Unique mobile elements and scalable gene flow at the prokaryote-eukaryote boundary revealed by circularized Asgard archaea genomes

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Eukaryotic genomes are known to have garnered innovations from both archaeal and bacterial domains but the sequence of events that led to the complex gene repertoire of eukaryotes is largely unresolved. Here, through the enrichment of hydrothermal vent microorganisms, we recovered two circularized genomes of *Heimdallarchaeum* species that belong to an Asgard archaea clade phylogenetically closest to eukaryotes. These genomes reveal diverse mobile elements, including an integrative viral genome that bidirectionally replicates in a circular form and aloposons, transposons that encode the 5,000 amino acid-sized proteins *Otus* and *Ephialtes*. Heimdallarchaeal mobile elements have garnered various genes from bacteria and bacteriophages, likely playing a role in shuffling functions across domains. The number of archaea- and bacteria-related genes follow strikingly different scaling laws in Asgard archaea, exhibiting a genome size-dependent ratio and a functional division resembling the bacteria- and archaea-derived gene repertoire across eukaryotes. Bacterial gene import has thus likely been a continuous process unaltered by eukaryogenesis and scaled up through genome expansion. Our data further highlight the importance of viewing eukaryogenesis in a pan-Asgard context, which led to the proposal of a conceptual framework, that is, the Heimdall nucleation-decentralized innovation-hierarchical import model that accounts for the emergence of eukaryotic complexity.

To chronicle the emergence of evolutionary innovation is a long-standing pursuit in biology. Due to scant record of reliable microscale fossils, resolving evolutionary history at the cellular scale relies primarily on molecular comparisons across present-day life, provided that phylogenetic relatives can be well delineated. Culture-independent metagenomics has substantially expanded our access to the Earth’s diverse biomes, including lineages carrying genetic imprints of critical evolutionary events through deep time. The Heimdallarchaeota, previously referred to as the ancient archaea group (AAG), are one such group and the closest known relative of eukaryotes as suggested by phylogenomics. Heimdallarchaeotes and their related lineages collectively called the Asgard archaea contain a sizeable repertoire of eukaryotic signature proteins (ESPs). However, the genetic make-up of Heimdallarchaeota has so far only been inferred from a few metagenome-assembled genomes (MAGs), which are fragmented and suffer from uncertainty in their completeness and accuracy. Mobile (genetic) elements, including transposons, viruses and plasmids, which are known to play dominant roles in evolution, are frequently misassembled, omitted or misassigned during MAG assembly and binning. These drawbacks propagate into uncertainties in the resolution of archaeal lineages related to eukaryotes and can obscure the drivers of evolutionary crosstalk and divergence between eukaryotes and their prokaryotic relatives.

**Results**

**Circular Heimdallarchaeota genomes.** Recovering contiguous genomes from environmental samples is notoriously challenging due to their enormous biodiversity and strain-level heterogeneity, while most known lineages have been hard to isolate due to their unresolved metabolism and/or poorly understood partner-dependent growth. We overcame these limitations by combining cultivation methods with molecular community profiling to progressively dissect environmental microbial enrichment cultures where a clonal expansion of our species of interest was accompanied by a reduction in diversity (Extended Data Fig. 1 and Methods). Using anaerobic cultivation methods, we enriched a member of the Heimdallarchaeota AAG clade from a barite-rich rock retrieved in 2017 from the Auka hydrothermal vent field (23° 57’ N, 108° 51’ W) located in the southern Pescadero Basin near the southern tip of the Gulf of California at a water depth of 3,674 m (ref. 15). While initially below detection, this rock-associated AAG phylotype emerged at 1–4% of the 16S ribosomal RNA gene relative abundance in 3 lactate-supplemented, anaerobic enrichment cultures incubated at 40°C after 7 months (Extended Data Fig. 1, Supplementary Tables 1–3 and Supplementary Note 1). In an independent set of enrichments inoculated with sediments collected from the Auka site in 2018 (23° 53’ N, 108° 48’ W), alkane-supplemented anaerobic incubations at 37°C additionally yielded a second AAG phylotype that increased in 16S rRNA gene relative abundance from 0.03 to 4–7% after 9 months (Supplementary Tables 4 and 5 and Supplementary Note 1).

De novo assembly of Nanopore long-read and Illumina paired-end sequencing of genomic DNA recovered from these enrichments (Supplementary Table 6) resulted in complete circularized genomes of the two AAG species from the barite and sediment enrichment cultures, with genome sizes of 3.32 and 3.08 million base pairs.
Fig. 1 Complete genomes of Ca. Heimdallarchaeum spp. provide insights for eukaryogenesis. a. Illustration depicting the enrichment procedure of a microbial community associated with a barite-rich rock no. NA091-45R retrieved from the southern Pescadero Basin Auka hydrothermal vent field at a water depth of 3,700 m. Successive transfers of rock and media (mixed) retained the Ca. H. endolitica while lactate-supplemented enrichment media alone (planktonic) did not. A similar strategy was used to enrich for Ca. H. aukensis from the nearby sediment, substituting alkanes for lactate. b. Maximum-likelihood phylogeny of 57 Heimdall group Asgard archaea based on 76 concatenated archaeal marker genes. The two circular genomes of Ca. Heimdallarchaeum spp. are highlighted in purple. AB_125 in bold is a MAG initially described that represents the clade. c. A schematic illustration depicting cytoplasmic SHY and MBH operons encoded by Ca. Heimdallarchaeum spp. (top) and their hypothetical roles in hydrogen-based syntrophy during eukaryogenesis (bottom). For SHY operons, the four required subunits are followed by a maturation protease. For MBH operon, the electron transport genes are in blue and the maturation factors in purple. The rectangle depicts an ancient archaeon related to the Ca. Heimdallarchaeum; the kidney shapes depict ancient bacteria that may have formed syntrophic relations with the archaeon extracellularly or intracellularly and ultimately evolved into mitochondria. d. Maximum-likelihood phylogeny of 40 genomes of representatives based on a concatenation of 56 archaea–eukaryote markers from 40 genomes showing the relationship with eukaryotes followed by select genome characteristics, marker gene coverage and the presence/absence of genes encoding TCA cycle enzymes, eukaryotic signature proteins and ester-linked lipid synthesis. The genomes constructed in this study are coloured purple, with the circularized genomes indicated in bold italic. Presence/absence and gene copy number are colour-coded. α-KG, α-ketoglutarate; NA, not applicable; OAA, oxaloacetate. For b and d, a list of genomes and markers can be found in Supplementary Tables 8, 16 and 17.

**Taxonomy and metabolism.** The taxonomy of Asgard archaea is yet to reach consensus. The initial Heimdallarchaeota1, despite remaining monophyletic in all phylogenomic analyses, was proposed to either split into four phyla (Heimdall-, Gerd-, Kari-, Hodarharchaeota)7 or alternatively grouped under a single order named the Heimdallarchaeia21. In this study, we collectively refer to them as ‘the Heimdall group’. Phylogenomic analyses based on 76 concatenated ribosomal proteins show that the Heimdallarchaeota spp. constitute a deeper-branching clade related to the previously described MAG AB_125 (ref. 9), well placed under ‘Heimdall’ in all proposed classification strategies (Fig. 1b and Extended Data Fig. 2). Additionally, we also identified a fragmented MAG B53_G1622 (299 contigs, 1.67 Mbp, approximately 50% complete) from the Guaymas Basin, formerly assigned under the Pacearchaeota, which we now designate as a strain of Ca. H. endolitica, with an average ANI of 97.5% compared with our PR6 strain. 

