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

Laurence Garczarek*, Wolfgang R. Hess†, Julia Holtzendorff†, Georg W. M. van der Staay*‡, and Frédéric Partensky*§

*Observatoire Océanologique de Roscoff, Centre National de la Recherche Scientifique et Université Paris 6, Station Biologique, B.P. 74, F-29682 Roscoff Cedex, France; and †Humboldt-Universität zu Berlin, Institut für Biologie/Genetik, Chausseestrasse 117, D-10115 Berlin, Germany

Communicated by Hewson Swift, University of Chicago, Chicago, IL, January 31, 2000 (received for review December 14, 1999)

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.

The 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 *pcb* 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 ± 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 μmol Q $m^{-2}{\cdot}s^{-1}$ (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-G-pcb-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 λGEM-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*)].

‡Present address: Universität zu Köln, Botanisches Institut, Lehrstuhl III, Gyrhofstrasse 15, 50931 Köln, Germany.

§To whom reprint requests should be addressed. E-mail: partensky@sb-roscoff.fr.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.070040897. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.070040897

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)

	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	—

*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

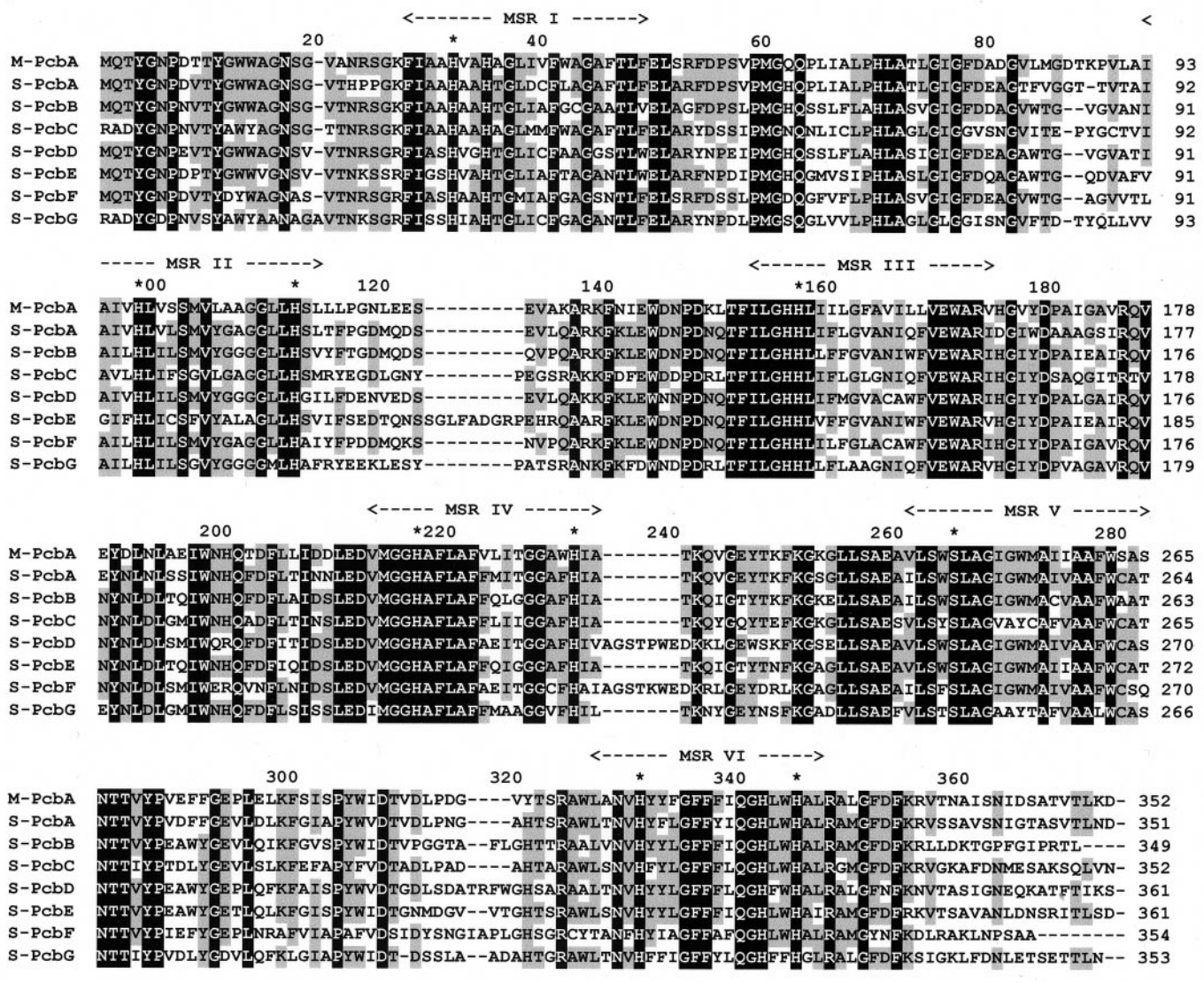

Fig. 1. Alignment of the deduced protein sequences from the *Prochlorococcus* MED4 *pcbA* gene (M-PcbA) and from *Prochlorococcus* SS120 *pcbA* to *pcbG* genes (S-PcbA to S-PcbG). Identical residues are shown in white type on a black background. Black type on gray squares indicates that the percentage 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.

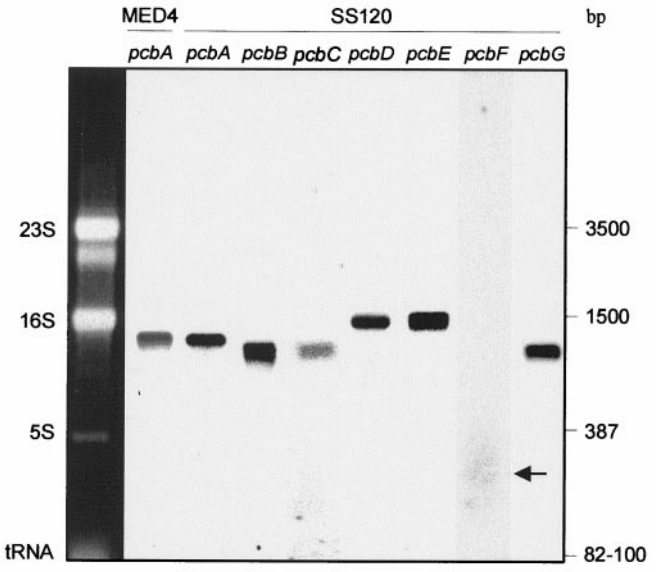

Fig. 2. Detection of antenna gene transcripts by Northern blotting from *Prochlorococcus* MED4 (*pcbA*) and SS120 (*pcbA* to *pcbG*) strains. Transcripts of *pcbF* have been indicated by an arrow. The sizes of major RNA molecules are indicated.

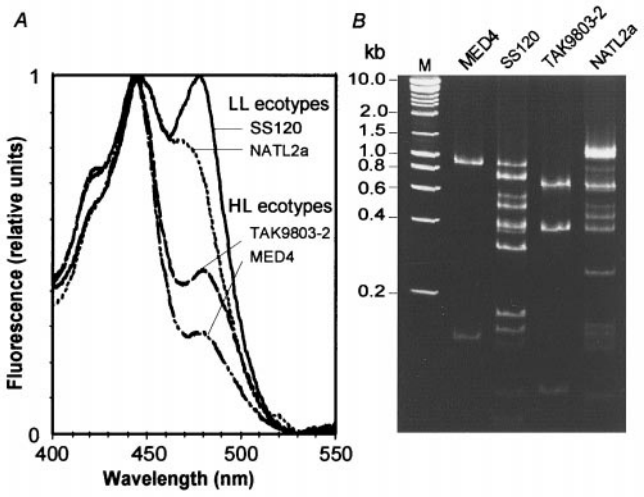

Fig. 3. Characterization of different low-light (LL)- and high-light (HL)-adapted *Prochlorococcus* ecotypes grown at 20 μmol Q $m^{-2}{\cdot}s^{-1}$. (*A*) Excitation fluorescence spectra of whole cells normalized to the divinyl-Chl *a* peak (444 nm). The variable height of the second peak, mainly monovinyl-Chl *b* (468 nm) for NATL2a and divinyl-Chl *b* (478 nm) for the other strains, indicates the widely different Chl *b* to Chl *a* ratios between strains. (*B*) Pattern of amplified *pcb* fragments obtained on polyacrylamide gels after cleavage by *Hae*III. Lane M, size markers.

