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Review

Major players on the microbial stage: why archaea are important

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As microbiology undergoes a renaissance, fuelled in part by developments in new sequencing technologies, the massive diversity and abundance of microbes becomes yet more obvious. The Archaea have traditionally been perceived as a minor group of organisms forced to evolve into environmental niches not occupied by their more 'successful' and 'vigorous' counterparts, the bacteria. Here we outline some of the evidence gathered by an increasingly large and productive group of scientists that demonstrates not only that the Archaea contribute significantly to global nutrient cycling, but also that they compete successfully in 'mainstream' environments. Recent data suggest that the Archaea provide the major routes for ammonia oxidation in the environment. Archaea also have huge economic potential that to date has only been fully realized in the production of thermostable polymerases. Archaea have furnished us with key paradigms for understanding fundamentally conserved processes across all domains of life. In addition, they have provided numerous exemplars of novel biological mechanisms that provide us with a much broader view of the forms that life can take and the way in which micro-organisms can interact with other species. That this information has been garnered in a relatively short period of time, and appears to represent only a small proportion of what the Archaea have to offer, should provide further incentives to microbiologists to investigate the underlying biology of this fascinating domain.

Introduction

When Carl Woese first proposed that the tree of life encompassed three distinct lineages, including a new prokaryotic one initially designated Archaebacteria (later Archaea), it would have been hard to imagine the broad spectrum of novel findings that study of these remarkable organisms would bring to light. Indeed, in their ground-breaking paper, in which Woese & Fox (1977) proposed the third Kingdom [later Domain (Woese *et al.*, 1990)] of Archaebacteria, they were represented solely by the methanogens. These were quickly supplemented by the addition of extreme halophiles and thermoacidophiles (Magrum *et al.*, 1978; Woese *et al.*, 1978), but at that time one of the characteristics of archaebacteria was 'their occurrence only in unusual habitats'. By the 1980s, many new hyperthermophilic organisms that grew optimally above 80 °C, and often optimally above 100 °C, had been isolated and shown also to be archaea (Tu *et al.*, 1982; Zillig *et al.*, 1981). Later studies showed by molecular rather than culture methods that archaea are cosmopolitan, and in certain habitats, such as the oceans, are significant contributors to the biomass (DeLong & Pace, 2001; Olsen *et al.*, 1986; Robertson *et al.*, 2005). Over the years, archaea have gone from microbial extremophilic oddities to organisms of universal importance and have been used to elucidate fundamental biological questions. Studies of archaea have proven to be enormously fruitful: unique traits found nowhere else in nature have been revealed in archaea, and there are many instances of archaeal processes that combine a mosaic of bacterial and eukaryal features with unique archaeal ones to create a third functioning mechanism. Archaea are useful model systems for processes in both eukarya and bacteria, and their major roles in various ecosystems, not just extremophilic ones, continue to be uncovered. They have useful biotechnology/commercial applications, and may yet prove to affect human health in significant ways. There is continued study and debate about their role in evolution and, due to their ability to thrive at the limits of life on Earth, the possible presence of organisms that resemble the archaea in extraterrestrial environments. In this review, we highlight some of the many key findings in archaeal research.

Archaea and evolution

The third Domain, Archaea

In 1977, Woese and Fox proposed the Archaea as a third domain of life based on small subunit rRNA (ssrRNA)

sequence cataloguing. This represented a profound paradigm shift from the dichotomy of eukaryotic and prokaryotic life forms that had previously existed. Not only does archaeal ssrRNA differ in primary sequence from that of the other two domains (Bacteria and Eukarya) but also each domain was shown to have specific 'signatures' in this conserved molecule, both in primary sequence and in the secondary or tertiary structure (Roberts *et al.*, 2008; Winker & Woese, 1991; Woese *et al.*, 1990). Correlations between ssrRNA signatures and ribosomal protein signatures were found, indicating that the RNA component and the domain-specific and universal ribosomal proteins evolved in tandem (Roberts *et al.*, 2008). 16S rRNA sequences were used to design domain-specific oligonucleotides for use as probes (Burggraf *et al.*, 1994; Embley *et al.*, 1992) and PCR primers (Hugenholtz *et al.*, 1998) that have been used to determine the presence of archaea in environments even when the organisms themselves remained unculturable.

In addition to ssrRNA signatures, evidence for an archaeal 'genomic signature' has been presented. The archaeal genomic signature presented by Graham *et al.* (2000) is a set of over 350 genes found in archaea but lacking homologues in either bacteria or eukaryotes. This relatively high number of archaeal-specific genes (most of which have no known function as yet) represents about 15 % of archaeal genomes and supports the belief that the archaea are an ancient lineage of major evolutionary importance (Graham *et al.*, 2000). While this archaeal genome signature was based almost entirely on euryarchaeota sequence data, the subsequent availability of more archaeal genome sequences has led to the identification of numerous signature insertion/deletions (indels) and signature proteins in Crenarchaeota and the proposed new Phylum Thaumarchaeota [previously classified as low-temperature marine crenarchaeotes (Gupta & Shami, 2011)]. The development of large numbers of signature patterns for archaeal phyla should greatly assist future classification, with the large number of Thaumarchaeota signatures lending support to their classification as a novel phylum.

The extremophilic properties of many archaea have made them a favourite starting point for theories concerning how life may have evolved in the hostile conditions of early Earth. Stetter (2006) has suggested that only hyperthermophiles, which were likely anaerobic chemolithoautotrophs (Berg *et al.*, 2010), could have inhabited the early hot anaerobic Earth, and that they may have been present as early as 3.9 Gyr ago. This would be consistent with the abilities of many extant archaea to thrive in extreme thermal environments, such as hydrothermal vents (Auguet *et al.*, 2010). This notion is also supported by the location of hyperthermophiles as short, deep branches near the root of the tree of life, although other views suggest that the Archaea evolved relatively recently (Cavalier-Smith, 2006).

Archaea and eukarya

Although a close evolutionary relationship between eukarya and archaea is widely accepted, the origin of the Domain Eukarya is one of the most controversial areas of evolutionary biology (Embley & Martin, 2006). Variations of two broad theories on the origin of eukaryotic cells involving the Archaea have been presented. The first posits that the Archaea and Eukarya shared an ancient common ancestor to the exclusion of the bacteria, while the second is based upon the fusion of an archaeon with a bacterium (Forterre, 2010; Gribaldo *et al.*, 2010). Many theories propose an archaeal origin of the eukaryotic nucleus in a fusion event with a bacterium, with the archaeal endosymbiont later developing into a nucleus, although the archaeal and bacterial partners in the event have varied (Forterre, 2010). Most recently, the archaeal partner has been suggested to be a member of the Thaumarchaeota, since these organisms possess a number of eukaryotic features not found in other archaeal phyla (Forterre, 2010). However, other models suggest that the nucleus is not derived from bacteria or archaea (Pace, 2006).

Horizontal gene transfer (HGT) is also cited as a major mechanism to explain how life on Earth evolved from one universal common ancestor. Evidence exists for HGT among members of the same domain and across domains (Boto, 2010), but its importance in the evolution of more complex organisms is disputed (Koonin & Wolf, 2009). Significant HGT appears to have occurred between archaeal and bacterial hyperthermophiles, as a much higher percentage of genes from the bacterial hyperthermophiles *Thermotoga* and *Aquifex* appear to be archaea-derived than those of mesophilic bacteria (Aravind *et al.*, 1998; Nelson *et al.*, 1999). A well-studied example of HGT is reverse gyrase, which is found in all hyperthermophiles, suggesting a critical role for this enzyme in organisms with high temperature growth optima (Brochier-Armanet & Forterre, 2006). It has been argued that reverse gyrase was likely transferred via one or two ancient HGT events from archaea to bacteria (Brochier-Armanet & Forterre, 2006). More recent analyses support the notion that HGT from bacteria to archaea has actually occurred more often than transfer from archaea to bacteria (Kanhere & Vingron, 2009).