Ca. Heimdallarchaeum spp. are predicted to garner energy by anaerobically oxidizing organic substrates via processes involving a partial tricarboxylic acid (TCA) cycle and, given the absence of discernible terminal electron accepting pathways, dissipating electrons via H2 production (Extended Data Fig. 3a). They each encode one membrane-bound hydrogenase (MBH) complex and two cytosolic sulfuroxygenases complexes (SHYI and SHYII) (Fig. 1c). Hydrogen has been hypothesized to act as a syntrophic intermediate bridging archaea and bacteria before the engulfment of mitochondrial ancestor by an (Asgard) archaeal ancestor of eukaryotes24-25. Indeed, in the recent description of Ca. Prometheoarchaeum syntrophicum, MBH associated with unusual membrane extensions were hypothesized to facilitate cell–cell contact and hydrogen exchange with syntrophic partner bacteria25. Following from this concept, we postulate that cytosolic hydrogen generation by SHY, as found in the Ca. Heimdallarchaeum spp., could impose a selective advantage for a hydrogen-dependent endosymbiotic strategy (Fig. 1c).

**Eukaryotic signatures.** One of the many challenges of resolving the relationship between archaea and eukaryotes is the cura-

tion of representative, high-quality genomes across lineages at their interface. To this end, we verified the complete marker gene
coverage of the Ca. Heimdallarchaeum spp. as well as six other highly contiguous Asgard archaea genomes (Extended Data Fig. 4a, Methods and Supplementary Note 2). They include three previously described19,24 and three assembled in this study from our enrichment cultures—a Lokiaarchaeote that we have named Ca. Harpocratesius repetitus FW102, a Thorarchaeote FW25 and a Heimdall group Gerdaarchaeote AC18 (Fig. 1d). Notably, the dual-contig assembly Ca. H. repetitus FW102, which relates to Ca. P. syntrophicum MK_D1 at the family level, contains two complete sets of 16S/23S rRNA genes, potentially relevant to their growth strategies in the environment17.

These complete genomes confirmed that many of the previously described ESPs46 are distributed universally across known Asgard phyla (Fig. 1d), specifically genes involved in (1) membrane remodeling (endosomal sorting complexes required for transport components VPS4/VPS22/VPS25), (2) cytokoskeleton organization (actin, profilin and gelsolin (except in Odin LCB_4)), (3) protein N-linked glycosylation (OST3/STT3/ribophorin) and (4) intracellular trafficking (roadblock/LC7/dynein family and a large repertoire of small GTPases). On the other hand, enzymes involved in the synthesis of ester-linked phospholipids, which are critical for closing the ‘lipid divide’ between the Archaea and Eukaryota domains25,26, show a mosaic distribution across the Asgard archaea lineages (Fig. 1d). For example, both Ca. Heimdallarchaeum spp. in our study lack 1-acetyl-sn-glycerol-3-phosphate acetyltransferase involved in the attachment of the second fatty acid chain to the glycerol backbone27.

Maximum-likelihood analysis using a previously described approach based on the SR4 model28–30 and a concatenation of a complete set of 56 single-copy markers, indicates a close relationship between the Heimdall group archaea, which include the Heimdallarchaeum spp. and eukaryotes (Fig. 1d). This supports a parsimonious topology, reported in multiple studies31–33. We additionally produced a set of customized Asgard-specific Hidden Markov Models (HMMs) (Supplementary Data 1) that complement existing Archaea-specific HMMs along with a set of filtering parameters (Methods and Supplementary Tables 8 and 9) as resources. Maximum-likelihood analyses of a greater diversity of Asgard archaea47–49 that were selected through the framework described above (19 of 282 evaluated MAGs shown in Extended Data Fig. 2) further verified the phylogenetic topology, placing the Heimdall group closest to eukaryotes (Extended Data Fig. 4b). We note that statistical model selection, taxonomic evenness and assumptions with rooting represent ongoing debates for deep phylogeny34. The circularized genomes and resources described in this study may assist with future analyses of the Asgard archaea.

Abundant repetitive features. Our approach retained a substantial number of non-tandem repeats (3% of genome lengths) and tandem CRISPR or intragenic repeats (212 and 262 counts) within the circular Ca. Heimdallarchaeum spp. genomes (Fig. 2a,b). This is notably more prominent relative to the recently constructed circular genomes of Ca. P. syntrophicum35, where no tandem repeats and only 1% of non-tandem repeats were observed.

Non-tandem repeats in the Ca. Heimdallarchaeum spp. overlap prominently with one of the most pervasive mechanisms of gene transfer within and between genomes, that is, a total of 11 families of transposases/integrases, 7 of which have multiplied and transposed to result in up to 27 copies within an individual genome (Fig. 2a). These and other transposases/integrases found in Asgard archaea primarily cluster with various small families within the 96,367 transposase/integrate sequences recovered from the prokaryotic Genome Taxonomic Database (GTDB)40 (Fig. 2c). Despite the under-representation of archaeal sequences in public databases and in the transposase/integrate dataset in this study, they have representatives in almost all clusters. The intermingled evolutionary relationship between archaeal and bacterial transposases/integrases documented in this study can potentially be both the result of, and contributor to, the gene flow observed between these two domains35–37.

The circular genomes of Ca. Heimdallarchaeum spp. contain seven CRISPR–Cas systems (Fig. 2b), including five complete operons (labelled C1–3, 5, 6), one array-free operon (C7) and one orphan array (C4) (see Extended Data Fig. 5 for the complete gene organizations). Contrasting the overall gene conservation between the two genomes, these CRISPR–Cas systems exhibit strong variability and site-specific integration (Fig. 2d). For example, C5 and C6 exhibited a complete local operon swap, while C3 and C4 were integrated immediately next to transfer RNA genes, a feature often exploited by bacteriophages38 and other Heimdallarchaeal mobile elements (see examples in Fig. 3 below).

CRISPR–Cas-guided discovery of mobile elements. We recruited a total of 1,565 Heimdall-associated CRISPR spacers in our Pescadero metagenomes constructed in this study and previously published Guaymas metagenomes (Methods). They revealed eight protospaters within four distinct mobile elements, which are hosted by Ca. Heimdallarchaeum spp. and are unrelated to any previously reported mobile elements (outlined in Fig. 3a). We named them Heimdallarchaeal mobile elements HeimM1 and HeimM2 and Heimdallarchaeal viruses HeimV1 and HeimV2, respectively.

HeimM1, detected within the sediment-hosted Ca. H. aukensis, is a C2-associated small defense island encoding an efflux pump CcmA and contains a protospacer that matches a spacer at the same genomic locus in the rock-hosted Ca. H. endolithica PR6 C1 (Fig. 3b). Such a territorial dispute within the genome, as well as the site-specific integrations of CRISPR–Cas outlined above, exemplify the emergent view that defence systems are mobile elements themselves39 and contribute to gene flow between habitats.

HeimM2 (8 kbp) encodes an internalin-like, leucine-rich repeat peptide and an enzyme homologous to rRNA self-splicing homing endonucleases (Fig. 3c). The latter are typically found as group I introns embedded within rRNA genes and are considered selfish elements. In this study, this gene was part of a mobile element inserted exactly between the only copy of the 16S rRNA gene and the tRNA gene ArgTCT, suggesting that it has likely been co-opted by HeimM2 for site-specific integration at this site.

