Archaeal imaging: leading the hunt for new discoveries

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ABSTRACT Since the identification of the archaeal domain in the mid-1970s, we have collected a great deal of metagenomic, biochemical, and structural information from archaeal species. However, there is still little known about how archaeal cells organize their internal cellular components in space and time. In contrast, live-cell imaging has allowed bacterial and eukaryotic cell biologists to learn a lot about biological processes by observing the motions of cells, the dynamics of their internal organelles, and even the motions of single molecules. The explosion of knowledge gained via live-cell imaging in prokaryotes and eukaryotes has motivated an ever-improving set of imaging technologies that could allow analogous explorations into archaeal biology. Furthermore, previous studies of essential biological processes in prokaryotic and eukaryotic organisms give methodological roadmaps for the investigation of similar processes in archaea. In this perspective, we highlight a few fundamental cellular processes in archaea, reviewing our current state of understanding about each, and compare how imaging approaches helped to advance the study of similar processes in bacteria and eukaryotes.

BACKGROUND The direct observation of bacteria and eukaryotes has yielded many insights into how these cells grow in given shapes, divide, and partition contents both within themselves and into their daughters. In contrast, our understanding of these same processes in archaea remains limited, even though there is a lot to explore: electron-microscopy of archaea revealed that there is a wide variety of different shapes, internal cellular organization, and previously unobserved structures (Figure 1). The lag in archaeal cell biology arises not from lack of interest but rather from challenges of imaging these extremophiles, which grow in high saline environments, extreme temperatures, or anaerobic conditions. Owing to recent technical developments in nanofabrication and microfluidics (Hol and Dekker, 2014; Wu and Dekker, 2016; Qi et al., 2017; Eun et al., 2018), some of these challenges may no longer be limiting. Within these customized fabricated microenvironments, we are now able to observe archaea grow and divide in their preferred extreme conditions. Likewise, it is becoming increasingly easy to label and observe archaeal proteins inside cells due to the development of cell-permeable photostable dyes (Grimm et al., 2017) and brighter, more photostable, and thermostable fluorescent proteins (Aliye et al., 2014; Rodriguez et al., 2017). As these innovations are combined with improvements in camera sensitivity and super-resolution microscopy, the field is poised to make huge leaps in the understanding of archaeal biology.

MAINTENANCE AND PROPAGATION OF CELL SHAPE The definition of organismal shape is a fundamental problem in biology; in most (but not all) cases, once cells define their overall geometry, they can then organize their contents within it. Microscopy has revealed that archaea encode a diversity of cell shapes, rods, squares, triangles, needlelike shapes, and nearly everything in between (Figure 1), raising the following question: What molecular processes generate and propagate these shapes? The simplest start to understanding archaeal shape formation is to watch cells grow and divide. However, even the seemingly simple
has two modes of growth; material is added either
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have been used to track the sites of insertion in fungal growth (May
36x30
otes by using labeled probes that incorporate or bind to the cell
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surface; fluorescent lectins, that bind to sugars on the cell surface
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ing growth. Are new subunits inserted all around the envelope, or is
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where new material is inserted at specific regions like the mid-cell or the
125
ing growth. Are new subunits inserted all around the envelope, or is
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morphology (Engelhardt, 2007; Jarrell
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self-assembling glycosylated proteins (Albers and Meyer, 2011; Ro
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encapsulating, tightly packed, proteinaceous array composed of
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cells are coated by a rigid monolayer structure called the S-layer, an
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that holds cells in shape as they grow and divide. Many archaeal
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samples. Adapted from Conklin et al., 2006. (E) Cryoelectron tomograph of a Thermococcus kodakaraensis cell showing a
corn basal body (bottom structure) anchoring the archaellum (top structure) to the cytoplasm. Figure adapted from
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was found that the coccoid Haloarcula japonica cells. Image adapted from Nakamura et al., 1992. (B) The square and flat Haloquadratum
278
parts. Adapted from unpublished data provided by Mike Dyall-Smith. (C) Contrast-phase of rods and “golf clubs” cells of Thermoproteus
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mediated by observing the insertion, turnover, and movement of the material
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commercial microfluidics like the CellASIC (EMD Millipore) can provide
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extremophile growth and division (Figure 2C). Microfluidics are especially useful for halophilic archaea as media evaporation and salt
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that holds cells in shape as they grow and divide. Many archaeal
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the traditional prokaryotic method of immobilizing cells under agarose pads (Eun et al., 2018), as even slight pressure caused cells to
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was unable to use material needed for growth at the cell poles (Wendland and Walther, 2017). While the S-layer is essential for cell
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Growth patterns in bacteria (Kuru et al., 2015; Pande et al., 2015). Similar pulse–chase experiments in archaea indicate they also
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and Mitchison, 1986). Likewise, gold-conjugated \( \delta \)-cysteine incorporation into bacterial cell walls allowed the discovery that Escherichia coli has two modes of growth; material is added either throughout the entire length of the cell or exclusively at the poles (de Pedro et al., 1997). More recently, pulse–chase experiments of fluorescently labeled \( \delta \)-amino acids (FDAAs) revealed several different growth patterns in bacteria (Kuru et al., 2015; Pande et al., 2015). Similar pulse–chase experiments in archaea indicate they also contain a variety of growth modes (Wirth et al., 2011); by incubating cells with dyes that react with primary amines on the cell surface, it was found that the coccoid Pyrococcus furiosus grows primarily at the division site, while the rodlike Methanopyrus kandleri adds new material everywhere along its cell length.

