ANNUAL REVIEWS

Annual Review of Microbiology

Horizontal Gene Transfer in Archaea—From Mechanisms to Genome Evolution

Uri Gophna[1,*] and Neta Altman-Price[1,2,*]

[1]The Shmunis School of Biomedicine and Cancer Research, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel; email: urigo@tauex.tau.ac.il, neta.altman@gmail.com

[2]Department of Natural and Life Sciences, The Open University of Israel, Raanana, Israel

Annu. Rev. Microbiol. 2022. 76:481–502

First published as a Review in Advance on June 6, 2022

The *Annual Review of Microbiology* is online at micro.annualreviews.org

https://doi.org/10.1146/annurev-micro-040820-124627

*These authors contributed equally to this article

Keywords

archaea, horizontal gene transfer, lateral gene transfer, evolution, mating, recombination

Abstract

Archaea remains the least-studied and least-characterized domain of life despite its significance not just to the ecology of our planet but also to the evolution of eukaryotes. It is therefore unsurprising that research into horizontal gene transfer (HGT) in archaea has lagged behind that of bacteria. Indeed, several archaeal lineages may owe their very existence to large-scale HGT events, and thus understanding both the molecular mechanisms and the evolutionary impact of HGT in archaea is highly important. Furthermore, some mechanisms of gene exchange, such as plasmids that transmit themselves via membrane vesicles and the formation of cytoplasmic bridges that allows transfer of both chromosomal and plasmid DNA, may be archaea-specific. This review summarizes what we know about HGT in archaea, and the barriers that restrict it, highlighting exciting recent discoveries and pointing out opportunities for future research.

Contents

INTRODUCTION

Archaea were first proposed to be a separate and coherent phylogenetic group in the late 1970s and later established as the third domain of life by Carl Woese (153). This shattered the prokaryote-eukaryote divide, and it is now widely accepted that archaea are the most likely progenitors of most molecular machinery observed in extant eukaryotes (68). The past decade has seen tremendous progress in our understanding of archaeal genome diversity (for a comprehensive recent review see Reference 7). Studies in various ecological habitats based primarily on metagenomics (63, 64, 71, 90, 93, 107, 119, 132, 147, 151, 160) have revolutionized the archaeal tree of life and have revealed new lineages, some of which have been cultivated more recently (60, 130). A better taxonomic representation, a fuller sampling of the gene family repertoire, and better phylogenetic methods have all contributed to more accurate representation of archaeal evolutionary processes. In combination with new molecular biology observations and insights, these discoveries represent an unprecedented opportunity for the study of the role of gene exchange, also known as horizontal gene transfer (HGT) in archaeal evolution. Knowing as we do today that HGT is the major driver of evolutionary innovation in the genomes of bacteria, it is reasonable to assume a similar role in archaea, and according to a few studies, several archaeal lineages may owe their existence to large-scale HGT events (98, 99) (see the section titled Massive Gene Transfer as a Driver for the Divergence of Major Archaeal Groups). Thus, HGT in archaea has become an exciting frontier in microbial genetics, with major discoveries continuously being made.

Research questions about archaeal HGT are plentiful: What are the major mechanisms of exchange, and how conserved are they across archaeal phyla? What are the relative contributions of homolog recombination and site-specific recombination by mobile genetic elements? How much DNA is typically gained in each event at a time? Do frequently transferred genes confer niche-specific benefits, or are they mostly neutral or even deleterious? While there has been substantial advance in addressing such questions for bacterial model organisms, such as *Escherichia coli* (111, 141), there are wide gaps of knowledge for the rest of the microbial world, including even

the best-studied model archaeal genera, such as *Haloferax* (phylum *Euryarchaeota*) and *Sulfolobus* (phylum *Crenarchaeota*). The purpose of this review is to summarize what is currently known about HGT in archaea and to highlight major opportunities for future research.

MOLECULAR MECHANISMS OF GENETIC EXCHANGE IN ARCHAEA

Natural Competence and Transformation

Natural transformation is a phenomenon that has been studied for almost a century (51) and a trait shared by many bacteria: the ability to take up naked environmental DNA that is mostly linear and double-stranded. In almost all natural competent bacteria, DNA uptake requires an active mechanism involving type IV pili (92), a system present in nearly all phyla of bacteria and archaea (106). The ComEA/ComEC mechanism is well characterized in gram-negative bacteria as responsible for the Brownian ratchet that ensures a unidirectional flow of DNA while one strand of DNA is degraded and the other enters the cytoplasm, where it provides nutrients or a substrate for homologous recombination. This mechanism is believed to operate in a similar manner in gram-positive bacteria (34). No ComEC homologs have been found in archaea that have been shown to be naturally competent.

Natural competence in archaea is scarce and was reported for a limited number of species, the first being the methanogens *Methanothermobacter marburgensis* (formerly *Methanobacterium thermautotrophicum*) and *Methanococcus voltae*. Works published in the late 1980s (13) showed reversion of induced mutations using $CaCl_2$ transformation procedures versus natural transformation procedures, namely, the addition of purified DNA to the culture in *M. voltae*. Worrell and colleagues (156) were able to show a gain of drug resistance using homologous DNA (but not DNA from *Haloferax volcanii* or *E. coli*) on solid media containing the purified DNA.

Natural competence is not to be confused with artificial transformation such as the polyethylene glycol (PEG)-mediated transformation methods that are efficiently used in the study of halophilic archaea (27, 28) and that induce a spheroplast (also known as a protoplast) state, which allows the taking up of both plasmid (circular) and linear DNA. Even those archaea that are naturally transformable typically show low transformation frequencies when compared to their bacterial counterparts, and artificial methods such as electroporation of spheroplasts have been shown to generate higher frequencies of transformation. For example, in *M. voltae*, transformation efficiency was improved by three orders of magnitude compared to natural transformation by means of electroporation of protoplasts (103). In halophilic archaea (haloarchaea), nutritional competence was convincingly demonstrated, but there was no evidence for genetic competence (23). An interesting study by Chen et al. (21) suggests that salt concentrations may play a significant role in DNA uptake in haloarchaea in nature. After determining the minimal salt concentration that allows growth for *Haloferax* and *Halorubrum* cells, cultures were suspended in different concentrations of NaCl and incubated with plasmid DNA, after which they were spread on solid medium with the appropriate selection. Plasmid DNA uptake was highest at close to minimum salt conditions, and although the transformation efficiencies reported were much lower (10^3 per microgram of plasmid DNA) than those reported for the PEG method, this natural-like situation suggests some form of natural transformation. Halocin H4, a kind of proteinaceous antiarchaeal agent (97), was suggested to increase membrane permeability, and thus facilitate DNA uptake (20). It is worth noting that NaCl concentrations also affect the S-layer *N*-glycosylation in *H. volcanii* (65). S-layer *N*-glycosylation and salinity affect DNA transfer by mating (see the section titled Mating by Cell Fusion), which is efficient when salt concentrations are higher. Nonetheless, lower salt concentrations might be optimal for uptake of environmental DNA by haloarchaea.

Recently, some light was shed on the DNA uptake mechanism of methanogenic archaea with the discovery of two additional naturally competent members: *Methanococcus maripaludis* and *Methanoculleus thermophilus*. Fonseca et al. (40) reported successful attempts at transformation using a shuttle vector in *M. maripaludis* and an integrative vector in *M. thermophilus*. In both cases transformants were obtained after incubation with DNA and without additional chemicals or specific treatment of the cells, and in both species the type IV pili (but not archaella, the archaeal analogs of flagella) were essential for DNA uptake. The authors concluded, therefore, that pili involvement in natural competence may also be conserved in archaea. The conserved Ups (UV-inducible pili of *Sulfolobus*) system is another example of type IV pili involvement in the uptake of DNA through cell aggregation. The Ups and Ced (crenarchaeal exchange of DNA) systems of *Sulfolobales* are discussed separately below.

Natural competence was also shown in the hyperthermophile *Thermococcus kodakarensis*, one of the best-studied hyperthermophilic archaea, previously known as *Pyrococcus* sp. KOD1. *T. kodakarensis* is conventionally transformed using a simple method that does not require $CaCl_2$ treatment. The culture is harvested at a mid-log phase and kept on ice with the donor DNA and then elevated to 85°C, the optimal growth temperature for this species (113). While it was shown that *T. kodakarensis* can also take up plasmid DNA (59), there is a strong preference for linear fragments that can yield high transformation rates (128). Direct transformation of linear DNA (i.e., PCR products) was also demonstrated in *Pyrococcus yayanosii* (128).

Another hyperthermophilic archaeon from the same family as *T. kodakarensis* that shows evidence for natural competence is *Pyrococcus furiosus*, in which the same standard methods of DNA transformation work well. Notably, a genetic manipulation aimed at creating a Δ*pyrF* strain of *P. furiosus* resulted in a mutant of remarkable natural competence (79). This mutant can take up DNA spotted on a lawn growing on solid medium without any chemical or physical intervention. That DNA can be genomic, plasmid, or linear. Genomic DNA is the most efficiently incorporated, suggesting that the DNA is imported into the cell via an active mechanism (54).

The extent, importance, and distribution of archaeal natural competence remain unknown, especially in newly discovered phyla, where genetic tools, and often even cultivated species, are lacking. It is nonetheless possible that a combination of enrichment cultures, metagenomics, and long-read-sequencing technologies will allow us to finally address such questions.

DNA Transfer Mechanisms that Require Cell-Cell Contact

Archaea possess various DNA transfer mechanisms. Some resemble those of bacteria, while others are archaea-specific, or even restricted to particular archaeal lineages.

Conjugation and conjugation-like systems. Conjugation, transduction, and natural transformation are considered the major HGT mechanisms in the domain Bacteria. Conjugation, which was first described by J. Lederberg and E.L. Tatum in 1947 (138), is the only mode of genetic exchange that requires cell-to-cell contact, but in contrast to cell fusion, or eukaryotic sexual reproduction, conjugation is unidirectional. In this process, the "male" partner bearing the transmissible DNA transfers integrative-conjugative-element DNA, plasmid DNA, or, in case of F-factor integration into the donor genome, parts of the donor chromosome to the recipient. Conjugation requires a set of mobilization genes and is very common among bacteria (17), where it is thought to be the main agent for the spread of antibiotic resistance (81) and has contributed to virulence (8). Interestingly, research done in *E. coli* demonstrates the ability of these bacteria to transfer DNA to genetically distant recipients ranging from yeast (10) to Chinese hamster ovary CHO K1 cells (152) to archaea (32, 39). Sato et al. (114) were able to integrate large genome fragments (up to 75 kb) from *P. furiosus* into the genome of *T. kodakarensis* using what they called the pellet

method, with whole cells as donors of genomic DNA. Unfortunately, in that case there was no differentiation between a possible active conjugation mechanism and simple DNA uptake from lysed cells by naturally competent archaea.

