



**HAL**  
open science

## Extracellular membrane vesicles and nanotubes in Archaea

Junfeng Liu, Nicolas Soler, Aurore Gorlas, Virginija Cvirkaite-Krupovic, Mart  
Krupovic, Patrick Forterre

► **To cite this version:**

Junfeng Liu, Nicolas Soler, Aurore Gorlas, Virginija Cvirkaite-Krupovic, Mart Krupovic, et al..  
Extracellular membrane vesicles and nanotubes in Archaea. *microLife*, 2021, 2, pp.uqab007.  
10.1093/femsml/uqab007 . pasteur-03264764v2

**HAL Id: pasteur-03264764**

**<https://pasteur.hal.science/pasteur-03264764v2>**

Submitted on 16 Jul 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

## SHORT REVIEW

# Extracellular membrane vesicles and nanotubes in Archaea

Junfeng Liu<sup>1</sup>, Nicolas Soler<sup>2</sup>, Aurore Gorlas<sup>3</sup>, Virginija Cvirkaite-Krupovic<sup>1,†</sup>, Mart Krupovic<sup>1,‡</sup> and Patrick Forterre<sup>3,4,\*,#</sup>

<sup>1</sup>Archaeal Virology Unit, Institut Pasteur, 75015 Paris, France, <sup>2</sup>Université de Lorraine, INRAE, DynAMic, F-54000 Nancy, France, <sup>3</sup>Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198 Gif-sur-Yvette, France and <sup>4</sup>Département de Microbiologie, Institut Pasteur, 75015 Paris, France

\*Corresponding author: Institut Pasteur, 25 rue du Docteur Roux, 75005 Paris, France. Tel: 33 6 74 08 68 46; E-mail: [patrick.forterre@pasteur.fr](mailto:patrick.forterre@pasteur.fr)

**One sentence summary:** Extracellular vesicles and nanotubes could play a profound role in archaeal physiology, evolution and environmental adaptation by promoting intercellular transfer of viral and cellular nucleic acids and proteins.

<sup>†</sup>Virginija Cvirkaite-Krupovic, <http://orcid.org/0000-0002-0876-9397>

<sup>‡</sup>Mart Krupovic, <http://orcid.org/0000-0001-5486-0098>

<sup>#</sup>Patrick Forterre, <http://orcid.org/0000-0002-1843-7214>

## ABSTRACT

Membrane-bound extracellular vesicles (EVs) are secreted by cells from all three domains of life and their implication in various biological processes is increasingly recognized. In this review, we summarize the current knowledge on archaeal EVs and nanotubes, and emphasize their biological significance. In archaea, the EVs and nanotubes have been largely studied in representative species from the phyla Crenarchaeota and Euryarchaeota. The archaeal EVs have been linked to several physiological processes such as detoxification, biomineralization and transport of biological molecules, including chromosomal, viral or plasmid DNA, thereby taking part in genome evolution and adaptation through horizontal gene transfer. The biological significance of archaeal nanotubes is yet to be demonstrated, although they could participate in EV biogenesis or exchange of cellular contents. We also discuss the biological mechanisms leading to EV/nanotube biogenesis in Archaea. It has been recently demonstrated that, similar to eukaryotes, EV budding in crenarchaea depends on the ESCRT machinery, whereas the mechanism of EV budding in euryarchaeal lineages, which lack the ESCRT-III homologues, remains unknown.

**Keywords:** extracellular vesicles; nanotubes; archaea; vesiduction; extremophiles; DNA transfer

## INTRODUCTION

Archaea have been recognized as a separate domain of life, besides Bacteria and Eukarya, only in 1977 by Carl Woese and colleagues who compared the ribosomal RNA (rRNA) gene sequences from diverse organisms (Woese and Fox 1977). The name Archaea, instead of archaeobacteria, was proposed later on by Carl Woese to emphasize the fact that Archaea and Bacteria form two distinct lineages in the universal tree of life (Woese, Kandler and Wheelis 1990). Although archaeal

cells are of the prokaryotic type, their informational systems (DNA replication, transcription, translation), as well as several membrane-associated machineries, such as the ATP synthase complex, the Sec secretion system and the signal recognition particles, are much more similar to those of eukaryotes. Similar to bacteria and eukaryotes, archaeal cells commonly secrete extracellular vesicles (EVs) and some produce tubular structures resembling bacterial nanopods and/or nanotubes (Gill, Catchpole and Forterre 2019).

Received: 13 April 2021; Accepted: 8 June 2021

© The Author(s) 2021. Published by Oxford University Press on behalf of FEMS. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

The production of various types of EVs (apoptotic bodies, exosomes, microvesicles, etc.) and nanotube-like structures (tunnelling nanotubes, etc.) has been extensively studied in Eukarya (for reviews, see Yanez-Mo et al. 2015; Gill, Catchpole and Forterre 2019; Cordero Cervantes and Zurzolo 2021). In Bacteria, EVs were observed for the first time by electron microscopy in *E. coli* in 1966 (Knox, Vesik and Work 1966), but their biological importance was first dismissed and, hence, microbial EVs have been considered as artefacts of cell growth or lysis for many years (Coelho and Casadevall 2019). However, bacterial EVs are increasingly recognized to play important roles in many processes from pathogenesis, bacterial communication and biofilm formation, to horizontal gene transfer and protection against viral infections (Brown et al. 2015; Jan 2017; Gill, Catchpole and Forterre 2019). Nanotubes have been described in Bacteria more recently (Baidya et al. 2018; Gill, Catchpole and Forterre 2019), but their physiological role remains controversial (Baidya, Rosenshine and Ben-Yehuda 2020; Pospíšil et al. 2020). In Archaea, EVs have been first described over two decades ago when they were found to carry protein toxins (Prangishvili et al. 2000). Although until recently, archaeal EVs received relatively little attention, perhaps due to the fact that their discovery was from the very beginning linked to a defined function (i.e. toxin transfer), archaeal EVs were not dismissed by the archaeal community as cellular ‘junk,’ as in some other branches of microbiology (Coelho and Casadevall 2019). The research on archaeal EVs has primarily focused on two orders of hyperthermophilic species, Sulfolobales and Thermococcales, whereas nanotubes have been primarily described in Thermococcales and Haloarchaea.

A major difference between Archaea and Bacteria that probably influences the respective mechanisms of EV and nanotube production is the structure of their cell envelopes. Similar to most eukaryotic cells, most Archaea are monoderms, i.e. their envelope consists of a single membrane (Ellen et al. 2010; Albers and Meyer 2011). Important exceptions are *Ignicoccus hospitalis* and members of the order Methanomassilicoccales that are diderm archaea with an outer membrane and a periplasmic space (Dridi et al. 2012; Klingl 2014; Heimerl et al. 2017). Most archaea lack rigid cell wall and this may facilitate the production of EVs and nanotubes. The exceptions are methanogenic archaea of the orders Methanobacteriales and Methanopyrales in which the membrane is surrounded by a peptidoglycan-like polymer, referred to as pseudomurein layer (Steenbakkers et al. 2006; Albers and Meyer 2011; Klingl 2014), and halophilic archaea of the genus *Halococcus* that are surrounded by a complex and rigid heteroglycan cell wall (Steber and Schleifer 1975). The cytoplasmic membrane is surrounded by a paracrystalline protein surface (S-) layer usually composed of a single main glycoprotein (40–200 kDa) that is capable of self-assembly into highly ordered structures (Rodrigues-Oliveira et al. 2017). As in eukaryotes, the outer surfaces of archaea are also often covered with abundant glycoproteins and the protein N-glycosylation pathways exhibit important similarities between Archaea and Eukarya, suggesting a common origin (Nikolayev, Cohen-Rosenzweig and Eichler 2020).