The putative integrated viruses HeimV1 and HeimV2 are both found in Ca. H. endolithica. Each encodes proteins with homologues preferentially found in the viral database IMG/VR v.340 compared to the microbial genome database GTDB v.202, and viral structural proteins predicted by machine learning-based annotations (PhANNs41) (Fig. 3d,e and Extended Data Fig. 6). HeimV2 (44 kbp), integrated at the same site as HeimM2, may be a hybrid between a virus and a previously undescribed class of transposons, which we tentatively call aloposons, in reference to the twin giants Aloadae in Greek mythology. They share the following features (Fig. 3d). First, they all contain tandem genes encoding proteins 3,000–6,000 amino acids in size, which we refer to as Otus and Ephialtes, the Aloadae twins. Second, they all integrate at different RNA sites downstream of the giant genes. Alaposon2 in Ca. H. endolithica and Alaposon3 in Ca. H. aukensis represent a highly conserved element that has transposed from one tRNA site to the other during its coevolution with its host. Third, they all encode four consecutive genes upstream of the giant genes, including a gene encoding a bacterial MinD/ParA-like AAA family ATPase. Additionally, we found tandem giant genes in two Thorarchaeota MAGs showing distant homology to the Heimdallarchaeum giant proteins, as well as many unrelated giant genes across the Asgard archaea, some of which may also be part of Asgard mobile elements (Extended Data Fig. 7).
Putative virus HeimV1 (30 kbp) is a circular element with a highly polycistronic gene arrangement and an enrichment in nucleic acid-processing enzymes, viral structural proteins and viral gene homologues (Fig. 3e). As shown in Fig. 3f, HeimV1 exists in two states. Besides the genome-integrated lysogenic state found in one of the incubations, where its sequencing read abundance was at the same level as its genomic neighbourhood, in another enrichment incubation, HeimV1 showed an anomalously high read abundance relative to the host Ca. H. endolithica, suggestive of active replication. PCR and Sanger sequencing further confirmed the circularized state of HeimV1 as well as its integration between the host transposase and tRNA genes. Furthermore, the detailed sequencing read abundance profile across HeimV1 shows the characteristic V shape of an unsynchronized, bidirectionally self-replicating population of circular DNA elements (Fig. 3f). Such a well-defined profile can only emerge if the replications in each HeimV1 circular element initiate at a defined origin of replication.

The mobile elements described above also influence ecosystems beyond the southern Pescadero Basin vent system. CRISPR spacers targeting HeimV1 and HeimV2 were detected in metagenomes from the Guaymas Basin22, a hydrothermal vent site 400 km northwest of the southern Pescadero Basin. The Pescadero-derived mobile element HeimM1 in Ca. H. aukaensis also exists in the Ca. H. endolithica B53_G16 MAG assembled from the Guaymas Basin. Furthermore, HeimV1-related proviruses encoding tail fibre protein homologues are also found in the Heimdall group MAGs from the Gulf of Mexico (Gerdarchaeota clade E44_bin34 (ref. 9)) and from the South China Sea (Hodarchaeota clade B3_Heim10) on the other side of the Pacific (Fig. 3e). Notably, the contig in the E44_bin34 MAG maintains the same gene synteny around the tail fibre gene as in HeimV1, albeit with only approximately 30% sequence homology. These observations indicate the expansive distribution of these mobile elements in diverse lineages of Heimdall group archaea across a large geographical range in deep sea ecosystems.

Fig. 2 | Circular Heimdallarchaeum genomes reveal abundant repeats belonging to complex networks of transposases/integrase and CRISPR–Cas operons. a, Representation of the circularized genomes of Ca. H. endolithica and Ca. H. aukensis where the black bars in the outer rings denote non-tandem repeat sequences identified using a cut-off of 100 bp alignment length and 95% sequence identity. Inner networks connect the transposases/integrase belonging to the same family, with the copy numbers of each family (a–k) shown in the bar chart using the same colour scheme. b, Schematic showing the genomic distribution of CRISPR–Cas operons (C1–C7) and intragenic tandem repeats (ig1–3) across the two circular genomes of Heimdallarchaeum spp. c, Alignment score matrix clustering of diverse transposases/integrase showing their evolutionary exchange across archaeal and bacterial domains. Each marker represents a sequence that has been colour-coded by its taxonomic affiliation with the Bacteria domain in pink and the Archaea domain in blue. Highlighted in the open circles are the identified transposases/integrase associated with Heimdallarchaeota, Gerdarchaeota, Lokiarchaeota and Thorarchaeota. d, The specific operon structures of CRISPR–Cas and their mobile element signatures, including integration at tRNA genes (C3 and C4) and complete local displacement (C5 and C6), are shown to the right. The text in the purple boxes indicates the Cas operon types; the numbers in the grey boxes denote the number of repeats. Yellow indicates neighbouring unconserved genes; blue indicates flanking sequences conserved between two Ca. Heimdallarchaeum genomes.
Diverse evolutionary origins of Heimdallarchaeal viruses. Phylogenetic analyses of viral genes indicate that HeimV1 and HeimV2 share their evolutionary origins with bacteriophages. As shown in Fig. 4a, the viral integrase of HeimV1 is phylogenetically most closely related to integrases found in environmental bacteriophages identified to be hosted by the phylum Bacteroidetes, along with integrases found in seven families of Bacteroidetidae and other viruses with microbial hosts that are unidentified. Similarly, independent phylogenetic analyses of homologues of proteins affiliated with prophy transcriptional regulators, IbrA and IbrB, which are encoded by HeimV2 simultaneously found their closest relatives in phylom Firmicutes (Fig. 4b and Extended Data Fig. 8).

While most viruses encoding genes related to HeimV1 and HeimV2 are unclassified, several belong to the order Caudovirales, including members of the family Siphoviridae. Well-studied members of Caudovirales are known to be tailed bacteriophages packaging double-stranded DNA, in line with the machine learning-based predictions of tail fibres in both HeimV1 and HeimV2 (>90% confidence; Fig. 3d,e).

Heimdallarchaeal viruses and other mobile elements associated with the Heimdall group archaea are predicted to have origins in both bacteria and archaea. For example, HeimV1 encodes a protein with two unknown domains flanking a full-length CTPase homologous to Noc/ParB/SpoJ-like proteins that bind DNA and regulate bacterial cell division (Fig. 4c). On the other hand, the HeimV1 methylase gene appears to have evolved from the Asgard archaea and is potentially involved in evading host detection (Fig. 4d). Phylogenetic analysis suggests that divergence of this viral methylase from its host was an ancient event that occurred before the divergence between the Heimdall and Loki group archaea, estimated to have taken place around two billion years ago.

A survey of Heimdallarchaeum-associated protospecs within the entire Pescadero/Guaymas metagenomic dataset yielded 56 total contigs belonging to the putative Heimdall group mobile elements (Supplementary Data 2). Most coding sequences (76.9%) have no apparent homology with known microorganisms and viruses, while another 13.1% have homologues in diverse bacteria (Fig. 4e), which is higher than the 8.9% archaeal fraction. This further suggests that mobile elements and viruses may play a prominent role in shaping...
the evolution of Heimdallarchaeota by introducing functional innovations of bacterial origin.

**Asgard–eukaryote parallelism in bacterial gene import.** To understand the consequence of cross-domain gene flow in the evolution of Asgard archaea, we performed protein orthology-based functional and taxonomic profiling of the proteomes encoded by the complete genomes in this study. Functional analyses of the Asgard archaeal proteome based on clusters of orthologous groups (COGs) revealed distinct categories of genes that are associated with different taxonomic groups (Fig. 5a). The Archaea-related proteins in Asgard archaea were predominantly represented by information processing functions, including translation (K), transcription (K) and replication and repair (L), which is similar to the key archaeal modules inherited by eukaryotes. By contrast, the bacterial origin proteins in Asgard archaea were predominantly represented by transcription (K) and replication and repair (L), which is similar to the bacterial origins of bacterial gene import.
metabolic functions, including energy production and conversion (C) and the metabolism and transport of amino acids (E), carbohydrates (G) and inorganic ions (P). Different from both the above groups, nearly half of eukaryote-related proteins within the Asgard genomes were dedicated to intracellular trafficking and secretion (U), and cytoskeleton (Z) and protein modification (O) functions.

