MICROBIOLOGY PIONEERS — ESSAY

The legacy of Carl Woese and Wolfram Zillig: from phylogeny to landmark discoveries

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Abstract | Two pioneers of twentieth century biology passed away during the past decade, Wolfram Zillig in April 2005 and Carl Woese in December 2012. Among several other accomplishments, Woese has been celebrated for the discovery of the domain Archaea and for establishing rRNA as the 'Rosetta Stone' of evolutionary and environmental microbiology. His work inspired many scientists in various fields of biology, and among them was Wolfram Zillig, who is credited with the discovery of several unique molecular features of archaea. In this Essay, we highlight the remarkable achievements of Woese and Zillig and consider how they have shaped the archaeal research landscape.

The discovery of a deep phylogenetic split within the prokaryotes by Carl Woese and George Fox at the University of Illinois at Urbana-Champaign, USA, in 1977 marked a major transition in the modern era of microbiology[1]. Woese used the small-subunit rRNA gene (16S rRNA of bacteria and 18S rRNA of eukaryotes) as a universal marker for phylogenetic reconstructions at a time when DNA-sequencing methods were not yet available (FIG. 1). This gene was regarded as a suitable phylogenetic yardstick to approximate the tree of life owing to its presence in all organisms and its high conservation[2,3]. Tedious biochemical analysis of oligonucleotide catalogues obtained by RNase T1 digestion led Woese to recognize that in addition to bacteria, there existed a second, fundamentally distinct prokaryotic life form, the archaebacteria[4].

The concept of three domains (formerly termed urkingdoms) of life — comprising eubacteria (later termed bacteria), archaebacteria (later termed archaea) and eukaryotes (BOX 1; FIG. 2) — was highly controversial and not immediately accepted by the scientific community. However, two German scientists, Otto Kandler and Wolfram Zillig, were highly supportive of Woese's new classification scheme. Kandler, a specialist of bacterial envelopes, was gratified to learn from Woese that methanogens, which lack the typical murein-containing cell wall of bacteria[5], were indeed not bacteria after all, but archaea. He strongly encouraged Zillig, a leading specialist in RNA polymerases (RNAPs), to begin investigating these microorganisms. Fifty-five-year-old Zillig, a biochemist and, at the time, director of the Max Planck Institute for Biochemistry in Munich, Germany, then turned his attention to archaea. Together with Karl Stetter, Zillig expanded the archaeal domain with several previously uncultured members[6–11]. Zillig also collaborated with Woese for many years, sending pellets of archaeal cells to Urbana University for 16S rRNA cataloguing[12].

Through his extensive work on archaeal RNAP, Zillig was the first to discover an evolutionary connection between archaea and eukaryotes[13]. In addition, he isolated the first archaeal viruses of hyperthermophiles[14]. His achievements inspired, and continue to inspire, the work of many molecular biologists. For those of us working in the archaeal research field, the foundations laid down by Woese and Zillig (FIG. 1) have provided several model microorganisms, the study of which continues to yield startling discoveries.

In this Essay, we briefly discuss some of the major breakthroughs made by Woese and Zillig and consider from our own perspectives how these achievements have paved the way forwards for more recent research in the field.

An unexpected Archaea–Eukarya link

Zillig's discovery of the distinct subunit composition of archaeal RNAP, which differs dramatically from that of the less complex bacterial RNAP, was an important piece of evidence that strengthened the validity of Woese's phylogenetic classification[15,16]. The new method of molecular phylogeny used by Woese was not yet widely accepted, and his suggestion of a tripartite division of all life forms was highly controversial. Therefore, the demonstration that a central cellular machine of this proposed new group of prokaryotes had a completely different molecular composition compared with that of bacteria "put molecular flesh on the phylogenetic skeleton", as Woese himself acknowledged. Not only was the archaeal RNAP distinct from its bacterial counterpart, but the subunit composition also resembled that of eukaryotic RNAPs, providing the first hint that archaea might share specific features with eukaryotes[17].

Before this fundamental discovery by Zillig, biologists had assumed that the more complex composition of eukaryotic RNAPs reflected the increased complexity of transcription in 'higher' organisms (eukaryotes) compared with the more 'primitive' nature of prokaryotes, as reflected in the small number of subunits of the bacterial RNAP. Thus, the finding that the archaeal RNAP had the same number of subunits as the eukaryotic enzyme came as a big surprise, as it suggested that the extra subunits were not required for the complex regulation of RNAP in eukaryotic organisms. Further work using antibodies showed that the archaeal RNAP does not simply resemble the eukaryotic

Figure 1 | **The late Carl Woese and the late Wolfram Zillig, two pioneers of the archaeal field.** **a**–**c** | Carl Woese, photographed by Patrick Forterre at the University of Illinois at Urbana-Champaign, USA. **a** | Carl Woese looking through the original stored films of rRNA oligonucleotide catalogues obtained by RNaseT1 digestion; each box contains the film corresponding to one species. **b** | Carl Woese holding one of his first autoradiography films for an archaeal species; each black spot corresponds to an rRNA oligonucleotide, providing a 'fingerprint' for the particular species. **c** | Carl Woese pointing at an oligonucleotide spot containing a modified base that is specific for archaea and has changed the electrophoretic behaviour of the oligonucleotide. **d** | Wolfram Zillig photographed by Sonja-Verena Albers in his laboratory at the Max Planck Institute for Biochemistry in Munich, Germany. **e** | Wolfram Zillig during a sampling tour in Iceland. Part **e** photograph courtesy of Arnulf Kletzin, Darmstadt University of Technology, Germany.

counterpart but is in fact evolutionarily related to it[13], a conclusion later confirmed by sequence analysis[18].

The work of Zillig on RNAPs encouraged others in the field to examine the composition of other central enzymes, such as DNA polymerases and DNA topoisomerases[19,20]. The Archaea–Eukarya connection re-emerged with the discovery that DNA replication in haloarchaea (formerly called halobacteria) is sensitive to aphidicolin, a specific inhibitor of eukaryotic DNA polymerases[21]. Further work revealed that archaea and eukaryotes indeed share DNA polymerases of the B family (several of which are sensitive to this drug), although most archaea (except crenarchaeotes) also contain a unique DNA polymerase, PolD, which is absent in bacteria and eukaryotes[17,22].

