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Review

Spotlight on FtsZ-based cell division in Archaea

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Compared with the extensive knowledge on cell division in model eukaryotes and bacteria, little is known about how archaea divide. Interestingly, both endosomal sorting complex required for transport (ESCRT)-based and FtsZ-based cell division systems are found in members of the Archaea. In the past couple of years, several studies have started to shed light on FtsZ-based cell division processes in members of the Euryarchaeota. In this review we highlight recent findings in this emerging field of research. We present current knowledge of the cell division machinery of halophiles which relies on two FtsZ proteins, and we compare it with that of methanobacteria, which relies on only one FtsZ. Finally, we discuss how these differences relate to the distinct cell envelopes of these two archaeal model systems.

Highlights

Archaea employ either endosomal sorting complex required for transport (ESCRT)-III homologs or FtsZ homologs as main players in cell division.

Two types of FtsZ-based cell division exist in archaea: one-FtsZ-based, occurs in a few lineages, and the other, more common, type, which is two-FtsZ-based.

FtsZ homologs have different functions in the two-FtsZ-based cell division: FtsZ1 is involved mainly in ring assembly while FtsZ2 is involved in constriction.

The one-FtsZ-based system co-occurs in the presence of a cell wall made of pseudopeptidoglycan and MreB homologs in some methanogens.

SepF is the anchor for FtsZ in archaea.

The two FtsZs are the result of an ancient duplication followed by loss of one or the other FtsZ during archaeal diversification.

Diversity of cell division mechanisms in archaea

Cell division is one of the most fundamental biological processes allowing the maintenance of species by passing on the genomic material from mother to progeny. For this, several actions must be spatiotemporally coordinated and regulated to allow for DNA replication and segregation, cell membrane and envelope growth, cell constriction during **cytokinesis** (see Glossary), and finally, physical separation of daughter cells (**abscission**). Thanks to the advancement of fluorescence and high-resolution light microscopy over the past decades, these mechanisms have been extensively studied in model eukaryotes and bacteria. The final stages of cell division require remodeling of the **cell envelope** to complete abscission. In model eukaryotes, this is carried out by ESCRT, which is recruited at the division furrow [1]. Among the different components of this machinery, ESCRT-III assembles as a dynamic polymer which is disassembled by the ATPase Vps4 and is proposed to be the driving force for membrane scission [2]. In most bacteria the main coordinator of cell division is the GTP-dependent tubulin homolog FtsZ [3,4], which polymerizes into a ring-like structure (**Z-ring**) at the division plane [5,6].

While some exceptions exist, the machinery responsible for cell division is overall conserved in bacteria (FtsZ-based) and in eukaryotes (actin- and ESCRT-based). However, in archaea, both FtsZ- and ESCRT-based (Cdv) cell division systems are found, and, given their largely complementary phylogenetic distribution (Figure 1), either one or the other seems to be involved in cell division [7,8]. ESCRT-III homologs are present in members of the TACK superphylum and Asgard [9,10]. The crenarchaeal model organisms *Sulfolobus acidocaldarius* and *Saccharolobus solfataricus* harbor a Cdv (cell division) system containing the archaea-specific protein CdvA; the ESCRT-III homologs CdvB, CdvB1, and CdvB2; and the Vps4 homolog CdvC (for a detailed review on archaeal Cdv-based cell division we refer readers to [10]). It was experimentally shown that CdvA first forms a ring at mid-cell and then recruits CdvB; the latter, in turn, forms a scaffold for recruitment of CdvB1 and CdvB2 [11]. Triggered by proteasome-mediated degradation of CdvB, CdvB1/2 proteins perform the actual final stages of cytokinesis [12]. Similar to eukaryotic Vps4, CdvC might catalyze the disassembly of CdvB1/2 during this process [10].

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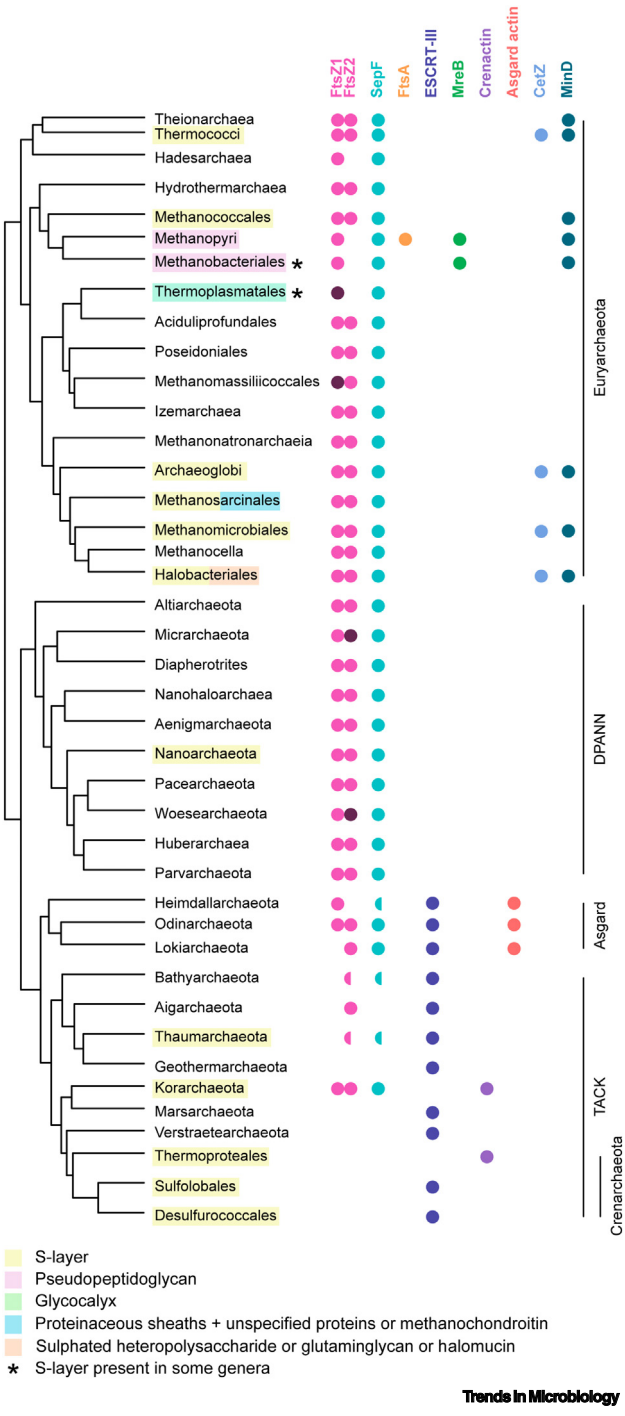

