

MiniReview

Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*

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Abstract

Oxygenic photoautotrophs of the genera *Synechococcus* and *Prochlorococcus* contribute significantly to primary production and are now widely accepted as the most abundant members of the picophytoplankton in the world's oceans. Since they represent one of the few cultured and representative groups of marine microorganisms, study of their physiology and biochemistry has progressed rapidly since their discovery. The recent and on-going sequencing of the complete genomes of representative strains will further hasten our understanding, and allow a complete interrogation, of the metabolism of these organisms. Moreover, since they inhabit a relatively simple environment they provide an excellent model system to begin to identify the underlying molecular mechanisms which allow their success in water columns with large vertical gradients of light and nutrients. Such work should provide novel insights into the genetic adaptations of these important marine microbes to their environment. We review here molecular ecological methods that are already available or which are currently being developed for these organisms. Such methods allow community structure, growth rate and nutrient status analysis, potentially at the single cell level, and can be used to define the niches, or identify the biotic or abiotic factors, which might control the productivity of specific genotypes. These techniques will undoubtedly provide the tools for answering more discerning questions concerning their ecology. How the complete genome sequence information is providing insights, and can further facilitate our understanding, of the ecology of these organisms is also discussed. © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

1. Introduction

The discovery of tiny, single-celled cyanobacteria as ubiquitous and abundant components of the marine microbiota has radically changed our view of the functioning and composition of marine ecosystems. It is now clear that the two genera *Prochlorococcus* and *Synechococcus* dominate the photoautotrophic picoplankton over vast tracts of the world's oceans where they occupy a key position at the base of the marine food web and contribute significantly to global primary productivity [1,2].

Although they often co-occur, they exhibit different spatial distributions on a worldwide and a local scale. *Synechococcus* are distributed ubiquitously throughout oceanic regions, ranging from polar through temperate to tropical waters and are generally more abundant in nutrient-rich

surface waters, whilst *Prochlorococcus* are largely confined to a 40°N–40°S latitudinal band, being generally absent from brackish or well-mixed waters. *Prochlorococcus* also generally extend deeper in the water column than *Synechococcus* [3].

Synechococcus was first detected earlier than *Prochlorococcus* by virtue of their intense orange phycoerythrin fluorescence [4], and it was only in the late 1980s that sensitive flow cytometers were able to detect the dim red fluorescence emitted by the unique divinyl derivatives of chlorophyll *a* and *b* (chl *a*₂ and chl *b*₂) of *Prochlorococcus* [5,6]. Since their discovery, several studies of cultures and natural populations of *Prochlorococcus* and *Synechococcus* have indicated that these populations could be both genetically and physiologically diverse. A wide variety of molecular approaches have subsequently been developed to (i) better understand this diversity, (ii) to define the niches occupied by specific populations and (iii) to define how populations, and indeed single cells, are affected spatially and temporally by biological and physical factors of the ocean environment.

We focus here then on reviewing recent progress in the

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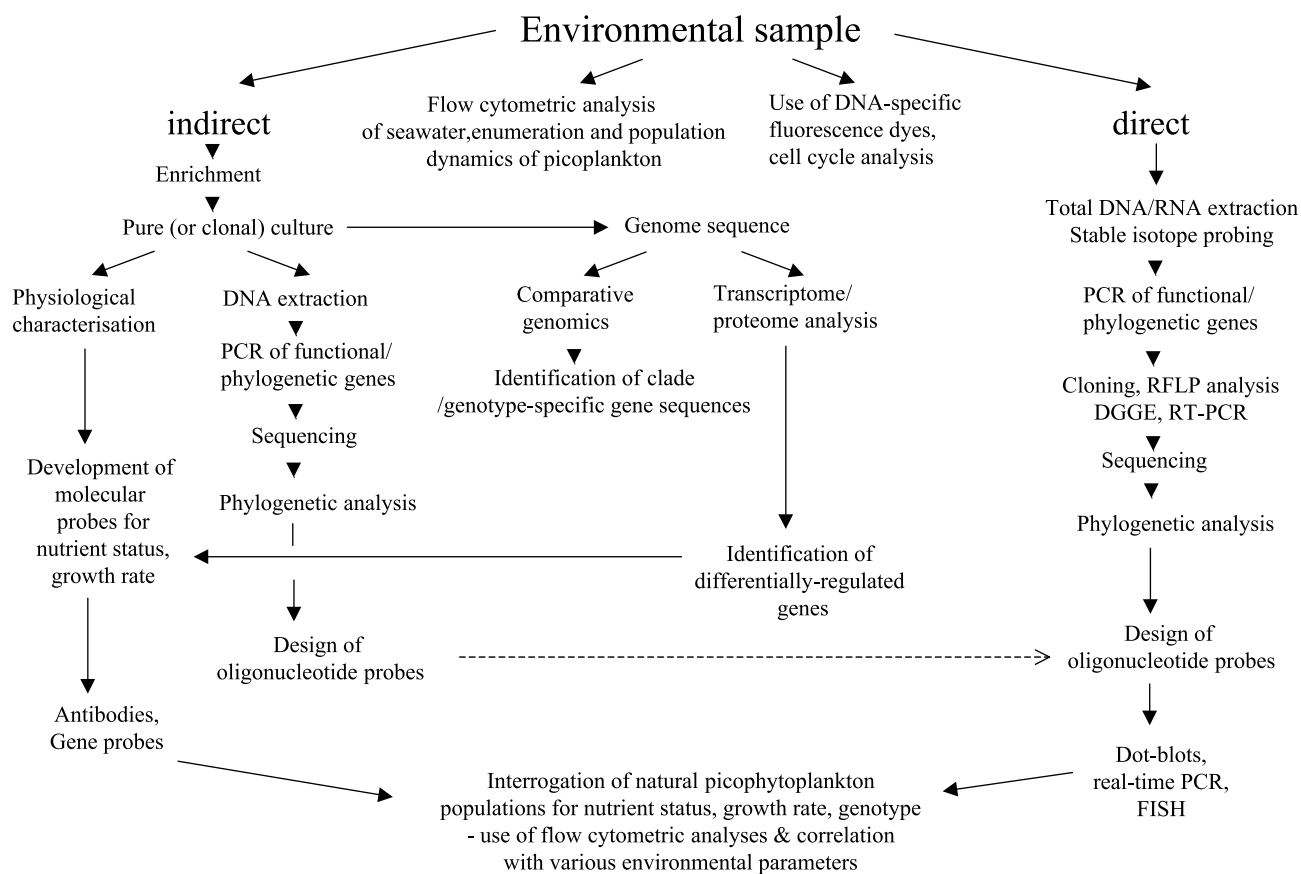