The first evidence for conjugation in archaea was observed in *Sulfolobus* in the mid-1990s, by Zillig's group (115). They observed exchange of the plasmid pNOB8 through visible, close donor-recipient contact. DNA transfer was polar, and the plasmid conferred to the recipient the ability to serve as a conjugation donor. In the following year Grogan (52) showed similar processes for chromosomal DNA. In both cases, other possible explanations, such as transduction by virus particles, cross-feeding, and genetic complementation by way of diploid heterozygous cell formation, were ruled out and in both cases cell-cell contact, including actual cell aggregation, was visible. Shortly after these observations it was noted that exposure to UV radiation greatly increases DNA exchange (117, 155).

Several other plasmids have been isolated since then, all from the same archaeal family (*Sulfolobaceae*). These plasmids seem to have a specific integration site into the recipient's genome owing to an integrase encoded by this family of plasmids (121). In their review of HGT mechanisms in archaea, Wagner et al. (149) discuss the comparative analysis of this family of plasmids that share sequence similarities with the bacterial VirB4 and VirD4 genes, part of the type IV secretion system (T4SS) that is thought to have evolved from an ancestral conjugation system in bacteria. The type IV pili of bacteria can be involved in conjugation or in environmental DNA uptake (19); the VirB/VirD4 translocation machine is employed by *Agrobacterium tumefaciens* to deliver transfer DNA and effector proteins to plant cells (3). Wagner et al. (149) state that the lack of a known archaeal relaxase gene homolog, required for nicking the conjugated DNA and rejoining it in the recipient cell, suggests that an alternative mechanism for conjugation exists in archaea.

A comparative analysis of 47 *Sulfolobus acidocaldarius* genomes from two hot springs in different geothermal basins in Yellowstone National Park revealed, among other variations, two different integrative plasmids with 82% identity along nearly half their sequence length to the conjugative plasmid pNOB8 isolated from *Sulfolobus* strain NOB8H2 from Japan. This is strong evidence that such integrative plasmids are common in these archaea worldwide (5).

Abby et al. (1) observed putative conjugative elements in the ammonia-oxidizing, extremely thermophilic archaeon "*Candidatus* Nitrosocaldus cavascurensis" of the *Thaumarchaeota* phylum. Notable deviations from the average G+C content of the chromosome suggested that "*Ca*. N. cavascurensis" might have acquired several islands integrated into tRNA genes by HGT. These regions showed homology to several Vir genes (*virD4*, *virB4*, *virB6*, and *virB2*), leading the authors to suggest that the conservation of both the integration site and the Vir-like elements indicates that the integration elements and conjugative elements of these systems are likely still active. The authors also reported that all genes that are required for the assembly of a type IV pilus are present in the genome of the archaeon and bear sequence similarity to those encoding the *Sulfolobales* and *Desulfurococcales* pili. Eight additional integrative-conjugative elements were later observed in a large-scale genomic analysis of *Thaumarchaeota* (73), most of which carry genes that are VirD4 and VirB4 homologs. Once more, no relaxases were detected in that study, supporting the hypothesis that archaea have an alternative mechanism for DNA transfer via conjugation.

The conjugation-like Ups system. A decade after the first experimental observation of conjugation in *Sulfolobus* spp. and the findings that UV radiation exposure increased its yield, Frols et al. (44) confirmed the formation of live cell aggregates and utilized microarray hybridization techniques to assess the general UV transcript response of *Sulfolobus solfataricus*. Interestingly, the UV-induced cells observed in aggregates also had pili formation, in line with the transcript upregulation of a putative type II/IV secretion system or pilus formation that they observed. Later,

in follow-up studies, the operon responsible for encoding these pili was named the *ups* operon, for UV-inducible pili operon of *Sulfolobus*, and the process of pilus formation was shown to be dose-dependent and dynamic (42). Ajon et al. (2) demonstrated the UV-induced pili in *S. solfataricus*, *S. tokodaii*, and *S. acidocaldarius*, all of which had similar pilus diameters but differed in pilus length. Cellular aggregation was also observed for all species. Interestingly, the aggregates were highly species-specific, strongly suggesting some cell-cell recognition mechanism. It was also noted that one cellular partner with an intact *ups* operon could promote conjugation to a strain in which *ups* had been deleted.

Similar to the findings in *E. coli* (15), double-strand DNA breaks were also observed following UV radiation. A review of the whole *Sulfolobus* reaction to UV radiation is presented in Reference 45. More recently it has been shown that five additional UV-induced genes in *S. acidocaldarius* are responsible for the in vivo response to UV-induced DNA damage (*cdc6-2*, *tfb3*, *rio1*, *Saci*_0951, and *Saci*_1302) (135). Furthermore, pilus formation in *Sulfolobales* was reported to be essential for DNA transfer, a process that increases cellular fitness after UV radiation (42), indicating importance for DNA repair. The *ups* operon that is common in *Sulfolobales* was not found in other species, and it encodes five genes that are important for pilus formation and DNA transfer: *upsA*, *upsB*, *upsE*, *upsF*, and *upsX*. UpsA and UpsB are signal peptide–pilin subunits that facilitate species-specific aggregation in a mechanism involving surface layer (S-layer) *N*-glycosylation (145). A specific low-conservation region of UpsA seems to be adapted to the glycan structure of the same species, possibly in order to ensure species-specific aggregation. UpsE is an ATPase, essential for cellular aggregation (42), UpsF is a membrane protein, and UpsX was proposed to promote DNA exchange (144).

The Ced system. Another independent UV-induced machinery that is essential for DNA import was detected in *S. acidocaldarius*. Homologs of the genes discovered were detected only in *Crenarchaeota* and were therefore named crenarchaeal exchange of DNA (*ced*) (146). The *ced* cluster encodes two small (CedA1, CedA2) and one large (CedA) transmembrane proteins and a VirB4/HerA homolog (CedB). The CedA proteins are hypothesized to form a membrane channel for passage of DNA, while CedB is suggested to be the ATPase essential for DNA transfer. Since DNA transfer was demonstrated from a Δ*ced* (*upsE*$^+$) strain to the Δ*upsE* (*ced*$^+$) strain, it was concluded that this system imports rather than exports DNA.

Although the *Sulfolobales* machinery for DNA transfer is not fully understood, the current model suggests that UV radiation leads to helix-distorting DNA damage, such as cyclobutane pyrimidine dimers (CPDs). Such damage is then removed; however, as a side effect, DNA lesions might also cause cell cycle arrest. The pilus formations that are responsible for formation of species-specific aggregates increase the chance of DNA repair using homologous DNA taken up by the Ced system. Since Ups pili have also been observed in biofilms (67, 162), this system might also promote DNA transfer in other conditions (25). Whether homologous systems exist in other archaeal phyla remains to be determined.

Mating by cell fusion. The intriguing phenomenon of HGT mediated by cell fusion (mating) in halophilic archaea was first reported in 1985 (94). In that study, two auxotrophic mutants of *H. volcanii* (then named *Halobacterium volcanii*) were grown in liquid medium, mixed, placed on filters, and incubated for 96 h on rich solid medium, and then transferred to selective media. The resulting prototrophic colonies were not the result of cross-feeding or reversion, and viral transduction was ruled out using a filtered supernatant. Agitation of the culture in the process abolished the complementation, and close contact between the cells was an essential prerequisite. Surprisingly, treatment with DNase was not only harmless but actually increased the number of prototrophs detected (mating efficiency). No such prototrophs were formed when one of the

auxotrophs in the pairs was heat-killed before the procedure, indicating an active mechanism. Mevarech & Werczberger (94) claimed that this mechanism was different from the previously observed conjugation, as the transfer was successful for any pair of the different mutants chosen and hence appeared not to be polar or unidirectional.

Soon after this discovery Rosenshine et al. (110) reported the presence of multiple cytoplasmic bridges visible under scanning electron microscopy. The dimensions of these bridges were up to 2 μm long and 0.1 μm in diameter, and since many pairs of cells were connected by more than one such bridge, it was concluded that this phenomenon is not a by-product of a failed cell division but a way of creating a network between multiple *H. volcanii* cells. Initially, it was thought that only chromosomal DNA could move through the bridges whereas plasmids could not, but that claim was later countered (139). It is now clear that there is high DNA transfer efficiency of both plasmid DNA and chromosomal DNA in haloarchaeal mating. In fact, in a recent study employing cryo–electron tomography (122), the in vivo, de novo formation of cell-cell bridges showed that the cytoplasms of the mated cells were connected to each other via a continuous S-layer. Remarkably, the cell-cell bridges were observed to form rather quickly (within 0.5 h) and were able to connect two cells 1.5 μm apart without any initial direct contact, suggesting that cell-cell bridge formation is an active process. The length of the cell-cell bridge was shown to decrease over time, indicating a contraction that brought both cells together while the bridge between them grew thicker and wider. The cytoplasms fused shortly after the cell-cell bridge formed. These cell fusions occurred in liquid media, and it is likely that more mating events would have been observed in solid media. DNA transfer by mating was also observed between *H. volcanii* and *Haloferax mediterranei* but not with *H. volcanii* and *Halobacterium salinarum* (formerly named *Halobacterium halobium*) or *Haloarcula marismortui* (139).

Cell fusion in the genus *Haloferax* has also been studied using genomics. Naor et al. (96) showed that this mechanism creates hybrid heterodiploid cells harboring chromosomal DNA from both parents. After resolution and subsequent cell separation, the resulting stable cells contain a single chromosome type. Whereas 62% of the observed intraspecies fusion events resulted in a chromosome showing evidence of chromosomal recombination, only 8% of the interspecies fusions resulted in a recombinant chromosome. Interspecies recombination rates were strikingly higher in these experiments compared to interspecies DNA transfer recombination events in bacteria. Moreover, the *H. volcanii–H. mediterranei* interspecies recombination events spanned very large DNA fragments (310–530 kb). Creation of the stable hybrids suggests a low barrier for DNA transfer between haloarchaea. CRISPR (clustered regularly interspaced short palindromic repeats) spacers matching chromosomal genes were later shown to be acquired during interspecies mating (143).