Figure 1. Types of archaeal cell envelope and the distribution of the genes involved in archaeal cell division and spatial organization. Distribution of FtsZ1, FtsZ2, SepF, FtsA, ESCRT-III, MreB, Crenactin, Asgard actin, CetZ, and MinD homologs on a schematic reference phylogeny of the archaea together with indication of cell envelope types. FtsZ1 and 2 homologs are present in most archaeal lineages (magenta), and frequently in two or more copies each (dark magenta). Semicircles indicate that the corresponding protein could not be identified in all the taxa of the corresponding clade and this may be due either to true absences or partial genomes. The presence of SepF (turquoise) correlates to that of FtsZ in most taxa. An FtsA homolog was identified only in *Methanopyri* (orange). Homologs of ESCRT-III (CdvB and homologs) (dark purple) are present only in the Asgard and in most representatives of the TACK superphylum, except for Korarchaeota and Thermoproteales. MreB (green) is present only in archaea that have a cell wall made from pseudopeptidoglycan (pPG) (highlighted in pink). Crenactin (light purple) is present only in Korarchaeota and Thermoproteales. Asgard actin (salmon) is widely distributed among the Asgard. One or multiple CetZ homologs (violet) are present in a few Euryarchaeota. MinD homologs (teal) are present only in some Euryarchaeota. The presence of FtsZ1, FtsZ2, SepF, and FtsA is based on [8,13], and the presence of ESCRT-III is based on [8,77]. The presence of MreB is based on unpublished data and [20]. The presence of Crenactin is based on [20]. The presence of Asgard actin is based on [78]. The presence of CetZ is based on [14,32]. The presence of MinD is based on [37,79]. Archaeal envelope types, materialized by different text highlight colors, are based on [53,80]. Abbreviation: ESCRT, endosomal sorting complex required for transport.

Glossary

Abscission: physical separation of cells during cell division, the last stage of the cytokinesis.

Cell envelope: includes the inner membrane and all the structures built on it such as the cell wall, the outer membrane, or the S-layer. Many archaeal cell envelopes consist of a cytoplasmic membrane covered with a proteinaceous S-layer or various surface decorations. Some have a cell wall made of pseudopeptidoglycan (pPG).

Cell wall: a structure that confers protection and shape on the cell. For bacteria, 'cell wall' refers mainly to the peptidoglycan (PG). Only members of the Methanobacteriales and Methanopyrales have a cell wall built from pPG.

Cytokinesis: the stage during cell division when the contractile ring induces invagination of the cell envelope into a cleavage furrow.

Divisome: mid-cell macromolecular machinery including all the components participating in cell division. The divisome is well described in bacteria but poorly understood in archaea.

Elongasome: a protein complex that, in bacteria, directs lateral insertion of PG along the long axis of the cell, allowing elongation. It is present only in rods and is absent in spherical bacteria. It is totally unknown in the Archaea.

***Haloferax volcanii*:** a halophilic Euryarchaeon formerly named *Halobacterium volcanii,* isolated and described by M.F. Mullakhanbhai and H. Larsen in 1975 and named after Benjamin Elazari Volcani who discovered halophiles in the Dead Sea in the 1930s.

***Methanobrevibacter smithii*:** a methanogenic Euryarchaeon formerly named *Methanobacterium ruminantium* strain PS Smith 1961(suldge strain), isolated from human feces in 1982. Since then, many studies have identified this species as the dominant methanogen in the animal and human gut.

Z-ring: the characteristic localization pattern of FtsZ which forms a discontinuous ring at the division plane of the cells. By extension, it can refer to the entire divisome. The Z-ring has been visualized in archaea that divide by using FtsZ.

With the exception of Crenarchaeota, FtsZ homologs are widespread across archaea, (Figure 1; [7,8,13]). Intriguingly, as opposed to bacteria, archaea can have either one or two FtsZ homologs: FtsZ1 and FtsZ2 (Figure 1; [7,8,13,14]). Moreover, some recently identified lineages in the TACK superphylum and Asgard encode both FtsZ and ESCRT-III homologs (Figure 1; [7,15]), leading to the question: which of these systems is actually involved in cytokinesis? It was shown in

Nitrosopumilus maritimus (Thaumarchaeota) that CdvA and CdvC are found at the division plane, similar to the arrangement in *S. acidocaldarius* and *S. solfataricus*. However, its highly divergent FtsZ is uniformly distributed in the cytoplasm, leading to the assumption that an ESCRT-based machinery, rather than an FtsZ-based one, is responsible for cell division in this archaeon [15,16]. This is consistent with significant differences between the thaumarchaeal FtsZ GTP binding and catalytic domains compared with other archaeal and bacterial FtsZ, further suggesting that it cannot polymerize, and its actual role remains unknown [14].

Additionally, homologs of the actin protein superfamily which function in bacteria and eukaryotes as cytoskeletal elements that are involved in cell shape, cell division, or DNA segregation, among other roles [17,18], have been identified in archaea (Figure 1) [19]. This includes the archaeal-specific type Crenactin that is found in members of the TACK superphylum and which assembles as filaments in a manner similar to that of eukaryotic actins [20–22], or the Asgard actins, which are phylogenetically closely related to eukaryotic actins [23]. Interestingly, members of the Thermoproteales do not encode homologs of FtsZ or ESCRT but encode Crenactins and their associated proteins Arcadins, suggesting a major role of these proteins in cell division within the order [20]. Conversely, members of the Asgard encode the most diverse range of cell-division-related proteins, including FtsZ, ESCRT-III, and actin homologs, but understanding which of these proteins is involved in cell division in these organisms require further studies (Figure 1).

