Prochlorococcus: the structure and function of collective diversity

Steven J. Biller[1], Paul M. Berube[1], Debbie Lindell[2] and Sallie W. Chisholm[1,3]

Abstract | The marine cyanobacterium *Prochlorococcus* is the smallest and most abundant photosynthetic organism on Earth. In this Review, we summarize our understanding of the diversity of this remarkable phototroph and describe its role in ocean ecosystems. We discuss the importance of interactions of *Prochlorococcus* with the physical environment, with phages and with heterotrophs in shaping the ecology and evolution of this group. In light of recent studies, we have come to view *Prochlorococcus* as a 'federation' of diverse cells that sustains its broad distribution, stability and abundance in the oceans via extensive genomic and phenotypic diversity. Thus, it is proving to be a useful model system for elucidating the forces that shape microbial populations and ecosystems.

Phytoplankton
Free-floating aquatic photosynthetic microorganisms that require sunlight and inorganic nutrients for growth

Euphotic zone
The sunlit upper region of the ocean water column that receives sufficient light energy to sustain photosynthesis. The depth can vary depending on local conditions, but it is generally the upper ~200 m in oligotrophic waters.

[1]*Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.*
[2]*Faculty of Biology, Technion – Israel Institute of Technology, Haifa 32000, Israel.*
[3]*Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.*
Correspondence to S.J.B., S.W.C.
e-mails: sbiller@mit.edu; chisholm@mit.edu
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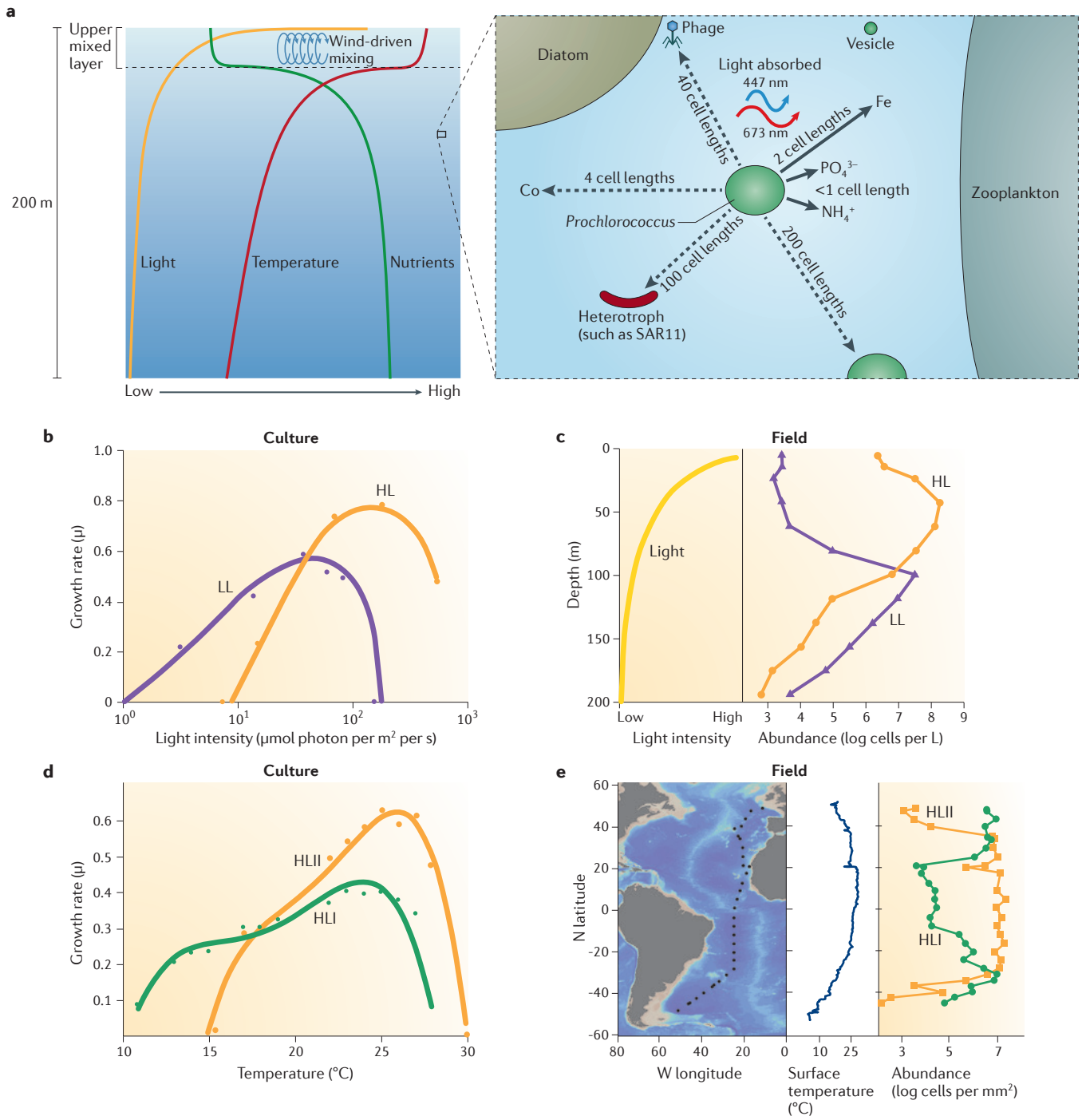
Since the discovery of *Prochlorococcus* in 1985[1], considerable progress has been made in understanding the characteristics that make this bacterium unique in the microbial world. It is the smallest (the cell diameter is 0.5–0.7 μm)[2] and most abundant photosynthetic organism on the planet, with an estimated global population of ~10^{27} cells[3–5]. *Prochlorococcus* has the smallest genome of any free-living phototroph[6]; some isolates have genomes as small as 1.65 Mbp, with only ~1,700 genes[7]. It is the only type of marine phytoplankton that uses the divinyl form of chlorophyll *a* and chlorophyll *b* to harvest light energy[8], which causes a slight red shift in its absorption spectra[2,9]. This unique pigmentation has made it possible to determine that *Prochlorococcus* accounts for 50% of the total chlorophyll in vast stretches of the surface oceans[10,11]. Collectively, this cyanobacterium produces an estimated 4 gigatons of fixed carbon each year[3], which is approximately the same net primary productivity as global croplands[12].

Prochlorococcus thrives throughout the euphotic zone of the tropical and subtropical oligotrophic ocean[1,13]. The daily light–dark cycle synchronizes cell division in *Prochlorococcus*[14] and is an important driver of highly choreographed gene expression patterns throughout the day[15–19]. The euphotic zone is shaped by continuous macroscale gradients of light, temperature and nutrients (FIG. 1); both light intensity and temperature are highest at the surface and decrease with depth, whereas nutrient levels are typically low at the surface and gradually increase with depth. Although there is fine-scale variation within the water column, the physical and chemical environment of the ocean as a whole tends to be constrained and less variable than many other microbial habitats (such as the soil), and exhibits gradual changes on monthly to annual timescales.

From the perspective of its microbial inhabitants, the oligotrophic ocean is an extremely dilute environment in terms of both its chemistry and biology (FIG. 1a). For example, the average *Prochlorococcus* bacterium may be hundreds of cell diameters away from another cell of any type, and even a few cell diameters away from essential nutrients, which are found at picomolar to nanomolar concentrations. By contrast, well-studied model microorganisms, such as *Escherichia coli*, tend to reside in relatively nutrient-rich and densely populated environments, such as the gut. Studies have shown that, to overcome the challenges associated with their dilute environment, some marine microorganisms attach to particles[20] or form close associations with other bacteria[21]. Although microscale patchiness of some form may contribute to the survival of *Prochlorococcus*, direct evidence to support or reject this hypothesis is lacking. Nevertheless, the dilute nature of oligotrophic ecosystems clearly imposes a unique set of selective pressures on microbial life.

Prochlorococcus has several traits that make it well-suited to this dilute habitat. Compared with other phytoplankton, *Prochlorococcus* has a low phosphorus requirement (it has high C/P and N/P ratios)[22–24], partly owing to its relatively small genome[22] and the substitution of sulpholipids for phospholipids in the cell membrane[25]. Its small size results in a high surface-to-volume ratio that facilitates efficient nutrient acquisition and enhances light absorption, which, when combined with its unique



pigmentation[9], make it the most efficient light absorber of any photosynthetic cell; *Prochloroccocus* is the only phytoplankton known to absorb more light than it scatters[2]. Thus, *Prochlorococcus* can thrive at lower light intensities than those required by most other phytoplankton[9] and its populations extend deeper in the water column than almost any other phototroph[26], essentially defining the lower boundary of photosynthetic life in the oceans.