Mating of *H. volcanii* is independent of type IV pili and archaella (142); however, biofilms form in a wide variety of haloarchaeal species, including *H. volcanii* (43). Biofilm formation in *H. volcanii* promotes DNA exchange in frequencies similar to those created by mating by fusion on membrane filters (24). Shalev et al. (120) postulated that such events rely on S-layer recognition, since surface glycosylation defects hampered fusion (mating) success (see the section titled Surface Recognition and Glycosylation). Recent *H. volcanii* glycoproteome analysis revealed the largest number of glycoproteins identified in any archaeon (118).

Formation of cell-cell bridges or cell fusion events might not be restricted to haloarchaea. Other than the above fusion mechanism in halophilic archaea and the proposed conjugation and conjugation-like mechanisms in *Crenarchaeota*, two species of *Thermococcus* (class *Thermococci*, phylum *Euryarchaeota*), for which no genetic tools currently exist, were also shown to fuse in the presence of a DNA-interchelating dye (74, 75). Similar bridges were also observed between *Nanoarchaeales* and *Thermoplasmatales* (30), hinting that the phenomenon of DNA transfer via

cell-cell fusion might be more widespread in archaea than generally appreciated. Estimating the extent of the phenomenon remains a challenging endeavor, as even in the model archaeon *H. volcanii*, little is known regarding the regulation and molecular mechanisms involved in membrane and S-layer remodeling, which are likely to be required for cytoplasmic bridge formation. Addressing this knowledge gap will be critical for the understanding of the role that this mechanism of HGT plays in archaeal ecology and evolution.

DNA Transfer by Viruses, and Related Elements

Studies on archaeal and bacterial viruses led to the elucidation of the many aspects of viral infection and have laid the basis for the development of modern virology and molecular biology (for a recent review see Reference 78). Archaea and bacteria share a variety of defense systems (see below) against mobile genetic elements. Hence, it is only logical that they should also have similar mobile genetic elements. However, while bacterial and archaeal insertion-sequence elements and some DNA viruses share common features, other archaeal viruses seem to greatly differ from those of their bacterial counterparts (for recent reviews of archaeal viruses see Reference 72).

Phage and virus life cycles dictate their role in bacterial and archaeal biology. Three major life stages of phages have been reported: lytic, lysogenic, and chronic (134). Archaeal viruses were initially thought to be divided based on host phylum. Euryarchaeal viruses were considered lytic and crenarchaeal viruses were considered nonlytic, having a chronic life cycle in which a small number of particles were continuously released from the cell without causing cell death or lysis. Later, lytic crenarchaeal viruses were described (108, 124), as were chronic infecting euryarchaeal viruses (136).

During virus reproduction within the host cell, host DNA fragments might be captured and packaged into the newly made virion particles. The mature virus might then serve as the transport vehicle for these DNA fragments. Such DNA delivery through viruses or viral particles is called transduction. Two types of transduction are known: specialized transduction, in which a virus packages only a specific region of the host genome, and generalized transduction, in which the virus packages a nonspecific portion of the host genome (76).

Most known archaeal viruses were isolated from either hyperthermophilic *Crenarchaeota* or halophilic *Euryarchaeota* (72). Some archaeal viruses were shown to be temperate and integrate into their host genomes. However, very few archaeal viruses were shown to serve as agents of HGT. The first archaeal viruses that were shown to facilitate the transfer of other particles' DNA were SSV1 and SSV2 (116). SSV1 is a fusiform virus integrated into a tRNA gene in the genome of its *S. solfataricus* host but could also be kept as an episome. UV irradiation of lysogenized *S. solfataricus* was followed by virus production but not cell lysis, and SSV1 showed budding, similar to some eukaryotic viruses and unlike any tailed bacteriophages. Its mechanism of budding is reviewed in Reference 149. SSV2 is a close relative of SSV1 discovered shortly after with the interesting plasmid/satellite virus pSSx in *Sulfolobus islandicus* (6). While no true generalized transduction was detected with the SSV1 and SSV2 (or any other archaeal virus), both are known for their ability to package and transfer the pSSVx and pSSVi satellite viruses. pSSVx and pSSVi require the presence of SSV2 or SSV1 as helpers for spreading and cannot infect hosts on their own (16, 116, 150). Such satellite viruses are also known in bacteria (26). They are suggested to be evolutionary bridges between phages and other mobile genetic elements, such as plasmids (69, 104).

Gene Transfer Agents—Machines for Generalized Transduction

Gene transfer agents (GTAs) are small virus- or phage-like entities produced by bacteria and archaea that are not infective in the usual sense; rather, they package random pieces of the

producing cell's genome. Genes for the phage-like GTAs are at a specific locus within the producing cell. While the occasional GTA can contain some GTA-encoding genes, the capsids are too small to contain DNA encoding the entire structure. GTAs are released from the producing cell by lysis and inject their genetic material into cells and are thus agents of gene delivery that were suggested to confer advantages associated with HGT (38).

Strain PS of the methanogen *M. voltae* is the only archaeon that has been reported to produce GTAs. The *M. voltae* GTA particles were named VTA (voltae transfer agent) and described in 1999 as agents of a generalized transduction process in which the viral component is absent. Examination by electron microscopy of concentrated preparations of VTAs revealed virus-like particles with isometric heads, about 40 nm in diameter, and with 61-nm-long tails (27). The authors reported that these DNase-resistant (but heat-sensitive) entities, obtained by filtration, reverted three different auxotrophies at a frequency much higher than that of natural reversion or natural transformation as previously described for this archaeon (see above), and that the same strain could be used as either a donor or a recipient. The purified VTA particles were found to contain 4.4-kb DNA fragments derived exclusively or almost exclusively from the archaeal chromosome and too small to encode all the viral genes necessary for the formation of a structurally complex particle (12). Thus VTAs are GTAs in the sense that they perform generalized transduction that is nonselfish.

How common GTAs are in archaea is an open question. One way to address it is to study so-called metaviromes, sequences derived from virus-like particles after they are separated from the cellular fraction, and look for chromosomal genes that can be identified as originating from particular archaeal species. This will enable the discovery of GTAs even in lineages known to resist cultivation, such as members of the *Asgardarchaeota* superphylum.

Horizontal Gene Transfer Mediated by Membrane Vesicles

Extracellular membrane vesicles (EMVs) that are secreted by members of Eukarya, Bacteria, and Archaea are widespread in nature (for a recent review see Reference 47). EMVs exist in a variety of ecological niches, from the bovine rumen to the oceans. They enclose a variety of metabolites, ranging from toxins to quorum-sensing molecules. Interestingly, EMVs can harbor not only metabolites but also DNA fragments and thereby play a role in HGT. Soler & Forterre (125), whose group was the first to describe virus-like vesicle particles in cultures of hyperthermophilic archaea of the order *Thermococcales* (127), suggested distinguishing between vesiculation, a term used to describe the production of EMVs that do not carry DNA fragments, and vesiduction. The latter is a process in which vesicles bud from cell membranes and protect DNA. The term has similarities to the word transduction, which refers to the mechanism by which cellular DNA is protected and delivered by a virus or virus-like capsid protein. For a recent review on vesiduction, see Reference 125.

Among *Euryarchaeota*, vesiduction has been reported in *Thermococcales* (126) and in an arctic haloarchaeon (37). Recently, it was shown that the well-described ESCRT (endosomal sorting complex required for transport) EMVs in *Sulfolobus islandicus* of the *Crenarchaeota* harbor chromosomal and plasmid DNA that can be transferred to recipient cells (80).

Vesiduction in *Thermococcales*. Vesicles of *Thermococcales* appeared to be strongly associated with DNA that proved to be thermostable. They were found in 26 of 34 strains in a *Thermococcales* strain collection as well as in various laboratory strains, including strains of *T. kodakarensis*, *Thermococcus gammatolerans*, *Pyrococcus abyssi*, and *Pyrococcus horikoshii*. The vesicles were usually very abundant, resembled the archaeal S-layer in structure, and exhibited a heterogenous size range (127).

Thermococcales EMVs are similar in structure to the *Thermococcales* cell membrane. The most abundant protein as well as the only protein enriched in EMVs compared with cell membranes is TK1804, a homolog of oligopeptide permease A (OppA), which is a peptide receptor of ABC (ATP-binding cassette) transporter systems. OppA is an extracellular oligopeptide-binding protein that is a part of the oligopeptide permease Opp. Opp is highly conserved in bacteria, poorly described in archaea, and generally absent from eukaryotes. Work to determine the structure of *T. kodakarensis* OppA is now in progress (159). EMVs of *Thermococcales* are produced by a budding process and may form a structure resembling bacterial nanopods (89).

EMVs from *Thermococcus nautilus* were shown to contain the *T. nautilus* plasmid pTN1 (126). In later studies another plasmid, pTN3, was also detected (46), but no incorporation of pTN2, a third *T. nautilus* plasmid, was observed. This suggested a role for thermococcal EMVs in specific plasmid transfer. pTN3 was found to encode an integrase of the SSV1 family, and a copy of the plasmid is indeed integrated into the *T. nautilus* genome. The vesicle nevertheless harbored no capsid proteins (46). Interestingly, *T. kodakarensis* transformed by a shuttle vector created by ligating pTN1 from *T. nautilus* to the commercial vector pCR2.1-TOPO (112) produced EMVs containing this plasmid (89). It remains unclear why these three plasmids can be packaged in the vesicles while pTN2 cannot. Treatment of the vesicles with DNase followed by DNA extraction revealed that both plasmids were still present; however, their amounts were significantly reduced. Since the DNase concentrations used were sufficient to degrade free DNA completely, it was concluded that while some DNA particles were indeed located inside the vesicle and hence protected, a portion of the DNA molecules are probably adsorbed onto the surface of the vesicle (46).