Archaeal FtsZ-based cell division

To understand archaeal FtsZ-based cell division it is important to briefly summarize what is known about this process in extensively studied model bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Caulobacter crescentus,* and *Staphylococcus aureus*. During cell division, **cell wall** growth and constriction need to be spatiotemporally coordinated. In bacteria, this is done by two macromolecular machineries called the **elongasome** and the **divisome** [24]. In bacteria, FtsZ polymerizes into a ring and recruits other proteins of the divisome that are responsible for generating the nascent poles [6,25]. In *E. coli* the divisome is formed by ten essential core cell division proteins (FtsA, B, I, K, L, N, Q, W, Z, and ZipA) that localize to the Z-ring, and more than 20 additional non-core proteins (for excellent recent reviews on bacterial cell division see [5,6]). In *E. coli* and *B. subtilis* several auxiliary proteins are necessary to positively or negatively regulate the Z-ring formation (EzrA, ZapA, B, C, and D) and its tethering to the membrane (FtsA, ZipA, and SepF). Proper Z-ring localization to the mid-cell is ensured by either nucleoid occlusion (SlmA, Noc) [26,27] or the Min system (MinC, D) [27].

Before the availability of its genome sequence, studies using DNA hybridization approaches in ***Haloferax volcanii*** were amongst the first to identify the presence of an archaeal *bona fide* FtsZ homolog that localized at mid-cell [28]. This led to the assumption that archaea might divide by FtsZ-based fission similar to bacteria [3]. The availability of more sequenced archaeal genomes, starting with *Methanocaldococcus jannaschii* [29], and other genomes including that of *H. volcanii* [30], revealed the presence of two FtsZ copies in many archaea alongside a large variety of other tubulin homologs such as the recently discovered archaeal tubulin family CetZ (Figure 1) [14,31–33]. Phylogenetic analysis showed that archaeal FtsZ1 and FtsZ2 are members of two distinct clades, indicating that they arose from an early gene duplication [8,13]. Both FtsZ copies share an overall similar domain organization with their bacterial counterpart, namely, a highly conserved core domain including the GTP-binding and polymerization domains and the less-conserved N-terminal and C-terminal tails [13,31]. However, differences are observed in their GTP binding and longitudinal subunit interfaces [13], suggesting different roles and/or mechanistic aspects in archaeal cell division.

Other than FtsZ, only a few other homologs of the bacterial divisome have been identified in archaea (Figure 1). These are SepF, which, in Actinobacteria and Cyanobacteria, functions as the main membrane tether for the Z-ring [34,35] and the negative regulator of FtsZ MinD [6,27] (Figure 1). In most archaea, SepF co-occurs with at least one copy of FtsZ and is absent from genomes that exclusively use an ESCRT-III-based system for cell division (Figure 1; [7,8]), indicating a strong functional link between FtsZ and SepF. This was confirmed by two recent studies described later, which have shown that archaeal SepF affects the anchoring of FtsZ to the cell membrane [8,36]. Interestingly, MinD is found only in a few members of the Euryarchaeota where FtsZ is present [37], which suggested a similar role in FtsZ regulation as in bacteria. However, *in vivo* studies in *H. volcanii* did not find a link between MinD homologs and cell division, as deletion mutants of none of the four MinD homologs, nor combinations of multiple deletion mutants, led to cell division or FtsZ localization defects [37]. This suggests that, in archaea, MinD might be involved in processes other than Z-ring positioning. Indeed, deletion of the *minD4* gene in *H. volcanii* reduced swimming motility and compromised correct formation of the motility machinery at the cell pole, leading to the proposal that MinD4 might be involved in spatiotemporal development of cell motility [37]. The mechanism by which the Z-ring is positioned at the mid-cell in archaea is currently unknown.

One or two Z-rings to divide

As mentioned earlier, archaea can possess one or two FtsZ homologs, suggesting different ways to divide. These have been recently investigated in ***Methanobrevibacter smithii*** as a model for 'one-FtsZ-based cell division' and in *H. volcanii* as a model for 'two-FtsZ-based cell division' (Box 1), providing a unique opportunity to understand their commonalities and differences.

Box 1. Archaeal model organisms

To date, genetic tools have been developed for only four groups of archaea: thermophilic Euryarchaeota (Thermococcales), methanogens and halophiles (both Euryarchaeota), and Crenarchaeota (Sulfolobales). The reason for the low number of available model organisms might be that many archaeal species are resistant to conventional antibiotics used for selection in genetic systems, which is a limitation to the establishment of good screening methods [54]. Also, the conditions in which many archaea grow, ranging from high temperature (>80°C) to high salinity, extreme pH and anaerobiosis, add further difficulties to the establishment of archaeal model organisms. Most studies of genetically tractable archaea have focused on metabolic processes such as nitrogen cycling and methanogenesis [81,82], physiological functions like regulation and transcription [83], as well as DNA replication and repair [84,85]. Only very few have dealt with cell division, shape, and the cell envelope.

- Thermococcocales: marine, aerobe hyperthermophiles. Most studies were performed with *Thermococcus kodakarensis*, but also *Thermococcus onnurineus* NA1. Shuttle vector transformation has been established for *Pyrococcus furiosus* COM1 [86], *Pyrococcus abyssi* [87], and *Pyrococcus yayanosii* [88].
- Methanogens: obligate anaerobes that produce methane. Genetic techniques have been developed for the mesophilic *Methanococcus maripaludis* [89] and *Methanosarcina acetivorans* [90], and many were also successfully applied to *Methanococcus voltae*, *Methanosarcina barkeri*, and *Methanosarcina mazei*. Recently, a shuttle-vector system that allows heterologous gene expression was reported for the thermophilic *Methanothermobacter thermoautotrophicus* [91]. Despite the lack of genetic tools, *M. smithii* can be considered a model organism as many studies have been conducted on its influence on the animal microbiome, the impact of antimicrobial agents, the composition of its pPG cell wall, and recently, cell division (Box 2).
- Haloarchaea: mostly mesophilic aerobes that favor salty environments. Their transformation with foreign DNA was accomplished early on [92,93]. The main model species are *Halobacterium salinarum* [94] and *Haloferax volcanii* [40], but *Haloarcula marismortui* can be also transformed with a shuttle vector from *H. salinarum* or *H. volcanii.* For cell biological studies, many fluorescent fusion proteins are available for *H. volcanii* (Box 2).
- Sulfolobales: aerobic, acidophilic, thermophilic sulfur-oxidizers. Genetically tractable species are *Saccharolobus solfataricus*, *Saccharolobus islandicus,* and *Sulfolobus acidocaldarius* [95–97]. The recent development of the 'Sulfoscope' has allowed imaging of *S. acidocaldarius* at 75°C, revealing the coordination of DNA segregation and cytokinesis in dividing cells [11,98].