The import of bacterial genes into archaea and eukaryotes have been independently explored31,32,41,42. In this study, we show that the inheritance of information processing from the Archaea and metabolic functions from the Bacteria domain in the Asgard archaea is very similar to the signature of the eukaryotic genome profile. Strikingly, the archaeal:bacterial gene ratio forms an inverse relation with the genome size in Asgard archaea that is quantitatively comparable with previous characterizations across eukaryotes41 (Fig. 5b).

Domain-specific scaling of gene flow. Different scaling laws appear to govern the fluidity of genes with different taxonomic origins within the Asgard archaea. The total number of genes with closest orthologues in Archaea were remarkably invariable at approximately 900 genes across all Asgard archaeal representatives that span a threefold difference in genome size, from 1.5 Mbp in Odin LCB_4 to 4.4 Mbp in Lokiarchaeotes (Fig. 5c). While the archaeal reference database is currently significantly smaller than the bacterial one, which likely caused an underestimation of the exact number of archaea-related genes, the trend cannot be explained by such a database bias. One the other hand, we found that genome completeness and accuracy is key to capturing this feature since

Fig. 5 | Functional and taxonomic profiling of gene content cross Asgard archaea. a. COG classification of genes within the Asgard archaea subdivided into closest taxonomic groups using eggNOG. The expanded wedges in each pie chart highlight the top categories preferentially enriched in the taxonomic group than other groups. They respectively indicate translation (J), transcription (K), replication and repair (L), energy production and conversion (C), the metabolism and transport of amino acids (E), carbohydrates (G) and inorganic ions (P), intracellular trafficking and secretion (U) and cytoskeleton (Z) and protein modification (O). The remaining categories can be found in Tatusov et al.40. The numbers indicate the percentages. Note that proteins with unknown function are excluded from each pie chart. b. The archaeal:bacterial gene ratio decreases with increasing genome size in both Asgard archaea (this study) and eukaryotes (data from Alvarez-Ponce et al.41). c. Numbers of genes related to different taxonomic groups in relation to the total number of genes in the representative genomes of the Asgard archaea indicate different scaling properties. The solid lines represent the linear fit of the data. The dashed lines represent the extrapolated base number of archaeal genes. Unassigned means that no homology was found in the genome database. Inset, Expanded view of the genes encoding ERPs.
Fig. 6 | Distribution of ERP genes and the hypothesized HDH model for eukaryotic origin. a. Presence of various ERP gene families across the selected representatives as shown in Fig. 1d, which belong to five candidate Asgard archaeal phyla—Heimdallarchaeota, Gerdarchaeota, Lokiarchaeota, Thorarchaeota and Odinarchaeota. Inset, Total gene numbers belonging to the gene families shown in a. b. Venn diagrams showing the ERP gene families shared between lineages of different phylogenetic distances, including three circular genomes (left), two Thorarchaeota members related at the family level (middle) and two members of the Lokiarchaeota related at the family level (right). c. The proposed HDH model provides a conceptual framework for the process of genome acquisition during early eukaryotic evolution. Key steps include a Heimdall-like ancestral archaeon with a simple genome engaged in endosymbiosis with a bacterium to establish the FECA. FECA then acquired innovations across the tree of life via an extensive gene import, most frequently, and often indirectly, through close closely related Asgard archaea, to ultimately orchestrate the LECA. The pink arrows indicate several major phases during early eukaryotic evolution. The dark arrows indicate horizontal transfer events from or via Asgard archaea into the eukaryotic genomes. The grey arrows indicate other horizontal transfer events that occurred and contributed to the eukaryotic genomes, although to a lesser extent.

Decentralized eukaryotic innovation. Eukaryote-related proteins (ERPs) capture present-day Asgard–Eukaryota protein orthologues that are estimated to be most closely related to each other. They include, but are not restricted to, previously investigated ESPs3,6,7—loosely defined as eukaryotic proteins with no archaeal or bacterial homologues in the predicted last eukaryotic common ancestor (LECA)44. Our analyses show that the scaling property of ERPs is similar to bacteria-related but not archaea-related proteins (Fig. 5c), prompting us to explore their evolutionary fluidity across the Asgard archaea lineages. Fewer ERPs overall, despite their closer phylogenetic relationship with eukaryotes (Fig. 6b). Furthermore, even species related at the genus (Ca. Heimdallarchaeum spp.) or family levels (within Thorarchaeota/Lokiarchaeota) have apparent differences in their ERP pools (Fig. 6b). Such a high mobility of ERPs in the recent evolutionary history of Asgard archaea suggests that many of these genes are involved in the auxiliary but not core cellular functions. They are likely, or could have been during their evolutionary history, shuffled as part of their mobilomes. Hence, the evolutionary entanglement between the Asgard archaea and the Eukaryota must be understood in the pan-Asgard space and in the context of genome size expansion.

Thus, our analyses collectively suggest a plausible scenario where an ancestral Heimdall group archaeon with a small genome engaged in endosymbiosis with a bacterium and established the archaeal basis of information processing in the first eukaryotic common ancestor (FECA). The remaining defining features of eukaryotes are a result of decentralized innovations across the tree of life that became hierarchically imported, most frequently and often indirectly, through Asgard archaea lineages closest to FECA, to ultimately orchestrate LECA (Fig. 6c). As such, it is possible that the acquired non-essential genes were later co-opted to serve essential functions as the archaeon–bacterium symbiont expanded its regulatory complexity. We refer to this conceptual framework as the Heimdall nucleation–decentralized innovation–hierarchical import (HDH) model for future implementation and debate.
Discussion
The contiguous and complete genomes of Asgard archaea constructed in this study allowed us to resolve the composite origins of their genetic repertoires and identify diverse, unique mobile elements as their drivers. One important facet to be considered is timescale. While the pivotal role of horizontal transfer in the diversification of Asgard archaea is evidenced by the high number of bacteria-related genes found in this study, a considerable fraction of these genes is likely now stable in their respective lineages and only a certain fraction is a part of their present-day mobileomes—the entire set of mobile elements in a genome. However, the uncharted features, such as the extraordinarily large proteins in alaposos and Asgard-specific host range of mobile elements found in this study, suggest that the Asgard archaea mobileome may still hold ancient signatures inherited around the time of eukaryogenesis. Expanding the repertoire of complete genomes in a broader Asgard archaea taxonomic range, pan-genomic analyses of the same or closely related species and molecular clock approaches will together help chronicle the horizontal transfer events across their evolutionary history. Given that the presence of bacterial genes is prevalent in both branches of the Asgard–eukaryote sisterhod, it will be particularly exciting to explore the extent to which bacterial genes have been transferred into their shared ancestors before eukaryogenesis.

Genome size variability in both eukaryotes and prokaryotes have been attributed to rapid expansion driven by mobile elements followed by gradual erosion under natural selection (such as nutrient availability)\(^1\). It is therefore reasonable to assume that such expansion–erosion cycles would have occurred around the time of eukaryogenesis. While the mechanism of genome expansion around eukaryogenesis is genetic, which will be further elucidated by future discoveries of more Asgard archaea mobile elements, the selection pressure for these traits is ecophysiological. In this study, we showed that the influx of genes into the Asgard archaea is highly constrained by genome size in a similar fashion as in eukaryotes. Hence, resolving the ecophysiological drivers of genome size stratification across Asgard archaea lineages may help us unlock the origin of eukaryotic genome complexity.

Etymology. Ca. *H. endolithica* PR6. Heimdall, watchman of the gods in Norse mythology; archaios (Greek), ancient, primitive; endo- (Greek), within; lithos (Greek), rock). Proposed classification: Ca. Hydindarchaeum, order Ca. Hydindarchaeales, family Ca. Hydindarcheaceae, genus Ca. *Hydindarchaeum*.