Archaea and extraterrestrial life

As many archaea have been found at the limits to life on this planet, they have often been proposed to resemble what life may be like if found outside our planet. The nature of Earth-like organisms that could exist on other planets has varied, with methanogens often mentioned due to their adaptation to anaerobic niches with little or no organic carbon (Moissl-Eichinger, 2011), and especially with respect to the possible biogenic formation of the methane detected on Mars (Formisano *et al.*, 2004; Mumma *et al.*, 2009; Sanderson, 2010). Some experiments suggest that terrestrial methanogens could survive under

Mars-like conditions (Chastain & Kral, 2010; Kendrick & Kral, 2006). It has recently been suggested that methane-oxidizing archaea (anaerobic methane-oxidizing archaea; ANME) may be able to use the methane on Mars, whatever its source, as a carbon and energy source, since these organisms have been detected in hypersaline permafrost methane seeps on Earth (Niederberger *et al.*, 2010). Landis (2001) has argued that extreme halophiles may be present on Mars and surviving trapped in salt crystals, where it is known that they may persist, essentially indefinitely, on Earth (McGenity *et al.*, 2000).

Unique structural features of archaea

Cell envelopes

The Archaea have long been known to contain unusual cell wall structures and components. Indeed, one of the first major distinguishing features of archaea used to separate them from bacteria was the lack of murein in their cell envelopes (Woese *et al.*, 1978). The cell walls of the various groups of archaea are chemically and structurally diverse. Murein is found almost ubiquitously in bacterial cell walls but never in archaea, although some methanogenic archaea do contain a related, but archaeal-specific polymer, called pseudomurein (Kandler & König, 1978; König *et al.*, 1989). A very common wall type in archaea is one never found in the bacterial domain, where the sole wall component lying outside the cytoplasmic membrane is a two-dimensional array of protein or glycoprotein termed the S-layer (Sleytr & Beveridge, 1999). Many bacteria have S-layers as their external envelope component, but bacterial S-layers are always separated from the cytoplasmic membrane by at least a layer of murein (as in Gram-positives) or murein plus an outer membrane (as in Gram-negatives). The S-layers of archaea have a historic place in glycobiology, as the S-layer of *Halobacterium salinarum* (cell surface glycoprotein; CSG) was the first prokaryotic protein to be shown to be glycosylated (Mescher & Strominger, 1976). Until that time, this post-translational modification was thought to be restricted to eukaryotic cells.

Unusual appendages

Archaea, like bacteria, may have a variety of appendages extending from the cell surface. Several appear to be unique to archaea (Fig. 1), while others, such as flagella and pili, appear superficially like organelles in bacteria but have archaea-unique features (Ellen *et al.*, 2010; Ng *et al.*, 2008). Among these features are the grappling hook appendages called hami (Fig. 1a, b), found in large abundance on the surface of a euryarchaeon discovered in marshes in Germany (Moissl *et al.*, 2005). A second are the hollow tubes, called cannulae, which connect cells of the hyperthermophile *Pyrodictium abyssi* (Fig. 1c) (Nickell *et al.*, 2003). Among the appendages found in archaea that resemble bacterial counterparts, the best studied are archaeal flagella (Jarrell & McBride, 2008; Thomas *et al.*, 2001). These differ fundamentally from their bacterial namesakes, with genetic and structural evidence suggesting that archaeal flagella are related to bacterial type IV pili, organelles that mediate the surface motility called twitching (Ng *et al.*, 2006). Recent work on type IV-like pili of archaea has shown that the structure is unlike that of any bacterial pilus yet described (Wang *et al.*, 2008).

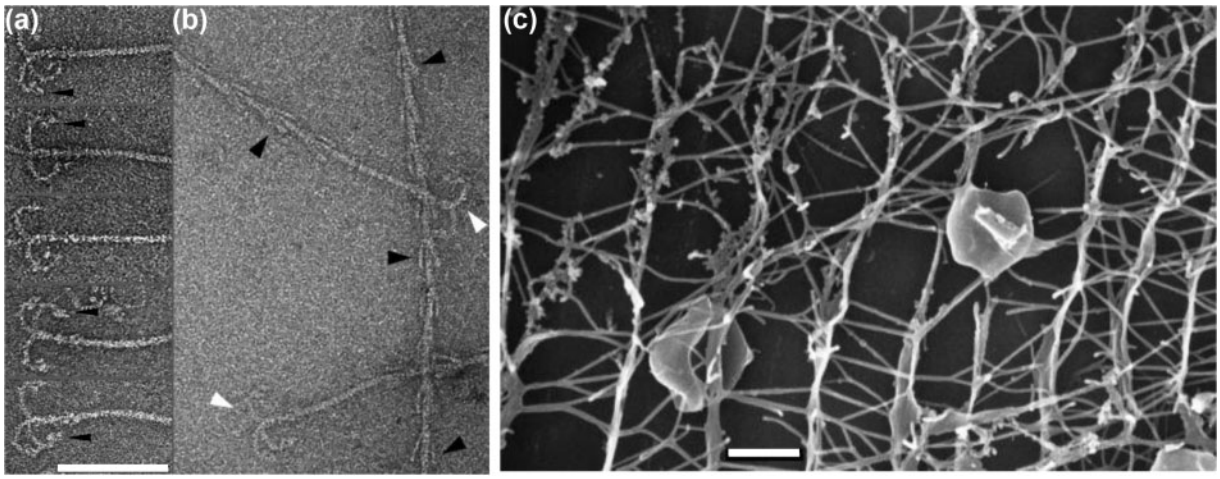

Fig. 1. Unusual appendages of selected archaea. (a) Ultrastructure of hami from the SM1 euryarchaeon; negative staining. Bar, 100 nm. Electron micrographs of grappling hooks, located at the distal ends of the hami. Arrowheads indicate location of the barbs. Reprinted from Moissl *et al.* (2005) with permission. (b) Electron micrograph of high level structured SM1 hami. The hami show prickles (black arrowheads) and grappling hooks (white arrowheads). Reprinted from Moissl *et al.* (2005) with permission. (c) Scanning electron micrograph of part of a network of *Pyrodictium* cells and cannulae, with tubules in a regular array. Bar, 1 μm. Reprinted from Rieger *et al.* (1995) with permission.

Archaeal ether-linked lipids

Another fundamental trait identified early in the study of the Domain Archaea was the presence of ether-linked lipids in the cytoplasmic membrane (Woese, 2004; Zillig, 1991). Phospholipids in the other two domains consist of linear fatty acids ester-linked to a glycerol backbone. Archaeal cytoplasmic membranes are typically composed of diphytanylglycerol diethers (containing phytanyl chains consisting of 20 carbons) which form a lipid bilayer, although in some cases, such as those of some thermoacidophiles, the membranes can consist of diphytanyldiglycerol tetraethers (phytanyl chains of 40 carbons) which span the cytoplasmic membrane in a very stable lipid monolayer (Matsumi *et al.*, 2011). Mixtures of the two types can be found in individual species, and other variations, such as cyclopentane-containing lipids, are also found. Besides these fundamental structural differences, the stereochemistry of archaeal lipids is different from those of both bacteria and eukarya. In archaea, 2,3-*sn*-glycerol backbones are used, while in the other two domains, 1,2-*sn*-glycerol backbones are employed (Matsumi *et al.*, 2011). The ether linkage is much more resistant than the ester linkage to hydrolysis upon exposure to the extremes of pH and temperature found in many archaeal habitats (van de Vossenberg *et al.*, 1998), and it was originally thought that the unique archaeal lipids were a specific adaptation to extreme environments, before it was realized that these lipids are a defining trait of the entire archaeal domain, regardless of their environmental niche.