Archaea turned out to be a goldmine for scientists with an interest in DNA topoisomerases. The first reward was the discovery of a reverse gyrase in the hyperthermophilic archaeon *Sulfolobus acidocaldarius*, an unusual type I DNA topoisomerase that introduces positive supercoils into DNA[23–25]. DNA isolated from the archaeal virus *Sulfolobus shibatae* virus 1 (SSV1) by Zillig was later used to demonstrate the presence of positively supercoiled DNA *in vivo*[26]. Reverse gyrase was later found to be present in all hyperthermophilic archaea, and also in hyperthermophilic bacteria, such as *Thermotoga maritima*, probably reflecting horizontal gene transfer from archaea to bacteria[27]. This amazing enzyme, which combines helicase activity with the classical topoisomerase activity in a single polypeptide, is in fact the only protein unique to hyperthermophiles[28].

The connection between archaea and eukaryotes also led to the discovery of the archaeal type II DNA topoisomerase, Topo VI[29], in Patrick Forterre's laboratory, although this finding was not based on shared homology. In fact, Topo VI is not homologous to the classical eukaryotic Topo II enzyme and represents the prototype of a new Topo II family. Thus, the discovery of archaeal Topo VI had a profound impact on eukaryotic molecular biology. Owing to its homology to one of the two archaeal Topo VI subunits, the endonuclease SPO11 was also discovered. This fundamental eukaryotic protein, which cleaves homologous chromosomes to initiate meiotic recombination[29], had previously eluded the scientific community working on meiosis. Following this, homologues of archaeal Topo VI were discovered in plants and were shown to be involved in the determination of plant size, such that mutants lacking Topo VI have stunted growth (resembling bonsai plants)[30]. These unexpected links between archaea, eukaryotes and plants illustrate how archaeal systems have become important and useful models in many aspects of eukaryotic molecular biology.

More recently, a new small single-stranded DNA-binding protein (SSB) was discovered in crenarchaeotes using classical biochemistry. This led to a previously unannotated gene in the human genome being identified as a gene encoding a novel SSB[31,32] and to the identification of a new protein complex (SOSS1) that has a major role in DNA damage recognition, DNA repair and recombination in mammals[33,34]. Considering the huge number of laboratories working on these processes in eukaryotes, it is amazing that such a fundamental complex remained unknown until 2009 and was finally discovered thanks to scientists who were initially searching for new SSBs in archaea.

Notably, the number of eukaryotic traits known to be present in archaea has increased following the sequencing of more archaeal genomes belonging to previously unknown or poorly studied phyla. For instance, a close relative of eukaryotic DNA topoisomerase IB, the main enzyme involved in relaxing positive DNA supercoils during replication and transcription in eukaryotes, and an important antitumour drug target, was discovered in Thaumarchaeota, a newly recognized archaeal phylum[35,36]. Importantly, homologues of the eukaryotic ESCRT-III (endosomal sorting complex required for transport III) system (the functional roles of which include protein sorting, virus budding and cytokinesis) and of the eukaryotic

cytoskeleton (actin and tubulin) were detected in the genomes of crenarchaeotes and a thaumarchaeote[37–40]. Furthermore, a ubiquitin protein modifier system was discovered in '*Candidatus* Caldiarchaeum subterraneum' (REF. 41). These discoveries emphasize the fact that the similarities between archaea and eukaryotes extend well beyond informational proteins to operational proteins.

Surprises on the archaeal cell surface

The study of archaeal motility has also revealed a few surprises. Despite the presence of visible structures resembling bacterial flagella in many archaeal species, flagellum proteins have not been found in archaea. Recent studies in the Sonja Albers laboratory, among others, have revealed that the archaeal equivalent of the bacterial flagellum (recently renamed the archaellum[42]) is a unique motility apparatus that structurally resembles a rotating bacterial type IV pilus. For the bacterial flagellum, rotation is driven by the proton motif force, whereas for the archaellum, assembly and movement is driven by a single ATPase[43,44]. Moreover, the archaellum is assembled by a system that is similar to that used to assemble bacterial type IV pili and is located at the base of the archaellum, in the cell membrane. By contrast, the bacterial flagellum is assembled by a type III secretion system that transports individual subunits from the cytoplasm, through the interior of the flagellum for addition to the tip of the growing filament.

Although the cell envelopes of bacteria and archaea are very different (archaea lack a murein cell wall, and most of them have a single membrane surrounded by a glycoprotein S-layer[45]), both domains use type IV pili for a wide variety of functions, such as adhesion, surface motility, cell aggregation and DNA exchange[46,47]. However, there are marked differences between the archaeal and bacterial structures. For example, assembly of archaeal type IV pili is more straightforward than assembly in bacteria, as the components required to build the archaeal pilus need to cross only one membrane during assembly. Moreover, twitching motility, in which the pilus extends and subsequently disassembles to achieve surface motion, has been found only in bacteria to date. Finally, the archaellum is capable of rotation, whereas evidence showing that bacterial type IV pili can rotate is lacking. Together, these findings indicate that the bacterial flagellum and the archaellum are two distinct motility structures and can be regarded as domain-determining features.

