Limnol. Oceanogr., 56(5), 2011, 1907–1916

doi:10.4319/lo.2011.56.5.1907

Crenarchaea and phytoplankton coupling in sedimentary archives: Common trigger or metabolic dependence?

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Abstract

The concentrations of chlorins (chlorophyll transformation products indicative of phytoplankton production) and crenarchaeol (a marker for Crenarchaea abundance) are significantly positively correlated (Spearman's rank correlation coefficient $r_s > 0.75$) in four core records from freshwater (Lake Baikal) and marine settings (Southern, Atlantic, and Arctic Oceans). This suggests a close relationship between Crenarchaea abundance and phytoplankton production. Degradation and transport mechanisms, as well as a common environmental trigger, may in part account for our observations, but these mechanisms alone cannot fully explain them. Instead our findings point to a metabolic dependence of Crenarchaea on resources released by phytoplankton, such as organic carbon or ammonium.

Phytoplankton and bacteria abundance and production consistently co-vary in different kinds of aquatic systems (Gasol and Duarte 2000). This coupling is generally explained by the dependence of heterotrophic bacteria on organic matter supplied by the autotrophic phytoplankton. The interactions between phytoplankton and Archaea are less studied, despite the fact that planktonic Archaea, and specifically Crenarchaea, constitute a large part (up to 30%) of the picoplankton (Karner et al. 2001) and are ubiquitously distributed from tropical to polar settings (Fuhrman et al. 1992; DeLong et al. 1994; Murray et al. 1998).

Different dominant metabolic pathways have been suggested to operate in Archaea, notably heterotrophy (Ouverney and Fuhrman 2000; Teira et al. 2006) and also mixotrophy utilizing both CO_2 and organic carbon (Hallam et al. 2006; Ingalls et al. 2006). Other studies suggest that Crenarchaea can be chemoautotrophs with light-independent inorganic carbon fixation metabolism (Pearson et al. 2001; Herndl et al. 2005), but their energy source remained unknown until Könneke et al. (2005) grew a pure Crenarchaea culture on ammonium and CO_2 alone. Subsequent molecular surveys provided further evidence for the widespread occurrence of ammonium-oxidizing Crenarchaea (Francis et al. 2005; Wuchter et al. 2006*a*; Church et al. 2010). All three major metabolic pathways are currently discussed for Archaea, but the exact nature of the phytoplankton–Archaea relationship remains poorly understood.

An inverse relationship between phytoplankton productivity and crenarchaeal abundance has been reported for different modern aquatic systems (e.g., Antarctic Ocean [Murray et al. 1998], Santa Barbara Channel [Murray et al. 1999], and North Sea [Herfort et al. 2007]). This suggests that Crenarchaea do not necessarily feed on newly produced organic matter (e.g., extracellular organic carbon), although they may use their decomposition products (e.g., ammonia) to fuel their activity. It is possible that the ammonium-oxidizing Crenarchaea may be direct competitors with phytoplankton for ammonium (Martens-Habbena et al. 2009), because ammonium concentrations and crenarchaeal abundance were negatively correlated in an enrichment culture (Wuchter et al. 2006*a*). In contrast, Wuchter et al. (2006*b*) showed that the seasonal abundances of Crenarchaea and phytoplankton biomarkers are coupled in sediment-trap material from the Northeast Pacific and Arabian Sea. In lake sediments, Bechtel et al. (2010) also found higher crenarchaeol concentrations for periods of high phytoplankton productivity.

Therefore, it remains debatable whether Crenarchaea are dependent on phytoplankton productivity and whether the derived organic matter is an important carbon or energy resource. Here we show that crenarchaeal and phytoplankton biomarkers in sedimentary archives display a significant coupling over centennial and millennial timescales. These sedimentary archives integrate much larger time spans than modern water-column and sediment-trap studies and, therefore, overcome any short-term decoupling processes, such as Crenarchaea not feeding directly on phytoplankton-released compounds but on decomposition or recycled products. We use chlorins, the diagenetic transformation products of chlorophyll, as a marker of total phytoplankton productivity (Harris et al. 1996), and crenarchaeol as a marker for the abundance of mesophilic Crenarchaea (Sinninghe Damsté et al. 2002*b*).

Methods

Study areas and core descriptions—The sedimentary archives discussed in this study were selected opportunis-

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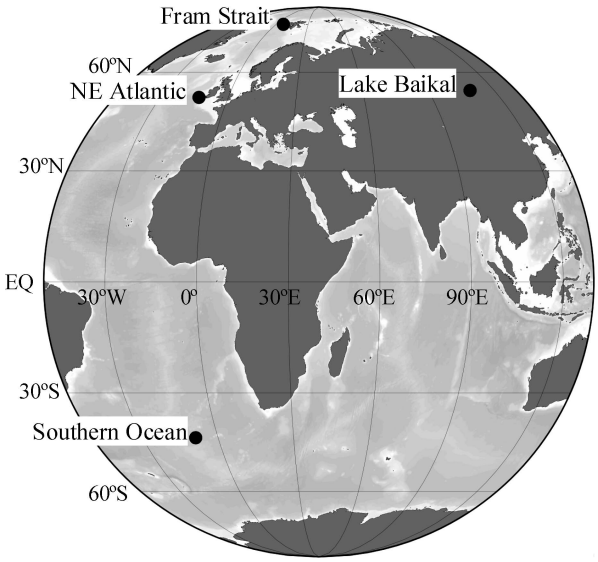

Fig. 1. Sample location map, including Ocean Drilling Program (ODP) Leg 177 drilling site in the Southern Ocean, MD01-2461 drilling site in the Northeast (NE) Atlantic, MSM05/05-712 site in the Fram Strait, and Lake Baikal's Northern Basin (Continent Project drilling site CON01-603).

tically while being the target of parallel paleoclimatological and paleoecological investigations by the authors.