The ability of *Prochlorococcus* to occupy the entire euphotic zone can be largely explained by its microdiversity, as different subgroups are adapted to different light optima for growth[9,27]. Strains isolated from deep waters grow optimally at substantially lower light intensities (termed low-light (LL)-adapted ecotypes) than those isolated from the surface (termed high-light (HL)-adapted ecotypes) (FIG. 1b), which results in niche-partitioning in the water column[28–30]: HL-adapted cells are orders of magnitude more abundant in surface waters[31–33] but are outnumbered by LL-adapted cells at the base of the euphotic zone (FIG. 1c). Despite the complexity of ocean dynamics, these distinct groups of *Prochlorococcus* shift in relative abundance in reproducible annual cycles[34,35], which demonstrates the remarkable robustness of *Prochlorococcus* populations. The

Oligotrophic
A term used to describe an environment with low concentrations of available nutrients.

Ecotypes
Genetically and physiologically differentiated subgroups of a species that occupy a distinct ecological niche.

Figure 1 | **The *Prochlorococcus* habitat. a** | *Prochlorococcus* inhabits the entire euphotic zone, which is characterized by gradients of light, temperature and nutrients. The ocean water column is divided into an upper 'mixed' layer (where wind and heat-driven turbulence homogenizes the distribution of nutrients and cells) and the stratified and less turbulent deeper waters, where gradients in nutrients form as a result of biogeochemical activity. Light levels decrease exponentially with depth; gradients of temperature and nutrient concentrations are largely similar in the mixed layer, whereas temperature decreases, and nutrient concentrations increase, with depth. The oligotrophic ocean represents an extremely dilute environment in terms of organisms and nutrients. Some key players in oligotrophic marine communities are shown, along with the distances between them as estimated from their average concentrations. According to these metrics, each *Prochlorococcus* bacterium is hundreds of cell diameters away from other members of its 'federation' and even a few cell diameters away from essential nutrients. The closest phage might be tens of cell lengths away. Mean inter-particle distances between a component of the ocean and *Prochlorococcus* are based on average concentrations in the North Pacific[35]. Distances that are to scale are marked with a solid line, whereas those that are not to scale are represented by dashed lines. Light wavelengths represent the two major absorption peaks of *Prochlorococcus* strain MED4 (REF. 133). **b–e** | The relationship between growth optima of cultured isolates in the laboratory and their abundance in the field has been most clearly shown by both longitudinal and depth niche partitioning of ecotypes as functions of light (b,c) or temperature (d,e). The relative abundance of high-light (HL)- and low-light (LL)-adapted strains in the water column is consistent with light optima in the laboratory, and the longitudinal abundance of two HL-adapted clades is consistent with the temperature optima for growth of representative strains, in which cells from the HLII clade grow maximally at higher temperatures than HLI strains. Although growth rate and abundance need not be related in wild bacterial populations, so far these patterns are correlated in *Prochlorococcus*, making it easier to interpret distribution patterns in the wild in the context of the physiology of the organism. Figure part b is modified from REF. 27, Nature Publishing Group. Figure part c is modified from REF. 35, Nature Publishing Group. Figure parts d and e are reproduced, with permission, from Johnson, Z.I., Zinser, E.R., Coe, A., McNulty, N.P., Woodward, E.M.S., and Chisholm, S.W. Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. *Science* **311**, 1737–1740 (2006). Reprinted with permission from AAAS.

distinction between HL- and LL-adapted cells forms the basis of our understanding of *Prochlorococcus* diversity, but, as discussed in this Review, light is just one of many factors that has driven the diversification of this bacterial group[36]. Here, we discuss the genomic diversity of *Prochlorococcus*, the factors that contribute to this diversity and its consequences for the ecology of this marine cyanobacterium.

Deeply rooted evolutionary diversity

The 16S rRNA sequences of all *Prochlorococcus* isolates do not differ by more than ~3%, which is the traditional boundary for defining a microbial species. Thus, this genus maintains a coherent identity, although it has extraordinary diversity in other traits (see below). Given the conservation of 16S rRNA, the ITS sequence (internal transcribed spacer sequence) between the 16S and 23S sequences is typically used to provide increased phylogenetic resolution[37]. In addition, many other marker genes (including *rpoC1*[38,39], *petB–petD*[40], *ntcA*[41] and *gyrB*[42]) provide similar insights into its evolutionary history. *Prochlorococcus* is a monophyletic group that is closely related to marine *Synechococcus*[28,37,40,43], although key physiological and ecological features distinguish the two genera (BOX 1). ITS-based trees of *Prochlorococcus* are surprisingly consistent with those derived from whole-genome protein-coding sequences, which makes this a useful sequence marker for exploring evolutionary relationships[44,45].

Although the initial partitioning of *Prochlorococcus* into the broad categories of HL- and LL-adapted strains was based solely on phenotype[9,27,46], molecular phylogenetic analyses have shown that this division is consistent with the earliest phylogenetic split within the *Prochlorococcus* lineage[31,37,38,40]. HL-adapted *Prochlorococcus* strains form a coherent, monophyletic group that resolves into at least six clades (HLI–HLVI)[9,27,31,46], whereas the LL-adapted strains are polyphyletic and partition into at least six clades (LLI–LLVII)[36,47,48] (FIG. 2a). Although alternative nomenclature has been proposed, the HL and LL notation has emerged as the most consistent way to refer to the ever-growing diversity within *Prochlorococcus* (TABLE 1).

What do we know about the physiological and ecological distinctions among these clades? HLI and HLII clades are distinguished by their temperature optima[30,33] (FIG. 1d,e), which suggests that temperature-dependent adaptations probably had a role in their divergence. Clades HLIII–HLVI[47–51] lack cultured representatives and are termed HL solely owing to their phylogenetic grouping with the HLI and HLII clades. On the basis of their distinctive distributions along ocean transects as well as genomic and metagenomic data, it has been proposed that members of the HLIII, HLIV and HLV clades thrive in regions characterized by high nitrogen and phosphorus, but low iron availability[47–50]. There is evidence that the HLIII and HLIV clades have adapted to these iron-limited environments by decreasing cellular iron requirements[49] (BOX 2) and acquiring siderophore transporters for efficient scavenging of this element[48]. Data on the HLVI clade are limited, but given that members of this clade are detected in the middle to lower euphotic zone, it has been proposed that they might be adapted to lower light levels than the HLI and HLII clades[47].

Environmental factors associated with the diversification of LL-adapted clades are less well understood, mostly because there are fewer cultured representatives[36,39,42,52]. However, inferences can be made from ecological, physiological and genomic data. For example, the LLI clade has characteristics that are intermediate between HL-adapted and the other LL-adapted clades: LLI bacteria are more abundant closer to the surface and during deep mixing events in the wild[30,35] than other LL-adapted cells[29,52], and they can better tolerate fluctuating light intensities[35]. They are also the only LL clade known to encode photolyase (a photoprotective enzyme[53]) and have more HL-inducible (*hli*) genes (which encode proteins that protect cells during light shock and other stresses[54]) than any other *Prochlorococcus* clade[44,53].

In contrast to the LLI clade, the LLII/III and LLIV clades are more restricted to the lower euphotic zone and decrease in relative abundance during deep mixing events. Among the cultured *Prochlorococcus* lineages, members of the LLIV clade are the most closely related to *Synechococcus* and have the largest and most diverse genomes. They are often physically larger than HL-adapted cells and seem to produce a wide range of secondary metabolites[55]. The LLV and LLVI clades lack cultured representatives and have only been detected

ITS sequence
(Internal transcribed spacer sequence). A non-functional rRNA sequence located between the 16S and 23S ribosomal RNA genes in bacteria, which is a useful phylogenetic marker.

Clades
Coherent phylogenetic groups of organisms, each of which comprises all the descendents of a single ancestor.

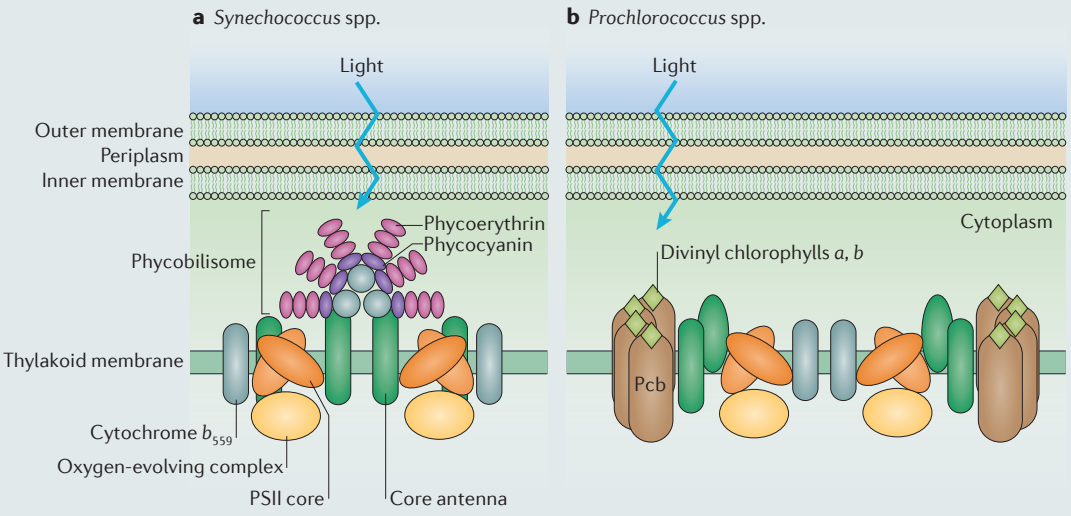
Siderophore
A molecule that can bind iron; it is often used by microorganisms to facilitate the acquisition of iron from the environment.