Ca. *H. aukensis* PM71. Heimdall, watchman of the gods in Norse mythology; archaios (Greek), ancient, primitive; Auka, the local hero of eukaryotic genome complexity.

Methods
Hydrothermal vent rock and sediment sample collection. Rock no. NA091-R045 (source of *Ca. H. endolithica* PR6. Ca. *H. repetitus* FW102 and Thorarchaeote FW25) and rock no. NA091-R008 (source of Heimdall archaea PM71). were retrieved from the Auka hydrothermal vent site situated on the margin of the southern Pescadero Basin of the Gulf of California using remotely operated vehicle *Hercules* during research expedition NA091 on E/V *Nautilus* on 2 November 2017. Local venting fluids have a measured temperature approaching 300°C, contain hydrocarbons and hydrogen and are precipitating minerals, such as calcite and barite. R045 was collected during dive H1654 at coordinates 23.95668776° N, 108.86227922° W at a water depth of 3.674 m, near shimmering water, a sign of locally focused hydrothermal fluid discharge. R008 was collected during dive H1657 at coordinates 23° 57′ N, 108° 52′ W at a water depth of 3.651 m. After shipboard recovery, rock samples were placed in Mylar bags, rinsed with 0.2 μm filtered bottom seawater collected during the same dive, flushed with N₂ gas for 10 min, and stored at 4°C until preparation for incubations in the laboratory.

Sediment sample no. FK181031-0019-PC3 (source of *Ca. H. aukensis*) was collected during the research expedition FK181031 on R/V *Falkor* to the southern Pescadero Basin on 14 November 2018. The sample was collected during dive S193 at the Auka hydrothermal vent site (23.954822° N, 108.863009° W, water depth of 3.657 m), near the site where rocks nos. NA091-R045 and NA091-R008 were collected in 2017. The sediment push core was extruded upwards and sectioned into discrete 3 cm depth horizons on board immediately after recovery, transferred into sterile Whirl-Pak bags and sealed in a larger Mylar bag, flushed with argon gas and stored at 4°C until use in the laboratory.

Sample collection permits for the expedition were granted by the Dirección General deOrdenamiento Pesquero y Acuícola, Comisión Nacional de Acuacultura y Pesca (Permiso de Pesca de Fomento no. PPF/DFOPA-200/18) and the Dirección General de Geografía y Medio Ambiente, Instituto Nacional de Estadística y Geografía (authorization no. IG10212018), with the associated diplomatic note no. 18-2083 (CTC/07345/18) from the Secretaría de Relaciones Exteriores-Agenzia Mexicana de Cooperación Internacional para el Desarrollo/Dirección General de Cooperación Técnica y Científica.

Artificial seawater medium recipe. Artificial seawater was prepared as described in Scheller et al. with minor modifications. Briefly, 11 of artificial seawater (ASW) medium contained 46.6 mM MgCl₂, 9.2 mM CaCl₂, 485 mM NaCl, 7 mM KCl, 20 mM Na₂SO₄, 1 mM K₂HPO₄, 2 mM NH₄Cl, 1 ml of 1,000x trace element solution, 1 ml of 1,000x vitamin solution and 0.5 mg of resazurin and was buffered with 25 mM HEPES buffer adjusted to pH 7.5. One litre of 1,000x trace element solution contained 50 mM nitric acid, 5 mM HCl, 3.5 mM BaCl₂, 1.3 mM CoCl₂, 1.5 mM ZnCl₂, 0.32 mM H₂BO₃, 0.38 mM NiCl₂, 0.03 mM Na₂SeO₃, 0.01 mM CuCl₂, 0.21 mM Na₂MoO₄ and 0.02 mM Na₂WO₄. One litre of 1,000x vitamin solution contained 82 μM thiamine, 45 μM folic acid, 490 μM pantothenic acid, 150 μM choline chloride, 410 μM nicotinic acid, 210 μM biotin, 310 μM para-aminobenzoic acid, 240 μM pyridoxine, 14 μM choline chloride and 7.4 μM vitamin B₁₂.

Enrichment cultivation. Rock no. NA091-R005 was anaerobically fragmented; then, approximately 5 g wet weight was crushed using a sterile agate mortar and pestle on 8 November 2018 and immediately immersed in anaerobic ASW medium in 25–125 ml of butyl rubber-stoppered serum bottles supplemented with different carbon/energy sources, including lactate, H₂/CO₂, hexane and decane and incubated in the dark at 40°C (Extended Data Fig. 1a). The headspace for all cultures was flushed and overpressurized with N₂ gas (2 atm). For the H₂-containing cultures, the N₂ gas headspace was replaced with H₂/CO₂ at an 80:20 mixture by flushing for 1 min and subsequent equilibration at 2 atm. After 33 days of incubation, the lactate-fed first-generation culture produced 5 mM sulphide, indicating active sulphate reduction. This enrichment was mixed by gentle shaking and diluted 1:100 vol/vol into fresh anaerobic ASW medium containing the same suite of carbon/energy sources as described above (Extended Data Fig. 1b). A transfer using the liquid fraction–rock particle fractions from the primary lactate enrichment was also included to enrich for members of the planktonic community along with lactate as the carbon and energy source. This enrichment was later found to be devoid of the AAG (Heimdall) phylotype. Third- and fourth-generation cultures were set up in the following months through 1:100 dilution (Extended Data Fig. 1b). Further details of microbial community development in these enrichments are provided in Supplementary Note 1 and Supplementary Tables 1–3.

R008 was prepared as above except using 2 atm of methane in the headspace as the sole carbon source and electron donor. The culture was passaged twice using a 1:100 dilution under the same culturing conditions; the cell fraction was collected by centrifugation after a total of 22 months for metagenomic sequencing (described below).

For sediment enrichment cultivation, the top 3 cm section of the sediment core was mixed with anaerobic ASW at a 1:4 vol/vol ratio; a total of 60 ml volume each was dispensed into seven 125 ml glass serum bottles sealed with butyl rubber stoppers. The headspace was replaced by ethane (2 atm) in 2 bottles (Supplementary Table 5), while the headspace in 1 bottle was replaced by 100% N₂ gas (2 atm). The cultures were incubated at 37°C in the dark. Further details on microbial community development are provided in Supplementary Note 1 and Supplementary Table 4.

Mineralogical analyses. The mineralogical composition of rocks NA091-R045 and R008 was characterized on a PANalytical X’Pert Pro X-Ray diffractometer. A dried rock aliquot was finely powdered using a clean agate mortar and pestle and scanned from 3 to 75° (2θ angle) at a 0.0167° step size. Mineral identification was performed with the XPert HighScore software v4.1 using the search and march algorithm.
DNA extraction. Combined cells with rock or sediment substrate were pelleted through centrifugation at 13,000 r.p.m. for 3 min. For amplicon sequencing, unless specified in Supplementary Table 6, DNA was extracted using the Qiagen DNeasy PowerSoil kit (catalogue no. 69104) according to the manufacturer’s instructions as described previously\(^8\) with a minor modification, where mechanical shearing was carried out using the MP Biomedicals FastPrep-24 system (catalogue no. 116004500) at level 5 for 45 s. For genomic sequencing, incubated rock and sediment cultures were extracted using multiple approaches, including the Qiagen DNeasy PowerSoil kit, ZymoBIOMICS 96 MagBead DNA Kit (catalogue no. D4302; Zymo Research Corporation), Quick-DNA 96 Kit (catalogue no. D3010; Zymo Research Corporation), ZymoBIOMICS DNA MicroPrep Kit (catalogue no. D4301; Zymo Research Corporation) and a standard phenol/chloroform-based protocol. The list of samples and their extraction methods are provided in Supplementary Table 6.