Lake Baikal is located in Siberia, Russia (Fig. 1). It is the deepest and one of the largest freshwater lakes of the world, and a unique ecosystem with many endemic species. The lake surface freezes for 4–6 months each year, but the water column remains oxygenated throughout. It has been estimated that in summer $>$ 50% of the chlorophyll *a* (Chl *a*) may result from autotrophic picoplankton, but overall diatoms form the dominant phytoplankton group, occasionally forming extensive blooms (Popovskaya 2000). Diatoms also dominate the sinking phytodetritus (Fietz et al. 2005). Sediment core CON01-603-3 was retrieved from the North Basin (53°57′N, 108°55′E; Fig. 1) at 386-m water depth. The core covers a time span from ~ 107 to 130 thousand yr (kyr) before present, which means that it includes an interglacial period (roughly corresponding to marine isotope stage [MIS] 5). The age model is based on magnetostratigraphy dating on a parallel core and both cores are correlated by comparing their high-resolution density profiles (*see* Fietz et al. 2007).

The record from the Atlantic Southern Ocean analyzed in this study (PS2489-2) is located in the present day Subantarctic Zone, between the Subantarctic and Subtropical Fronts (42°52′S, 8°58′E; Fig. 1), and retrieved from 3700-m water depth (Martínez-Garcia et al. 2009). The site is characterized by relatively low phytoplankton export production during interglacial periods and high export production during glacial stages, essentially stimulated by atmospheric supply of iron (Martínez-Garcia et al. 2009). The record studied covers a time span from the mid-Pleistocene to the Holocene, encompassing several glacial and interglacial cycles (MIS 1 to MIS 12, ~ 500 kyr before present). In this study, we use the age model published by Martínez-Garcia et al. (2009) for core PS2489-2.

Core MD01-2461 was collected from the northwestern flank of the Porcupine Seabight in the Northeast (NE) Atlantic (51°45′N, 12°55′W; Fig. 1) at a water depth of 1153 m. The area is characterized by seasonal phytoplankton succession involving diatom dominance in the early spring. Downward fluxes of organic matter, including chlorophyll and its transformation products, peak in spring and early summer (Fabiano et al. 2001). The analyzed core section spans from 24.5 kyr to 14.5 kyr before present, across the last glacial maximum and Heinrich events 1 and 2 (H1 and H2). The age model was based on 15 monospecific foraminifera accelerator mass spectrometry (AMS) ^{14}C dates, and by correlation of the relative abundance of the polar planktonic foraminifera *Neogloboquadrina pachyderma* sin. to the second Greenland Ice Sheet Project (GISP II) δ^{18}O ice record (Peck et al. 2006).

Core MSM05/05-712-1 was retrieved from the Fram Strait in the western continental margin of Svalbard at 1490-m water depth (78°55′N, 6°46′E; Fig. 1). Most of the heat and mass exchange between the North Atlantic and the Arctic Ocean occur through the Fram Strait because it is the passageway through which freshwater and sea ice are exported southward and warm saline waters are transported northward. The pelagic community structure is strongly influenced by the Atlantic water inflow and the sea-ice extent in this region (Hop et al. 2006). Diatoms dominate the spring bloom and the primary production on an annual basis (Hop et al. 2006). The core from the Fram Strait spans approximately the last 2000 yr. The age model for core MSM05/05-712-1 is based on five AMS ^{14}C age measurements (Spielhagen et al. 2011).

Total lipid extraction—One to two grams of homogenized freeze-dried sediment were extracted by microwave-assisted extraction using a mixture of dichloromethane : methanol (3 : 1, v : v). The temperature of the microwave was increased to 70°C over 5 min, held at 70°C for 5 min, and then allowed to cool down to 30°C. Extracts were filtered through a glass pipette filled with sodium sulphate to remove water, then taken to dryness under nitrogen flow and stored frozen (−20°C) until further analysis.

Chlorin analysis—For the Southern Ocean, Fram Strait, and NE Atlantic records, the total dry extracts were redissolved in acetone and analyzed using a Thermo high-performance liquid chromatography (HPLC) system using acetone as eluent. A 17-cm flow restrictor (Pickering Laboratories) was used to stabilize the pressure. The HPLC was operated in off-column mode with an isocratic flow of 1 mL min^{-1}. The total abundance of chlorins was measured as absorbance at 662 nm using a photodiode array detector (Thermo Surveyor). Pyropheophorbide *a* was used as external standard for quantification. The remaining extracts were taken to dryness under a gentle stream of nitrogen, sealed and stored frozen (−20°C) until further fractionation. Chlorophyll transformation products in the Lake Baikal record were measured using a different procedure in an earlier study, and the methods are described below after lipid fractionation and crenarchaeol analysis descriptions.

Lipid fractionation—The Lake Baikal and NE Atlantic extracts were fractionated with preparative column chromatography using activated alumina and a sequential eluent mixture of hexane : dichloromethane (9 : 1, v : v) and dichloromethane : methanol (1 : 1, v : v [Sinninghe Damsté et al. 2002*b*; Escala et al. 2009]). The Fram Strait extracts were redissolved in 100 μL hexane : dichloromethane (50 : 50, v : v) prior to manual injection in a Thermo Surveyor HPLC system equipped with a Lichrosphere Silicon dioxide column (4.6 × 250 mm, 5 μm; Teknokroma). Compound class fractionation was achieved by elution with n-hexane (0–2.7 min), dichloromethane (2.7–5.7 min), acetone (5.7–9.2 min), and n-hexane (9.2–13.5 min) with a 2-mL min^{-1} flow. The respective fractions containing the crenarchaeol were evaporated and stored at −20°C until analysis. The Southern Ocean record extracts were pure enough to avoid interferences in the mass spectra and were analyzed for crenarchaeol without further fractionation.

