39 Acosta-Serrano, A. *et al*. (2001) The surface coat of procyclic *Trypanosoma brucei*: programmed expression and proteolytic cleavage of procyclin in the tsetse fly. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1513–1518
40 Ruepp, S. *et al*. (1997) Survival of *Trypanosoma brucei* in the tsetse fly is enhanced by the expression of specific forms of procyclin. *J. Cell. Biol.* 137, 1369–1379
41 Ruepp, S. *et al*. (1999) Glutamic acid/alanine-rich protein from *Trypanosoma congolense* is the functional equivalent of 'EP' procyclin from *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* 98, 151–156
42 Nagamune, K. *et al*. (2000) Critical roles of glycosylphosphatidylinositol for *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 10336–10341
43 Vickerman, K. *et al*. (1988) Biology of African trypanosomes in the tsetse fly. *Biol. Cell.* 64, 109–119
44 Bütikofer, P. *et al*. (2002) Glycosylphosphatidylinositol-anchored surface molecules of *Trypanosoma congolense* insect forms are developmentally regulated in the tsetse fly. *Mol. Biochem. Parasitol.* 119, 7–16
45 Bayne, R.A. *et al*. (1993) A major surface antigen of procyclic stage *Trypanosoma congolense*. *Mol. Biochem. Parasitol.* 61, 295–310
46 Beecroft, R.P. *et al*. (1993) Identification and characterization of an acidic major surface glycoprotein from procyclic stage *Trypanosoma congolense*. *Mol. Biochem. Parasitol.* 61, 285–294
47 Vlachou, D. *et al*. (2001) *Anopheles gambiae* laminin interacts with the P25 surface protein of *Plasmodium berghei* ookinetes. *Mol. Biochem. Parasitol.* 112, 229–237
48 Brennan, J.D. *et al*. (2000) *Anopheles gambiae* salivary gland proteins as putative targets for blocking transmission of malaria parasites. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13859–13864
49 Lal, A.A. *et al*. (2001) Anti-mosquito midgut antibodies block development of *Plasmodium falciparum* and *Plasmodium vivax* in multiple species of *Anopheles* mosquitoes and reduce vector fecundity and survivorship. *Proc. Natl. Acad. Sci. U. S. A.* 98, 5228–5233
50 Schneider, D. and Shahabuddin, M. (2000) Malaria parasite development in a *Drosophila* model. *Science* 288, 2376–2379
51 Boulanger, N. *et al*. (2001) Immune response of *Drosophila melanogaster* to infection with the flagellate parasite *Crithidia* spp. *Insect. Biochem. Mol. Biol.* 31, 129–137
52 Hao, Z. *et al*. (2001) Tsetse immune responses and trypanosome transmission: implications for the development of tsetse-based strategies to reduce trypanosomiasis. *Proc. Natl. Acad. Sci. U. S. A.* 98, 12648–12653
53 Van Den Abbeele, J. *et al*. (1999) *Trypanosoma brucei* spp. development in the tsetse fly: characterization of the post-mesocyclic stages in the foregut and proboscis. *Parasitology* 118, 469–478

Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light-harvesting strategies

Claire S. Ting, Gabrielle Rocap, Jonathan King and Sallie W. Chisholm

***Prochlorococcus* and *Synechococcus* are abundant unicellular cyanobacteria and major participants in global carbon cycles. Although they are closely related and often coexist in the same ocean habitat, they possess very different photosynthetic light-harvesting antennas. Whereas *Synechococcus* and the majority of cyanobacteria use phycobilisomes, *Prochlorococcus* has evolved to use a chlorophyll a_2/b_2 light-harvesting complex. Here, we present a scenario to explain how the *Prochlorococcus* antenna might have evolved in an ancestral cyanobacterium in iron-limited oceans, resulting in the diversification of the *Prochlorococcus* and marine *Synechococcus* lineages from a common phycobilisome-containing ancestor. Differences in the absorption properties and cellular costs between chlorophyll a_2/b_2 and phycobilisome antennas in extant *Prochlorococcus* and *Synechococcus* appear to play a role in differentiating their ecological niches in the ocean environment.**

Approximately one-half of the total primary production on Earth occurs in the oceans [1]. Although hundreds of species contribute to oceanic productivity, members of two cyanobacterial genera – *Prochlorococcus* [2,3] and *Synechococcus* [4] – are responsible for a significant fraction of the primary production in open ocean waters of the subtropical and tropical Atlantic and Pacific [5,6]. In recognition of their global significance, the genomes of three representative isolates of these two groups have recently been sequenced as part of the DOE Microbial Genome Project (http://www.jgi.doe.gov/JGI_microbial/html/index.html).

A characteristic feature of the majority of extant cyanobacteria is their supramolecular light-harvesting antenna, the PHYCOBILISOME (see Glossary). Although *Prochlorococcus* and marine A-cluster *Synechococcus* form sister clades within the cyanobacteria [7,8], *Prochlorococcus* has evolved to use a chlorophyll a_2/b_2 (Chl a_2/b_2) light-harvesting complex rather than the phycobilisome (Fig. 1; Box 1). Phylogenetic trees based on 16S rDNA sequences suggest a rapid diversification of these two genera from a common phycobilisome-containing ancestor [9].

The close phylogenetic relationship between *Prochlorococcus* and *Synechococcus* combined with the recent availability of whole genome sequences makes it possible to examine how the evolution of a key protein complex – the light-harvesting antenna – has proceeded along different paths in these two genera and might contribute to their ability to dominate specific oceanic habitats. Here, we describe major characteristics of the two antennas and present a scenario for the evolution of a *Prochlorococcus* Chl *a*/*b*-based antenna from a Chl *a*-binding, iron-stress-induced protein (IsiA or CP43′). This latter chlorophyll-binding protein might have been maintained constitutively in an ancestral cyanobacterium inhabiting iron-limited oceans if it provided a selective advantage over the phycobilisome

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Glossary

Chromophore: a chemical group capable of light absorption at selective wavelengths.
CP43 and CP47: proteins encoded by *psbC* and *psbB*, respectively, which bind chlorophyll *a* and form an antenna associated with PSII in oxygenic photosynthetic organisms.
Ecotype: within a single genus, a genetically and physiologically distinct population (or group of isolates) adapted to a particular set of environmental conditions.
IsiA (or CP43′): protein encoded by an iron-stress-induced gene (*isiA*) in *Synechococcus* and *Synechocystis* that binds chlorophyll *a* and forms a PSI-associated antenna.
Pcb: protein that binds chlorophylls and possibly carotenoids, and forms the major light-harvesting antenna of chlorophyll *a*/*b*-containing cyanobacteria.
Phycobilin: a chromophore consisting of an open-chain tetrapyrrole that is covalently attached to phycobiliproteins and functions in the selective absorption of light energy. Examples include phycoerythrobilin (PEB), phycourobilin (PUB), phycocyanobilin (PCB) and phycobiliviolin (PXB).
Phycobiliprotein: a water-soluble protein that covalently binds phycobilins and is one of the major structural components of the phycobilisome. Examples include phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC).
Phycobilisome: a supramolecular light-harvesting complex that functions as the primary antenna of PSII in red algae and the majority of cyanobacteria.
Prochlorophyte: original name used to describe chlorophyll *a*/*b*-containing photosynthetic prokaryotes, now known to be dispersed among three different lineages within the cyanobacteria (Box 1).

complex in a particular oceanic environment. We then focus on the key environmental factors, light and nitrogen, and examine how differences in the major light-harvesting antennas of extant *Prochlorococcus* and *Synechococcus* contribute to niche differentiation in these cyanobacteria.