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. 3*A*) 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 ≈1,000 bp were amplified from the *pcb* genes of these two strains. The amplification products subsequently were cleaved with the restriction endonuclease *Hae*III, 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. 3*B* 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. 3*B*, top band of lane 5).

Discussion

Our results show that part of the genetic and likely ecophysiological differentiation between high-light- and low-light-adapted *Prochlorococcus* strains can be explained by a multiplication of *pcb* genes in the latter strains. Because these genes are 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 μmol Q $m^{-2}{\cdot}s^{-1}$ (2, 11).

All higher plants studied to date possess 6 to 15 Chl *a*/*b* binding 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 *Prochlorococcus*, but its further analysis may provide fundamental insights in the evolution and adaptation of light-harvesting

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

We thank Prof. S. W. Chisholm and Dr. L. Moore for providing culture material for use in this study and Ms. F. Legall for taking care of the cultures. We also thank Drs. D. Vaulot and S. Loiseaux-de-Goër for critically reading the manuscript. This study was funded by the European Community program MAS3-CT97–0128 (Promolec program) and supported by the Deutsche Forschungsgemeinschaft to W.R.H. (SFB 429). L.G. was supported by a Ph.D. grant cofunded by the Centre National de la Recherche Scientifique and the Région Bretagne, and G.W.M-.v.d.S. was supported by the Centre National de la Recherche Scientifique–Institut National des Sciences de l'Univers.

1. Chisholm, S. W., Frankel, S. L., Goericke, R., Olson, R. J., Palenik, B., Waterbury, J. B., West-Johnsrud, L. & Zettler, E. R. (1992) *Arch. Microbiol.* **157,** 297–300.
2. Moore, L. R., Rocap, G. & Chisholm, S. W. (1998) *Nature (London)* **393,** 464–467.
3. Moore, L. R. & Chisholm, S. W. (1999) *Limnol. Oceanogr.* **44,** 628–638.
4. West, N. J. & Scanlan, D. J. (1999) *Appl. Env. Microbiol.* **65,** 2585–2591.
5. La Roche, J., van der Staay, G. W., Partensky, F., Ducret, A., Aebersold, R., Li, R., Golden, S. S., Hiller, R. G., Wrench, P. M., Larkum, A. W. & Green, B. R. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 15244–15248.
6. Durnford, D. G., Deane, J. A., Tan, S., McFadden, G. I., Gantt, E. & Green, B. R. (1999) *J. Mol. Evol.* **48,** 59–68.
7. van der Staay, G. W. M., Yurkova, N. & Green, B. R. (1998) *Plant Mol. Biol.* **36,** 709–716.
8. Urbach, E., Scanlan, D. J., Distel, D. L., Waterbury, J. B. & Chisholm, S. W. (1998) *J. Mol. Evol.* **46,** 188–201.
9. Partensky, F., LaRoche, J., Wyman, K. & Falkowski, P. G. (1997) *Photosynth. Res.* **51,** 209–222.
10. Partensky, F., Hess, W. R. & Vaulot, D. (1999) *Microbiol. Mol. Biol. Rev.* **63,** 106–127.
11. Moore, L. R., Goericke, R. & Chisholm, S. W. (1995) *Mar. Ecol. Prog. Ser.* **116,** 259–275.
12. Smith, S. W., Overbeek, R., Woese, C. R. & Gilbert, W. (1994) *Comput. Appl. Biosci.* **10,** 671–675.
13. Hess, W. R., Weihe, A., Loiseaux-de Goër, S., Partensky, F. & Vaulot, D. (1995) *Plant Mol. Biol.* **27,** 1189–1196.
14. West, N., Schönhuber, W. A., Amann, R. I., Rippka, R. & Scanlan, D. J. (2000) *Appl. Environ. Microbiol.*, in press.
15. Strehl, B., Holtzendorff, J., Partensky, F. & Hess, W. R. (1999) *FEMS Microbiol. Lett.* **181,** 261–266.
16. Kotani, H. & Tabata, S. (1998) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49,** 151–171.
17. Schaefer, M. R. & Golden, S. S. (1989) *J. Bacteriol.* **171,** 3973–3981.
18. Bustos, S. A. & Golden, S. S. (1992) *Mol. Gen. Genet.* **232,** 221–230.