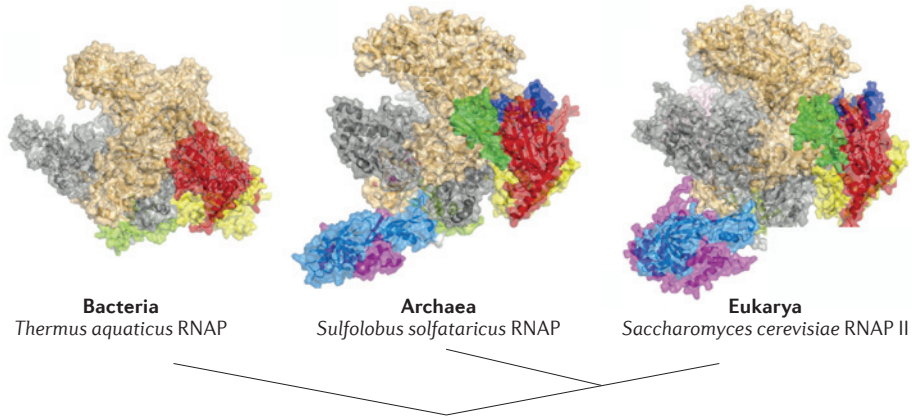


Figure 2 | **RNA polymerase structure in the three domains of life.** The structures of RNA polymerases (RNAPs) in bacteria (*Thermus aquaticus*; Protein Data Bank (PDB) accession 1I6V), archaea (*Sulfolobus solfataricus*; PDB accession 2PMZ) and eukaryotes (*Saccharomyces cerevisiae* RNAP II; PDB accession 1NT9). Homologous subunits are colour-coded and clearly demonstrate the high degree of similarity between all three transcription engines and, in particular, how the archaeal RNAP closely resembles eukaryotic RNAP II. Image is modified, with permission, from REF. 87 © (2008) Elsevier.

Box 1 | Archaea and the universal tree of life

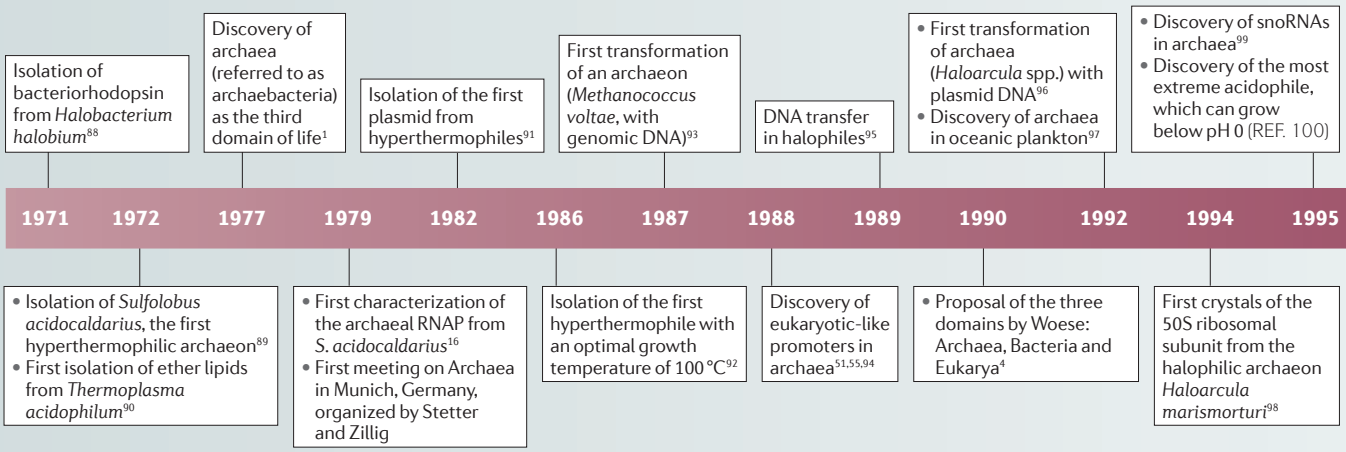
The discovery of archaea opened a Pandora's box and converted many biochemists, molecular biologists and microbiologists into fervent evolutionary biologists. Early on, Woese proposed that the large number of shared features between archaea and eukaryotes was evidence that these two domains were sister groups, as suggested by early phylogenetic analyses of universal proteins[4,77,78]. This led to the classic 'universal tree of life', often dubbed the Woese tree. However, this tree was contested by several researchers. One of them was Wolfram Zillig, who, well before the advent of genomics, discovered one of the first discrepancies between protein-based and rRNA-based trees[79]. Noticing that eukaryotic RNA polymerase (RNAP) I, RNAP II and RNAP III do not form a monophyletic group, but rather branch in-between archaeal and bacterial RNAPs in phylogenetic analyses, Zillig was the first to propose that a fusion of an archaeon and a bacterium[80] led to the origin of a proto-eukaryote with a nucleus. Woese strongly rejected such a scenario, arguing that "modern cells are sufficiently complex, integrated and 'individualized' that further major change in their designs does not appear possible" (REF. 81). However, fusion hypotheses are still very popular, although controversial[82–84].

The phylogeny of RNAPs remains puzzling, and the situation becomes even more complicated when RNAPs encoded by giant viruses (including members of the family *Megaviridae*) are added to the picture. These giant-virus RNAPs branch in between eukaryotic and archaeal RNAPs in phylogenetic trees. This observation, together with the nature of these viruses (which have a larger physical size and more complex genomes than any previously identified viruses), was interpreted by some scientists as evidence for the existence of a fourth domain of life[85]. Others argue that viral RNAPs were recruited from ancient eukaryotes or that some eukaryotic RNAPs were derived from giant-virus RNAPs[83]. The proposal of new domains and the controversies surrounding them testify to the importance of the domain concept itself and remind us that Pandora's box is far from being closed. Furthermore, the recent discovery of Pandoraviruses, the genomes of which are larger than those of some parasitic eukaryotes, adds even more complexity to the domain concept discussions[86].

Something viral in your research

Viruses were for a long time collateral victims of Woese's work, as they lack ribosomes and are therefore not amenable to the rRNA classification system introduced by Woese. However, thanks to the work of Zillig, archaeal viruses eventually found their way into the limelight. With a particular interest in the functional characterization of archaeal RNAPs and the exploration of transcriptional regulation in archaea, Zillig envisaged that viruses would be the most suitable models for such studies (extrapolating from his experience with eukaryotic viruses). Therefore, he started hunting specifically for viruses of hyperthermophilic archaea, none of which were known at that time. This led to the discovery of a number of viruses with diverse

Timeline | **Benchmark breakthroughs in the archaeal field**



ESCRT, endosomal sorting complex required for transport; RNAP, RNA polymerase; snoRNAs, small non-coding RNAs.

life cycles and unique morphotypes, and to the description of four novel virus families, *Fuselloviridae*, *Lipothrixviridae*, *Rudiviridae* and *Guttaviridae*[48]. For example, two viruses that infect *Sulfolobus* spp., *Sulfolobus islandicus* rod-shaped virus 2 (SIRV2) and *Sulfolobus* turreted isocahedral virus (STIV), generate a unique seven-sided, pyramid-like structure that is composed of a single protein and protrudes from the host cell membrane prior to lysis. To date, however, the mechanism used for the release of mature virions is unknown[49,50].

