were found more than once, and of these 16, only 12 appeared in more than one sample. The results of this study were consistent with the hypothesis that populations from different depths of stratified water columns are derived from a single gene pool but that gene frequencies vary at different depths, resulting in populations with different ratios of Chl b_2 to Chl a_2 . The main difference between this study and the previous ones based on *rpoC1* (29, 124) is that in the latter, DNA sequences were classified as *Prochlorococcus* or *Synechococcus* on the basis of the G+C content of their third codon position and their phylogenetic proximity to the two *Prochlorococcus* isolates MED and SS120 or to *Synechococcus* isolates WH7803, WH7805, and 8103. However, it is possible that a number of the sequences classified as “*Synechococcus*-like” by this method (e.g., clones 1CNB, 1CNI, and 1CNJ in reference 124) were in fact from *Prochlorococcus* cells. This inference can be drawn from the fact that *Prochlorococcus* strain MIT9303 has 55% G+C at third codon positions and is more closely related to *Synechococcus* strain WH8103 than to any known *Prochlorococcus* strains (166). There is also the (wholly theoretical) possibility that some of the “*Prochlorococcus*-like” *rpoC1* clones were in fact from *Synechococcus* cells. Urbach and Chisholm (164) safely classified as *Prochlorococcus* a large number of clones, which could have been classified as *Synechococcus* because of their high G+C content; the correct classification occurred because the cells were initially purified by flow cytometric cell sorting. If some of *Synechococcus*-like clones in the study by Palenik (124) were indeed *Prochlorococcus* and none of the *Prochlorococcus*-like clones were *Synechococcus*, this strengthens the conclusion that the *Prochlorococcus* population in the sample analyzed was more genetically diverse than the *Synechococcus* population.

The apparently limited genetic difference observed between near surface *Prochlorococcus* from remote areas, a conclusion which comes mainly from culture studies (145, 166) and remains to be confirmed in the field, raises a number of questions. For instance, surface populations appear to be physiologically different between mesotrophic areas, such as the northwestern Mediterranean Sea in winter, and very oligotrophic subtropical areas. Growth of the former populations seems to be limited by inorganic nitrogen (177), whereas the latter populations are able to sustain high growth rates in the nutrient-depleted mixed layer (94). It is surprising that these physiologically different populations may correspond to the same genotype (or species), since they probably have very

different nutrient acquisition systems and metabolisms. Along the same lines, temporal changes in the dominant *Prochlorococcus* species may also occur at a given site and depth, even over the short term, as suggested by observations of changes in scatter or fluorescence characteristics of natural *Prochlorococcus* populations from the Equatorial Pacific following iron addition (192) or the passage of a tropical instability wave (7). In both cases, authors have put forward the hypothesis that these artificial or natural events induced physiological changes within a homogeneous population (i.e., growth and/or photosynthesis), but a change in the *Prochlorococcus* population structure is also possible. Although never applied in such studies, use of molecular markers should be able to answer a number of such questions on population dynamics in the field.

Phylogeny

The original pigment complement found in *Prochlorococcus* cells led Chisholm et al. (21) to call this organism a prochlorophyte, which was the name previously given to the only two other known oxyphototrophic prokaryotes possessing Chl *b* but no phycobilins. The first of these organisms to be discovered was *Prochloron* sp., which is an obligate symbiont of marine ascidians (80). The second was *Prochlorothrix hollandica*, a free-living, filamentous prokaryote found in some freshwater lakes (12). Hence, the three known “prochlorophytes” appear to differ by many aspects. Although they were considered for some time to belong to a distinct phylum, the *Prochlorophyta* (79), or order, the *Prochlorales* (113), molecular studies involving, e.g., 16S rRNA (165), *rpoC1* (125), or *psbA* (57) gene alignments have clearly demonstrated the very distinct origins of these organisms. For example, *Prochlorothrix* lacks seven amino acids near the C terminus of the otherwise very conserved D1 protein, a characteristic it shares with green plastids (109), whereas *Prochlorococcus* SS120 does not lack this region (57). All three “prochlorophytes” are emerging within the cyanobacteria but on clearly separate branches. Thus, there is no phylogenetic unity within “prochlorophytes” (165), and these should soon be reclassified as *Oxyphotobacteria* (135), together with the Chl *d*-containing *Acaryochloris marina* (103) and all classical cyanobacteria. *Prochlorococcus* is phylogenetically nearest to *Synechococcus* of the marine A cluster, such as WH 8103, a typical oceanic species (165). In fact, the fuzzy phylogenetic limits between *Prochlorococcus* and marine *Synechococcus* suggest a near simultaneous diversification of these groups from a common ancestor (166). Among *Prochlorococcus* strains, those belonging to the “high-light clade” (Fig. 11), which inhabit the nutrient-depleted surface layer, appear to be the most recent to diverge (166). This observation is consistent with the idea that the ancient oceans might have been less nutrient limited than they are nowadays.

