Chapter 1

Introduction

1.1 Archaea: the third domain of life

Besides the Bacteria and the Eukarya, a third domain of life on Earth is formed by the Archaea [*Woese et al*., 1990]. The Archaea are prokaryotes like Bacteria and also have some genes and metabolic sequences in common. However, Archaea also share some similarities in the genome replication and expression systems with the Eukarya and, like the Eukarya, Archaea lack muramic acid in their cell wall [see *Woese et al*., 1990 and references therein]. The cell membrane of the Archaea is unique and differs from the cytoplasm membrane of both Bacteria and Eukarya (see 1.3).

Based on the composition of the 16S ribosomal (rRNA) gene the domain of the Archaea is subdivided in two major phyla, the Crenarchaeota and Euryarchaeota [*Woese et al*., 1990] and one smaller phylum the Korarchaeota [*Barns et al*., 1996] (Fig. 1). Traditionally Archaea were viewed as organisms that thrive under extreme conditions, such as anoxic, hypersaline, extremely warm (> 60 ºC) and acidic (< pH 2) environments [*Tindal et al*., 1992]. About a decade ago, however, phylogenetic analyses of environmental 16S rRNA genes showed that Archaea are far more widespread than previously thought and that they occur in more temperate environments [*Fuhrman et al*., 1992; *DeLong*, 1992]. This discovery changed the traditional view on the Archaea dramatically and over the following years, small-subunit archaeal rRNA genes and unique archaeal membrane lipids were retrieved from the pelagic realm of tropical, temperate and polar seas [*DeLong*, 1994; 1998; *Hoefs et al*., 1997; *Sinninghe Damsté et al*., 2002], marine sediments [*Vetriani et al*., 1999; *Schouten et al*., 2000], lake waters and sediments [*Keough et al*., 2003; *McGregor et al*., 1997; *Powers et al*., 2004], soils [*Buckley et al*., 1998; *Ochsenreiter et al.*, 2003; *Pesaro et al*., 2002] and peat bogs [*Weijers et al*., 2004]. Thus, Archaea are organisms occurring ubiquitously on this planet in a wide variety of settings ranging from ice seas to hydrothermal vents.