Crenarchaeol analysis—The dry fractions were redissolved in hexane : n-propanol (99 : 1, v : v) and filtered through 0.50-μm PTFE filters (Advantec). A Dionex P680 HPLC system coupled to a Thermo Finnigan TSQ Quantum Discovery Max quadrupole mass spectrometer with an atmospheric pressure chemical ionization (APCI) interface was used. The samples were eluted in a Tracer Excel CN column (0.4-cm diameter, 20-cm length, 3-μm particle size; Teknokroma) equipped with a precolumn filter and a guard column. The solvent program is modified from Schouten et al. (2007; *see* Escala et al. 2009). Samples were eluted with hexane : n-propanol at 0.6 mL min^{-1}. The amount of n-propanol was held at 1.5% for 4 min, increased gradually to 5.0% during 11 min, then increased to 10% during 1 min and held at 10% for 4 min, then decreased to 1.5% during 1 min and held at 1.5% for 9 min until the end of the run. The parameters of the APCI were set as follows to generate positive ion spectra: corona discharge 3 μA, vaporizer temperature 400°C, sheath gas pressure 49 mTorr, auxiliary gas (N_2) pressure 5 mTorr, and capillary temperature 200°C. Crenarchaeol was monitored in selected ion-monitoring mode at *m*/*z* 1292. The synthetic tetraether lipid GR was used as external standard. Compound GR has a *m*/*z* of 1208, a structure typical of neutral archaeal membrane lipids, and presumably does not occur in the environment (Réthoré et al. 2007).

Lake Baikal chlorin and organic carbon analyses—Chlorin and total organic carbon (TOC) analyses for the Lake Baikal record were carried out on parallel samples (from the same core) to the above-described crenarchaeol analyses within an earlier study at Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany (Fietz et al. 2007). Shortly, for chlorins, the freeze-dried sediment was extracted with dimethylformamide and an ion pairing reagent (Fietz et al. 2007). Pigments were separated through a non–end-capped Waters Resolve C18 column with a gradient system from a solvent mixture consisting of methanol, acetonitrile, and the ion pairing reagent to a solvent mixture consisting of acetonitrile and

Table 1. Chlorins (Chl.) vs. Crenarchaeol (Cren.): fits (shown in Fig. 2), concentrations, and ratios. To calculate the best fits for the NE Atlantic and Fram Strait, the three-point running averages were considered (cf. Figs. 5B and 6B), while the original data set was used for all sites to calculate chlorins and crenarchaeol average concentrations and ratios in order to assess the full extent of variation. Coefficients of determination (r^2) are given additionally for the non–log-transformed, linear fits (not shown in Fig. 2). Spearman's rank correlations were calculated for all records on the non–log-transformed data and resulted in significant correlations ($p < 0.001$). The respective ranges for chlorin and crenarchaeol concentrations and ratios are plotted in Figs. 3–6. Abbreviations: Av = average, SD = standard deviation, CV = coefficient of variation, G = average over glacial periods, IG = average over interglacial periods.

Region	Best fit	Chl. (μg g^{-1}) Av±SD	Cren. (μg g^{-1}) Av±SD	Chl. : Cren. Av±SD (CV)
Lake Baikal	log(Cren.)=0.46×log(Chl.)−0.68, r^2=0.90	1.09±1.25 (G: 0.07; IG: 2.00)	0.40±0.40 (G: 0.06; IG: 0.71)	1.56±1.60(1.0); (G: 0.42; IG: 2.54)
	Cren.=0.28×Chl.+0.10, r^2=0.74, n=72, p<0.001	—	—	—
Southern Ocean	log(Cren.)=0.89×log(Chl.)−1.20,r^2=0.78	0.20±0.36 (G: 0.40; IG: 0.01)	0.07±0.10 (G: 0.14; IG: 0.004)	3.88±6.00(1.5); (G: 2.92; IG: 4.85)
	Cren.=0.20×Chl.+0.03, r^2=0.59, n=125, p<0.001	—	—	—
NE Atlantic	log(Cren.)=1.37×log(Chl.)+0.19, r^2=0.65	0.14±0.07	0.09±0.05	1.70±0.73(0.4)
	Cren.=0.64×Chl.−0.003, r^2=0.63, n=65; p<0.001	—	—	—
Fram Strait	log(Cren.)=0.52×log(Chl.)−1.42, r^2=0.55	2.64±0.70	0.40±0.10	6.71±1.58(0.2)
	Cren.=0.08×Chl.+0.18, r^2=0.58, n=41; p<0.001	—	—	—