PROBING CYTOSKELETAL ELEMENTS INVOLVED IN CELL SHAPE AND CELL DIVISION

All domains of life use self-assembling filaments to create and propagate their shape. Fungi use actin cables or microtubules oriented along the cell length as highways for the transport of material needed for growth at the cell poles (Wendland and Walther, 2005; Chang and Martin, 2009). In most rod-shaped bacteria, insertion of new cell wall material for both growth and division is controlled by short, mobile polymers that move circumferentially around the rod width. The actin homologue MreB orients to the greatest membrane curvature (Hussain et al., 2018), constraining enzyme activity so that new peptidoglycan is built in hoops around the rod (Dominguez-Escobar et al., 2011; Garner et al., 2011; van Teeffelen et al., 2011). During cell division, the tubulin homologue FtsZ treadsills around the division site, guiding the enzymes responsible for septal synthesis (Bisson-Filho et al., 2017; Yang et al., 2017).
While several different actin and tubulin homologues have been identified in archaea (Makarova and Koonin, 2010; Yutin and Koonin, 2012; Spang et al., 2015; Stoddard et al., 2017), the dynamics and function of these polymers are mostly unknown. Similarly to the early studies of microtubules (Kirschner and Mitchison, 1986; Borisy et al., 2016), observing their dynamics in vivo and correlating these motions to the processes they control could elucidate their function. Furthermore, the internal dynamics of filaments can be probed by sparsely labeling monomers within them (also known as “speckling”), as pioneered in microtubules (Waterman-Storer et al., 1998). As recently demonstrated by studies of FtsZ in bacteria, combining both of these approaches can lead to new insights; while fully labeled FtsZ filaments move directionally around the cell (Figure 3B), single monomers are immobile, indicating that this directional motion arises via filament treadmilling (Bisson-Filho et al., 2017; Yang et al., 2017).

Archaea also have tubulin homologues, including the FtsZ and CetZ families (Aylett and Duggin, 2017). While FtsZ is widespread among prokaryotes, CetZs appears to be unique to archaea. Interestingly, most archaeal genomes contain multiple ftsZ and cetZ homologues (Vaughan et al., 2004). The FtsZ1 homologue in the archaeon *Haloferax volcanii* localizes to the division site (Figure 3A) (Duggin et al., 2015). However, it is not known whether FtsZ2 and other homologues also localize to the division site, and if so, whether they coassemble with FtsZ1 into one filament or whether it forms independent structures recruited to the division site at different stages of the cell cycle. It is also not known whether, like their bacterial counterparts, archaeal FtsZ filaments treadmill or whether their dynamics regulate S-layer insertion. Interestingly, CetZ also localizes to the division site in *H. volcanii* but is not involved in cell division; rather CetZ appears to be required for both the rod shape of cells and their motility (Duggin et al., 2015). This suggests CetZ filaments may control where the cell adds new S-layer material for growth, a hypothesis that could be further investigated by correlating the localization and dynamics of CetZ during the transition to rod shape from other morphologies.

The most extensively studied archaeal actin homologue is crenactin (Lindås et al., 2014; Izoré et al., 2016). The presence of crenactin is correlated with rodlike shapes (Ettema et al., 2011). Furthermore, immunofluorescence of crenactin in the archaeon *Pyrobaculum calidifontis* shows spiral structures (Figure 3C), again hinting at a potential role in cell-shape control. This could be clarified by live-cell imaging of crenactin filaments; if crenactin and bacterial MreB are functionally equivalent, then crenactin filaments might show a directional motion linked to the insertion of S-layer (Figure 3D).