Sulfolobus islandicus ESCRT-derived EMVs. In eukaryotes, EMV formation that relies on the ESCRT machinery has been studied in depth (47). Unlike members of *Euryarchaeota* (including *Thermococcales* and haloarchaea), most archaea of the TACK (*Thaumarchaeota*, *Aigarchaeota*, *Crenarchaeota*, and *Korarchaeota*) and Asgard superphyla encode the ESCRT machinery. The ESCRT machinery plays a pivotal role in cell division in *Sulfolobales* and was also shown to mediate EMV formation. ESCRT-III, ESCRT-III-2, and VPS4 are present within EMVs secreted by *S. acidocaldarius*, *S. solfataricus*, and *S. tokodaii* (35), and EMVs from *S. islandicus* were shown to contain all six components of the *Sulfolobus* ESCRT machinery. *S. islandicus* EMVs demonstrated an ability to support the heterotrophic growth of *Sulfolobus* when added to growth media containing only mineral salts and a mix of vitamins, and their production was suggested to be regulated to the cell cycle–linked changes in ESCRT-III expression (80).

In relation to HGT, *S. islandicus* EMVs were shown to carry chromosomal as well as plasmid DNA. The DNA that was found in association with the vesicles was partially resistant to treatment with DNase, implying that the DNA content of the vesicles is heterogenic. Vesicles were purified by the same method from a strain previously artificially transformed with the plasmid pSeSD, designed to carry the *pyrEF* locus (the uracil biosynthesis pathway). The vesicles from this strain were treated with DNase and then incubated with a plasmid-free strain, deleted for this locus. Half of the colonies of the incubated strain became plasmid positive and able to grow on media lacking uracil when freshly inoculated, unlike the growth of the plasmid-free colonies that were able to grow due to the nutrients imported in the vesicles (80).

Plasmid vesicles from the Antarctic haloarchaeon _Halorubrum lacusprofundi_. Erdmann et al. (37) proposed the term plasmid-vesicle (PV) to describe a virus-like particle harboring a 50,329 bp plasmid named pR1SE, discovered in the Antarctic haloarchaeon *Halorubrum lacusprofundi* strain R1S1. These virus-like vesicles are unique in the sense that their membrane contains 10 proteins that are encoded by the pR1SE plasmid they harbor and that these proteins are absent from the host cell membrane of which they are produced.

A plasmid-free strain of *H. lacusprofundi*, ACAM34, also produces vesicles. However, these contain little or no DNA and are more irregular in size, and their membrane resembles that of the host. Plasmid-vesicles released from the plasmid-vesicle-producing *H. lacusprofundi* R1S1 were able to infect that plasmid-free strain, and the newly infected strain in turn began to produce plasmid-vesicles (37). Sequencing of the 50,329 bp of the pR1SE plasmid revealed 48 putative ORFs (open reading frames), out of which ORF6 has five predicted transmembrane domains. The large non-transmembrane domain of this protein had a WD40 domain, a domain known in eukaryotes to serve as a scaffold for protein interactions and that is commonly found in proteins involved in vesicle formation. Other predicted ORFs also showed similarities to known vesicle-associated proteins such as the Sar1/Arf family GTPases.

Importantly, none of the 48 putative ORFs of pR1SE were reported to resemble strictly viral genes, but one did encode an integrase. pR1SE indeed eventually became integrated into the chromosome of strain R1S1 after three months of continuous culturing. The strain with integrated pR1SE plasmid showed plasmid-vesicles containing a larger DNA fragment than usual that also contained a small segment of host chromosomal DNA (37). Thus, vesiduction in this case could serve as a plasmid-packaging mechanism and potentially also as a transducing agent.

Symbiosis-Related Gene Transfer

A special case of HGT is the transfer of genes between closely symbiotic partners. The symbiotic interaction between *Nanoarchaeum equitans* and *Ignicoccus hospitalis*, two hyperthermophilic archaea, was first discovered in a hydrothermal vent by the group of Karl Stetter. It involves transport of metabolite from the latter (the host) to the former (55, 62). When both genomes became available for phylogenomic analysis, it became apparent that despite the fact that the two archaea belong to different phyla, for 13 genes the orthologs from *N. equitans* and *I. hospitalis* were the closest homologs; these included genes for aminoacyl tRNA synthetases and recombination and repair (41, 105). Thus, despite the fact that *I. hospitalis* has two membranes, and the fact that *N. equitans* clings to it from the outside, there must have been DNA transfer between the two symbionts that has resulted in HGT and fixation. Indeed, more recent work has shown that the cytoplasms of the ecto-symbiont and its host can get into direct contact with one another (58). It remains to be determined whether other symbiotic archaea pairs such as "*Candidatus* Nanohaloarchaeum antarcticus" and its host *Halorubrum lacusprofundi* (56) or even "*Candidatus* Prometheoarchaeum syntrophicum" and its associated methanogen (60) have also shared genes during recent evolution.

Inteins and Intein-Induced Gene Conversion and Their Effects on Horizontal Gene Transfer

Inteins are selfish genetic elements within protein-coding genes that are spliced out posttranslationally using an autocatalytic protein-splicing reaction that leaves behind an intact and functional protein. Most inteins harbor a homing endonuclease (HEN) domain, which encodes a nuclease that targets a highly specific recognition sequence (hence "homing"), that corresponds to an allele that does not have the intein within it. The HEN cleaves the DNA of the intein-less allele when such an allele becomes available (a gene transfer event) and generates a double-strand break. When the break is repaired by homologous recombination, the selfish element invades the empty allele via gene conversion (9). Although inteins are found in all domains of life, they are most prevalent in archaea, where nearly 50% of genomes have at least one intein (100). This is in stark contrast to group I introns that can also encode HENs (57) and have not been detected in archaea (140). Since inteins are nearly always found in housekeeping genes, often essential ones, intein invasion (either by HEN activity or by a rare HEN-independent homologous recombination

event) requires transfer of chromosomal DNA and may therefore reveal gene exchange networks between species and strains (131). Although most intein transfer in archaea has occurred within species or between closely related ones (131), there have been distant transfer events in archaeal evolution, as was shown for the vacuolar ATPase gene *vma-1* (137). This raises a question: What mechanisms of HGT provide an opportunity for intein invasion?

Inteins are far more abundant in *Euryarchaeota* than in other archaeal phyla (100), suggesting that the DNA transfer mechanisms that underlie intein invasion are those known in multiple members of that phylum, namely natural transformation and cell fusion. Until fairly recently there were no reports showing intein homing in vivo in archaea. Naor et al. (95) have demonstrated that when haloarchaeal cells mate via cell fusion, inteins can invade intein-free alleles of the *polB* gene in *H. volcanii*. An interesting observation was that homing activity also increased homologous recombination (and by extension capacity for HGT) at sites distant from the *polB* locus (95). Thus, similar to other selfish genetic elements such as plasmids and viruses, HEN-containing inteins can increase HGT between lineages.

Barriers to Genetic Exchange in Archaea

HGT is never without limitations, since without some barriers to gene exchange there would not be distinct lineages that are the product of speciation processes that are largely vertical.

Homologous recombination limitations. Among diverse archaea, homologous recombination contributes more to allele variation than mutations do (18, 36, 102), indicating the importance of HGT within efficiently recombining species (leaving aside the difficulties in defining microbial species). The homologous recombination machinery in bacteria requires not just a stretch of homologous DNA but also a relatively high level of sequence identity between the pieces of DNA to be recombined (86, 87, 148, 161). Such recombination barriers may in fact maintain the tree-like pattern of organismal evolution (4, 48). Similar observations have been made of members of the phylum *Euryarchaeota*, where homologous recombination between divergent species occurred only at loci with unusually high sequence similarity, such as tRNA genes (36, 96). This has the interesting side effect of very large recombined regions that can exceed 500 kb (96). However, this was not the case in a crenarchaeote (18), perhaps due to the lack of a mismatch repair system that can impede recombination with more dissimilar DNA fragments (86, 148).

Archaeal defense systems against foreign DNA. Similar to bacteria, archaea can have diverse defenses against selfish elements such as viruses, many of which primarily target DNA (70). Below we review the defense systems that are known to degrade or recognize target foreign DNA and elaborate on their potential effects on HGT.

Restriction-modification systems. Restriction-modification systems recognize a relatively short sequence of DNA; therefore, they are likely to be present on any selfish element that is longer than about 10 kb. Discrimination between self and nonself, which is required to avoid digestion of self-DNA, is based on the methylation status of the invading DNA. Only incorrectly methylated or nonmethylated DNA is cut on both strands.

BREX systems. Similar to restriction-modification systems, bacteriophage exclusion (BREX) systems discriminate between self-DNA and nonself DNA based on methylation of the self-DNA (50, 61). BREX systems have been detected in both *Euryarchaeota* and *Crenarchaeota* (49). While they do not degrade foreign DNA, they can prevent viral lysogenization, and hence reduce gene transfer (49).

Phosphorothioate modification-based systems. DNA degradation and SspABCD–SspE systems modify the DNA backbone by replacing a nonbridging oxygen atom with a sulfur atom as a means of marking self-DNA (61). In bacteria, this is generally based on degradation of foreign DNA. However, the system that has recently been characterized in halophilic archaea does not share this activity (157) and thus does not necessarily impede HGT. Curiously, this subtype of DNA degradation systems is also found in several bacteria and appears to have been transferred horizontally between bacteria and archaea in evolution (157). The SspABCD–SspE system, which unlike DND only modifies a single strand of DNA and only nicks the foreign DNA, has thus far been identified only in bacteria (158).

CRISPR-Cas systems. CRISPR-Cas systems sample invader nucleic acids and generate immune memory against invaders that is stored as DNA arrays of spacers interspersed with repeats. These arrays are transcribed, and the processed RNA, known as crRNA, is incorporated into a ribonucleic-protein complex that can base-pair with matching DNA or RNA of invaders and lead to degradation of the foreign genetic material. Since CRISPR-Cas nucleases ignore DNA methylation, immune memory against self-DNA must be somehow avoided to prevent autoimmunity. Consequently, there is often tight downregulation of spacer acquisition when invading mobile elements are absent. CRISPR-Cas systems are very common in nearly all archaeal groups and are encoded by >85% of archaeal genomes (88). These systems are often themselves encoded on mobile elements, especially archaeal plasmids (133), and have been often transferred and exchanged via HGT among archaeal species.

The Gabija system. The Gabija system is relatively rare in archaea compared to bacteria and has thus far been detected only in methanogenic *Euryarchaeota* (33). This system has been recently shown to cut DNA in bacteria only upon the drastic depletion of NTPs and dNTPs that occurs during late infection of lytic viruses (22). If this is also the case in archaea, then Gabija is unlikely to limit HGT substantially in that domain of life.