For further reading on the genetic systems available for archaea, refer to reviews [99–101].

M. smithii – one-FtsZ-based cell division

M. smithii is the most abundant archaeal species in the human gastrointestinal tract, but very little is known about its role in health and disease (for a recent review see [38]), and even less about how it grows and divides. Currently, no genetic tools are available for this organism (Box 1), but thanks to the development of immunolabeling protocols in *M. smithii* (Box 2), it was shown that FtsZ1, the only FtsZ present in this ovococcoid archaeon, forms a discontinuous and contracting ring at the division plane, similarly to the process in bacteria [5], and is therefore most likely involved in cell division (Figure 2A,B) [8]. Before cell constriction is fully completed, two new Z-rings form in the prospective daughter cells at the future division site, a pattern similar to what is observed in the bacterium *Streptococcus pneumoniae* [39] (Figure 2A,B). However, unlike in *S. pneumoniae*, in *M. smithii*, FtsZ initially colocalizes with its membrane anchor, SepF, at the septum, but then SepF relocates to the future division planes of the future daughter cells before FtsZ. Such priming of the division site has never been reported before, neither for the archaeal nor the bacterial homologs of SepF. Structural analysis revealed that the SepF functional dimer, which is defined by being in a complex with FtsZ, differs between *M. smithii* SepF, where it is formed via a β–β interface, and the bacterial SepF (from *Corynebacterium glutamicum)*, where it is formed via an α–α interface. Additionally, the binding mode of the C-terminal domain of FtsZ to the SepF dimer is only partially conserved between bacteria and archaea. Archaeal SepF also seems unable to polymerize as it is missing a crucial glycine residue (Gly114 in *C. glutamicum* SepF) that is important for the α–α interface interaction. Finally, a comprehensive evolutionary analysis of SepF and FtsZ in bacteria and archaea shows that these two proteins date back to the Last Universal Common Ancestor (LUCA) where they, possibly helped by some auxiliary proteins, may have formed a minimal cytokinesis system. Based on these results, it was

Box 2. Fluorescence microscopy techniques for studying archaeal cell biology

Recently, several tools have been developed for *in vivo* localization studies of archaeal proteins. Genetically encoded fluorescently tagged proteins have been mainly developed for Haloarchaea and used to study diverse cellular processes such as DNA replication [102,103], cell shape [32], cell division [13,36], S-layer biosynthesis [69], and cell motility [37,104,105]. Fluorescent protein (FP) reporters have been successfully used, including soluble, red-shifted variant smRS-GFP [106] and its brighter variant mGFP6 [107], red-FP mCherry [13,32,36], and monomeric superfolder green FP (msf-GFP) [43,69]. Single-molecule localization microscopy (SMLM) has been established in *H. volcanii* with the codon optimization of the photoactivatable/photoconvertible FPs, PAmCherry1Hfx and Dendra2Hfx, and the generation of an autofluorescence-free strain by the deletion of *crtI* (HVO_2528) [108].

Various archaeal cell structures are visualized with fluorescent dyes: acridine orange (AO), Hoechst 33342, propidium iodide (PI, fixed/permeabilized cells only) [109], SYTO 9 for internal DNA staining and the combination of AO/PI for LIVE/DEAD assays whereas DAPI penetration inside the cells is limited [110]; cell envelope structures are visualized with FM1-43, cell mask orange, the lectin concanavalin A (ConA) [110], Congo red and fluorescently labeled wheat germ agglutinin (WGA) dyes for planktonic cells and/or biofilms [111].

Live imaging has been developed for Haloarchaea based on tools developed for bacteria, such as nanofabricated soft lithographic microchambers [46], the mother machine microfluidic device, and the CellASIC® system. Such devices are crucial as Haloarchaea have a naturally malleable cell envelope and are sensitive to pressure and mechanical forces applied while immobilized on soft agar pads [112].

The advancement in imaging of hyperthermophiles [11,98] has triggered efforts in the development of thermostable FPs for *in vivo* localization studies, for example, *in vivo* selection of an sf-GFP variant in thermophilic bacteria [113,114] and the development of a new generation of SNAP-like tags adapted to thermophilic archaea [115].

In archaeal species without genetic manipulation systems, such as *M. smithii*, microscopy approaches have proven to be powerful tools in unraveling cellular processes. Recently, immunolabeling techniques have been adapted for *M. smithii* using the phage endoisopeptidase PeiW to permeabilize the pPG cell wall, allowing the penetration of antibodies targeted against cell division proteins (FtsZ, SepF) [8]. Epifluorescence and 3D high-resolution microscopy could then be used to reveal the localization of these cytoskeletal proteins inside the cells. Although live-cell imaging is not possible with immunolabeling, this technique provides ground for future discoveries in nongenetically tractable archaea and increases our knowledge in many areas of archaeal cell biology.