Fig. 1. Strategies for the development of molecular probes for use in interrogating natural picophytoplankton populations, a coupled molecular and ecological analysis of cultures.

development and use of molecular ecological tools, and including recent genomic information (see http://www.jgi.doe.gov/JGI_microbial/html/index.html), that will allow further insights into the ecology of the marine *Prochlorococcus* and *Synechococcus* genera to be made. For more extensive literature on the ecology, biology and physiology of *Synechococcus* and *Prochlorococcus* readers should refer to earlier comprehensive reviews [1–3,7,8].

2. The tools of molecular ecology: whole community analysis and culture studies

We use the term molecular ecology as a general one to denote the use of molecular biological techniques to address issues of ecology, i.e. the interrelationships between the organism and its environment. With respect to marine cyanobacterial molecular ecology such techniques have largely been used following two contrasting pathways, being either direct or indirect approaches to addressing ecological questions (Fig. 1).

The ability to routinely isolate and maintain in culture marine bacteria is relatively rare and so the relative ease with which both *Synechococcus* and *Prochlorococcus* strains can be isolated has not only allowed development of phylogenetic probes but also those molecular markers

where precise physiological information was needed (e.g. see [9]). We describe in detail now the recent progress that has been made elucidating marine cyanobacterial diversity taking culture studies and natural populations in turn. We focus on diversity studies since these underpin our knowledge of the oceanic distribution of these organisms, whilst they also introduce the subtleties of their physiology, specifically with regard to adaptation to their environment.

3. *Synechococcus* and *Prochlorococcus* genetic and physiological diversity

3.1. Culture studies

With both culture studies and environmental analyses several different gene loci have been used for marine cyanobacterial genetic diversity analysis which can make direct comparison of in situ population structure data difficult. Recent work in our laboratory has attempted to obviate this problem by direct comparison of several genes in the same strain, a so-called multi-locus approach, which suggests relatively good congruence phylogenetically using different genetic markers [10]. The most well-studied loci have been the SSU 16S rRNA, *rpoC1* encoding a subunit of the DNA-dependent RNA polymerase, the *petB–D* in-

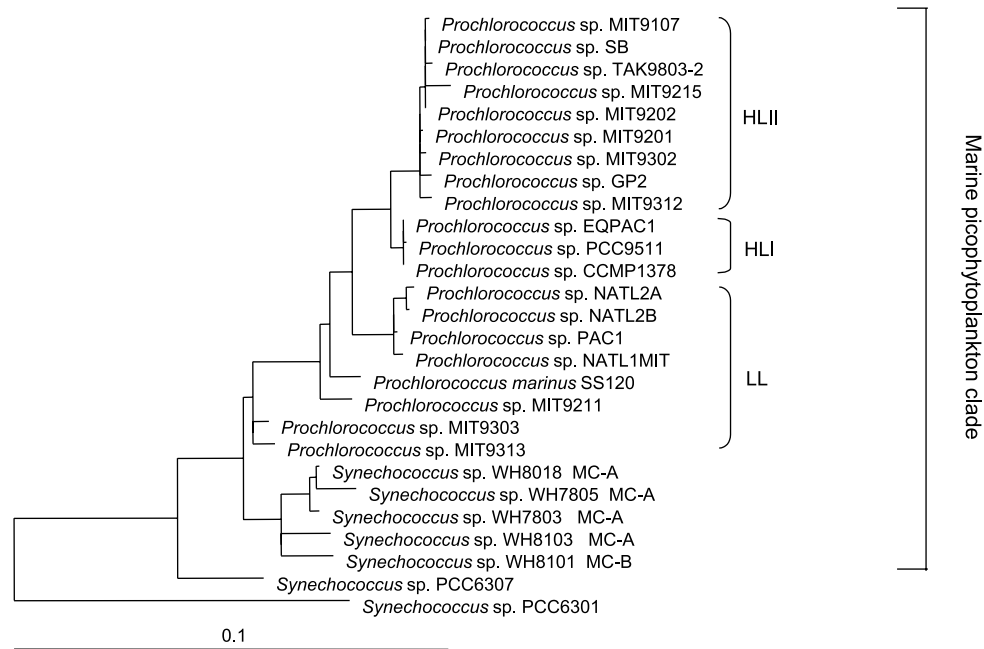


Fig. 2. Consensus tree showing the phylogenetic relationships amongst *Prochlorococcus* sp. and *Synechococcus* sp. inferred from 16S rDNA gene sequences. The initial analysis was based on 1364 positions, and shorter sequences were subsequently added by the maximum-parsimony method. The scale bar represents 10% estimated sequence divergence. Redrawn from [34] with permission.

tergenic spacer which includes partial coding sequences for cytochrome *b* and subunit IV of the photosynthetic *b₆f* complex, and *rbcL*. Using such genetic markers and from various physiological studies some general comments regarding 'ecotypic diversity' in the two genera can be made, although it should be emphasised that we are still far from obtaining a complete understanding of the genetic diversity within marine cyanobacterial populations.