Box 1 | ***Prochlorococcus* and *Synechococcus*: what's in a name?**

Prochlorococcus and marine *Synechococcus* are thought to have diverged from a common ancestor ~150 million years ago[76], but most members of the two groups would be considered to be the same species on the basis of their 16S rRNA sequences. Although differences in cell size and photosynthetic pigments yield distinct flow cytometry profiles, they still share many phenotypic and ecological traits. Whole-genome phylogenies clearly separate *Prochlorococcus* from *Synechococcus*[44], but the phylogenies of many individual gene families cluster low-light (LL)-adapted *Prochlorococcus* more closely with *Synechococcus* than with high-light (HL)-adapted *Prochlorococcus*[37,132]. Although these data question the distinction between these two genera, several physiological and ecological factors justify their separation.

The clearest difference between the two groups is in their photosynthetic apparatus (see the figure). Similarly to most cyanobacteria, the main light-harvesting antenna in *Synechococcus* is the phycobilisome, which comprises phycobiliproteins (for example, phycoerythrin and phycocyanin), each of which binds one or several light-harvesting chromophores, such as phycoerythrobilin and phycocyanobilin[133]. This antenna complex collects light and transfers the energy to the photosystem II (PSII) core antenna proteins (CP43 and CP47) and then into the PSII reaction centre (comprising multiple proteins and cytochrome b_{559}). *Prochlorococcus* is one of the few cyanobacteria (together with *Prochloron* and *Prochlorothrix*) that lack phycobilisomes; instead, its main light-harvesting antenna complex is made up of prochlorophyte chlorophyll-binding protein (Pcb), which binds divinyl chlorophyll *a* and divinyl chlorophyll *b*. *Prochlorococcus* also uses monovinyl chlorophyll *b* as an accessory pigment in the antenna complex[9,58,133]. Together, these unique pigments increase the absorption of blue light — which is the dominant wavelength in deep waters — by *Prochlorococcus*[1,8].

The geographic distributions of *Prochlorococcus* and *Synechococcus* provide clues about the forces that mediate their niche partitioning. *Synechococcus* is present in almost all marine environments, whereas *Prochlorococcus* is restricted to warmer, oligotrophic oceans, such as subtropical gyres and the eastern Mediterranean Sea, and is absent from colder, nutrient-rich waters at high latitude as well as in most nutrient-rich coastal waters. What might explain these differences? *Synechococcus* can tune its phycobilisome antenna systems to acclimate to changing temperatures, which may contribute to its greater geographical range[134,135]. It is also less susceptible to copper toxicity than *Prochlorococcus*[136], which might explain, in part, its dominance in coastal waters. Furthermore, *Synechococcus* strains have higher maximum growth rates[9] than *Prochlorococcus* and they are prey for many of the same predators. As the growth rate of predators is coupled to that of their prey[137], it may be impossible for *Prochlorococcus* to achieve net positive growth rates when *Synechococcus* is growing maximally — it would simply be 'grazed away' (REF. 137). Consistent with this hypothesis, *Prochlorococcus* can be cultivated in the laboratory using seawater collected from coastal sites[138] even though it is essentially absent from such regions in the wild. Adapted from *Trends Microbiol.*, **10**, Ting, C.S., Rocap, G., King, J., and Chisholm, S.W., Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light-harvesting strategies., 134–142, Copyright (2002), with permission from Elsevier.



Oxygen minimum zones
Subsurface ocean regions that are deficient in oxygen owing to poor ventilation and high rates of respiration.

Gyres
Ocean systems bounded by circular rotating winds and currents. The five major ocean gyres are found in the North Atlantic, North Pacific, South Atlantic, South Pacific and Indian Oceans.

in oxygen minimum zones (OMZs), where the oxygen-depleted layer meets the euphotic zone. This suggests that they are adapted to the unique redox conditions, and associated microbial community, of this habitat[47,56].

Diversity at the genomic level

The analysis of whole genomes has greatly increased our understanding of the vast amount of genomic variation within each of the deeply branching HL- and LL-adapted clades, which is evident across many levels, ranging from genome size to gene content to fine-scale allelic variation. However, there are clear patterns in how this diversity is organized.

Characteristics of the* Prochlorococcus *genome. *Prochlorococcus* is a prime example of an organism with a 'streamlined' genome[57]; the genomes of these bacteria are smaller than those of other cyanobacteria, which reflects a rapid decrease in genome size following divergence from a common ancestor with *Synechococcus*[58]. Initially,