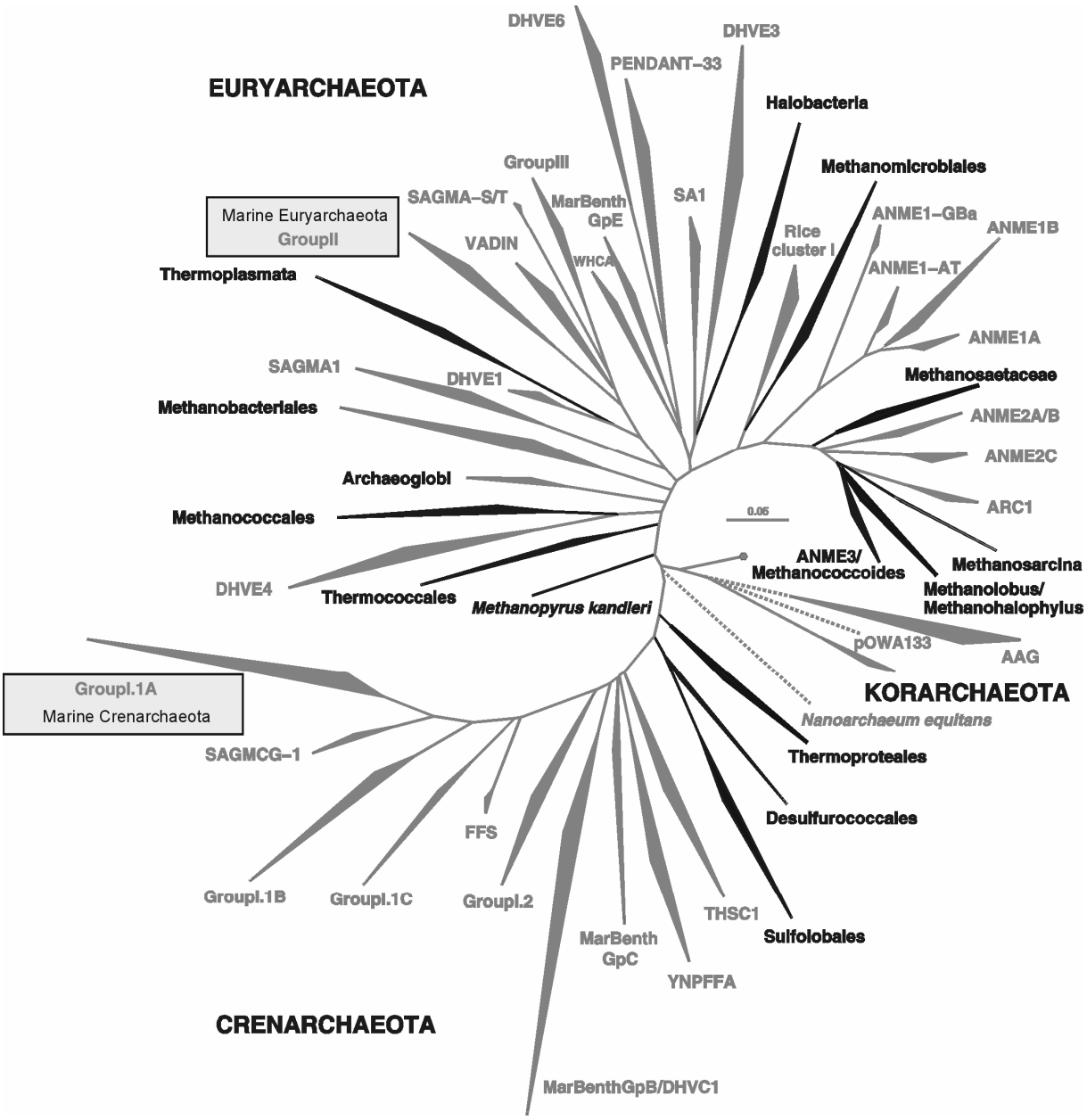

Figure 1. Phylogenetic tree based on the 16S rRNA gene of Achaea showing the three phyla Crenarchaeota, Euryarchaeota and Korarchaeota [after *Schleper et al.*, 2005]. Triangles in grey represent branches containing exclusively uncultivated species. The black triangles correspond to branches containing at least one cultivated species. Overall, the tree reveals the predominance of environmental clones representing groups for which no cultured close relatives are available. The marine Crenarchaeota group and the marine Euryarchaeota group are highlighted.

1.2 Ecology and physiology of marine mesophilic Archaea

Archaea in temperate aquatic environments appear to be widespread and a very abundant group. In the marine environment they form two major planktonic archaeal groups in the crenarchaeotal and euryarchaeotal phyla (Fig. 1). Recent studies revealed that marine Crenarchaeota comprise almost 20% of the picoplankton in the world oceans [*Karner et al*., 2001]. Generally, marine Crenarchaeota are relative more abundant than marine Euryarchaeota in deeper layers of the neritic waters and in the meso- and bathypelagic zone of the ocean [*DeLong et al*., 1999; *Karner et al*., 2001; *Church et al*., 2003; *Herndl et al*., 2005] (Fig. 2). In contrast, marine Euryarchaeota are relatively more abundant than the marine Crenarchaeota in surface waters of open oceans and coastal systems [*Murray et al*., 1999; *Massana et al*., 2000; *Pernthaler et al*., 2002].