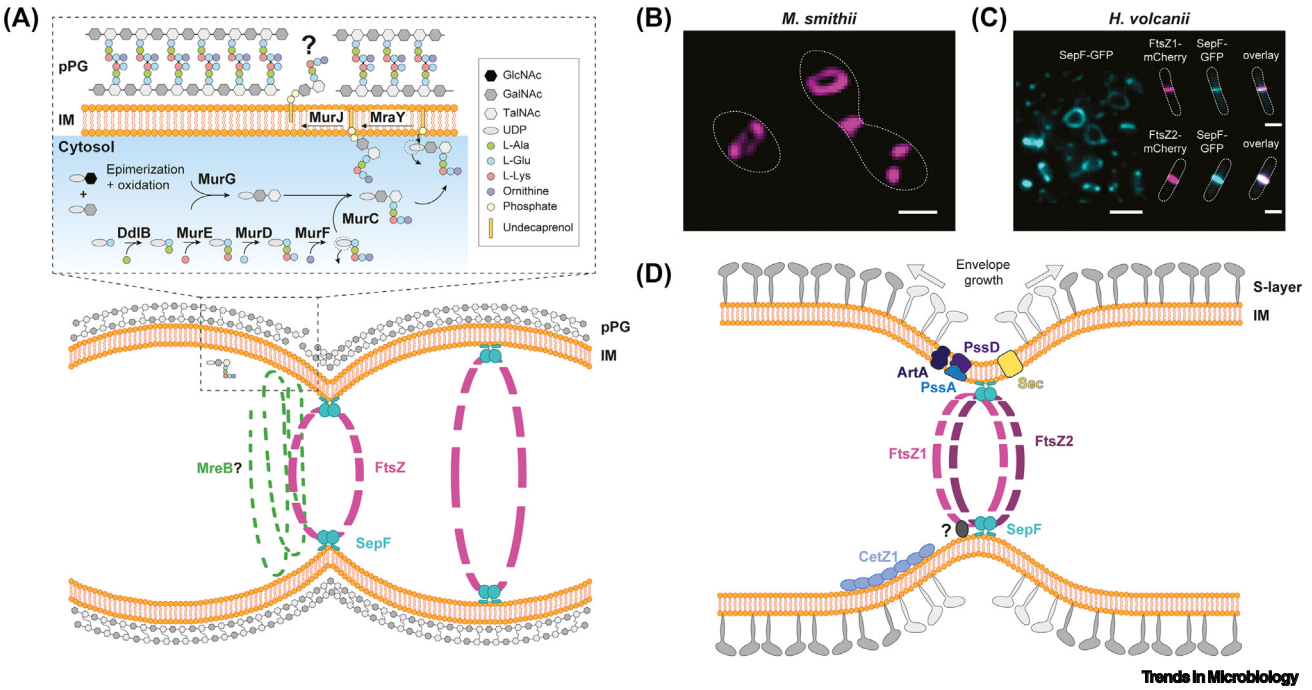

Figure 2. Schema of hypothetical and known factors involved in cell division in the two model archaea *Methanobrevibacter smithii* and *Haloferax volcanii*. (A) A dividing ovococcoid cell of *M. smithii*. FtsZ and SepF colocalize at the division plane, but also at the future division site in the prospective daughter cells. The hypothetical localization of MreB near the current division plane is shown, potentially guiding the pseudopeptidoglycan (pPG) synthesis machinery. In the enlarged inlay, the pathway for pPG synthesis is shown as proposed by [52], and the potential function in the pPG biosynthesis pathway of the proteins, found by our comparative phylogenomic analysis, is indicated. (B) 3D structured illumination microscopy (SIM) images of FtsZ-rings in *M. smithii* immunolabeled with a specific anti-*M. smithii* FtsZ antibody. The left panel shows a nondividing cell with one Z-ring, and the right panel shows a cell where division is almost completed with three Z-rings, two corresponding to the prospect division plane of the daughter cells. (C) Fluorescent images of SepF–GFP and FtsZ1– and FtsZ2–mCherry signal in *H. volcanii*. The left panel shows multiple SepF–GFP rings in tilted cells, the right upper panel shows the localization of FtsZ1–mCherry and SepF–GPF, and the lower row shows FtsZ2–mCherry and SepF–GPF localization. (D) A dividing *H. volcanii* cell. FtsZ1 and FtsZ2 colocalize as two rings at the division plane at mid-cell. SepF anchors FtsZ2 to the membrane, whereas the anchor for FtsZ1 is unknown (dark gray protein with question mark). The newly synthesized S-layer (light gray) is incorporated at mid-cell via the Sec pathway and anchored to the lipids by the archeosortase ArtA and assisting proteins PssA and PssD. FtsZ2 is involved mainly in cell constriction whereas FtsZ1 is most likely at the central interface between cell division, the S-layer synthesis, and the cell-shape determination coordinated by CetZ1. Scale bars are 1 μm in B and 2 μm in C. Abbreviation: IM, inner membrane.

proposed that the distinct SepF/FtsZ binding interface of archaea may reflect ancestral features, while the bacterial SepF/FtsZ binding interface diverged to accommodate the emergence of the more complex divisome machinery and of other proteins required for coordinating cell division and peptidoglycan (PG) cell wall growth [8]. However, many questions remain open, notably: what localizes SepF to the division site before FtsZ, and what are the other components of the *M. smithii* divisome?

H. volcanii – two-FtsZ-based cell division

The halophilic euryarchaeon *H. volcanii* is one of the most studied archaeal model organisms with a large set of genetic tools and methods developed for both *in vivo* and *in vitro* studies [40] (Boxes 1 and 2). Like most archaea, *H. volcanii* encodes two phylogenetically distinct FtsZ homologs, FtsZ1 and FtsZ2, alongside a large variety of other tubulin homologs [13,14] (Figure 1). Although initial localization studies of archaeal FtsZs were conducted as early as the 1990s [28,41,42], breakthroughs in understanding the *in vivo* role of these two FtsZs have been provided only recently [13]. FtsZ1 and FtsZ2 both colocalize as a ring to mid-cell (Figure 2C,D) and they are both essential for proper cell division. Deletion of one or both FtsZs leads to severe cell division defects, including an enlarged cell volume, cell shape defects, and

accumulation of DNA, but the mutants remain partially viable. FtsZ1 was shown to form a ring in the absence of FtsZ2, whereas FtsZ2 could polymerize into a ring only in the presence of a functional FtsZ1, suggesting that FtsZ1 plays a major role in scaffolding the Z-ring. However, cell constriction appeared to be dependent on the presence of FtsZ2, as FtsZ1 alone was not sufficient to initiate it. Additionally, mutations that affected the GTPase activity of FtsZ2 affected the ability of FtsZ1 to form condensed rings, revealing an intricate feedback between the interaction of FtsZ1 and FtsZ2 [13].