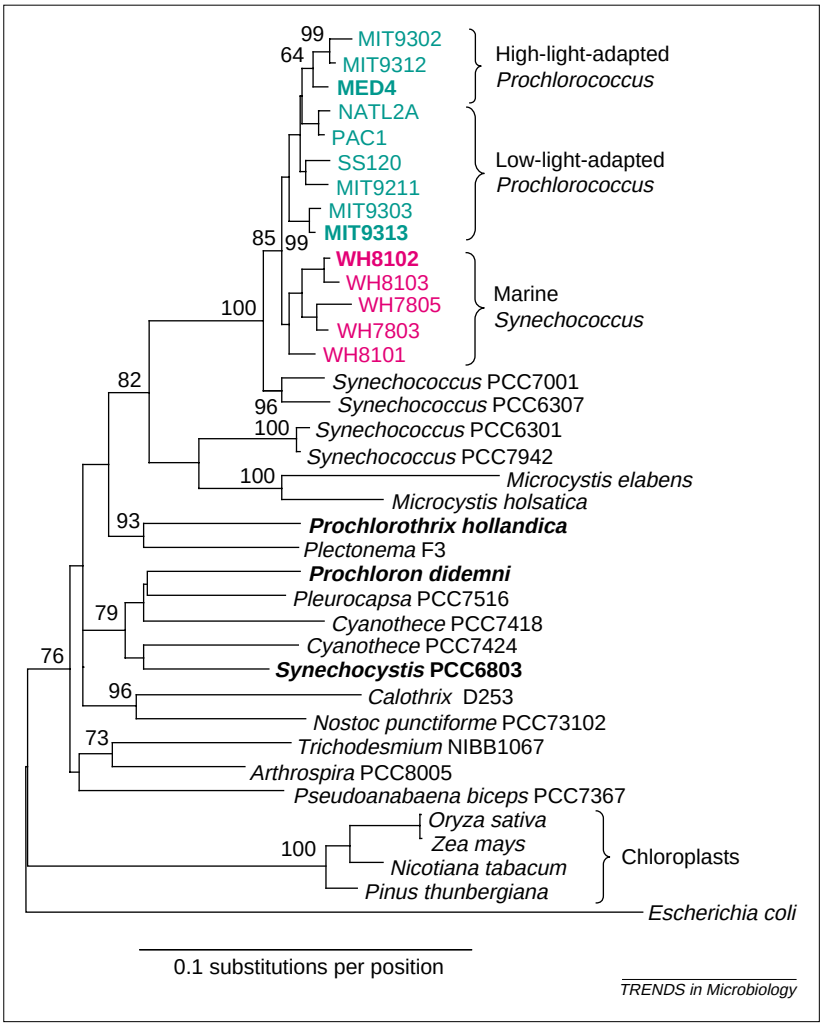

Fig. 1. A phylogenetic tree constructed from 16S rDNA sequences depicts the close relationship between *Prochlorococcus* and marine *Synechococcus*, which together form sister clades within the cyanobacteria. The two other chlorophyll (Chl) *a*/*b*-containing cyanobacteria, *Prochloron* and *Prochlorothrix* (shown in bold), form separate lineages and are not as closely related to *Prochlorococcus* as marine *Synechococcus* are. Furthermore, none of the three Chl *a*/*b*-containing cyanobacteria (prochlorophytes) are specifically related to the chloroplasts of higher plants. Within the *Prochlorococcus* lineage, a recently arisen clade consists of isolates that are relatively better adapted for growth at higher irradiance levels than the other isolates. Note, however, that the low-light-adapted isolates do not form a single clade. The distance tree shown was constructed with minimum evolution as the objective criterion and logdet (paralinear) distances. The bootstrap values displayed are also based on these settings and were generated following 100 resamplings.

Divergent light-harvesting complexes

A diverse set of pigment-protein complexes has evolved in photosynthetic organisms to absorb light and transfer excited-state energy to the reaction centers where the photochemical and electron-transfer reactions of photosynthesis occur. These light-harvesting antennas increase the effective absorption cross-section of the reaction centers. Proteins that constitute antennas do not all belong to the same family and their associated pigments (PHYCOBILINS, chlorophylls, bacteriochlorophylls and carotenoids) absorb light at different wavelength maxima. The ability to absorb and use efficiently the specific wavelengths enriched in a particular environment would provide a photosynthetic organism with a competitive advantage in that habitat.

Phycobilisomes – major light-harvesting antennas of Synechococcus

In the majority of cyanobacteria, the major antenna complex associated with the Photosystem II (PSII) reaction center consists of PHYCOBILIPROTEINS, a unique family of water-soluble polypeptides that covalently bind CHROMOPHORES (i.e. pigments). The phycobiliproteins associate with linker polypeptides to form a supramolecular antenna, the phycobilisome, on the outer surface of the intracytoplasmic membranes (Fig. 2) [10]. Differences in the chromophore composition of phycobiliproteins result in wavelength-specific differences in light absorption among diverse cyanobacteria (Box 2).

The phycobilisomes of marine *Synechococcus* (marine A-cluster) are rich in the chromophores phycoerythrobilin (PEB) and phycourobilin (PUB), which have absorption maxima in the blue-green region of the visible spectrum (Fig. 3a; Box 2) [11]. Comparisons between open ocean *Synechococcus* WH 8103 and freshwater *Synechocystis* PCC 6701, which uses phycocyanobilin (PCB) and PEB as its major chromophores, suggest that the former is nine times more effective at absorbing blue-green light (490 nm) than the latter [12]. Furthermore, marine *Synechococcus*, like many cyanobacteria, have the ability to acclimate to changes in light quantity by

Box 1. Prochlorophytes are members of the cyanobacterial lineage

A distinguishing characteristic of the majority of extant cyanobacteria is their supramolecular light-harvesting antenna, the phycobilisome. In fact, this group derives its name from the blue-green or cyan color of many of its members, which is caused by the phycobiliprotein chromophore, phycocyanin (Box 2). Thus the discovery of oxygenic photosynthetic prokaryotes lacking phycobilisomes and containing chlorophylls *a* and *b* (Chl *a* and *b*), a combination previously observed only in eukaryotic green algae and higher plants, generated much speculation regarding the endosymbiont theory of chloroplast evolution. The novel prokaryotes were thought to be the missing link between cyanobacteria and plant chloroplasts and were thus named 'Prochlorophytes'.

