Evolution of Sub-micron-Size Cyanobacteria in Polar Ice over 50 Million Generations

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Using fluorescence spectrometry to map autofluorescence of chlorophyll (Chl) in polar ice cores, followed by flow cytometry (FCM) and epifluorescence microscopy (EFM) of melts of that ice, we found that sub-micron size picocyanobacteria (*Prochlorococcus* and *Synechococcus*) were responsible for most if not all of the Chl in the cores. In ice melts from 2 Arctic and 6 Antarctic sites passed through a 1.2 µm filter to remove large cells, we calibrated the FCM patterns of Chl (red) *vs* phycoerythrin (PE, orange) with FCM patterns of cultures of *Pro* and *Syn*. From the FCM measurements we concluded that *Pro* and *Syn* are the dominant sub-micron ecotypes in all polar ice samples, sometimes with 2 to 3 strains of *Syn* in the same sample. FCM plots of Chl *vs* side scattering (SSC) showed that *Pro* are usually 0.5 to 0.8 µm and *Syn* are ~0.6 to ~1.5 µm, consistent with the sizes of those genera in the oceans. We measured concentrations from ~2 to 2 x 10^4 *Pro* cells/cm^3 of ice (median 70 *Pro*/cm^3) and a median value 2.6 for the ratio *Pro/Syn*. Chl and PE autofluorescence intensities showed no apparent decrease per cell with time during 150,000 years of storage in glacial ice. From our fluorimetric scans of Chl concentrations in ice cores we found values up to ~30% higher at depths corresponding to local summers than to local winters. Figure 1 shows examples of our fluorimetry compared with SeaWiFS satellite Chl measurements for the North Atlantic and the Antarctic Oceans. Taking into account this annual modulation, together with ocean temperatures and winds, we inferred that both *Pro* and *Syn* cells are wind-transported from mid-latitude, temperate waters onto polar ice.

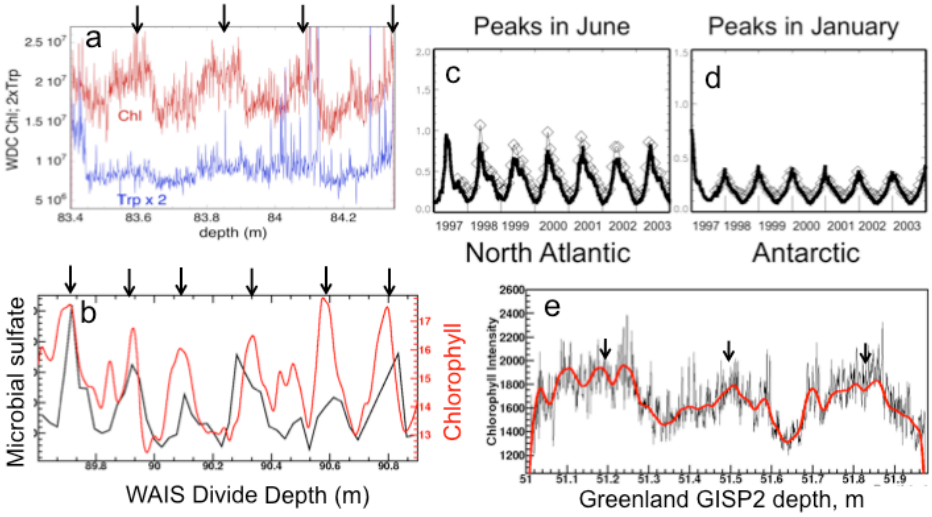

Figure 1. Summer maxima of Chl autofluorescence in ice cores scanned by the Berkeley Fluorescence Spectrometer. (a) Chl and Trp over 4 years in 1-meter length of West Antarctic Ice Sheet (WAIS) Divide ice; (b) Chl and microbial sulfate maxima in WAIS Divide ice are in phase; (c) and (d) are data from SeaWiFS satellite showing that Chl in North Atlantic Ocean surface are maxima in northern summer, and in Antarctic Ocean are maxima in southern summer; (e) Chl peaks for 3 summers in Greenland GISP ice core.

