Multiplication of antenna genes as a major adaptation to low light in a marine prokaryote

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Two ecotypes of the prokaryote Prochlorococcus adapted to distinct light niches in the ocean have been described recently. These ecotypes are characterized by their different (divinyl-) chlorophyll (Chl) a to Chl b ratios and 16S rRNA gene signatures, as well as by their significantly distinct irradiance optima for growth and photosynthesis [Moore, L. R., Rocap, G. & Chisholm, S. W. (1998) Nature (London) 393, 464-467]. However, the molecular basis of their physiological differences remained, so far, unexplained. In this paper, we show that the low-light-adapted Prochlorococcus strain SS120 possesses a gene family of seven transcribed genes encoding different Chl a/b-binding proteins (Pcbs). In contrast, Prochlorococcus sp. MED4, a high-light-adapted ecotype, possesses a single pcb gene. The presence of multiple antenna genes in another low-light ecotype (NATL2a), but not in another high-light ecotype (TAK9803-2), is demonstrated. Thus, the multiplication of pcb genes appears as a key factor in the capacity of deep Prochlorococcus populations to survive at extremely low photon fluxes.

he ecological distribution of photosynthetic organisms is strongly controlled by the availability of solar light. A remarkable example of the role of light in modeling natural communities in the ocean was provided recently by the discovery of two ecotypes of the prokaryote Prochlorococcus living at different depths (1). These ecotypes, characterized by their different (divinyl-) chlorophyll (Chl) a to Chl b ratios and 16S rRNA gene signatures, were shown to have significantly distinct irradiance optima for growth and photosynthesis (2-4). Thus, major differences can be expected between the light-harvesting complexes of these ecotypes. The nature of the antenna genes of Prochlorococcus, as well as the two other prokaryotes that contain Chl b as the major accessory pigment, has been identified recently (5). The encoded proteins, named Pcbs for prochlorophyte Chl b-binding proteins, seem to be phylogenetically and structurally different from the antenna proteins found in photosynthetic eukaryotes (6). Although Prochlorothrix was shown to possess three different *pcb* genes, a single gene copy was found in *Prochloron* (5, 7). Up to now, only one $pc\bar{b}$ gene was reported for each of two Prochlorococcus clones (5), representative of the high-light- and low-light-adapted ecotypes (1, 8), MED4, from surface waters of the Mediterranean Sea, and SS120, isolated from the deep waters (120 m) of the Sargasso Sea, respectively. However, the biochemical characterization of their thylakoid proteins revealed a much more prominent band of antenna proteins extending over a wider range of molecular masses in SS120 (31-38 kDa) than in MED4 (31-32.5 kDa) at low light (9), suggesting that the former strain might in fact possess several proteins. In this study, we reexamined these strains and other representatives of high- and low-light-adapted ecotypes and found that the latter but not the former had multiple antenna genes. The evolutionary and ecological implications of this discovery are discussed.

Materials and Methods

Culturing and Spectrofluorimetry. *Prochlorococcus marinus* clone SS120 (CCMP1375; ref. 1), *Prochlorococcus* sp. clone MED4

(CCMP1378; ref. 1), and the unicyanobacterial *Prochlorococcus* sp. strains NATL2a (isolated at 30 m in the North Atlantic Ocean, 38° 59' N, 40° 33' W) and TAK9803–2 (isolated at 20 m in the Takapoto lagoon, 14° 30' N, 145° 20' W) were grown at 24 \pm 1°C in PCR-S11 medium (10). To obtain comparable fluorescence excitation spectra using a Perkin–Elmer LS50 spectrofluorimeter, all strains were maintained under blue light at ~20 μ mol Q m⁻²·s⁻¹ (Q is Quanta), a value intermediate between the growth irradiance maxima of MED4 and SS120 (11).