Despite the apparent similarities in their photosynthetic antennas, phylogenetic analyses using 16S rDNA and *rpoC1* sequence data revealed that the Prochlorophytes do not form a natural assemblage, but are spread in three different lineages within the cyanobacteria [a,b]. Furthermore, none of the three is specifically related to chloroplasts of higher plants (Fig. 1). The three genera are found in quite different habitats. *Prochloron didemni* is a symbiont of a marine ascidian and, despite considerable efforts, has not yet been brought into culture [c]. *Prochlorothrix hollandica* is a filamentous organism, isolated from a freshwater lake [d], and *Prochlorococcus* is found as single cells living in open oceans [e]. The independent origins of the Chl *a*/*b*-containing cyanobacteria suggest that the ability to synthesize Chl *b* and to bind it in antenna complexes has also evolved independently at least four times. Recently, Tomitani *et al.* [f] put forth an alternative proposal involving an ancestral cyanobacterium containing both Chl *b* and phycobilins, and they suggested that Chl *b* was lost in most cyanobacteria and phycobilins were lost in the four lines leading to the prochlorophytes and chloroplasts. This scenario was based on analyses of *Prochloron* and *Prochlorothrix* Chl *b* synthetase (or Chl *a* monooxygenase, *cao*) gene sequences, which are related to those of green algae and higher plants. However, there are several problems with this scenario. First, it is more parsimonious to hypothesize acquisition of the *cao* genes via lateral gene transfer, rather than via numerous independent losses among members of the cyanobacterial lineage [g]. Second, the fully sequenced genomes of two strains of *Prochlorococcus* contain genes encoding putative Chl *a* monooxygenases, whose predicted amino acid sequences share low (<20%) similarity with the Chl *a* monooxygenases of other Chl *b*-containing cyanobacteria. Thus, in *Prochlorococcus*, Chl *b* is likely to be synthesized using an unrelated monoxygenase [h].

References

a Urbach, E. *et al.* (1992) Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. *Nature* 355, 267–270

b Palenik, B.P. and Haselkorn, R. (1992) Multiple evolutionary origins of prochlorophytes, the chlorophyll *b*-containing prokaryotes. *Nature* 355, 265–267

c Christen, G. *et al.* (1999) Isolation and characterization of oxygen evolving thylakoids from the marine prokaryote *Prochloron didemni*. *FEBS Lett.* 449, 264–268

d Bullerjahn, G.S. and Post, A.F. (1993) The prochlorophytes: are they more than just chlorophyll *a*/*b*-containing cyanobacteria? *Crit. Rev. Microbiol.* 19, 43–59

e Chisholm, S.W. *et al.* (1988) A novel free-living prochlorophyte occurs at high cell concentrations in the oceanic euphotic zone. *Nature* 334, 340–343

f Tomitani, A. *et al.* (1999) Chlorophyll *b* and phycobilins in the common ancestor of cyanobacteria and chloroplasts. *Nature* 400, 159–162

g Green, B.R. (2001) Was 'molecular opportunism' a factor in the evolution of different photosynthetic light-harvesting pigment systems? *Proc. Natl. Acad. Sci. U. S. A.* 98, 2119–2121

h Hess, W. *et al.* (2001) The photosynthetic apparatus of *Prochlorococcus*: insights through comparative genomics. *Photosynth. Res.* 70, 53–71

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adjusting the pigment-protein content of their phycobilisomes. For example, when grown at low irradiance levels, marine *Synechococcus* increase their light absorption capacity primarily by increasing their cellular phycoerythrin levels, rather than their phycocyanin or chlorophyll content [13].

The Pcb antenna of *Prochlorococcus*

Pigments and proteins

Prochlorococcus possesses an antenna complex different from that found in most cyanobacteria including marine *Synechococcus*, its close relative. It lacks organized phycobilisomes, although it still possesses genes (*cpeB* and *cpeA*) encoding phycoerythrin, a phycobiliprotein (Box 2) [14–17]. The major pigments of *Prochlorococcus* include divinyl Chl *a* (Chl a_2) (*in vivo* A_{max} = ~443–450 nm), and divinyl (*in vivo* A_{max} = ~476–480 nm) and monovinyl (*in vivo* A_{max} = ~468 nm) Chl *b* [11,18,19]. Thus, the *Prochlorococcus* light-harvesting antenna is better at absorbing blue wavelengths of light than green wavelengths (Fig. 3a). The suite of divinyl Chl *a* and *b* pigments found in *Prochlorococcus* is unique among photosynthetic organisms; additional pigments include zeaxanthin, α-carotene and low levels of a Chl *c*-like pigment, which might not contribute significantly to light harvesting [11,18–20].

The *Prochlorococcus* antenna proteins (PCB) possess as many as six transmembrane helices [21] that bind divinyl chlorophylls *a* and *b*. Sequence analyses suggest that there are at least eight [22] and perhaps as many as 11 conserved His residues that might participate in pigment binding (Fig. 4; available as supplementary information online at http://archive.bmn.com/supp/tim/chisholm.pdf). This is similar to the number of conserved His residues associated with the membrane-spanning regions of CP43 (ten His residues) and CP47 (12 His) [23] and additional residues (i.e. asparginyl and glutamyl) might also serve as chlorophyll ligands, as in the higher-plant light-harvesting complex (LHCII) [23,24]. The Pcb proteins do not belong to the light-harvesting complex (LHC) superfamily [21], which includes the membrane-intrinsic, Chl *a*/*b*-binding (Cab) proteins of green algae and plant chloroplasts, the Chl *a*/*c*-binding proteins and other proteins involved in protection against photooxidative stress [25,26].

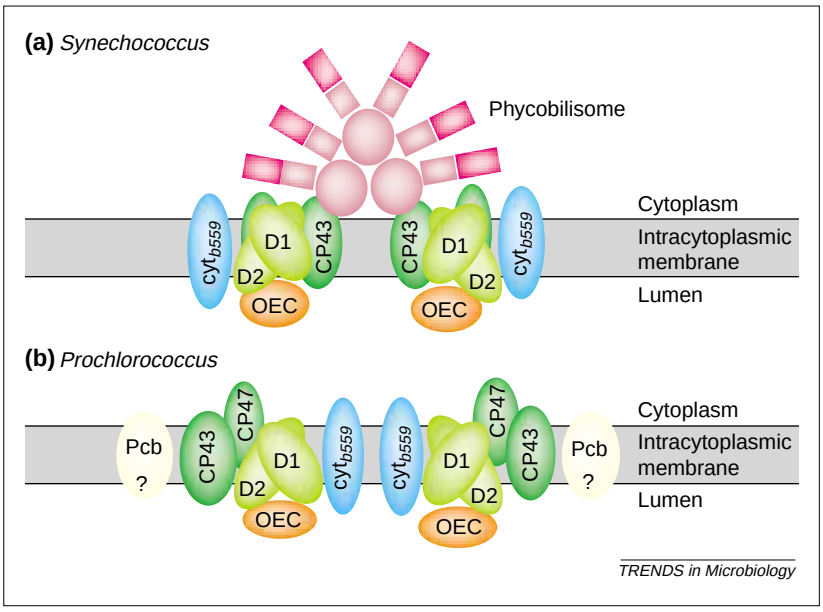

Fig. 2. Schematic illustration of some of the major proteins associated with the Photosystem II (PSII) reaction center and their possible organization in *Synechococcus* and *Prochlorococcus*. Although the major PSII antenna of *Synechococcus* consists of phycobilisomes, *Prochlorococcus* lacks organized phycobilisomes. The structural association of the *Prochlorococcus* chlorophyll a_2/b-binding Pcb proteins with PSII is unknown, although they are known to form a ring around Photosystem I in *Prochlorococcus* SS120. Abbreviations: D1 and D2, core proteins of the PSII reaction center; CP43 and CP47, chlorophyll *a*-binding core antenna proteins associated with the PSII reaction center; OEC, oxygen evolving complex; cyt b_{559}, cytochrome b_{559}; Pcb, prochlorophyte chlorophyll-binding protein.

Instead, they are members of a family that includes the Chl *a*-binding proteins CP43 and CP47, which form the core antenna associated with the PSII reaction center in all oxygenic photosynthetic organisms, and the cyanobacterial Chl *a*-binding IsiA protein (CP43′), which is induced under conditions of iron stress [21,27,28]. It has been suggested that the genes encoding the Pcb antenna proteins of PROCHLOROPHYTES, such as *Prochlorococcus* (Box 1), might have evolved independently from ancestral genes encoding IsiA-like and CP43-like proteins [29].