The Druantia system. The very large Druantia system remains uncharacterized, but since it has a helicase domain protein, it might target DNA and thus affect HGT. It, too, has been found exclusively in methanogenic *Euryarchaeota* (33).

The Wadjet system. The Wadjet system has been shown to protect exclusively against plasmids and is found exclusively in methanogenic *Euryarchaeota*, primarily in *Methanosarcina* (33). If its anti-plasmid activity that was demonstrated in bacteria indeed proves similar in archaea, then this system will be a major barrier to HGT.

DISARM. The defense island system associated with restriction-modification (DISARM) can be found in two alternative configurations known as class I and II (101). Class II DISARM systems have been detected in the genomes of *Euryarchaeota*, *Crenarchaeota*, and *Thaumarchaeota* (101) and generally contain a helicase and a cytosine-specific methyltransferase (61). This methyltransferase was shown in bacteria to methylate host DNA, and deletion of it proved to be toxic. This implies that discrimination between self and nonself could be similar in principle between this system and restriction-modification systems. If this is indeed the case, DISARM may prevent the acquisition of DNA that is not correctly methylated and hence reduce HGT of such DNA.

Surface recognition and glycosylation. Some mechanisms of HGT, such as generalized transduction, require specific recognition of cell surface molecules for subsequent genetic exchange. All archaeal cell walls lack bacterial peptidoglycan, but nearly all archaea have one or more S-layer proteins that have important roles in maintaining cell shape and integrity (for a recent review see References 14, 109, and 123). Mating by gene fusion has been shown to be highly dependent on

surface protein glycosylation (120). This is to be expected, since such a mechanism whose benefit lies in the transfer of alleles and genes within species or between related species should have some "mating-preference" specificity factor. Specifically, cells of *H. volcanii* that had impaired surface glycosylation showed markedly reduced mating by cell fusion. This was especially severe when both mating partners had glycosylation defects (120).

EVOLUTIONARY IMPLICATIONS OF ARCHAEAL HORIZONTAL GENE TRANSFER

Massive Gene Transfer as a Driver for the Divergence of Major Archaeal Groups—A Big Bang or Gradual Gene Transfer?

HGT between archaea and bacteria, while much rarer than gene transfer between archaea, has occurred throughout archaeal evolution—often with dramatic consequences. As soon as more than 100 archaeal genomes became available for analysis (although still outnumbered by at least an order of magnitude by bacterial genomes), several phylogenomics studies looking at interdomain HGT were conducted. Such studies consistently found that transfers from bacteria to archaea were much more frequent (3- to 10.7-fold) than transfers from archaea to bacteria. Archaea especially were found to acquire bacterial genes whose products contribute to metabolism (66, 99). A more controversial conclusion was that since the emergence of several ancient archaeal groups (such as *Halobacteriales*, *Archaeoglobales*, and *Thermoplasmatales*, all of which are thought to be derived from methanogenic ancestors) coincided with major gene acquisitions (involving hundreds of genes in each lineage) from bacteria in the phylogenetic reconstruction, it is more likely that those lineages have emerged from mass transfers involving symbiotic associations, such as the one that resulted in eukaryogenesis, rather than independent HGT events (99). The results of these studies were later challenged, since they included many genes that have a narrow and patchy distribution and are very rare in bacterial genomes. It was claimed that in most cases there were actually more bacteria-to-archaea gene transfer events that occurred after the divergence of those groups rather than before they emerged, consistent with continuous gene-exchange events throughout evolution (53). Such a continuous gene flow from bacteria to archaea has been detected in genomes of uncultured group II and group III marine *Euryarchaeota* and *Thaumarchaeota*, where both recent and ancient transfers were common, contributing >20% of coding genes in those lineages (31). Notably, acquisitions from bacteria are thought to have facilitated the transition from a hyperthermophilic lifestyle to lower temperatures, which is reflected in convergent acquisition of the same gene families by different archaeal lineages (82). In agreement with the adaptive nature of these genes, the functions of the encoded proteins tend to involve energy metabolism, amino acid transport and metabolism, and lipid or membrane biogenesis (82).

Nevertheless, even if only about 25% of the genes that Nelson-Sathi and colleagues (99) originally inferred to have been imported from bacteria prior to the emergence of those archaeal orders (53) were indeed to some extent acquired en masse, this would still constitute landmark events in archaeal evolution and demonstrate the power of HGT as a driver of diversification and speciation.

Perhaps the best example for archaeal lineage emergence is that of the haloarchaea (order *Halobacteriales*), which are almost certainly derived from a halophilic, methanogenic (and thus anaerobic) group but have adopted a largely aerobic lifestyle. It has been proposed that this evolutionary leap was fueled by the acquisition of 1,089 genes from bacteria in the haloarchaeal common ancestor (98). This idea was later questioned when a more extensive sampling of haloarchaeal genomes became available and revealed that over two-thirds of bacterially derived gene families were not acquired by the common ancestor and often were acquired multiple times by different

lineages and derived from different bacterial sources (11). Nonetheless, as noted above for the origin of other bacterial orders, even if the original number reported by Nelson-Sathi and colleagues (98) was inflated, many bacteria-to-archaea transfers remain well-supported and could have made a large impact on the emergence of haloarchaea. The fact that bacterial genes have been continuously acquired and the fact that bacterial gene fragments are present in 126 gene families that constitute haloarchaeal genomic innovations (91) highlight the importance of bacteria-to-archaea HGT for the haloarchaea.

Horizontal Gene Transfer from Bacteria and Its Contribution to Adaptation

Two species of methanogenic archaea, *Methanosphaera stadtmanae* and *Methanobrevibacter smithii*, that are associated with the human colon were shown to have acquired many genes for adhesins (84), ABC transporters, and glycosyl transferases (85) from anaerobic bacteria, presumably niche neighbors. These laterally acquired genes are thought to have played a substantial role in the adaptation to mammalian hosts (83). It should be noted that not every evolutionary transition necessarily involves HGT, let alone between-domain HGT. Indeed, a large comparative analysis of *Methanococcales*, where some lineages diverged from a hyperthermophilic ancestor and later became mesophiles, showed no evidence for higher rates of HGT in the internal branches corresponding to the thermoadaptation process (77).

While bacterial genomic contributions to archaea via HGT are common, the opposite is much rarer. The transfer of the structural maintenance of chromosomes (SMC) genes from archaeal methanogens to *Cyanobacteria* (29, 129) is an exception to the rule that has been recently used to date the divergence of methanogens (and methanogenesis) to no later than 3.51 Ga (154).

CONCLUSION

Archaeal evolution has been impacted to a great extent by HGT from distant lineages. Additionally, all archaea that have been studied intensively in the laboratory have mechanisms that enable continuous and efficient gene exchange among genetically related species or strains. The biggest leap this field of research can make in the next few years will be the study of HGT in the many novel lineages discovered in the last decade that are currently nearly impossible to cultivate. By combining advanced microscopy and mass spectrometry techniques with novel genomics approaches, this hurdle may be partly overcome, likely resulting in many exciting discoveries.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

U.G. acknowledges funding from the European Research Council (grant ERC-AdG 787514) that helped to support the preparation of this manuscript. We thank Uri Neri and J. Peter Gogarten for their helpful suggestions.

LITERATURE CITED

1. Abby SS, Melcher M, Kerou M, Krupovic M, Stieglmeier M, et al. 2018. *Candidatus* Nitrosocaldus cavascurensis, an ammonia oxidizing, extremely thermophilic archaeon with a highly mobile genome. *Front. Microbiol.* 9:28

2. Ajon M, Frols S, van Wolferen M, Stoecker K, Teichmann D, et al. 2011. UV-inducible DNA exchange in hyperthermophilic archaea mediated by type IV pili. *Mol. Microbiol.* 82:807–17
3. Amrani N, Gao XD, Liu P, Edraki A, Mir A, et al. 2018. NmeCas9 is an intrinsically high-fidelity genome-editing platform. *Genome Biol.* 19:214
4. Andam C, Gogarten JP. 2013. Biased gene transfer contributes to maintaining the tree of life. In *Lateral Gene Transfer in Evolution*, ed. U Gophna, pp. 263–74. New York: Springer
5. Anderson RE, Kouris A, Seward CH, Campbell KM, Whitaker RJ. 2017. Structured populations of *Sulfolobus acidocaldarius* with susceptibility to mobile genetic elements. *Genome Biol. Evol.* 9:1699–710
6. Arnold HP, She Q, Phan H, Stedman K, Prangishvili D, et al. 1999. The genetic element pSSVx of the extremely thermophilic crenarchaeon *Sulfolobus* is a hybrid between a plasmid and a virus. *Mol. Microbiol.* 34:217–26
7. Baker BJ, De Anda V, Seitz KW, Dombrowski N, Santoro AE, Lloyd KG. 2020. Diversity, ecology and evolution of Archaea. *Nat. Microbiol.* 5:887–900
8. Bartke K, Garoff L, Huseby DL, Brandis G, Hughes D. 2021. Genetic architecture and fitness of bacterial interspecies hybrids. *Mol. Biol. Evol.* 38:1472–81
9. Barzel A, Naor A, Privman E, Kupiec M, Gophna U. 2011. Homing endonucleases residing within inteins: evolutionary puzzles awaiting genetic solutions. *Biochem. Soc. Trans.* 39:169–73
10. Bates S, Cashmore AM, Wilkins BM. 1998. IncP plasmids are unusually effective in mediating conjugation of *Escherichia coli* and *Saccharomyces cerevisiae*: involvement of the tra2 mating system. *J. Bacteriol.* 180:6538–43
11. Becker EA, Seitzer PM, Tritt A, Larsen D, Krusor M, et al. 2014. Phylogenetically driven sequencing of extremely halophilic archaea reveals strategies for static and dynamic osmo-response. *PLOS Genet.* 10:e1004784
12. Bertani G. 1999. Transduction-like gene transfer in the methanogen *Methanococcus voltae*. *J. Bacteriol.* 181:2992–3002
13. Bertani G, Baresi L. 1987. Genetic transformation in the methanogen *Methanococcus voltae* PS. *J. Bacteriol.* 169:2730–38
14. Bharat TAM, von Kugelgen A, Alva V. 2021. Molecular logic of prokaryotic surface layer structures. *Trends Microbiol.* 29:405–15
15. Bonura T, Smith KC. 1975. Enzymatic production of deoxyribonucleic acid double-strand breaks after ultraviolet irradiation of *Escherichia coli* K-12. *J. Bacteriol.* 121:511–17
16. Bruno A, Dovizio M, Tacconelli S, Contursi A, Ballerini P, Patrignani P. 2018. Antithrombotic agents and cancer. *Cancers* 10:253
17. Cabezon E, Ripoll-Rozada J, Pena A, de la Cruz F, Arechaga I. 2015. Towards an integrated model of bacterial conjugation. *FEMS Microbiol. Rev.* 39:81–95
18. Cadillo-Quiroz H, Didelot X, Held NL, Herrera A, Darling A, et al. 2012. Patterns of gene flow define species of thermophilic Archaea. *PLOS Biol.* 10:e1001265
19. Chen I, Christie PJ, Dubnau D. 2005. The ins and outs of DNA transfer in bacteria. *Science* 310:1456–60
20. Chen S, Sun S, Korfanty GA, Liu J, Xiang H. 2019. A halocin promotes DNA uptake in *Haloferax mediterranei*. *Front. Microbiol.* 10:1960
21. Chen S, Tulloss RE, Liu Y, Feng B, Zhao Z, Yang ZL. 2012. Lateral gene transfer occurring in haloarchaea: an interpretative imitation study. *World J. Microbiol. Biotechnol.* 28:2913–18
22. Cheng R, Huang F, Wu H, Lu X, Yan Y, et al. 2021. A nucleotide-sensing endonuclease from the Gabija bacterial defense system. *Nucleic Acids Res.* 49:5216–29
23. Chimileski S, Dolas K, Naor A, Gophna U, Papke RT. 2014. Extracellular DNA metabolism in *Haloferax volcanii*. *Front. Microbiol.* 5:57
24. Chimileski S, Franklin MJ, Papke RT. 2014. Biofilms formed by the archaeon *Haloferax volcanii* exhibit cellular differentiation and social motility, and facilitate horizontal gene transfer. *BMC Biol.* 12:65
25. Chimileski S, Papke RT. 2015. Getting a hold on archaeal type IV pili: an expanding repertoire of cellular appendages implicates complex regulation and diverse functions. *Front. Microbiol.* 6:362
26. Christie GE, Dokland T. 2012. Pirates of the Caudovirales. *Virology* 434:210–21
27. Cline SW, Doolittle WF. 1987. Efficient transfection of the archaebacterium *Halobacterium halobium*. *J. Bacteriol.* 169:1341–44

