technological advances as we continue to explore their physiological role in health and disease.

FURTHER READING

Chastney, M.R., Lawless, C., Humphries, J.D., Warwood, S., Jones, M.C., Knight, D., Jorgensen, C., and Humphries, M.J. (2020). Topological features of integrin adhesion complexes revealed by multiplexed proximity biotinylation. J. Cell Biol. *219*, e202003038.

Conway, J.R.W., and Jacquemet, G. (2019). Cell matrix adhesion in cell migration. Essays Biochem. *63*, 535–551.

Couchman, J.R. (2003). Syndecans: proteoglycan regulators of cell-surface microdomains? Nat. Rev. Mol. Cell Biol. *4*, 926–937.

Ezratty, E.J., Partridge, M.A., and Gundersen, G.G. (2005). Microtubule-induced focal adhesion disassembly is mediated by dynamin and focal adhesion kinase. Nat. Cell Biol. *7*, 581–590.

Green, H.J., and Brown, N.H. (2019). Integrin intracellular machinery in action. Exp. Cell Res. *378*, 226–231.

Hamidi, H., and Ivaska, J. (2018). Every step of the way: integrins in cancer progression and metastasis. Nat. Rev. Cancer *18*, 533–548.

Hoffmann, J.-E., Fermin, Y., Stricker, R.L., Ickstadt, K., and Zamir, E. (2014). Symmetric exchange of multi-protein building blocks between stationary focal adhesions and the cytosol. eLife *3*, e02257.

Horton, E.R., Byron, A., Askari, J.A., Ng, D.H.J., Millon-Frémillon, A., Robertson, J., Koper, E.J., Paul, N.R., Warwood, S., Knight, D., *et al*. (2015). Definition of a consensus integrin adhesome and its dynamics during adhesion complex assembly and disassembly. Nat. Cell Biol. *17*, 1577–1587.

Kanchanawong, P., Shtengel, G., Pasapera, A.M., Ramko, E.B., Davidson, M.W., Hess, H.F., and Waterman, C.M. (2010). Nanoscale architecture of integrin-based cell adhesions. Nature *468*, 580–584.

Kechagia, J.Z., Ivaska, J., and Roca-Cusachs, P. (2019). Integrins as biomechanical sensors of the microenvironment. Nat. Rev. Mol. Cell Biol. *20*, 457–473.

Lock, J.G., Baschieri, F., Jones, M.C., Humphries, J.D., Montagnac, G., Strömblad, S., and Humphries, M.J. (2019). Clathrin-containing adhesion complexes. J. Cell Biol. *218*, 2086–2095.

López-Ceballos, P., Herrera-Reyes, A.D., Coombs, D., and Tanentzapf, G. (2016). In vivo regulation of integrin turnover by outside-in activation. J. Cell Sci. *129*, 2912–2924.

Michael, M., and Parsons, M. (2020). New perspectives on integrin-dependent adhesions. Curr. Opin. Cell Biol. *63*, 31–37.

Moreno-Layseca, P., Icha, J., Hamidi, H., and Ivaska, J. (2019). Integrin trafficking in cells and tissues. Nat. Cell Biol. *21*, 122–132.

Schiller, H.B., Hermann, M.-R., Polleux, J., Vignaud, T., Zanivan, S., Friedel, C.C., Sun, Z., Raducanu, A., Gottschalk, K.-E., Théry, M., *et al*. (2013). β1- and αv-class integrins cooperate to regulate myosin II during rigidity sensing of fibronectin-based microenvironments. Nat. Cell Biol. *15*, 625–636.

Spiess, M., Hernandez-Varas, P., Oddone, A., Olofsson, H., Blom, H., Waithe, D., Lock, J.G., Lakadamyali, M., and Strömblad, S. (2018). Active and inactive β1 integrins segregate into distinct nanoclusters in focal adhesions. J. Cell Biol. *217*, 1929–1940.