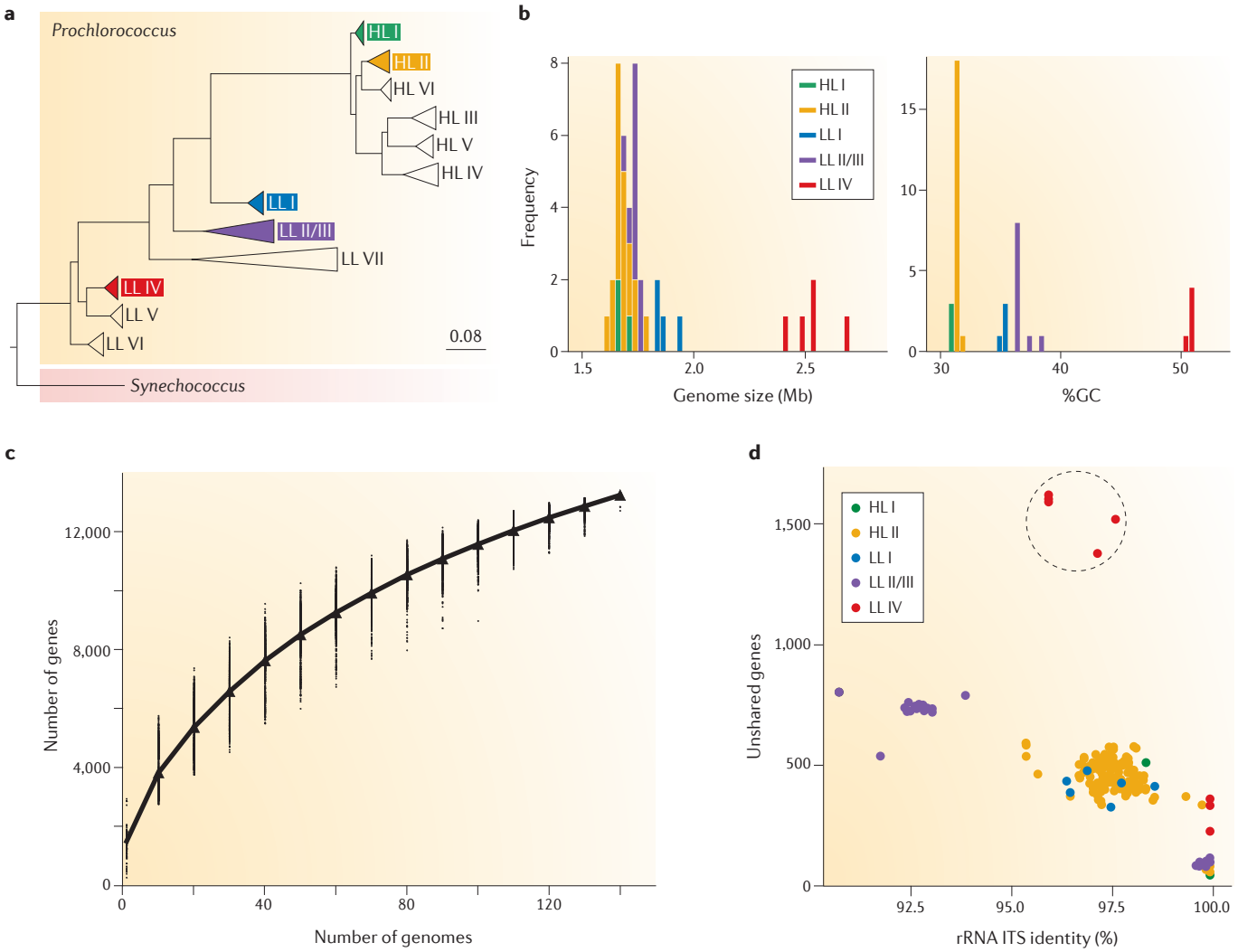


Figure 2 | **Phylogenetic and genomic diversity of *Prochlorococcus*.** **a** | The phylogenetic distribution of *Prochlorococcus* clades as determined by rRNA internal transcribed spacer (ITS) sequence diversity. Of the 12 major clades that are known, five (shown in colour) have cultured representatives; the rest have been identified using environmental sequence data only. The division into high-light (HL)- and low-light (LL)-adapted clades according to different light optima correlates with rRNA phylogeny. **b** | Distributions of genome size (left-hand graph) and GC content (right-hand graph) of cultured *Prochlorococcus* isolates shows that these characteristics correlate with phylogeny. The genomes of HL-adapted cells have, on average, the smallest and least GC-rich genomes among *Prochlorococcus*. Genomes from members of the LLIV clade are the largest and most GC-rich, and genomes from the LLI and LLII/III clades fall between these two extremes. **c** | Analysis of the pan-genome shows that the number of novel genes in the *Prochlorococcus* federation increases with the contribution of each additional genome (calculated as in REF. 44). Data include cultured isolates, single-cell genomes and consensus metagenomic assemblies. **d** | Gene content diversity varies among *Prochlorococcus* clades. Compared with the HL-adapted strains, LL-adapted strains contribute more new genes on average to the *Prochlorococcus* pan-genome. Each point represents a pairwise comparison of two genomes in each clade (as indicated by colour key). Overall, the number of unique genes found in any pair of genomes is inversely correlated with ITS identity (although strains with identical ITS sequences still differ in gene content). LLIV genomes (red; indicated by the dashed circle) are clearly unique among *Prochlorococcus*: they have the greatest average gene diversity of any clade, which cannot be explained by their ITS diversity. Data in part **b** taken from REF. 60, data in part **c** taken from REFS 45,60 and data in part **d** taken from REF. 60.

this reduction was probably driven by strong, genome-wide selection for the removal of genes with only a small fitness benefit that was outweighed by the associated costs[59]. Following initial streamlining, genome diversity that correlated with the deeply branching HL- and LL-adapted physiologies began to emerge. The genomes of HL-adapted strains are generally smaller and have a lower GC content than the genomes of LL-adapted strains (FIG. 2b). Variation in these basic characteristics is also observed among LL-adapted genomes, with members of the LLIV clade having the largest (2.4–2.7 Mbp) and most GC-rich (~50%) genomes[7,44,60].

Extensive genome diversity is also apparent at the level of gene content. Although each individual isolate

Table 1 | **The main clades of *Prochlorococcus* as defined by rRNA internal transcribed spacer sequences**

Clade	Alternative names for the same ribotype	Representative cultured strains*	Habitat‡
HLI	eMED4 (REF. 29), low-B/A *Prochlorococcus* clade I (REF. 37)	MED4, MIT9515	• Isolated from the upper-middle euphotic zone, typically in the subtropical ocean • Distribution is shifted to higher latitudes consistent with their lower optimum growth temperature relative to HLII cells[31,33–35]
HLII	eMIT9312 (REF. 29), low-B/A *Prochlorococcus* clade II (REF. 37)	AS9601, MIT9215, MIT9312, SB	• Often found throughout the euphotic zone • Typically among the most abundant *Prochlorococcus* group in the water column • Especially abundant at lower latitudes consistent with their higher optimum growth temperature relative to HLI cells[31,33–35]
HLIII	HNLC1 (REFS 47,50), HNLC2‖(REF. 49)	None	• Sequences derived from this clade are found in high-nutrient but low-chlorophyll-containing equatorial waters • These regions are generally limited in iron, and it has been suggested that these cells have adapted to lower iron requirements[47–50]
HLIV	HNLC1 (REF. 49), HNLC2‖ (REFS 47,50)	None	• Sequences derived from this clade are found in high-nutrient but low-chlorophyll-containing equatorial waters • These regions are typically limited in iron, and it has been suggested that these cells have adapted to lower iron requirements[47–50]
HLV	NA	None	• Sequences derived from this clade are found in surface equatorial waters that are typically limited in iron • Physiological distinctions between the HLIII, HLIV and HLV clades are not known[47]
HLVI	NA	None	• Sequences derived from this clade are found in the middle or lower euphotic zone (75–150 m) of the South China Sea • Postulated to have an intermediate light optimum[47]
LLI	eNATL2A[29], high-B/A *Prochlorococcus* clade I (REF. 37)	NATL1A, NATL2A, PAC1	• Typically most abundant in the middle euphotic zone of stratified waters • Unlike other LL clades, they often remain abundant in mixed waters throughout the water column owing to their ability to tolerate light shock[35,37]
LLII/III§	eSS120/eMIT9211 (REF. 29), high-B/A *Prochlorococcus* clade II/III (REF. 37)	MIT9211, SS120	Usually found in the middle-lower euphotic zone[35,37,44]
LLIV	eMIT9313 (REF. 29), high-B/A *Prochlorococcus* clade IV (REF. 37)	MIT9303, MIT9313, MIT0701	Typically most abundant near the base of the euphotic zone; highly sensitive to light shock[35,37]
LLV	NA	None	Maximum abundance in the lower euphotic zone of oxygen minimum zones, where oxygen-depleted layers extend into the upper water column[56]
LLVI	NA	None	Maximum abundance in the lower euphotic zone of oxygen minimum zones, where oxygen-depleted layers extend into the upper water column[56]
LLVII	NC1 (REF. 36)	None	• Sequences derived from this clade are found in the lower euphotic zone of subtropical waters • Little is known about this clade[36]

NA, not applicable. *For more information on these and other strains, see[10,44,60]. ‡Refers to the type of environment in which this clade is most abundant and/or where it was isolated. §Originally defined as separate clades[37], the LLII and LLIII are now grouped because their separation is not well resolved phylogenetically. ‖Two publications[49,50] assigned the names HNLC1 and HNLC2 to different clades; in the future, we suggest the use of the HLIII and HLIV nomenclature to refer to these clades[47,48].

contains only a few thousand genes, the *Prochlorococcus* genus has a huge pan-genome[44,60,61]. All *Prochlorococcus* isolates that have been sequenced so far share ~1,000 genes (the 'core' genome), which make up about one-half of the average *Prochlorococcus* genome and often encode basic housekeeping functions[44]. The remaining genes, known as the 'flexible' genome, are found in only one or a few *Prochlorococcus* genomes and presumably contribute to the relative fitness of each distinct lineage within its local environment[44,62].

The *Prochlorococcus* genome can be understood, at least in part, through this lens of core and flexible gene content. In contrast to other cyanobacteria (such as *Microcystis aeruginosa*), in which repeat sequences are common and genes are added and lost at a similar rate throughout the genome[63], the flexible genes of *Prochlorococcus* tend to be clustered in hypervariable islands of the chromosome[44,62]. Such genomic islands have been observed in the metagenomes of wild *Prochlorococcus* populations[62,64] and are also found in *Synechococcus*[65,66]. Although gene loss has clearly played an important part in its evolution, gene gains have also occurred in all *Prochlorococcus* lineages; this is particularly evident in the LLIV clade[44] (see below). Genes gained by horizontal gene transfer (HGT) commonly occur in genomic islands, as deduced from gene occurrence patterns, homology to genes from other microorganisms and GC content.

The clustering of genomic hypervariability into genomic islands probably contributes to the maintenance of gene order in the core genome. Examination of available *Prochlorococcus* genome sequences[44,60] indicates that 45% of core genes are locally syntenic (meaning that the same genes are located immediately upstream

Pan-genome
The complete set of genes that is encoded by all the genomes of a defined group of organisms.

Box 2 | Nutrient acquisition genes as signatures of the local environment

Prochlorococcus strains vary in their ability to use different inorganic nutrient sources, and much of this physiological diversity is clearly reflected in their underlying genomic diversity[58]. Adaptations linked to the availability of phosphorus, nitrogen and trace metals do not follow the ribotype-defined phylogeny, as observed for light and temperature[36], and are better interpreted as signatures of the local environment in which a given strain is found[69]. Thus, much can be learned about the environment and the selective pressures experienced by a given *Prochlorococcus* bacterium from the composition of its genome and, in some cases, from the composition of the bacterium itself.

Phosphorous

Genomic and metagenomic analyses have revealed that *Prochlorococcus* populations in phosphorus-limited environments, as well as the cyanophages that infect them, contain more genes involved in phosphorus acquisition than populations from environments where phosphorus is more abundant[68,69,71,139,140]. *Prochlorococcus* genes involved in phosphite and phosphonate assimilation are also prevalent specifically in *Prochlorococcus* populations from phosphorus-limited environments[140–142]. Phosphorus-starvation experiments in the laboratory have shown that, in addition to known phosphorus-starvation response genes, several genes of unknown function, all of which are clustered in a hypervariable genomic island, are highly upregulated[62,68]. Unravelling the functions of these genes will shed light on the response of these bacteria to phosphorous stress.