Imaging the in vivo dynamics of archaeal polymers faces challenges similar to their study in other prokaryotes, as these cytoskeletal filaments are close to the diffraction limit of light, often only a few nanometers thick. Imaging systems in live archaeal cells thus face the additional challenges of maintaining cell viability and providing appropriate optical access to the cell. Recent advances in microfluidics and live-cell imaging have enabled the study of archaeal cell dynamics in vitro and in vivo, providing new insights into the role of actin and tubulin homologues in archaeal cell biology. However, the full extent of the role of these polymers in the regulation of cell shape and motility remains to be elucidated.
FIGURE 3: Archaeal machineries compared with bacterial and eukaryotic systems under the microscope. (A) Midcell localization of FtsZ1-GFP in the archaeon *H. volcanii* cells. Adapted from Duggin et al., 2015. (B) Localization and dynamics of bacterial FtsZ filaments. Left, three-dimensional structured illumination microscopy (3D-SIM) maximum intensity projection (MIP) of mNeonGreen-FtsZ in the bacterium *Bacillus subtilis*. Center and right, TIRF-SIM time-lapse showing FtsZ filaments moving directionally inside and outside Z rings. Images are 10 s apart (unpublished data). Arrows indicate the direction of the motion. (C) Immunofluorescence showing Crenactin filaments in *Pyrobaculum calidifontis* cells. Note the similarity with the MreB filaments in D. Adapted from Ettema et al., 2011. (D) Localization and dynamics of bacterial MreB filaments. Left, 3D-SIM MIP of MreB-HaloTag-JF549 in *Bacillus subtilis*. Center and right, TIRF-SIM time-lapse showing MreB filaments moving directionally around the rod circumference. Images are 5 s apart (unpublished data). Arrows indicate the direction of the motion. (E) Immunofluorescence showing the fluorescence of PCNA foci (red) over the chromosomes (blue) in Sulfolobus acidocaldarius cells (differential interference contrast [DIC]). Image adapted from Gristwood et al., 2012. (F) Time-lapse showing assembly and disassembly dynamics of DnaN-GFP in the bacterium *B. subtilis*. In minimal media, where growth is significantly slowed, predivisional cells contain two to four replication forks. Images are 60 min apart (unpublished data). Arrows indicate the location of the active replication forks reported by DnaN. (G) Localization and dynamics of mRuby-PCNA inside mammalian nuclei throughout different stages of the cell cycle. Concomitantly with DNA replication, PCNA foci appear during S-phase and then disassemble during mitosis. Note the oscillation in fluorescence from G1 through mitosis. Figure adapted from Zerjatke et al., 2017. All images were reused with permission.
few hundred-nanometers in length or often too dense to be resolved. The use of illumination minimizing super-resolution techniques like total internal reflection fluorescence structured illumination microscopy, known as TIRF-SIM (Kner et al., 2009), or minimal emission fluxes, known as MINFLUX (Balzarotti et al., 2017), may allow better resolution of the structure and dynamics of archaeal cytoskeletal elements in live cells.

TRACKING CHROMOSOME SEGREGATION

One of the most essential biological processes is the partitioning of genetic material into daughter cells. In eukaryotes, the direct observation of chromosome and microtubule dynamics revealed that microtubules not only capture chromosomes but also measure the tension across the kinetochore to ensure proper copy number (Rieder and Alexander, 1990). Likewise, tracking chromosome dynamics in bacteria has revealed that both the replication origins and the replication machinery are spatially organized (Wang et al., 2013) and undergo directional motions. In Caulobacter crescentus, the newly replicated origin undergoes a biased, directional motion from one pole to the other (Viollier et al., 2004). This motion is driven by the Par system, a frequently occurring machinery that partitions chromosomes and plasmids (Gerdès et al., 2010), and of three components: 1) parS, a DNA sequence recognized by 2) ParB, and 3) ParAs, which pull the parS sites apart.

In contrast to eukaryotes and bacteria, we have a limited understanding of DNA segregation in archaea. The one exception is Sulfolobus, which provides the only known example of active DNA segregation in archaea. Sulfolobus contains only one chromosome that, following duplication, is segregated by two proteins, SegA and SegB (Kallioma-Sanford et al., 2012). SegA is a ParA homologue, and SegB binds to specific DNA sequences (Kallioma-Sanford et al., 2012). DNA-loci labeling in concert with SegAB tracking will reveal whether the SegAB system is pulling or pushing chromosomes to opposite poles.