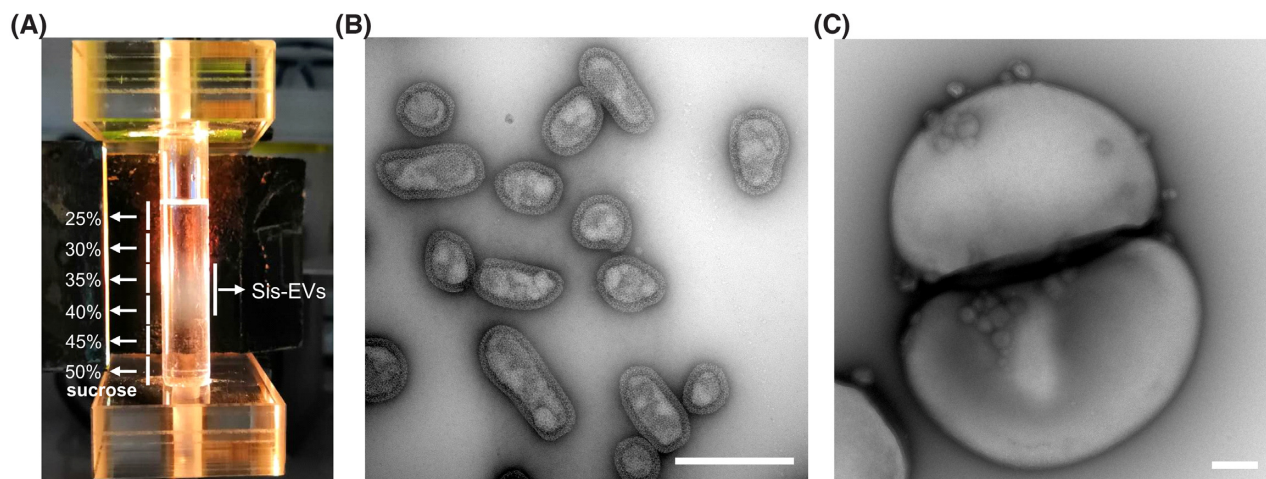
For a long time, Archaea have been divided into two major phyla based on rRNA sequence comparisons: Crenarchaeota and Euryarchaeota (Woese, Kandler and Wheelis 1990). Crenarchaeota includes thermophilic or hyperthermophilic species, whereas members of Euryarchaeota are phenotypically very diverse, including (hyper)thermophiles, mesophiles, methanogens and halophiles. Most cultivated species belong to these two phyla and all experimental studies on archaeal

EVs have been performed either on Crenarchaeota (order Sulfolobales) or Euryarchaeota (order Thermococcales and class Halobacteria). In 2008, a third major archaeal phylum was proposed, the Thaumarchaeota (Brochier-Armanet et al. 2008). Many thaumarchaeal species, either mesophilic or thermophilic, have now been cultivated, but they all turned out to be very fastidious and the production of EVs has not yet been studied in this phylum.

Although many eukaryotic features are common to all archaea, others are specific to only some lineages, phyla or superphyla. This is the case for the endosomal sorting complexes required for transport (ESCRT) machinery proteins that are responsible not only for cell division but also for EV biogenesis in eukaryotes (Vietri, Radulovic and Stenmark 2020). Both eukaryotic-like proteins of the ESCRT machinery, ESCRT-III and Vps4 ATPase, are conserved in Crenarchaeota (except for Thermoproteales). Members of the Thaumarchaeota also encode the ESCRT proteins that are very similar to those of Crenarchaeota (Caspi and Dekker 2018; Lu et al. 2020). However, despite producing EVs, members of the order Euryarchaeota, such as Thermococcales and Halobacteriales, do not encode the ESCRT machinery but only Vps4 homologues. Instead, the euryarchaeal lineage relies on the bacterial-like FtsZ-based system for cell division. This suggests that EV biogenesis in different archaeal lineages occurs by very different mechanisms. Thus, mechanistic comparisons of EV budding in archaea and eukaryotes might provide insights into the evolution and diversification of this important process in different cellular lineages.

In recent years, a wealth of new archaeal phyla have been described from the reconstructions of metagenome-assembled genomes, vastly expanding the known diversity of Archaea and the range of their ecological distribution (Adam et al. 2017; Spang, Caceres and Ettema 2017). Several new phyla corresponding to small archaea have been grouped in the DPANN superphylum (referring to the first described constituent lineages, Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota and Nanohaloarchaeota) (Rinke et al. 2013). ESCRT machinery proteins and many other eukaryotic-like proteins missing in Euryarchaeota are also absent in DPANN archaea. These miniature archaea have reduced genomes and most of them lack essential metabolic pathways, such as lipid biosynthesis, suggestive of parasitic lifestyles. Consequently, most DPANN members are probably ectosymbionts of larger archaeal hosts (Dombrowski et al. 2019). The best characterized of these symbiotic interactions is the association of *Nanoarchaeum equitans* (a member of the Nanoarchaeota) with its host, the diderm crenarchaeon *Ignicoccus hospitalis*. Remarkably, membrane vesicles that might be implicated in the interactions between *Nanoarchaeum* and *Ignicoccus* have been observed budding from the internal cytoplasmic membrane of *I. hospitalis* (see later) (Junglas et al. 2008).

Another major superphylum, the Asgardarchaeota, has attracted much attention because some phylogenetic analyses of universal protein concatenation have suggested that eukaryotes emerged from within this phylum (Spang et al. 2015; Zaremba-Niedzwiedzka et al. 2017; Williams et al. 2020). This hypothesis is still debated because Archaea are monophyletic, with Asgard archaea branching between euryarchaea and crenarchaea in other universal protein phylogenies (Da Cunha et al. 2017, 2018; Jay et al. 2018). Interestingly, the genomes of Asgardarchaeota encode more eukaryotic-like proteins than most other archaeal phyla (Zaremba-Niedzwiedzka et al. 2017), including actin, tubulin and additional components of the ESCRT system (Caspi and Dekker 2018; Lu et al. 2020). Moreover, these



**Figure 1.** Extracellular vesicles produced by *Sulfolobus islandicus*. **(A)** EV purification by ultracentrifugation in the 25–50% sucrose gradient. EVs form an opalescent band in the region corresponding to 30–40% sucrose. The figure is modified from Liu et al. (2021b). **(B)** Transmission electron micrograph of negatively stained EVs. **(C)** EVs budding from a dividing *Sulfolobus* cell. Scale bars: 400 nm.

proteins are also more closely related to their eukaryotic homologues than those from other Archaea. Asgard archaea should therefore serve as valuable models to study the role of these proteins in the biogenesis of archaeal EVs and/or nanotubes. However, only one Asgard archaeon, *Candidatus Prometheoarchaeum syntrophicum*, has been successfully cultivated yet, and only in symbiosis with other microorganisms (Imachi et al. 2020). Interestingly, electron microscopy analyses have suggested that this organism produces both EVs and nanotubes (see later).

## PRODUCTION OF EVS BY ARCHAEA OF THE PHYLUM CRENARCHAEOTA

Nearly all studies dealing with the production of EVs in crenarchaea have been performed in organisms of the order Sulfolobales. These archaea are thermoacidophiles thriving in acidic (pH 2–3) terrestrial hot springs that have been studied as model organisms by biochemists and molecular biologists from the very beginning of archaeal research, mainly because they are aerobes and are easy to cultivate (Schocke, Brasen and Siebers 2019). Moreover, many genetic tools are now available for several *Sulfolobus* species (Peng et al. 2017). The earliest reports about archaeal EVs came from studies carried out on *Sulfolobus islandicus* (Prangishvili et al. 2000). These EVs, 90–230 nm in diameter and coated with the S-layer, were shown to be associated with an antimicrobial protein, termed ‘sulfolobacin,’ that inhibits the growth of related *Sulfolobus* species. Subsequently, EV-associated toxins were identified in some other *Sulfolobus* species and were shown to be encoded by a two-gene operon (Ellen et al. 2011). Disruption of these genes has shown that the two sulfolobacin proteins, dubbed Sula and SulB, are required for the antimicrobial activity (Ellen et al. 2011). Notably, their association with EVs was not necessary for the antimicrobial activity since purified sulfolobacins were still active once extracted from EVs by alkaline carbonate treatment.

Characterization of EVs produced by *Sulfolobus acidocaldarius*, *Saccharolobus solfataricus* and *Sulfurisphaera tokodaii* showed that lipid and protein profiles of the parental cells were different from those of the corresponding EVs (Ellen et al. 2009). Interestingly, the protein content analyses have shown that EVs from all three species carry some components of the ESCRT machinery, namely, ESCRT-III-1 and ESCRT-III-2 and the Vps4 ATPase (also

called CdvC) (Ellen et al. 2009). All three proteins play a key role in the *Sulfolobus* cell division (Lindås et al. 2008; Samson et al. 2008; Liu et al. 2017; Tarrason Risa et al. 2020). Given that in eukaryotes the ESCRT machinery is responsible not only for the cytokinesis but also among other functions, for EV budding (Vietri, Radulovic and Stenmark 2020), it has been hypothesized that production of *Sulfolobus* EVs also depends on the ESCRT machinery.

