Current Biology
Magazine

CellPress

Primer

DPANN archaea

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Archaea are one of the two primary domains of life alongside Bacteria. Extant archaea play an important role in global nutrient cycles and comprise members that were crucial for the evolution of life on Earth including the origin of eukaryotic cells through a symbiotic integration of an archaeal and bacterial partner. Despite their importance in ecology and evolution, our knowledge of archaeal diversity and function remains limited in part because it has proven challenging to cultivate archaea in the laboratory. Over the last two decades, the use of novel cultivation-independent approaches such as metagenomics has not only led to the discovery of a vast diversity of previously unknown archaeal lineages but also provided a window into their genomic content, allowing researchers to make predictions about metabolic functions and lifestyles. For example, by combining genomics approaches with phylogenetic analyses (that is, the reconstruction of species trees) researchers have uncovered several phylum-level lineages of putative genome-reduced archaea referred to as the 'DPANN' archaea, whose members were shown to have limited metabolic capabilities, indicating their dependency on symbiotic partners. These findings are consistent with observations from cultivation-based studies that have succeeded in enriching some of these small-cell symbionts in co-cultures with their hosts. Although they were initially discovered in extreme environments, DPANN archaea have now been shown to be widespread across a variety of environments and may thus play an important role in not only host evolution but also ecology. Herein, we aim to highlight DPANN archaea by providing an overview of their diversity, genomic and metabolic features, unique cell biology and interactions, and evolutionary origins. We also underscore several fascinating topics that remain underexplored.

The growing family tree of DPANN archaea

Initially, archaeal symbionts were thought to comprise predominantly syntrophic, metabollically co-dependent organisms. This perspective changed in 2002, with the discovery of tiny spherical cells (~400 nm) in a co-culture established from hydrothermal vent samples at the Kolbeinsey Ridge off the coast of Iceland by Harald Huber and coworkers. This small organism could not be grown without its host, a hyperthermophilic archaeon called *Ignicoccus hospitalis*, suggesting a strict host dependency. Further investigations confirmed that this organism with its tiny genome of just 490 kbp has an ectosymbiotic lifestyle requiring direct cell–cell contact with its host for growth at around 90°C. As a result, it was named *Nanoarchaeum equitans*, meaning 'a dwarf archaeon riding on a fireball'. *N. equitans*'s 16S rRNA gene sequence turned out to be significantly distinct from the known archaeal homologues at the time, suggesting that it represented a previously unknown and potentially deep-branching archaeal phylum — the Nanoarchaeota. Indeed, its divergence prior to the split between the formerly recognized Crenarchaeota and Euryarchaeota in phylogenetic analyses and highly reduced genome led to the speculation of *N. equitans*'s primitive state, which, however, was contested early on.

The subsequent use of microscopy combined with cultivation-independent methods to explore the vast diversity of microorganisms has led to the discovery of many additional nano-sized archaea. For example, ultrasmall archaeal cells referred to as ARMAN were discovered in low-complexity acidic biofilms (pH 0.5 – 1.5) and shown to form ectosymbiotic interactions with archaea affiliated with the Thermoplasmatales order, resembling the dwarf archaeon and its fireball. Shortly thereafter, archaea with small genomes and limited metabolic capacities were also found in metagenomes from hypersaline environments and were named Nanohaloarchaeota. Indeed, it soon became evident that Nanoarchaeota, ARMAN, and Nanohaloarchaeota are part of a much larger diversity of nano-sized archaea. Using single-cell genomics and metagenomics approaches, respectively, Rinke and coworkers as well as Castelle and coworkers reconstructed genomes from non-extreme environments and uncovered four additional lineages that were affiliated with these nano-sized archaeal clades: the Diapherotrites, Aenigmarchaeota, Woesearchaeota and Pacearchaeota. Collectively, these microbes are now assigned to a group of archaea referred to as DPANN (ICNP syn. Nanobdellati), in reference to the first members of this group: Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota and Nanohaloarchaeota. Remarkably, we now know that the DPANN archaea display an unparalleled breadth of diversity that greatly improves our grasp of the archaeal domain of life. Conservatively speaking, more than 26% of identified archaeal representative species belong to the DPANN, spanning 11 phyla and 53 orders as recognized by the Genome Taxonomy Database (release 220) (Figure 1).