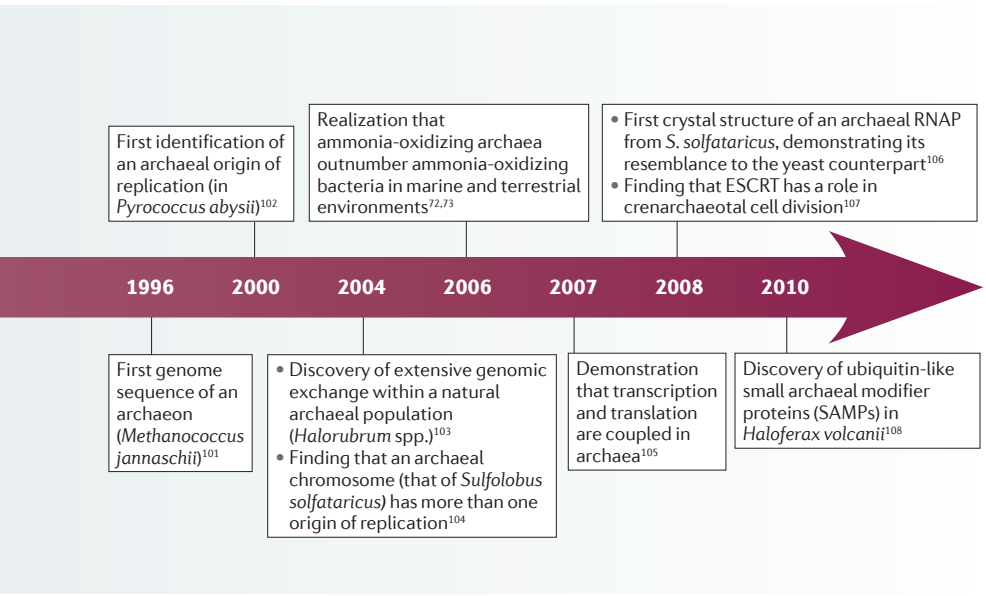
The archaeal virus SSV1 was used to identify the first archaeal transcriptional promoters, regulatory sequences and transcriptional terminators in the Zillig laboratory[51–55], and for the development of an *in vitro* transcription system[56] and the first genetic system based on recombinant vectors in a hyperthermophilic archaeon[57,58]. David Prangishvili became 'infected' by Zillig's passion for virus hunting, and his work resulted in the isolation and description of members of six other novel archaeal virus families[59], and eventually to the recognition of the virosphere of Archaea as one of the distinct features of this domain[60]. The work on archaeal viruses thus completes the initial work of Woese by showing that three viral worlds overlap with the three cellular domains[61,62]. This observation ruined the traditional dichotomy between viruses and bacteriophages, which was used to distinguish between viruses infecting eukaryotes and prokaryotes, respectively. It is noteworthy to point out that Zillig was infuriated by the use of the term bacteriophages to describe archaeal viruses merely because archaea are considered prokaryotes. To prevent any potential confusion, we recently suggested that viruses from the three domains be referred to as bacterioviruses, archeoviruses and eukaryoviruses[60]. In addition to the discovery of giant viruses[63] (BOX 1), research on archaeal viruses has greatly contributed to the renewed interest of biologists for the origin, nature and complexity of viruses, and their major role in biological evolution[64,65].

The expanding archaeal universe

All the organisms that Woese initially identified as archaea were extremophiles or organisms that were restricted to anaerobic niches (such as thermophiles, acidophiles, halophiles and anaerobic methanogens). This was the major reason which led him to argue that archaea are close descendants of the first living organisms, hence the name Archaea. This initial observation inspired scientists to search for life in environments that were thought to be beyond the limits of life.

During their first expeditions to hot springs in southern Italy, Stetter and Zillig used thermos flasks to minimize temperature loss from their samples, worrying that the organisms contained in the samples would not be able to survive moderate temperatures. Although Thomas Brock had isolated *S. acidocaldarius* in 1972 from hot springs in Yellowstone National Park (USA), Stetter and Zillig were the first to realize that hot springs contain a huge number of live cells, suggesting that such extreme environments can harbour as many microbial cells as moderate environments. They were also the first to discover life forms thriving at temperatures of 80 °C and above, which were subsequently termed hyperthermophiles by Stetter. Most, but not all, of these hyperthermophiles were archaea. The record-setting *Pyrodictium* spp. (which was isolated from a thermal vent in the ocean floor) and several other archaeal species are capable of thriving at temperatures far above the boiling point of water[66]. Owing to the discoveries made by Stetter and Zillig, many present-generation researchers are appreciative of the importance of field work and are acutely aware of the treasures to be found in extreme environments.

Woese's approach of using rRNA genes as a phylogenetic marker has not only become a standard technique in taxonomy and phylogeny, but also paved the way for modern microbial ecology. Progress in this area has strongly relied on culture-independent amplification of small-subunit rRNA genes directly from environmental samples to obtain an inventory of naturally occurring microorganisms. This new research field has tremendously changed our perception of the diversity and abundance of microorganisms and their role in biogeochemical cycles[67]. It has also brought about a fundamentally new view of archaea: that not all members of this domain are limited to growth in extreme environments. With molecular techniques, including 16S rRNA sequencing, many novel archaeal lineages and even phyla have now been discovered in commonplace environments. It took roughly 20 years from Woese's discovery



of the third domain to realize that archaea encompass not only exotic organisms that live in extreme niches, but also organisms that are part of our daily lives and occur in large numbers in moderate and aerobic environments, from the ocean to the soil and even our skin[68].