Nitrogen

Productivity in many regions of the oligotrophic ocean is limited by nitrogen availability; indeed, the average amount of nitrogen in the *Prochlorococcus* proteome (as estimated on the basis of amino acid sequence) is reduced compared with that of coastal bacteria[143]. Nitrogen minimization is due, in part, to the low GC composition of *Prochlorococcus* genomes: the amino acids encoded by low GC codons have a lower nitrogen content (reduced N/C ratio) than those encoded by GC-rich codons[144]. Surface waters tend to be more nitrogen-limited than deeper waters; this correlates with the fact that high-light (HL)-adapted strains, which are typically most abundant near the surface, have a lower GC content — and thus require less nitrogen — than low-light (LL)-adapted strains[145]. Some strains have additional signatures of selection for nitrogen minimization in the particularly reduced nitrogen content of many nitrogen stress-responsive proteins[145].

Although all *Prochlorococcus* strains can use ammonium, and none can fix dinitrogen, they differ in their ability to assimilate other forms of nitrogen, including urea, cyanate, nitrite, nitrate and amino acids[53,106,126,146–149]. Genes for the uptake of nitrite, cyanate and amino acids seem to be subject to horizontal gene transfer (HGT), as indicated by their positioning in genomic islands of some strains[7,62]. Genomic analysis of recently identified nitrite and nitrate assimilation genes suggests that nitrate assimilation may have been maintained in distinct lineages of the LLI and HLII clades for some time[106,147]. Nevertheless, genes associated with nitrate assimilation also seem to be subject to HGT, as suggested by the discovery of common mobility elements surrounding the nitrate assimilation genes in one genome[106].

Iron

Because of its importance in photosynthetic reaction centres and its low concentration in ocean waters, iron availability seems to exert substantial selective pressure on *Prochlorococcus* niche differentiation. Cultured strains show large variations in their iron requirements; for example, the LLIV strain MIT9313 can grow at an iron concentration that is an order of magnitude lower than that required by the HLI strain MED4 (REF. 150). Cells from the uncultured HLIII and HLIV clades, which have been found in iron-limited regions, may have reduced their iron requirements by dispensing with several iron-containing proteins, including cytochrome c_m, two ferrodoxins and the plastoquinol terminal oxidase[49]. There is also evidence that these cells may use siderophores to increase iron acquisition[48]. The diversity in iron acquisition and the requirement for iron among different *Prochlorococcus* strains is consistent with the observation that many genes exhibiting differential expression during iron starvation have signatures of HGT[150].

and downstream in all isolates), compared with 30% in *Synechococcus*. In addition, the genomes of HL-adapted strains have maintained core genome synteny to a greater extent than those of LL-adapted strains. Although the loss of paralogous genes is more common in *Prochlorococcus* than in *Synechococcus*, selection pressure to remove duplicate genes seems to be lower among the larger LL-adapted genomes than the HL-adapted genomes[67].

Synteny
The conserved ordering of genes along a chromosome.

The* Prochlorococcus *pan-genome. The sequencing of each new *Prochlorococcus* genome adds, on average, 160 novel genes (~5–8% of the genome) to the *Prochlorococcus* pan-genome[44,60]. But what is the total number of different genes distributed throughout the global *Prochlorococcus* population? Genomic and metagenomic data show that at least 12 major clades exist (FIG. 2a), which contain more than 13,000 genes (FIG. 2c). These genes are thought to have important roles in tailoring *Prochlorococcus* physiology to its local environment. Consistent with this hypothesis, isolates that occupy similar habitats, irrespective of HL or LL status, frequently have similar sets of flexible genes, which are often associated with nutritional adaptations (BOX 2). This indicates that flexible genes are maintained under selection and are thus likely to contribute to fitness in these environments[68–71]; indeed, mutations in some flexible genes have been shown to decrease growth rate[72].

The *Prochlorococcus* pan-genome continues to grow as more strains are sequenced; in addition, it is dynamic, as genes are continually gained and lost from lineages. Theoretical projections based on 13 published genomes predicted that the global *Prochlorococcus* population contains 57,792 genes and 18 distinct clades[61]. Applying an updated version of this model[73] to 41 cultured *Prochlorococcus* genomes[44,60] increases the pan-genome size estimate to 84,872 genes, which is four times the size of the human genome. This analysis suggests that we have so far identified only a small fraction of the genes in the *Prochlorococcus* pan-genome.

Understanding the distribution of diversity among known clades can guide the search for 'missing' genes; for example, the genomes of LL-adapted strains contain, on average, more unique genes than HL-adapted strains (FIG. 2d). Specifically, of the genes found in any pair of LL-adapted strains, approximately 30% are unique to each genome (measured as in REF. 74), whereas for pairs of HL-adapted strains, only 13% of genes are unique. There is a correlation between ITS similarity and gene content similarity among *Prochlorococcus* genomes; however, LLIV strains have disproportionately more unique genes per genome than any other clade of cultured *Prochlorococcus* (FIG. 2d). Although the LL-adapted genomes are also the largest (FIG. 2b), these trends are independent of genome size. These data suggest that our knowledge of the *Prochlorococcus* pan-genome will expand through single-cell genomics, metagenomics and targeted isolation of LL-adapted cells.

But what is the cause of the increased gene content diversity among LL-adapted strains compared with HL-adapted strains? One possibility is that LL-adapted strains can acquire new genes via HGT at a higher rate than HL-adapted strains. Alternatively, this difference may reflect the selective pressures of stable and strong environmental gradients in deeper waters (the primary habitat of LL-adapted strains), which create additional

potential niche space to select for a greater diversity of novel functions. By contrast, a substantial fraction of HL-adapted cells is found in the more homogeneous environments of the well-mixed surface waters. The large population sizes of HL-adapted strains and their relatively high growth rates[9,14,27,75] combine to impose strong selective pressures[45] on the relatively few niche dimensions available in this habitat, driving the system towards small variations among closely related cells.

The *Prochlorococcus* pan-genome has provided many insights into the contribution of *Prochlorococcus* to ocean processes, but major gaps in gene annotations limit our ability to interpret these data. Although the metabolic functions of many core and some flexible genes are known, nearly 75% of the genes that are currently part of the *Prochlorococcus* pan-genome are of unknown function. In terms of understanding the biogeochemical role of *Prochlorococcus*, it is helpful that the pan-genome of the more abundant HL-adapted strains is better characterized than that of the LL-adapted cells. That said, genomes of LL-adapted strains contain a higher number of novel genes and therefore have the potential to provide clues about the functional capabilities and evolutionary history of *Prochlorococcus*. Although they are less abundant than their HL-adapted relatives, it seems evident that these populations have important ecological roles.

Fine-scale variation. In addition to differences in gene content, there is a layer of fine-scale sequence diversity that results in extensive allelic variation among bacteria. Even putatively 'clonal' *Prochlorococcus* strains can have hundreds of stably selected single nucleotide polymorphisms[45,60]. This raises the question of where the baseline of ecologically meaningful diversity lies; for example, what is the cell-to-cell diversity in a single water sample, and how does this change in response to environmental variability? Single-cell genomic analyses have recently shown that *Prochlorococcus* populations in the same milliliter of water comprise hundreds of distinct coexisting and stably maintained subpopulations[45]. Each subpopulation is associated with a unique 'genomic backbone' (a set of shared core alleles that is linked to a defined set of flexible genes) that seems to be shaped by selection. Such backbones contain alleles that define deeply rooted adaptations as well as genes that contribute to local environmental adaptations. Even when comparing cells that have identical ITS sequences, extensive allelic and gene content diversity is observed, which seems to contribute to ecological differentiation. Population structure, as defined by genomic backbone composition, can vary over seasonal timescales but seems to reflect ancient and stable niche partitioning, implying that this structuring of microdiversity contributes to the resilience of *Prochlorococcus*[45].

What are the mechanisms that have generated and shaped the observed variation? Although *Prochlorococcus* cells are exposed to potentially high amounts of ultraviolet radiation and lack several key DNA repair enzymes[76], the mutation rate of *Prochlorococcus* is similar to that of *E. coli* (on the order of 10^{-7} mutations per gene per generation)[77]. Thus, sequence diversity is not simply due to a high mutation rate and probably reflects the impact of the selective pressures that are imposed by the many different environments, at both the microscale and macroscale, that this genus is exposed to. From a population genetics perspective, *Prochlorococcus* has a massive effective population size that is estimated to be between 10^{11} and 10^{13} cells[45,61], which is at least four orders of magnitude larger than that estimated for *E. coli*[61] and is probably among the largest on the planet[45]. Despite the small genome size and typical bacterial mutation rate, the population size alone should minimize the impact of genetic drift and provide extensive genetic variation for selection to act on[45,59,78], thus leading to selection for minute fitness differences between strains[45].