Gene Isolation and Sequencing. pcb fragments were amplified directly from *Prochlorococcus* cells by using several sets of primers: P-G-pcb-1-a-S-20, 5'-ATCGARACNTAYGGNAAYCC-3'; P-G-pcb-25-a-S-20, 5'-TNACNTAYGGNTGGTGGGC-3'; P-Gpcb-760-a-A-20, 5'-GCNGCNATDATNGCCATCCA-3'; and P-G-pcb-998-a-A-20, 5'-ACNCKYTTRAARTCRAANCC-3'. PCR was performed with the Ready-to-Go PCR Beads kit (Amersham Pharmacia). pcb fragments of 730 to 1014 bp were purified with the Wizard kit (Promega) and cloned by using the TOPO-TA cloning kit (Invitrogen). Complete pcb genes were obtained by screening a genomic library of Prochlorococcus SS120 established in λ GEM-12 (Promega), and DNA sequences were determined on a Vistra 725 automated sequencer (Molecular Dynamics). Protein sequences were aligned by using PILEUP (Wisconsin Package, Version 8; Genetics Computer Group, Madison, WI), followed by manual refinement on the GENETIC DATA ENVIRONMENT software package (12).

Northern Blotting. Total RNA was isolated and analyzed as described previously (13). Gene-specific *pcb* fragments of 271 to 298 bp were amplified by PCR with the cloned genes as templates. From these, $[^{32}P]$ UTP-labeled specific RNA probes were synthesized by using the Lig'nScribe and MAXIscript kits (Ambion, Austin, TX). After hybridization under stringent conditions (61.5°C), detection was performed by using a Storm PhosphorImager (Molecular Dynamics). The absence of cross-hybridization between each specific RNA probe and transcripts of all other *pcb* genes was verified by RNase protection assay experiments (data not shown).

Abbreviations: Chl, chlorophyll; Pcb, prochlorophyte Chl b-binding protein.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. AF198526 (*pcbB*), AF198527 (*pcbC*), AF198528 (*pcbD*), AF198529 (*pcbE*), AF198530 (*pcbF*), and AF198531 (*pcbG*)].

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	M-PcbA	S-PcbA	S-PcbB	S-PcbC	S-PcbD	S-PcbE	S-PcbF	S-PcbG
M-PcbA		75	64	63	63	63	57	54
S-PcbA	87	_	71	66	66	66	61	58
S-PcbB	77	80	_	61	70	71	65	56
S-PcbC	78	79	73	_	56	57	53	66
S-PcbD	77	80	80	70	_	66	67	55
S-PcbE	78	80	79	71	78	_	57	55
S-PcbF	74	76	76	68	80	71	_	50
S-PcbG	72	75	70	80	71	71	68	_

Table 1. Percentages of sequence identities (upper right triangle) and sequence similarities (lower left triangle) between the different Pcb proteins from *Prochlorococcus* MED4 (M-PcbA) and SS120 (S-PcbA to S-PcbG)

*Sequence similarities comprise identical sequences plus conservative substitutions within the groups DN, EQ, ST, KR, FYW, and LIVM.

Results

Fragments of the *pcb* gene were amplified by PCR directly from whole *Prochlorococcus* MED4 and SS120 cells. We confirmed that the high-light-adapted genotype contained a single *pcb* gene. Only one PCR product with the expected sequence (5) was

obtained, and hybridization of restriction digests of chromosomal DNA with *pcb* probes at low stringency resulted only in the banding pattern predicted from the reported *pcb* sequence (data not shown). However, from the low-light strain, seven different *pcb* genes were recovered. We arbitrarily called them *pcbA* to

