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# Split decision: A thaumarchaeon encoding both FtsZ and Cdv cell division proteins chooses Cdv for cytokinesis

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## Abstract

Cytoskeletal proteins play a pivotal role in cytokinesis in prokaryotes and eukaryotes. Most bacteria and a major branch of the archaea called the Euryarchaeota harbor a tubulin homolog, FtsZ, which assembles into a dynamic polymeric ring structure required for cytokinesis. However, Crenarchaeota, another branch of the archaea, lack FtsZ and instead use Cdv proteins, which are homologs of the ESCRT-III-like system involved in vesicular sorting and cytokinesis in eukaryotes, for cell division. Recently, a group of Crenarchaeota that grow in non-extreme environments was found to be sufficiently divergent to warrant its own branch of the archaea called the Thaumarchaeota. Notably, Thaumarchaeota have both Cdv and FtsZ homologs, which begs the question of which system is used for cell division. In this issue of *Molecular Microbiology*, Pelve and colleagues tackle this question. They found that cells of the thaumarchaeon *Nitrosopumilus maritimus* likely divide using the Cdv system and not FtsZ, based on localization of Cdv proteins but not FtsZ to division sites. The authors also provide evidence that the cell cycle during growth of *N. maritimus* differs significantly from those of other archaea.

Despite the diversity represented by the three domains of life, surprisingly few distinct modes of cytokinesis exist. In the Eukarya domain, for instance, nearly all animal and fungal cells use an actin-myosin ring to separate daughter cells (Pollard, 2010). Plant cells use actin as well as microtubules to position a cell plate at the division site but not for driving growth of the cell plate (Smith, 1999). Many bacteria and some archaea also use tubulin, in the form of the FtsZ protein, for cytokinesis (Margolin, 2005). However, Crenarchaeota possess neither tubulin nor FtsZ homologues, and at least one genus, *Sulfolobus*, uses a completely different system for cytokinesis called the Cdv system (Cell division) (Bernander & Ettema, 2010). Two of the three Cdv proteins share significant homology with the eukaryotic endosomal sorting complex required for transport (ESCRT) system, a complex of proteins that act to pinch membranes, which are involved in formation of multivesicular bodies, virus budding, and the final abscission stage of cytokinesis in eukaryotic cells (Saksena & Emr, 2009, Guizetti *et al*., 2011). Specifically, archaeal proteins CdvB and CdvC resemble the eukaryotic ESCRT-III and Vps4 proteins, respectively, whereas the CdvA protein does not have a eukaryotic counterpart (Lindas *et al*., 2008, Samson *et al*., 2008).

Recently, an additional branch of archaea was proposed, called Thaumarchaeota (Brochier-Armanet *et al*., 2008). Among the many intriguing surprises from characterizing organisms in this branch was the discovery of both *cdv* and *ftsZ* in their genomes. In this issue, Pelve et al. test experimentally whether the thaumarchaeon they study, *Nitrosopumilus maritimus*, uses the Cdv system, FtsZ, or both to divide its cells. They find that Cdv seems to be active in cytokinesis but FtsZ is not, suggesting that this may be true for other members of the

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Thaumarchaeota. They also characterize the cell cycle of *N. maritimus* and identify key differences from the cell cycle parameters of Crenarchaeota.

Organisms belonging to the Archaea domain were historically classified, along with bacteria, as members of the now outdated prokaryotic kingdom Monera. Although archaea share morphological similarities with bacteria, the evolutionary lineage of archaea is distinct from bacteria, and many of the molecular mechanisms used by archaea more closely resemble those found in eukaryotes. These differences prompted the separation of archaea and bacteria into two separate domains (Woese & Fox, 1977). The Archaea domain encompasses two major phyla, the Crenarchaeota and the Euryarchaeota, however additional phyla, including the Thaumarchaeota phylum, have been proposed (Woese *et al*., 1990, Brochier-Armanet et al., 2008). The Thaumarchaeota phylum consists of archaeal species that were previously classified as mesophilic and psychrophilic crenarchaea. Creation of this new phylum was recommended based on gene content analyses and phylogenetic evidence that suggest hyperthermophilic crenarchaea and euryarchaea emerged later in evolutionary history than thaumarchaea (Brochier-Armanet et al., 2008).