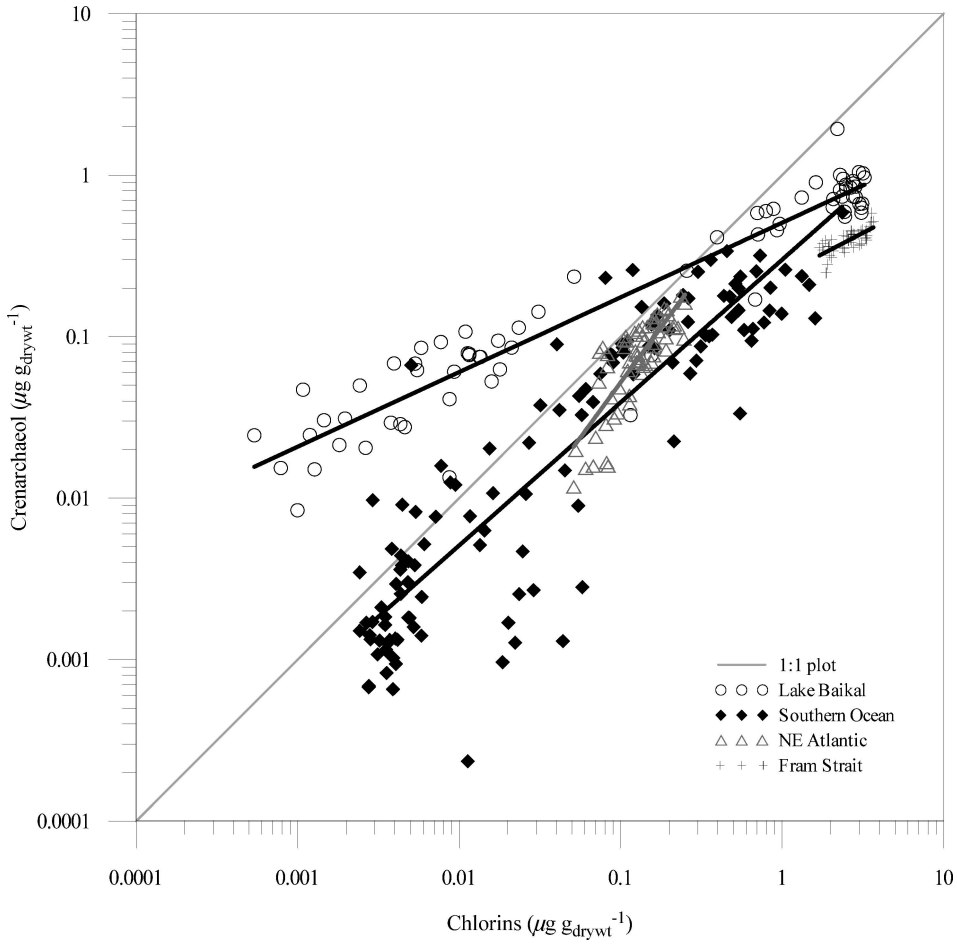

Fig. 2. Correlation between chlorins (chlorophyll transformation products) and crenarchaeol in the four sedimentary records. Sites are shown in Fig. 1 and corresponding power fits given in Table 1. Abbreviation: g_{drywt} = grams dry weight.

acetone. The eluting peaks were monitored using a photodiode array detector and a fluorescence detector. All Chl *a* transformation products singled out in the Fietz et al. (2007) study were combined in the present study into the term 'chlorins' (Harris et al. 1996). The TOC was analyzed with a Vario EL CHNOS elemental analyzer (Elementar Analysesysteme GmbH, Germany) after acidification with 0.2 N hydrochloric acid (HCl) and drying at 105°C in order to remove inorganic carbon (Fietz et al. 2007).

Results

Site intercomparison—The average chlorin and crenarchaeol concentrations in the four sites range over one order of magnitude (0.14–2.64 μg g^{-1} and 0.07–0.40 μg g^{-1}, respectively), with the Lake Baikal and Fram Strait sites having much higher concentrations than the Southern Ocean and NE Atlantic sites (Table 1). Intriguingly, all records show a significant ($p < 0.001$) positive correlation between chlorins and crenarchaeol (Table 1; Fig. 2). The strongest correlation between chlorins and crenarchaeol is found in Lake Baikal, where the chlorin : crenarchaeol ratio is lowest (Table 1). In the Fram Strait, in contrast, where the chlorin : crenarchaeol ratio is highest, the coupling is weaker (Table 1). The correlation is based on the non-transformed data (Spearman's rank correlation, r_s), but data are log-transformed for visualization in Fig. 2. The slopes of these power fits vary greatly among the four studied systems; the NE Atlantic has the highest slope value, while Lake Baikal and Fram Strait have the lowest (Table 1). These slopes indicate the proportional change of the crenarchaeol compared to the chlorin concentrations (i.e., the higher the slope the higher the proportional change of the Crenarchaea abundance compared to the phytoplankton productivity).

Site-specific evolution over time—In Lake Baikal, the core spans from ~ 107 kyr to 130 kyr before present, including an interglacial period (corresponding to MIS 5) from ~ 113 kyr to 126 kyr before present and parts of the preceding and following glacial periods. Average chlorin concentrations are 2.7 times higher than average crenarchaeol concentrations (1.09 μg g^{-1} and 0.40 μg g^{-1}, respectively; Table 1), but both show a similar evolution over time (r_s = 0.99; Fig. 3A). Chlorin concentrations increase