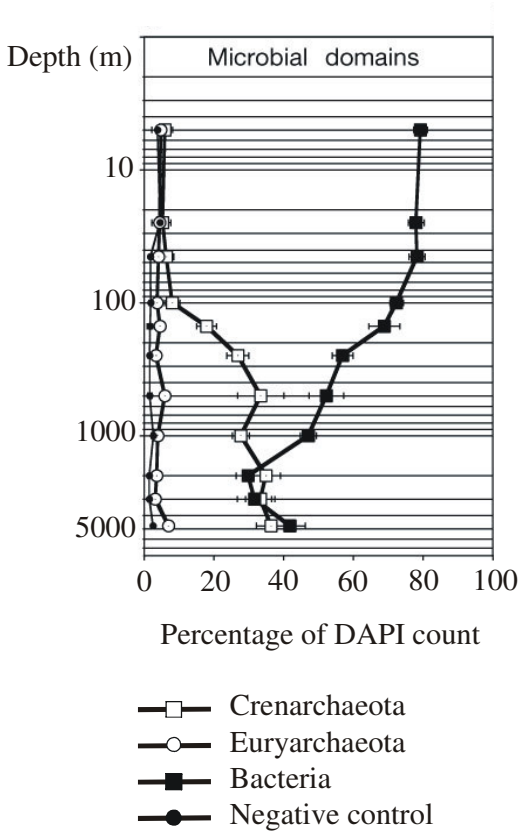

Figure 2. The mean annual depth profile of microbial domains from the North Pacific subtropical gyre obtained by fluorescence *in situ* hybridization [after *Karner et al*., 2001]. Depth profiles for bacteria (solid squares), pelagic Crenarchaeota (open squares), pelagic Euryarchaeota (open circles) and non-specific control probe ('negative', solid circles) are shown.

Despite the fact that marine Archaea constitute a substantial fraction of the world ocean picoplankton, little is known about the basic physiology of these organisms. A positive correlation between the abundance of Crenarchaeota and nitrite concentrations was reported from field studies in the Santa Barbara Channel [*Murray et al*., 1999], the Arabian Sea [*Sinninghe Damsté et al*., 2002] and with particulate organic nitrogen in Arctic waters [*Wells and Deming*, 2003]. This suggested that marine Crenarchaeota may in some way be involved in the marine nitrogen cycle.

Stable isotope analyses of the archaeal membrane lipids (see 1.3) showed that the $\delta^{13}C$ values of these lipids are relatively invariant in comparison to phytoplankton lipids [*Hoefs et al*., 1997; *Schouten et al*., 1998]. These findings suggested that marine Crenarchaeota may utilize bicarbonate as their carbon source [*Hoef et al*., 1997; *Kuypers et al*., 2001]. Radiocarbon analyses, done on biphytanes derived from sedimentary archaeal lipids, suggested that Archaea utilize "old" ^{14}C depleted dissolved inorganic carbon from below the photic zone in the water column [*Pearson et al*., 2001]. Direct experimental evidence for the metabolic requirements of pelagic Archaea is, however, limited. Microautoradiography showed that some marine Crenarchaeota and Euryarchaeota are capable to utilize bicarbonate or CO_2 as their carbon source [*Herndl et al*., 2005] and that some marine Archaea are capable to take up amino acids [*Overney and Fuhrman*, 2000, *Teira et al*., 2004]. Thus, the metabolic requirement of pelagic Archaea remains still enigmatic.

1.3 Archaeal membrane lipids and their application as paleothermometer

The Archaea biosynthesize unique membrane lipids compared to Bacteria and Eukarya. This has been, in addition to their unique gene composition, a major argument to propose the Archaea as the third domain of life [*Woese et al*., 1990]. While the core membrane lipids of Bacteria and Eukarya consist predominantly of fatty acids esterified to glycerol, the Archaea synthesize lipids with ether-linkages, which consist of long-chain isoprenoid diphytanyl glycerol diethers (DGDs) and glycerol dibiphytanyl glycerol tetraethers (GDGTs) (Fig. 3).