The remarkable amount of stably maintained, coexisting genomic diversity in *Prochlorococcus* populations cannot easily be explained by classic ecotype models, in which adaptive mutations are predicted to lead to whole-genome selective sweeps, resulting in a homogenous genomic population structure[79]. Selective pressures from predators (particularly phages; see below) probably play an important part in maintaining this diversity, as predicted by models incorporating density-dependent fluctuating selection such as the 'kill-the-winner' and 'constant diversity' hypotheses[80]. They are based on the idea that, as a microbial lineage increases in abundance, so will the predation pressures that act on it. Thus, predation would have a disproportionately larger effect on the dominant lineage, ultimately resulting in a population of diverse genotypes with different susceptibilities to the predator. This model seems to be consistent with the observation of diverse genomic backbone lineages within *Prochlorococcus*; in some instances, genomic backbones link alleles of genes known to influence predation together with those affecting other physiological adaptations[45,72]. However, not all genes affecting predation are necessarily associated with a backbone lineage. Many such alleles are found in hyperdiverse genomic islands[72] and may recombine at a relatively high rate; if so, they would become unlinked from the backbone, and selection against these alleles would not explain variation across the genome[81]. Thus, it is likely that predator-mediated fluctuating selection explains only part of the story. The maintenance of diversity within *Prochlorococcus* populations must depend on the complex and poorly understood interplay of many forces including predation, recombination, selection, population structure and environmental complexity.

A federation of diverse cells. *Prochlorococcus* can be viewed as a federation of coexisting cells: a large collection of many groups, each of which exhibits different adaptations to specific environmental variables and represents combinatorial arrangements of alleles that reflect important niche dimensions. In turn, each of these groups contains subgroups with adaptations to slightly different selective pressures, ultimately filling out the total niche space that is occupied by *Prochlorococcus* (FIG. 3a). The immense diversity of *Prochlorococcus*, and particularly the combinatorial nature of this diversity,

Paralogous genes
A pair of similar genes that were created by a duplication event.

Effective population size
In population genetics, the size of an idealized population that would be expected to behave in the same manner as the actual population in terms of the effects of selection and genetic drift.

Genetic drift
The change in the frequency of an allele in a population due to chance or random events.

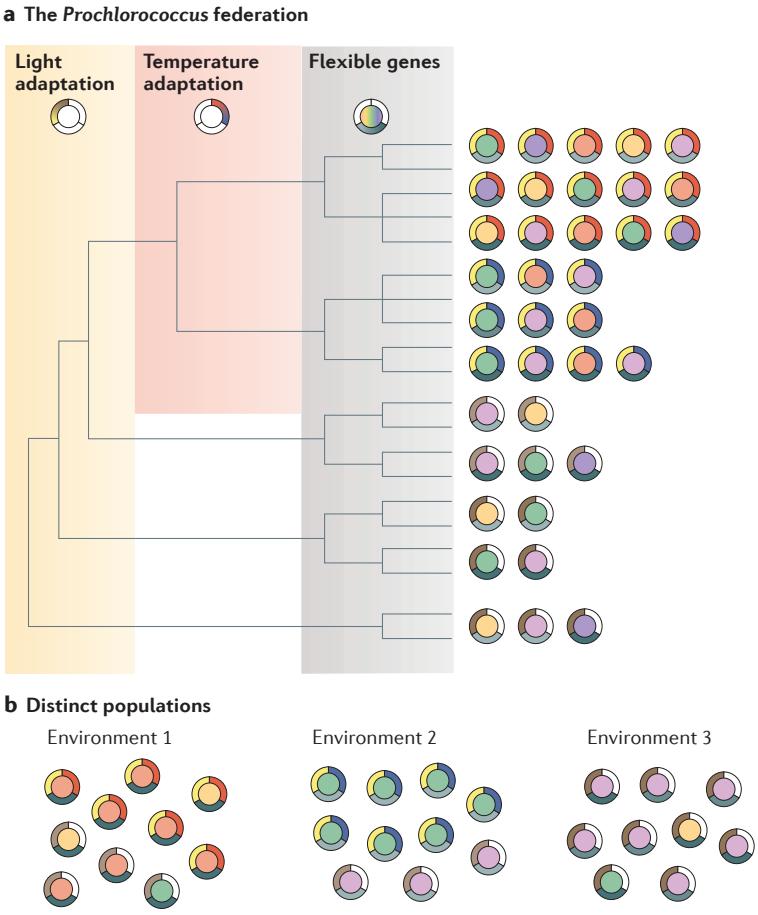


Figure 3 | **The *Prochlorococcus* federation. a** | The *Prochlorococcus* federation is composed of groups of cells, each representing different combinatorial arrangements of genes that are required for adaptation to their distinct ecological niches. Each circle represents an individual *Prochlorococcus* bacterium or clonal lineage. The outer coloured ring represents the genomic backbone of the bacterium (which contains both core and flexible genes) and the inner circle represents a unique set of flexible gene content. The genomic backbone consists of alleles that determine adaptation to basic, deeply divergent traits such as light and temperature optima for growth, together with a subset of flexible genes that contribute to niche adaptation[45]. The composition of the backbone generally correlates with whole-genome phylogeny, but the flexible gene content varies markedly according to the local environment. **b** | The diversity found within the federation contributes to the stability and resilience of global *Prochlorococcus* populations by providing an extensive pool of diverse traits that different environmental conditions can select for.

results in niche partitioning and the robustness of populations across time and space. Different environmental conditions will select for different combinations of adaptive alleles encoded by some members of the federation, thus changing the relative abundance of different subpopulations but ensuring that the overall *Prochlorococcus* meta-population remains stable (FIG. 3b). Thus, it could be argued[82] that the entire *Prochlorococcus* federation, which comprises cells with a backbone of core and flexible genes, might function as its own selectable unit in microbial communities.

Interactions with phages and heterotrophs

The diversity of the *Prochlorococcus* federation can only be understood in the context of the surrounding microbial community. In this section, we focus on our understanding of the interactions between *Prochlorococcus* and the cyanophages and abundant heterotrophic bacteria with which it has co-evolved, and discuss how these interactions contribute to *Prochlorococcus* physiology and diversity.

Phages as a vehicle for* Prochlorococcus *genome diversification. Cyanophages that infect *Prochlorococcus* are lytic double-stranded DNA tailed phages that belong to the T4, T7 and lambdoid groups[83–87], and they are suggested to represent a notable fraction of the total viral population in some parts of the ocean[88]. Lysogenic phages have not been found in *Prochlorococcus* genomes, even though phage integrases are present (see below) and a partial phage sequence has been detected in a partial single-cell genome[48]. The apparent absence of lysogens may be related to genomic streamlining, which could lead to the rapid loss of prophages from *Prochlorococcus* genomes.

Cyanophages have played an integral part in the evolution and diversification of *Prochlorococcus* genomes. Several lines of evidence suggest that phage-mediated HGT is important for gaining flexible genes in genomic islands (FIG. 4a). For example, tRNA genes, which are common sites for the integration of phages[89], often flank genomic islands in *Prochlorococcus*[62]. In addition, several genes, including those encoding integrases, DNA methylases and stress-response proteins, are found in both genomic islands and cyanophage genomes[62]. The upregulation of several of these genes during phage infection has led to the hypothesis that host genes expressed during infection have been stably incorporated into phage genomes, which increases their opportunity for transfer back to *Prochlorococcus*[90]. A striking example of this is the expansion of the *hli* gene family of stress-response genes in *Prochlorococcus,* which was most probably mediated by phages[62,90,91]. The influence of phages on gene sequence diversity is not limited to the flexible genome. Intragenic recombination between core photosynthesis genes that are shared by both *Prochlorococcus* and their phages seems to accelerate the diversification of the genes that encode proteins involved in this key metabolic process[92,93]. This could be a general phenomenon that affects genes that are found both in *Prochlorococcus* and in their phages.

Prochlorococcus and almost all marine *Synechococcus* lack CRISPR–Cas (clustered, regularly interspaced short palindromic repeats–CRISPR-associated proteins) systems for phage resistance[94], perhaps because it is too costly to maintain these genes or because CRISPR–Cas systems may be ineffective given the high level of phage diversity that is predicted for cyanophages[95]. In addition, most *Prochlorococcus* and *Synechococcus* strains lack restriction–modification systems, which suggests that restriction is also not a widespread mechanism of defence against cyanophages in these groups[94]. Instead, resistance to cyanophages in *Prochlorococcus* is typically conferred by mutations in genes that encode cell surface molecules, which impair phage attachment to the cell surface[72]. These genes are part of the flexible genome, have

Cyanophages
Phages that infect cyanobacteria.

Lysogenic phages
Bacteriophages that are capable of integrating their genome into the host genome and are replicated along with the cell, without killing it.