Another recent study revealed that the SepF homolog present in *H. volcanii* localizes together with FtsZ1 and FtsZ2 at mid-cell (Figure 2C,D) [36]. Although SepF is an essential gene in *H. volcanii*, and deletion mutants could not be generated, SepF depletion experiments using an *in situ* conditional expression system showed severe cell division defects similar to the FtsZ mutants. Interestingly, although FtsZ1 seems to direct the assembly of the Z-ring [13], SepF was shown to interact only with FtsZ2, suggesting that SepF is a membrane anchor for FtsZ2, and that another yet unidentified membrane anchor could exist for FtsZ1 [36]. Additionally, following SepF depletion, FtsZ2 did not form as many rings as FtsZ1 and was mainly localized towards the poles of the filamentous cells, which could not properly divide. These results suggest that the depletion of SepF limits the number of FtsZ2 rings and/or the integration of FtsZ2 to the FtsZ1 ring, and that the few localized FtsZ2 rings are not efficient in cell constriction, implying that the role of FtsZ2 in constriction may depend on the presence of SepF.

Apart from proteins directly acting at the divisome, control of cell division may also occur at the level of transcriptional regulation. Remarkably, in many archaeal genomes, the *ftsZ2* gene is part of an operon with the gene coding for the transcriptional regulator CdrS [8,43,44]. Recent studies in *H. volcanii* [44] and *Halobacterium salinarum* [43] have shown that CdrS is required for proper cell division and control of FtsZ2 expression but does not affect FtsZ1 levels in *H. salinarum* and only moderately affects FtsZ1 levels in *H. volcanii*. *H. volcanii* cells depleted of CdrS showed a characteristic cell division phenotype with misshaped and enlarged cells. In *H. salinarum,* cells lacking *cdrS* continued to elongate, but division occurred asymmetrically, revealing that perturbation of FtsZ2 expression results in a misregulation of cytokinesis. This further supports the essential role of FtsZ2 in constriction [13]. Additionally, it was shown in *H. salinarum* that FtsZ2 levels increase throughout the exponential phase and diminish in the stationary phase, whereas FtsZ1 fluctuations are more subtle along the growth phases, suggesting regulation of division via the regulation the FtsZ2 levels [43].

Finally, unlike in most bacteria, where FtsZ is essential, both FtsZ1 and FtsZ2 can be deleted in *H. volcanii* [13], individually and together, and despite severe cell division phenotypes, the cells maintain some viability. This might suggest the existence of an alternative, and perhaps ancient, FtsZs-independent division mechanism that could be linked to the absence of a rigid cell wall in most archaea.

Coordination of archaeal cell growth and shape with cell division

Archaea, just like bacteria, must grow and constrict their cell envelopes to divide. Different models of cell growth, depending on the parameter that regulates cell size homogeneity in the population, have been proposed [45]: the sizer model in which cells divide only after reaching a certain size (fission yeast), the timer model, in which cells divide after a certain time of growth (*C. crescentus*), and the adder model, in which cells divide after a certain increase in cell size (e.g., cell volume, cell surface area) independent of their initial size (*E. coli, B. subtilis,* budding yeast). Recently, single-cell studies of different growth parameters of the euryarchaeon *H. salinarum* have shown that cells divide after growing a certain length, consistent with the adder model [46].

During cell growth, the new cell wall needs to be synthesized. In bacteria, the mesh-like PG layer is an essential cellular component that provides cell shape and mechanical strength to resist, for example, osmotic challenges [47]. Spherical and coccoid bacterial species like *S. aureus* integrate the new PG only at the septum [48,49] whereas, in rod and vibrioid bacteria, in addition to septal new PG synthesis, a phase of lateral elongation occurs before division. This stage involves many proteins that are part of the macromolecular complex called the elongasome. In *E. coli*, *B. subtilis*, and *C. crescentus,* the actin homolog MreB, the main protein of the elongasome, interacts with conserved inner-membrane proteins (MreC, MreD, RodZ) as well as lipid II synthesis enzymes (MraY, MurG) [24,47] and guides the directional movement of the Rod complex (MreB, RodZ, RodA, PBP2) for lateral PG insertion into the cell wall (for recent reviews about the role of MreB and its importance for cell shape see [50,51]).

Methanobacteriales (which includes *M. smithii*) and Methanopyrales are currently the only archaeal orders in which a cell wall made of pseudopeptidoglycan (pPG) has been identified (Figure 1). The overall structure of archaeal pPG is similar to bacterial PG but shows significant differences in its composition, namely, the oligosaccharide backbone is composed of L-N-acetyltalosaminuronic acid with a β-1,3 linkage to N-acetylglucosamine, and the stem peptide consists only of L-amino acids [52,53] (Figure 2A). These differences make archaeal pPG resistant to β-lactam antibiotics, most lysozymes and proteases [54]. Based on biochemical experiments [52] and more recently on genome analysis [55], a potential biosynthetic pathway for pPG synthesis was proposed more than two decades ago. Consistent with this, genomes of members of the Methanobacteriales and Methanopyrales encode homologs of bacterial lipid-II precursor synthesis proteins (MurC, D, E, Ddl, and MraY), which are located in a conserved gene cluster [55,56], and furthermore encode homologs of the lipid-II flippase (MurJ) (Figure 2A) [55]. It is likely that pPG cell wall synthesis was acquired via horizontal gene transfer from bacteria in the ancestor of Methanopyrales and Methanobacteriales, although an earlier origin followed by multiple losses in archaea has been recently proposed [56]. Moreover, the functions of these enzymes should still be experimentally validated, and the pPG remodeling enzymes such as hydrolases, glycosyltransferases, and transpeptidases have not yet been identified.