Evolution

The evolution of a major *Prochlorococcus* Pcb antenna from an iron-stress-induced, IsiA-like protein is plausible, given the role iron could have played over the course of cyanobacterial evolution. In the reduced environment of the ancient ocean, iron was highly soluble and available for uptake and use by microorganisms. With the advent of oxygenic photosynthesis approximately 3.5 billion years ago, however, iron became oxidized and precipitated into forms that are not readily available. Hence, it is believed that iron limitation could have been a selective force in the evolution of cyanobacteria. Indeed, in today's oceans, biologically available iron is scarce, and has been shown to limit the growth of phytoplankton, including cyanobacteria [30–32].

A direct link between iron limitation and the light-harvesting capabilities of phycobilisome-containing cyanobacteria was established recently by studies demonstrating that under conditions of iron depletion, an additional chlorophyll antenna system is synthesized and associates specifically with Photosystem I (PSI). When freshwater *Synechocystis* PCC 6803 and *Synechococcus* PCC 7942 are grown under iron-depleted conditions, cellular levels of PSI are reduced and the phycobilisome, which functions as the major light-harvesting antenna of the PSII reaction center, is targeted for rapid degradation [27,28,33,34]. Concomitantly, a major protein that is synthesized is the Chl *a*-binding IsiA (or CP43′) polypeptide [27,28,33,34]. In these *Synechococcus* and *Synechocystis* cells, IsiA increases the light-harvesting capacity of the remaining PSI reaction centers by forming a ring consisting of 18 molecules and about 180 chlorophylls around the PSI trimer [33,34].

At present, it is not known whether a similar PSI antenna is formed in extant marine *Synechococcus* under iron-depleted conditions. When marine *Synechococcus* are grown under conditions of iron stress, a major protein synthesized is IdiA, which is localized to the periplasm and is thought to function in iron uptake as part of an ATP-binding cassette (ABC) transporter [35]. Interestingly, in freshwater *Synechococcus* PCC 7942, IdiA might play a role in protecting PSII from oxidative stress [36]. Thus, the mechanisms of acclimation to iron limitation could be very different in marine and freshwater *Synechococcus*.

Recent structural studies on the PSI antenna of *Prochlorococcus* (isolate SS120) indicate that the Pcb proteins and associated pigments are organized in a ring around the PSI reaction center [37], and thus form an antenna with a structure similar to that of the iron-stress-induced IsiA proteins of freshwater *Synechocystis* [33] and *Synechococcus* [34]. Further studies are required to determine whether this type of antenna organization is associated with PSI in other *Prochlorococcus* isolates and to characterize the association of the Pcb antenna with PSII.

These structural data lend credence to a model for the evolution of one of the major *Prochlorococcus* antennas from an iron-stress-induced, IsiA-like protein. In this scenario, an IsiA-like antenna complex could have been maintained constitutively in an ancestral cyanobacterium if it provided a selective advantage over the phycobilisome complex in a particular light or nutrient regime. The ability to synthesize divinyl Chl *a* and both monovinyl and divinyl Chl *b* must also have been acquired (Box 1), although the details and sequence of these events are unknown. As the Chl *a*/*b* antenna became a primary light-harvesting complex, the genes encoding the phycobilisome proteins experienced altered selective pressure, resulting in the loss of some of these genes from the genome. Differences in the properties of chlorophyll-based and phycobilisome antennas could have led to the proliferation of this ancestral cyanobacterium in specific oceanic niches, thus resulting in the diversification of the *Prochlorococcus* and marine *Synechococcus* lineages from a common, phycobilisome-containing ancestor.

Box 2. Cyanobacterial phycobiliproteins

All phycobiliproteins are thought to have evolved from a common ancestor, possibly a globin-like protein [a]. Cyanobacterial and red algal phycobiliproteins are heteromonomers, consisting of two subunits (α and β) that share similar tertiary structures and bind chromophores by one or two cysteinyl thioether bonds [a–d].

Phycobiliproteins have been classified based on their absorption properties and include phycoerythrin (PE; A_{max} = ~565–575 nm), phycocyanin (PC; A_{max} = ~615–640 nm), phycoerythrocyanin (PEC; A_{max} = ~575 nm) and allophycocyanin (APC; A_{max} = ~650–655 nm). Two types of PE (PEI and PEII) exist that differ in the number and type of associated chromophores [e]. In marine *Synechococcus* WH 8020 and WH 8103, which possess both PEI and PEII, the PEI sequences are homologous to well-characterized rhodophytan (R-PE), Bangiophycean (B-PE) and other cyanobacterial (C-PE, soil and freshwater) phycoerythrins [e]. Five chromophores are covalently linked to PEI, two to the α-subunit and three to the β-subunit [e]. By contrast, PEII binds an additional chromophore and is associated with a total of six pigments, three on each subunit [e]. Chromophores associated with both PEI and PEII include phycoerythrobilin (PEB; A_{max} = ~550 nm) and, in several cases, phycourobilin (PUB; A_{max} = ~490 nm) [e].

Although *Prochlorococcus* lacks organized phycobilisomes, it possesses genes encoding phycoerythrin (*cpeB* and *cpeA*). These genes most likely encoded proteins that were remnants of an ancestral phycobilisome-like complex, rather than having been acquired *de novo* by horizontal gene transfer [f,g]. Whole genome comparisons among *Synechococcus* WH 8102, *Prochlorococcus* MIT 9313 and *Prochlorococcus* MED4 indicate that there is a progressive reduction in the number of phycobilisome-related genes from *Synechococcus* to the more deeply branching *Prochlorococcus* isolate (MIT 9313; Fig. 1) to the more recently evolved isolate (MED4) [h]. Selective forces shaping the evolution of the phycoerythrin gene set have not been uniform in the *Prochlorococcus* lineage, as illustrated by the loss of *cpeA* and the presence of a degenerate form of *cpeB* in MED4 [g]. Interestingly, the phycoerythrin genes are expressed at low levels in *Prochlorococcus* [f,i], although the functional significance of this protein, in light-harvesting and/or photoreception, has not been determined.

References

a Apt, K.E. *et al.* (1995) Evolution of the phycobiliproteins. *J. Mol. Biol.* 248, 79–96

b Ficner, R. and Huber, R. (1993) Refined crystal structure of phycoerythrin from *Porphyridium cruentum* at 0.23 nm resolution and localization of the γ-subunit. *Eur. J. Biochem.* 218, 103–106

c Grossman, A.R. *et al.* (1993) The phycobilisome, a light-harvesting complex responsive to environmental conditions. *Microbiol. Rev.* 57, 725–749

d Ritter, S. (1999) Crystal structure of a phycourobilin-containing phycoerythrin at 1.9 Å resolution. *J. Struct. Biol.* 126, 86–97

e Ong, L.J. and Glazer, A.N. (1991) Phycoerythrins of marine unicellular cyanobacteria. I. Bilin types and locations and energy transfer pathways in *Synechococcus* spp. phycoerythrins. *J. Biol. Chem.* 266, 9515–9527

f Hess, W.R. *et al.* (1999) The phycoerythrins of *Prochlorococcus marinus* are associated to the thylakoid membrane and are encoded by a single large gene cluster. *Plant Mol. Biol.* 40, 507–521

g Ting, C.S. *et al.* (2001) Phycobiliprotein genes of the marine photosynthetic prokaryote *Prochlorococcus*: evidence for rapid evolution of genetic heterogeneity. *Microbiology* 147, 3171–3182

h Hess, W. *et al.* (2001) The photosynthetic apparatus of *Prochlorococcus*: insights through comparative genomics. *Photosynth. Res.* 70, 53–71

i Hess, W.R. *et al.* (1996) Coexistence of phycoerythrin and a chlorophyll *a*/*b* antenna in a marine prokaryote. *Proc. Natl. Acad. Sci. U. S. A.* 93, 11126–11130