DPANN archaea have small genomes and limited metabolic capabilities

Culture-independent approaches have assembled an extensive repository of DPANN archaeal genomes, providing a valuable window into the genomic and metabolic characteristics of these enigmatic organisms. Many available DPANN archaeal genomes, although encoding canonical information machinery, are characterised by limited metabolic potential, indicating that many representatives are obligate symbionts, consistent with findings from the small number of co-cultured systems. However, the genome sizes of DPANN archaea vary among and within lineages, with some representatives having larger genomes and more complete pathways, potentially enabling a less host-dependent lifestyle. For example, although members of the Nanoarchaeales (referred to previously as Nanoarchaeota) harbour highly reduced genomes (0.45 – 0.66 Mbp, median 0.55 Mbp), the genomes of Woesearchaeales members range from 0.64 to 2.06 Mbp (median 1.22 Mbp), indicating considerable variation in metabolic potential amongst DPANN archaea. Furthermore, Altiarchaeota, which appear to belong to the DPANN radiation, comprise host-independent organisms that can even serve as hosts to members of another DPANN lineage, the Huberarchaeota (Figure 1). Many DPANN genomes lack many genes encoding components of electron transport chains, central carbon

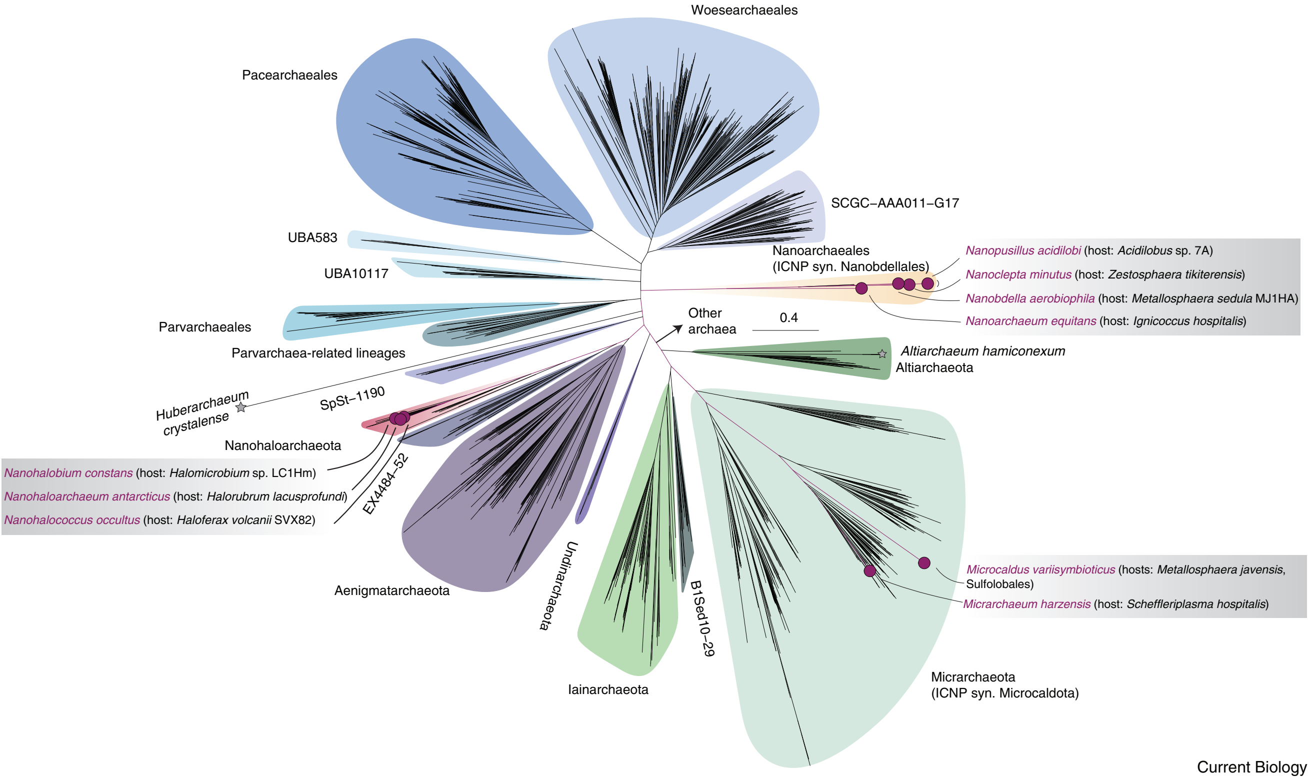

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Figure 1. Phylogenetic diversity of the DPANN group archaea (ICNP sym. Nanobdellati).
Representative DPANN archaeal genomes were sampled from the genome taxonomy database (release 220). Branches leading to successfully co-cultivated DPANN symbionts are highlighted in red, contrasting them from the diverse uncultivated DPANN members. Known host names (in parentheses) are given alongside their respective DPANN symbionts (in dark red text). The host–symbiont relationship between *Altiarchaeum hamiconexum* and *Huberarchaeum crystalense* was based on co-occurrence profiles and FISH microscopy and is hence denoted with grey stars. Most lineages are shown and coloured at the phylum level, whereas lineages from within Nanoarchaeota (ICNP sym. Nanobdellota) are shown at the order level, due to their huge diversity. Please note that most of the cultivated symbionts have Candidatus status.