Unusual cell structure

The Domain Archaea contains many isolates with extremely unusual structural features (Fig. 2). A short list would include: *Methanospirillum hungatei*, covered with a proteinaceous sheath composed of individual hoops, and with complex multilayered spacer and end plugs separating individual cells within chains (Fig. 2a) (Beveridge *et al.*, 1985, 1991); the rectangular, ultrathin (as thin as 0.1 μm thickness) *Haloquadratum walsbyi* (Fig. 2b), which appears to divide at right angles, producing the appearance of sheets of postage stamps (Burns *et al.*, 2007; Walsby, 2005); and *Thermoproteus tenax*, one of the first hyperthermophiles isolated, which has the unusual morphology of long, thin, aseptate rods which have true branching and often end in spherical bodies to give a golf club appearance (Zillig *et al.*, 1981). However, the most unusual archaeon may well be the hyperthermophilic *Ignicoccus hospitalis*. It possesses the smallest genome of any known free-living organism, at only 1.3 Mb (Podar *et al.*, 2008). In addition, it is the only cultivated archaeon known to have two membranes, and has an enormous intermembrane space, with a volume larger than that of the cytoplasm, filled with unusual vesicles (Fig. 2c) (Junglas *et al.*, 2008). Furthermore, unlike any other known archaeon or bacterium, the ATP synthase is localized in the outer membrane, indicating that the outer membrane is energized and that ATP is formed in the periplasmic space (Küper *et al.*, 2010), all of which cause us to rethink basic tenets about energy generation. *I. hospitalis* is also one of the components of the only known interaction between two archaeal species (Huber *et al.*, 2002). It forms a special interaction with the very small *Nanoarchaeum equitans* (about 1 % of the volume of *Escherichia coli*), which seems unlike symbiosis, commensalism or parasitism (Jahn *et al.*, 2008). The connection between the two organisms can be via unusual structures, including fine fibres at the site of

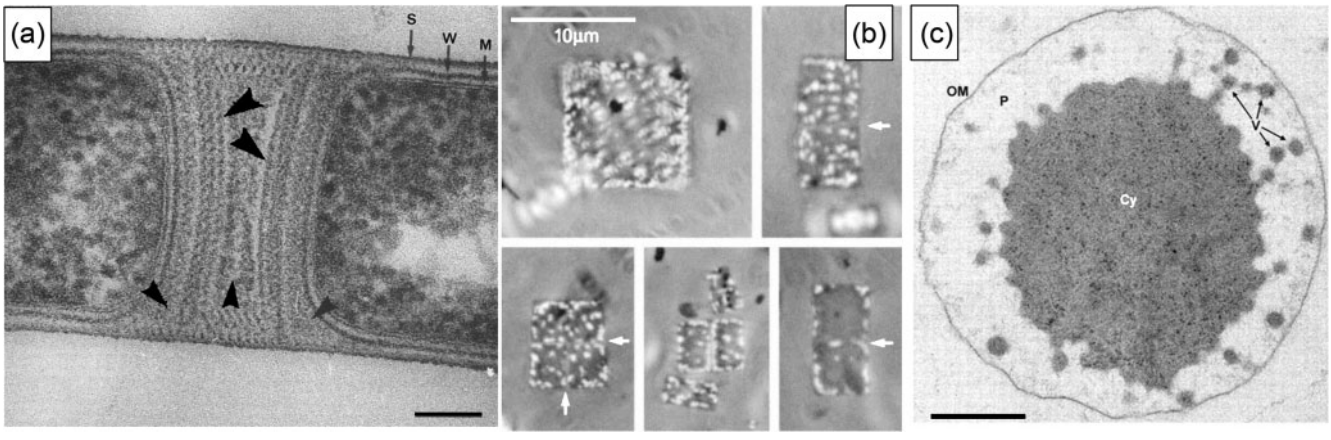

Fig. 2. Unusual structural features of selected archaea. (a) Thin section of *Methanospirillum hungatei* showing the unusual multilayered spacer plugs (large arrowheads). S, sheath; W, cell wall; M, plasma membrane; small arrowheads point out amorphous material between wall and plug and within the cell spacer. Bar, 100 nm. Reproduced from Southam & Beveridge (1992) with permission. (b) Phase-contrast light micrographs of the square archaeon from the Sinai. Division lines are visible in some cells (arrows). Bar, 10 μm. Reproduced from Walsby (2005) with permission. (c) Ultrathin section of *I. hospitalis* strain KIN4/I^T showing the large periplasm containing vesicles. Cy, cytoplasm; P, periplasm; V, vesicle; OM, outer membrane; bar, 1 μm. Reproduced from Paper *et al.* (2007) with permission.

contact, or apparently by direct contact of the surfaces of the two organisms (Burghardt *et al.*, 2009). *N. equitans* also has an extremely small genome of only 490 kb, encoding 552 genes, the smallest of any exosymbiont (Waters *et al.*, 2003). Other nanosized (<500 nm diameter), uncultured archaea have been identified, called ARMAN (archaeal Richmond Mine acidiphilic nano-organisms), that have estimated volumes near the theoretical lower limit for life (0.009–0.04 μm^3). ARMAN contain a unique intracellular tubular structure of unknown composition and function that can extend to 200 nm in length (Baker *et al.*, 2010; Comolli *et al.*, 2009).

Novel archaeal virus families

Many viruses that infect various archaeal species also have unique structural characteristics. Examination of high-temperature biomes, and more recently mesophilic, highly halophilic environments, has led to the identification of a large variety of archaeal viruses that, both in ultrastructure and in the genetic makeup of their genomes, are unlike any observed in either of the two other domains (Fig. 3) (Comeau *et al.*, 2008; Prangishvili *et al.*, 2006a). Genome analysis has revealed that up to 90 % of the genes of some of these viruses lack homologues. Indeed, it has been suggested that all three domains of life may have a set of unique dsDNA viruses (Prangishvili *et al.*, 2006b). In archaeal viruses, many unusual morphotypes have been reported, including bottle-shaped (ampulla), fusiform, droplet, linear and spherical forms, leading to the classification of many of these viruses as novel virus families (http://www.ictvonline.org/virusTaxonomy.asp?version=2009). One of the more unusual of the archaeal viruses is the two-tailed virus (ATV), which is a lytic virus active on the thermoacidophile *Acidianus* [75 °C, pH 3 (Häring *et al.*, 2005)]. ATV is released as a tailless fusiform virus, but then the virus undergoes a morphological change, independent of the host cell and exogenous energy sources or cofactors, by forming tails at each end (Häring *et al.*, 2005). Another virus of *Acidianus* with an exceptional morphology, AFV1, is a flexible filament with claw-like ends that attach to pili on the surface of target cells (Bettstetter *et al.*, 2003).

Unique biochemical features of archaea

Biochemistry of methanogenesis

Methanogenic archaea are strict anaerobes, usually existing in complex communities of microbial consortia, vital for the degradation of complex organic compounds and thus for carbon cycling. Methanogens make their living through the complex and archaea-unique process of methanogenesis, which involves a number of unusual cofactors and a unique biochemical pathway (DiMarco *et al.*, 1990; Thauer *et al.*, 2008; Weiss & Thauer, 1993). The conversion of CO_2 to CH_4 occurs in a well-known step-wise process of successive two-electron reductions, with the C_1 group bound to a carrier at each step (Weiss & Thauer, 1993). Three different carriers are involved: methanofuran (MFR), tetrahydromethanopterin (H_4MPT) and co-enzyme M (CoM-SH), with CoM-SH unique to methanogens and the others found in a limited number of other organisms. Methanogenesis begins with the reduction of CO_2 and its attachment to MFR, producing formyl-MFR, followed by the transfer of the formyl group to H_4MPT. A further two reduction steps of formyl-H_4MPT generate methylene-H_4MPT and finally methyl-H_4MPT. The methyl group is then transferred to CoM-SH, producing methyl-S-CoM. In the final stage of methanogenesis, methyl-S-CoM is reduced to CH_4 by methyl CoM reductase, an enzyme containing the coenzyme F_{430}, another factor unique to methanogens, as a prosthetic group. The other product of the methyl-CoM reductase reaction is a heterodisulfide of

