Oceanographic Basis of the Global Surface Distribution of Prochlorococcus Ecotypes

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By using data collected during a continuous circumnavigation of the Southern Hemisphere, we observed clear patterns in the population-structure of Prochlorococcus, the most abundant photosynthetic organism on Earth, between and within the three Southern Subtropical Gyres. The same mechanisms that were previously invoked to account for the vertical distribution of Prochlorococcus ecotypes at local scales accounted for the global (horizontal) patterns we observed. Basin-scale and seasonal variations in the structure and strength of vertical stratification provide a basis for understanding large-scale horizontal distribution in genetic and physiological traits of Prochlorococcus, and perhaps of marine microbial communities in general.

Prochlorococcus is the smallest and most abundant phytoplankton in the global ocean and contributes significantly to the primary productivity of tropical and subtropical oceans (1). That the genus thrives throughout a wide range of photic zone conditions has been explained by the discovery of genetically and physiologically distinct populations, commonly referred to as high light (HL)– and low light (LL)–adapted ecotypes (2). Prochlorococcus ecotypes partition themselves according to depth in a stratified water column (3); however, the coexistence of multiple ecotypes (2) and phylogenotypes (4–6) has also been reported and attributed to vertical mixing in response to local physical forcing. But the effect of physical forcing on Prochlorococcus ecotypes at the global scale has not been explored. By using data collected during a circumnavigation of the Southern Hemisphere, we investigated whether the genetic structure of Prochlorococcus populations changes in response to vertical mixing within and between the major ocean basins of the world. Samples were collected during the Blue Earth Global Expedition (BEAGLE) (Fig. 1A) during the winter (averaging 1.00 \( C_i \) values observed in the strongly stratified Indian Ocean, a pattern that is consistent with our current understanding of the distribution of this genus (1, 10, 11). However, the concentration of divinyl chlorophyll a is high in the Pacific Basin (except near 140\(^{\circ}\)W) and low in the Atlantic and Indian Basins. This is perhaps counterintuitive; it can be explained as follows. Because all samples were collected within the top 10 m of the water column, vertical mixing would be an important mechanism altering the growth conditions (light and nutrients) of the phytoplankton cells. Thus, the high divinyl chlorophyll a concentrations in the Pacific may arise from photoacclimatory (physiological) or photoadaptive (genetic) response of the cells to a decrease in mean light intensity. Basin-scale patterns in the intracellular concentration of divinyl chlorophyll a (\( C_i \)) for Prochlorococcus are evident (Fig. 2B), with low \( C_i \) values observed in the strongly stratified Indian Ocean during the summer (averaging 0.14 fg DV Chla per cell), consistent with those found in the surface waters of the subtropical North Atlantic (12), and high values observed in the well-mixed Archipelagic Deep Basins Province (8) during the winter (averaging 1.00 fg DV Chla per cell), similar to those typically found deeper in the water column in subtropical gyres (12).

Because light decreases exponentially with depth, phytoplankton cells mixed deeper in the water column would experience a lower mean daily irradiance than if they remained at the sea surface. Phytoplankton respond to this reduction in irradiance by increasing the concentration of pigment per cell. An inverse relation between \( C_i \) and
and daily mean irradiance in the mixed layer ($I_m$) was seen (Fig. 3A), consistent with the physiological response of Prochlorococcus in culture experiments (13). The ratio of the sum of photoprotective accessory pigments to the sum of both photoprotective and photosynthetic accessory pigments ($D$) can also be used as an index of the response of the phytoplankton community to ambient light conditions and has been shown to be strongly correlated with available irradiance within the mixed layer (14). Again, this pigment index is significantly correlated with $C_i$ (Fig. 3B).

The cellular properties of Prochlorococcus, in particular cell fluorescence, have been known to change in response to vertical mixing (15). Thus, in the absence of genetic information, the relationship between the $C_i$ and vertical mixing could be interpreted as simply the response of a single genotype to changes in light history. But vertical variation in both cell fluorescence and $C_i$ has also been associated with a change in genotype (3, 12). In fact, the unique fluorescence properties of surface and deep populations of Prochlorococcus led to the isolation and identification of HL and LL ecotypes (2).