Direct evidence that budding of *Sulfolobus* EVs depends on the ESCRT machinery has been recently provided by an in-depth characterization of the EVs from *S. islandicus* (Liu et al. 2021b). Proteomic analysis has shown that highly purified *S. islandicus* EVs (Sis-EVs; Fig. 1A and B) carry 413 proteins, including all six components of the *Sulfolobus* ESCRT machinery, with ESCRT-III-2 and ESCRT-III-1 being in the top-10 of the most abundant EV proteins. Western blot analysis confirmed that both proteins were present and strongly enriched in the Sis-EVs. Using a CRISPR-based knockdown system, it was demonstrated that the four archaeal ESCRT-III homologues and the AAA+ ATPase Vps4 were all required for EV production, whereas the archaea-specific component CdvA appeared to be dispensable (Liu et al. 2021b). Importantly, using synchronized *S. islandicus* cultures, it was shown that EV production is linked to cell division (Fig. 1C) and coincides with the natural, cell cycle-linked changes in the expression of ESCRT-III homologues, in particular, ESCRT-III-1 and ESCRT-III-2. Consistently, overexpression of ESCRT-III-1 and ESCRT-III-2 from a plasmid resulted in 200–250% increase in vesiculation, while overexpression of other ESCRT machinery components had little effect on EV production (Liu et al. 2021b). Based on these findings, it has been suggested that ESCRT-mediated EV biogenesis has deep evolutionary roots and pre-dates the divergence of eukaryotes and archaea. Interestingly, it has been recently shown that in virus-infected *S. islandicus* cells, ESCRT machinery mediates asymmetric cell division, whereby normal-sized cells are budding from the giant virus-infected cells (Liu et al. 2021a), topologically resembling the EV budding.

A hypervesiculation phenotype was obtained in cells overexpressing the CdvA protein (Liu et al. 2021b). The latter protein does not appear to participate in normal EV budding, because CRISPR-mediated depletion of the *cdvA* transcripts had little effect on the EV production. However, when the CdvA was overexpressed from a plasmid, the EV production was boosted by several folds. It was suggested that the hypervesiculation is the

result of excessive binding of CdvA to the membrane (Liu *et al.* 2021b). Regardless of the exact mechanism, it appears that EVs can be produced by different mechanisms even in the same organism. The normal EV production is evidently linked to cell division in *Sulfolobus* but it is possible that under different physiological conditions and upon exposure to different stressors EVs could be produced through different pathways.

Besides ESCRT machinery components, Sis-EVs carry a diverse subset of the *S. islandicus* proteome, including diverse proteases and nucleases (Liu *et al.* 2021b). However, highly purified Sis-EVs showed no toxicity against other *Sulfolobus*, *Saccharolobus* and *Sulfurisphaera* species tested, suggesting that EV-mediated transfer of sulfolobocins might not be a general phenomenon. Notably, proteins carried by Sis-EVs were not randomly included from the *S. islandicus* proteome. Indeed, comparison of the Sis-EV and *S. islandicus* proteomes showed that Sis-EV protein fraction is strongly enriched in membrane proteins as well as proteins from particular functional categories, including the cell division (as discussed earlier), cell motility, posttranslational modification, protein turnover and secretion as well as energy production and conversion, and inorganic ion transport and metabolism (Liu *et al.* 2021b).

Sis-EVs cargo includes not only diverse proteins but also chromosomal and plasmid DNA. Importantly, Sis-EVs protect the cargo DNA from nucleases as well as the harsh physicochemical conditions of the extracellular milieu and can transfer it to recipient cells (Liu *et al.* 2021b). The possibility to transfer DNA via EVs was previously observed with archaea of the order Thermococcales (see later) and also in bacteria (Domingues and Nielsen 2017). The term 'vesiduction' has been proposed for DNA transfer mediated by EVs as a fourth way of DNA transfer, besides transformation, transfection and conjugation (Soler and Forterre 2020).

Moreover, Sis-EVs can also support the heterotrophic growth of *S. islandicus* in minimal medium, implicating EVs in carbon and nitrogen fluxes in extreme environments. Thus, it is becoming clear that EVs play an important role in horizontal gene transfer and nutrient cycling in extreme environments. Indeed, S-layer-covered EVs have been detected directly in an environmental sample collected from a terrestrial hot spring (Baquero *et al.* 2020; Liu *et al.* 2021b), providing evidence that EVs are not a laboratory artefact.

It has been suggested that *Sulfolobus* EVs also promote biomineralization (Kish *et al.* 2016). Whilst S-layers have long been implicated in mineral formation, the underlying mechanisms remained unresolved. A study using *Sulfolobus acidocaldarius*, a hyperthermophilic archaeon isolated from metal-enriched environments, demonstrated a passive process of iron phosphate nucleation and growth within the S-layer of cells and cell-free S-layer 'ghosts' during incubation in a Fe-rich medium. In addition, EVs of ~175 nm in diameter were formed and released in response to S-layer encrustation by minerals. These EVs were fully encrusted by minerals, even when cells were only partially encrusted (Kish *et al.* 2016). The authors proposed that these EVs are produced in an attempt to remove sections of damaged S-layer.

Besides *Sulfolobales*, the production of EV vesicles in Crenarchaeota was reported in *I. hospitalis*, a member of the order Desulfurococcales. *Ignicoccus hospitalis* is a diderm archaeon with an inner cytoplasmic membrane and an outer membrane separated by a fairly large periplasmic space (20–1000 nm in width). Vesicles are produced by budding from the inner membrane and numerous vesicles can accumulate in the periplasm and

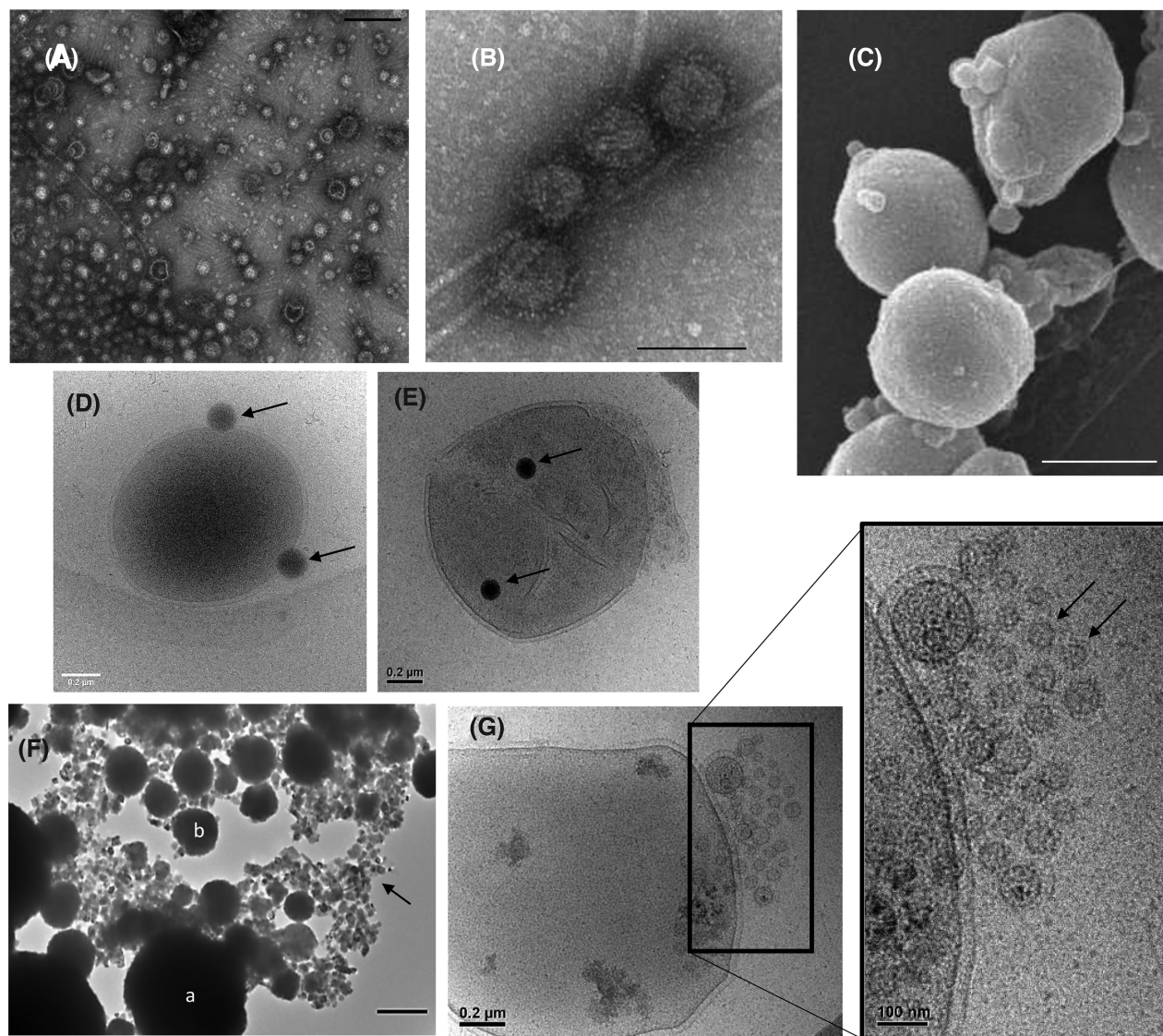
fuse with the outer membrane (Näther and Rachel 2004). *Ignicoccus hospitalis* often hosts cells of the tiny archaeon *N. equitans* attached to its surface (Huber *et al.* 2002; Küper *et al.* 2010). *Nanoarchaeum equitans* has the smallest known genome for an archaeon (0.49 Mb) and cannot synthesize many essential components, including lipids (Waters *et al.* 2003). It is assumed that these components could be delivered from the cytoplasm of *I. hospitalis* to *N. equitans* via vesicles that reach the outer membrane at the position of the symbiont attachment (Junglas *et al.* 2008). Unfortunately, there are presently no genetic tools available to address the mechanism of vesicle production in this fascinating system.