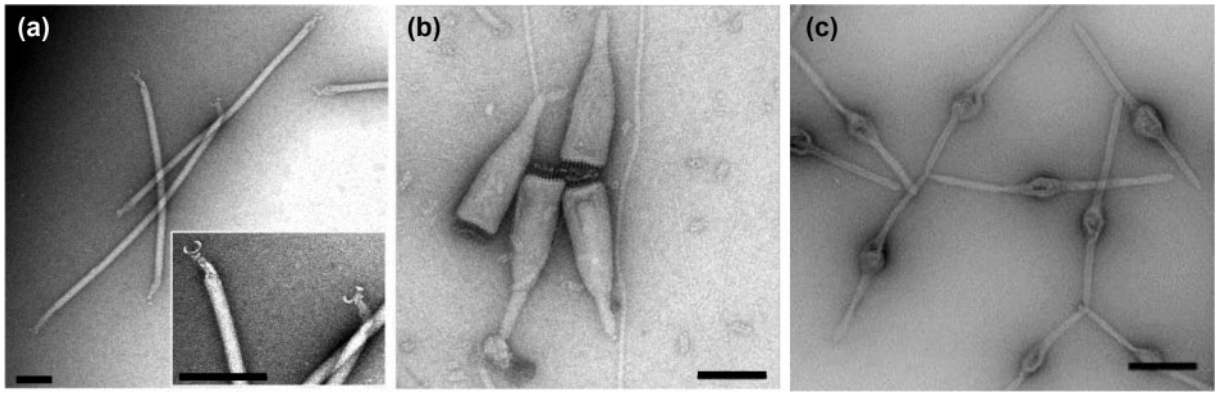

Fig. 3. Unusual structural features of selected archaeal viruses. (a) Electron micrograph of particles of AFV1 with tail structures in their native conformation, negatively stained with 3 % uranyl acetate. Bars, 100 nm. Reprinted from Bettstetter *et al.* (2003) with permission. (b) Electron micrograph of *Acidianus* bottle-shaped virus (ABV) particles attached to each other with their thin filaments at the broad end. Bar, 100 nm. Reprinted from Häring *et al.* (2005) with permission. (c) Electron micrograph of negatively stained (2 % uranyl acetate) two-tailed virions. Bar, 200 nm. Reprinted from Prangishvili *et al.* (2006) with permission.

CoM and another unique factor, coenzyme B. The heterodisulfide is the substrate for heterodisulfide reductase, which regenerates CoM and coenzyme B. While the methanogenesis pathway is well described, the steps that actually lead to net energy generation are still the subject of investigation. However, there is now evidence which suggests that in hydrogenotrophic methanogens the heterodisulfide reductase step, an exergonic reaction which appears to drive the initial endogonic step in the pathway, may occur through a flavin-based electron bifurcation, as suggested by Thauer *et al.* (2008), likely at the FAD-containing subunit in the heterodisulfide reductase (Kaster *et al.*, 2011). These two steps have recently been physically linked in a protein complex isolated from *Methanococcus maripaludis* (Costa *et al.*, 2010) and *Methanothermobacter marburgensis* (Kaster *et al.*, 2011).

Glycolytic pathways

Investigations using a variety of enzymic studies, genome sequence analysis, ^{13}C-NMR, crystal structures and microarrays conducted mainly on hyperthermophilic archaea and extreme halophiles have shown that archaea use novel variations of the Embden–Meyerhof (EM) and Entner–Doudoroff (ED) pathways, prevalent in bacteria and eukarya, for glycolysis (Siebers & Schönheit, 2005; Verhees *et al.*, 2003). Unexpectedly, while most of the intermediates of the EM pathway in archaea are the same as in the classic version of the pathway found in the other domains, the archaea generate these intermediates with a series of unusual enzymes involved in various phosphorylation and isomerization steps as well as the oxidation of glyceraldehyde 3-phosphate, the latter catalysed by glyceraldehyde-3-phosphate ferredoxin oxidoreductase or a nonphosphorylating glyceraldehyde-3-phosphate dehydrogenase (Reher *et al.*, 2007; Siebers & Schönheit, 2005; Verhees *et al.*, 2003). For example, while the classic EM pathway contains 10 enzymes, only four have orthologues in *Pyrococcus furiosus*, with the remaining steps catalysed by novel enzymes, including a unique glucokinase and phosphofructokinase enzymes that are ADP-dependent rather than ATP-dependent (Sakuraba *et al.*, 2004). The conversion of acetyl-CoA to acetate in anaerobic fermentative hyperthermophiles is the major energy-conserving reaction for these organisms, and this reaction is catalysed by an unusual enzyme, ADP-forming acetyl-CoA synthetase, also found in some halophiles (Siebers & Schönheit, 2005). In contrast, bacteria use a combination of phosphotransacetylase and acetate kinase to do the same conversion.

Archaeal modifications have also been recognized in the ED pathways of extreme halophiles and certain thermoacidophiles. Three different versions have been reported with variations mainly in the early steps of the pathways: semiphosphorylative, found in extreme halophiles; nonphosphorylative, found in certain thermoacidophiles; and a branched ED pathway in which there is simultaneous operation of both the semiphosphorylative and nonphosphorylative pathways, as recently suggested for *Sulfolobus* and *Thermoproteus* (Reher *et al.*, 2010; Zaparty *et al.*, 2008). In the semiphosphorylative version, the unusual step is the conversion of 2-keto-3-deoxygluconate (KDG) to 2-keto-3-deoxy-6-phosphogluconate (KDPG) via phosphorylation by KDG kinase before its further conversion to pyruvate and glyceraldehyde 3-phosphate by KDPG aldolase (Verhees *et al.*, 2003). In the nonphosphorylative ED variation, a key enzyme is a novel KDG-specific aldolase which cleaves KDG to form pyruvate and glyceraldehydes (Reher *et al.*, 2010). No phosphorylated hexose derivatives are generated, and it is only later in the pathway that phosphorylation of glycerate occurs by a specific kinase to generate 2-phosphoglycerate. No net ATP synthesis occurs via this route.

These studies on archaeal central metabolism and the enzymes involved show the metabolic diversity of the Archaea, which is considered greater than that of either the Bacteria or the Eukarya (Siebers & Schönheit, 2005), while at the same time contributing to a more general knowledge about novel enzyme families and their mechanism of action.

Other unique features of archaea

Archaea are also characterized by many other unique features (Table 1), including the composition of their DNA-dependent RNA polymerase (Werner, 2007), ribosome structure and composition (Lecompte *et al.*, 2002), unusual resistance to antibiotics (Bock & Kandler, 1985) (itself a reflection of unusual walls, membranes and ribosomes) and a variety of modifications to tRNAs (Edmonds *et al.*, 1991; Gupta & Woese, 1980). There are novel twists on lipoylation (Posner *et al.*, 2009), histones which lack the N- and C-terminal extensions found in eukaryotic histones that are sites for post-translational modifications important for regulation (Sandman & Reeve, 2005), and an *N*-linked glycosylation system which, in archaea, is an amalgam of the processes observed in the other two domains (Jarrell *et al.*, 2010). *Ferroplasma acidiphilum* is unique in possessing the vast majority of its proteins (86 % of the investigated total) in the form of iron-metalloproteins, including many that have not been described as metalloproteins in other organisms (Ferrer *et al.*, 2007).