Metagenomic and cultivation studies of soil samples in Christa Schleper's laboratory[69,70] , as well as the cultivation of a marine archaeon[71], led to the recognition that these archaea (now termed thaumarchaeotes) are autotrophic ammonia oxidizers that occur in huge numbers in marine and soil environments[72,73]. They carry out an important step in the global nitrogen cycle by oxidizing mineralized ammonia to nitrite. This first and rate-limiting step in nitrification was considered for more than 100 years to be carried out exclusively by certain proteobacteria. Owing to the high numbers of thaumarchaeotes and their ubiquity, it is now becoming increasingly clear that these organisms are the predominant ammonia oxidizers, particularly in pristine environments, and that they even rank among the most abundant microorganisms on the planet. Together with the methanogens and the as-yet-uncultured anaerobic methane-oxidizing archaea[74], they form the third group of archaea that successfully colonize a wide range of habitats and have a crucial role in global biogeochemical nutrient cycles.

The legacy

At the Gordon Research Conference on Archaea, which celebrated its thirtieth anniversary in 2013, we realized how inspiring the multidisciplinary research field of archaea has been to every attendant, as it unifies research of the highest quality from a diverse array of disciplines, including molecular and structural biology, physiology, ecology and evolution. Through the integration of research from these disciplines over a period spanning more than three decades (FIG. 3 (TIMELINE)), we now have a broad knowledge base of the archaeal domain and are finally beginning to unravel the mysteries that this domain initially presented. Those of us already in the field continue to promote the work and legacy of Woese and Zillig in the hope that their achievements will also inspire the upcoming generations of biologists. It is hoped that these researchers will appreciate that the biosphere offers many more hidden treasures than just the classical model organisms of today.

Projects such as the Human Microbiome Project have raised the profile of microbiology in general, including archaea, and highlight the importance of human–microorganism interactions. A recent study suggests that the absence of methanogens is linked to obesity in humans[75]; such interactions are likely to be the tip of the iceberg and are open to future exploration. Moreover, it will be crucial to study the metabolic activities of the poorly characterized ammonia- and methane-oxidizing archaea in more detail, as these species are likely to have important roles in the global cycling of nitrogen and carbon. Importantly, these microorganism-based cycles influence the emission of the greenhouse gases methane and nitrous oxide[76].

Given the amount of data collected and the astonishing discoveries made over the past 30 years, it is surprising that the battle to support the recognition of Archaea as a separate domain, rather than as a curious branch of Bacteria, is still continuing today. We predict that this century will see the rise of archaea, with many more surprising discoveries of their unique biology.

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1. Woese, C. R. & Fox, G. E. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc. Natl Acad. Sci. USA* **74**, 5088–5090 (1977).
2. Fox, G. E., Magrum, L. J., Balch, W. E., Wolfe, R. S. & Woese, C. R. Classification of methanogenic bacteria by 16S ribosomal RNA characterization. *Proc. Natl Acad. Sci. USA* **74**, 4537–4541 (1977).
3. Pace, N. R., Sapp, J. & Goldenfeld, N. Phylogeny and beyond: scientific, historical, and conceptual significance of the first tree of life. *Proc. Natl Acad. Sci. USA* **109**, 1011–1018 (2012).
4. Woese, C. R., Kandler, O. & Wheelis, M. L. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl Acad. Sci. USA* **87**, 4576–4579 (1990).
5. Kandler, O. & Konig, H. Chemical composition of the peptidoglycan-free cell walls of methanogenic bacteria. *Arch. Microbiol.* **118**, 141–152 (1978).
6. Schleper, C. *et al. Picrophilus* gen. nov., fam. nov.: a novel aerobic, heterotrophic, thermoacidophilic genus and family comprising archaea capable of growth around pH 0. *J. Bacteriol.* **177**, 7050–7059 (1995).
7. Woese, C. R., Gupta, R., Hahn, C. M., Zillig, W. & Tu, J. The phylogenetic relationships of three sulfur dependent archaebacteria. *Syst. Appl. Microbiol.* **5**, 97–105 (1984).
8. Zillig, W. *et al.* The archaebacterium *Thermofilum pendens* represents, a novel genus of the thermophilic, anaerobic sulfur respiring *Thermoproteales*. *Syst. Appl. Microbiol.* **4**, 79–87 (1983).
9. Zillig, W. *et al. Hyperthermus butylicus*, a hyperthermophilic sulfur-reducing archaebacterium that ferments peptides. *J. Bacteriol.* **172**, 3959–3965 (1990).
10. Zillig, W., Holz, I., Janekovic, D., Schafer, W. & Reiter, W. D. The archaebacterium *Thermococcus celer* represents, a novel genus within the thermophilic branch of the archaebacteria. *Syst. Appl. Microbiol.* **4**, 88–94 (1983).
11. Zillig, W., Tu, J. & Holz, I. Thermoproteales—a third order of thermoacidophilic archaebacteria. *Nature* **293**, 85–86 (1981).
12. Stetter, K. O. A brief history of the discovery of hyperthermophilic life. *Biochem. Soc. Trans.* **41**, 416–420 (2013).
13. Huet, J., Schnabel, R., Sentenac, A. & Zillig, W. Archaebacteria and eukaryotes possess DNA-dependent RNA polymerases of a common type. *EMBO J.* **2**, 1291–1294 (1983).