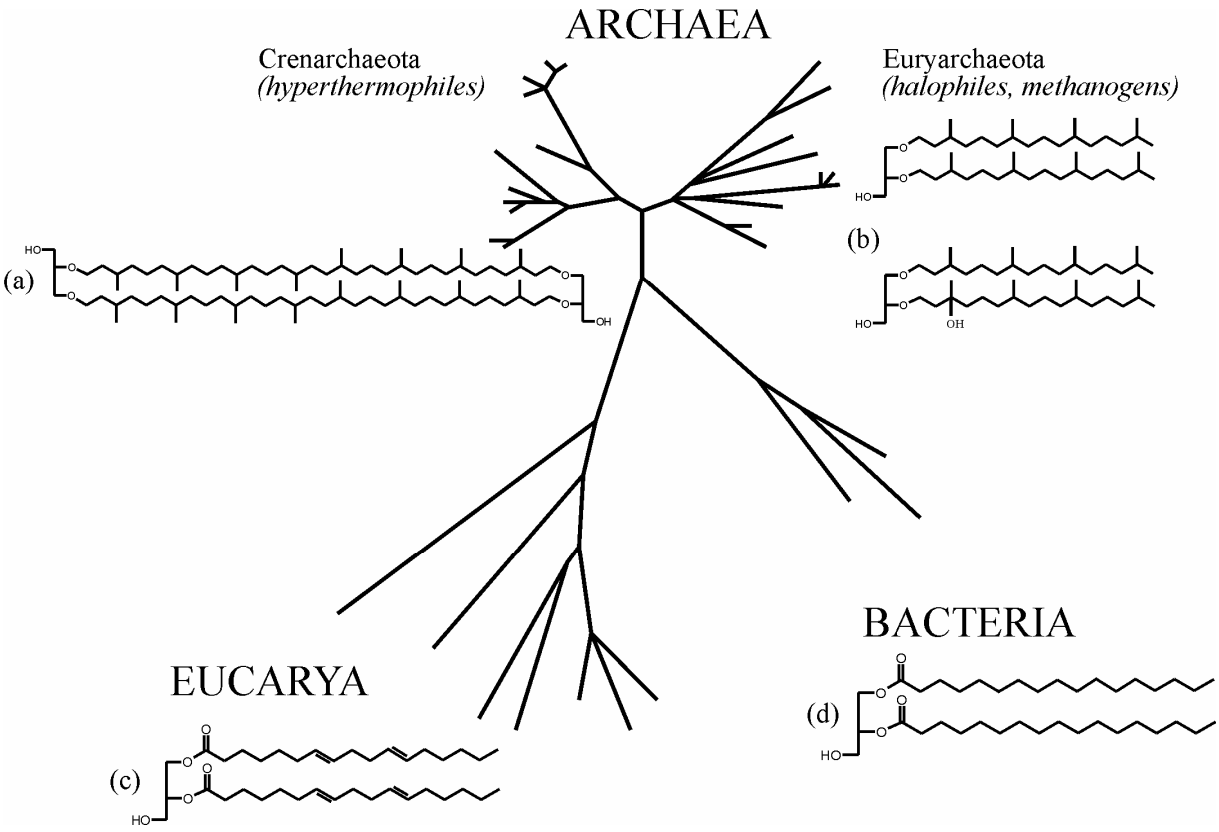

Figure 3. The different membrane lipid composition in the three domains of life (a) glycerol dibiphytanyl glycerol tetraethers, (b) diphytanyl glycerol diethers, (c) and (d) fatty acids glycerol diesters.

Some Archaea, especially the hyperthermophilic Crenarchaeota, biosynthesize only GDGTs, which form a lipid monolayer in the membrane. In the membrane this lipid monolayer is less sensitive to denaturation at high temperatures than a lipid bilayer composed from for example DGDs [*van de Vossenberg*, 1999 and references therein]. The GDGTs of Archaea can contain cyclopentane moieties (Fig. 4). Hyperthermophilic Crenarchaeota can increase the amount of cyclopentane ring-containing GDGTs as a response to changes in temperature [*Gliozzi et al*., 1983; *DeRosa and Gambacorta* 1988; *Uda et al*., 2001] to keep their cytoplasmic membrane at a liquid crystalline state and reduce their proton permeabilisation rate [*Albers et al*., 2000]. The biosynthesis of cyclopentane moieties in the membrane lipids of hyperthermophilic Archaea is, therefore, generally considered to be a temperature adaptation mechanism of the membrane.