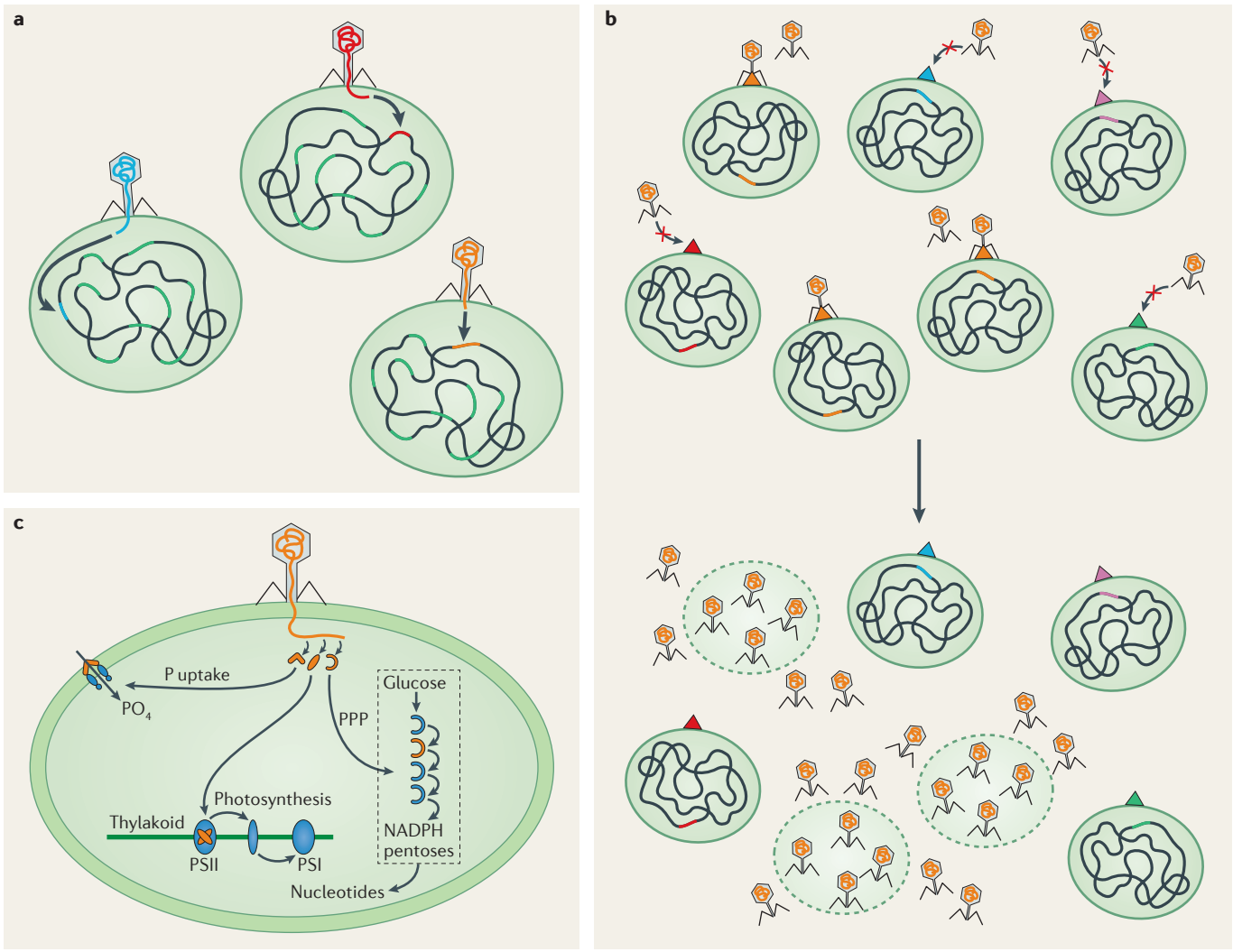


Figure 4 | **The influence of phage predation on *Prochlorococcus* gene content, population diversity and physiology.** **a** | *Prochlorococcus* bacteria have a common core genome (shown in dark grey) that encodes basic housekeeping functions, in addition to a diverse set of flexible genes that are primarily located on genomic islands (other colours of the genome), which seem to be under strong selection pressure. Phages alter the genetic content of these islands by transferring genetic material (which may have been acquired from another bacterium; indicated by coloured DNA injected by the phage) from the phage into the infected bacterium. **b** | To infect and subsequently lyse a bacterium (as indicated by the dashed outlines of some cells), phages attach to cell surface molecules, which results in positive selection for bacteria with surface molecules that are not recognized by abundant phages in the surrounding environment. Thus, predation by phage can prevent any one genotype with a particular set of cell surface molecules from becoming dominant within a population, which promotes population diversity. Genes encoding cell surface proteins are often found in genomic islands. Selection for diverse genes encoding cell surface proteins together with horizontal gene transfer of genes into genomic islands leads to a large *Prochlorococcus* population with a common core genome but an assortment of subpopulations with different flexible genomes[72,80]. **c** | Some cyanophage proteins can participate in cellular metabolic processes together with host proteins. These host–phage hybrid complexes presumably facilitate the acquisition of energy and materials needed for DNA synthesis and the production of large numbers of phage progeny. The host processes that seem to be temporarily boosted by phage genes (on the basis of gene expression analysis) include photosynthesis for energy production, transport of phosphate (PO_4) for nucleotide biosynthesis and pentose phosphate pathway (PPP) proteins for the generation of reducing power and nucleotide precursors. Proteins derived from the host genome are shown in blue and phage-encoded proteins are shown in orange. P, phosphate; PSI, photosystem I; PSII, photosystem II.

been laterally acquired from other bacterial phyla, are located in genomic islands and account for the greatest genomic differences between closely related *Prochlorococcus* strains[44,62,72]. This strongly suggests that selection pressure from phages, and potentially from grazers (see below), influences both sequence diversification and the presence of genes encoding cell surface molecules and their biosynthesis (FIG. 4b). Furthermore, phage selection

presumably leads to the loss of such genes and the gain of non-orthologous genes with equivalent functionality to compensate for such losses[72].

Mutations that confer phage resistance often have a fitness cost, which manifests as either a reduction in growth rate or an enhanced susceptibility to other phages[72]. For example, the mutation of cell surface molecules provides resistance to a subset of phages but can also confer enhanced susceptibility to other phages. In this way, phages contribute to the diversity and population structure of *Prochlorococcus*: each population is composed of an assortment of subpopulations that differ in their susceptibility to the range of phages found in the oceans[72,96] (FIG. 4b). This variability probably leads to density-dependent fluctuations in the abundance of host and phage subpopulations, which prevents a high degree of infection at the population level and thus facilitates stable coexistence of *Prochlorococcus* spp. and their phages[72,80,96]. These host–phage dynamics suggest that phages may have a limited ability to control the size of *Prochlorococcus* populations but have a strong influence on population structure and diversification.

Phage influences on host metabolism. Most cyanophage genomes encode auxiliary metabolic genes, many of which have been acquired from their cyanobacterial hosts[84,86,91,97,98]. These host-like genes are often found in islands on the phage genome[86,87,98] and probably influence the infection process (FIG. 4c). For example, although phage infection disrupts *Prochlorococcus* gene expression[90], key metabolic processes such as photosynthesis are sustained, in part because cyanophages encode and express homologues of key genes in photosynthetic pathways[91,97,99]. In addition, phage genes encoding proteins that inhibit the Calvin cycle or that are involved in the pentose phosphate pathway are transcribed together with genes involved in photosynthesis, DNA replication and metabolism[70,90]. This suggests that phages direct the energy derived from photosynthesis away from carbon fixation and towards the pentose phosphate pathway to produce pentoses and reducing power for nucleotide biosynthesis. Indeed, the ratio of NADPH to NADP is higher in phage-infected *Prochlorococcus* cells than in non-infected cells[70]. Similarly, host-derived phosphate-acquisition genes are transcribed from phage genomes during infection, are upregulated during infection of phosphate-deprived host cells and are even regulated in the phage by the phosphate two-component regulatory system of the host[100].

The role of heterotrophs. *Prochlorococcus* has a central role in supplying photosynthetically fixed carbon to the marine heterotrophs with which it coexists. For example, members of the abundant SAR11 clade require glycine or serine for growth, a requirement that can also be fulfilled by their metabolic precursor glycolate[101], which *Prochlorococcus* releases in substantial amounts[102]. The heterotrophic community, in turn, influences the fitness of *Prochlorococcus*, as evidenced by the difficulty of removing heterotrophic 'contaminants' from *Prochlorococcus* cultures in the early days of its cultivation[103]. Since then, axenic strains have been generated by various approaches[104–107], which have enabled the systematic study of interactions between *Prochlorococcus* and co-cultured heterotrophs. The presence of some heterotrophic bacteria can increase the growth rate of *Prochlorococcus*, the final culture density and the longevity of cultures, whereas other heterotrophs have inhibitory or neutral effects on growth[107,108]. Although the mechanisms underlying the inhibitory interactions are not understood, some insights into the beneficial interactions have emerged.

An elegant set of laboratory and field experiments has shown that *Prochlorococcus* grows better in the presence of some heterotrophs because they reduce the concentrations of toxic reactive oxygen species (ROS; such as hydrogen peroxide), which compensates for the absence of genes encoding catalase and peroxiredoxin in *Prochlorococcus*[107,109]. This led to the 'Black Queen' hypothesis[110], which posits that free-living microbial communities evolve and sustain a division of labour for certain essential functions. In this scenario, a subset of cells carries out an essential function that becomes a 'public good', which enables non-producing cells to benefit from this activity and to avoid the cost of carrying it out themselves. Because there is strong selective pressure on all cells to avoid damage by ROS, cells that dispense with the costly expression of defence mechanisms have an advantage as long as they can rely on other cells for protection. In this case, *Prochlorococcus* does not produce catalase but is protected from ROS by nearby heterotrophs[110].