Culture experiments have shown that HL ecotypes have optimal growth rates at higher irradiance levels than their LL counterparts (13). Pronounced differences in the regulation of light harvesting between HL and LL ecotypes have also been observed (16). Genomic comparison between HL- and LL-adapted strains of Prochlorococcus has shown that the HL strain has a photolyase gene, which serves to repair ultraviolet damage, and which is absent in the LL-adapted strain (17). Both culture experiments and genomic analysis have revealed that HL ecotypes must exclusively rely on reduced forms of nitrogen, whereas several LL ecotypes can use both nitrite and ammonium (17, 18). The relatively high nitrate concentration and low mean irradiance in the Pacific Basin caused by seasonal mixing, compared with those of the Atlantic and Indian Oceans (Fig. 4A), imply conditions resembling those found at depth in stratified water columns. Thus, we would anticipate major differences in the population genetic structure of Prochlorococcus

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**Fig. 1.** (A) Map of the biological sampling stations for the BEAGLE and the biogeochemical provinces defined by Longhurst (9). The gray dots indicate routine sampling stations where all regular biological and physicochemical data were obtained. The red triangles represent stations from which DNA samples were used for dot blot hybridization analysis; each analysis was done on combined samples of three stations. (B) Contour plot of the strength of the vertical density gradient index, $N$ (9), with corresponding estimates of the depth of the mixed layer ($z_m$) using conductivity-temperature-depth (CTD) density profiles represented by the yellow triangles. Values of the maximum observed $N$ for the water column ($N_w$) for the Indian Ocean were high, averaging $14.2 \pm 3.45$ cycles per hour, whereas in the Pacific and Atlantic Basins, average values of $N_w$ were $7.3 \pm 1.70$ and $8.9 \pm 2.29$ cycles per hour, respectively. The average ($\pm 1$ SD) $z_m$ in the Pacific, Atlantic, and Indian Ocean Basins were $126 \pm 46.1$ m, $52.1 \pm 26.3$ m, and $29.5 \pm 9.3$ m, respectively.

**Fig. 2.** (A) Distribution of divinyl chlorophyll a (DV Chla) concentration and cell abundance; the black dots indicate the concentration of divinyl chlorophyll a and the open triangles indicate the Prochlorococcus abundance. (B) The intracellular concentration of divinyl chlorophyll a ($C_i$). All samples were collected within the top 10 m of the water column.

**Fig. 3.** Relationship between the intracellular concentration of divinyl chlorophyll a ($C_i$) for Prochlorococcus. (A) The average daily irradiance within the mixed layer ($I_m$) and (B) the ratio of the sum of the concentration of photoprotective pigments to the total pigment concentration ($D$) for samples collected during the BEAGLE expedition.
between the three ocean basins as a result of changes in vertical mixing.

To test this expectation, polymerase chain reaction (PCR) amplified oxygenic phototroph 16S ribosomal DNA (rDNA) sequences derived from environmental DNA were hybridized with genotype-specific oligonucleotide probes (3, 19) designed to detect the two HL adapted clusters (HLI and HLII), the LL adapted strains, and the specific LL adapted genotype SS120 (fig. S2). This method has been shown to yield similar results to those obtained using quantitative PCR (qPCR) (20). The analysis was conducted on surface samples selected to cover a broad range in the Cj and mixing conditions. To ensure ample DNA for the dot blot hybridization analysis, filters from three alternate stations were combined (Fig. 1A).

Prochlorococcus probes hybridized faintly but significantly at stations located at the edge of the Pacific Basin and more strongly in the central Pacific, Atlantic, and Indian Oceans (Fig. 4B). This pattern reflects changes in the relative contribution of Prochlorococcus to the total phytoplankton biomass, which can also be represented by the ratio of the concentration of divinyl chlorophyll a, a pigment found exclusively in Prochlorococcus, to the sum of the concentration of divinyl and monovinyl chlorophyll a (DV Chla/TChla) (Fig. 4B). At the eastern and western Pacific Basin, where nutrient concentrations were high because of winter mixing, we found a low percent hybridization and DV Chla/TChla, revealing high concentrations of Synechococcus and eukaryotic picoplankton (fig. S3), whereas Prochlorococcus cell abundance decreased.

Several factors may contribute to the observed distribution of Prochlorococcus. First, under strong vertical mixing, Prochlorococcus is believed to have limited ability for chromatic adaptation, compared with that of Synechococcus (21). Furthermore, the higher chlorophyll concentrations in nitrate-rich regions also result in a light field rich in green photons, which has the accessory pigments in Synechococcus cells absorb preferentially (21). Second, no known strain of Prochlorococcus is able to use nitrate, a nutrient associated with mixed waters (17, 18). Third, vertical mixing reduces the temperature of surface waters, which may affect the relative contribution of Prochlorococcus to total biomass, given that the dominant ecotypes of Prochlorococcus achieve their temperature optimal for growth at ~25°C (22).

Our observations harmonized with these expectations. For example, we found the abundance of HLI was positively correlated with D, Im, and temperature, but negatively associated with the concentration of nitrate and phosphate (table S1). At lower temperatures, LL was less abundant. However, the abundance of other Prochlorococcus genotypes (HLI and SS120) was related only weakly to the covariates of physical forcing. Our results also revealed that within the LL and HL groupings there is further niche partitioning among genotypes. For example, HLII dominates in surface waters of regions with high stratification (Indian Ocean), whereas HLI is more prevalent in surface waters with moderate stratification and mixed-layer depth (middle of the Pacific Basin and Atlantic Basin). By contrast, LL and SS120 disassociate, which may be explained in part by the inability of SS120 to use nitrite (23), whereas several LL-adapted strains can use this form of nitrogen (18).