Interestingly, all pPG-bearing archaea, including *M. smithii*, also possess *bona fide* homologs of the bacterial MreB [20] (Figure 1), which may also have been acquired through horizontal gene transfer. Although nothing is known about the function of archaeal MreB, its exclusive presence in pPG-bearing archaea suggests that, similar to its function in bacteria, it might be involved in cell shape maintenance and in guiding the pPG synthesis machinery during growth (Figure 2A). This hypothesis, however, remains to be experimentally validated. Also, no other components of the elongasome have been identified in archaea so far, suggesting the existence of a different, but yet to be discovered, machinery. Interestingly, bacterial spherical species (e.g., *Staphylococcus, Streptococcus*, *and Deinococcus* [48]*)* do not possess MreB, *questioning the role of the MreB homolog in the ovococcoid M. smithii.* The mode of growth and cell shape maintenance, and its coordination with cell division in *M. smithii*, remain a priority in future studies.

However, only a minority of archaeal species contain a pPG layer, and most archaeal cell envelopes are constituted of a proteinaceous surface (S-) layer, which forms a 2D crystal-like layer around the cells [53]. Other cell-envelope types are found sporadically across archaeal lineages (Figure 1) (for a recent review on archaeal cell envelopes see [53]). For example, in some archaea a second outer S-layer or a protein sheath can be found, and some members of the Thermoplasmatales have a flexible protective coat called the glycocalyx along with an S-layer [57]. Many methanogenic and halophilic members of the Euryarchaea have a polysaccharide

layer that can be composed of either glutaminylglycan [58], heterosaccharide [59,60], or methanochondroitin [61]. The *H. volcanii* cell envelope consist of only the S-layer glycoprotein (SLG) (Figures 1 and 2D) [62,63]. Although many studies have described the pathway for S-layer glycosylation [64] and its lipid anchoring to the cell membrane [65,66], little is known about the dynamics of its synthesis during growth and division. Recently, the archaeosortase ArtA, essential for the lipidation of the S-layer [67] and its assisting proteins PssA and PssD, which are proposed to be involved in lipid biosynthesis [68,69], have been shown to localize as a band at mid-cell together with the newly synthesized SLG, and to rapidly relocalize to the division plane of the daughter cells after cytokinesis [69]. Another study showed that the loss of ArtA, and therefore proper lipidation of the S-layer, induces a reduced growth of the cell that might be due to a thicker and weaker S-layer as observed by transmission electron microscopy [67]. Cells devoid of ArtA did not show severe morphological defects as, for example, FtsZ mutants, but showed an overall reduced length of the rod-shaped cells compared with the wild-type (WT) strain [67]. Similar phenotypes are also observed in *pssA* and *pssD* deletion mutants, suggesting that the ArtA/PssA/PssD-dependent lipid anchoring of the S-layer protein is essential for proper cell envelope growth and cell elongation but not directly for cell division [69]. These results strongly suggest that cell growth happens from the division plane and that ArtA, PssA, and PssD are part of a cell-elongation pathway in *H. volcanii* (Figure 2D) that most likely interacts with the divisome.

Generally, the synthesis of the new cell envelope and cell constriction must be spatiotemporally coordinated. In bacteria, recent studies in *E. coli* and *B. subtilis* have shown that FtsZ treadmilling around the circumference of the cell facilitates PG synthesis at the division site [5,70,71]. However, this is less clear in coccoid bacteria, such as *S. pneumoniae*, where the PG synthesis rate does not correlate with FtsZ treadmilling [72,73]. So far, no direct link between archaeal FtsZs and envelope synthesis has been shown but several observations point toward a role (direct or indirect) for FtsZ1 in this process. Intriguingly, in *H. volcanii*, FtsZ1 and FtsZ2 have different impacts on cell shape [13]. Overexpression of FtsZ1 induced the formation of spindle-like cells and, in contrast to FtsZ2-deficient cells, almost no filamentous cells were observed in the absence of FtsZ1. This suggests that FtsZ1 could play a major role in coordinating cell growth and envelope synthesis, cell shape determination, and division. Recently, CetZ1, a member of a new archaeal tubulin family, has been correlated with cell shape in members of the Euryarchaea and is involved in cell shape transition (Box 3), especially the formation of rods [32]. Indeed, CetZ1 mutants cannot form rods under the conditions tested, and therefore CetZ1 seems to be an essential protein in cell shape development in the Euryarchaeota [32]. Remarkably, CetZ1 transitionally localizes to mid-cell [32], suggesting that the mid-cell area could be a central hub for cell growth, cell division, but also cell shape determination (Figure 2D) [13]. The pathways and players underlying an interplay between FtsZ-dependent division, SLG secretion, and ArtA-dependent SLG lipidation, and CetZ1 cell shape determination as well as the sequential order of these events, await further studies. Interestingly, in *H. salinarum cetZ1* and *ftsZ2* are among the genes that are regulated by the transcriptional regulator CdrS [43], further underlining the likely coordination and intricate regulation of cell shape, growth and division in archaea.

Presently, little is known about the synthesis pathways of SLG or pPG in *H. volcanii* and *M. smithii*, respectively, nor about their role in cell shape determination, the proteins involved in these processes, and how this is coordinated with cytokinesis. However, due to their striking differences in envelope composition and their different FtsZ-based cytokinesis mechanisms, research on these topics in *H. volcanii* and *M. smithii* will provide major insights into two differing archaeal cell growth and division processes.

Box 3. Haloarchaea: cell shape variability and cell division

In addition to ensuring cell volume conservation during growth, cell shape maintenance is an equally important part of the cell growth process. Interestingly, many Haloarchaea have been shown to undergo a growth-phase-dependent cell shape transition during their life cycle [105,116,117]. During the first phase, starting from early log and extending more or less into the exponential phase depending on the species, cells are rods. This is a cellular shape that seems to coincide with a motile phase in all the organisms tested. Then, the cells start to transition to a disk-like cell shape until the population is composed of only plate cells of a smaller volume in stationary phase. It must be noted that many other cell shapes have been observed, together or without the rods and plates, including all the shapes between a perfect rod and a perfect disk or even triangular or squared cells which reflect the high malleability of Haloarchaea cells in general. Cell shape variability is probably linked to the observed noisier growth, size distribution, and division site placement compared with bacteria [46]. To ensure the correct placement of the division plane, cells must have developed sensing strategies to probe their own shapes and organization.