Niche differentiation in *Prochlorococcus* and *Synechococcus*

In contemporary oceans, many biological and environmental factors (including surface area to volume ratios, mortality from grazing and viral lysis, light intensity and quality, nutrient and/or trace metal concentration, and temperature) interact to determine the observed distributions of *Prochlorococcus* and *Synechococcus*. How do differences in the major light-harvesting antennas of these two cyanobacteria specifically contribute to niche differentiation between them? Analysis of their distributions in the oceans, comparisons of the known functional and/or structural properties of their antennas, and examination of their growth responses to two key environmental factors, light and nitrogen, give us clues.

Prochlorococcus and *Synechococcus* are easily identified and differentiated in the field using flow cytometric techniques, and their global distributions and seasonal dynamics have been well described [38,39]. *Synechococcus* is found in nearly all surface waters of the ocean, whereas *Prochlorococcus* distribution is restricted between 40°N and 30°S [38]. In addition, *Prochlorococcus* is often in low abundance or absent from coastal waters where *Synechococcus* thrives [38,40]. In open ocean regions where *Prochlorococcus* and *Synechococcus* co-occur, *Prochlorococcus* has generally been found to extend deeper in the water column than *Synechococcus* and is often present at depths of 100–200 m [38]. *Prochlorococcus* concentrations frequently reach 10^5 cells ml^{-1} or higher and can exceed those of *Synechococcus* by an order of magnitude or more throughout the water column and particularly in deeper waters (100–200 m) (Fig. 3b) [38,41,42].

The role of light

In the oceans, the wavelengths of solar radiation available for fuelling photosynthesis differ from those of terrestrial ecosystems. Water rapidly attenuates longer wavelengths of light, so red light does not penetrate below ~15 m [43]. In the open ocean, near-surface waters (i.e. above 30 m) are illuminated primarily with blue and green wavelengths and deeper waters (100–200 m) are enriched with blue light (400–500 nm) [43]. In coastal areas, green wavelengths generally predominate owing to the composition of the sea water, which has higher concentrations of humic materials and phytoplankton [43]. Thus, the ability to absorb and use efficiently the specific wavelengths enriching a particular region and depth of the ocean would provide a photosynthetic organism with a competitive advantage in that environment.

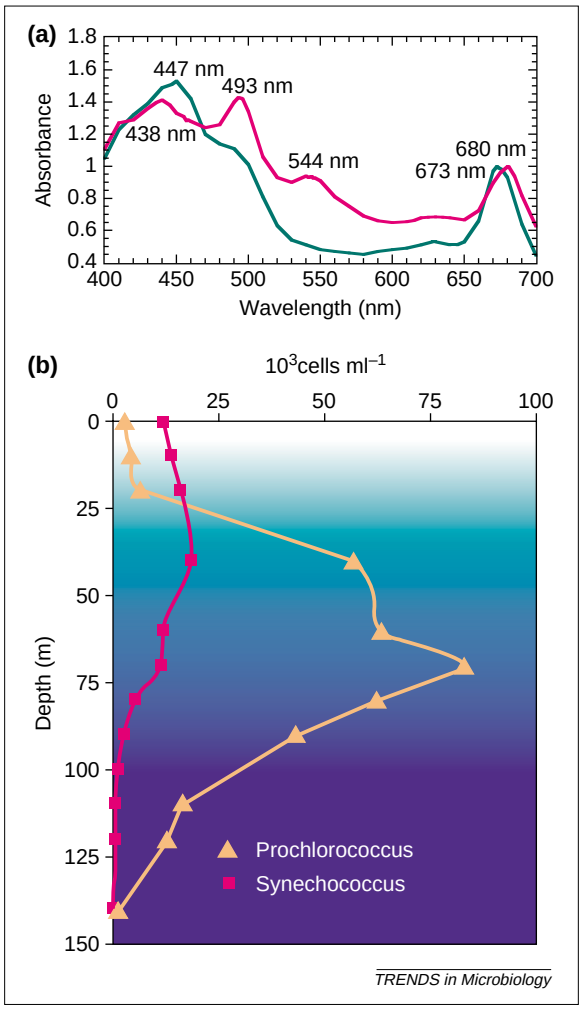

Fig. 3. (a) The *in vivo* absorption spectra of *Prochlorococcus* MED4 (green) and marine *Synechococcus* WH8102 (pink) grown under identical conditions (21°C, 14 hr light/10 hr dark, 30 μmol photons m^{-2} s^{-1}, blue light) depict major differences in their absorption of visible light, particularly in the green region of the spectrum (~500–560 nm). In *Synechococcus*, the peaks at 493 nm and 544 nm are attributable primarily to the chromophores phycourobilin and phycoerythrobilin, respectively. The divinyl chlorophyll (Chl) *a* and *b* pigments of *Prochlorococcus* absorb maximally in the blue region (447 nm,~485 nm) of the visible spectrum. Note that the red peak of *Prochlorococcus* divinyl Chl *a* (673 nm) is shifted approximately 7 nm towards the blue region of the spectrum compared with the monovinyl Chl *a* (680 nm) of *Synechococcus*. Both absorption spectra have been normalized to a value of one at this red peak. (b) Typical vertical distributions of *Prochlorococcus* (orange triangles) and *Synechococcus* (pink squares) at a station in the subtropical Atlantic (32°08' N, 70°02' W) during the summer (10 June, 1996) shows *Prochlorococcus* cell concentrations exceeding those of *Synechococcus*, particularly deeper (80–120 m) in the water column. Cell concentrations were measured by flow cytometry using a modified FACScan (Becton Dickinson). Approximate depths of the open ocean water column that are enriched with blue-green and blue wavelengths of light are shown.

Prochlorococcus is particularly efficient at absorbing blue wavelengths of light (Fig. 3a). Although smaller than *Synechococcus* (~0.6 μm vs 0.9 μm, average), its absorption cross-section is similar (~0.1 μm^2, blue maximum), and thus it can potentially achieve a greater photosynthetic rate per unit biomass [11]. Furthermore, as a result of its unique antenna pigment content (divinyl chlorophyll) and small size, photons of blue wavelengths have a greater chance of being absorbed rather than scattered by *Prochlorococcus* cells, and its average ratio of energy absorbed to energy striking its geometrical cross-section is almost twice that of *Synechococcus* [11]. *Prochlorococcus*'s advantage in blue light is consistent with its dominance of deep waters at depths of 100–200 m [38,44], which are enriched with blue light. In particular, in the Atlantic and Pacific, as well as the Mediterranean and Arabian seas, *Prochlorococcus* populations extend deeper in the water column than *Synechococcus* (Fig. 3b), and have been found as far as 230 m below the surface [20,38,41,45].