The presence or absence of *ftsZ* and *cdv* genes correlates well with archaeal phyla. Most Euryarchaea, for example, encode FtsZ but not the Cdv proteins, whereas all Crenarchaeota characterized to date lack FtsZ and, except for the Thermoproteales branch, contain Cdv proteins (Fig. 1A). Thaumarchaeota differ further, encoding both FtsZ and Cdv proteins, thus raising the question of which is used for cytokinesis in these organisms. To address this, Pelve *et al*. asked where the Cdv proteins localized in *N. maritimus* cells by using immunofluorescence microscopy with antibodies raised against CdvA, CdvC, and three CdvB homologues. They found that CdvA and CdvC localized to the cell midpoint between segregated chromosomes, mimicking the pattern observed in *Sulfolobus acidocaldarius* and *Sulfolobus solfataricus* (Lindas et al., 2008, Samson et al., 2008), as well as the typical medial FtsZ ring in bacteria (Bi & Lutkenhaus, 1991) and euryarchaea (Wang & Lutkenhaus, 1996). In addition, two of the three CdvB homologues were detected almost exclusively in cells with segregated chromosomes, although the CdvB proteins did not localize specifically between the chromosomes in these cells. In support for the Cdv system as a protein machine for cytokinesis, cells staining for CdvB also stained for CdvA and CdvC, whereas cells that lacked detectable staining for CdvB also lacked staining for CdvA and CdvC.

When Pelve et al. then checked for FtsZ staining in *N. maritimus* cells using immunofluorescence with anti-FtsZ, they saw strong fluorescence intensities, but the staining was generally uniform, with no FtsZ banding patterns between segregated chromosomes as observed with CdvA and CdvC. Moreover, FtsZ staining was observed in a majority of cells regardless of cell cycle state. Together, these results suggest that *N. maritimus* cells divide using the Cdv system and not FtsZ (Fig. 1A).

Pelve *et al*. also provide the first cell cycle characterization of a thaumarchaeon. Using flow cytometry and theoretical simulations, the authors discovered that *N. maritimus* cells display a long $G_1$ phase and a relatively short post-replicative phase. This is in sharp contrast to the cell cycle organization of crenarchaeal species, which typically have a short $G_1$ phase and long post-replicative phase. The rate of chromosome replication calculated for *N. maritimus* was also much slower than the rates observed in crenarchaea. The authors hypothesize that the differences in cell cycle organization and replication rate may be attributed to the distinct lifestyles of the Thaumarchaeota relative to the Crenarchaeota. Thaumarchaeota live in extremely oligotrophic environments in the deep sea, and *N. maritimus* has an impressively slow 33-hour generation time, which could explain the skew in its cell cycle parameters. In any case, the differences in cell cycle and cell division mechanisms between *N. maritimus*

and *Sulfolobus* species further support the separation of these organisms into two separate phyla.

The current study of *N. maritimus* cell division and cell cycle properties addresses an important question in archaeal cell biology, yet many puzzles remain. Notably, what function, if any, does FtsZ perform in *N. maritimus* cells? One clue comes from the observation that FtsZ orthologs from *N. maritimus* and other Thaumarchaeota have divergent carboxy termini relative to other FtsZs, including those from their nearest euryarchaeal relatives (Fig. 1B). The short C termini are highly negatively charged, perhaps to form electrostatic bonds with other proteins. In addition, and perhaps more importantly, whereas FtsZ and tubulin are characterized by a highly conserved GGGTG(S/T)G signature sequence in the T4 loop for GTP binding that is crucial for FtsZ polymerization, *N. maritimus* FtsZ has AGKAGSA at this position instead (Fig. 1B). Other thaumarchaeal FtsZs have similarly divergent tubulin/FtsZ signature sequences. This suggests that thaumarchaeal FtsZ proteins may not form GTP-dependent filaments or need extra factors to form them, which might explain the lack of an FtsZ banding pattern in the immunofluorescence experiments.