Currently, little is known about proteins involved in the spatial organization of archaeal cells. Through live imaging and prediction models in *H volcanii* and *Haloarcula japonica*, the division plane placement has been shown to be consistent with a Turing pattern [118] similar to MinD localization patterns involved in the Z-ring placement in bacteria. In *H. volcanii*, MinD4 has been shown to oscillate from pole to pole in rod cells and to localize the motility machineries but not the FtsZ ring [37], but future studies might reveal that other proteins are localized via the MinD4 Turing pattern.

This variation in cell shape in Haloarchaea raises intriguing questions about how cell shape and cell poles are determined when cells transition from disks to rods and vice versa, how cell-division mechanisms can cope with such shape variation, and what is the link between cell division and cell shape mechanisms and regulation.

Archaeal cell biology studies for understanding the origin of cell division mechanisms

Although mainly focused on a handful of model organisms, cell division in bacteria has been studied for many decades. By contrast, we are only beginning to scratch the surface of this area of research in the archaea. FtsZ [8,13], and its membrane anchor SepF [8,36], represent the key conserved elements between the two prokaryotic domains, indicating that they have an ancient evolutionary origin [31] and were likely part of a minimal division system in the LUCA. In this respect, further research into archaeal cell division and its comparison with bacterial mechanisms will bring key insights into our most ancient past. Indeed, archaea seem to have developed different cell division strategies and do not have the vast majority of the bacterial divisome and elongasome components [24,25]. It has been proposed that a main event in evolution was the duplication of FtsZ, leading to two copies [8,13] which appear to have distinct functions and regulation (as shown in *H. volcanii,* [13]). In comparison to bacteria with a single FtsZ and cell wall, the emergence of this second FtsZ copy in archaea may be linked to characteristic features of the cell envelope such as the presence of an S-layer [13]. Interestingly, during the evolution of archaea, FtsZ2 was lost in only a few clades, in particular the pPG-bearing methanogens (Methanobacteriales and Methanopyrales, including *M. smithii*) [13]. It is tempting to speculate that such loss was linked to the emergence of a cell wall structurally and functionally homologous to the bacterial cell wall, a striking event of convergent evolution that occurred only once in the archaea and surely had important consequences on cell shape and division.

Given its systematic co-occurrence with FtsZ (Figure 1) [7,8] and its essentiality in *H. volcanii* [36], SepF is most likely an essential protein of the archaeal divisome. However, its role and interaction with archaeal FtsZ seem to differ in the two archaeal FtsZ-based cell division models. In *M. smithii* SepF interacts with the only FtsZ (FtsZ1) and is most likely the major membrane anchor of the entire divisome [8], while in *H. volcanii* SepF interacts with FtsZ2, but not FtsZ1, and is proposed to anchor it to the divisome and/or to assist in its constriction activity [36]. Finally, some archaea have lost FtsZ altogether and replaced it with an ESCRT-III-based system [7]. Further studies of the lineages that encode homologs of both systems, such as Thaumarchaeota and Asgard, will bring new insights into this important transition.

Despite all the recent data on the role of archaeal FtsZ in cell division, many questions remain unanswered. For example, it is unknown whether archaeal FtsZ, as in bacteria, is capable of treadmilling, and whether there is a direct link between archaeal FtsZ and cell wall synthesis [70,71]. Furthermore, in analogy to bacteria, the archaeal divisome must include proteins that positively and negatively control the function of the one or two FtsZ(s). However, FtsZ-containing archaea lack most of the bacterial divisome protein homologs. Therefore, currently unidentified proteins are expected to be part of the archaeal divisome. As bioinformatical searches for archaeal counterparts of bacterial divisome proteins have their limits, large-scale studies of essential genes, cell-division mutants, and cell-division regulators, such as CdrS and their targets [43,44], will need to be performed to identify these proteins. In parallel to cell growth and cytokinesis, cell division implies proper transmission of the genome to the daughter cells. Not much is known on how replication and chromosome segregation are coordinated with cell elongation and division, and what proteins are involved in these processes. Finally, many players in the cell cycle of archaea remain to be uncovered, especially in the Euryarchaeota where it has not been as well characterized as in the Crenarchaeota [74,75]. Concerning cell-walled archaea, the specific machinery involved in pPG synthesis and modification (hydrolases, peptidases) remains to be identified.

Concluding remarks

The recent discoveries highlighted in this review underline the variety and originality of cell division mechanisms in the archaea. Our current knowledge of archaeal FtsZ-based division suggests two different mechanisms: the most widespread two-FtsZ-based system, as in *H. volcanii* [13,36] and *H. salinarum* [43], and the one-FtsZ-based mechanism as in *M. smithii* [8] which shares more similarities with bacterial FtsZs. The increasing knowledge of archaeal cell division systems can also initiate new insights in bacterial cell division systems. An important example of this is that the first crystal structure of an FtsZ protein, obtained as early as 1998 [76], was from an archaeon (FtsZ1 from *Methanocaldococcus jannashii*), which led to major discoveries in the bacterial cell division field.

To conclude, continuous efforts in further developing existing and new archaeal model organisms (Box 1) with the elaboration of new imaging techniques and methods suitable for extremophilic archaea (Box 2) will certainly lead to major discoveries and new paradigms to fill the current gaps in our knowledge of archaeal cell biology (see Outstanding questions).

Outstanding questions

The recent findings described in this review provide foundations for new discoveries in archaeal cell biology. Although they shed some light on the variety of archaeal cell division, many questions need further investigation. Amongst these are:

- What regulates the placement of the Z-rings at mid-cell in archaea?
- Does archaeal FtsZ treadmill as in bacteria?
- How is cell envelope constriction promoted in archaea?
- What are the other components of the archaeal divisome?
- What are the archaeal hydrolases and peptidase involved in modification of the pPG cell wall?
- How are cell envelope synthesis, growth, cell division, and chromosome segregation coordinated?
- Is the archaeal MreB homolog involved in cell shape in cell-walled methanogens?

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Declaration of interests

No interests are declared.

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