Although *Synechococcus* does not absorb blue light as well as *Prochlorococcus*, it is capable of absorbing green wavelengths (~500–550 nm; Fig. 3a) owing to the PEB pigments of its phycobilisomes [11,12,19]. Thus, marine *Synechococcus* are equipped for growing in green light (500–550 nm), which predominates in coastal waters. In the upper water column of the open ocean (~0–30 m), green light penetrates to a similar extent as blue [43], and this could contribute to the ability of *Synechococcus* to proliferate at these depths [11].

The role of nitrogen

Many of the open ocean regions where *Prochlorococcus* and *Synechococcus* co-occur are characterized as oligotrophic – that is, containing very low levels of essential plant nutrients such as nitrogen, phosphorus and iron. *Prochlorococcus* is particularly abundant in such waters [38]. For example, in the upper 100 m of the North Pacific, although *Synechococcus* is a minor contributor, *Prochlorococcus* contributes a large fraction (~40%) of the total prokaryotic carbon biomass [46]. Differences in cellular nitrogen requirements between the two genera could also be a factor in determining their distributions.

Cyanobacterial phycobilisomes represent a major cellular investment, with phycobiliproteins constituting as much as 50%–60% of the soluble protein of the cell [47,48]. Under conditions of nutrient (nitrogen or sulfur) or trace metal (iron) starvation, the phycobilisome is targeted for rapid degradation [10,47]. As a result, amino acids could become available for the synthesis of proteins essential for survival under nutrient stress [47].

Given that both *Prochlorococcus* and *Synechococcus* rely on photosynthesis as their primary form of energy metabolism, a lower protein (nitrogen) investment per tetrapyrrole (e.g. chlorophyll and phycobilin) in their major light-harvesting complex could also result in a significant advantage in nutrient-limited, open oceans. Recent calculations suggest that phycobilisome-based antennas have a greater protein investment than Chl *a*/*b*-based antennas [49]: the protein investment (kDa per tetrapyrrole) of C-phycoerythrin (cyanobacterial, PEI) is 7.5, whereas for LHCII of green plants it is 2.25 [49]. For marine cyanobacterial PEII, which contains an additional bilin molecule,

Questions for future research

- How do the chlorophyll *a*/*b*-binding proteins of *Prochlorococcus* (high- and low-light-adapted ecotypes) and other prochlorophytes interact structurally and functionally with the PSII and PSI reaction centers? Is the structural organization conserved under different environmental conditions? Are there differences in the pigment-binding properties and structure of the different Pcb proteins?
- Do the multiple *pcb* genes in low-light-adapted *Prochlorococcus* MIT 9313 and SS120 differ in their regulation of expression and do their products differ in their inherent pigment-binding properties or structural associations? How would the deletion of one or more of the multiple *pcb* genes affect their overall photosynthetic capacity and ability to acclimate to low irradiance levels? Is the possession of multiple *pcb* genes a characteristic that is conserved among low-light-adapted *Prochlorococcus* isolates?
- What is the function of phycoerythrin in *Prochlorococcus*? Does this phycobiliprotein function as a photoreceptor? Is its function conserved among high- and low-light-adapted *Prochlorococcus* ecotypes?
- Given the presence of phycobiliprotein genes in chlorophyll *a*/*b*-containing *Prochlorococcus* isolates, are there extant marine cyanobacteria that possess both functional Pcb-based antennas and organized phycobilisomes?

similar estimates yield a slightly smaller value of approximately 6.5. A conservative estimate can also be made for the *Prochlorococcus* Pcb protein, based on the number of highly conserved histidine residues present in the membrane-spanning regions of the protein (Fig. 4; available as supplementary information online at http://archive.bmn.com/supp/tim/chisholm.pdf). If we assume at least nine tetrapyrroles are bound to each Pcb protein (average calculated molecular mass of 39 kDa), a protein investment of 4.3 is calculated. This suggests that the protein investment per tetrapyrrole of the *Prochlorococcus* Pcb antenna is approximately 1.5–1.7 times less than that of PEI and PEII of cyanobacteria. In open oceans, where nitrogen can be limiting, this could contribute directly to the competitive edge of *Prochlorococcus*.

The specific form of nitrogen present in the environment could also play a role in the different distributions of the two genera. In the near-surface, mixed layers of oligotrophic oceans, although regenerated forms of nitrogen [i.e. ammonium (NH_4^+)] are available, nitrate (NO_3^-) and nitrite (NO_2^-) are often limiting and their concentrations increase only in deeper waters (>100 m) [41,50]. By contrast, both coastal waters and polar oceans contain measurable concentrations of nitrate and nitrite [50]. Interestingly, analyses of the complete genome sequences of two *Prochlorococcus* isolates (MED4 and MIT 9313) indicate that both lack several genes required for nitrate utilization in cyanobacteria, including genes encoding a nitrate transporter (*napA* or *nrtABC*) and nitrate reductase (*narB*) [51]. Furthermore, to date, not a single *Prochlorococcus* isolate is capable of growth on nitrate. However, some *Prochlorococcus* isolates are capable of growth on nitrite, and analyses of the MIT 9313 genome indicate it possesses a putative nitrite reductase gene (*nirA*) [51]. By contrast, the majority of marine *Synechococcus* are capable of growth on all three forms of nitrogen [51], and genes encoding putative nitrate and nitrite reductases and proteins involved in nitrate transport are found in the genome of *Synechococcus* WH 8102.

The role of ecotypes

Isolates of *Prochlorococcus* differ significantly from one another in their physiology, as do isolates of *Synechococcus*, and we refer to genetically and physiologically distinct groups of isolates that appear to be adapted to a particular set of environmental conditions as 'ECOTYPES'. Differences among ecotypes of a single genus could also be important in determining the relative distributions of these cyanobacteria in the water column and throughout the oceans. The most striking example is within the *Prochlorococcus* lineage (Fig. 1), where 16S rDNA phylogenies have identified a recently arisen clade whose members are capable of growth at greater irradiance levels (> 250 μmol photons m^{-2} s^{-1}) and possess a lower Chl *b*:a_2 ratio at a given light level (and hence are called 'high-light-adapted' or 'low B/A' ecotypes) than members of more deeply branching clades ('low-light-adapted' or 'high B/A' ecotypes) [9,44,52,53]. The recent divergence of the *Prochlorococcus* lineage into a distinct clade that is better adapted for growth at higher irradiance levels (Fig. 1) could contribute to the ability of this genus to dominate at several different depths in the water column [44,53,54].

High- and low-light-adapted *Prochlorococcus* ecotypes differ in the number of genes encoding light-harvesting antenna proteins (Pcb). Garczarek *et al*. [22] have suggested that the presence of multiple *pcb* genes in low-light-adapted ecotypes (seven in SS120) compared with high-light-adapted ecotypes (one in MED4), could be an important factor in acclimation to low irradiance levels. However, not all low-light-adapted isolates possess this many *pcb* genes, as the genome of the low-light-adapted isolate MIT 9313 has only two *pcb* genes (http://www.jgi.doe.gov/JGI_microbial/html/index.html). In MIT 9313 the two *pcb* genes do not form an operon but are separated on the chromosome by >500 000 bp, suggesting they could be differentially expressed. Future work is necessary on the expression of different *pcb* genes and how expression patterns can vary among high- and low-light-adapted ecotypes exposed to different environmental conditions. In SS120, it has been suggested that the turnover rates of different *pcb* transcripts might be dissimilar, although they do not appear to be regulated differentially by light intensity [22,55], and in MED4, transcript levels of the single *pcb* gene exhibit strong diel variations [56].