Marine *Synechococcus* isolates likely comprise a complex taxon and have been classified by their major light-harvesting accessory pigment profiles, growth requirements, and their ability to carry out swimming motility, hence the term likely includes several species [1,11–13]. Thus, both halotolerant strains that possess phycocyanin but lack phycoerythrin and appear confined to coastal waters (marine cluster B (MC-B) mol% G+C=63–69.5), and phycoerythrin-containing strains have been described. It is the latter that have an elevated salt (Na^+ , Cl^- , Mg^{2+} and Ca^{2+}) requirement for growth and that occur abundantly within the euphotic zone of both open-ocean and coastal waters, the so-called marine cluster A (MC-A) group (mol% G+C=55–62). A further cluster, marine cluster C (MC-C) has been distinguished by its low % G+C (47.5–49.5) containing strains from brackish or coastal marine waters [14]. These latter environments have been relatively poorly studied so far and are likely under-represented in cultured *Synechococcus* isolates and hence within phylogenetic trees. For the basis of this review, however, we include only MC-A and MC-B strains since not only do most cultured isolates fall within these clusters but they also appear to form a coherent phyloge-

netic group together with *Prochlorococcus* (see Fig. 2) representing a single marine picophytoplankton clade [11].

Studies so far have focused on relatively few oceanic regions with perhaps most information being obtained from the California Current off the coast of California. Using *rpoC1* sequence data of isolated strains, seven or more genetically distinct groups of marine *Synechococcus* could be identified (Fig. 3) [15,16]. This correlates well with recent work using the internal transcribed spacer of the ribosomal operon and 16S rRNA gene sequencing of cultured isolates from various ocean provinces (Rocap et al., submitted for publication; N. Fuller and D. Scanlan, unpublished data), as well as earlier restriction fragment length polymorphism analysis using various gene sequences [17] which all suggest that a relatively large genetic diversity exists within the marine *Synechococcus* group.

So, does this genetic diversity correlate with specific physiological adaptation in defined lineages, and hence occupancy of a specific environmental niche? We are only part way to providing any clear answer to this question. Thus, it is well known that the MC-A *Synechococcus* group is additionally diverse in that ratios of phycoerythrin (PUB) to phycoerythrobilin (PEB) chromophores differ among phycoerythrins of different strains [18]. However, it was long thought that strains did not alter their PUB/PEB ratio in response to light quality. We now know though, that indeed some strains are capable of chromatic adaptation, increasing their PUB/PEB chromophore ratio when growing under blue light [15]. This latter trait seems to present phylogenetic coherence amongst those non-motile strains so far studied but not within motile strains where

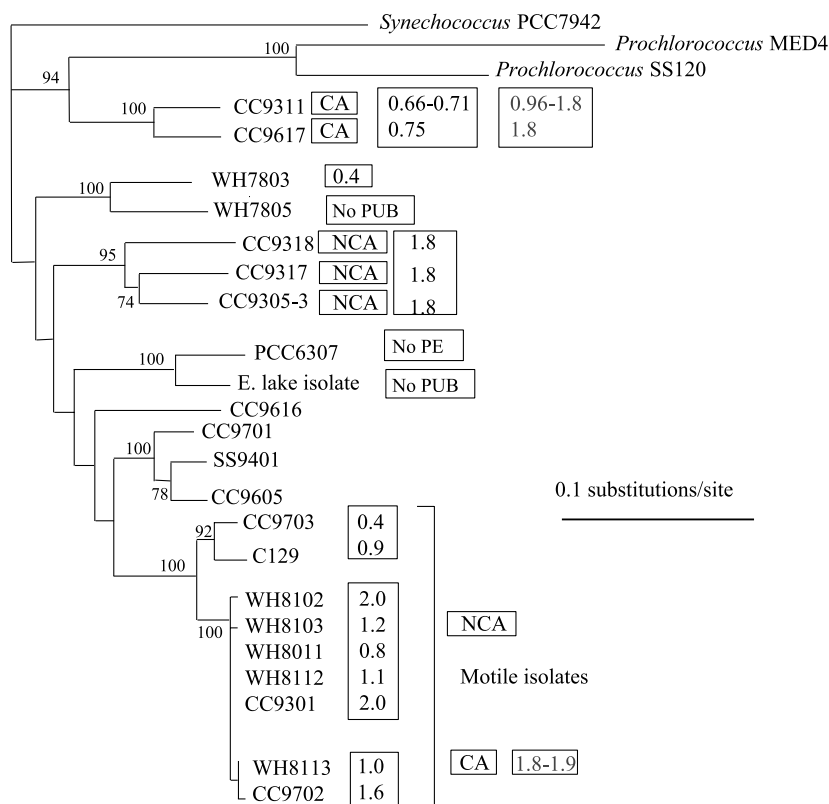


Fig. 3. Neighbour-joining tree constructed with Jukes–Cantor corrected distances by using a 612-bp fragment of the DNA-dependent RNA polymerase (*rpoC1*) gene sequence of different *Synechococcus*-like isolates. Bootstrap values based on 100 replicates are shown at each node. Scale bar = 0.1 substitutions per site. The values in boxes are PUB/PEB fluorescence ratios in white light (dark grey) or blue light relative to white light conditions (pale grey). NCA indicates no chromatic adaptation. CA indicates chromatic adaptation. Redrawn from [13] with permission and including information from [15].

both adapters and non-adapters exist (Fig. 3). It is possible that nutrient conditions as well as light intensity, rather than quality, effects may act as triggers of PUB/PEB acclimation in these motile strains and further work is required to clarify this.

The only physiological trait so far that demonstrates true phylogenetic coherence is the non-flagellar swimming motility phenotype which comprises strains forming a monophyletic group [13]. Other known physiological differences between strains include their response to nitrogen depletion [19], preference for nitrate or urea for growth [20], and cell cycle behaviour [21]. Although only a few strains have been analysed with regard to these properties the fact that *Synechococcus* strains WH7803 and WH7805 show contrasting abilities to utilise urea and differences in their cell cycle behaviour but are phylogenetically closely related, suggests no clear phylogenetic coherence of these physiological properties will exist. Perhaps this is not so surprising, since the fact that swimming motility appears to have arisen only once during the evolution of the marine *Synechococcus* group may reflect a putative complexity in the swimming mechanism and/or that the ability imparts a distinct ecological advantage, especially as it is known that some motile strains are chemotactic towards a variety of nitrogenous sources [22]. This may ultimately allow a specific niche to be occupied by these motile

strains, or since the motile cluster comprises strains with widely varying PUB/PEB ratios, have conversely allowed them to occupy a range of other niches with distinct light intensities/qualities or nitrogen regimes. The development of specific antibody or oligonucleotide probes to the motile cluster will ultimately resolve this issue by determining the exact distribution of this lineage in various water columns, and particularly whether they might be more associated with microaggregates as might be expected if they can seek out patches of nutrient enrichment.