Marine Crenarchaeota are close relatives to the hyperthermophilic Crenarchaeota and chemical degradation studies suggested that marine Crenarchaeota also biosynthesize GDGTs

[*Hoefs et al*., 1997; *DeLong et al*., 1998]. A new high performance liquid chromatography mass spectrometry (HPLC/MS) method was developed to analyze intact core GDGT membrane lipids of Archaea [*Hopmans et al*., 2000] and this method allowed a rapid detection of intact GDGTs in all kinds of environmental samples. With this HPLC/MS method intact GDGTs were found in *Cenarchaeum symbiosum* [*Sinninghe Damsté et al*., 2002], in marine water samples [*Sinninghe Damsté et al*., 2002] and sediments [*Schouten et al*., 2002]. A specific GDGT which contains four cyclopentane rings and one cyclohexane ring was detected with this new method (Fig. 4). This GDGT is exclusively attributed to the mesophilic Crenarchaeota and was, therefore, named crenarchaeol [*Sinninghe Damsté et al*., 2002]. It has been suggested that marine Crenarcheota have evolved from hyperthermophilic Crenarchaeota by building an additional ”kink” in their membrane lipid, i.e. the cyclohexane ring [*Sinninghe Damsté et al*., 2002]. Crenarchaeol is thought to have lowered the transition point of the membrane lipids and allowed the Crenarcheota to live in temperate environments such as the marine water column.

Analyses of core top sediments from different geographic settings showed that the distribution of marine crenarchaeotal GDGTs varies with temperature [*Schouten et al*., 2002]. In surface sediments of warmer parts of the ocean the GDGT distribution is dominated by crenarchaeol and relatively higher amounts of the 1-3 cyclopentane-containing GDGTs [*Schouten et al*., 2002] (Fig. 4). The GDGT distribution in surface sediments from cold areas consisted almost completely of GDGT I and crenarchaeol. Thus, it seems that marine Crenarchaeota inherited a temperature adaptation mechanism for their cytoplasm membrane similar to that of their hyperthermophilic relatives.

The change in the GDGT distribution in core top sediments was expressed in an index of GDGT isomers [*Schouten et al*., 2002]. This index was named Tetraether Index of lipids with 86 carbon atoms (TEX_{86}) and is defined as followed:

$$TEX_{86} = (III+IV+VI)/ (II+III+IV+VI) \quad (1)$$

The correlation of this index to the annual mean sea surface temperature (SST) at the position of the surface sediments gave the following linear correlation:

$$TEX_{86} = 0.015 * T + 0.28, (r^2 = 0.92) \quad (2)$$

With T = annual mean SST (in °C) (Fig. 5). The membrane lipids of marine Crenarchaeota are relatively stable components, and are found in sediments up to 140 million years old [*Kuypers*

et al., 2001; *Carrillo- Hernandez et al*., 2003]. Thus, by analysis of ancient GDGT distribution the TEX_{86} may have the potential to calculate ancient SST and may allow reconstruction of climatic changes in the past. SST is one of the most important parameter needed to reconstruct ancient environments [*Fischer and Wefer*, 1999].