Synechococcus also consists of numerous genetically distinct clades, of which the best characterized is the motile clade, whose members are all capable of a unique form of swimming motility [57]. Another

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genetically distinct clade consists of isolates that lack the chromophore, PUB, which is present in members of all other clades [53]. The no-PUB ecotype might be the dominant phycoerythrin-containing *Synechococcus* in coastal waters, whereas the clades that possess PUB dominate in the open ocean [58]. The relative amounts of PUB and PEB vary among members of these clades, and some isolates acclimate to fluctuations in growth light quality by changing the ratio of these chromophores [59]. These isolates increase their PUB:PEB ratios when grown under blue light compared with white light [59] in an acclimation process similar to what has traditionally been called complementary chromatic adaptation in other cyanobacteria [47]. This acclimation could be related to differential expression of the two forms of phycoerythrin, which bind different amounts of PUB and PEB. The ability of at least some *Synechococcus* to acclimate chromatically to fluctuating light regimes could explain the dominance of *Synechococcus* over *Prochlorococcus* in winter mixed regimes in the Sargasso Sea [59].

Concluding remarks

Cyanobacteria are one of the most ubiquitous and diverse groups of photosynthetic organisms, and the versatility of their light-harvesting systems has contributed to their ability to proliferate in widely different environments. *Prochlorococcus* and *Synechococcus* are among the most abundant members of the cyanobacterial lineage and are important contributors to primary production in the world's oceans. Given their close phylogenetic relationship, it is possible to examine how the evolution of key protein complexes has proceeded along different paths and contributes to their ability to dominate specific oceanic regions. Further analysis of the structural organization of *Prochlorococcus* Pcb antennas and their energy-transfer properties will provide exciting new information on how this light-harvesting system enables *Prochlorococcus* to dominate regions of the water column characterized by very low irradiance levels. In addition, with the completion of whole genome sequencing projects and progress in transcriptome and proteome analysis, detailed characterization of the coordinated expression of these major photosynthetic apparatus proteins with other cellular components will be possible. The utilization of vastly different antennas in two globally significant marine cyanobacteria provides an important system for understanding the origins and scope of phylogenetic diversity in ocean ecosystems.

References

1 Whitman, W.B. *et al.* (1998) Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6578–6583
2 Chisholm, S.W. *et al.* (1988) A novel free-living prochlorophyte occurs at high cell concentrations in the oceanic euphotic zone. *Nature* 334, 340–343
3 Chisholm, S.W. *et al.* (1992) *Prochlorococcus marinus nov.* gen. *nov.* sp.: an oxyphototrophic marine prokaryote containing divinyl chlorophyll *a* and *b*. *Arch. Microbiol.* 157, 297–300
4 Waterbury, J.B. *et al.* (1979) Widespread occurrence of a unicellular marine phytoplankton cyanobacterium. *Nature* 277, 293–294
5 Goericke, R. and Welschmeyer, N.A. (1993) The marine prochlorophyte *Prochlorococcus* contributes significantly to phytoplankton biomass and primary production in the Sargasso Sea. *Deep Sea Res.* 40, 2283–2294
6 Liu, H.B. *et al.* (1997) *Prochlorococcus* growth rate and contribution to primary production in the equatorial and subtropical North Pacific Ocean. *Aquat. Microb. Ecol.* 12, 39–47
7 Palenik, B.P. and Haselkorn, R. (1992) Multiple evolutionary origins of prochlorophytes, the chlorophyll *b*-containing prokaryotes. *Nature* 355, 265–267
8 Urbach, E. *et al.* (1992) Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. *Nature* 355, 267–270
9 Urbach, E. *et al.* (1998) Rapid diversification of marine picoplankton with dissimilar light-harvesting structures inferred from sequences of *Prochlorococcus* and *Synechococcus* (cyanobacteria). *J. Mol. Evol.* 46, 188–210
10 Sidler, W.A. (1994) Phycobilisome and phycobiliprotein structures. In *The Molecular Biology of Cyanobacteria* (Bryant, D.A., ed.), pp. 139–216, Kluwer Academic Publishers
11 Morel, A. *et al.* (1993) *Prochlorococcus* and *Synechococcus*: a comparative study of their optical properties in relation to their size and pigmentation. *J. Mar. Res.* 51, 617–649
12 Ong, L.J. and Glazer, A.N. (1991) Phycoerythrins of marine unicellular cyanobacteria. I. Bilin types and locations and energy transfer pathways in *Synechococcus* spp. phycoerythrins. *J. Biol. Chem.* 266, 9515–9527
13 Kana, T.M. and Gilbert, P.M. (1987) Effect of irradiances up to 2000 μE m^{-2} s^{-1} on marine *Synechococcus* WH7803. I. Growth, pigmentation, and cell composition. *Deep Sea Res.* 34, 479–495
14 Hess, W.R. *et al.* (1996) Coexistence of phycoerythrin and a chlorophyll *a*/*b* antenna in a marine prokaryote. *Proc. Natl. Acad. Sci. U. S. A.* 93, 11126–11130
15 Ting, C.S. *et al.* (1999) Characterization of phycoerythrin genes in the chlorophyll a_2/b_2 containing prokaryote, *Prochlorococcus* sp. MIT 9303. In *Photosynthesis: Mechanisms and Effects* (Vol. 1) (Garab, G., ed.), pp. 225–228, Kluwer Academic Publishers
16 Ting, C.S. *et al.* (2001) Phycobiliprotein genes of the marine photosynthetic prokaryote *Prochlorococcus*: evidence for rapid evolution of genetic heterogeneity. *Microbiology* 147, 3171–3182
17 Penno, S. *et al.* (2000) Presence of phycoerythrin in two strains of *Prochlorococcus* (cyanobacteria) isolated from the subtropical north pacific ocean. *J. Phycol.* 36, 723–729
18 Goericke, R. and Repeta, D. (1992) The pigments of *Prochlorococcus marinus*: the presence of divinyl chlorophyll *a* and *b* in a marine prokaryote. *Limnol. Oceanogr.* 37, 425–434
19 Moore, L.J. *et al.* (1995) The comparative physiology of *Synechococcus* and *Prochlorococcus*: influence of light and temperature on growth, pigments, fluorescence, and absorptive properties. *Mar. Ecol. Progr. Ser.* 116, 259–275
20 Goericke, R. *et al.* (2000) A novel niche for *Prochlorococcus* sp. in low-light suboxic environments in the Arabian Sea and the Eastern Tropical North Pacific. *Deep Sea Res.* 147, 1183–1205
21 LaRoche, J. *et al.* (1996) Independent evolution of the prochlorophyte and green plant chlorophyll *a*/*b* light-harvesting proteins. *Proc. Natl. Acad. Sci. U. S. A.* 93, 15244–15248
22 Garczarek, L. *et al.* (2000) Multiplication of antenna genes as a major adaptation to low light in a marine prokaryote. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4098–4101
23 Barber, J. *et al.* (2000) Revealing the structure of the photosystem II chlorophyll-binding proteins, CP43 and CP47. *Biochim. Biophys. Acta* 1459, 239–247
24 Bassi, R. *et al.* (1999) Mutational analysis of higher plant antenna protein provides identification of chromophores bound into multiple sites. *Proc. Natl. Acad. Sci. U. S. A.* 96, 10056–10061
25 Green, B.R. and Kuhlbrandt, W. (1995) Sequence conservation of light-harvesting and stress-response proteins in relation to the three-dimensional molecular structure of LHCII. *Photosynth. Res.* 44, 139–148
26 Green, B.R. and Durnford, D.G. (1996) The chlorophyll-carotenoid proteins of oxygenic photosynthesis. *Annu. Rev. Plant Physiol.* 47, 685–714
27 Leonhardt, K. and Straus, N.A. (1994) Photosystem II genes *isiA*, *psbD1* and *psbC* in *Anabaena* sp. PCC 7120: cloning, sequencing and the transcriptional regulation in iron-stressed and iron-repleted cells. *Plant Mol. Biol.* 24, 63–73
28 Park, Y. *et al.* (1999) Expression of the *isiA* gene is essential for the survival of cyanobacterium *Synechococcus* sp. PCC 7942 by protecting photosystem II from excess light under iron limitation. *Mol. Microbiol.* 32, 123–129