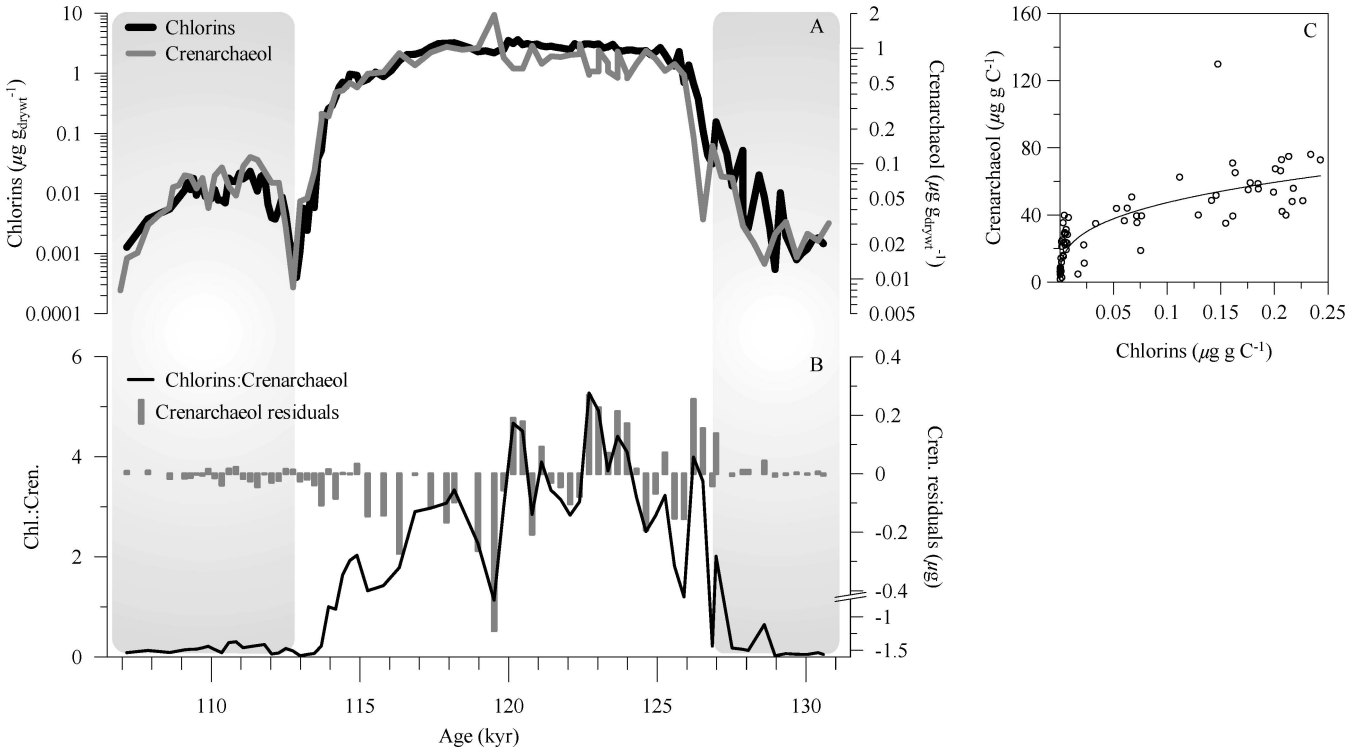

Fig. 3. Coupling of chlorins and crenarchaeol in Lake Baikal. (A) chlorins and crenarchaeol concentrations per gram dry weight; (B) chlorins : crenarchaeol concentrations ratio and calculated crenarchaeol residuals. The crenarchaeol residuals were calculated subtracting the measured crenarchaeol from the calculated one using the regression equation of chlorins vs. crenarchaeol concentrations shown in Fig. 2 and given in Table 1: $Cren_{residual}$ = [exp (0.46 × log($Chl_{measured}$) − 6.78)] − [$Cren_{measured}$]. Positive residuals indicate overestimation of calculated crenarchaeol based on chlorins; negative residuals indicate underestimation. Shaded areas indicate transitions to glacial periods. (C) Correlation between chlorins and crenarchaeol concentrations per gram organic carbon (r^2 = 0.72).

from an average of 0.065 μg g^{-1} during the preceding and subsequent glacial periods to an average of 2.0 μg g^{-1} during the interglacial, and crenarchaeol concentrations from 0.056 μg g^{-1} to 0.71 μg g^{-1} (Fig. 3A; Table 1).

The low slope value (0.46; Table 1) indicates that the average change of Crenarchaea abundance is smaller than the change of phytoplankton productivity. The power regression between the chlorin and crenarchaeol concentrations (Fig. 2; Table 1) allows the calculation of the residuals of the regression that is the deviation of a particular point from the regression line or its predicted value. In Lake Baikal, the residuals are positive for crenarchaeol concentration when the chlorin : crenarchaeol ratios increase and the residuals are negative when the chlorin : crenarchaeol ratios decrease (Fig. 3B). This provides further evidence that crenarchaeal abundance changes were less pronounced than changes of phytoplankton productivity. The significant correlation persists whether both biomarkers are plotted in concentration relative to dry weight of sediment, or normalized to TOC (Fig. 3C).

The Southern Ocean record spans from the mid-Pleistocene to the Holocene, encompassing several glacial and interglacial cycles (MIS 1 to MIS 12; Fig. 4A). The correlation between chlorins and crenarchaeol concentrations is high (r_s = 0.88, $p < 0.001$), but in contrast to Lake Baikal, much higher concentrations are recorded for both markers during the glacial periods instead of the interglacials (Fig. 4A; Table 1). The chlorin : crenarchaeol ratio is also higher (4.85) during the productive glacial periods compared to the unproductive interglacial periods (2.92; Table 1) and peak chlorin : crenarchaeol ratios are found mainly during deglaciations (Fig. 4B). The larger positive residuals from the power fit lay within the productive glacial periods, but not at times of peak chlorin : crenarchaeol concentration ratios during the deglaciations (Fig. 4B).

During the investigated late Pleistocene period in the NE Atlantic, off the Great Britain coast, common trends are indicated by both markers when a three-point running average is performed on the time series (Fig. 5A; r_s = 0.78, $p < 0.001$). Such a running average reduces the resolution of the record and highlights the underlying trend over the high-frequency signal (Fig. 5A). Chlorins and crenarchaeol both show a series of increasing and decreasing trends over time, notably a considerable shift in concentrations at ~ 24.5 kyr before present and then a peak between ~ 23.5–22.5 kyr before present (Fig. 5A). The chlorin : crenarchaeol ratio is similarly low (1.70; Table 1) than the one found in Lake Baikal and much lower than in the Southern Ocean (Table 1; Fig. 5B). The slope of the power regression, in contrast, is the highest of our four records (1.37; Table 1), indicating that at this site the crenarchaeal abundance changes were more pronounced than those of