The discovery of phycobilins and phycobiliproteins in *Prochlorococcus* strain SS120 (55) invalidates even more strongly the original definition of the term “prochlorophytes” as being oxyphototrophic prokaryotes possessing Chl *b* but no phycobilins. The phylogenetic origin of these phycobilins has not yet been fully resolved. There is no evidence that these genes were acquired through a lateral gene transfer. Instead, the characteristics of *Prochlorococcus* phycoerythrins are consistent with the supposed evolution of the organism based on 16S rRNA or *rpoC1* phylogenies. The two structural genes encoding the α and β subunits of phycoerythrin are part of a larger gene cluster that also contains a putative linker polypeptide as well as several open reading frames and genes frequently found clustered in cyanobacterial phycobiliprotein operons (54). This gene arrangement resembles that of cyanobacteria such as *Syn-*

echococcus strain WH8020, and phylogenetic analyses place *Prochlorococcus* type III phycoerythrin slightly closer to the marine type II phycoerythrin than to type I (54). That these genes are absent in at least one member of the phylogenetically recent high-light clade indicates that these strains may have eliminated from their genome any genes that had become useless, a process consistent with the relatively small size of *Prochlorococcus* genome.

The assertion of a common origin of *Prochlorococcus* and *Synechococcus* has been challenged, however, by one study that used the *rbcL* gene, encoding the large subunit of Rubisco (155). In this analysis, *rbcL* of strain GP2 was found to resemble more closely the Rubisco form IA of the γ subdivision of the *Proteobacteria* than that of most cyanobacteria (form IB). From these data, the authors concluded that *Prochlorococcus* was the most primitive oxygenic prokaryote. However, later analysis of the *rbcL* gene in two other *Prochlorococcus* strains (MED and PAC) did not support this conclusion, since they were found to group with form IB (133). Furthermore, the marine *Synechococcus* strain WH7803 possesses a Rubisco form IA (186), as does *Prochlorococcus* strain GP2. Thus, the Rubisco form is probably not a useful marker for the phylogeny of *Prochlorococcus* and *Synechococcus*. Since the *rbcL* gene is often transferred horizontally, such an event probably occurred between ancestors of *Prochlorococcus* strain GP2 and purple bacteria in the γ subdivision of the *Proteobacteria* (26). The close clustering between the GP2 and MED strains based on 16S rRNA (Fig. 11) suggests that this transfer could be fairly recent. It is interesting that the GP2 Rubisco is very similar to the corresponding protein of *Hydrogenovibrio marinus*, an aerobic chemolithotrophic bacterium which is present in similar environments to those favored by *Prochlorococcus* and *Synechococcus* and thus would be an example of a possible donor for such a lateral gene transfer to recent ancestors of GP2 and WH7803. A remaining question to be answered by a more detailed study is whether in *Prochlorococcus* strains such as GP2, Rubisco genes are organized in a tricistronic operon together with a *ccmK* homologue that encodes a component of the inorganic carbon-concentrating mechanism (186), as is the case in *Synechococcus* strain WH7803.

CONCLUSION

In the decade since its discovery, *Prochlorococcus* has revealed itself to be a truly fascinating organism. It is unambiguously one of the most important components of marine phytoplankton in terms of biomass, although its real importance in terms of global production is still only very approximately estimated (130). The analysis of its natural distribution provides many clues to interpret the physiological and genetic features it displays in the laboratory and vice versa. Its most spectacular traits include its extremely small size, its unique pigmentation, and its ability to proliferate in nutrient-depleted areas. From a phylogenetic viewpoint, it is unfortunately not the long-sought-after direct ancestor of the green chloroplast. Still, the evolution of *Prochlorococcus*, *Prochloron*, and *Prochlorothrix*, which have evolved independently from different cyanobacterial ancestors and converged to recruit the same protein to build a novel antenna to replace the more complex phycobilisomes (77), is a very exciting topic. Much remains to be discovered about *Prochlorococcus*, and this review has pointed out some directions for future research. In particular, there is a clear need for more axenic strains isolated from a variety of ecosystems where *Prochlorococcus* proliferates. Because of its many intriguing features, but even more importantly because of its true significance from a global environ-

mental perspective, *Prochlorococcus* could establish itself in the near future as a standard model in photosynthetic prokaryote research, warranting a concerted effort to sequence its genome.

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