Genetic diversity studies within the *Prochlorococcus* genus are perhaps slightly more advanced than those we have described above for *Synechococcus*, particularly with regard to development and in situ use of oligonucleotide probes specific for particular genotypes. This has been greatly facilitated by the availability of 16S rRNA sequences of cultures from a wide range of ocean provinces and from different depths down the water column, and is corroborated by analysis of *psbB*, *petB/D* and *rpoC1* sequences of selected cultures whose phylogenies show congruence with 16S rRNA [11,23]. The most striking observation from such phylogenetic studies is that strains that are genetically most similar generally cluster as a function of their depth of isolation rather than their geographic origin (though see Section 3.2 which describes caveats to this statement), suggesting that it is the vertical

light and nutrient gradients found in stratified water columns that act as the key selective pressure mediating speciation in this genus [24]. Indeed, this genetic clustering of surface and deep strains is mirrored especially by the photosynthetic properties that these clades possess, most notably differences in Chl *b*/Chl *a*₂ ratio and the light intensities at which they become photoinhibited [25], as well as possession of single or multiple antenna genes [26]. Such properties have led to the designation of so-called high light-adapted (HL) (low chl *b/a*₂ ratio) and low light-adapted (LL) (high chl *b/a*₂ ratio) ecotypes of *Prochlorococcus* [11], the HL ecotype being further subdivided into HLI and HLII sub-groups [27] (Fig. 2). Caution should be attached to use of this terminology though, since physiologically it appears that all *Prochlorococcus* strains are low light-adapted, but that LL ecotypes are restricted to growth at low light levels whereas the HL ecotype can grow at low and higher light levels. Certainly no strains capable of growth at the light intensities typical of the uppermost layers of the ocean have been isolated thus far.

The relative position of the different ecotypes in phylogenetic trees suggests that members of the HL cluster are the most recently evolved of the *Prochlorococcus* strains and recent genome data suggests that members of this ecotype have undergone genome reduction and specialisation (see Section 6). The LL ecotypes then, are genetically more diverse than their HL counterparts and this may reflect the availability of several different niches at the bottom of the euphotic zone and distinct evolutionary adaptation to the available light and nutrient fields.

3.2. *In situ* studies

Since isolation and culturing may miss ecologically important genotypes it has been important in analysis of marine cyanobacterial diversity to complement culture studies with direct analysis of field populations. For *Synechococcus* such studies have included analysis of phycobiliprotein-associated fluorescence characteristics [28], immunofluorescence [29], and *rpoC1* sequencing from clone libraries [23,30]. Thus, flow cytometry studies have shown that populations with low PUB/PEB ratios tend to dominate in mesotrophic or coastal green waters, whereas high PUB/PEB ratios are common in oligotrophic areas [28] and hence it is likely that optical parameters are important in defining niche dimensions for marine *Synechococcus*. However, as we have seen with culture studies there seems to be no general correlation of pigment composition with phylogenetic position though individual clades can contain solely high or low PUB strains (Fig. 3). The construction of *rpoC1* clone libraries and sequencing of individual clones has certainly reinforced the idea that several genetically defined *Synechococcus* clades can co-exist at the same site. It has also shown the likelihood that one or more of these clades may be specific to surface waters since at least at one site, a stratified water body off the

Californian coast, sequences from these clades could not be found in deep samples taken from the same water column [30]. Indeed, using polyclonal antibodies raised against one strain, *Synechococcus* sp. CC9605, a distribution biased towards oligotrophic surface waters has been shown (B. Palenik, personal communication). Another clade, containing a cluster of strains including CC9311 and CC9617, seems more indicative of organisms that appear following recent mixing or the influence of coastal waters since they are absent from the same site under highly stratified oligotrophic conditions [15]. Furthermore, a study which used immunofluorescence assays to investigate the community structure of *Synechococcus* in the Mediterranean Sea reported a differential distribution of the WH7803 and WH5701 serogroups in a water column [29].

Flow cytometry studies have been more useful in describing the distribution of *in situ* *Prochlorococcus* populations, largely because of the ability to distinguish, and the frequent observation of, bimodal red fluorescence distributions [31]. Thus, dim and bright populations, which we now know will usually correspond to HL and LL ecotypes, respectively, though there can be overlap in the fluorescence per cell ranges of the two ecotypes, have been found to co-occur around the deep chlorophyll maximum, but with the bright populations tending to dominate below this and the dim populations largely confined to surface waters, certainly in stratified waters. That these high and low fluorescence cells can correspond to genetically and physiologically distinct strains was cleverly demonstrated by sorting and bringing into culture representatives of these dim and bright cells [32]. Not only did the cultures stably maintain their fluorescence characteristics but their 16S rRNA sequences fell into distinct lineages (the co-isolates had 97% similarity in their 16S rRNA sequences). This gave rise to the idea of microdiversity within *Prochlorococcus* populations allowing growth over a much broader range of light and nutrient conditions than could be possible by a single homogeneous population. The presence and differential distribution of genetically defined *Prochlorococcus* populations has now been unequivocally demonstrated using several different molecular methods, including 16S rRNA, *rpoC1* and *petB/D* sequencing from environmental clone libraries [27,30,33], as well as denaturing gradient gel electrophoresis, dot-blot hybridisation (see Fig. 4) [27] and fluorescent *in situ* hybridisation (FISH) technologies [34]. The latter two methods provide semi-quantitative or quantitative enumeration of individual genotypes, respectively, whilst FISH also circumvents the need for polymerase chain reaction (PCR) and hence avoids any inherent biases associated with it. FISH has also been carried out by using a chemically synthesised peptide nucleic acid (PNA) general eubacterial probe targeted to the 16S rRNA gene for the detection of *Prochlorococcus* and *Synechococcus* cells by flow cytometry [35]. This could be a very promising technique for the