The influence of vertical mixing on the population structure of Prochlorococcus becomes clear when we examine the relative proportions of HL and LL ecotypes occurring in the three ocean basins (Fig. 4C). In the well-mixed Pacific Basin, relative hybridization for HL and LL genotypes is similar. In contrast, in the Atlantic Basin hybridization showed greater abundance of HL compared with LL. This is accentuated in the highly stratified Indian Ocean, where HL ecotypes dominate over their LL counterparts, with HLII genotypes being particularly abundant. However, the highly stratified, summer conditions present in the Indian Ocean would likely result in a dominance of LL ecotypes near the base of the photic zone (3, 12, 22). One could argue that the high relative abundance of LL ecotypes in the Pacific is the result of resuspension of cells from depth toward the sea surface. However, given that Prochlorococcus division rates are on the order of 1 day, and given that the samples were collected in August, which was months after the initiation of deep winter mixing and after the maximum in mixed-layer depth (fig. S1), the population structure of Prochlorococcus is likely the result of a successional change to environmental conditions.

In the surface ocean, the occurrence of the LL ecotype appears to be significantly correlated not only with temperature, but also with Im and D. The abundances of the ecotypes are correlated positively (LL) and negatively (HLII) with phos-
Wnt Gradient Formation Requires Retromer Function in Wnt-Producing Cells

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Wnt proteins function as morphogens that can form long-range concentration gradients to pattern developing tissues. Here, we show that the retromer, a multiprotein complex involved in intracellular protein trafficking, is required for long-range signaling of the Caenorhabditis elegans Wnt ortholog EGL-20. The retromer functions in EGL-20–producing cells to allow the formation of an EGL-20 gradient along the anteroposterior axis. This function is evolutionarily conserved, because Wnt target gene expression is also impaired in the absence of the retromer complex in vertebrates. These results demonstrate that the ability of Wnt to regulate long-range patterning events is dependent on a critical and conserved function of the retromer complex within Wnt-producing cells.

During C. elegans early larval development, EGL-20 is expressed by a group of cells located at the posterior end of the animal (1). EGL-20 controls the anterior migration of the HSN neurons (2) and the polarity of the division of the epidermal seam cell V5 (3). In addition, EGL-20 regulates the left/right asymmetric migration of the more distantly located Q neuroblasts (2) (fig. S1A). EGL-20 generates this asymmetry by specifically activating the expression of the Hox gene mab-5 in the left Q cell (QL) (1). MAB-5 in turn directs the migration of the QL daughter cells (QL,d) toward the posterior (4). The Q daughter cells on the right side (QR,d) migrate in the default anterior direction. We used this asymmetric migration as an assay to identify novel components of the EGL-20 pathway. In a genome-wide RNA-mediated interference (RNAi)–based screen (5), we found that an ortholog of the yeast retromer complex subunit Vps35p (table S1) is required for posterior localization of the QL,d. In yeast, the retromer directs endosome-to-Golgi retrieval of proteins such as the carboxypeptidase Y receptor Vps10p (6). In vertebrate epithelial cells, it physically interacts with the immunoglobulin receptor (IgR) and mediates basal-to-apical transcytosis of the IgR-IgA complex (7).

We isolated a deletion allele that likely represents the 19s-35 null phenotype (fig. S1B).

References and Notes

9. Materials and methods are available as supporting material on Science Online.
34. The authors would like to thank the instructors of BEAGLE Bio-optics Program (B. Irwin, Leg 1; G. Alarcon, Leg 2; and P. Bonham, Leg 5) for the collection of samples and the many students and researchers who participated in the bio-optics training program funded by the Partnership for Observation of the Global Oceans, the International Ocean-Colour Coordinating Group, and the Intergovernmental Oceanographic Commission. The assistance and support of the research scientists and crew onboard the R.V. Mirai, in particular the captain, M. Akamine, is also appreciated. We also thank E. Devred and the Japan Agency for Marine-Earth Science and Technology administration, in particular T. Hirano. This research was also funded by the Department of Fisheries and Oceans Strategic Science Fund, the Chilean National Commission for Scientific and Technological Research through the Funds for Advanced Research in Priority Areas Program, and by Natural Environment Research Council (D.J.S.). H.A.B. was supported by a Natural Sciences and Engineering Research Council of Canada Postdoctoral Fellowship and Canadian Space Agency Postdoctoral Supplement.

Supporting Online Material

www.sciencemag.org/cgi/content/full/312/5775/918/DC1
Materials and Methods
Figs. S1 to S4
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References

16 November 2005; accepted 11 April 2006
10.1126/science.1122692