	<> MSR I> <	
	20 * 40 60 80	
M-PcbA	MQTYGNEDTTYGWWAGNSG-VANRSGKFIAAHVAHAGLIVEWAGAFHLFELSRFDPSVEMGQOPLIALPHLATLCIGFDADGVLMGDTKPVLAI	93
S-PcbA	MQTYGNEDVTYGWWAGNSG-VTHPPGKFIAAHAAHTGLDCELAGAFTLFELARFDPSVPMGHQPLIALPHLATLCIGFDEAGTFVGGT-TVTAI	92
S-PcbB	MQTYGNENVTYGWWAGNSG-VTNRSGKFIAAHAAHTGLIAFGCCAATLVELAGFDPSLPMCHQSSLFLAHLASVCICFDDAGVWTGVGVANI	91
S-PcbC	RADYGNENVTYAWYAGNSG-TTNRSGKFIAAHAAHAGLMMEWAGAFTLFELARYDSSIPMGNONLICLPHLAGLCIGGVSNGVITE-PYGCTVI	92
S-PcbD	MQTYGNEEVTYGWWAGNSV-VTNRSGRFIASHVGHTGLICEAAGGSTLWELARYNPEIPMGHOSSLFLAHLASICIGFDEAGAWTGVGVATI	91
S-PcbE	MQTYGNDDPTYGWWVGNSV-VTNKSSRFIGSHVAHTGLIAFTAGANTLWELARFNPDIPMGHQGMVSIPHLASLCIGFDQAGAWTGQDVAFV	91
S-PcbF	MQTYGNDDVTYDYWAGNAS-VTNRSGRFIASHAAHTGMIAFGAGSNTLFELSRFDSSLPMGDQGFVFLPHLASVCIGFDEAGVWTGAGVVTL	91
S-PcbG	RADYGDENVSYAWYAANAGAVTNKSGRFISSHIAHTGLICEGAGANTLFELARYNPDLPMGSQGLVVLPHLAGLCLGGISNGVFTD-TYQLLVV	93
	MSR II> < MSR III>	
	*00 * 120 140 *160 180	
M-PcbA	aiv <mark>hi</mark> vs <mark>mulaagelih</mark> slllpgnleesevakarkeniemonpokl <mark>neilghhu</mark> iilgeavill <u>vewar</u> vhevyopaigavRov	178
S-PcbA	AIVHLVLSMYYGAGGLLHSLTFPGDMQDSEVLQARKEKLEWDNPDNQTFILGHHLIFLGVANIQFVEWARIDGIWDAAAGSIRQV	177
S-PcbB	AILHLILSMYYGGGGLLHSVYFTGDMQDSQVPQARKEKLEWDNPDNQTFILGHHLLFFGVANIWFVEWARIHGIYDPAIEAIRQV	176
S-PcbC	AVLHLIFSGULGAGGLLHSMRYEGDLGNYPEGSRAKKFDFEWDDPDRLTFILGHHLIFLGLGNIQFVEWARIHGIYDSAQGITRTV	178
S-PcbD	aivhlilsmyyggggllhgilfdenvedsevloakkeklemnnpdnotfilghhuifmgvacawfvewarihgiydpalgairov	176
S-PcbE	GIFHLICSFWIALAGLLHSVIFSEDTQNSSGLFADGRPEHROAARFKLEWDNPDNQTFILGHHLVFFGVANIWFVEWARVHGIYDPAIEAIROV	185
S-PcbF	AILHLILSMYYGAGGLLHAIYFPDDMQKSNVPQARKFKLEWDNPDNQTFILGHHLILFGLACAWFVEWARIHGIYDPAIGAVRQV	176
S-PcbG	AILHLILSGWYGGGGMLHAFRYEEKLESYPATSRANKEKFDWNDPDRLTFILGHHLLFLAAGNIQFVEWARVHGIYDPVAGAVRQV	179
	< MSR IV> < MSR V>	
	200 *220 * 240 260 * 280	
M-PcbA	EYDINDAEIWNHQTDFLLIDDDEDVMGGHAFLAFVLITGCAWHIATKQVGEYTKFKCKGLLSAEAVLSWSLAGIGWMAIIAAFWSAS	265
S-PcbA	EYNINISSIWNHQFDFLTINNHEDVMGGHAFLAFFMITGGAFHIATKQVGEYTKFKGSGLLSAEAILSWSLAGIGWMAIVAAFWCAT	264
S-PcbB	NYNLDITQIWNHQFDFLAIDSLEDVMGGHAFLAFFQLGGGAFHIATKQIGTYTKFKCKELLSAFAILSWSLAGIGWMACVAAFWAAT	263
S-PcbC	NYNLDIGMIWNHQADFLTINSLEDVMGGHAFLAFFLIIGCAFHIATKQYGQYTEFKCKGLLSAESVLSYSLAGVAYCAFVAAFWCAT	265
S-PcbD	NYNLDISMIWOROFDFITTDSLEDVMGGHAFLAFAEITGGAFHIVAGSTPWEDKKLGEWSKFKGSELLSAEAVLSWSLAGIGWMAIVAAFWCAS	270
S-PcbE	NYNLDLTOUWNHOEDFIOIDSUEDVMCGHAELAFFOIGCCAFHIATKOICTYTNEKCACLLSAEAVLSWSLACIGWMAITAAEWCAT	272