16S rRNA gene amplicon sequencing. For amplicon (iTAG) sequencing of 16S rDNA genes, extracted DNA was amplified using primer pair 515/806 (GTGCTACGGGNGGCAGCAG/ GTGACCTCACGACGCTTTTGCT (reverse)) as described by Bahram et al.\(^51\), multiplexed as described by Pach et al.\(^77\) v.2.1, which yielded a 30 Mbp assembly, including a 3.4 Mbp contig. Using Canu\(^17\) v.2.1, the approximate 40 kilobase (kb) regions at two ends of an approximate 3.4 Mbp genome was annotated, it was rotated such that the genomic sequence ended with the edges (approximate 50 bp region), the two ends of the genomic sequence were joined as described above to place the tRNA gene GlyCCC at the end. The final assembly was performed using the ONG Guppy software v.3.4.5. For error correction through pilon\(^54\) v.1.22. To account for the reduced mapping at the ends of the genome, a de novo genome assembly was performed using SPAdes\(^55\) v.3.14.1 with the ‘-meta’ option and Sanger sequencing was performed using the primers GAGTTTTTTCAATCTTATAATGCCAAACTAAAAAATAG (forward), CAGTCAGATTTGACACAAAAATTTGTC (reverse) and GCTGGACTCAACCTATAACTAATAGT (reverse). It was then binned using metabat2 v.2.15 (ref. \(^7\)) with default parameters. The bin was then used to recruit long reads using minimap2 v.2.17 and reassembled and binned again. We then used LRScaf to scaffold the contigs and used ten iterations of pilon v.1.24 to achieve error correction and resolve ambiguous bases.

The metagenome containing the Lokiarchaeota Ca. H. repetitus FW102 was assembled using Canu v.2.1, as described for the Ca. H. endolthiarchae gene, and then binned using metabat2 v.2.15 (ref. \(^7\)) with default parameters. The MAG bin was then used to recruit long reads using miniasm v.2.17 and reassembled and binned again. We then used LRScaf to scaffold the contigs and used ten iterations of pilon v.1.24 to achieve error correction and resolve ambiguous bases.

The Thorarcheote FW25 MAG was assembled using the hybrid assembly of illumina reads and nanopore reads using SPAdes v.3.14.1 with k-mers 21,33,55,77,99, and then binned using metabat2 v.2.15 with default parameters. The MAG bin was then used to recruit reads through MIRAbat in the MIRA v.4 pipeline (https://mira-assemble.sourceforge.net/docs/DefinitiveGuideToMIRA.html#chap_intro). These reads were then used for hybrid assembly with nanopore long reads via SPAdes v.3.14.1 with k-mers 21,33,55,77,99. It was then binned again using metabat2 v.2.15 with default parameters. The MAG bin was then used to recruit reads through MIRAbat in the MIRA v.4 package and then reassembled and binned using SPAdes and metabat2 to yield the final Gerarchaeota AC18 bin.

Alignment fraction, ANI and AAI. ANI and alignment fraction values, independently calculated for rRNA, tRNA and coding gene sequences were obtained using ANICalculator\(^57\) 2014-127, v.1.0 (https://ani.jgi.doe.gov/html/download.php). Note that Lokiarchaeote FW102 contains 2 copies of 16S rDNA genes at 99% identity with each other, and Thorarcheote BC has a partial 16S rRNA gene. The alignment of the 16S rRNA was carried out using the SINA\(^1\) v.2.1.2. The AA1 values of translated proteomes were obtained with the enveomics package v.1.8.0\(^8\). The final output is shown in Supplementary Table 7.

Genome and mobilome annotations. Gene calling was done using a combination of Prodigal v.2.6.3 and Glimmer v.3.0.2 using translation code 11 within the RAST\(^8\) pipeline, now under the PATRIC package v.1.03\(^2\). Translated coding sequences were annotated and domain-assigned using eggNOG mapper\(^8\) v.2. The tRNA, 16S rRNA and 23S rRNA genes were identified using RNaMMer\(^1\) v.1.2 embedded in RAST. Thus far, 55 rRNA gene sequences could not be predicted through the existing HM using various approaches, non-tandem repeats were identified using RASTk with the default cut-off of 95% identity and 100 bp. Random repeat sequences were identified using RASTk, Prokka v.1.14.6 and CRISPReCAsTyper 1.1.44. Prokka and CRISPReCAsTyper both employ MinCED (https://github.com/cstennerton/minced) to identify repeats and detect intragenic tandem repeats, which were manually removed from the CRISPRe-Cas analyses.

The genome was annotated using CRISBCaTyper. All identified Heimdallarchaeum mobilomes were further analysed using PSI-BLAST 1.10.0\(^9\), CDD search v.3.19\(^9\) and PhANNS webserver (version March 2021)\(^9\).

Genome evaluation and HMM construction. Marker coverage was carried out using a two-step process. First, we used the automated marker analyses via CheckM\(^5\) v.1.1.3 with the lineage, wf option and the default HM H. E value cut-off, which included the 149 standard archaeal single-copy marker set. Next, each of the missing markers was examined with hammer\(^5\) v.3.3.2 using the hmmsearch option with manual inspection of alignment regions and bit scores. This rescued markers unidentified through the default cut-offs by CheckM as well as divergent variants that most likely functionally replace the genuinely missing marker. The detailed description of markers missed by CheckM can be found in Supplementary Note 2 and the final evaluation of marker presence is displayed in Extended Data Fig. 4a and Supplementary Table 15. Next, we constructed an updated HMM for each marker unidentified through the default cut-offs by CheckM as well as divergent variants that most likely functionally replace the genuinely missing marker. The updated HMM was used to evaluate the 282 Asgard archaea genomes used for the phylogenomic analyses shown in Extended Data Fig. 4b. We applied the following filtering criteria: \(\leq 100\) contigs, \(\geq 96%\) marker completeness and \(\leq 8%\) marker redundancy. We also
Phylogenomics. A phylogenomic tree of Acidarchaeota was constructed with IQ-TREE v2.1.2 (ref. 95) using a partitioned analysis96 with model selection using ModelFinder97 and 1,000 ultrafast bootstrap replicates using UFBoot29 on a concatenated alignment generated from MUSCLE3 v.3.8.1551 alignments of 76 archaeal marker genes identified in the genomes using HHMMS included with antis v6.2 (ref. 98). The phylogenetic tree was visualized using iTOL10 and rooted with the TACK superphylum.

The archaea–Eukaryota phylogenomic tree, including the Acidarchaeota discussed in this study, was constructed based on the 56 archaea–Eukaryota ribosomal proteins used by Zaremba-Niedzwiedzka et al. using reference sequences from the corresponding Dryad DRYAD84. The HeimV1 archaea identified in this study, additional sequences of the most complete genomes representing different lineages of the TACK superphylum were added to the dataset. Sequences of 56 archaeal COGs obtained from the Dryad repository were used as reference databases to retrieve homologous sequences from target genomes and CRISPR sets. In total, the final de-replicated, putative CRISPR arrays were aligned using MUSCLE v3.8.1551 and inspected and trimmed manually. Manually trimmed alignments were then further trimmed using BMGE92, recoded to four-state SR4 using a custom script (https://github.com/dspeth/bioinfo_scripts/tree/master/phylogeny) and finally concatenated and converted to PHYLIP format using catfasta2phyml v1.1.0 (https://github.com/nylander/catfasta2phyml).

The final concatenated, recoded alignment was used to calculate phylogenies using IQ-TREE v2.1.2.12 (ref. 100) using a C60 model adapted for SR4 recoded data by Zaremba-Niedzwiedzka et al. and 1,000 ultrafast bootstrap replicates using UFBoot. The phylogenetic tree was visualized using iTOL10 and rooted with Euryarchaeota as the outgroup. The genomes and conserved genes used for the phylogenomic analyses are listed in Supplementary Tables 16 and 17.