metabolism, and various biosynthesis pathways. Whereas some may be able to conserve energy through fermentative processes, others may be entirely dependent on their hosts or other external sources for the acquisition of ATP and cellular building blocks, including amino acids, purines, pyrimidines, lipids and vitamins.

Established and putative host–symbiont systems

To date, only a few DPANN archaea have been enriched in co-culture with their hosts (Figure 1), namely, symbionts belonging to Nanoarchaeales, Micrarchaeota and Nanohaloarchaeota. Although cultivated members of the Nanoarchaeales seem to rely on interactions with a single host strain, the Micrarchaeota might be able to interact with multiple distinct host strains. Notably, *Microcaldus variisymbioticus* was found to interact with not only *Metallosphaera javensis* but also various thermoacidophilic Sulfolobales species, showing an even more flexible range of hosts.

Although these co-cultures have allowed for the unequivocal identification of viable hosts of these DPANN, little is known about the interaction partners for the majority of the other DPANN organisms. In the absence of enrichments, co-occurrence networks can be used to identify putative host–symbiont lineages. For instance, *Huberarchaeum crystalense* was suggested to interact with Altiarchaeota based on co-abundance profiles and microscopy, representing the first documented example of a host–symbiont relationship with both partners being affiliated with the DPANN. However, network-based approaches have been less informative for predicting hosts of other DPANN, and conflicting results have been obtained for putative partners of the extremely diverse Woesearchaeota.

Unusual cell biology and host interactions

The characterisation of some of these co-cultured DPANN archaea has started to yield fascinating insights into their cell biology (Figure 2). All experimentally investigated DPANN archaea are symbionts that require cell–cell contacts with a host for growth and survival. Attachment to host cells may allow for the acquisition of key metabolites that the DPANN cannot synthesise. Microscopy data from one of the most-studied systems, the ectosymbiotic *N. equitans* with its *I. hospitalis* host, unveiled two modes of interactions that might facilitate metabolite exchange: direct cytoplasmic contact between two cell surfaces and indirect interconnection via thin fibres (Figure 2A). Although the detailed transfer process remains largely enigmatic, several membrane-bound proteins have been suggested to mediate transfer. Similarly, electron micrographs of *Nanobdella aerobiophila*