## PRODUCTION OF EVS BY ARCHAEA OF THE PHYLUM EURYARCHAEOTA

Most studies dealing with the production of EVs in Euryarchaeota have been performed with archaea of the genus *Thermococcus* (order Thermococcales). Members of the Thermococcales are strictly anaerobic, hyperthermophilic sulfur reducers, which are placed at the base of the Euryarchaeota in most archaeal phylogenies (Adam *et al.* 2017; Da Cunha *et al.* 2017). They are rather easy to cultivate under laboratory conditions, requiring typical equipment for the cultivation of anaerobes, and are typically abundantly present in environmental samples from hydrothermal vents, both terrestrial and marine. Massive production of EVs was first observed in the course of screening for viruses a collection of Thermococcales isolated from hydrothermal deep-sea vents (Soler *et al.* 2008). Subsequently, EVs were also observed in several reference strains widely used as model organisms for the study of hyperthermophiles, such as *Thermococcus kodakarensis* (Gaudin *et al.* 2013; Marguet *et al.* 2013). These EVs (50–150 nm) are covered with the S-layer and are apparently produced by budding (Fig. 2A–C).

Since the genomes of Thermococcales and of other Euryarchaeota do not encode for ESCRT-III homologues (Makarova *et al.* 2010), the mechanism of EV production in this phylum is likely to be different from that postulated for Sulfolobales (Liu *et al.* 2021b). Indeed, the genomes of Thermococcales encode three putative homologues of the Vps4 ATPase, but disruption of any of their genes in *Thermococcus kodakarensis* did not affect EV production (Gill, Catchpole and Forterre, unpublished result). In contrast with the result obtained with *Sulfolobales*, the biochemical characterization of purified EVs from three species of Thermococcales (*T. kodakarensis*, *T. gammatolerans* and *Thermococcus* sp. 5-4) revealed that protein and lipid profiles of EVs and cell membranes from the same species have a similar composition (Gaudin *et al.* 2013). However, the major protein present in both cell membranes and EVs of *Thermococcus* species, the oligopeptide binding protein OppA, was also found in *Sulfolobus* EVs (Ellen *et al.* 2009; Liu *et al.* 2021b).

In addition to the typical EVs, some members of the Thermococcales, such as *T. prieurii* or *T. kodakarensis*, produce numerous intracellular dark vesicles that bud from the host cells (Gorlas *et al.* 2015) (Fig. 2E and F). Energy-dispersive X-ray spectroscopy analyses revealed that these dark vesicles are filled with sulfur, and hence they have been termed 'sulfur vesicles' (SVs). The presence of SVs was exclusively observed when elemental sulfur S(0) is added into the growth medium, suggesting that these SVs could be produced to prevent the toxic intracellular accumulation of S(0) and/or polysulfides, thus playing a key role in sulfur detoxification. Surprisingly, the SVs are not produced



**Figure 2.** Extracellular vesicles produced by Thermococcales. Transmission electron microscopy images of purified EVs produced by (A) *T. nautili* showing the size diversity (scale bar, 200 nm) and (B) by *T. gammatolerans* (scale bar, 100 nm). (C) Scanning electron micrograph of *T. kodakarensis* cells producing EVs (scale bar, 500 nm). Cryo-electron micrographs of (D) *Thermococcus* sp. 15-2 and (E) *T. prierii* cells associated with dark vesicles named sulfur vesicles (SVs). The arrows indicate SVs. (F) Transmission electron microscopy image of *T. kodakarensis* cells (a) and EVs (b) entirely mineralized within  $\text{FeS}_2$  pyrite. The EVs are dispersed among  $\text{Fe}_3\text{S}_4$  greigite nanocrystals (indicated by arrow). Scale bar, 500 nm. (G) Cryo-electron micrographs of a *T. prierii* cell producing very small EVs (indicated by an arrow) grouped into a spherical structure at the cell surface.

by all species of Thermococcales, suggesting significant differences in the sulfur metabolic pathways (Gorlas et al. 2015). More recently, it has been observed that Thermococcales SVs and EVs are actively involved in the production of iron-sulfide biominerals (Gorlas et al. 2018), suggesting a defensive function of EVs that might allow Thermococcales to survive in a broad range of extreme environments characterized by the high iron and sulfide contents (Gorlas et al. 2018) (Fig. 2F and G).

The EVs produced by members of the Thermococcales were shown to be often associated with either chromosomal or plasmid/viral DNA (Soler et al. 2008, 2011; Gaudin et al. 2013, 2014; Choi et al. 2015). As recently observed with *Sulfolobus* EVs, DNA enclosed within EVs produced by Thermococcales is more resistant to thermodenaturation than free DNA, suggesting a protective role of the EVs (Soler et al. 2008), which is likely to be vital for

horizontal gene transfer in extreme geothermal environments. The EVs of Thermococcales can indeed transfer DNA between cells. It was demonstrated that EVs of *T. kodakarensis* can be used to transfer plasmid DNA into plasmid-free cells (Gaudin et al. 2013).

*Thermococcus onnurineus* cells produce heterogeneous populations of EVs, which differ in terms of size and DNA content (Choi et al. 2015). EVs always encapsidate ~14-kb-long DNA fragments. However, sequencing of the packaged DNA revealed that all regions of the *T. onnurineus* genome are represented in EVs, except for a 9.4-kb region. The authors speculated that this region might participate in DNA packaging and/or EV production (Choi et al. 2015). Interestingly, a *T. onnurineus* mutant in which the 9.4-kb region has been deleted still produces EVs but without associated DNA, supporting the original hypothesis (Kim, pers.

comm.). This 9.4-kb region encodes various enzymes involved in sulfur metabolism and/or hydrogen production, with the possible roles of these enzymes in DNA packaging being unclear.

Remarkably, EVs produced by *T. nautili*, which contains three plasmids, selectively incorporate only two of these plasmids, pTN1 and pTN3, but not pTN2 (Soler et al. 2011; Gaudin et al. 2014). The reason for this specificity is unknown and purified EVs did not contain proteins encoded by pTN1 or pTN3. Notably, pTN3 is a defective virus belonging to the viral realm *Varidnaviria* (formerly the PRD1-Adenovirus lineage). This observation reinforces the idea that EVs of Thermococcales can serve as vehicles for the intercellular transport of extrachromosomal DNA (Soler et al. 2011; Gaudin et al. 2014). EVs containing viral DNA have also been detected in Bacteria and Eukarya (Gill, Catchpole and Forterre 2019) and the term ‘viral vesicle’ has been proposed for these biological entities. Interestingly, *in silico* analysis of the DNA associated with bacterial EVs isolated in diverse marine environments has revealed the presence of many viral genes, suggesting that such ‘viral vesicles’ are abundant in nature, alongside true virions and EVs containing cellular DNA (Soler et al. 2015).