14. Martin, A. *et al.* SAV 1, a temperate u.v.-inducible DNA virus-like particle from the archaebacterium *Sulfolobus acidocaldarius* isolate B12. *EMBO J.* **3**, 2165–2168 (1984).
15. Zillig, W., Stetter, K. O. & Tobien, M. DNA-dependent RNA polymerase from *Halobacterium halobium*. *Eur. J. Biochem.* **91**, 193–199 (1978).
16. Zillig, W., Stetter, K. O. & Janekovic, D. DNA-dependent RNA polymerase from the archaebacterium *Sulfolobus acidocaldarius*. *Eur. J. Biochem.* **96**, 597–604 (1979).
17. Pisani, F. M., De Martino, C. & Rossi, M. A. DNA polymerase from the archaeon *Sulfolobus solfataricus* shows sequence similarity to family B DNA polymerases. *Nucleic Acids Res.* **20**, 2711–2716 (1992).
18. Langer, D., Hain, J., Thuriaux, P. & Zillig, W. Transcription in archaea: similarity to that in eucarya. *Proc. Natl Acad. Sci. USA* **92**, 5768–5772 (1995).
19. Prangishvili, D. A. Molecular biology of archaebacteria. *Mol. Biol.* **17**, 234–248 (1983) (in Russian).
20. Forterre, P., Squali, F. Z., Hughes, P. & Kohiyama, M. Studies on the role of dam methylation at the *Escherichia coli* chromosome replication origin (*oriC*). *Adv. Exp. Med. Biol.* **179**, 543–549 (1984).
21. Forterre, P., Elie, C. & Kohiyama, M. Aphidicolin inhibits growth and DNA synthesis in halophilic arachaebacteria. *J. Bacteriol.* **159**, 800–802 (1984).
22. Ishino, Y., Komori, K., Cann, I. K. & Koga, Y. A novel DNA polymerase family found in Archaea. *J. Bacteriol.* **180**, 2232–2236 (1998).
23. Forterre, P., Mirambeau, G., Jaxel, C., Nadal, M. & Duguet, M. High positive supercoiling *in vitro* catalyzed by an ATP and polyethylene glycol-stimulated topoisomerase from *Sulfolobus acidocaldarius*. *EMBO J.* **4**, 2123–2128 (1985).
24. Kikuchi, A. & Asai, K. Reverse gyrase—a topoisomerase which introduces positive superhelical turns into DNA. *Nature* **309**, 677–681 (1984).
25. Nadal, M., Mirambeau, G., Forterre, P., Reiter, W. D. & Duguet, M. Positively supercoiled DNA in a virus-like particle of an archaebacterium. *Nature* **321**, 256–258 (1986).
26. Jaxel, C. *et al.* Reverse gyrase binding to DNA alters the double helix structure and produces single-strand cleavage in the absence of ATP. *EMBO J.* **8**, 3135–3139 (1989).
27. Brochier-Armanet, C. & Forterre, P. Widespread distribution of archaeal reverse gyrase in thermophilic bacteria suggests a complex history of vertical inheritance and lateral gene transfers. *Archaea* **2**, 83–93 (2007).
28. Forterre, P. A hot story from comparative genomics: reverse gyrase is the only hyperthermophile-specific protein. *Trends Genet.* **18**, 236–237 (2002).
29. Bergerat, A. *et al.* An atypical topoisomerase II from Archaea with implications for meiotic recombination. *Nature* **386**, 414–417 (1997).
30. Yin, Y. *et al.* A crucial role for the putative *Arabidopsis* topoisomerase VI in plant growth and development. *Proc. Natl Acad. Sci. USA* **99**, 10191–10196 (2002).
31. Robbins, J. B. *et al.* The euryarchaeota, nature's medium for engineering of single-stranded DNA-binding proteins. *J. Biol. Chem.* **280**, 15325–15339 (2005).
32. Richard, D. J. *et al.* Single-stranded DNA-binding protein hSSB1 is critical for genomic stability. *Nature* **453**, 677–681 (2008).
33. Skaar, J. R. *et al.* INTS3 controls the hSSB1-mediated DNA damage response. *J. Cell Biol.* **187**, 25–32 (2009).
34. Yang, S. H. *et al.* The SOSS1 single-stranded DNA binding complex promotes DNA end resection in concert with Exo1. *EMBO J.* **32**, 126–139 (2013).
35. Brochier-Armanet, C., Boussau, B., Gribaldo, S. & Forterre, P. Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nature Rev. Microbiol.* **6**, 245–252 (2008).
36. Brochier-Armanet, C., Gribaldo, S. & Forterre, P. A. DNA topoisomerase IB in Thaumarchaeota testifies for the presence of this enzyme in the last common ancestor of Archaea and Eucarya. *Biol. Direct* **3**, 54 (2008).
37. Makarova, K. S., Yutin, N., Bell, S. D. & Koonin, E. V. Evolution of diverse cell division and vesicle formation systems in Archaea. *Nature Rev. Microbiol.* **8**, 731–741 (2010).
38. Makarova, K. S. & Koonin, E. V. Two new families of the FtsZ-tubulin protein superfamily implicated in membrane remodeling in diverse bacteria and archaea. *Biol. Direct* **5**, 33 (2010).
39. Yutin, N. & Koonin, E. V. Archaeal origin of tubulin. *Biol. Direct* **7**, 10 (2012).
40. Yutin, N., Wolf, M. Y., Wolf, Y. I. & Koonin, E. V. The origins of phagocytosis and eukaryogenesis. *Biol. Direct* **4**, 9 (2009).
41. Nunoura, T. *et al.* Insights into the evolution of Archaea and eukaryotic protein modifier systems revealed by the genome of a novel archaeal group. *Nucleic Acids Res.* **39**, 3204–3223 (2011).
42. Jarrell, K. F. & Albers, S. V. The archaellum: an old motility structure with a new name. *Trends Microbiol.* **20**, 307–312 (2012).
43. Reindl, S. *et al.* Insights into FlaI functions in archaeal motor assembly and motility from structures, conformations, and genetics. *Mol. Cell* **49**, 1069–1082 (2013).
44. Streif, S., Staudinger, W. F., Marwan, W. & Oesterhelt, D. Flagellar rotation in the archaeon *Halobacterium salinarum* depends on ATP. *J. Mol. Biol.* **384**, 1–8 (2008).
45. Albers, S. V. & Meyer, B. H. The archaeal cell envelope. *Nature Rev. Microbiol.* **9**, 414–426 (2011).
46. Lassak, K., Ghosh, A. & Albers, S. V. Diversity, assembly and regulation of archaeal type IV pili-like and non-type-IV pili-like surface structures. *Res. Microbiol.* **163**, 630–644 (2012).
47. Pohlschroder, M., Ghosh, A., Tripepi, M. & Albers, S. V. Archaeal type IV pilus-like structures—evolutionarily conserved prokaryotic surface organelles. *Curr. Opin. Microbiol.* **14**, 357–363 (2011).
48. Zillig, W. *et al.* Genetic elements in the extremely thermophilic archaeon *Sulfolobus*. *Extremophiles* **2**, 131–140 (1998).
49. Bize, A. *et al.* A unique virus release mechanism in the Archaea. *Proc. Natl Acad. Sci. USA* **106**, 11306–11311 (2009).
50. Brumfield, S. K. *et al.* Particle assembly and ultrastructural features associated with replication of the lytic archaeal virus *Sulfolobus* turreted icosahedral virus. *J. Virol.* **83**, 5964–5970 (2009).
51. Reiter, W. D., Hudepohl, U. & Zillig, W. Mutational analysis of an archaebacterial promoter: essential role of a TATA box for transcription efficiency and start-site selection *in vitro*. *Proc. Natl Acad. Sci. USA* **87**, 9509–9513 (1990).