S-PcbF		
	NYNLDLSMIWERQVNFLNIDSLEDVMGGHAFLAFAEIIGCCFHAIAGSTKWEDKRLGEYDRLKCAGLLSAEAILSFSLAGIGWMAIVAAFWCSQ	270
S-PcbG	NYNLDLSMIWERQVNFLNIDSLEDVMGGHAFLAFAEITGGCFHAIAGSTKWEDKRLGEYDRLKGAGLESAEAILSFSLAGIGWMAIVAAFWCSQ EYNLDLGMIWNHQFDFLSISSLEDIMGGHAFLAFFMAAGGVFHILTKNYGEYNSFKGADLLSAEFVLSTSLAGAAYTAFVAALWCAS	270 266
S-PcbG	NYNLDLSMIWERQVNFLNIDSLEDVMGGHAFLAFAEITGGCFHAIAGSTKWEDKRLGEYDRLKGAGLLSAEAILSFSLAGIGWMAIVAAFWCSQ EYNLDLGMIWNHQFDFISISSLEDIMGGHAFLAFFMAAGGVFHILTKNYGEYNSFKGADLLSAEFVLSTSLAGAAYTAFVAALWCAS	270 266
S-PcbG	NYNLDLSMIWERQVNFLNIDSLEDVMGGHAFLAFAEITGGCFHAIAGSTKWEDKRLGEYDRLKGAGLLSAEAILSFSLAGIGWMAIVAAFWCSQ EYNLDLGMIWNHQFDFLSISSLEDIMGGHAFLAFFMAAGGVFHILTKNYGEYNSFKGADLLSAEFVLSTSLAGAAYTAFVAALWCAS < MSR VI>	270 266
S-PcbG	NYNLDLSMIWERQVNFLNIDSLEDVMGGHAFLAFAEITGGCFHAIAGSTKWEDKRLGEYDRLKGAGLLSAEAILSFSLAGIGWMAIVAAFWCSQ EYNLDLGMIWNHQFDFLSISSLEDIMGGHAFLAFFMAAGGVFHILTKNYGEYNSFKGADLLSAEFVLSTSLAGAAYTAFVAALWCAS < MSR VI> 300 320 * 340 * 360	270
S-PcbG M-PcbA	NYNLDLSMIWERQVNFLNIDSLEDVMGGHAFLAFAEITGECFHAIAGSTKWEDKRLGEYDRIKGAGLLSAEAIDSFSLAGIGWMATVAAFWCSQ EYNLDLGMIWNHQFDFLSISSLEDIMGGHAFLAFFMAAGGVFHILTKNYGEYNSFKCADLLSAEFVLSTSLAGAAYTAFVAALWCAS < MSR VI> 300 320 * 340 * 360 NTTVYEVEFFGEPLELKFSISEYWIDTVDLPDGVYTSRAWLANVHYYFGFFFIQGHLWHALRALGFDFKRVTNAISNIDSATVTLKD- 352	270 266
S-PcbG M-PcbA S-PcbA	NYNLDLSMIWERQVNFLNUDSLEDVMGGHAFLAFAE I TGECFHAIAGSTKWEDKRLGE YDRIKCAGLLSAEA I DSFSLAGI GWMATVAAFWCSQ EYNLDLGMIWNHOFDFLSISSLEDIMGGHAFLAFFMAAGGVFHILTKNYGEYNSFKCADLLSAEFVLSTSLAGAAYTAFVAALWCAS < MSR VI> 300 320 * 340 * 360 NTTVYEVEFFGE PLELKFSISEYWIDTVDLPDGVYTSFAWLANVHYYFGFFFIQGHLWHALRALGFDFKRVTNAISNIDSATVTLKD-352 NTTVYEVEFFGE PLELKFSISEYWIDTVDLPDGAHTSFAWLANVHYFGFFFIQGHLWHALRAMGFDFKRVSSAVSNIGTASVTLND-353	270 266 2
S-PcbG M-PcbA S-PcbA S-PcbB	NYNLDLSMIWERQVNFLNLDSLEDVMGGHAFLAFAE I TGECFHAIAGSTKWEDKRLGE YDRLKCAGLLSAEA I LSFSLAGIGWMATVAAFWCSQ EYNLDLGMIWNHOFDFLSISSLEDIMGGHAFLAFFMAAGGVFHILTKNYGEYNSFKCADLLSAEFVLSTSLAGAAYTAFVAALWCAS MSR VI> 300 320 * 340 * 360 NTTVYEVEFFCEPLELKFSISEYWIDTVDLPDGVYISRAWLANVHYYFGFFFIQGHLWHALRALGFDFKRVTNAISNIDSATVTLKD-355 NTTVYEVDFFCEVLDLKFGIAPYWVDTVDLPNGAHTSRAWLTNVHYFIGFFIQGHLWHALRAMGFDFKRVSSAVSNIGTASVTLND-355 NTTVYECHDEVLDLKFGIAPYWVDTVDLPNG	270 266 2 1 9
S-PcbG M-PcbA S-PcbA S-PcbB S-PcbC	NYNLDUSMIWERQVNFLNUDSLEDVMGGHAFLAFAE I TGECFHAIAGSTKWEDKRLGE YDRIKCAGLLSAEA I LSFSLAGI GWMATVAAFWCSQ EYNLDUGMIWNHOFDFLSISSLEDIMGGHAFLAFFMAAGGVFHILTKNYGE YNSFKCADLLSAEFVLSTSLAGAAYTAFVAALWCAS <pre></pre>	270 266 2 1 9