Some EVs have been recently detected in cultures of *Methanocaldococcus fervens*, a methanogenic hyperthermophile belonging to the order Methanococcales (Thiroux et al. 2021). The natural isolate of *M. fervens* is a lysogen producing a head-tailed virus MFTV1 (Krupovic, Forterre and Bamford 2010; Thiroux et al. 2021). *Methanocaldococcus fervens* cultures exposed to copper displayed greater production of EVs, but lower virus production, suggesting an interplay between EV production and virus life cycle (Thiroux et al. 2021). Notably, *Methanocaldococcus* and *Thermococcus* species inhabit the same deep-sea hydrothermal vent ecosystems and were shown to share several groups of non-conjugative plasmids, some of which could have been exchanged horizontally through EVs (Krupovic et al. 2013). EVs and structures resembling nanotubes were observed in cultures of *Aciduliprofundum boonei* (Reysenbach et al. 2006; Reysenbach and Flores 2008), a thermoacidophilic euryarchaeon distantly related to Thermococcales and Methanococcales.

Haloarchaea appear to represent a very promising model for the study of EVs in Archaea. Haloarchaea are halophilic and aerobic microorganisms that thrive in up to 5.5 M NaCl, i.e. salt concentrations approaching saturation, and are responsible for the pink colour of many hypersaline seas and lakes around the globe due to the specific carotenoid pigments that they produce. A seminal study describing EVs produced by *Halorubrum* has uncovered entities blurring the canonical frontiers between plasmids and viruses (Erdmann et al. 2017). Indeed, in contrast to plasmid vesicles produced by *T. nautili*, the membranes of EVs from *Halorubrum* carrying the plasmid pR1SE contain mostly proteins encoded by this plasmid, resembling the packaging of viral genomes by capsid proteins. Many of these plasmid-encoded proteins were found in EVs by mass spectrometry analysis. These peculiar ‘plasmid vesicles’ were proposed to be the prototype of a new type of biological entities called ‘plasmidions,’ standing for membrane vesicles mimicking virions (Forterre, Da Cunha and Catchpole 2017). Similar to EVs of *Sulfolobus* (Liu et al. 2021b) and *Thermococcus* (Soler et al. 2008; Gaudin et al. 2014), *Halorubrum* EVs can mediate the transfer of plasmid DNA (pR1SE and derivatives). Notably, the plasmids carried by *Halorubrum* EVs can integrate into haloarchaeal replicons (note that Haloarchaea harbour several circular replicons), and subsequent excision from these replicons generates plasmid derivatives with different segments of the host chromosome, possibly

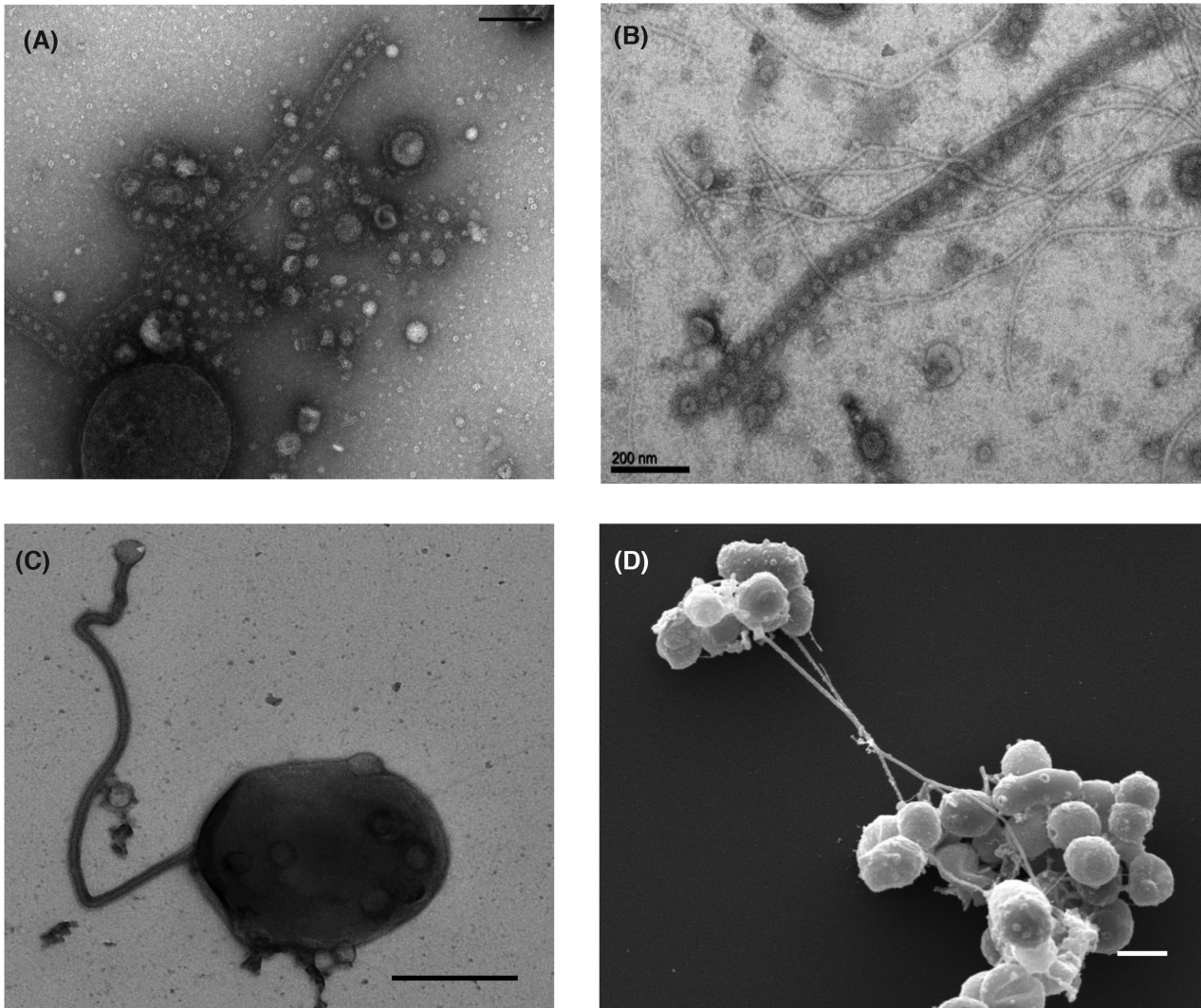
leading to the horizontal transfer of the host genes by vesiduction (Erdmann et al. 2017).

## NANOTUBES IN ARCHAEA

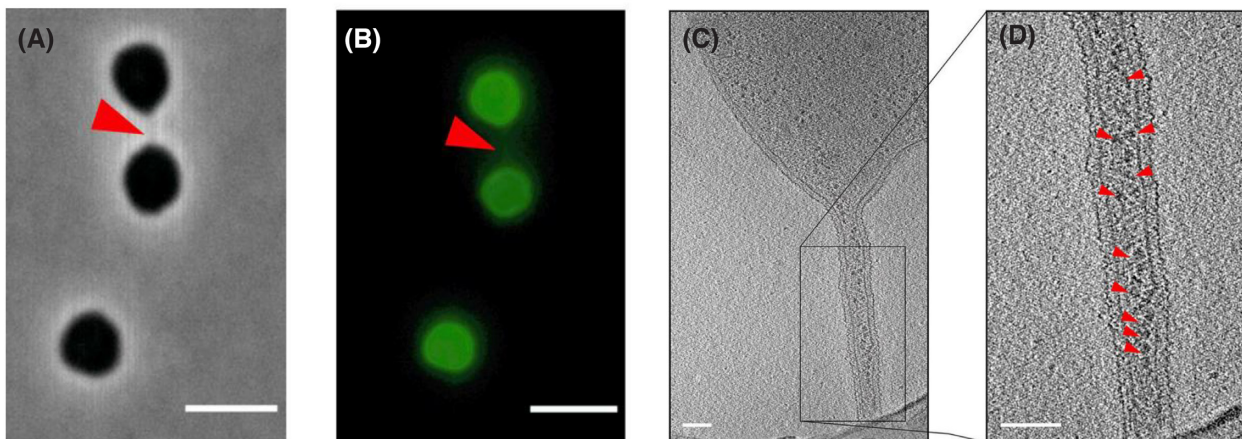
It has been known for a long time that eukaryotic cells can produce long tubular structures, often known as tunnelling nanotubes or sometimes microvillus (Lou et al. 2012; Rustom 2016; Nawaz and Fatima 2017; Cordero Cervantes and Zurzolo 2021). These nanotubes, formed by extrusion from the cytoplasmic membrane, can have very different lengths and thickness and can contain actin and/or tubulin filaments (for review, see Gill, Catchpole and Forterre 2019). They can connect different cells across substantial distances and have different physiological roles. Nanotubes (also called nanopods) were reported more recently in Bacteria (Baidya et al. 2018). It has been proposed that they could play a role in transferring nutrients, electrons or genetic material between cells. Bacterial nanotubes have been extensively studied in *Bacillus subtilis* (Baidya, Rosenshine and Ben-Yehuda 2020 and references therein). More recently, it has been suggested that these structures are not physiologically relevant since ‘they are exclusively extruded from dying cells as a result of biophysical forces’ (Pospíšil et al. 2020). The production of nanotubes by bacteria, especially Gram-positive, indeed raises questions because extrusion of nanotubes cannot take place without formation of apertures in the thick peptidoglycan layer. Considering the high number of studies that have emphasized important roles for nanotubes in bacteria during these last few years, the question of their physiological relevance should become a hot topic.