52. Reiter, W. D. *et al.* Putative promoter elements for the ribosomal RNA genes of the thermoacidophilic archaebacterium *Sulfolobus* sp. strain B12. *Nucleic Acids Res.* **15**, 5581–5595 (1987).
53. Reiter, W. D., Palm, P., Yeats, S. & Zillig, W. Gene expression in archaebacteria: physical mapping of constitutive and UV-inducible transcripts from the *Sulfolobus* virus-like particle SSV1. *Mol. Gen. Genet.* **209**, 270–275 (1987).
54. Reiter, W. D., Palm, P. & Zillig, W. Transcription termination in the archaebacterium *Sulfolobus*: signal structures and linkage to transcription initiation. *Nucleic Acids Res.* **16**, 2445–2459 (1988).
55. Reiter, W. D., Palm, P. & Zillig, W. Analysis of transcription in the archaebacterium *Sulfolobus* indicates that archaebacterial promoters are homologous to eukaryotic pol II promoters. *Nucleic Acids Res.* **16**, 1–19 (1988).
56. Hudepohl, U., Reiter, W. D. & Zillig, W. *In vitro* transcription of two rRNA genes of the archaebacterium *Sulfolobus* sp. B12 indicates a factor requirement for specific initiation. *Proc. Natl Acad. Sci. USA* **87**, 5851–5855 (1990).
57. Schleper, C., Kubo, K. & Zillig, W. The particle SSV1 from the extremely thermophilic archaeon *Sulfolobus* is a virus: demonstration of infectivity and of transfection with viral DNA. *Proc. Natl Acad. Sci. USA* **89**, 7645–7649 (1992).
58. Jonuscheit, M., Martusewitsch, E., Stedman, K. M. & Schleper, C. A reporter gene system for the hyperthermophilic archaeon *Sulfolobus solfataricus* based on a selectable and integrative shuttle vector. *Mol. Microbiol.* **48**, 1241–1252 (2003).
59. Prangishvili, D. The wonderful world of archaeal viruses. *Annu. Rev. Microbiol.* **67**, 565–585 (2013).
60. Prangishvili, D., Forterre, P. & Garrett, R. A. Viruses of the Archaea: a unifying view. *Nature Rev. Microbiol.* **4**, 837–848 (2006).
61. Prangishvili, D., Garrett, R. A. & Koonin, E. V. Evolutionary genomics of archaeal viruses: unique viral genomes in the third domain of life. *Virus Res.* **117**, 52–67 (2006).
62. Pina, M., Bize, A., Forterre, P. & Prangishvili, D. The archeoviruses. *FEMS Microbiol. Rev.* **35**, 1035–1054 (2011).
63. La Scola, B. *et al.* A giant virus in amoebae. *Science* **299**, 2033 (2003).
64. Forterre, P. & Prangishvili, D. The major role of viruses in cellular evolution: facts and hypotheses. *Curr. Opin. Virol.* http://dx.doi.org/10.1016/j.coviro.2013.06.013 (2013).
65. Koonin, E. V. & Dolja, V. V. A virocentric perspective on the evolution of life. *Curr. Opin. Virol.* http://dx.doi.org/10.1016/j.coviro.2013.06.008 (2013).
66. Stetter, K. O., Konig, H. & Stackebrandt, E. *Pyrodictium* gen. nov., a new genus of submarine disc-shaped sulphur reducing archaebacteria growing optimally at 105 °C. *Syst. Appl. Microbiol.* **4**, 535–551 (1983).
67. Pace, N. R. A molecular view of microbial diversity and the biosphere. *Science* **276**, 734–740 (1997).
68. Probst, A. J., Auerbach, A. K. & Moissl-Eichinger, C. Archaea on human skin. *PLoS ONE* **8**, e65388 (2013).
69. Schleper, C., Jurgens, G. & Jonuscheit, M. Genomic studies of uncultivated archaea. *Nature Rev. Microbiol.* **3**, 479–488 (2005).
70. Tourna, M. *et al. Nitrososphaera viennensis*, an ammonia oxidizing archaeon from soil. *Proc. Natl Acad. Sci. USA* **108**, 8420–8425 (2011).
71. Konneke, M. *et al.* Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**, 543–546 (2005).
72. Wuchter, C. *et al.* Archaeal nitrification in the ocean. *Proc. Natl Acad. Sci. USA* **103**, 12317–12322 (2006).
73. Leininger, S. *et al.* Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**, 806–809 (2006).
74. Boetius, A. *et al.* A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* **407**, 623–626 (2000).
75. Angelakis, E., Armougom, F., Million, M. & Raoult, D. The relationship between gut microbiota and weight gain in humans. *Future Microbiol.* **7**, 91–109 (2012).
76. Offre, P., Spang, A. & Schleper, C. Archaea in biogeochemical cycles. *Annu. Rev. Microbiol.* http://dx.doi.org/10.1146/annurev-micro-092412-155614 (2013).
77. Iwabe, N., Kuma, K., Hasegawa, M., Osawa, S. & Miyata, T. Evolutionary relationship of archaebacteria, eubacteria, and eukaryotes inferred from phylogenetic trees of duplicated genes. *Proc. Natl Acad. Sci. USA* **86**, 9355–9359 (1989).
78. Gogarten, J. P. *et al.* Evolution of the vacuolar H^+-ATPase: implications for the origin of eukaryotes. *Proc. Natl Acad. Sci. USA* **86**, 6661–6665 (1989).
79. Puhler, G. *et al.* Archaebacterial DNA-dependent RNA polymerases testify to the evolution of the eukaryotic nuclear genome. *Proc. Natl Acad. Sci. USA* **86**, 4569–4573 (1989).
80. Zillig, W. Comparative biochemistry of Archaea and Bacteria. *Curr. Opin. Genet. Dev.* **1**, 544–551 (1991).
81. Woese, C. R. Interpreting the universal phylogenetic tree. *Proc. Natl Acad. Sci. USA* **97**, 8392–8396 (2000).
82. Gribaldo, S., Poole, A. M., Daubin, V., Forterre, P. & Brochier-Armanet, C. The origin of eukaryotes and their relationship with the Archaea: are we at a phylogenomic impasse? *Nature Rev. Microbiol.* **8**, 743–752 (2010).
83. Forterre, P. Giant viruses: conflicts in revisiting the virus concept. *Intervirology* **53**, 362–378 (2010).
84. Martijn, J. & Ettema, T. J. From archaeon to eukaryote: the evolutionary dark ages of the eukaryotic cell. *Biochem. Soc. Trans.* **41**, 451–457 (2013).
85. Boyer, M., Madoui, M. A., Gimenez, G., La Scola, B. & Raoult, D. Phylogenetic and phyletic studies of informational genes in genomes highlight existence of a 4 domain of life including giant viruses. *PLoS ONE* **5**, e15530 (2010).
86. Philippe, N. *et al.* Pandoraviruses: amoeba viruses with genomes up to 2.5 Mb reaching that of parasitic eukaryotes. *Science* **341**, 281–286 (2013).
87. Werner, F. Structural evolution of multisubunit RNA polymerases. *Trends Microbiol.* **16**, 247–250 (2008).
88. Oesterhelt, D. & Stoeckenius, W. Rhodopsin-like protein from the purple membrane of *Halobacterium halobium*. *Nature New Biol.* **233**, 149–152 (1971).
89. Brock, T. D., Brock, K. M., Belly, R. T. & Weiss, R. L. *Sulfolobus*: a new genus of sulfur-oxidizing bacteria living at low pH and high temperature. *Arch. Microbiol.* **84**, 54–68 (1972).
90. Langworthy, T. A., Smith, P. F. & Mayberry, W. R. Lipids of *Thermoplasma acidophilum*. *J. Bacteriol.* **112**, 1193–1200 (1972).
91. Yeats, S., McWilliam, P. & Zillig, W. A plasmid in the archaebacterium *Sulfolobus acidocaldarius*. *EMBO J.* **1**, 1035–1038 (1982).