Archaea as model organisms

The 21st and 22nd amino acids

Selenocysteine (Sec) and pyrrolysine (Pyr) are known as the 21st and 22nd amino acids, and have been shown to be co-translationally inserted into proteins by virtue of specialized tRNA complexes that recognize the UGA and UAG stop codons, respectively. Among archaea, a restricted number of genera of methanogens are the only known members to incorporate Sec and Pyr residues into

Table 1. Selected unique features of archaea

Feature	Reference
Ether-linked lipids	Matsumi *et al.* (2011)
Unique cell envelopes	Kandler & König (1998)
Novel surface appendages	Ng *et al.* (2008)
New virus families	Comeau *et al.* (2008)
ssrRNA signatures	Winker & Woese (1991)
tRNA modifications	Gupta & Woese (1980)
Ribosome structure	Harauz & Musse (2001)
Ribosome composition	Lecompte *et al.* (2002)
Antibiotic sensitivity pattern	Bock & Kandler (1985)
RNA polymerase composition	Werner (2007)
Growth above 100 °C	Stetter (2006)
Methanogenesis and unique coenzymes	Thauer *et al.* (2008)
Sulfur-oxidizing pathways	Rohwerder & Sand (2007)
Hyperthermophilic nitrogen fixation	Mehta & Baross (2006)
Ammonia oxidation mechanism	Walker *et al.* (2010)
Lipoylation	Posner *et al.* (2009)
Modified sugar-degrading pathways	Verhees *et al.* (2003)

proteins, almost exclusively into enzymes involved in methanogenesis (Rother & Krzycki, 2010). Sec is present in proteins from all three domains of life, and while it was first observed in bacterial formate dehydrogenase, studies in archaea were crucial in elucidating the route of Sec formation in eukarya (Su *et al.*, 2009). Of all the amino acids, Sec is unique in not having its own aminoacyl-tRNA synthetase. In bacteria, selenocysteine synthase (SelA) catalyses the direct conversion of Ser-tRNA^{Sec} to Sec-tRNA^{Sec}. However, in archaea and eukarya, Sec synthesis proceeds through an intermediate, selenophosphate (Sep)-tRNA^{Sec}, generated through the activity of two enzymes, phosphoseryl-tRNA^{Sec} kinase (PSTK) and Sep-tRNA : Sec-tRNA synthetase (SepSecS), and no SelA homologues have been detected. When archaeal versions of these two enzymes were expressed in a *selA* mutant of *E. coli,* active selenoprotein formate dehydrogenase was produced, proving the involvement of these proteins in the two-step synthesis of Sec. A repeat of this experiment with the eukaryotic SepSecS yielded the same result, indicating a shared pathway for Sec formation in archaea and eukarya (Su *et al.*, 2009; Yuan *et al.*, 2006).

Pyr has only been identified in the proteins of some Methanosarcinales and an extremely limited number of bacteria, such as the Gram-positive bacterium *Desulfitobacterium hafniense* (Rother & Krzycki, 2010; Srinivasan *et al.*, 2002). In archaea, this residue is found almost exclusively in enzymes involved in methanogenesis, e.g. methylamine methyltransferases in *Methanosarcina barkeri*. A small gene cluster (*pylTSBCD*) is sufficient for the biosynthesis (*pylBCD*) and incorporation of Pyr (*pylST*). Distinct from the situation with Sec, Pyr is ligated directly onto a specific tRNA by PylS, pyrrolysyl tRNA synthetase. The adjoining gene, *pylT*, encodes the specialized tRNA^{Pyl} with unique elements, such as an elongated anticodon stem. Novel features associated with the incorporation of Sec into proteins (e.g. motifs in the mRNA secondary structure and a specific elongation factor) appear not to used for the incorporation of Pyr (Rother & Krzycki, 2010).

Transcription

The core transcriptional apparatus in archaea is homologous to that of eukaryotes (Hausner *et al.*, 1996), with RNA polymerase (RNAP) in archaea showing high similarity to eukaryotic RNAP II (Werner, 2007). Although the first structure of RNAP II was elucidated in yeast (Cramer *et al.*, 2001), functional studies of the whole complex are highly challenging in eukaryotic systems. The stability of recombinant RNAP subunits from the hyperthermophilic archaeon *Methanocaldococcus jannaschii* allowed the first *in vitro* reconstitution of an active eukaryotic-type RNAP (Werner & Weinzierl, 2002). Successful reconstitution of the 13 subunits of RNAP from *M. jannaschii* (Werner & Weinzierl, 2002) and later *P. furiosus* (Naji *et al.*, 2007) has allowed functional analysis of individual subunits and residues within the complex (Naji *et al.*, 2008; Nottebaum *et al.*, 2008). Interestingly, most of the transcriptional regulators in archaea are homologous to bacterial proteins (Bell & Jackson, 2001), raising some intriguing questions over how the core and regulatory elements of transcription interact in archaea.

DNA replication

DNA replication is another process in which archaea have proven to be useful, simplified models for eukaryotic processes. The DNA binding, ATPase and helicase activities of the predicted replicative helicase in eukaryotes and archaea, MCM (minichromosome maintenance), were first demonstrated using recombinant protein from *Methanothermobacter thermautotrophicus* (Chong *et al.*, 2000; Kelman *et al.*, 1999). Subsequently, detailed biochemical analysis of archaeal MCMs has provided significant insight into the mechanism of action of this key replication protein (Barry *et al.*, 2007; Jenkinson & Chong, 2006; Kasiviswanathan *et al.*, 2004). The first high-resolution structure of the N-terminal domain of an MCM protein came from *Methanothermobacter thermautotrophicus* (Fletcher *et al.*, 2003), and more recently the structure has been solved for the near full-length protein from *Sulfolobus solfataricus* (Brewster *et al.*, 2008) and *Methanopyrus kandleri* (Bae *et al.*, 2009). The origin binding protein in archaea also shows significant homology to the origin recognition complex (ORC) proteins in eukaryotes. The elucidation of the structure of archaeal ORC bound to a DNA replication origin sequence showed that the interaction between ORC and DNA introduces a significant bending and unwinding of the DNA, and provided insight into the mechanistic details of the initiation of DNA replication (Dueber *et al.*, 2007; Gaudier *et al.*, 2007). As with transcription, DNA

replication seems to consist of an interesting mix of eukaryotic and bacterial features. The identification of a single origin of replication in *Pyrococcus abyssi* indicated that archaeal DNA replication occurs in a bacterial-like manner, using eukaryotic-like machinery (Myllykallio *et al.*, 2000). However, multiple functioning origins have subsequently been identified in *Sulfolobus* and halophilic species, proving that some archaea utilize multiple replication origins, as in eukaryotes (Coker *et al.*, 2009; Lundgren *et al.*, 2004; Norais *et al.*, 2007; Robinson *et al.*, 2004).

Proteasome

Proteolysis is another process in which the proteins involved are similar in archaea and eukaryotes (for a review, see Maupin-Furlow *et al.*, 2006). The first crystal structure of the 20S proteasome was from *Thermoplasma acidophilum*, and revealed that the proteasome is a barrel-shaped particle made from a stack of four heptameric rings (Löwe *et al.*, 1995). Subsequent studies of archaeal proteasomes and regulatory particles have provided details of the mechanism of substrate entry (Rabl *et al.*, 2008; Religa *et al.*, 2010) and the interaction between the substrate and the proteasome antechamber (Ruschak *et al.*, 2010). Studies of the archaeal proteasome regulatory particle, PAN, which is a hexameric ATPase ring complex, have provided useful mechanistic insight into the regulation of eukaryotic proteolysis. Structural and functional studies of PAN from *M. jannaschii* have identified key residues and domains involved in substrate unfolding and translocation (Zhang *et al.*, 2009a, b). The structure of PAN bound to the 20S proteasome in *T. acidophilum* was recently solved (Yu *et al.*, 2010), and provides further details of how the regulatory particle and the core proteasome interact in archaea and eukaryotes.