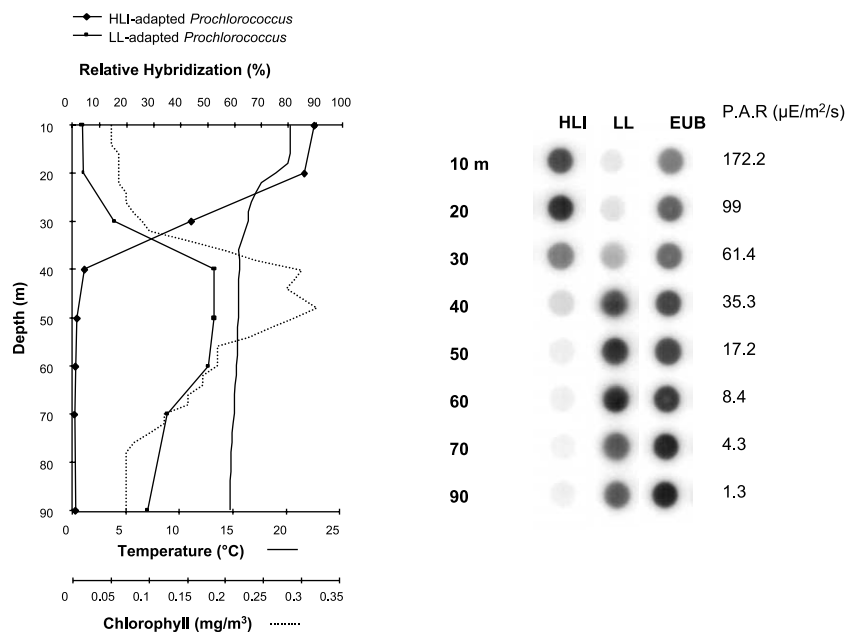


Fig. 4. Vertical distribution of HLI- and LL-adapted *Prochlorococcus* 16S rDNA genotypes in a stratified water column in the eastern North Atlantic, revealed by dot-blot hybridisation. Differential hybridisation of the HLI and LL probes to oxygenic phototroph 16S rDNA sequences PCR-amplified from different depths together with the temperature and fluorescence data (left panel). The corresponding dot-blot from which the relative hybridisation of each genotype-specific probe was quantified, together with the photosynthetically active radiation (P.A.R.) measured at each depth (right panel). EUB, eubacterial probe. From [27] with permission.

analysis of natural populations especially if genotype-specific PNA probes can be designed.

Recent studies using genotype-specific 16S rDNA oligonucleotides for probing environmental DNA on dot-blot [27] and 16S rRNA-targeted probes for FISH [34] has revealed that geographical factors may be important in determining the distribution of *Prochlorococcus* genotypes. Thus, in samples taken from the eastern North Atlantic during 1996, HLI and LL genotypes were partitioned into surface and deep waters, respectively, and the HLII genotype was absent [27]. Similar observations of a partitioning of HLI and LL genotypes have been noted in stratified waters in the Mediterranean Sea, during a transect of several stations (L. Garczareck, N.J. West, D.J. Scanlan, F. Partensky, unpublished data). In contrast, in the western North Atlantic, specifically the Sargasso Sea, using samples taken during 1997, HLII and LL genotypes dominated a well-mixed water column (N.J. West, D.J. Scanlan, unpublished data). The general presence of HLII genotypes in the Sargasso Sea is further supported by the isolation of several *Prochlorococcus* strains from this region whose 16S rRNA sequences cluster within the HLII clade [32], and by the retrieval of *rpoC1* sequences [23], termed the A2 cluster in Ferris and Palenik [30], which likely correspond to this 16S rRNA HLII cluster. *Prochlorococcus* cells belonging to the HLII cluster were also dominant in the Red Sea and interestingly were found to extend throughout a stratified water column down to 100 m, at which depth they even outnumbered LL genotypes [34]. Finally, in the Pacific Ocean off the coast of California, from *rpoC1* clone library data and sequence comparisons,

it seems likely that the HLI ecotype is dominant [30]. Thus, *Prochlorococcus* genotypic distribution should take account of this differential distribution of the closely related HLI and HLII genotypes which may be driven by environmental factors such as temperature and nutrients. Indeed, since data from culture studies suggests differences in primary production contribution at depth for HL and LL genotypes [32], and this may be complicated by differential distribution of HLI and HLII genotypes, such diversity data will be critical for defining accurate estimates of depth-integrated productivity especially in waters where these organisms dominate. Finally, the apparent ability of HLII genotypes to colonise deeper waters reiterates caution with use of the term HL-adapted genotype.

4. Nutrient acquisition capacity of marine cyanobacteria

Uptake of key macro- and micro-nutrients is clearly critical for an organism's survival, and it is surprising therefore that there is relatively little information on acquisition mechanisms by marine cyanobacteria, especially considering their proliferation in oligotrophic systems. Although much is known about nutrient transport mechanisms in heterotrophic bacteria, though perhaps not from marine oligotrophs (and as far as we know no marine cyanobacteria have been isolated that only grow on especially 'poor' medium so we might consider them as facultative oligotrophs), it has only been with the availability of an axenic *Prochlorococcus* strain that some data on N and P substrates utilised has appeared in that genus.