29 Van der Staay, G.W.M. *et al.* (1998) The 38 kDa chlorophyll *a*/*b* protein of the prokaryote *Prochlorothrix hollandica* is encoded by a divergent *pcb* gene. *Plant Mol. Biol.* 36, 709–716
30 Martin, J.H. *et al.* (1994) Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. *Nature* 371, 123–129
31 Coale, K.H. *et al.* (1996) A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. *Nature* 383, 495–501
32 Mann, E. and Chisholm, S.W. (2001) Iron limits the cell division rate of *Prochlorococcus* in the Eastern Equatorial Pacific. *Limnol. Oceanogr.* 45, 1067–1076
33 Bibby, T.S. *et al.* (2001) Iron deficiency induces the formation of an antenna ring around trimeric photosystem I in cyanobacteria. *Nature* 412, 743–745
34 Boekema, E.J. *et al.* (2001) A giant chlorophyll-protein complex induced by iron deficiency in cyanobacteria. *Nature* 412, 745–748
35 Webb, E.A. *et al.* (2001) Iron stress in open-ocean cyanobacteria (*Synechococcus*, *Trichodesmium*, and *Crocosphaera* spp.): identification of the IdiA protein. *Appl. Environ. Microbiol.* 67, 5444–5452
36 Exss-Sonne, P. *et al.* (2000) The IdiA protein of *Synechococcus* sp. PCC 7942 functions in protecting the acceptor side of photosystem II under oxidative stress. *Photosyn. Res.* 63, 145–157
37 Bibby, T.S. *et al.* (2001) Oxyphotobacteria. Antenna ring around photosystem I. *Nature* 413, 590
38 Partensky, F. *et al.* (1999) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiol. Mol. Biol. Rev.* 63, 106–127
39 DuRand, M.R. *et al.* (2001) Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. *Deep Sea Res.* II 48, 1983–2003
40 Carr, N.G. and Mann, N.H. (1994) The oceanic cyanobacterial picoplankton. In *The Molecular Biology of Cyanobacteria* (Bryant, D.A., ed.), pp. 27–48, Kluwer Academic Publishers
41 Olson, R.J. *et al.* (1990) Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. *Deep Sea Res.* 37, 1033–1051
42 Campbell, L. *et al.* (1997) Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991–1994 ENSO event. *Deep Sea Res.* 44, 167–192
43 Kirk, J.T.O. (1994) The nature of the underwater light field. In *Light and Photosynthesis in Aquatic Ecosystems,* pp. 509, Cambridge University Press
44 Moore, L.R. *et al.* (1998) Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. *Nature* 393, 464–467
45 Johnson, Z. *et al.* (1999) Energetics and growth of a deep *Prochlorococcus* spp. population in the Arabian Sea. *Deep Sea Res.* II 46, 1719–1743
46 Campbell, L. *et al.* (1994) The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. *Limnol. Oceanogr.* 39, 954–961
47 Grossman, A.R. *et al.* (1993) The phycobilisome, a light-harvesting complex responsive to environmental conditions. *Microbiol. Rev.* 57, 725–749
48 Apt, K.E. *et al.* (1995) Evolution of the phycobiliproteins. *J. Mol. Biol.* 248, 79–96
49 Scheer, H. The pigments. In *Light-harvesting Antennas in Photosynthesis* (Green, B.R. and Parson, W.W., eds), Kluwer Academic Publishers (in press)
50 Cavender-Bares, K.K. *et al.* (2001) Nutrient gradients in the western North Atlantic Ocean: relationship to microbial community structure and comparison to patterns in the Pacific Ocean. *Deep Sea Res.* I 48, 2373–2395
51 Moore, L.R. *et al.* Utilization of different nitrogen sources by marine cyanobacteria, *Prochlorococcus* and *Synechococcus*. *Limnol. Oceanogr.* (in press)
52 Moore, L.R. and Chisholm, S.W. (1999) Photophysiology of the marine cyanobacterium *Prochlorococcus*: ecotypic differences among cultured isolates. *Limnol. Oceanogr.* 44, 628–638
53 Rocap, G. *et al.* Resolution of *Prochlorococcus* and *Synechococcus* ecotypes using 16S-23S rDNA internal transcribed spacer (ITS) sequences. *Appl. Environ. Microbiol.* (in press)
54 West, N.J. *et al.* (2001) Closely related *Prochlorococcus* genotypes show remarkably different depth distributions in two oceanic regions as revealed by *in situ* hybridization using 16S rRNA-tagged oligonucleotides. *Microbiology* 147, 1731–1744
55 Garczarek, L. *et al.* (2001) Expression and phylogeny of the multiple antenna genes of the low light adapted strain *Prochlorococcus marinus* SS120 (Oxyphotobacteria). *Plant Mol. Biol.* 46, 683–693
56 Garczarek, L. *et al.* (2001) Differential expression of antenna and core genes in *Prochlorococcus* PCC 9511 (Oxyphotobacteria) grown under a modulated light-dark cycle. *Environ. Microbiol.* 3, 168–175
57 Toledo, G. (1999) Swimming marine *Synechococcus* strains with widely different photosynthetic pigment ratios form a monophyletic group. *Appl. Environ. Microbiol.* 65, 5247–5251
58 Wood, A.M. *et al.* (1998) Water column transparency and the distribution of spectrally distinct forms of phycoerythrin-containing organisms. *Mar. Ecol. Prog. Ser.* 162, 25–31
59 Palenik, B.P. (2001) Chromatic adaptation in marine *Synechococcus* strains. *Appl. Environ. Microbiol.* 67, 991–994

Hijacking the host: survival of pathogenic mycobacteria inside macrophages

Jean Pieters and John Gatfield

Mycobacteria are among the most persistent pathogens known today: one-third of the global population is latently infected with *Mycobacterium tuberculosis*, an organism that is responsible for ~2.5 million deaths each year. One key to the pathogenic potential of the mycobacteria lies in their capacity to resist destruction by macrophages. Although it has long been recognized that mycobacteria achieve such resistance by interfering with phagosome–lysosome fusion, recent work has shed some more light on the molecular basis of this highly efficient survival strategy.

The interaction of *Mycobacterium tuberculosis* with its host is a perfect example of a balanced biological system: *M. tuberculosis* uses host macrophages exclusively for replication yet the macrophages must remain viable to host the mycobacteria. Indeed, *M. tuberculosis*, as well as other pathogenic mycobacteria, can survive within macrophages for very long periods of time without being lethal to the infected host. Outgrowth of the mycobacteria within the host is kept under control by innate and adaptive immune responses that apparently are well equipped to deal with mycobacteria that have invaded host macrophages. It is problematic, however, that, as a result of the successful establishment of mycobacterial infections, these microorganisms have

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