In their first studies on the Thermococcales EVs, Forterre and colleagues noticed the presence of strings of EVs enclosed within an elongated membrane structure covered by the S-layer (Soler et al. 2008), resembling the nanopods or nanotubes later observed in bacteria (Fig. 3). It remains to be definitively demonstrated that such structures are normally produced by living cells in natural habitats, as in the case of EVs from *Sulfolobales*. However, nanotubes from Thermococcales are sometimes produced in abundance (Soler et al. 2008) and throughout different growth phases (Gauliard, pers. comm.), suggesting that they could be physiologically relevant. Some species of Thermococcales are able to produce giant nanotubes that can reach several micrometers in length and are often filled with EVs (Fig. 3). EVs present within these nanotubes are usually smaller than free EVs (Fig. 3A and B) and larger vesicle-like structure are sometimes located in the extremities of the nanotubes (Fig. 3C), suggesting that these structures could be involved in the transport and/or formation of EVs. Nanotubes often connect cells of Thermococcales together (Fig. 3D) and it was suggested that they could be involved in transfer of materials (nucleic acids and proteins) between cells (Marguet et al. 2013).

During the 80s, the Mevarech group has described an original mechanism of gene transfer between species of the genus *Haloferax* (order Halobacteriales) (Mevarech and Werczberger 1985; Rosenshine, Tchelet and Mevarech 1989). These transfers can be interspecific and were shown to be bidirectional, leading to genetic hybrids through DNA recombination between the parental genomes (Naor et al. 2012). They do not seem to involve EVs but instead cell–cell bridges were recently observed by electron cryo-tomography (Sivabalasarma et al. 2020) (Fig. 4). The S-layer-covered nanotube-like protrusions are 100 nm in diameter and connect the cells that are up to 2 µm apart (Rosenshine, Tchelet and Mevarech 1989; Sivabalasarma et al. 2020) (Fig. 4C).



**Figure 3.** Nanotubes produced by Thermococcales. (A–C) Transmission electron microscopy images: (A) *T. priurii* cell producing both EVs and nanotubes containing EVs (scale bar, 200 nm); discrete EVs are surrounded by the cellular S-layer forming the nanotube structure. (B) Long nanotubes containing EVs produced by *T. priurii* (scale bar, 200 nm). (C) A cell of *Thermococcus* sp. 15-2 producing a long nanotube. (D) Scanning electron microscopy image of long nanotubes connecting clusters of *Thermococcus* sp. 15-2 cells. EVs can be also observed at the surface of most cells (scale bar, 1 μm) (Marguet and Forterre, unpublished observations).



**Figure 4.** Nanotubes produced by *Haloferax volcanii*. Cells of *H. volcanii* are linked by nanotubes as observed by (A) phase-contrast and (B) fluorescence microscopy. In panel (B), *H. volcanii* cells are labeled with Alexa Fluor 488. The cell-cell bridge is indicated by a red arrow. Scale bar, 4 μm. (C, D) Electron cryo-tomography images of *H. volcanii* nanotubes. In panel (D), ribosomes are indicated by small red arrows. Scale bar, 100 nm. All images are reproduced from Sivabalasarma et al. (2020).



These structures were shown to allow the diffusion of cellular materials, such as ribosomes (Fig. 4D).

A hyperthermophilic crenarchaeon of the genus *Pyrodictium* was found to form extracellular tubules with an outer diameter of around 25 nm, which interconnected the cells and led to the formation of extensive networks (Horn et al. 1999; Nickell et al. 2003). These extracellular tubes formed by *Pyrodictium* are much thinner than those produced by *Thermococcus* spp. (Soler et al. 2008; Marguet et al. 2013), and it is not known whether their biogenesis is mechanistically related to the EV production.

Finally, very long tubular structures similar to those observed in Thermococcales have been occasionally observed to be associated with cells of the first cultivated Asgard archaeon, *Prometheoarchaeum syntrophicum* (Imachi et al. 2020). However, these nanotubes do not connect cells together, suggesting divergence in physiological role. Imachi and co-workers have suggested that similar structures present in the Asgard ancestor of eukaryotes have facilitated the engulfment of an aerobic bacterial symbiont at the onset of eukaryogenesis (Imachi et al. 2020).

## PERSPECTIVES

Although the research on archaeal EVs and nanotubes is still in its infancy, it is already clear that these structures, especially EVs, play a profound role in archaeal physiology and environmental adaptation. Archaeal EVs were shown to contain cellular, plasmid or viral DNA. Vesiduction has now been demonstrated for several archaeal phyla (Thermococcales, Haloarchaea and Sulfolobales). As a result, archaeal EVs probably play an important role in the evolution and plasticity of cellular archaeal genomes and their mobilome, thus enabling archaeal adaptation in very diverse and often extreme environments. Future investigations on archaeal EVs should now more systematically test their ability to facilitate horizontal gene transfers by vesiduction in order to have a more complete overview of their importance in archaeal evolution. Moreover, the molecular mechanism of DNA recruitment into EVs is yet to be understood.

The existence of diverse mechanisms of EV production in Archaea is exemplified by the abundant production of EVs in Crenarchaeota and Euryarchaeota that encode and lack the ESCRT system, respectively. The discovery of proteins involved in EV production in Thermococcales and Haloarchaea is a major challenge right now. Hopefully, the availability of powerful genetic tools for these organisms will help to identify such proteins in the near future. One possibility is that some proteins involved in cell division in Euryarchaeota are also involved in EV production, as is the case for ESCRT proteins in Crenarchaeota. Another interesting possibility is that EV formation depends on proteins involved in polar lipids biosynthesis, especially those involved in the modification of the polar head groups. It was suggested that EV production in Bacteria and Eukarya is controlled, at least partly, by the regulation of enzymes involved in the incorporation of polar lipids in order to create asymmetry between the outer and inner membrane leaflets (Gill, Catchpole and Forterre 2019). However, this hypothesis cannot be directly transposed to Archaea that often have monolayer membranes formed by long tetraether lipids. In that case, the classical models of membrane fission and fusion that involve the transient opening of the bilayer cannot be applied (Relini et al. 1996). Notably, monolayer membranes are especially preva-

lent in Sulfolobales and Thermococcales, which have been more thoroughly studied for EV production. Indeed, in many Sulfolobales species, 95–100% of lipids in the membrane are membrane-spanning C<sub>40</sub> glycerol dibiphytanyl glycerol tetraethers (Quemin et al. 2015; Kasson et al. 2017; Liu et al. 2018; Wang et al. 2019). One possibility is that membrane curvature in organisms with monolayer membranes is induced by the accumulation of larger polar head groups in the outer surface of the monolayer. Alternatively, patches of membrane could adopt bilayer structure by favouring the local clustering of diether lipids or that of tetraether lipids adopting the horseshoe conformation, as recently observed in the membranes of some archaeal viruses (Kasson et al. 2017). In the future, a better knowledge of the archaeal lipid biosynthetic pathways, especially that of the formation of polar head group, would enable the genetic manipulations necessary to test different hypotheses.

The production of nanotubes observed in some Archaea is another aspect worthy of further studies. Curiously, although nanotubes have not yet been observed in Sulfolobales, some species of Thermococcales produce abundant nanotubes that could be linked to EV production. Studying the still mysterious mechanisms for EV production in these archaea thus should also bring information on nanotubes, their relevance and physiological roles. Interestingly, it was shown that some nanotubes in eukaryotes are formed from EVs (Rustom 2016). This suggests that nanotubes could be also relevant structure in Archaea and studying their connection to EVs is likely to become a hot topic of research in the near future. It will be especially interesting to see whether, besides Lokiarchaeota, other Asgard archaea produce nanotubes and whether the eukaryotic-like actins encoded by these archaea are involved in the formation of these structures. A possible evolutionary connection between the pathway for nanotube formation in Archaea and Eukaryotes will be worth exploring in Asgard archaea or Bathyarchaea, which encode actin and/or tubulin homologues.

## ACKNOWLEDGEMENTS

The authors would like to thank Emilie Gauliard and Evelyne Marguet for electron micrographs of Fig. 3C and D.