A feature of eukaryotic cells critical for protein turnover is the ubiquitination system, whereby ubiquitin (Ub) is covalently bound to proteins, targeting them for degradation at the proteasome. Recently, pupylation, which involves the covalent attachment of pup proteins [prokaryotic ubiquitin-like protein (Pup)], was shown to be a functionally equivalent but not homologous system in bacteria (Burns & Darwin, 2010). In archaea, ubiquitin-like proteins have also been reported, and recently covalent attachment of SAMP (small archaeal modifier protein, SAMPylation) to proteins of *Haloferax volcanii* was observed (Humbard *et al.*, 2010). Such proteins accumulate in proteasome-deficient mutants, although there is no direct evidence yet to show that SAMPs are involved in targeting proteins to the proteasome like their eukaryotic and bacterial counterparts, Ub and Pup, respectively (Darwin & Hofmann, 2010). SAMPs conjugate to proteins via Gly (as in Ub) and not Glu (as in Pup). It appears that archaea also have a eukaryotic E1 homologue (Ub-activating enzyme) that could be involved in SAMPylation (Ranjan *et al.*, 2011), although homologues of other critical eukaryotic enzymes involved in ubiquitination are missing. These data point to an archaeal system that is more like the eukaryotic Ub system than the bacterial Pup system (Darwin & Hofmann, 2010).

ESCRT proteins

Homologues of the eukaryotic ESCRT proteins Vps4 and ESCRT-III were recently identified in some crenarchaea (Obita *et al.*, 2007). In eukaryotes, the ESCRT system is required to couple cargo sorting to vesicle formation (Williams & Urbé, 2007). Studies in *Sulfolobus acidocaldarius* have established that archaeal ESCRT homologues are involved in cell division (Lindås *et al.*, 2008; Samson *et al.*, 2008). The structural basis of the interaction between Vsp4 and ESCRT-III proteins is the same in archaea and eukaryotes, indicating that this partnership predates the divergence of the archaeal and eukaryotic lineages (Obita *et al.*, 2007). The common ancestry of the archaeal and eukaryotic ESCRT proteins means that the proteins are likely to function by similar mechanisms, albeit in different cellular processes.

CRISPR

The recently discovered CRISPR (clustered, regularly interspaced short palindromic repeats) system in bacteria and archaea is a small RNA-based defence mechanism against phages and plasmids (for a review, see Karginov & Hannon, 2010). CRISPR sequences are found in more than 90 % of archaeal genomes (Grissa *et al.*, 2007), and several archaeal species have been used as models for elucidating the mechanism by which the CRISPR system functions. The single unit transcription of CRISPR repeats and spacers before processing into small RNAs was first demonstrated in *Archaeoglobus fulgidus* (Tang *et al.*, 2002). Recent work has shown that as in the eukaryotic RNAi system, in *P. furiosus* the CRISPR/Cas system uses guide RNAs to specifically target foreign RNA for destruction (Hale *et al.*, 2009; van der Oost & Brouns, 2009).

Functional genomics

Archaea have been used as models for studying specific cellular processes in eukaryotes and bacteria. As the age of functional genomics has arisen, the utility of archaea in structural genomics and systems biology projects has become increasingly clear (Albers *et al.*, 2009; Bonneau *et al.*, 2007; Facciotti *et al.*, 2007). The stability of recombinant proteins from thermophilic archaea has been exploited for some time, with many key crystal structures being elucidated using archaeal homologues of universal proteins. For example, the first bacteriorhodopsin (Henderson & Unwin, 1977) and high-resolution ribosome (Ban *et al.*, 2000) structures were elucidated using archaeal homologues. A more recent success story was the discovery of a new motif in the oligosaccharyltransferase (homologue

of the STT3 catalytic subunit of the eukaryotic oligosaccharyltransferase complex) of *P. furiosus* near the known catalytic domain (Igura *et al.*, 2008). Subsequent mutation of the so-called DK motif in yeast STT3 revealed its essential role in catalysis. The relative ease of carrying out structural studies in thermophilic and hyperthermophilic archaea has led to their use in major structural genomics projects, in which vast numbers of proteins are purified and crystallized using high-throughput systems. One of the pioneering organisms in the field of functional genomics was *Methanothermobacter thermautotrophicus*, followed by hyperthermophiles such as *Pyrococcus* species and *M. jannaschii*, with a large number of crystal structures of their proteins being solved and deposited in databases (Christendat *et al.*, 2000; Jenney & Adams, 2008). Not only are the structures that emerge from these projects informative, but the small archaeal genomes are useful for developing efficient technologies that can be applied to structural genomics projects using higher organisms.

Additional insights into eukaryotic type II chaperonins (Ditzel *et al.*, 1998; Zhang *et al.*, 2010a), novel DNA binding (Bell *et al.*, 2002; Luo *et al.*, 2007; Wardleworth *et al.*, 2002) and DNA repair mechanisms (Kvaratskhelia & White, 2000; Rudolf *et al.*, 2006), and protein translocation (Mandon *et al.*, 2009; Ng *et al.*, 2007; Pohlschröder *et al.*, 2005; Ring & Eichler, 2004; Van den Berg *et al.*, 2004) have also been provided using model archaeal systems.

Expanded ecological significance of archaea

Originally, archaea were considered to be organisms relegated to life in extreme environments, such as salt brines, hot water springs, hydrothermal vents, extremely acidic niches and anoxic environments, where they contributed significantly to the ecology (Bini, 2010; Chaban *et al.*, 2006; Gittel *et al.*, 2009; Liu & Whitman, 2008; Macalady *et al.*, 2007). However, with the advent of culture-independent analysis techniques it has become increasingly evident that archaea are much more widespread. Sequencing results imply that they are also more metabolically diverse than initially thought (Fig. 4). As such they represent a sizeable proportion of the microbial population in a wide variety of 'non extreme' environments, such as soil, oceans and lakes (DeLong & Pace, 2001; Schleper *et al.*, 2005). However, the relative abundance of archaea varies greatly in different habitats, being particularly important in marine ecosystems, where archaea reach an abundance of 5–30 % of the total planktonic cell population (DeLong *et al.*, 1999; Schleper *et al.*, 2005). In a recent study using a global analytical approach to reveal the diversity and abundance of archaea in various habitats, it was observed that despite their high abundance, the diversity of archaea in oceans and soils is far lower than that in hydrothermal vents and freshwater ecosystems. Also, salinity rather than temperature was found to be responsible for this variable distribution

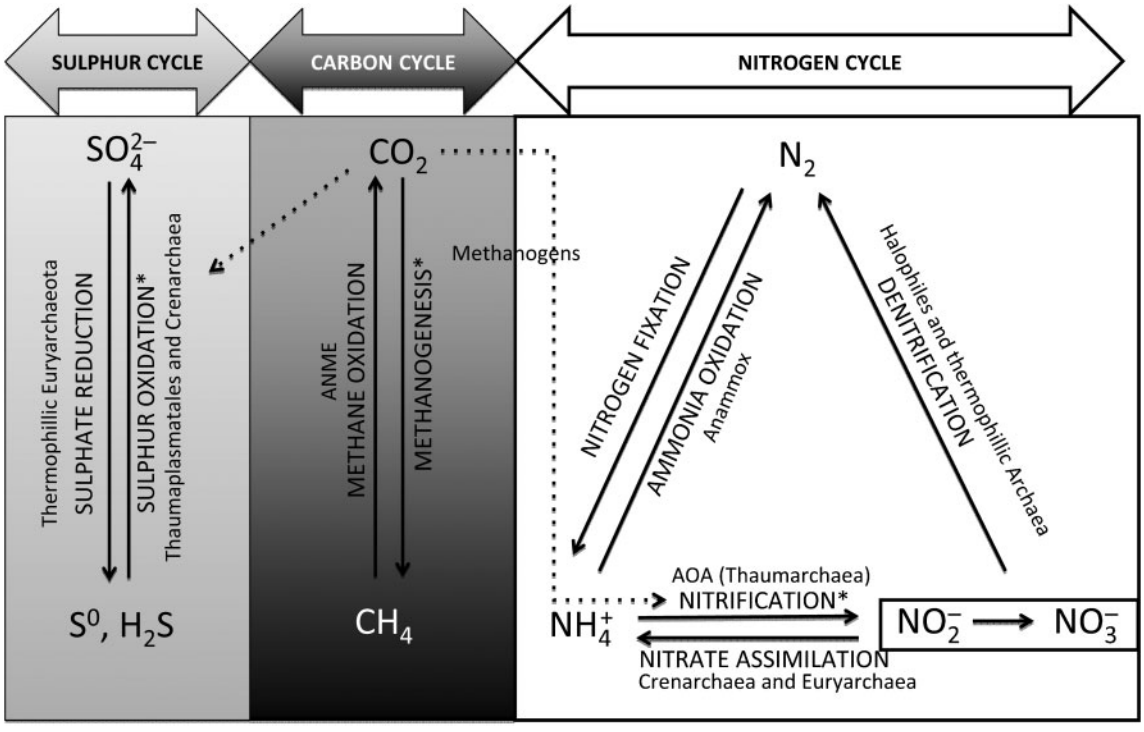

Fig. 4. Illustration of the role of archaea in the global biogeochemical cycles. The element cycle pathways and the archaea involved in these pathways indicate the contribution of this domain to the global cycles. Asterisks indicate the pathways in which archaea play a major role.