Prochlorococcus undoubtedly affects heterotrophs in other ways; for example, certain strains produce a remarkable diversity of lanthipeptide secondary metabolites[55]. Although their function in *Prochlorococcus* is unknown, similar compounds have functions ranging from antibiotics to surfactants, which could affect the heterotrophic community. [111]. In addition, *Prochlorococcus* continually releases small (~100 nm diameter) extracellular membrane vesicles[112] that contain a wide range of components, including lipids, proteins and small fragments of DNA and RNA. Although the ecological function of these vesicles is currently unknown, they might function as vehicles for the movement of carbon through marine food webs, as vectors for HGT or possibly as decoys for predators and phages.

Distributing the genome through a community. The concept of the pan-genome is based on the idea that the total genetic repertoire of a bacterial group is greater than the number of genes encoded in any single strain[113]. Considering the Black Queen hypothesis and the impact of phage-encoded homologues of bacterial proteins on cellular physiology, should the pan-genome of *Prochlorococcus* be broadened to include heterotroph-encoded genes that supply essential functions for *Prochlorococcus* survival? Should it also include phage-encoded genes that function in the host? In keeping with the view that entire microbial communities are relevant units of biological organization[114], certain heterotrophs and phages could be part of the same selectable unit as *Prochlorococcus*[82], which would strengthen arguments

Calvin cycle
The biochemical process that converts CO_2 into organic carbon.

Reducing power
In redox chemistry, the availability of compounds that can supply electrons.

Axenic
A term used to describe a pure culture of a single organism that is free of any other contaminating organism.

Reactive oxygen species
(ROS). Oxygen-containing compounds, such as H_2O_2, that readily react with and damage cellular components.

Extracellular membrane vesicles
Small (~20–200 nm diameter) spherical structures enclosed by a lipid bilayer. In Gram-negative cells, they are thought to be derived from the outer membrane.

for their inclusion in the same pan-genome. Although it is not clear where to draw boundaries to define the complete metabolic repertoire of one organism, co-evolutionary selective pressures undoubtedly lead to some tight associations. Identifying discontinuities in the network of interactions could help to reveal these associations and expand the *Prochlorococcus* pan-genome.

Prochlorococcus and ocean carbon cycling

Prochlorococcus is an important global primary producer, especially in the oligotrophic ocean, where dissolved organic carbon from this group contributes up to 40% of total bacterial production[102]. *Prochlorococcus* releases a diverse range of organic molecules into the surrounding seawater[115] using many different mechanisms. These include direct secretion (sometimes termed 'leakage') from the cell[102,112] and cell lysis, which is mediated either by phages or by grazers[116]. *Prochlorococcus* also directly supports the carbon and nutrient requirements of other trophic levels as it is prey to a wide range of eukaryotic predators, including tunicates[117,118], ciliates[119,120], flagellates[120,121], prymnesiophytes[122], stramenopiles and dinoflagellates[123]. It is possible that mixotrophic eukaryotes that have primarily autotrophic lifestyles feed on *Prochlorococcus* as a source of nitrogen and phosphorus. As acquisition of these essential elements is limited by diffusion in large cells, direct transport may be insufficient to support their nutrient requirements, whereas engulfing concentrated 'packets' of nutrients in the form of small cells such as *Prochlorococcus*[123,124] may provide an advantage in hyper-oligotrophic environments.

Much of the carbon that is fixed by *Prochlorococcus* in the euphotic zone is thought to be recycled in the upper waters through the microbial loop: it is taken up by heterotrophic bacteria and is either respired or incorporated into other compounds that move up the food web[116]. However, it has also been reported that *Prochlorococcus*-derived carbon is exported to deep waters by aggregation and sinking of biomass following trophic processing[125]. For example, degradation products of the unique *Prochlorococcus* divinyl chlorophyll *a* have been found in the faecal matter of salps recovered from deep waters[117]. The degree to which *Prochlorococcus* participates in this biological pumping of carbon from the atmosphere to the deep ocean is an open question.

Our understanding of the role of *Prochlorococcus* in marine carbon cycles has been complicated by a growing body of evidence suggesting that mixotrophy occurs in both cultured and wild *Prochlorococcus* populations. *Prochlorococcus* can import organic compounds for use as either nitrogen, phosphorus, energy or carbon sources. High uptake rates of amino acids, including both methionine and leucine, have been observed in wild *Prochlorococcus* populations[126–129], which are capable of assimilating nucleic acids, possibly functioning as a nitrogen source[127]. In addition, studies of both cultured and wild *Prochlorococcus* have shown that it can take up glucose[130,131]. This is particularly intriguing as glucose lacks both nitrogen and phosphorus; thus, it could only be used as a source of carbon or energy.

Prochlorococcus in a warming world

Advancing our understanding of the ecology and physiology of *Prochlorococcus* is particularly important in the face of global climate change. The rise in surface water temperatures and the expansion of ocean stratification will almost certainly affect the structure and function of bacterial populations. For example, models predict that in a world with ~650 ppm atmospheric CO_2, the global abundance of *Prochlorococcus* may increase by more than 25% and expand towards the poles as the waters increase in temperature[3]. The complexity of these scenarios in terms of the distribution of ecotypes is daunting, but hypothetical scenarios can be illuminating. An expansion of stratified waters will decrease nutrient input from the deep waters, making these regions more oligotrophic, which will almost certainly change the local ecotype distributions. We expect that members of the HLII clade, which are currently the most abundant group and have the highest optimum temperature for growth, will expand their habitat into higher latitudes. By contrast, the relative abundance of groups that have lower temperature optima would shift away from the equator.

As we consider such scenarios, it is important to recognize that selection for genome-wide adaptations, such as temperature optima, will simultaneously select for linked traits that are encoded by the same genomic backbone, such as specific nutrient assimilation capabilities. As *Prochlorococcus* biomass is a substantial fraction of total photosynthetic biomass, its biogeochemical contributions would then feed back to the environment and shift selection pressures in the entire ecosystem. Thus, *Prochlorococcus* subpopulations with different physiological abilities will arrive in different regions of the oceans as a result of a complex set of feedback loops. Although climate change may increase the abundance of *Prochlorococcus* worldwide, we cannot predict how the complicated relationships between the cell, its community and the environment will eventually play out; the system is simply too complex.

Future challenges

The pace of discovery of *Prochlorococcus* ecology and evolution increased considerably after the first genomes became available and continues to increase as a result of improvements in the technologies available for DNA sequencing. In the next decade, we should get closer to describing the global pan-genome of *Prochlorococcus* and the distribution of its genes among different regions and along vast oceanic gradients. Interpreting these data in an integrated physiological and ecological context is an enormous challenge that will ultimately require us to unravel the function of the large number of unannotated genes in microbial genomes. Deciphering the roles of genes of unknown function that are unique to *Prochlorococcus* is particularly important for illuminating the role of this group within the ocean ecosystem. Advances in this area will require the development of an efficient genetic system for *Prochlorococcus,* which has so far proven to be a challenge.

Full exploitation of the information from metagenomic, metatranscriptomic and single-cell genomic data

Mixotrophic
A term used to describe an organism that can use multiple metabolic modes for acquiring energy or carbon for growth. In the context of this Review, this refers to organisms that can use both CO_2 (autotrophy) and organic carbon (heterotrophy).

Autotrophic
A term used to describe an organism that can build complex, energy-containing organic molecules from CO_2 using either light or inorganic chemical reactions as an energy source. *Prochlorococcus* is capable of photoautotrophic growth and uses light energy to turn CO_2 into organic carbon via photosynthesis.

Microbial loop
The network of interactions among microorganisms at the base of the marine food web through which carbon and other nutrients move before they are supplied to larger organisms.

Salps
Marine tunicates that consume plankton by filter feeding.

Ocean stratification
The division of the water column into low-density and high-density zones, with a boundary layer (the pycnocline) defined by a gradient of densities across which water will not passively mix. Changes in density that lead to stratification are typically due to differences in temperature and salinity.

from field studies relies heavily on reference genomes and physiological studies of cultured strains. Continued efforts to obtain new isolates of *Prochlorococcus* from diverse regions of the ocean — along with the abundant oligotrophic and heterotrophic bacteria and phages with which it coexists — will be important in this regard. These cultures are essential for testing hypotheses about the forces that shape these co-evolved genomes and the global biogeochemical influence of *Prochlorococcus* and its metabolic partners. Finally, although we know by inference that the death rates of *Prochlorococcus* are high in the wild, our understanding of the impact of viral infection, predation and spontaneous cell death on these populations is in its infancy; there is much yet to learn!

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Competing interests statement

The authors declare no competing interests.