What might a non-ring forming FtsZ do? One possibility is to function in chromosome segregation. Support for this idea comes from the activity of a homolog of FtsZ called TubZ, which helps drive plasmid segregation in *Bacillus thuringiensis* and other related *Bacillus* species (Ni *et al*., 2010, Larsen *et al*., 2007). Assuming that *N. maritimus* FtsZ can assemble into transient mitotic filaments for only a short time, particularly given the short post-replicative phase of the cell cycle, it may have made localized FtsZ hard to detect. Another possibility is that FtsZ is involved in cell wall growth of *N. maritimus*, as it is in bacteria (Varma & Young, 2004, Aaron *et al*., 2007). This might explain why it seems to be localized throughout the cell and not necessarily at the cell division site. In any case, it will be fascinating to unravel the different roles of FtsZ in the archaea and its interactions with the Cdv system.

Mitochondria of primitive eukaryotes and plant chloroplasts also use FtsZ in combination with dynamins to promote organelle fission, with dynamin becoming dominant over FtsZ in mitochondria of higher eukaryotes. Perhaps the same evolutionary forces were at work in selecting the Cdv system over FtsZ for cytokinesis in Thaumarchaeota and Crenarchaeota. Interestingly, some Euryarchaeota have homologs of Vps4 (CdvC) in addition to FtsZ (Makarova *et al*., 2010), and it will be illuminating to know which system these species have chosen for their cytokinesis.

The question of why the Cdv system might be preferred over FtsZ for cytokinesis in Thaumarchaeota can best be addressed by further understanding the molecular mechanism of Cdv-mediated cytokinesis. Yeast two-hybrid, pull-down, and co-crystallization studies in *Sulfolobus spp*. have uncovered a number of protein-protein interactions among its ESCRT homologues that closely resemble the associations found in the eukaryotic ESCRT system, further emphasizing the degree of conservation shared between the Cdv and ESCRT machines (Samson *et al*., 2008). Recently, Cdv proteins from *Sulfolobus* were also shown to deform liposomes, demonstrating that these proteins can indeed pinch membranes, which could facilitate constriction at division sites (Samson *et al*., 2011). Whether *N. maritimus* FtsZ can also pinch membranes like *E. coli* FtsZ (Allard & Cytrynbaum, 2009, Osawa *et al*., 2008) remains an open question. Interestingly, CdvA was recently shown to assemble into filaments on DNA in a DNA-dependent manner (Moriscot *et al*., 2011), by analogy to the bacterial DNA partition protein ParA2. As CdvA is also part of the cytokinesis machine, perhaps it acts like the bacterial FtsK protein, which coordinates cytokinesis with chromosome segregation (Bigot *et al*., 2007). These findings and those of Pelve *et al*. have

provided valuable insight into the mechanism of Cdv-based cell division in archaea. Future genetic and biochemical studies of *N. maritimus* and other archaea, particularly those that have multiple candidates for cell division proteins, should certainly yield additional surprises.

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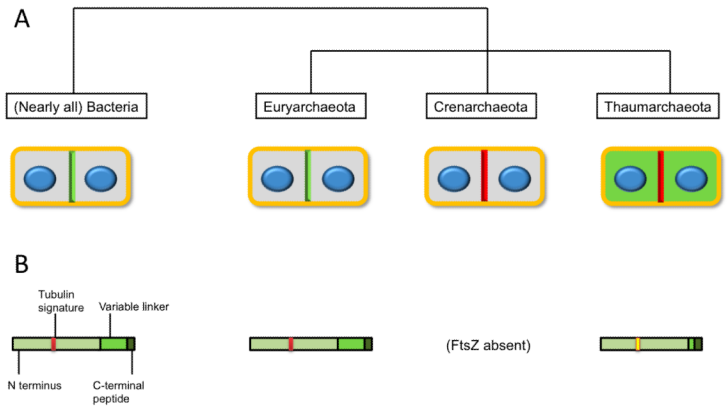


**Fig. 1.**

A. Use of FtsZ or ESCRT (Cdv) system in various prokaryotic lineages. Cells are shown at the initiation of cytokinesis, with separated daughter chromosomes in blue. A green line at the medial site of division denotes FtsZ, while a red line denotes Cdv. Green throughout the cell indicates that FtsZ is present but does not seem to localize to the site of division.

B. FtsZ protein domains present in the various prokaryotic lineages. Domains are defined in the bacterial FtsZ. The red tubulin signature motif involved in GTP binding is colored yellow for the Thaumarchaeota because of its significant divergence from consensus.