Perhaps the most surprising discovery with regard to substrate utilisation is the inability of the axenic *Prochlorococcus* sp. PCC9511, a HLI ecotype, to utilise nitrate or nitrite as a N source [36,37]. Given that nitrate is likely the major N substrate for new production in the world's oceans this is an important observation. This data is confirmed by the absence of the corresponding nitrate and nitrite reductase genes in the MED4 genome (which is genetically very close, if not identical, to strain PCC9511). Indeed, a recent screen of several *Prochlorococcus* isolates for growth on a range of N sources found that all grew well on ammonium but none of the ecotypes grew on nitrate (Moore et al., in press). Interestingly though, four LL isolates were capable of growth on nitrite, and this correlates with the presence of the *nirA* gene in the genome of the LL strain *Prochlorococcus* sp. MIT 9313. Thus it seems that HL *Prochlorococcus* strains at least, essentially rely on the use of reduced N sources for growth and this is supported by detection of urease activity [38] and high level expression of the *amt* gene, encoding a putative ammonium transporter in this ecotype (Lindell et al., submitted for publication). It is likely then this loss of genes for utilising nitrate and nitrite reflects the niche occupied by this ecotype, surface waters where oxidised forms of N are extremely scarce, providing a relatively strong selection for gene loss and ultimately contributing to a compact genome for this ecotype. LL strains though, in addition to utilising ammonium and urea, may be able to utilise nitrite, reflecting the often found nitrite maxima at the bottom of the euphotic zone and the likely niche of this ecotype. It remains to be seen whether *Prochlorococcus* strains capable of utilising nitrate can be isolated from deep water environments in oceanic waters where nitrate concentrations may be relatively high.

As well as the inability of HL *Prochlorococcus* strains to utilise nitrate it has been shown for the axenic strain at least that there are also unusual aspects of regulation of N metabolism. Thus, the enzyme glutamine synthetase (GS), which catalyses ammonium incorporation into glutamate, shows a slight drop in activity upon transfer of cells from ammonium to nitrate, nitrite or no N medium. This contrasts the standard increase in activity which is the common response of cyanobacteria and other photosynthetic organisms to nitrogen starvation. Moreover, phosphorus starvation appears to cause a marked decline in GS activity whilst dark incubation caused little change in activity [37]. Both these observations suggest unusual regulatory circuits for controlling the activity of this enzyme exist in this HL *Prochlorococcus* strain, likely a reflection of the fine-tuning of N metabolism to the oligotrophic environmental conditions these organisms inhabit.

In contrast to *Prochlorococcus*, virtually all marine *Synechococcus* strains that have been isolated so far are capable of utilising nitrate as a N source for growth ([1,16], N. Fuller and D.J. Scanlan, unpublished data). Interestingly, the nitrate uptake mechanism consists of a permease

(NapA/NrtP) of the major facilitator superfamily, rather than the *nrtABCD* operon encoding a typical ABC transporter found in freshwater cyanobacteria [39]. This suggests caution when extrapolating mechanistic features of nutrient uptake from data obtained with freshwater cyanobacteria to marine strains. One exception to the ability of marine *Synechococcus* strains to utilise nitrate has been reported. *Synechococcus* sp. MIT S9220, isolated from the equatorial Pacific, could not use nitrate for growth nor could be rescued from N limitation by nitrate (Moore et al., in press). This strain could however utilise nitrite, ammonium and urea as a N source, though growth on the latter source was significantly reduced. Lack of nitrate utilisation amongst marine *Synechococcus* strains thus appears generally rare. This difference in nitrate utilisation between *Synechococcus* and *Prochlorococcus* populations is reflected in observations made in situ, where natural populations of *Synechococcus* have been reported to respond rapidly to periodic nitrate input, and bloom [40], whilst *Prochlorococcus* is generally absent in high nitrate well-mixed winter waters (see e.g. [41]).

Prochlorococcus and *Synechococcus* appear to be more cosmopolitan with regard to utilisation of P sources, but this is based on screening of few strains. Thus, the axenic HLI *Prochlorococcus* PCC9511 is capable of growth on Na₂ β-glycerophosphate, Na₄-pyrophosphate, glucose-6-phosphate and ATP in addition to inorganic phosphate (Pi) [36], whilst the marine *Synechococcus* strain WH7803 has been shown to utilise Pi, dCTP, *p*-nitrophenyl phosphate, glucose-6-phosphate and glycerol phosphate as a sole P source [9]. Interestingly, the ability to utilise cAMP as sole P source may be a clade-specific trait amongst marine *Synechococcus* strains ([9]; L. Moore, personal communication), and whilst the one axenic *Prochlorococcus* isolate that has been investigated can utilise this substrate (L. Moore, personal communication) it will require further work to assess whether this reflects the genera as a whole. The ability to utilise nucleotides as a P source suggests hydrolysis by alkaline phosphatase or the presence of a specific 5'-nucleotidase, but certainly utilisation of cAMP would suggest the presence of a specific high affinity transport system for this substrate. Seemingly, the capacity for cAMP uptake appears to be relatively rare among marine bacterioplankton [42]. Further insights into the P physiology of these marine cyanobacterial genera and how differences in P physiology may provide an explanation for their phylogenetic separation, particularly in the *Prochlorococcus* genus, is discussed in relation to genomic information below (see Section 6).

So why have we focused on aspects of nutrient physiology? As we have already stated, nutrients are essential for growth, and if we are to begin to understand some of the control points for bacterial photosynthesis in marine systems it is only right that we should address the role of nutrient limitation in this scheme. Moreover, and arising sequentially out of these studies, has come the develop-

ment of molecular markers of the nutrient deplete state and it is to these we now turn.

5. Physiological response of cells to the environment as revealed by molecular markers

One way of revealing how cells are responding to their environment is by the use of molecular markers which act as proxies for a given physiological response. Such markers may indicate stress responses to low nutrient concentrations or changes in light intensity and can be detected at the gene transcript or protein level [43]. They allow an indication of the factors that can constrain productivity, factors which in turn may affect community structure and species succession. With respect to *Synechococcus* and *Prochlorococcus* and the macronutrient P, markers which are useful have been based on responses that either induce high affinity uptake systems for the preferred source of the nutrient [9,44] or utilise alternative sources of the nutrient, but which may be energetically more costly to acquire [45]. Alternatively, markers may target a component of the regulatory system itself. For instance, the N status of marine *Synechococcus* can be assessed using a protocol based on expression of the global N regulator *ntcA* [46]. In combination with P status markers this will allow dual nutrient status information to be obtained from the same organisms. In addition, the fact that for one of these P status markers, PstS, encoding a high-affinity P binding protein, a single cell immunofluorescence assay has been developed [9], whilst *ntcA* gene expression uses *ntcA* primers of different taxonomic specificity, allows assessment of N and P status at various taxonomic levels. Given the likely importance of clade-specific differences in nutrient physiology, which we have already seen with respect to utilisation of specific N and P substrates, such specificity is clearly important, though interpretation of this molecular data will require a clear understanding of the regulation of nutrient physiology across the genera.