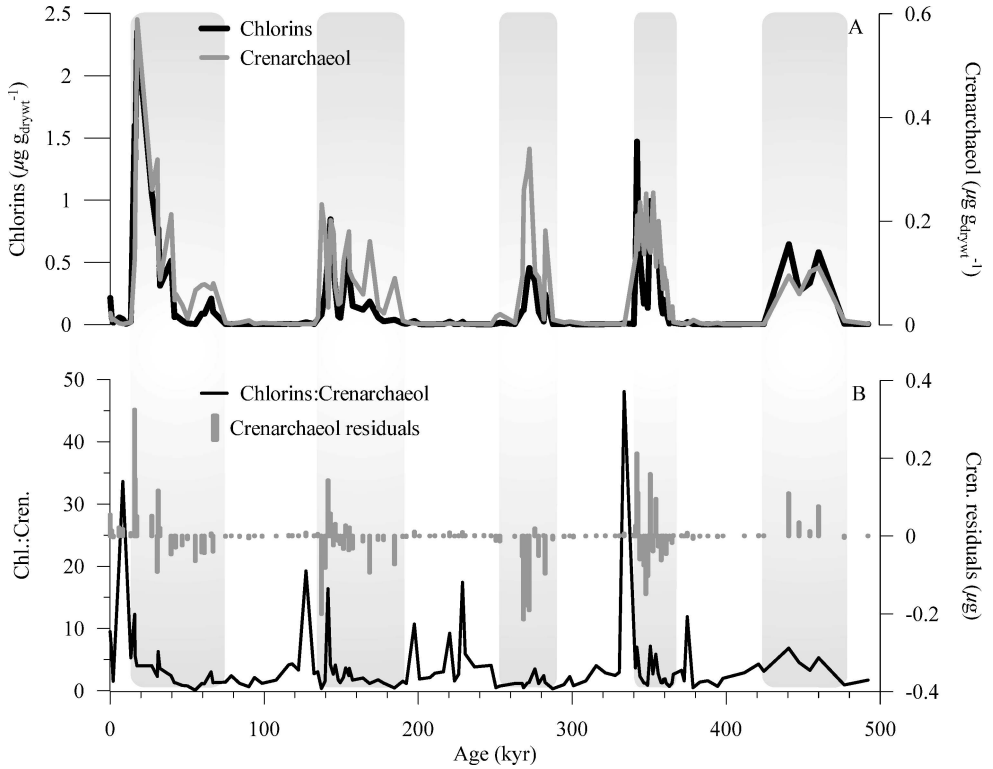

Fig. 4. Coupling of chlorins and crenarchaeol in the Southern Ocean. (A) chlorins and crenarchaeol concentrations per gram dry weight; (B) chlorins : crenarchaeol concentrations ratio and calculated crenarchaeol residuals (*see* Fig. 3 legend for residual calculation). Shaded areas indicate glacial periods.

the phytoplankton productivity. As in Lake Baikal, the residuals (Fig. 5B) in the NE Atlantic record are negative when the chlorin : crenarchaeol ratio is low (Fig. 5B).

Common trends are also indicated by both markers in the relatively modern record of the Fram Strait when a three-point running average is performed (Fig. 6A; r_s = 0.75, $p < 0.001$). This site has the highest chlorin and crenarchaeol concentrations but the lowest variability (Table 1; Fig. 6B). The strongest increase of both markers occurred between ~ 1000 yr and 700 yr ago (Fig. 6A). A second pronounced peak in both markers was found ~ 200 yr ago (Fig. 6A). The chlorin : crenarchaeol ratio is highest in the Fram Strait site (6.71; Table 1), while the power slope is low (0.52; Table 1), indicating again less pronounced changes of Crenarchaea abundance compared to those of the phytoplankton productivity.

Discussion

The present study considers sedimentary records from both freshwater and marine systems, from high-latitude to temperate climatic regions, and spanning glacial and interglacial periods. Despite this wide range of conditions, we observe a pervasive, statistically significant coupling between the sedimentary concentration of phytoplankton and Crenarchaea biomarkers in all investigated sites. This close coupling could arguably be attributed to depositional processes of the organic matter, or related to a common environmental trigger, or to a metabolic dependence between the two types of organisms.

Settling and degradation—Evidence of a close coupling between crenarchaeol and phytoplankton export production was previously reported in sediment traps from the Northeast Pacific and the Arabian Sea (Wuchter et al. 2006*b*). Wuchter et al. (2006*b*) proposed that this coupling reflected a depositional process of entrainment or scavenging of Crenarchaea cells and their biomarkers by the settling phytoplankton and fecal pellets. However, it is unlikely that this process can explain by itself the strong correlations that we observe at all four sites. If, for example, the crenarchaeal abundance in the water column decreases over a given time span, an increasing trend of phytoplankton export cannot result in an increasing Crenarchaea (and their biomarker) export over that time span. Even if crenarchaeal abundance remains constant over a certain time span, an increasing trend of phytoplankton export can only result in an increasing Crenarchaea export if there is always an excess of crenarchaeal cells that can be scavenged by the settling phytoplankton. However we find co-variation in all four sites with very different chlorin and crenarchaeol concentrations as well as chlorin : crenarchaeol ratios (Table 1).

Furthermore, different biomarkers have different preservation potentials depending on their chemical structure (Sinninghe Damsté et al. 2002*a*). Comparing crenarchaeol