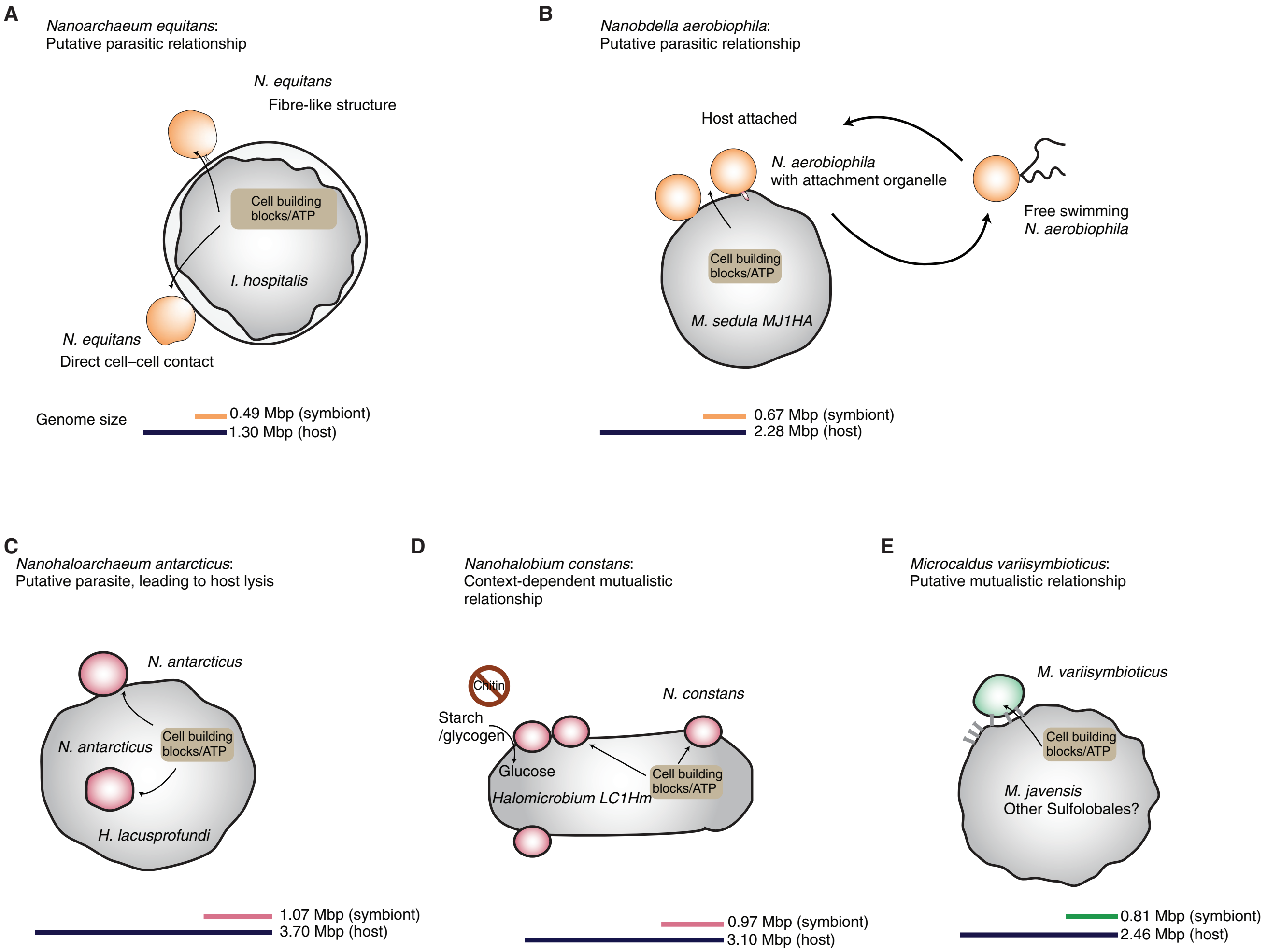

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Figure 2. Schematic cells depicting an array of host–symbiont interactions (from putative parasitic to mutualistic) based on selected systems. Genome sizes of hosts and symbionts are shown in scale. (A) *Nanoarchaeum equitans* interacts with its host *Ignicoccus hospitalis* via a fibre-like structure or through direct cell–cell contact. (B) *Nanobdella aerobiophila* has two life-cycle stages, a free-swimming and a host-attached stage, and uses a specialised ‘attachment organelle’ to interact with its host, *Metallosphera sedula*. (C) *Nanohaloarchaeum antarcticus* appears to enter its host and leads to host-cell lysis. (D) In contrast, *Nanohalobium constans* can break down starch/glycogen for its hosts in the absence of chitin, forming a context-dependent mutualistic relationship. (E) The interactions between *Microcaldus variisymbioticus* and its host *Metallosphera javensis* seem to be initiated by the host via protein nanotubes.

and *Nanofervidus parviconus* suggest that these DPANN cells directly connect to their hosts’ cytoplasm via thin filament-like structures that were proposed to form an ‘attachment organelle’ that might act as a cytoplasmic bridge for metabolite exchange (Figure 2B). Intriguingly, free and motile cells of these two strains have been seen in their co-cultures, suggesting an ectobiotic life cycle with two distinct stages (Figure 2B): first, a host-seeking stage in which unattached cells swim to their host using ATP either accumulated from earlier host interactions or conserved by substrate-level phosphorylation; and secondly, a host-attached stage in which the cell envelope is remodelled to form the attachment organelle.