(Auguet *et al.*, 2010). The large numbers of archaea in all ecosystems indicate that they act to a much greater extent than previously believed as major players in various biogeochemical cycles. Below we summarize a select few of these contributions.

Novel important roles for archaea in biogeochemical cycles

Nitrogen: ammonia oxidation and nitrogen fixation

Our understanding of the nitrogen cycle has been revised in the past few years by the discovery of ammonia oxidation carried out by archaea. Until this discovery, ammonia oxidation, the first nitrification step of the nitrogen cycle, was thought to be carried out only by bacterial autotrophs. Ammonia-oxidizing archaea (AOA) are members of the proposed novel Phylum Thaumarchaea, and are now recognized as a ubiquitous component of marine plankton (Gribaldo *et al.*, 2010), as well as being found in almost all environments. AOA typically greatly outnumber bacterial ammonia oxidizers in many common environments, and are among the most abundant micro-organisms on Earth (Schleper & Nicol, 2010). However, as they are difficult to cultivate, some aspects of their physiology and contribution to biogeochemical pathways are still speculative. Metagenomic studies of both seawater (Venter *et al.*, 2004) and soil have revealed the presence of putative ammonia monooxygenase genes (*amoA*) in uncultivated archaea, strongly suggesting that members of this domain possess the ability to oxidize ammonia (Francis *et al.*, 2007). Analysis of the growth in pure culture of the first marine archaea to be cultured confirmed chemolithoautrophic growth employing aerobic oxidation of ammonia to nitrite (Könneke *et al.*, 2005; Walker *et al.*, 2010). While more controversial, recent evidence suggests that soil AOA are chemolithoautotrophs as well (Zhang *et al.*, 2010b).

Analysis of sequenced genomes indicates that AOA may employ a unique biochemistry. Thaumarchaea contain the putative ammonia mono-oxygenase genes *amoA*, *amoB* and *amoC*, but lack the homologues used by bacteria to carry out the second step in the nitrification process, i.e. the components required for electron flow between hydroxylamine and ubiquinone (Prosser & Nicol, 2008; Walker *et al.*, 2010). Unexpectedly, it appears that *Nitrosopumilus maritimus* utilizes a copper-based system of electron transport rather than the typical iron-based one prevalent in bacteria (Walker *et al.*, 2010). Genome analysis has also indicated that AOA, although chemolithoautotrophs like ammonia-oxidizing bacteria, likely fix CO_2 in a different way from bacterial ammonia oxidizers, in which RuBisCo is the key enzyme. *Nitrosopumilus maritimus* probably employs a mechanism similar but not identical to the 3-hydroxypropionate/4-hydroxybutyrate pathway of the hyperthermophile *Metallosphaera sedula* for autotrophic carbon assimilation (Walker *et al.*, 2010).

AOA appear to be well adapted to oligotrophic environments with low oxygen, and *Nitrosopumilus maritimus* is uniquely capable of growth at the extremely low ammonia levels found in ocean waters (Walker *et al.*, 2010). Positive correlations of archaeal cell counts and *amo* genes with nitrite maxima in the oceans were initially suggestive that most ammonia oxidation in this environment is archaeal-derived (Wuchter *et al.*, 2006). Furthermore, the presence of AOA in extreme environments and various mesophilic biomes suggests that AOA are adapted to growth conditions that differ from those of ammonia-oxidizing bacteria, indicating niche separation (Schleper, 2010). AOA have been found to be the dominant ammonia oxidizers in most surface soils. As soil depth increases, the number of AOA remains constant, whereas the number of ammonia-oxidizing bacteria decreases dramatically (Schleper & Nicol, 2010). The archaeal community seems to be dominant in soils with low nitrogen and low nitrification rates (Schleper, 2010; Tourna *et al.*, 2008). According to Valentine (2007), the dominance of archaeal communities under limiting nutrition conditions can be attributed to their adaptation to chronic energy stress, and this might be a primary factor in differentiating bacterial and archaeal ecology.

Archaea are known to be involved in other parts of the nitrogen cycle. The discovery of nitrogen fixation in methanogens extended the distribution of this important activity to the archaeal domain, and more recently archaeal nitrogen fixation has been documented at hyperthermophilic temperatures (Mehta & Baross, 2006). Unusual regulatory mechanisms have been reported for archaeal nitrogen fixation (Leigh & Dodsworth, 2007).

Carbon: methanogenesis and anaerobic methane oxidation (reverse methanogenesis)

Methanogens have long been known to play an essential role in the decomposition of complex organic material in a variety of anaerobic habitats, such as peat bogs, digestors, rice paddies, landfill sites and ruminants (Liu & Whitman, 2008; Thauer *et al.*, 2008). Three major pathways of methanogenesis have been elucidated, with methane derived from the reduction of CO_2 with hydrogen or formate, from the methyl group of acetate or from methanol and methylamines. Aceticlastic-derived methane constitutes approximately two-thirds of the total produced annually in the biosphere, with most of the remaining one-third originating from the reduction of CO_2 with hydrogen or formate (Ferry, 2010). The other pathway, using methanol and methylamines as primary substrates, contributes comparatively minor amounts to the total methane production and is limited to a small subset of methanogens, such as the Methanosarcinales. After degradation of organic substrates by a variety of hydrolytic and fermentative bacteria, molecules generated by acetogenic and syntrophic bacteria are used by methanogens in the terminal step of degradation. As noted above, methanogenic archaea are unique in their ability to convert a limited number of simple carbon compounds to

methane. Estimates of 1 billion tonnes of methane per annum produced by methanogens attest to their important role in the global carbon cycle (Thauer *et al.*, 2008). Recent exciting experiments have reported the expression of a bacterial esterase in a methanogen that enables the host to efficiently convert to methane novel previously unutilized substrates, such as the industrial solvents methyl acetate and methyl propionate (Lessner *et al.*, 2010).

Ocean sediments contain vast quantities of solid methane hydrates (Knittel & Boetius, 2009). Most of the methane contained in these deposits does not reach the atmosphere due to the activities of the group of, so far, uncultured archaea called ANME (see above). There are currently three known clades of these methanogen-related archaea (ANME-1, ANME-2, ANME-3). ANME are believed to be widely distributed in all methane-containing environments (Knittel & Boetius, 2009). These methanotrophic euryarchaeota, likely in syntrophy with sulfate-reducing bacteria (or possibly alone in the case of ANME-1), oxidize methane to CO_2 through the reduction of sulfate. Anaerobic methane oxidation has been proposed to occur via a reversed methanogenesis pathway (Hallam *et al.*, 2004), a hypothesis supported by metagenomic analysis of ANME, showing the presence of most of the methanogenesis genes. More recently, and importantly for the support of the reverse methanogenesis hypothesis, the key final enzyme in methanogenesis, methyl CoM reductase, which converts methyl CoM to methane, has been shown to be able to carry out the reverse reaction as well (Scheller *et al.*, 2010).