92. Fiala, G. & Stetter, K. O. *Pyrococcus furiosus* sp. nov. represents anovel genus of marine heterotrophic archaebacteria growing optimally at 100°C. *Arch. Microbiol.*, 56–61 (1986).
93. Bertani, G. & Baresi, L. Genetic transformation in the methanogen Methanococcus voltae PS. *J. Bacteriol.* **169**, 2730–2738 (1987).
94. Thomm, M. & Wich, G. An archaebacterial promoter element for stable RNA genes with homology to the TATA box of higher eukaryotes. *Nucleic Acids Res.* **16**, 151–163 (1988).
95. Rosenshine, I., Tchelet, R. & Mevarech, M. The mechanism of DNA transfer in the mating system of an archaebacterium. *Science* **245**, 1387–1389 (1989).
96. Cline, S. W. & Doolittle, W. F. Transformation of members of the genus *Haloarcula* with shuttle vectors based on *Halobacterium halobium* and *Haloferax volcanii* plasmid replicons. *J. Bacteriol.* **174**, 1076–1080 (1992).
97. DeLong, E. F. Archaea in coastal marine environments. *Proc. Natl Acad. Sci. USA* **89**, 5685–5689 (1992).
98. Evers, U., Franceschi, F., Boddeker, N. & Yonath, A. Crystallography of halophilic ribosome: the isolation of an internal ribonucleoprotein complex. *Biophys. Chem.* **50**, 3–16 (1994).
99. Omer, A. D. *et al.* Homologs of small nucleolar RNAs in Archaea. *Science* **288**, 517–522 (2000).
100. Schleper, C., Puhler, G., Kuhlmorgen, B. & Zillig, W. Life at extremely low pH. *Nature* **375**, 741–742 (1995).
101. Bult, C. J. *et al.* Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. *Science* **273**, 1058–1073 (1996).
102. Myllykallio, H. *et al.* Bacterial mode of replication with eukaryotic-like machinery in a hyperthermophilic archaeon. *Science* **288**, 2212–2215 (2000).
103. Papke, R. T., Koenig, J. E., Rodriguez-Valera, F. & Doolittle, W. F. Frequent recombination in a saltern population of *Halorubrum*. *Science* **306**, 1928–1929 (2004).
104. Robinson, N. P. *et al.* Identification of two origins of replication in the single chromosome of the archaeon *Sulfolobus solfataricus*. *Cell* **116**, 25–38 (2004).
105. French, S. L., Santangelo, T. J., Beyer, A. L. & Reeve, J. N. Transcription and translation are coupled in Archaea. *Mol. Biol. Evol.* **24**, 893–895 (2007).
106. Hirata, A., Klein, B. J. & Murakami, K. S. The X-ray crystal structure of RNA polymerase from Archaea. *Nature* **451**, 851–854 (2008).
107. Samson, R. Y., Obita, T., Freund, S. M., Williams, R. L. & Bell, S. D. A role for the ESCRT system in cell division in archaea. *Science* **322**, 1710–1713 (2008).
108. Humbard, M. A. *et al.* Ubiquitin-like small archaeal modifier proteins (SAMPs) in *Haloferax volcanii*. *Nature* **463**, 54–60 (2010).

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