28. Cline SW, Lam WL, Charlebois RL, Schalkwyk LC, Doolittle WF. 1989. Transformation methods for halophilic archaebacteria. *Can. J. Microbiol.* 35:148–52
29. Cobbe N, Heck MM. 2004. The evolution of SMC proteins: phylogenetic analysis and structural implications. *Mol. Biol. Evol.* 21:332–47
30. Comolli LR, Banfield JF. 2014. Inter-species interconnections in acid mine drainage microbial communities. *Front. Microbiol.* 5:367
31. Deschamps P, Zivanovic Y, Moreira D, Rodriguez-Valera F, Lopez-Garcia P. 2014. Pangenome evidence for extensive interdomain horizontal transfer affecting lineage core and shell genes in uncultured planktonic Thaumarchaeota and Euryarchaeota. *Genome Biol. Evol.* 6:1549–63
32. Dodsworth JA, Li L, Wei S, Hedlund BP, Leigh JA, de Figueiredo P. 2010. Interdomain conjugal transfer of DNA from bacteria to archaea. *Appl. Environ. Microbiol.* 76:5644–47
33. Doron S, Melamed S, Ofir G, Leavitt A, Lopatina A, et al. 2018. Systematic discovery of antiphage defense systems in the microbial pangenome. *Science* 359:eaar4120
34. Dubnau D, Blokesch M. 2019. Mechanisms of DNA uptake by naturally competent bacteria. *Annu. Rev. Genet.* 53:217–37
35. Ellen AF, Albers SV, Huibers W, Pitcher A, Hobel CF, et al. 2009. Proteomic analysis of secreted membrane vesicles of archaeal *Sulfolobus* species reveals the presence of endosome sorting complex components. *Extremophiles* 13:67–79
36. Eppley JM, Tyson GW, Getz WM, Banfield JF. 2007. Genetic exchange across a species boundary in the archaeal genus *Ferroplasma*. *Genetics* 177:407–16
37. Erdmann S, Tschitschko B, Zhong L, Raftery MJ, Cavicchioli R. 2017. A plasmid from an Antarctic haloarchaeon uses specialized membrane vesicles to disseminate and infect plasmid-free cells. *Nat. Microbiol.* 2:1446–55
38. Esterman ES, Wolf YI, Kogay R, Koonin EV, Zhaxybayeva O. 2021. Evolution of DNA packaging in gene transfer agents. *Virus Evol.* 7:veab015
39. Fink C, Beblawy S, Enkerlin AM, Muhling L, Angenent LT, Molitor B. 2021. A shuttle-vector system allows heterologous gene expression in the thermophilic methanogen *Methanothermobacter thermautotrophicus* ΔH. *mBio* 12:e0276621
40. Fonseca DR, Halim MFA, Holten MP, Costa KC. 2020. Type IV-like pili facilitate transformation in naturally competent archaea. *J. Bacteriol.* 202:e00355-20
41. Forterre P, Gribaldo S, Brochier-Armanet C. 2009. Happy together: genomic insights into the unique Nanoarchaeum/Ignicoccus association. *J. Biol.* 8:7
42. Frols S, Ajon M, Wagner M, Teichmann D, Zolghadr B, et al. 2008. UV-inducible cellular aggregation of the hyperthermophilic archaeon *Sulfolobus solfataricus* is mediated by pili formation. *Mol. Microbiol.* 70:938–52
43. Frols S, Dyall-Smith M, Pfeifer F. 2012. Biofilm formation by haloarchaea. *Environ. Microbiol.* 14:3159–74
44. Frols S, Gordon PM, Panlilio MA, Duggin IG, Bell SD, et al. 2007. Response of the hyperthermophilic archaeon *Sulfolobus solfataricus* to UV damage. *J. Bacteriol.* 189:8708–18
45. Frols S, White MF, Schleper C. 2009. Reactions to UV damage in the model archaeon *Sulfolobus solfataricus*. *Biochem. Soc. Trans.* 37:36–41
46. Gaudin M, Krupovic M, Marguet E, Gauliard E, Cvirkaite-Krupovic V, et al. 2014. Extracellular membrane vesicles harbouring viral genomes. *Environ. Microbiol.* 16:1167–75
47. Gill S, Catchpole R, Forterre P. 2019. Extracellular membrane vesicles in the three domains of life and beyond. *FEMS Microbiol. Rev.* 43:273–303
48. Gogarten JP, Doolittle WF, Lawrence JG. 2002. Prokaryotic evolution in light of gene transfer. *Mol. Biol. Evol.* 19:2226–38
49. Goldfarb T, Sberro H, Weinstock E, Cohen O, Doron S, et al. 2015. BREX is a novel phage resistance system widespread in microbial genomes. *EMBO J.* 34:169–83
50. Gordeeva J, Morozova N, Sierro N, Isaev A, Sinkunas T, et al. 2019. BREX system of *Escherichia coli* distinguishes self from non-self by methylation of a specific DNA site. *Nucleic Acids Res.* 47:253–65
51. Griffith F. 1928. The significance of pneumococcal types. *J. Hyg.* 27:113–59

52. Grogan DW. 1996. Exchange of genetic markers at extremely high temperatures in the archaeon *Sulfolobus acidocaldarius*. *J. Bacteriol.* 178:3207–11
53. Groussin M, Boussau B, Szollosi G, Eme L, Gouy M, et al. 2016. Gene acquisitions from bacteria at the origins of major archaeal clades are vastly overestimated. *Mol. Biol. Evol.* 33:305–10
54. Guschinskaya N, Brunel R, Tourte M, Lipscomb GL, Adams MWW, et al. 2016. Random mutagenesis of the hyperthermophilic archaeon *Pyrococcus furiosus* using in vitro mariner transposition and natural transformation. *Sci. Rep.* 6:36711
55. Hamerly T, Tripet BP, Tigges M, Giannone RJ, Wurch L, et al. 2015. Untargeted metabolomics studies employing NMR and LC-MS reveal metabolic coupling between *Nanoarcheum equitans* and its archaeal host *Ignicoccus hospitalis*. *Metabolomics* 11:895–907
56. Hamm JN, Erdmann S, Eloe-Fadrosh EA, Angeloni A, Zhong L, et al. 2019. Unexpected host dependency of Antarctic Nanohaloarchaeota. *PNAS* 116:14661–70
57. Hausner G, Hafez M, Edgell DR. 2014. Bacterial group I introns: mobile RNA catalysts. *Mobile DNA* 5:8
58. Heimerl T, Flechsler J, Pickl C, Heinz V, Salecker B, et al. 2017. A complex endomembrane system in the archaeon *Ignicoccus hospitalis* tapped by *Nanoarchaeum equitans*. *Front. Microbiol.* 8:1072
59. Hileman TH, Santangelo TJ. 2012. Genetics techniques for *Thermococcus kodakarensis*. *Front. Microbiol.* 3:195
60. Imachi H, Nobu MK, Nakahara N, Morono Y, Ogawara M, et al. 2020. Isolation of an archaeon at the prokaryote-eukaryote interface. *Nature* 577:519–25
61. Isaev AB, Musharova OS, Severinov KV. 2021. Microbial arsenal of antiviral—Part I. *Biochemistry* 86:319–37
62. Jahn U, Gallenberger M, Paper W, Junglas B, Eisenreich W, et al. 2008. *Nanoarchaeum equitans* and *Ignicoccus hospitalis*: new insights into a unique, intimate association of two archaea. *J. Bacteriol.* 190:1743–50
63. Jay ZJ, Beam JP, Dlakic M, Rusch DB, Kozubal MA, Inskeep WP. 2018. Marsarchaeota are an aerobic archaeal lineage abundant in geothermal iron oxide microbial mats. *Nat. Microbiol.* 3:732–40
64. Jungbluth SP, Amend JP, Rappe MS. 2017. Metagenome sequencing and 98 microbial genomes from Juan de Fuca Ridge flank subsurface fluids. *Scientific Data* 4:170037
65. Kaminski L, Guan Z, Yurist-Doutsch S, Eichler J. 2013. Two distinct N-glycosylation pathways process the *Haloferax volcanii* S-layer glycoprotein upon changes in environmental salinity. *mBio* 4:e00716-13
66. Kanhere A, Vingron M. 2009. Horizontal gene transfers in prokaryotes show differential preferences for metabolic and translational genes. *BMC Evol. Biol.* 9:9
67. Koerdt A, Godeke J, Berger J, Thormann KM, Albers SV. 2010. Crenarchaeal biofilm formation under extreme conditions. *PLOS ONE* 5:e14104
68. Koonin EV. 2015. Origin of eukaryotes from within archaea, archaeal eukaryome and bursts of gene gain: eukaryogenesis just made easier? *Philos. Trans. R. Soc. Lond B.* 370:20140333
69. Koonin EV, Dolja VV. 2014. Virus world as an evolutionary network of viruses and capsidless selfish elements. *Microbiol. Mol. Biol. Rev.* 78:278–303
70. Koonin EV, Makarova KS, Wolf YI. 2017. Evolutionary genomics of defense systems in archaea and bacteria. *Annu. Rev. Microbiol.* 71:233–61
71. Kozubal MA, Romine M, Jennings R, Jay ZJ, Tringe SG, et al. 2013. Geoarchaeota: a new candidate phylum in the Archaea from high-temperature acidic iron mats in Yellowstone National Park. *ISME J.* 7:622–34
72. Krupovic M, Cvirkaite-Krupovic V, Iranzo J, Prangishvili D, Koonin EV. 2018. Viruses of archaea: structural, functional, environmental and evolutionary genomics. *Virus Res.* 244:181–93
73. Krupovic M, Makarova KS, Wolf YI, Medvedeva S, Prangishvili D, et al. 2019. Integrated mobile genetic elements in Thaumarchaeota. *Environ. Microbiol.* 21:2056–78
74. Kuwabara T, Minaba M, Iwayama Y, Inouye I, Nakashima M, et al. 2005. *Thermococcus coalescens* sp. nov., a cell-fusing hyperthermophilic archaeon from Suiyo Seamount. *Int. J. Syst. Evol. Microbiol.* 55:2507–14
75. Kuwabara T, Minaba M, Ogi N, Kamekura M. 2007. *Thermococcus celericrescens* sp. nov., a fast-growing and cell-fusing hyperthermophilic archaeon from a deep-sea hydrothermal vent. *Int. J. Syst. Evol. Microbiol.* 57:437–43