Sulfur and iron: acid mine drainage

Acid mine drainage (AMD), an acidic solution containing large quantities of sulphate, iron and a variety of toxic heavy metals such as arsenic, is an environment inhabited by a complex community of bacteria, archaea and even acidophilic eukaryotes. The collective metabolism of these organisms results in the release of heavy metals from sulfidic ores in a process termed bioleaching (Bruneel *et al.*, 2008). Historically, the dominant organisms thought to carry out sulphur and iron oxidation were bacteria, but more recent studies have indicated that thermoacidophilic archaea play an important role in the bioleaching process and are often the numerically dominant members of the community (Edwards *et al.*, 2000). Fluorescence *in situ* hybridization analysis of AMD sites has shown that archaea colonize more extreme regions and constitute numerically >50 % of the communities (Golyshina & Timmis, 2005). Microbial communities present at AMD sites are mainly composed of species belonging to the Thermoplasmatales (which includes *Ferroplasma* and *Thermoplasma*) (Bini, 2010), organisms which prefer low pH and oxidize sulphur and Fe(II).

Sulphur-oxidizing pathways have been best studied in archaea. These pathways are similar in bacteria and archaea except for the step of oxygen incorporation into the elemental sulphur. This reaction is catalysed by sulphur oxygenase reductase (SOR) in archaea, whereas bacteria use sulphur diooxygenase (SDO) for elemental sulphur conversion. Archaea similar to those found in AMD have been reported in acidic cave 'snottites', expanding their niche (Macalady *et al.*, 2007). Although extremely acidic sites are geographically limited, the archaea living in such environments may play a substantial role in the global cycles of sulphur, iron and toxic metals such as arsenic (Bruneel *et al.*, 2008; Edwards *et al.*, 2000; Huber *et al.*, 2000). There is huge potential for the use of acidophilic sulphur-metabolizing archaea for bioprocessing low-grade ore and the bioremediation of heavy metal-contaminated sites (Bini, 2010), as these organisms can carry out the removal of metal oxides even at very low pH.

Archaea in disease and health

The presence of methanogenic archaea in the mammalian gut, particularly in ruminants but also in humans, has provoked considerable interest. Whether archaeal pathogens exist remains unresolved due to a lack of definitive evidence (Cavicchioli *et al.*, 2003; Conway de Macario & Macario, 2009; Eckburg *et al.*, 2003; Reeve, 1999). Reports vary widely on the correlation or otherwise of breath methane with bowel conditions, while a number of reports correlate the presence of archaea with periodontal disease (Kulik *et al.*, 2001; Vianna *et al.*, 2006; Yamabe *et al.*, 2008). There is no evidence to date to support the notion that the growth of archaeal organisms at sites of disease is anything other than adventitious.

In contrast to the notion that archaea may be pathogenic is the possibility that the involvement of archaea in mutualistic relationships may provide health benefits or influence the metabolism of their hosts. This is best illustrated in a mouse model, where *Methanobrevibacter smithii* cocolonization with *Bacteroides thetaiotaomicron* produces a significant increase in host adiposity (Samuel & Gordon, 2006). These studies have shown significant parallels with the microbial populations described for obese or anorexic individuals (Armougom *et al.*, 2009). It appears that *Methanobrevibacter smithii* has adapted to persist in the distal gut (Samuel *et al.*, 2007), and that the resulting products of polysaccharide fermentation influence host cell behaviour (Samuel *et al.*, 2008). How much of this effect is direct or indirect is not clear. For example, short-chain fatty acids (SCFAs) are produced as a result of the fermentation of complex carbohydrates and can directly affect the growth of human colon carcinoma cells (Hinnebusch *et al.*, 2002), but SCFAs can also be utilized by methanogenic archaea as growth substrates. The balance of methanogens and other hydrogen-consuming microbes (such as sulfate reducers) has also been examined (Christl *et al.*, 1992; El Oufir *et al.*, 1996; Strocchi *et al.*, 1994). Thus the presence of methanogens may be a consequence rather than a cause of a healthy gut.

There is much variability in the reported prevalence of archaea (usually methanogens) in the human gut. This can

be attributed in part to the sensitivity of the techniques used to test breath methane (Levitt *et al.*, 2006) and stool samples. Improved methods suggest that organisms such as *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* are more prevalent than previously thought (Dridi *et al.*, 2009). Additional methanogenic clades, possibly related to ANME organisms, have also been identified in the human gut (Mihajlovski *et al.*, 2008). A number of studies have also identified the presence of methanogenic archaea in human guts as indicative of a healthy microbiota, with reports of reduced methanogen presence in individuals with inflammatory bowel or Crohn's disease (Scanlan *et al.*, 2008).

Archaea and biotechnological contributions

Despite the obvious potential of extremophilic archaea to yield many commercially appealing enzymes, thermostable DNA polymerases remain the only major class of molecule to have been effectively exploited in a wide range of PCR protocols. In addition to being a rich source of proof-reading repair polymerases which are still yielding improved processivity and fidelities (Kim *et al.*, 2007), additional 'enhancers', such as inorganic pyrophosphatase (Park *et al.*, 2010), dUTPase (Hogrefe *et al.*, 2002) and dITPase (Kim *et al.*, 2008), have been isolated and exploited. Error-prone versions of proof-reading enzymes have been developed to enhance mutagenic reactions (Biles & Connolly, 2004), and combinations of enzymes have been used to enhance processivity. The Y-family DNA polymerases unique to archaea can be used to replicate damaged and/or ancient DNA molecules that would otherwise be difficult to amplify (McDonald *et al.*, 2006).

Adjuvants

The ether-linked lipids from archaea were first examined and characterized as a possible adjuvant in antigen delivery by Sprott *et al.* (1997). Liposomes composed of lipids isolated from *Methanobrevibacter smithii* or *Methanosphaera stadtmanae* (both of which are normally found in the human gut) provoke a strong immune response in inoculated mice (Krishnan *et al.*, 2000) and have the potential to be useful in combination vaccines (Patel *et al.*, 2004). Polar lipids from different species of archaea provoke different responses, which are attributable to variations in glycosylation of the head groups (Sprott *et al.*, 2008). Despite their apparent potential, to date, archaeal adjuvants have not been used in commercial vaccine production.

Bacteriorhodopsin

Bacteriorhodopsin (bR) was first identified in halophilic archaea, where it is responsible for the 'purple membranes' characteristic of these organisms. bRs have been identified in numerous microbes, where they act as simple light-driven proton pumps driven by a *cis*/*trans* retinal isomerization, underpinning a primitive form of photosynthesis. bR readily forms two-dimensional crystalline arrays and has been extensively studied. The notion of bR as a light-modified information storage molecule has been around since the 1970s and the molecule has been commercially available for a number of years. To date, no commercial devices have been made available, but a number of recent publications indicate that the commercial potential of this molecule is still being actively pursued. Applications utilizing bR in solar cells (Thavasi *et al.*, 2009), radiation sensors (Ahmadi & Yeow, 2011) and as a rewritable storage system (Yao *et al.*, 2005a, b) have all been reported.

Conclusions

In 1998, Kandler and Konig wrote ''Perhaps archaeal research as a whole will remain the subject of a small circle of 'naturalists' and evolutionists, unless pathogenic archaea (archaebacteria) are discovered and evoke medical interest''. Thankfully, this has not happened and archaea, despite the lack of proven pathogens, have instead drawn the interest of a large contingent of scientists with a wide breadth of interests. Their combined efforts have resulted in a wealth of knowledge relevant not only to archaea but also to studies in bacterial and eukaryotic cells, as well as contributing significantly to larger questions in biology. A large number of inter-domain and archaea-specific genes are yet to be characterized, and it is likely that work in this area will continue to shed significant light on various aspects of biology.

We recommend that readers also consult Cavicchioli (2011), which was published during the preparation of this review.

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