S-PcbG M-PcbA S-PcbA S-PcbB S-PcbC S-PcbC	NYNLDUSMIWERQVNFLNUDSLEDVMGGHAFLAFAE I TGECFHAIAGSTKWEDKRLGE YDRIKCAGLLSAEA I LSFSLAGIGWMATVAAFWCSQ EYNLDUGMIWNHOFDFLSISSLEDIMGGHAFLAFFMAAGGVFHILTKNYGEYNSFKCADLLSAEFVLSTSLAGAAYTAFVAALWCAS <pre></pre>	270 266 2 1 9 2
S-PcbG M-PcbA S-PcbA S-PcbB S-PcbC S-PcbD S-PcbE	NYNLDUSMIWERQVNFLNUDSLEDVMGGHAFLAFAE I TGECFHA I AGSTKWEDKRL GE YDRLKCAGLLSAEA I LSFSLAG I GWMAT VAAFWCSQ EYNLDUGMIWNH OFDFLSISSLEDIMGGHAFLAFFMAAGGVFHILTKNYGE YNSFKCADLLSAE FVLSTSLAGAAYTAFVAALWCAS C MSR VI> 300 320 * 340 * 360 NTTVYFVEFFGE PLELKFSISEYWIDTVDLPDGVYTSRAWLANVHYYFGFFFIQGHLWHALRALGFDFKRVTNAISNIDSATVTLKD-352 NTTVYFVDFFGE VLDLKFGIAPYWVDTVDLPNGAHTSRAWLINVHYFIGFFIQGHLWHALRAMGFDFKRVSSAVSNIGTASVTLND-353 NTTVYPEAWYGEVLQIKFGVSPYWIDTVPGGTAFLGHTTRAALVNVHYYLGFFFIQGHLWHALRAMGFDFKRUSSAVSNIGTASVTLND-352 NTTVYPEAWYGEVLQIKFGVSPYWIDTVDGGTAFLGHTTRAALVNVHYYLGFFFIQGHLWHALRAMGFDFKRVGKAFDNMESAKSQLVN-352 NTTVYPEAWYGEVLQIKFGVSPYWIDTVDGDSDATRFWGHSARAALTNVHYYLGFFFLQGHLWHALRAMGFDFKRVTASIGNEQKAFFIKS-363 NTTVYPEAWYGE PLQFKFAISPYWVDTGDLSDATRFWGHSARAALTNVHYYLGFFFLQGHFWHALRALGFNFKNVTASIGNEQKATFTIKS-363 NTTVYPEAWYGE TLQLKFGISPYWIDTGNNDGVVTGHTSRAWLSNVHYYLGFFFLQGHFWHALRAMGFDFRKVTSAVANLDNSRITLSD-363	270 266 2 1 9 2 1
S-PcbG M-PcbA S-PcbA S-PcbB S-PcbC S-PcbD S-PcbE S-PcbF	NYNLDUSMIWERQVNELNUDSLEDVMGGHAFLAFAE I TGECFHA I AGSTKWEDKRL GE YDRLKCAGLLSAEA I LSFSLAG I GWMATVAAFWCSQ EYNLDUGMIWNHOFDELSISSLED I MGGHAFLAFFMAAGGVFHILTKNYGE YNSFKCADLLSAE FVLSTSLAG AYTAFVAALWCAS MSR VI> 300 320 * 340 * 360 NTTVYEVEFFGE PLELKFSISEYWIDTVDLPDGVYTSRAWLANVHYYFGFFFIQGHLWHALRALGFDFKRVTNAISNIDSATVTLKD-352 NTTVYEVEFFGE VLDLKFGIAFYWVDTVDLPNGAHTSRAWLINVHYFIGFFFIQGHLWHALRAMGFDFKRVSSAVSNIGTASVTLND-355 NTTVYEVEFFGE VLDLKFGIAFYWVDTVDLPNGAHTSRAWLINVHYFIGFFFIQGHLWHALRAMGFDFKRVSSAVSNIGTASVTLND-355 NTTVYEDAWYGEVLQIKFGVSEYWIDTVPGGTAFLGHTTRAALVNVHYYLGFFFIQGHLWHALRAMGFDFKRVSSAVSNIGTASVTLND-355 NTTVYEEAWYGEVLQIKFFGSEYWIDTVDADLPADAHTARAWLSNVHFYLGFFFIQGHLWHALRAMGFDFKRVGKAFDNMESAKSQLVN-355 NTTVYEEAWYGE PLQFKFAISEYWVDTGDLSDATRFWGHSARAALTNVHYYLGFFFLQGHLWHALRAMGFDFKRVTASIGNEQKATFTIKS-365 NTTVYEEAWYGE TLQLKFGISEYWIDTGMMDGVVTGHTSRAWLSNVHYLGFFFIQGHLWHALRAMGFDFRKVTSAVANLDNSRITLSD-365 NTTVYEIEFYGE PLNRAFVIAPAFVDSIDYSNGIAPLGHSGRCYTANFHYIAGFPFAQGHLWHALRAMGYNFKDLRAKLNPSAA354	270 266 2 1 9 2 1 1 4