To us, the annual modulation of Chl autofluorescence in terrestrial ice cores is reminiscent of the exciting finding by others that methane concentration in the Martian atmosphere increases during Martian summer and wanes in winter. It has still not been established whether the methane has a biogenic or abiogenic origin. By being able to obtain samples of terrestrial ice cores, we have concluded that the increase in Chl is due to the annual

warming of terrestrial oceans inhabited by *Pro* and *Syn* and to the transport of some of those cells from ocean surfaces onto the growing Arctic and Antarctic ice sheet. Similarly, by being able to return samples from Mars, it may be possible to pin down the origin of the periodic methane releases and possibly to discover indigenous life forms on Mars.

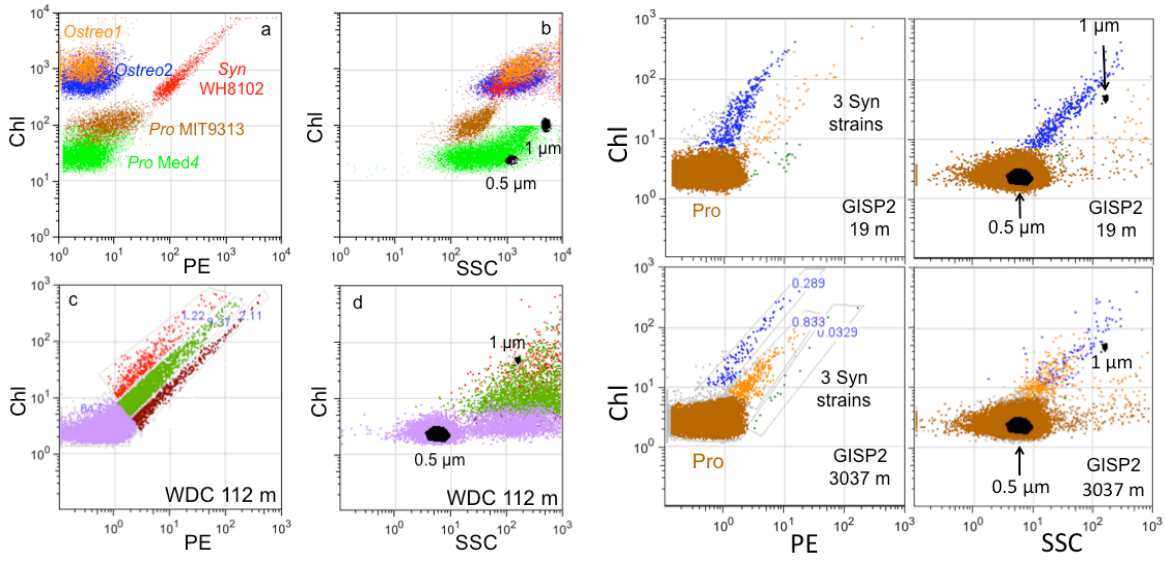

Figure 2. Examples of flow cytograms in pairs (Chl *vs* PE; Chl *vs* SSC). (a,b) show patterns for cultures of *Ostreococcus* strains 1 and 2, *Pro* strains MIT9313 and Med4, and *Syn* strain WH8102, using 0.5 and 1.0 µm fluorescent beads for size calibration; (c,d) WAIS Divide ice from 112 m depth; and GISP2 ice from depths of 19 m and 3037 m.

Finally, comparing the flow plots on the right sides of Fig. 2, we see that the concentrations of *Pro* and *Syn* cells are very similar for ice of ages 60 yrs (i.e., 19 m) and 150,000 yrs (i.e., 3037 m). There appear to be 3 strains of *Syn* at both depths. With a growth rate of 1 to 2 per day for *Pro* and *Syn* in mid-latitude oceans, and no growth for cells in glacial ice, we would like to do genomics on *Pro* and *Syn* at, say, 10 depths in Arctic ice and 10 depths in Antarctic ice, to study evolutionary changes over 50 million generations in the ocean. We would use the cells in ice at -30°C as a frozen proxy of the same ecotypes living in the ocean up to 150,000 years ago. It will be an intriguing problem in microbial ecology to tease out evolution of the same strains at different depths.

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