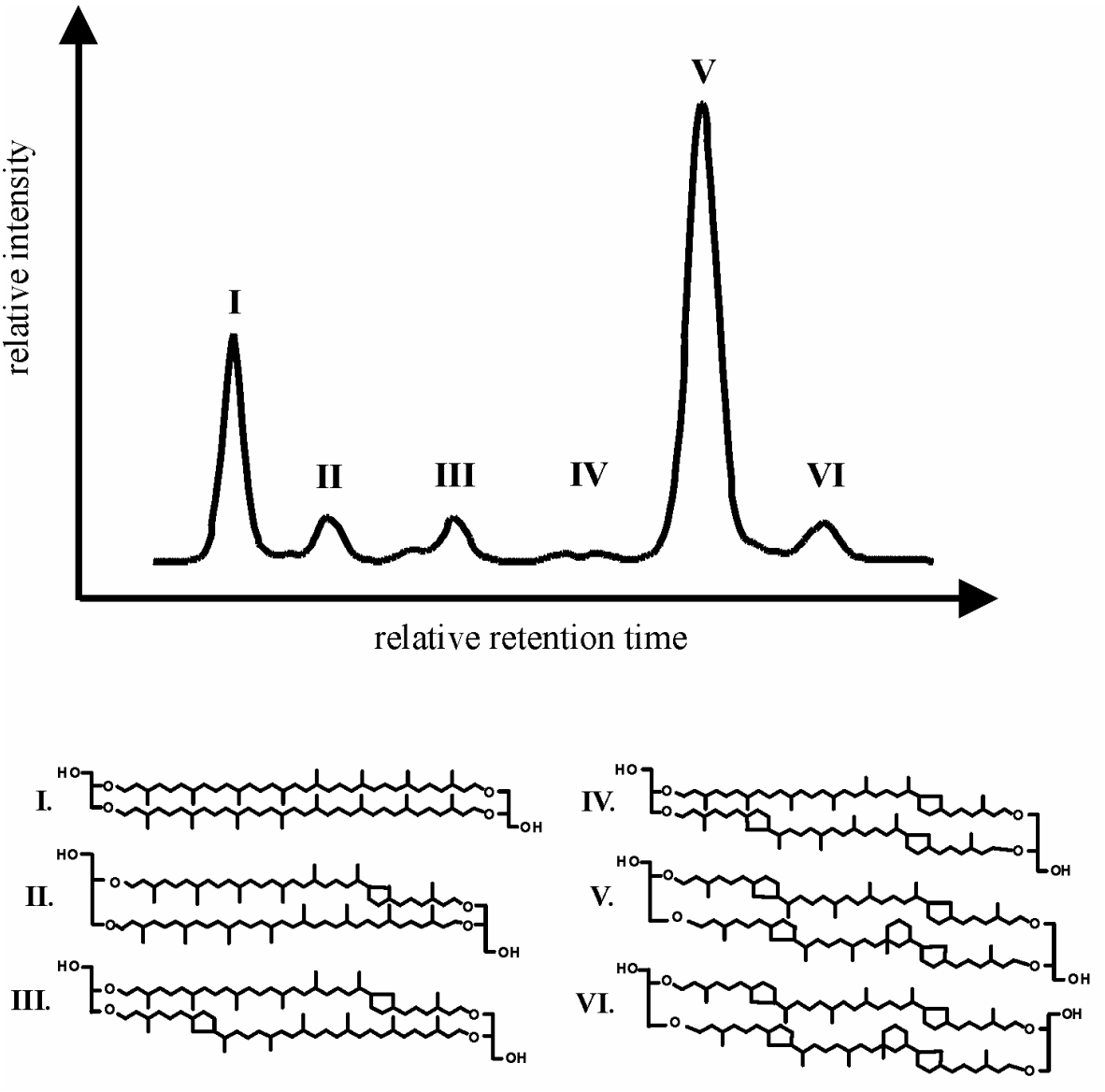

Figure 4. Partial HPLC/MS base peak chromatogram of an Arabian Sea surface sediment extract and structures of intact core tetraether membrane lipids of marine Crenarchaeota. Structure of the stereoisomer (VI) of crenarchaeol is likely a regio-isomer [*Sinninghe Damsté et al*, unpublished data 2004].