Although some Nanoarchaeales strains rely on their hosts for numerous cellular building blocks and may negatively impact host growth without providing known benefits, they can be stably maintained in co-culture. *Nanohaloarchaeum antarcticus* (Figure 2C), on the other hand, lyses its host cells and thus seems to be predatory. Microscopy data indeed suggest that *N. antarcticus* may enter the host cytoplasm by invasion or engulfment. By contrast, *Nanohalobium constans* and *Halomicrobium* sp., seem to form a mutualistic relationship under certain growth conditions. Although *Halomicrobium* sp. can use chitin to support its growth, it relies on *N. constans* to convert glycogen and starch into glucose, when these are the sole carbon sources (Figure 2D). More recently, transcriptomic and metabolomic evidence indicated that *Nanohalococcus occultus*, an ectosymbiont of *Haloferax volcanii*, accelerates xylan degradation by *Halorhabdus* sp., a non-host archaeon, by influencing its gene expression. Thus, symbiont–host relationships appear to vary drastically within Nanohaloarchaeota and are likely even more complex within natural communities.

The known host–symbiont interactions of micrarchaeal strains with larger genome size and hence greater metabolic flexibility differ somewhat from those observed in the Nanoarchaeales and Nanohaloarchaeota. Instead of building a cytoplasmic bridge or invading host cells, Micrarchaeota strains (ICNP syn. Microcladota) connect to their hosts via a pilin-like filament structure. Indeed, some representatives may remodel the cell surface at the interaction site, potentially through a filament structure produced by the host (Figure 2E). Further, proteinaceous nanotubes traverse through the surface layers of both the host and symbionts during interactions, presumably connecting their cytoplasm for metabolite exchange. Although proteomic data showed the downregulation of proteins in the translation processes of the host cells upon interaction with *M. variisymbioticus*, indicative of resource reallocation, the host origin of nanotubes and antenna structures may suggest a mutualistic ectosymbiotic host–symbiont relationship.

Collectively, these studies reveal important molecular aspects of the cell–cell interactions between DPANN symbionts and their hosts and provide insights into the different types of interaction, ranging from parasitism to mutualism. The remarkable diversity in symbiont–host interactions, even between closely related DPANN symbionts, indicates that prospective studies on DPANN archaea will lead to many novel insights into the biology of these enigmatic archaea and their hosts.

Evolutionary origin and similarities with CPR bacteria

Since the discovery of *N. equitans*, the phylogenetic placement and the monophyly of DPANN archaea in the archaeal tree of life have been a subject of debate. Although it has been proposed that the monophyly of DPANN may be an artefact, with some DPANN lineages being more closely related to other archaeal clades, most phylogenetic studies support the grouping of all DPANN lineages in an unrooted tree. However, the root placement in the archaea is contested, and it remains unresolved whether DPANN indeed forms a monophyletic clade. Additionally, although the evolution of DPANN archaeal genomes is generally thought to be characterised by reductive evolution, it is important to note that this is not a universal feature. For example, a recent study used a phylogenetic reconciliation approach and demonstrated genome expansion through horizontal gene transfer within the Woesearchaeales, driving an increase in metabolic versatility. Thus, future research into the placement of the various DPANN lineages within a rooted archaeal tree, combined with the reconstruction of genome evolution through time will be needed to resolve the origin and evolution of symbiotic lifestyles across these archaea and elucidate to what extent members exhibit parallel evolution and genome expansion in addition to reductive evolution.

Conclusions

The DPANN archaea comprise a vast diversity of putative archaeal symbionts, which are widespread in many environments on Earth. Even though much of our initial knowledge is derived from genome-based predictions and the study of a small number of symbiont–host systems, these organisms have already unearthed many new insights into archaeal biology. We are convinced that the future exploration and characterisation of members of these lineages will not only help to clarify the evolution and cell biology underlying archaeal symbioses but also to elucidate the role of these organisms in their natural microbial communities and ecology with their hosts.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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