Resolution of the genomic insertion and circularization of HeimV1. To capture the two different states during the life cycles of HeimV1 (Fig. 3), we used three primer sets to amplify the sequences around the two insertion sites of HeimV1 and confirmed them using gel electrophoresis and Sanger sequencing. Set 1 amplified the region between upstream tRNA GlyCCC in the Ca. H. endolitica genome and the first coding gene of the HeimV1 (GTGATTGAATAGCTGCAACATITC). Set 2 amplified the regions containing the transposase in the Ca. H. endolitica genome and the integrase in HeimV1 (CTTGATAGTATGCAGCTGATAGCTATGCGTTCTCTCCCTTCTAGTGTCC). Set 3 amplified the two ends of the circular HeimV1 (CTTAGATIGCTAGTGATAGCTATAGTGATTGATTAGCTGCAACATITC). Each primer set amplified approximately 2 kb of target regions with set 1 and set 2 indicating the presence of the integrated state of HeimV1 and set 3 indicating the circular state.

Protein clustering of integrases and transposases. Protein sequences showing integrase and transposase domains, identified using eggNOG mapper from the 8 Asgard archaea MAGs, were pooled and clustered at 90% sequence identity using cd-hit101 v.4.8.1. The resulting representative sequences were used for two sequential rounds of homology searches using DIAMOND102 v.2.0.6 against the protein sequences obtained in addition to the CRISPR sets. In the first round, the dataset was searched against the Asgard archaea integrase/transposase dataset91. To avoid self-matches, the CRISPR arrays containing the spacers were removed from the Eukaryota group using eggNOG mapper from the conserved domain database. This led to the recombination of the candidate ERP clusters into the functionally distinct 135 ERP families. To align with previous work103, all small GTPases were classified as one single ERP family, constituting 291 proteins from the 8 representative Asgard archaea MAGs.

Maximum-likelihood analyses of proteins encoded by HeimV1 and HeimV2. Homology search for all peptide sequences of HeimV1 through DIAMOND v.2.0.6 was carried out against the GTDB v.95, Pescadero Basin and Guaymas Basin assemblies, ReSeq virus database104, IMG/VR105 and huge phage106, giant virus107 and Lokí’s castle virus datasets108. The search outputs were pre-clustered with a 70% identity cut-off using cd-hit v.4.8.1 (ref. 109). The representative sequences were aligned using the MAFFT v.7.475 (ref. 110) option limsim and trimmed with trimAl v.1.4. The maximum-likelihood analyses were carried out with IQ-TREE v2.1.12 (ref. 111) using the LG4x model and ultrafast bootstrap with 2,000 replicates. The phylogenetic tree was visualized and prepared using iTOL10.
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Extended Data Fig. 1 | The emergence of *Ca. Heimdallarchaeum endolithica* belonging to the Ancient Archaea Group of Heimdallarchaeota in a series of incubations derived from the same rock originated from Pescadero basin. **a.** Maximum fraction of the AAG phylotype within the first-generation incubations from the rock detected within a 13-month period. **b.** Maximum fraction of the AAG phylotype within the serial dilution cultures of the initial lactate-fed culture. **c.** Amplicon sequencing of a hypervariable region in 16S rRNA gene showing the fraction of AAG phylotype in second-generation lactate-fed cultures. Mixed, mixture of rock and medium transferred from the first-generation incubation. Planktonic, only top-layer medium was transferred. **d.** Community complexity reduction over time as indicated by total operational taxonomic unit (OTU) counts (top) and the Shannon diversity index (bottom). **e.** Full-length 16S rRNA gene survey using universal archaea primers showing a single abundant AAG phylotype (*Ca. Heimdallarchaeum endolithica*) species above noise, and its 16S sequence dissimilarity (percent sequence identity difference) with other archaea in the community. Loki, a Lokiarchaeota phylotype; Thermopl, a Thermoplasmatota phylotype; Woese, a Woesearchaeia phylotype. **f.** Wide-field microscopy images of a large multispecies biofilm isolated from the lactate-fed 2nd-generation incubation, which was stained using DAPI (DNA), FM1-43 (membrane lipids), and concanavalin A (extracellular matrix). Imaging was repeated two times with similar observations. In c and d, error bars indicate SD. N=2, independent DNA samples extracted from the same incubation.
Extended Data Fig. 2 | Maximum-likelihood analyses of 282 Asgard Archaea MAGs and genomes rooted using 15 TACK archaea. The different clades are labeled in different colors, with clade names indicated in the same color. MAGs selected for detailed phylogenomics analyses are annotated, with published ones in black and those constructed in this study in bold blue. Jord and Wukong clades do not yet have representatives passing the genome selection filter based on Marker coverage and genome contiguity scores. Detailed descriptions of these genomes can be found in the Supplementary Tables 8 (All Asgard archaea), S9 (Selected Asgard archaea), and S16 (TACK), Markers used can be found in Supplementary Table 17.
Extended Data Fig. 3 | Genome-based metabolic predictions of Ca. Heimdallarchaeum spp. and comparisons with other contiguous, near-complete Asgard Archaea MAGs. a, Illustration of metabolic reconstruction highlighting hydrogen metabolism and tricarboxylic acid (TCA) cycle. Abbreviations: α-KG, α-ketoglutarate; OAA, oxaloacetate; SHy, sulfhydrogenase (cytosolic hydrogenase); MBH, membrane-bound hydrogenase; lac, lactate; carb.hyd., carbohydrate; Pyr, pyruvate; PEP, phosphoenolpyruvate; Ace, acetate; Eth, ethanol. b and c, Enzymes involved in TCA cycle reactions (b) and cytosolic hydrogen evolution (c) in each genome/MAG representatives of Asgard archaea.
Extended Data Fig. 4 | Marker determination and phylogeny of expanded representatives of Asgard archaea. **a**, Differential distributions of putatively single-copy archaea marker genes in initially selected genomes/MAGs, which show cross-asgard and clade-specific marker coverage features. **b**, Maximum-likelihood phylogeny of an expanded selection of asgard archaea MAGs in relation to Euryarchaeota, TACK, and Eukaryota. Ca. H. aukensis was omitted to improve evenness in the taxonomic selection here due to its close relation with Ca. H. endolithica. Detailed descriptions of the 51 genomes used in the analyses can be found in Supplementary Tables 9 (Selected Asgard archaea) and S16 (TACK+Eukaryotes). Markers used can be found in Supplementary Table 17. Purple indicates genomes and MAGs constructed in this study.
Extended Data Fig. 5 | CRISPR/Cas systems in Ca. Heimdallarchaeum spp. a–e. Schematic showing the gene synteny of the CRISPR/Cas systems (serial numbers and operon types are in bold pink) and their alignments between the two genomes. Genes conserved between the two genomes are labeled in various shades of blue and purple to assist visualization. Genes only appearing in one of the genomes are in yellow. Red indicates CRISPR arrays. Array sizes are indicated by the number of repeats such as [77x]. In b, The Aloposons with giant genes are also shown to illustrate their site-specific integration.
f. Size distribution of spacers in each CRISPR array.
Extended Data Fig. 6 | Numbers of sequences homologous to some of the proteins encoded by Heimdallarchaeal viruses HeimV1 and HeimV2. Magenta stars indicate enrichments in viral database. The homology search was carried out using diamond v2.0.6 using a e-value cutoff of 10^-3. ASG, asgard archaea genomes; FWA, in-house metagenomic assemblies of microbial communities in Pescadero basin incubations; PAA, publicly available and published metagenomic assemblies of microbial communities in Guaymas basin sediment; Vir, IMGVR3 viral database; Gtdb, genomic sequences from GTDB v202. See methods and supplementary tables for the details of these datasets.
Extended Data Fig. 7 | Giant proteins encoded by Asgard archaea. a. Gene synteny showing 1) an additional genomic region with truncated, fragmented sequences homologous to one of the giant genes in Alposons, and 2) tandem giant genes which show high homologies with their neighbors are found in Thorarchaeotes, and are distantly related to one of the two giant genes in Alposons. b. Giant proteins larger than 3000 a.a. encoded by selected Asgard archaea representatives. In dark grey are part of the Alposons. Functional domains as identified through conserved domain database (CDD) analyses are indicated on the right. Purple indicates genomes constructed in this study.
Extended Data Fig. 8 | Maximum-likelihood analyses of HeimV2 lbrA-like protein. The branch names are as follows: For viruses, serial numbers followed by viral taxonomy then followed by host taxonomy if available. For microbial genomes, serial numbers followed by taxonomy. In total, 147 proteins were included in the analyses.
Extended Data Fig. 9 | Scaling property of gene flow is obscured by fragmented genomes of varying quality. The plots show the number of Archaea-related genes in relation to the total gene counts in the Asgard archaea genomes. a, only the 8 genomes investigated in detail in this study. All genomes have less than 20 contigs and with verified coverage of all archaeal markers. b, In addition to a, an additional 12 genomes were added (in black), which contain no more than 100 contigs with a loosened completeness scores as shown in Supplementary Table 9. Since marker redundancy differs among lineages, contamination level is hard to assess. c, In addition to b, all other 262 published Asgard archaea genomes were added (in green). This indicates a severe deviation from the invariable relation shown in a, but instead show a near linear relation. This can be understood that in either incomplete or contaminated genomes, all types of genes have equal possibility to be retained. For example, the 1.5Mb Odinarchaeote genome contains the similar number of Archaea-related genes (~900) as a Lokiarchaeote genome sized 4.4Mb. However, if a Lokiarchaeote is fragmented into 300 contigs and only 1.5Mb in total length is randomly binned into a MAG, the latter will roughly contain ~300 Archaea-related genes. Hence, the type of relation shown in (a) can only be captured in highly confident, complete genomes. Legend for all panels is shown in c.
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- For null hypothesis testing, the test statistic (e.g. & , ƚ , ƌ) with confidence intervals, effect sizes, degrees of freedom and P value noted
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Software and code