76. Lang AS, Zhaxybayeva O, Beatty JT. 2012. Gene transfer agents: phage-like elements of genetic exchange. *Nat. Rev. Microbiol.* 10:472–82
77. Lecocq M, Groussin M, Gouy M, Brochier-Armanet C. 2021. The molecular determinants of thermoadaptation: *Methanococcales* as a case study. *Mol. Biol. Evol.* 38:1761–76
78. Letarov AV. 2020. History of early bacteriophage research and emergence of key concepts in virology. *Biochemistry* 85:1093–110
79. Lipscomb GL, Stirrett K, Schut GJ, Yang F, Jenney FE Jr., et al. 2011. Natural competence in the hyperthermophilic archaeon *Pyrococcus furiosus* facilitates genetic manipulation: construction of markerless deletions of genes encoding the two cytoplasmic hydrogenases. *Appl. Environ. Microbiol.* 77:2232–38
80. Liu J, Cvirkaite-Krupovic V, Commere PH, Yang Y, Zhou F, et al. 2021. Archaeal extracellular vesicles are produced in an ESCRT-dependent manner and promote gene transfer and nutrient cycling in extreme environments. *ISME J.* 15:2892–905
81. Llosa M, Gomis-Ruth FX, Coll M, de la Cruz F. 2002. Bacterial conjugation: a two-step mechanism for DNA transport. *Mol. Microbiol.* 45:1–8
82. Lopez-Garcia P, Zivanovic Y, Deschamps P, Moreira D. 2015. Bacterial gene import and mesophilic adaptation in archaea. *Nat. Rev. Microbiol.* 13:447–56
83. Lurie-Weinberger MN, Gophna U. 2015. Archaea in and on the human body: health implications and future directions. *PLOS Pathog.* 11:e1004833
84. Lurie-Weinberger MN, Peeri M, Gophna U. 2012. Contribution of lateral gene transfer to the gene repertoire of a gut-adapted methanogen. *Genomics* 99:52–58
85. Lurie-Weinberger MN, Peeri M, Tuller T, Gophna U. 2012. Extensive inter-domain lateral gene transfer in the evolution of the human commensal *Methanosphaera stadtmanae*. *Front. Genet.* 3:182
86. Majewski J, Cohan FM. 1998. The effect of mismatch repair and heteroduplex formation on sexual isolation in *Bacillus*. *Genetics* 148:13–18
87. Majewski J, Cohan FM. 1999. DNA sequence similarity requirements for interspecific recombination in *Bacillus*. *Genetics* 153:1525–33
88. Makarova KS, Wolf YI, Iranzo J, Shmakov SA, Alkhnbashi OS, et al. 2020. Evolutionary classification of CRISPR-Cas systems: a burst of class 2 and derived variants. *Nat. Rev. Microbiol.* 18:67–83
89. Marguet E, Gaudin M, Gauliard E, Fourquaux I, le Blond du Plouy S, et al. 2013. Membrane vesicles, nanopods and/or nanotubes produced by hyperthermophilic archaea of the genus *Thermococcus*. *Biochem. Soc. Trans.* 41:436–42
90. Martijn J, Schon ME, Lind AE, Vosseberg J, Williams TA, et al. 2020. Hikarchaeia demonstrate an intermediate stage in the methanogen-to-halophile transition. *Nat. Commun.* 11:5490
91. Méheust R, Watson AK, Lapointe F-J, Papke RT, Lopez P, Bapteste E. 2018. Hundreds of novel composite genes and chimeric genes with bacterial origins contributed to haloarchaeal evolution. *Genome Biol.* 19:75
92. Mell JC, Redfield RJ. 2014. Natural competence and the evolution of DNA uptake specificity. *J. Bacteriol.* 196:1471–83
93. Meng J, Xu J, Qin D, He Y, Xiao X, Wang F. 2014. Genetic and functional properties of uncultivated MCG archaea assessed by metagenome and gene expression analyses. *ISME J.* 8:650–59
94. Mevarech M, Werczberger R. 1985. Genetic transfer in *Halobacterium volcanii*. *J. Bacteriol.* 162:461–62
95. Naor A, Altman-Price N, Soucy SM, Green AG, Mitiagin Y, et al. 2016. Impact of a homing intein on recombination frequency and organismal fitness. *PNAS* 113:E4654–61
96. Naor A, Lapierre P, Mevarech M, Papke RT, Gophna U. 2012. Low species barriers in halophilic archaea and the formation of recombinant hybrids. *Curr. Biol.* 22:1444–48
97. Naor A, Yair Y, Gophna U. 2013. A halocin-H4 mutant *Haloferax mediterranei* strain retains the ability to inhibit growth of other halophilic archaea. *Extremophiles* 17:973–79
98. Nelson-Sathi S, Dagan T, Landan G, Janssen A, Steel M, et al. 2012. Acquisition of 1,000 eubacterial genes physiologically transformed a methanogen at the origin of Haloarchaea. *PNAS* 109:20537–42
99. Nelson-Sathi S, Sousa FL, Roettger M, Lozada-Chavez N, Thiergart T, et al. 2015. Origins of major archaeal clades correspond to gene acquisitions from bacteria. *Nature* 517:77–80
100. Novikova O, Jayachandran P, Kelley DS, Morton Z, Merwin S, et al. 2016. Intein clustering suggests functional importance in different domains of life. *Mol. Biol. Evol.* 33:783–99