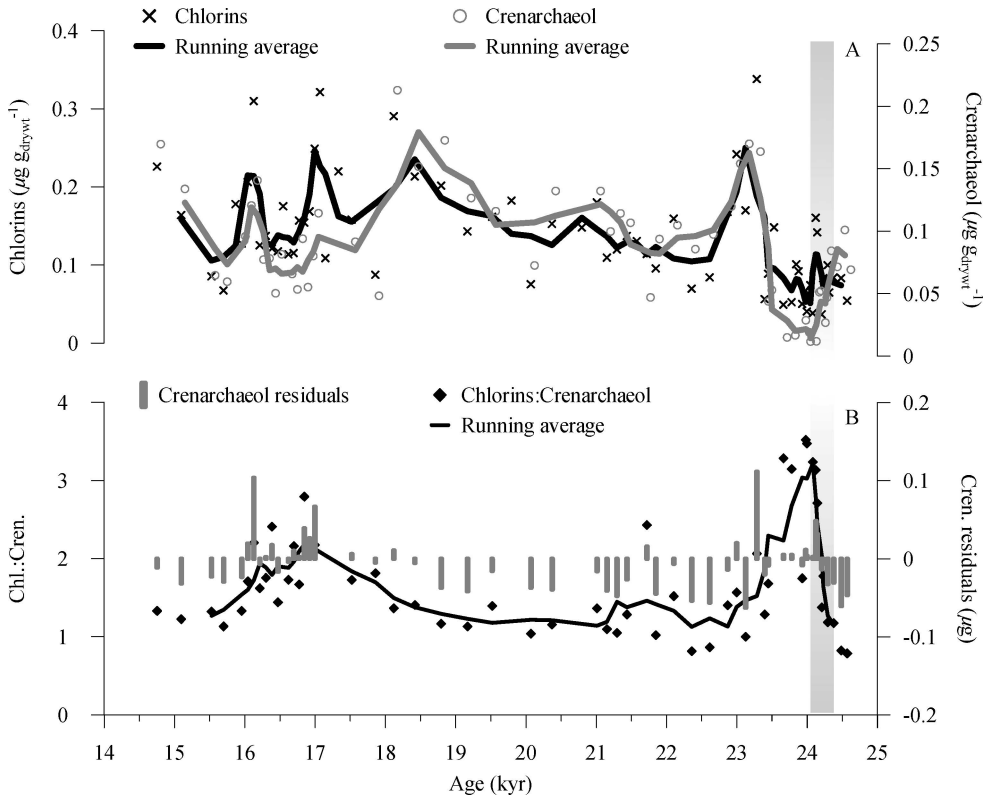

Fig. 5. Coupling of chlorins and crenarchaeol in the NE Atlantic. (A) chlorins and crenarchaeol concentrations per gram dry weight; (B) chlorins : crenarchaeol concentrations ratio and calculated crenarchaeol residuals (*see* Fig. 3 legend for residual calculation). (A, B) Symbols correspond to raw data and lines to simple three-point running averages. Shaded area indicates Heinrich event 2.

and chlorins, the isoprenoid chain, macrocyclic structure, and ether bonds of the crenarchaeol should guarantee their better preservation. In fact, chlorins are more strongly degraded than crenarchaeol in Lake Baikal's oxic surface sediments (Fietz et al. 2005; M. Escala, unpubl.). Potential differential degradation of the two markers would, hence, most likely result in decreased coupling between crenarchaeol and chlorins in the sediment record. Furthermore, the correlation between chlorins and crenarchaeol persists if normalized to the organic matter content (Fig. 3C), which accounts for differential degradation during certain periods. Consequently, settling and degradation could certainly play a role, but could not solely explain the tight coupling observed in our lake and ocean sediments. The correlation observed must also be due to a dependence of Crenarchaea on resources released by phytoplankton or that phytoplankton and Crenarchaea respond simultaneously to a common environmental factor.

Crenarchaea and phytoplankton respond to a common environmental trigger—The coupling of the two biomarker abundances in the sedimentary records could be driven by a common process, which could, for instance, influence temperature or nutrient availability. A climatic factor such as water temperature is debatable, because several modern monitoring studies have shown that Crenarchaea are often more abundant during winter months (Murray et al. 1998; Herfort et al. 2007), while in other environments Crenarchaea are less abundant in winter than during the rest of the year (Alonso-Sáez et al. 2007), indicating that the seasonal patterns are site dependent. Also, in our records, Crenarchaea and phytoplankton both increase during the warm interglacial in Lake Baikal and during the cool glacial periods in the Southern Ocean, showing that temperature changes cannot be the direct stimulating environmental factor, and that probably another trigger, such as nutrient input into the aquatic system, explains the correlation.

Könneke et al. (2005) were the first to show that marine mesophile Crenarchaea can grow chemoautotrophically by aerobically oxidizing ammonia to nitrite. Subsequently, several studies have reported the widespread presence of mesophile archaeal ammonium-oxidizers (Francis et al. 2005; Wuchter et al. 2006*a*; Church et al. 2010). These archaeal ammonium-oxidizers could be assumed to be metabolically dependent on phytoplankton ammonia release. However, they might also successfully compete with phytoplankton wherever nitrogen is a limiting factor, due to their high affinity for nitrogen (Martens-Habbena et al. 2009) and would then be metabolically independent. The distribution of ammonium-oxidizing Crenarchaea is, indeed, suggested to be determined by nutrient concentrations, especially ammonium supply (Wuchter et al. 2006*a*; Herfort et al. 2007). In Lake Baikal, warming is probably