## FUNDING

The work of extracellular vesicles and nanotubes was supported in our laboratories by a European Research Council grant from the European Union Seventh Framework Programme (FP/2007–2013)/Project EVOMOBIL-ERC Grant Agreement 340440 to PF, and Agence Nationale de la Recherche (#ANR-17-CE15-0005-01) and Ville de Paris Emergence(s) program (project MEMREMA) grants to MK. JL was partly supported through the PRESTIGE post-doctoral program from European Union Seventh Framework Programme. AG was supported by the Agence Nationale de la Recherche, project HYPERBIOMIN (ANR-20-CE02-0001-01).

**Conflict of Interest.** None declared.

## REFERENCES

- Adam PS, Borrel G, Brochier-Armanet C et al. The growing tree of Archaea: new perspectives on their diversity, evolution and ecology. *ISME J* 2017;11:2407–25.
- Albers SV, Meyer BH. The archaeal cell envelope. *Nat Rev Microbiol* 2011;9:414–26.

- Baidya AK, Bhattacharya S, Dubey GP et al. Bacterial nanotubes: a conduit for intercellular molecular trade. *Curr Opin Microbiol* 2018; **42**:1–6.
- Baidya AK, Rosenshine I, Ben-Yehuda S. Donor-delivered cell wall hydrolases facilitate nanotube penetration into recipient bacteria. *Nat Commun* 2020; **11**:1938.
- Baquero DP, Contursi P, Piochi M et al. New virus isolates from Italian hydrothermal environments underscore the biogeographic pattern in archaeal virus communities. *ISME J* 2020; **14**:1821–33.
- Brochier-Armanet C, Boussau B, Gribaldo S et al. Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nat Rev Microbiol* 2008; **6**:245–52.
- Brown L, Wolf JM, Prados-Rosales R et al. Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. *Nat Rev Microbiol* 2015; **13**:620–30.
- Caspi Y, Dekker C. Dividing the archaeal way: the ancient Cdv cell-division machinery. *Front Microbiol* 2018; **9**:174.
- Choi DH, Kwon YM, Chiura HX et al. Extracellular vesicles of the hyperthermophilic archaeon “*Thermococcus onnurineus*” NA1T. *Appl Environ Microbiol* 2015; **81**:4591–9.
- Coelho C, Casadevall A. Answers to naysayers regarding microbial extracellular vesicles. *Biochem Soc Trans* 2019; **47**:1005–12.
- Cordero Cervantes D, Zurzolo C. Peering into tunneling nanotubes: the path forward. *EMBO J* 2021; **40**:e105789.
- Da Cunha V, Gaia M, Gadelle D et al. Lokiarchaea are close relatives of Euryarchaeota, not bridging the gap between prokaryotes and eukaryotes. *PLoS Genet* 2017; **13**:e1006810.
- Da Cunha V, Gaia M, Nasir A et al. Asgard archaea do not close the debate about the universal tree of life topology. *PLoS Genet* 2018; **14**:e1007215.
- Dombrowski N, Lee JH, Williams TA et al. Genomic diversity, lifestyles and evolutionary origins of DPANN archaea. *FEMS Microbiol Lett* 2019; **366**:fnz008.
- Domingues S, Nielsen KM. Membrane vesicles and horizontal gene transfer in prokaryotes. *Curr Opin Microbiol* 2017; **38**:16–21.
- Dridi B, Fardeau ML, Ollivier B et al. *Methanomassiliicoccus luminyensis* gen. nov., sp. nov., a methanogenic archaeon isolated from human faeces. *Int J Syst Evol Microbiol* 2012; **62**:1902–7.
- Ellen AF, Albers S-V, Huibers W et al. Proteomic analysis of secreted membrane vesicles of archaeal *Sulfolobus* species reveals the presence of endosome sorting complex components. *Extremophiles* 2009; **13**:67–79.
- Ellen AF, Rohulya OV, Fusetti F et al. The *sulfolobin* genes of *Sulfolobus acidocaldarius* encode novel antimicrobial proteins. *J Bacteriol* 2011; **193**:4380–7.
- Ellen AF, Zolghadr B, Driessen AM et al. Shaping the archaeal cell envelope. *Archaea* 2010; **2010**:1.
- Erdmann S, Tschitschko B, Zhong L et al. A plasmid from an Antarctic haloarchaeon uses specialized membrane vesicles to disseminate and infect plasmid-free cells. *Nature Microbiology* 2017; **2**:1446–55.
- Forterre P, Da Cunha V, Catchpole R. Plasmid vesicles mimicking virions. *Nat Microbiol* 2017; **2**:1340–1.
- Gaudin M, Gauliard E, Schouten S et al. Hyperthermophilic archaea produce membrane vesicles that can transfer DNA. *Environ Microbiol Rep* 2013; **5**:109–16.
- Gaudin M, Krupovic M, Marguet E et al. Extracellular membrane vesicles harbouring viral genomes. *Environ Microbiol* 2014; **16**:1167–75.
- Gill S, Catchpole R, Forterre P. Extracellular membrane vesicles in the three domains of life and beyond. *FEMS Microbiol Rev* 2019; **43**:273–303.
- Gorlas A, Jacquemot P, Guigner JM et al. Greigite nanocrystals produced by hyperthermophilic archaea of Thermococcales order. *PLoS One* 2018; **13**:e0201549.
- Gorlas A, Marguet E, Gill S et al. Sulfur vesicles from Thermococcales: a possible role in sulfur detoxifying mechanisms. *Biochimie* 2015; **118**:356–64.
- Heimerl T, Flechsler J, Pickl C et al. A Complex Endomembrane System in the Archaeon *Ignicoccus hospitalis* Tapped by Nanoarchaeum equitans. *Front Microbiol* 2017; **8**:1072.
- Horn C, Paulmann B, Kerlen G et al. In vivo observation of cell division of anaerobic hyperthermophiles by using a high-intensity dark-field microscope. *J Bacteriol* 1999; **181**:5114–8.
- Huber H, Hohn MJ, Rachel R et al. A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature* 2002; **417**:63–7.
- Imachi H, Nobu MK, Nakahara N et al. Isolation of an archaeon at the prokaryote-eukaryote interface. *Nature* 2020; **577**:519–25.
- Jan AT. Outer membrane vesicles (OMVs) of Gram-negative bacteria: a perspective update. *Front Microbiol* 2017; **8**:1053.
- Jay ZJ, Beam JP, Dlakic M et al. Marsarchaeota are an aerobic archaeal lineage abundant in geothermal iron oxide microbial mats. *Nat Microbiol* 2018; **3**:732–40.
- Junglas B, Briegel A, Burghardt T et al. *Ignicoccus hospitalis* and *Nanoarchaeum equitans*: ultrastructure, cell-cell interaction, and 3D reconstruction from serial sections of freeze-substituted cells and by electron cryotomography. *Arch Microbiol* 2008; **190**:395–408.
- Kasson P, DiMaio F, Yu X et al. Model for a novel membrane envelope in a filamentous hyperthermophilic virus. *eLife* 2017; **6**:e26268.
- Kish A, Miot J, Lombard C et al. Preservation of archaeal surface layer structure during mineralization. *Sci Rep* 2016; **6**:26152.
- Klingl A. S-layer and cytoplasmic membrane: exceptions from the typical archaeal cell wall with a focus on double membranes. *Front Microbiol* 2014; **5**:624.
- Knox KW, Vesik M, Work E. Relation between excreted lipopolysaccharide complexes and surface structures of a lysine-limited culture of *Escherichia coli*. *J Bacteriol* 1966; **92**:1206–17.
- Krupovic M, Forterre P, Bamford DH. Comparative analysis of the mosaic genomes of tailed archaeal viruses and proviruses suggests common themes for virion architecture and assembly with tailed viruses of bacteria. *J Mol Biol* 2010; **397**:144–60.
- Krupovic M, Gonnet M, Hania WB et al. Insights into dynamics of mobile genetic elements in hyperthermophilic environments from five new *Thermococcus* plasmids. *PLoS One* 2013; **8**:e49044.
- Küper U, Meyer C, Müller V et al. Energized outer membrane and spatial separation of metabolic processes in the hyperthermophilic archaeon *Ignicoccus hospitalis*. *Proc Natl Acad Sci USA* 2010; **107**:3152–6.
- Lindås AC, Karlsson EA, Lindgren MT et al. A unique cell division machinery in the Archaea. *Proc Natl Acad Sci USA* 2008; **105**:18942–6.
- Liu J, Cvirkaite-Krupovic V, Baquero DP et al. Virus-induced cell gigantism and asymmetric cell division in archaea. *Proc Natl Acad Sci USA* 2021; **118**:e2022578118.
- Liu J, Cvirkaite-Krupovic V, Commere P-H et al. Archaeal extracellular vesicles are produced in an ESCRT-dependent manner and promote gene transfer and nutrient cycling in