While Sulfolobus actively partitions its single chromosome, the vast majority of identified archaea are polyploid, some of which have up to 55 chromosomes per cell (Hildenbrand et al., 2011; Barillà, 2016), leading to the suggestion that these archaea do not need machinery to actively segregate their DNA (Malandrin et al., 1999). However, these polyploid archaea are still able to maintain a given copy number when chromosomes are reduced to low numbers (Zerulla et al., 2014), suggesting they might contain a segregation mechanism. This could be tested by fluorescently labeling DNA loci and tracking their motions (Stracy et al., 2014). Alternatively, careful quantitation and analysis of chromosome number with single molecule fluorescence in situ hybridization (Wang et al., 2016) under different growth conditions could determine whether segregation is random or controlled.

TIMING DNA REPLICATION WITH THE CELL CYCLE

While the biochemical activity of the archaeal proteins involved in DNA replication is well characterized in vitro (Barry and Bell, 2006; Ausiannikava and Allers, 2017), little is known regarding their spatiotemporal regulation inside cells. In eukaryotes, the simultaneous visualization of DNA loci and the replication machinery revealed that DNA replication only takes place during one phase of the cell cycle (Kitamura et al., 2006). In contrast, visualizing the origins and replication machinery of bacteria revealed that, in most cases, replication occurs continuously throughout the cell cycle (Gorov et al., 2009; Kuzminov, 2013).

In many of these studies, fluorescent fusions to the sliding clamp were used to report both the location of DNA replication and the number of simultaneous replication forks. The sliding clamp, present in all domains of life, is a donutlike hexamer that stabilizes the DNA polymerase during replication (Matsumiya et al., 2001). Both the sliding clamps of bacteria (DnaN; Figure 3F) and eukaryotes (proliferating cell nuclear antigen [PCNA]; Figure 3G) localize as discrete foci over the chromosomes during replication and then disassemble when DNA synthesis is completed (Gorov et al., 2009; Yokoyama et al., 2016). Fluorescent fusions to the eukaryotic PCNA give the secondary benefit of providing a readout of the cell cycle (Zerjak et al., 2017): foci appear at the beginning of S-phase and then disappear when PCNA proteins are degraded when S-phase ends (Figure 3G).

PCNA immunofluorescence in Sulfolobus shows foci similar to eukaryotic and bacterial cells. Surprisingly, these foci localize to opposite ends of the cell (Figure 3E), suggesting that DNA replication is restricted to the periphery (Gristwood et al., 2012). Fluorescent fusions to the PCNA could allow the study of both the timing and spatial organization of archaeal DNA replication. It will be exciting to determine whether, as in eukaryotes, archaeal PCNA foci reveal a cell-cycle-like oscillation, indicating a synchronous replication of chromosomes.

Visualizing DNA replication with fluorescent fusions to the PCNA may also allow us to understand how H. volcanii can not only survive but also grow faster in the absence of any replication origins (Hawkins et al., 2013). This fast-growing phenotype requires the DNA recombinase RadA, suggesting that recombination might serve an alternative route to replication initiation. Simultaneous visualization of PCNA and RadA could illuminate whether PCNA and RadA act in different phases throughout the cell cycle or whether the RadA mechanism only occurs in the absence of replication origins.

FUTURE OUTLOOK

While this review touched on a few fundamental biological processes, archaea show many other behaviors that are just beginning to be studied, such as archaeal-driven motility (Kinosita et al., 2016) and mating behavior (Rosenshine et al., 1989; van Wolferen et al., 2016). However, the archaeal community is still technically limited by what organisms can be cultivated and observed under a microscope. For instance, the development of sufficiently thermal-tolerant microscopes would facilitate the live imaging of thermophiles, some of which grow at temperatures exceeding 80°C, which is problematic for both the microscope stages and microscope objectives. Given that no commercial objectives can tolerate temperatures above 60°C, the solution could come from different fields. Material scientists have been using nanocontact (air gapped) objectives for decades, as well as isolating their stages with heating devices and ceramic chambers. These setups have allowed them to image samples at temperatures above 1000°C (Boccaccini and Hamann, 1999). Adapting this technology for archaeal cell biology could open up an entire new field containing a multitude of new discoveries.

Metagenomics is rapidly increasing the number and diversity of existent organisms (Hug et al., 2016; Spang et al., 2017), leading to the recent discovery of the Asgard superphylum. These uncultured archaea include species containing a number of machineries specific to eukaryotes (Spang et al., 2015; Zarem-Biezadwiecka et al., 2017). Once we can culture these organisms and develop methods to visualize them, their biology becomes a wide-open frontier to probe the origins of eukaryotic cellular processes.

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