101. Ofir G, Melamed S, Sberro H, Mukamel Z, Silverman S, et al. 2018. DISARM is a widespread bacterial defence system with broad anti-phage activities. *Nat. Microbiol.* 3:90–98
102. Papke RT, Koenig JE, Rodriguez-Valera F, Doolittle WF. 2004. Frequent recombination in a saltern population of *Halorubrum*. *Science* 306:1928–29
103. Patel GB, Nash JH, Agnew BJ, Sprott GD. 1994. Natural and electroporation-mediated transformation of *Methanococcus voltae* protoplasts. *Appl. Environ. Microbiol.* 60:903–7
104. Pfeifer E, Moura de Sousa JA, Touchon M, Rocha EPC. 2021. Bacteria have numerous distinctive groups of phage-plasmids with conserved phage and variable plasmid gene repertoires. *Nucleic Acids Res.* 49:2655–73
105. Podar M, Anderson I, Makarova KS, Elkins JG, Ivanova N, et al. 2008. A genomic analysis of the archaeal system *Ignicoccus hospitalis-Nanoarchaeum equitans*. *Genome Biol.* 9:R158
106. Pohlschroder M, Esquivel RN. 2015. Archaeal type IV pili and their involvement in biofilm formation. *Front. Microbiol.* 6:190
107. Probst AJ, Ladd B, Jarett JK, Geller-McGrath DE, Sieber CMK, et al. 2018. Differential depth distribution of microbial function and putative symbionts through sediment-hosted aquifers in the deep terrestrial subsurface. *Nat. Microbiol.* 3:328–36
108. Quax TE, Lucas S, Reimann J, Pehau-Arnaudet G, Prevost MC, et al. 2011. Simple and elegant design of a virion egress structure in Archaea. *PNAS* 108:3354–59
109. Rodrigues-Oliveira T, Belmok A, Vasconcellos D, Schuster B, Kyaw CM. 2017. Archaeal S-layers: overview and current state of the art. *Front. Microbiol.* 8:2597
110. Rosenshine I, Tchelet R, Mevarech M. 1989. The mechanism of DNA transfer in the mating system of an archaebacterium. *Science* 245:1387–89
111. Rousset F, Cabezas-Caballero J, Piastra-Facon F, Fernandez-Rodriguez J, Clermont O, et al. 2021. The impact of genetic diversity on gene essentiality within the *Escherichia coli* species. *Nat. Microbiol.* 6:301–12
112. Santangelo TJ, Cubonova L, Reeve JN. 2008. Shuttle vector expression in *Thermococcus kodakaraensis*: contributions of *cis* elements to protein synthesis in a hyperthermophilic archaeon. *Appl. Environ. Microbiol.* 74:3099–104
113. Sato T, Fukui T, Atomi H, Imanaka T. 2003. Targeted gene disruption by homologous recombination in the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. *J. Bacteriol.* 185:210–20
114. Sato T, Takada D, Itoh T, Ohkuma M, Atomi H. 2020. Integration of large heterologous DNA fragments into the genome of *Thermococcus kodakarensis*. *Extremophiles* 24:339–53
115. Schleper C, Holz I, Janekovic D, Murphy J, Zillig W. 1995. A multicopy plasmid of the extremely thermophilic archaeon *Sulfolobus* effects its transfer to recipients by mating. *J. Bacteriol.* 177:4417–26
116. Schleper C, Kubo K, Zillig W. 1992. The particle SSV1 from the extremely thermophilic archaeon *Sulfolobus* is a virus: demonstration of infectivity and of transfection with viral DNA. *PNAS* 89:7645–49
117. Schmidt KJ, Beck KE, Grogan DW. 1999. UV stimulation of chromosomal marker exchange in *Sulfolobus acidocaldarius*: implications for DNA repair, conjugation and homologous recombination at extremely high temperatures. *Genetics* 152:1407–15
118. Schulze S, Pfeiffer F, Garcia BA, Pohlschroder M. 2021. Comprehensive glycoproteomics shines new light on the complexity and extent of glycosylation in archaea. *PLOS Biol.* 19:e3001277
119. Seitz KW, Dombrowski N, Eme L, Spang A, Lombard J, et al. 2019. Asgard archaea capable of anaerobic hydrocarbon cycling. *Nat. Commun.* 10:1822
120. Shalev Y, Turgeman-Grott I, Tamir A, Eichler J, Gophna U. 2017. Cell surface glycosylation is required for efficient mating of *Haloferax volcanii*. *Front. Microbiol.* 8:1253
121. She Q, Shen B, Chen L. 2004. Archaeal integrases and mechanisms of gene capture. *Biochem. Soc. Trans.* 32:222–26
122. Sivabalasarma S, Wetzel H, Nussbaum P, van der Does C, Beeby M, Albers SV. 2020. Analysis of cell-cell bridges in *Haloferax volcanii* using electron cryo-tomography reveal a continuous cytoplasm and S-layer. *Front. Microbiol.* 11:612239
123. Sleytr UB, Schuster B, Egelseer EM, Pum D. 2014. S-layers: principles and applications. *FEMS Microbiol. Rev.* 38:823–64
124. Snyder JC, Bolduc B, Young MJ. 2015. 40 years of archaeal virology: expanding viral diversity. *Virology* 479–480:369–78

125. Soler N, Forterre P. 2020. Vesiduction: the fourth way of HGT. *Environ. Microbiol.* 22:2457–60
126. Soler N, Gaudin M, Marguet E, Forterre P. 2011. Plasmids, viruses and virus-like membrane vesicles from Thermococcales. *Biochem. Soc. Transact.* 39:36–44
127. Soler N, Marguet E, Verbavatz JM, Forterre P. 2008. Virus-like vesicles and extracellular DNA produced by hyperthermophilic archaea of the order Thermococcales. *Res. Microbiol.* 159:390–99
128. Song Y, Zhu Z, Zhou W, Zhang YPJ. 2021. High-efficiency transformation of archaea by direct PCR products with its application to directed evolution of a thermostable enzyme. *Microb. Biotechnol.* 14:453–64
129. Soppa J. 2001. Prokaryotic structural maintenance of chromosomes (SMC) proteins: distribution, phylogeny, and comparison with MukBs and additional prokaryotic and eukaryotic coiled-coil proteins. *Gene* 278:253–64
130. Sorokin DY, Makarova KS, Abbas B, Ferrer M, Golyshin PN, et al. 2017. Discovery of extremely halophilic, methyl-reducing euryarchaea provides insights into the evolutionary origin of methanogenesis. *Nat. Microbiol.* 2:17081
131. Soucy SM, Fullmer MS, Papke RT, Gogarten JP. 2014. Inteins as indicators of gene flow in the halobacteria. *Front. Microbiol.* 5:299
132. Spang A, Saw JH, Jorgensen SL, Zaremba-Niedzwiedzka K, Martijn J, et al. 2015. Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature* 521:173–79
133. Stachler AE, Turgeman-Grott I, Shtifman-Segal E, Allers T, Marchfelder A, Gophna U. 2017. High tolerance to self-targeting of the genome by the endogenous CRISPR-Cas system in an archaeon. *Nucleic Acids Res.* 45:5208–16
134. Stone E, Campbell K, Grant I, McAuliffe O. 2019. Understanding and exploiting phage-host interactions. *Viruses* 11:567
135. Suzuki S, Kurosawa N. 2019. Participation of UV-regulated genes in the response to helix-distorting DNA damage in the thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius*. *Microbes Environ.* 34:363–73
136. Svirskaite J, Oksanen HM, Daugelavicius R, Bamford DH. 2016. Monitoring physiological changes in haloarchaeal cell during virus release. *Viruses* 8:59
137. Swithers KS, Soucy SM, Lasek-Nesselquist E, Lapierre P, Gogarten JP. 2013. Distribution and evolution of the mobile *vma-1*b intein. *Mol. Biol. Evol.* 30:2676–87
138. Tatum EL, Lederberg J. 1947. Gene recombination in the bacterium *Escherichia coli*. *J. Bacteriol.* 53:673–84
139. Tchelet R, Mevarech M. 1993. Interspecies genetic transfer in halophilic archaebacteria. *Syst. Appl. Microbiol.* 16:578–81
140. Tocchini-Valentini GD, Fruscoloni P, Tocchini-Valentini GP. 2011. Evolution of introns in the archaeal world. *PNAS* 108:4782–87
141. Touchon M, Perrin A, Moura de Sousa JA, Vangchhia B, Burn S, et al. 2020. Phylogenetic background and habitat drive the genetic diversification of *Escherichia coli*. *PLOS Genet.* 16:e1008866
142. Tripepi M, Imam S, Pohlschroder M. 2010. *Haloferax volcanii* flagella are required for motility but are not involved in PibD-dependent surface adhesion. *J. Bacteriol.* 192:3093–102
143. Turgeman-Grott I, Joseph S, Marton S, Eizenshtein K, Naor A, et al. 2019. Pervasive acquisition of CRISPR memory driven by inter-species mating of archaea can limit gene transfer and influence speciation. *Nat. Microbiol.* 4:177–86
144. van Wolferen M, Ajon M, Driessen AJ, Albers SV. 2013. Molecular analysis of the UV-inducible pili operon from *Sulfolobus acidocaldarius*. *MicrobiologyOpen* 2:928–37
145. van Wolferen M, Shajahan A, Heinrich K, Brenzinger S, Black IM, et al. 2020. Species-specific recognition of *Sulfolobales* mediated by UV-inducible pili and S-layer glycosylation patterns. *mBio* 11:e03014-19
146. van Wolferen M, Wagner A, van der Does C, Albers SV. 2016. The archaeal Ced system imports DNA. *PNAS* 113:2496–501
147. Vanwonterghem I, Evans PN, Parks DH, Jensen PD, Woodcroft BJ, et al. 2016. Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. *Nat. Microbiol.* 1:16170
148. Vulic M, Dionisio F, Taddei F, Radman M. 1997. Molecular keys to speciation: DNA polymorphism and the control of genetic exchange in enterobacteria. *PNAS* 94:9763–67

149. Wagner A, Whitaker RJ, Krause DJ, Heilers JH, van Wolferen M, et al. 2017. Mechanisms of gene flow in archaea. *Nat. Rev. Microbiol.* 15:492–501
150. Wang Y, Duan Z, Zhu H, Guo X, Wang Z, et al. 2007. A novel *Sulfolobus* non-conjugative extrachromosomal genetic element capable of integration into the host genome and spreading in the presence of a fusellovirus. *Virology* 363:124–33
151. Wang Y, Wegener G, Hou J, Wang F, Xiao X. 2019. Expanding anaerobic alkane metabolism in the domain of Archaea. *Nat. Microbiol.* 4:595–602
152. Waters VL. 2001. Conjugation between bacterial and mammalian cells. *Nat. Genet.* 29:375–76
153. Woese CR, Kandler O, Wheelis ML. 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *PNAS* 87:4576–79
154. Wolfe JM, Fournier GP. 2018. Horizontal gene transfer constrains the timing of methanogen evolution. *Nat. Ecol. Evol.* 2:897–903
155. Wood ER, Ghane F, Grogan DW. 1997. Genetic responses of the thermophilic archaeon *Sulfolobus acidocaldarius* to short-wavelength UV light. *J. Bacteriol.* 179:5693–98
156. Worrell VE, Nagle DP Jr., McCarthy D, Eisenbraun A. 1988. Genetic transformation system in the archaebacterium *Methanobacterium thermoautotrophicum* Marburg. *J. Bacteriol.* 170:653–56
157. Xiong L, Liu S, Chen S, Xiao Y, Zhu B, et al. 2019. A new type of DNA phosphorothioation-based antiviral system in archaea. *Nat. Commun.* 10:1688
158. Xiong X, Wu G, Wei Y, Liu L, Zhang Y, et al. 2020. SspABCD-SspE is a phosphorothioation-sensing bacterial defence system with broad anti-phage activities. *Nat. Microbiol.* 5(7):917–28
159. Yokoyama H, Kamei N, Konishi K, Hara K, Ishikawa Y, et al. 2021. Preparation, crystallization, and X-ray data collection of archaeal oligopeptide permease A. *Crystallogr. Rep.* 66:1300–5
160. Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Backstrom D, Juzokaite L, et al. 2017. Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature* 541:353–58
161. Zawadzki P, Roberts MS, Cohan FM. 1995. The log-linear relationship between sexual isolation and sequence divergence in *Bacillus* transformation is robust. *Genetics* 140:917–32
162. Zolghadr B, Klingl A, Koerdt A, Driessen AJ, Rachel R, Albers SV. 2010. Appendage-mediated surface adherence of *Sulfolobus solfataricus*. *J. Bacteriol.* 192:104–10