- extreme environments. *ISME J* 2021b, DOI: 10.1038/s41396-021-00984-0.
- Liu J, Gao R, Li C *et al.* Functional assignment of multiple ESCRT-III homologs in cell division and budding in *Sulfolobus islandicus*. *Mol Microbiol* 2017;**105**:540–53.
- Liu Y, Osinski T, Wang F *et al.* Structural conservation in a membrane-enveloped filamentous virus infecting a hyperthermophilic acidophile. *Nat Commun* 2018;**9**:3360.
- Lou E, Fujisawa S, Barlas A *et al.* Tunneling Nanotubes: a new paradigm for studying intercellular communication and therapeutics in cancer. *Commun Integr Biol* 2012;**5**:399–403.
- Lu Z, Fu T, Li T *et al.* Coevolution of Eukaryote-like Vps4 and ESCRT-III Subunits in the Asgard Archaea. *mBio* 2020;**11**:e00417–20.
- Makarova KS, Yutin N, Bell SD *et al.* Evolution of diverse cell division and vesicle formation systems in Archaea. *Nat Rev Microbiol* 2010; **8**:731–41.
- Marguet E, Gaudin M, Gaudiard E *et al.* Membrane vesicles, nanopods and/or nanotubes produced by hyperthermophilic archaea of the genus *Thermococcus*. *Biochem Soc Trans* 2013;**41**:436–42.
- Mevarech M, Werczberger R. Genetic transfer in *Halobacterium volcanii*. *J Bacteriol* 1985;**162**:461–2.
- Naor A, Lapierre P, Mevarech M *et al.* Low species barriers in halophilic archaea and the formation of recombinant hybrids. *Curr Biol* 2012;**22**:1444–8.
- Näther DJ, Rachel R. The outer membrane of the hyperthermophilic archaeon *Ignicoccus*: dynamics, ultrastructure and composition. *Biochem Soc Trans* 2004;**32**:199–203.
- Nawaz M, Fatima F. Extracellular vesicles, tunneling nanotubes, and cellular interplay: synergies and missing links. *Front Mol Biosci* 2017;**4**:50.
- Nickell S, Hegerl R, Baumeister W *et al.* Pyrodictium cannulae enter the periplasmic space but do not enter the cytoplasm, as revealed by cryo-electron tomography. *J Struct Biol* 2003;**141**:34–42.
- Nikolayev S, Cohen-Rosenzweig C, Eichler J. Evolutionary considerations of the oligosaccharyltransferase AglB and other aspects of N-glycosylation across Archaea. *Mol Phylogenet Evol* 2020;**153**:106951.
- Peng N, Han W, Li Y *et al.* Genetic technologies for extremely thermophilic microorganisms of *Sulfolobus*, the only genetically tractable genus of crenarchaea. *Sci China Life Sci* 2017;**60**:370–85.
- Pospíšil J, Vítovská D, Kofroňová O *et al.* Bacterial nanotubes as a manifestation of cell death. *Nat Commun* 2020;**11**:4963.
- Prangishvili D, Holz I, Stieger E *et al.* Sulfolobocins, specific proteinaceous toxins produced by strains of the extremely thermophilic archaeal genus *Sulfolobus*. *J Bacteriol* 2000;**182**:2985–8.
- Quemin ER, Pietila MK, Oksanen HM *et al.* *Sulfolobus* spindle-shaped virus 1 contains glycosylated capsid proteins, a cellular chromatin protein, and host-derived lipids. *J Virol* 2015;**89**:11681–91.
- Relini A, Cassinadri D, Fan Q *et al.* Effect of physical constraints on the mechanisms of membrane fusion: bolaform lipid vesicles as model systems. *Bophys J* 1996;**4**:1789–95.
- Reysenbach AL, Flores GE. Electron microscopy encounters with unusual thermophiles helps direct genomic analysis of *Aciduliprofundum boonei*. *Geobiology* 2008;**6**:331–6.
- Reysenbach AL, Liu Y, Banta AB *et al.* A ubiquitous thermoacidophilic archaeon from deep-sea hydrothermal vents. *Nature* 2006;**442**:444–7.
- Rinke C, Schwientek P, Szczyrba A *et al.* Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 2013;**499**:431–7.
- Rodrigues-Oliveira T, Belmok A, Vasconcellos D *et al.* Archaeal S-layers: overview and current state of the art. *Front Microbiol* 2017;**8**:2597.
- Rosenshine I, Tchelet R, Mevarech M. The mechanism of DNA transfer in the mating system of an archaeobacterium. *Science* 1989;**245**:1387–9.
- Rustom A. The missing link: does tunnelling nanotube-based supercellularity provide a new understanding of chronic and lifestyle diseases? *Open Biol* 2016;**6**:160057.
- Samson RY, Obita T, Freund SM *et al.* A role for the ESCRT system in cell division in archaea. *Science* 2008;**322**:1710–3.
- Schocke L, Brasen C, Siebers B. Thermoacidophilic *Sulfolobus* species as source for extremozymes and as novel archaeal platform organisms. *Curr Opin Biotechnol* 2019;**59**:71–7.
- Sivabalasarma S, Wetzel H, Nussbaum P *et al.* Analysis of cell-cell bridges in *Haloferax volcanii* using electron cryotomography reveal a continuous cytoplasm and S-layer. *Front Microbiol* 2020;**11**:612239.
- Soler N, Forterre P. Vesiduction: the fourth way of HGT. *Environ Microbiol* 2020;**22**:2457–60.
- Soler N, Gaudin M, Marguet E *et al.* Plasmids, viruses and virus-like membrane vesicles from *Thermococcales*. *Biochem Soc Trans* 2011;**39**:36–44.
- Soler N, Krupovic M, Marguet E *et al.* Membrane vesicles in natural environments: a major challenge in viral ecology. *ISME J* 2015;**9**:793–6.
- Soler N, Marguet E, Verbavatz JM *et al.* Virus-like vesicles and extracellular DNA produced by hyperthermophilic archaea of the order *Thermococcales*. *Res Microbiol* 2008;**159**:390–9.
- Spang A, Caceres EF, Ettema TJG. Genomic exploration of the diversity, ecology, and evolution of the archaeal domain of life. *Science* 2017;**357**:eaaf3883.
- Spang A, Saw JH, Jørgensen SL *et al.* Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature* 2015;**521**:173–9.
- Steber J, Schleifer KH. Halococcus morrhuae: a sulfated heteropolysaccharide as the structural component of the bacterial cell wall. *Arch Microbiol* 1975;**105**:173–7.
- Steenbakkens PJ, Geerts WJ, Ayman-Oz NA *et al.* Identification of pseudomurein cell wall binding domains. *Mol Microbiol* 2006;**62**:1618–30.
- Tarrason Risa G, Hurtig F, Bray S *et al.* The proteasome controls ESCRT-III-mediated cell division in an archaeon. *Science* 2020;**369**:eaaz2532.
- Thiroux S, Dupont S, Nesbø CL *et al.* The first head-tailed virus, MFTV1, infecting hyperthermophilic methanogenic deep-sea archaea. *Environ Microbiol* 2021.
- Vietri M, Radulovic M, Stenmark H. The many functions of ESCRTs. *Nat Rev Mol Cell Biol* 2020;**21**:25–42.
- Wang F, Liu Y, Su Z *et al.* A packing for A-form DNA in an icosahedral virus. *Proc Natl Acad Sci USA* 2019;**116**:22591–7.
- Waters E, Hohn MJ, Ahel I *et al.* The genome of *Nanoarchaeum equitans*: insights into early archaeal evolution and derived parasitism. *Proc Natl Acad Sci USA* 2003;**100**:12984–8.
- Williams TA, Cox CJ, Foster PG *et al.* Phylogenomics provides robust support for a two-domains tree of life. *Nat Ecol Evol* 2020;**4**:138–47.

- Woese CR, Fox GE. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc Natl Acad Sci USA* 1977;**74**:5088–90.
- Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci USA* 1990;**87**:4576–9.
- Yanez-Mo M, Siljander PR-M, Andreu Z et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 2015;**4**:27066.
- Zaremba-Niedzwiedzka K, Caceres EF, Saw JH et al. Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature* 2017;**541**:353–8.