Fig. 1. Alignment of the deduced protein sequences from the *Prochlorococcus* MED4 *pcbA* gene (M-PcbA) and from *Prochlorococcus* SS120 *pcbA* to *pcbG* genes (S-PcbA to S-PcbG). Identical residues are shown in white type on a black background. Black type on gray squares indicates that the percentage of conserved residues is >60% (i.e., at least 5 of 8 sequences). Predicted membrane-spanning regions (MSR) are indicated by arrows, and stars mark putative Chl-binding residues.



Fig. 2. Detection of antenna gene transcripts by Northern blotting from Prochlorococcus MED4 (pcbA) and SS120 (pcbA to pcbG) strains. Transcripts of pcbF have been indicated by an arrow. The sizes of major RNA molecules are indicated.

pcbG. The deduced proteins have lengths from 351 to 363 aa, with sequence identities ranging from 50% to 75% (Table 1). They all contain the six potential transmembrane helices found in other Pcbs (5), with conserved histidines that can serve as potential ligands for Chl molecules (Fig. 1).

All genes are expressed (Fig. 2) as monocistronic transcripts (1.35-1.5 kb). The transcript of *pcbF* most likely has a much faster turnover than those of the six other genes, because it is detected only as a degraded product on Northern blots, whereas rehybridization of the membrane with another probe (pcbG)attested the good quality of the mRNA. A partial physical mapping of the Prochlorococcus SS120 genome by pulse-field gel electrophoresis, sequence analysis, and Southern hybridization revealed that these genes are not clustered, but randomly spread throughout the genome (data not shown). The fairly wide distance between these genes suggests that different promoters most likely control their transcription. The independent regulation of their expression might provide a higher level of plasticity to the antenna system of this organism.