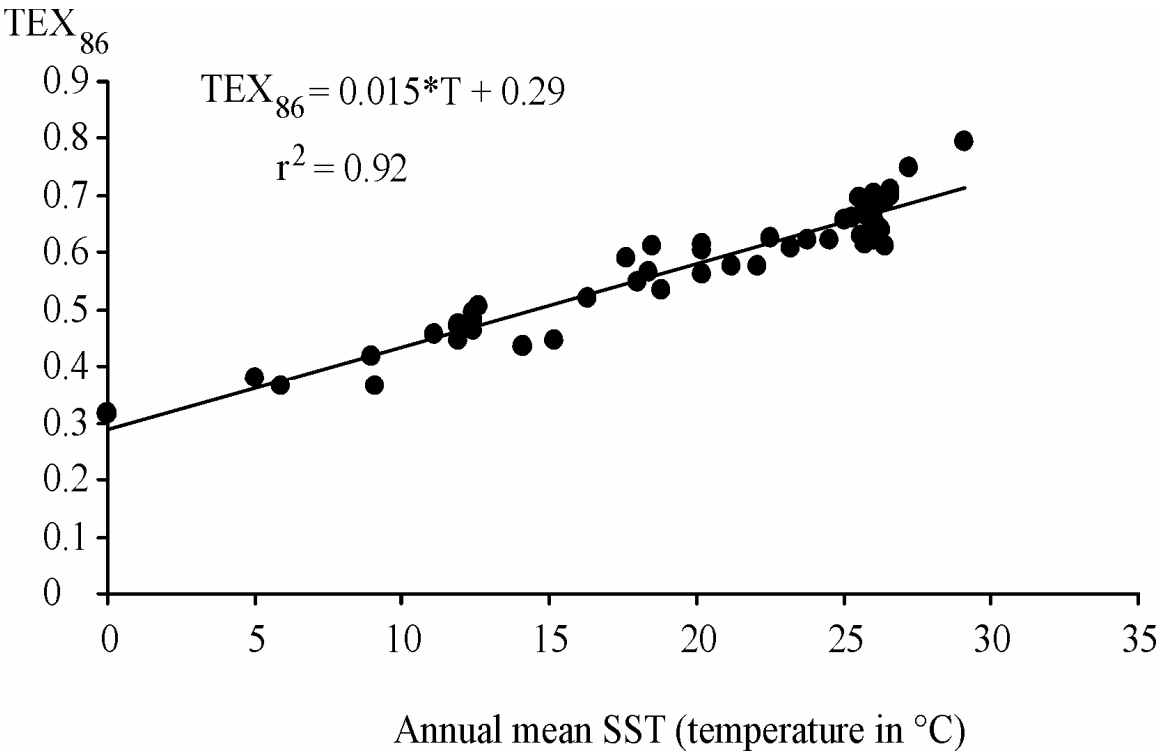

Figure 5. Updated correlation line of TEX_{86} in core top samples with annual mean SST [after *Schouten et al.*, 2002].

Marine Crenarchaeota may adjust their membrane lipids to temperature similar to their hyperthermophilic relatives but direct evidence that temperature is the only controlling factor is lacking. It is difficult to link the GDGT distribution only to temperature since little is known about the metabolic requirements of marine Crenarchaeota. For instance, salinity and nutrient levels can vary to a substantial degree in marine systems and may also influence the GDGT distribution. Furthermore, the new temperature proxy TEX_{86} was established on core top sediments and correlated best with SST. However, marine Crenarchaeota occur highly seasonal in surface waters and are generally relatively more abundant in the deeper layers of the neritic waters and in the meso-and bathypelagic zone of the ocean (see Fig. 2). It is as yet not clear during which season and at which depth in the water column the lipid signal used for TEX_{86} paleothermometry is biosynthesized. Thus, further research is required to validate the TEX_{86} as a new SST proxy.