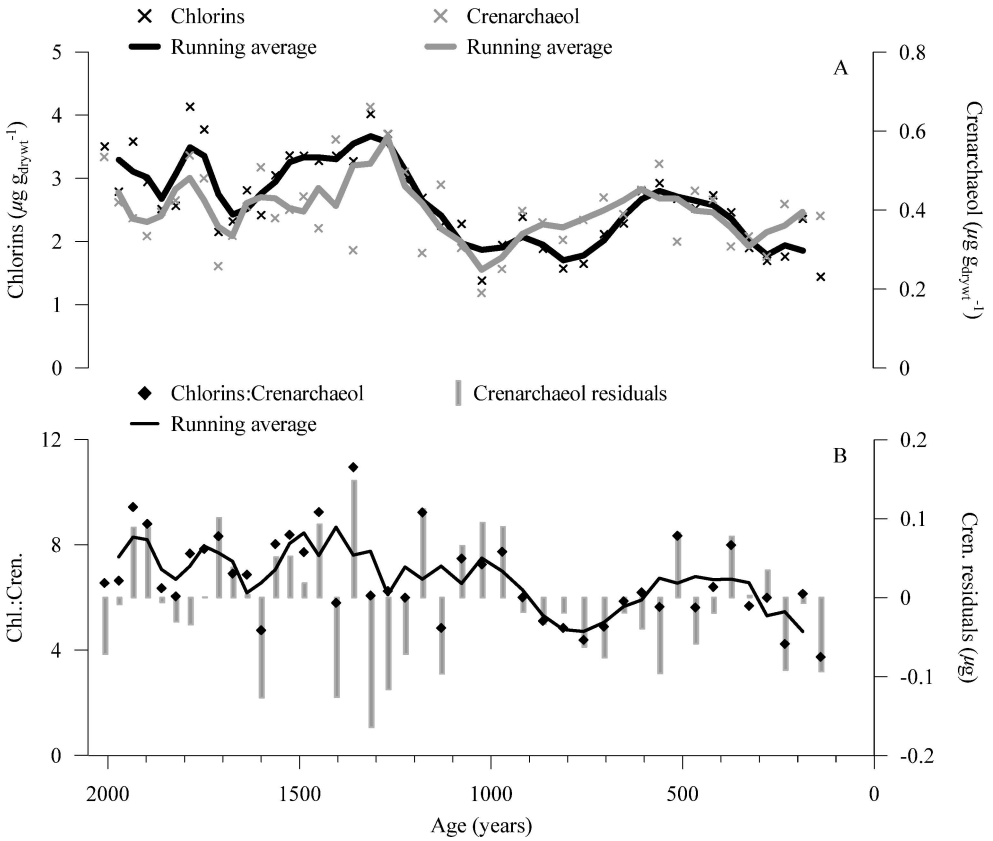

Fig. 6. Coupling of chlorins and crenarchaeol in the Fram Strait. (A) chlorins and crenarchaeol concentrations per gram dry weight; (B) chlorins : crenarchaeol concentrations ratio and calculated crenarchaeol residuals (*see* Fig. 3 legend for residual calculation). (A, B) Symbols correspond to raw data and lines to simple three-point running averages.

not sufficient to explain phytoplankton export productivity peaks, and nutrient availability is thought to be an important factor (Fietz et al. 2007). However, in the Southern Ocean, major nutrient concentrations are perennially high and the increase of phytoplankton export productivity during glacial stages is directly linked to an increase in iron availability (Martínez-Garcia et al. 2009). Assuming a common nutrient trigger would, therefore, require that mesophile Crenarchaea are also iron-limited, which so far has not been described. Hence, the observed correlation between phytoplankton and Crenarchaea biomarkers is unlikely to be solely explained by a common trigger related to temperature or nutrient availability.

Crenarchaea depend on resources released by phytoplankton—Such dependence can be explained by Crenarchaea having a heterotrophic metabolism that would use phytoplankton-derived organic carbon (Ouverney and Fuhrman 2000; Teira et al. 2006) or by Crenarchaea having a chemoautotrophic metabolism using phytoplankton decomposition products, such as ammonium, as energy source (Könneke et al. 2005). Nitrifying bacteria have been found to be associated to particles so that microbial-mediated nitrification rates increase with suspended particles concentrations (Karl et al. 1984). It is possible that ammonium-oxidizing chemoautotrophic Crenarchaea might also be attached to degrading phytoplankton particles using up the released ammonium. If Crenarchaea are heterotrophs, ammonium-oxidizing chemoautotrophs, or majorly mixotrophs (Hallam et al. 2006; Ingalls et al. 2006), crenarchaeal abundance changes might follow phytoplankton productivity patterns with a delay if Crenarchaea do not rely on resources released by exudation but by cell lysis or indirectly by egestion from phytoplankton grazers. Such delay, or seasonal succession, might cause the decoupling found between phytoplankton and Crenarchaea in snapshot water-column samples, but will be smoothed out in our time-integrated sedimentary material. This could explain the apparent contradiction between the strong correlations we find in our sedimentary archives and the decoupling found in the water-column and sediment-trap studies (Murray et al. 1998, 1999; Herfort et al. 2007).

However, in Lake Baikal, where the coupling is tightest, the phytoplankton productivity changes captured in the sediment are much more pronounced than those of the Crenarchaea abundance, and the chlorin : crenarchaeol ratio strongly increases during the interglacial. This indicates that Crenarchaea abundance is probably dependent on phytoplankton released resources, but is additionally limited by environmental factors. Furthermore, in the Southern Ocean, the phytoplankton–Crenarchaea correlation is high and the Crenarchaea response to phytoplank-

ton changes strong, which may indicate adequate supply of phytoplankton-derived carbon or ammonium to the Crenarchaea at this site. However, the chlorin : crenarchaeol ratio in the Southern Ocean peaked three times during the productive deglaciation periods, which may suggest, as in Lake Baikal, that Crenarchaea, on occasions, are limited by other factors than only phytoplankton-derived resources. Also, at the onset of the H2-event in the NE Atlantic, Crenarchaea seem to be more severally affected than phytoplankton by the sudden changes of the environmental conditions or by the ice-rafted debris input.

Based on the sediment cores throughout the ocean and in Lake Baikal, phytoplankton and Crenarchaea strongly co-vary through time. There is also a decoupling at certain times in the sediment records, which suggests that the response of these organisms is complex. Rather than a common driving environmental factor, we propose that the major driving process is a cascading effect of environmental changes through phytoplankton release fuelling Crenarchaea growth. This effect might be amplified due to increased entrainment of crenarchaeal cells by phytoplankton debris.

Acknowledgments

We thank N. Moraleda and N. Núñez for technical assistance, and A. Nicklisch and H. Winkler for their contributions in getting the pigment data of the Baikal record. We gratefully thank the collaboration of the reviewers for their constructive comments. S.F. thanks the Deutsche Forschungsgemeinschaft (DFG) for a Postdoctoral Fellowship FI 1466/1-1.

Support for this work was provided by the Spanish Ministry of Science and Innovation (MICINN) as research funds (POL2006-02990, CGL2008-03288-E, CGL2010-15000), Formación del Profesorado Universitario (FPU) studentships to A.M.G. and G.R. (AP2004-7151 and AP2008-00801, respectively), Juan de la Cierva fellowships to S.F. and C.H., and a European Commission Marie Curie-IOF (235626) to A.R.M.

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Associate editor: Wade H. Jeffrey

Received: 11 January 2011
Accepted: 05 July 2011
Amended: 11 July 2011