To generalize these observations, two other *Prochlorococcus* strains, TAK9803-2 and NATL2a, were studied. The phylogenetic analysis of the 16S rRNA genes of these strains shows that the former strain belongs to the high-light-adapted clade (14), as does MED4 (8), whereas NATL2a groups with SS120, outside of this cluster (2, 8). Furthermore, spectrofluorimetry (Fig. 3A) shows that the TAK9803-2 strain has a high ratio of divinyl-Chl a to b, which is characteristic of all high-light-adapted Prochlorococcus strains, whereas NATL2a displays a low Chl a to b ratio, typical for a low-light-adapted strain (2, 3). PCR fragments of \approx 1,000 bp were amplified from the *pcb* genes of these two strains. The amplification products subsequently were cleaved with the restriction endonuclease HaeIII, an enzyme that cuts all eight known pcb genes from Prochlorococcus between one and three times. Although the exact number of *pcb* genes cannot be determined with certainty by this method, summation of the sizes of the different bands shown in Fig. 3B suggests that there is a single gene in TAK9803-2 as there is in MED4, and there are at least five genes in NATL2a. In the latter, there could be even more *pcb* genes, as some of the PCR-products apparently were not cleavable by *Hae*III (Fig. 3B, top band of lane 5).



450

Wavelength (nm)

A

Fluorescence (relative units)

0 400

1

all expressed and show considerable sequence variation, these findings result in an extraordinary diversity of light-harvesting complexes of low-light ecotypes, probably associated with a diversification of their properties. A reexamination of thylakoid profiles obtained by Partensky et al. (9), which showed a thickening of the antenna band of SS120 from high to low light, suggests that the expression of some of the *pcb* genes might be specifically induced under low-light conditions. This phenomenon results in a relative increase in the size of antenna complexes, which could enable such a low-light-adapted strain to efficiently use the scarce photons reaching natural oceanic populations at depths below 100 m. Nevertheless, it is worth noting that despite the apparently higher plasticity of the antenna of SS120 as compared with MED4, its photosynthetic apparatus is more sensitive to photodamage at high light, as shown by the strong photoinhibition of these cells above 100 μ mol Q m⁻²·s⁻¹ (2, 11).

All higher plants studied to date possess 6 to 15 Chl a/bbinding proteins encoded by members of the Lhc gene family and having distinct functions within the photosynthetic apparatus (6). During acclimation to changes in photon fluxes, one of the major alterations exhibited by plants is a variation in their antennae size. This adaptation is attributable mainly to changes in the relative abundance of the individual proteins regulated at the transcriptional or translational level. However, there is no evidence, either from the literature or from the available sequence data, for a lower number of antenna genes in sun plants than there are in shade plants, nor is there evidence for a comparable degree of sequence variation within a given type of eukaryotic antenna gene (e.g., *Lhca* or *Lhcb*). Thus, multiplying the number of antenna genes is a specific adaptation mechanism to low light. This phenomenon seems to be unique to *Prochlo*rococcus, but its further analysis may provide fundamental insights in the evolution and adaptation of light-harvesting

NECA 55120 TAYO8032

в

LL ecotypes

SS120

NATL2a

HL ecotypes

500

TAK9803-2 MED4

kb 10.0

2.0

1.5

1.0 0.6

0.4

0.2

550

Fig. 3. Characterization of different low-light (LL)- and high-light (HL)-

adapted Prochlorococcus ecotypes grown at 20 µmol Q m⁻²·s⁻¹. (A) Excitation fluorescence spectra of whole cells normalized to the divinyl-Chl a peak (444

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systems that may also be relevant to higher plants. Such a multiplication is even more surprising in the case of this organism, because it has the smallest genome of all oxyphototrophic organisms and displays many examples of genome compactness (15). For instance, *Synechocystis* PCC6803, a cyanobacterium with a twice bigger genome (3.6 Mbp) as *Prochlorococcus* (15), has no photosynthetic genes in more than four copies (e.g., *petF*, encoding ferredoxin; ref. 16).

One intriguing question that remains to be solved is whether the multiple *pcb* gene copies are expressed differentially depending on the light conditions, as was previously shown for the

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different copies of *psbA* (17) and *psbD* (18) in *Synechococcus* PCC7942.

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