In addition to the use of diagnostic molecular markers to assess nutrient status, cell cycle variables have also been successful, particularly to monitor nutrient addition bioassays. The rationale is that cells that have been deprived of an essential nutrient usually arrest at the beginning of their cell cycle (G1 phase) but can initiate DNA synthesis (S phase) within a few hours of nutrient addition. Using

flow cytometry to discriminate the autotrophic populations such cell cycle analyses showed very clearly that *Synechococcus* populations in the Mediterranean Sea in summer were P- rather than N-limited [47]. Clearly the use of such alternative approaches to assess cellular nutrient status will add independent confirmation to any conclusions made using molecular marker studies and extend the range of techniques available to molecular ecologists to answer questions on control of picophytoplankton growth rates by external parameters.

6. Genomics: new insights into the ecology of *Prochlorococcus* and *Synechococcus*

The recent completion of the genome sequences of two *Prochlorococcus* and one marine *Synechococcus* strain allows an unprecedented overview of the genomic requirements for existence in oligotrophic marine waters, as well as providing a unique data set for comparative genomic analysis within and between these genera. We can thus begin to gain insights into the genetic and physiological basis relating to the ecology of these organisms.

The two *Prochlorococcus* genomes comprise strains MED4 and MIT 9313, representatives of HLI and LL ecotypes, respectively, whilst the single marine *Synechococcus* genome is a member of the 'motile' clade, a monophyletic trait within this genus. Some general properties of the three genomes are summarised in Table 1. Particularly noteworthy is that the size of the MED4 genome is the smallest known for a free-living photosynthetic prokaryote, significantly less than its LL counterpart. In addition, the number of genes unique to a particular genome hint at the possibility of niche-specific alleles, as well as genes responsible for known physiological differences between the two genera, e.g. motility, photosynthetic antenna, etc. Note however, that for *Prochlorococcus* MIT 9313 and *Synechococcus* WH8102 the information presented here derives from the annotation of genomes that at the time of writing are not yet fully closed. Comparison of the two *Prochlorococcus* genomes has already identified several differences that likely correlate with their light-dependent physiologies. Thus, MED4 possesses many more genes encoding high-light-inducible proteins (HLIPs) and photolyases to repair UV-induced DNA damage. In contrast, MIT 9313 contains more genes associated with the photosynthetic apparatus, including an extra copy of the

Table 1
General comparison of marine cyanobacterial genomes

Organism	Genome size (Mb) ^a	% GC ^a	Total No. of genes ^a	No. of genes unique to that genome ^b
<i>Prochlorococcus</i> MED4	1.67	30.9	1694	216
<i>Prochlorococcus</i> MIT 9313	2.40	50.7	2195	554
<i>Synechococcus</i> WH8102	2.42	59.5	2426	536

^aBased on annotation from the JGI.

^bThat is, not found in the other two marine cyanobacterial genomes (A. Dufresne, D. Vaultot, personal communication).

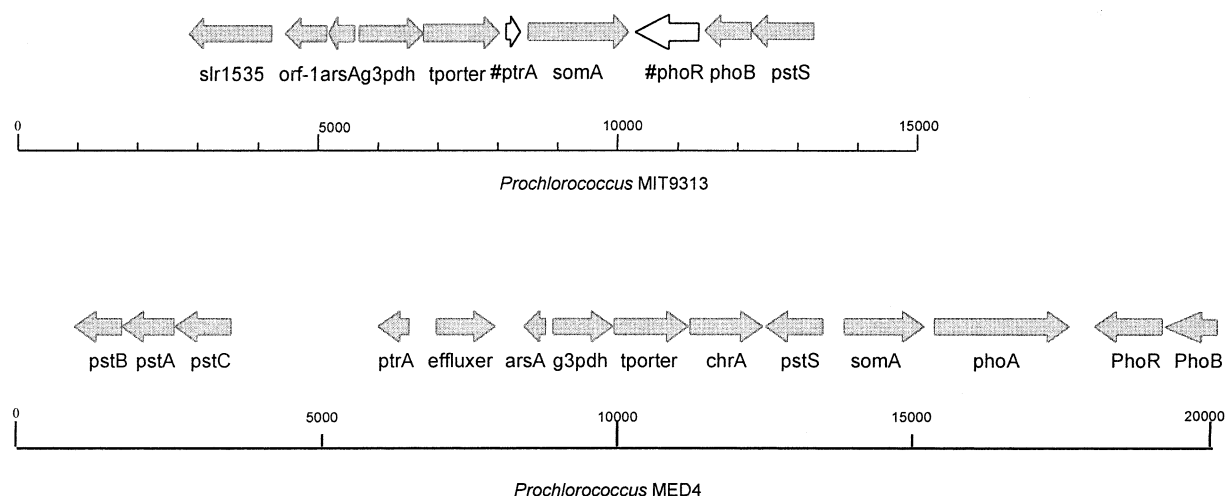


Fig. 5. Genomic regions of *Prochlorococcus* MED4 and MIT 9313 containing genes of the P acquisition and regulatory system. Filled arrows indicate the relative position of ORFs. Blank arrows indicate ORFs that appear to be non-functional based on sequence information provided by the JGI and by independent sequencing (D.J. Scanlan, unpublished data). Gene designation is based on the closest BLAST hit for each ORF. Abbreviations: PhoB and PhoR, P sensor and response regulator of a two-component system. PhoA, alkaline phosphatase. SomA, outer membrane protein, porin. PstS, P binding protein of a high affinity P transport system. ChrA, chromate transporter. Tporter, nutrient transporter. G3PDH, glyceraldehyde-3-phosphate dehydrogenase. ArsA, arsenate resistance regulator. PtrA, putative transcriptional regulator. PstABC, ABC components of the high affinity P acquisition system.