Policy information about availability of computer code

Data collection
Zen black version ELYRA was used for the acquisition of fluorescent images on Zeiss microscope.

Data analysis
DADA2 v1.9.1; R package(v3.6.0); canu v2.1; BamM v2.5.0; pilon v1.22; bedtools v2.29.2; LRScaf v1.1.10; SPAdes v3.14.1; metabat2 v2.15; MIRA v4 package; ANIcalculator v1.0; SINA v1.2.11; EggNOG mapper v2; cctyper 1.1.4; PSI-BLAST (https://blast.ncbi.nlm.nih.gov); CDD search (https://blast.ncbi.nlm.nih.gov/); PHANNs (https://edwards.sdsu.edu/phanns); CheckM v1.1.3; hmmer v3.3.2; IQtree v2.1.2; UFBoot v2; MUSCLE v3.8.1551, anvi'o v6.2; ASM-Clust v1; blast v2.2.26; MAFFT v7.475; trimAl v2.17; catfasta2phyml (https://github.com/nylander/catfasta2phyml); custom script for amino acid recoding (https://github.com/nylander/catfasta2phyml); custom script for amino acid recoding (https://github.com/dspeth/bioinfo_scripts/tree/master/phylogeny); custom matlab scripts under https://github.com/wufabai/genomics.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The assembled genomes and raw metagenomic sequencing reads can be found on NCBI database under BioProject PRJNA721962, which was made publicly available on November 8, 2021.
Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Study description       | Anaerobic laboratory cultivation using artificial sea water |
|-------------------------|-----------------------------------------------------------|
| Research sample         | Sediment and Rocks collected from hydrothermal vents. The samples were chosen due to their geographical proximity to the vents with diffusive venting, which provide nutrients that fuel the local ecosystem. |
| Sampling strategy       | Samples were collected in an anaerobic chamber using pipettes. Sample sizes were empirical determined, typically 1ml in volume, to allow extraction of sufficient amount of DNA while causing the least amount of disturbance to the existing microbiome. |
| Data collection         | 16S rRNA Amplicon sequencing data using Illumina MiSeq were collected by Laragen. Full-Length 16S rRNA Sequencing data using PacBio Sequel II were collected by Brigham Young University Sequencing Center. Metagenomic sequencing data via Illumina HiSeq2000 were collected by Novogen. Metagenomic sequencing data via Oxford Nanopore MinION were collected by author Igor A. Antoshechkin. |
| Timing and spatial scale| The sampling of the initial rock and sediment samples were respectively carried out at the Auka vent field, Pescadero basin, Mexico on November 2, 2017 and on November 14, 2018. The sampling of rock incubations were sampled inside of the anaerobic chamber at Caltech between November 8, 2018 and December 15, 2019 with an increasing interval from 3 weeks to 8 months. The exact dates are specified in Supplementary Table 2. The sediment incubations were sampled on date June 23, July 29, and September 23, 2019. |
| Data exclusions         | All sequencing data were used for analyses without exclusion. |
| Reproducibility         | The paper focuses on bioinformatics analyses, and all analyses can be reproduced using publicly available software packages provided in the Methods section. The DNA samples were analyzed twice during the rock incubation at 2-4 months around the time when the AAG phylotypes started to emerge. No specific incubation conditions had experimental replicates. |
| Randomization           | The experiments were designed to discover novel organisms from any possible condition. The work does not focus on the effect of environmental parameters. |
| Blinding                | We do not carry out randomized testing on experimental subjects, as the experiments were designed to discover novel organisms from any possible condition. There is no visual link between the samples and the microbes of interest, and there is a minimum of 2 months between the time of sampling and the time of sequencing data output, blinding neither increase nor decrease bias. |
| Did the study involve field work? | Yes | No |

Field work, collection and transport

| Field conditions       | Field sites are 3.6 km below sea level, collected at natural conditions on the dates and location provided in the Methods section. The local temperature were measure at around 40 °C, although with uncertainty due to the strong temperature gradient at the sampling site. |
| Location               | [23°57'N; 108°51'W] [23°57'N; 108°52'W] [23°53'N; 108°48'W] |
| Access & import/export | Sample collection accompanied by and under the construction local scientists under permission granted by local government. Sample collection permits for the expedition was granted by la Dirección General de Ordenamiento Pesquero y Acuícola, Comisión Nacional de Acuacultura y Pesca (CONAPESCA; Permiso de Pesca de Fomento No. PPFE/DGOPA-200/18) and la Dirección General de Geografía y Medio Ambiente, Instituto Nacional de Estadística y Geografía (INEGI; Autorización EG0122018), with the associated Diplomatic Note number 18-2083 (CTC/07345/18) from la Secretaría de Relaciones Exteriores - Agencia Mexicana de Cooperación Internacional para el Desarrollo / Dirección General de Cooperación Técnica y Científica. The permit EG0072017 for the 2017 cruise was granted on April 18, 2017. The permit EG0122018 for the 2018 cruise was granted on July 25, 2018. |
| Disturbance            | Samples were collected outside the major chimney area to result in minimal influence on the macrofauna and the structural integrity of the chimneys. |
We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ✓   | Antibodies            |
| ✓   | Eukaryotic cell lines |
| ✓   | Palaeontology and archaeology |
| ✓   | Animals and other organisms |
| ✓   | Human research participants |
| ✓   | Clinical data         |
| ✓   | Dual use research of concern |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ✓   | ChIP-seq              |
| ✓   | Flow cytometry        |
| ✓   | MRI-based neuroimaging |