1.4 Scope and Framework of the thesis

This thesis has two major objectives. The first objective was to shed more light on the basic physiology of marine Crenarchaeota, and the second objective was to validate the newly introduced temperature proxy $TEX_{86.}$ Hence, answers were sought for the following key questions:

(1) What is the basic physiology of marine Crenarchaeota and how does this determine their ecology?

(2) Is the GDGT distribution in the membrane lipids of marine Crenarchaeota mainly determined by temperature or are other factors such as nutrient levels or salinity also important?

(3) Does the GDGT signal which is found in the sediments reflect a physiological response to temperature of single species or is it an integrated signal of complex crenarchaeotal communities?

(4) Is the GDGT signal produced in the surface water over the whole annual cycle or is it a seasonal signal?

(5) Which GDGT signal is effectively transported in deeper waters and sediments and how does this influence the temperature calibration?

These questions address ecological and physiological aspects of the marine Crenarcheota and the mechanism and temperature calibration of the TEX_{86} proxy. Therefore, the thesis is divided into two parts. The first part of these thesis (**Chapter 2**, **3** and **4**) deals with studies concerning the physiology and ecology of marine Crenarchaeota. **Chapter 2** describes an *in situ* ^{13}C bicarbonate labeling experiment to study the carbon acquisition mechanism of marine Crenarchaeota and demonstrates that at least some pelagic marine Crenarchaeota are capable of light independent bicarbonate uptake and are thus autotrophic organisms. In **Chapter 3** an enrichment culture of marine Crenarchaeota obtained from mesocosm experiments showed that a crenarchaeotal species is involved in ammonium oxidation and is a chemolithoautotrophic organism. **Chapter 4** describes the seasonal dynamics of marine Archaea in costal North Sea surface waters during a 1.5 year long time series, using different molecular approaches, and demonstrates that cell densities of marine Crenarcheota strongly vary over the annual cycle and are highest during winter. This is in

agreement with the idea that marine Crenarchaeota are autotrophic ammonia oxidizing organismns.

The second part of this thesis (**Chapters 5**, **6** and **7**) deals with factors determining the GDGT distribution in marine Crenarchaeota and thus of the TEX_{86} proxy. **Chapter 5** describes the temperature dependent adaptation of marine crenarchaeotal GDGTs using mesocosm experiments, and shows that indeed temperature is the major controlling factor on the distribution of crenarcheotal tetraether membrane lipids. Nutrient levels and salinity do not play an important role in the GDGT distribution. The mesocosm temperature calibration shows a similar slope to the core top correlation but differs in the intersection probably caused by differences between the crenarchaeotal species enriched under laboratory conditions and the more complex crenarchaeotal communities in the field. In **Chapter 6,** lipid analysis of suspended particulate organic matter (POM) from surface and deep waters in different oceanic settings are presented. These data showed that the GDGT signal mainly reflects column temperatures of the upper 100 m. GDGTs from the upper parts of the ocean are probably more effectively transported to the sediment by grazing and repackaging in the upper water column since an active food-web exists there. The temperature calibration of the POM was similar to the core top correlation but with a lower correlation coefficient due to scatter of a mixed signal from living suspended Crenarchaeota and fossil sedimenting GDGTs. In **Chapter 7** sediment trap samples from the northeast Pacific and the Arabian Sea revealed that the TEX_{86} at all trap deployment depth reflects SST. At the shallow trap in the Arabian Sea the TEX_{86} followed the seasonal cycle, whereas the signal which ends up in the deeper traps reflects an integrated SST signal probably due lateral transport and mixing processes.

In summary, the experimental work and the field studies presented in the first part of the thesis revealed that, at least some, marine Crenarchaeota appear to be chemolithoautothrophic organisms and that they have a highly seasonal occurrence in surface waters. The experimental work and the field studies described in the second part of this thesis validates the newly introduced temperature proxy TEX_{86}. Thus, with this new proxy it is possible to reconstruct upper water column temperatures in ancient environments of up to 140 million years old.

1.5 References

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