pcb gene encoding the antenna chlorophyll-binding protein [48]. Interestingly, both the *Prochlorococcus* MIT 9313 and *Synechococcus* WH8102 genomes contain around 70% more ATP-binding components of ABC nutrient transport systems than the smaller MED4 genome (see Table 2). This suggests a much greater capacity for acquisition of a variety of substrates for MIT 9313 and WH8102, and correlates with a known location at the bottom of the euphotic zone, for MIT 9313 at least, where a more diverse array of substrates are likely available. Moreover, it suggests a compaction of the genome in the case of MED4 with regard to its capacity to acquire nutrients via ABC transporters although the possibility that MED4 possesses alternative novel systems for acquiring nutrients in the ultra-oligotrophic surface waters it inhabits remains. Indeed, we have recently identified a phosphate starvation-inducible polypeptide in *Prochlorococcus* sp. PCC9511, which is present in the MED4 and WH8102 genomes but not in MIT 9313 (N.J. West, D.J. Scanlan, unpublished data). This may represent an example of a niche-specific gene, referred to above.

Also of note is the relative lack of two-component sys-

tems in these marine cyanobacterial genomes (Table 2), responsible for sensing their environment and regulating nutrient transport and other cellular components, certainly compared to the freshwater cyanobacteria *Synechocystis* and *Nostoc*. Of particular interest, and which may be of general significance if it is typical of other LL *Prochlorococcus* strains, is the presence in the MIT 9313 genome of a homologue of the *phoR* gene encoding a histidine kinase responsible for P sensing, but which contains a stop codon and frameshift within the coding sequence (Fig. 5). Significantly, the first stop codon occurs just upstream of the conserved histidine residue of these sensor kinases. Since PhoR is the sensor that phosphorylates PhoB, the response regulator, which in turn is a transcriptional activator of several genes involved in P uptake, mutation of *phoR* would suggest an incapacity to phosphorylate PhoB, which though itself intact would result in an inability to regulate the P acquisition machinery. This loss of regulatory capacity is mirrored by the apparent degeneration of an open reading frame (ORF) which shares homology with a putative transcriptional activator, *ptrA*, from the marine *Synechococcus* sp. WH7803 and which

Table 2
Comparison of cyanobacterial genomes with specific respect to complexity of two-component systems and ABC transporters

Organism	Histidine kinases ^a	Response regulators ^a	ABC nutrient transport systems ^b
<i>Prochlorococcus</i> MED4	6	7	25
<i>Prochlorococcus</i> MIT9313	7	12	42
<i>Synechococcus</i> WH8102	6	14	42
<i>Synechocystis</i> sp. PCC6803	44	52	54
<i>Nostoc punctiforme</i>	146	102	94

^aBased on annotation from the JGI/Cyanobase.

^bBased on the number of genes encoding the ATPase component from JGI/Cyanobase annotation.

may also be involved in regulating genes involved in P utilisation [49]. This may reflect the relatively high P concentrations that are found in deep waters and hence little need for precise regulation of the P acquisition machinery in these LL *Prochlorococcus* strains like MIT 9313. In contrast the MED4 and WH8102 genomes contain intact copies of the *phoR* and *ptrA* genes. Curiously the *phoB* and *phoR* gene products appear translationally coupled in MED4 which again appears to be a slight variation on the typical two-component system paradigm. Other features of these genomes with specific regard to P utilisation are the apparent absence of a gene encoding alkaline phosphatase in MIT 9313 and the presence in WH8102 but not the *Prochlorococcus* genomes of a gene encoding SphX, which in a freshwater *Synechococcus* is a cytoplasmic membrane protein thought to play a role in P assimilation [50]. These data suggest specific differences with regard to P assimilation capacity and regulation in the marine cyanobacterial genera, and when taken together with information on N nutrition and light physiology already alluded to, we can begin to see how actual gene complement clearly reflects the ecology of the organism itself. Further experimentation will be required to provide confirmation of these *in silico* studies and will surely reveal much regarding the fine tuning of regulatory and acquisition systems to environmental niche.

7. Conclusions

Population structure in these organisms clearly reflects not only the obvious light gradient of the euphotic zone, but also the different nutrient regimes that also co-exist within this environment. Although we have largely only considered here the macronutrients N and P, micronutrient availability and partitioning likely also has some influence in dictating the niches and population structure of these organisms.

Given the ecological significance of these picophytoplankton it is perhaps of primary concern that we begin to identify and estimate the importance of the many factors that control their abundance and productivity. As we have seen molecular probes can be used to address the limiting nutrient or physiological state of the organism in single cells *in situ*, whilst diagnostic oligonucleotides or gene probes can be used to quantitate not only gross cyanobacterial numbers but also the abundance of specific genotypes, and potentially also cyanobacterial-specific virus numbers. The development of molecular probes that can unequivocally demonstrate the multitude of genotypes or ecotypes of these organisms that are present in a given water column is important because individual cells will always experience their own microenvironments giving rise to subtly different responses to environmental conditions. Molecular tools thus have the potential advantage of revealing the complexity and diversity of such responses

and give underlying structure to population-based measurements. This is particularly pertinent to modelling studies which generally treat the ocean as a homogeneous environment. Certainly, there are great advantages in the use of satellite imagery to monitor surface chlorophyll levels, but can we increase the accuracy of such depth-integrated productivity measurements using knowledge of phytoplankton community structure, particularly over temporal and spatial scales, and associated differences in cell-specific carbon fixation rates among specific genotypes?

Perhaps the major areas that will be addressed and extended using molecular tools are (i) the extent of genetic diversity that exists among the genera *Prochlorococcus* and *Synechococcus*, (ii) how this diversity is packaged into environmental niches and evolutionary groups, (iii) how we relate diversity to physiology and thus function and (iv) how we can use these tools to estimate growth rates and losses to grazing/viruses/environmental factors (including UV damage). Clearly genomic information will also play a big role in answering and setting new puzzles regarding the molecular ecology of these important organisms.

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