[1]Turku Bioscience Center, University of Turku and Åbo Akademi University, FIN-20520 Turku, Finland. [2]Department of Biochemistry, University of Turku, FIN-20520 Turku, Finland. [3]Equal contribution.
*E-mail: joivaska@utu.fi

Primer

Bacterial and archaeal cytoskeletons

Yue Liu and Jan Löwe*

All living cells depend on the intricate organization of molecular components in space and time. Although this notion was historically based on eukaryotic cells, with their structured intracellular architecture and cellular morphologies, it is now recognized that prokaryotes (that is, bacteria and archaea) also possess complex structures. A cytoskeleton is a network of intracellular protein filaments that play a structural or mechanical role (such as scaffolding, pushing, or pulling) in the spatiotemporal organization of cellular processes. Polymerization of protein monomers in a roughly linear fashion into filaments represents an effective means to establish long-range spatial order by bridging the gap between nanometer-sized molecules and micron-sized cells. It is now evident that bacteria and archaea possess numerous kinds of cytoskeletal proteins, including prokaryotic homologues of the eukaryotic actins, tubulins, and intermediate filaments, as well as other types that have been found primarily or exclusively in prokaryotes (Table 1). Understanding the diverse functions and mechanisms of the rapidly growing universe of prokaryotic cytoskeletal proteins will not only advance prokaryotic cell biology and reveal evolutionary principles, but also open up new avenues for the development of anti-microbial agents, *de novo* protein design, and the construction of minimal and synthetic cells.

Cytoskeletal proteins fulfill a wide range of functions essential for the reproduction of prokaryotes and for their adaptation to specific environments. These functions include, but are not limited to, cell division, cell-shape maintenance, DNA partitioning, organization of intracellular compartments, and cell motility. A common theme underlying this functional diversity is that evolutionarily distinct bacteria and archaea, as a group, have evolved a variety of specific strategies to achieve the flexible use of cytoskeletal building blocks in diverse functional contexts. Collectively, prokaryotes both employ one type of cytoskeletal protein for different functions and utilize different types of cytoskeletal proteins for similar functions. This means that a comprehensive understanding of the principles that govern cytoskeletal functions in prokaryotes necessitates the study of diverse organisms.

Cytoskeletal proteins in bacteria and archaea can be categorized into four major types: tubulin homologues, actin homologues, coiled-coil-rich proteins (CCRPs), and bactofilins (Table 1). CCRPs include prokaryotic intermediate-filament-like proteins that are closely related to the eukaryotic intermediate filaments as well as other coiled-coil proteins that are largely specific to bacteria and archaea, as is the case for bactofilins. Functional specificity is determined in large part by the biophysical properties of cytoskeletal filaments and by their interactions with binding partners. At the filament level, filaments exhibiting dynamic polymerization properties commonly serve as linear motors to move objects. Interestingly, these 'cytomotive filaments' are currently limited to actin homologues and tubulin homologues, whose activity involves nucleotide-binding-dependent polymerization and hydrolysis-driven depolymerization (Table 1). In contrast, most nucleotide-independent filaments, exemplified by filaments of CCRPs and bactofilins, are static and primarily play scaffolding roles (Table 1). At the level of higher order assemblies, lateral contact sites on individual filaments (also called 'protofilaments') facilitate the formation of bundles through self-interactions. The association with matrix-like structures, such as lipid membranes or other protein filaments and scaffolds, yields 'collaborative filaments'. Together with their accessory proteins, these higher-order assemblies determine function-specific subcellular localization of cytoskeletal filaments and also give rise to emergent properties, such as avidity, bending, and curvature sensing. In particular, avidity often promotes the assembly

Table 1. Major types of cytoskeletal proteins in bacteria and archaea.

Type	Examples	Typical functions
Tubulin homologues[a]	FtsZ, TubZ, PhuZ	Cell division, DNA partitioning, intracellular organization
Actin homologues[a]	MreB, ParM, MamK	Cell-shape generation, DNA partitioning, intracellular organization, cell motility
CCRPs[b]	Intermediate-filament-like[b]: crescentin, FilP, Spy; other coiled-coil proteins: DivIvA, CrvA/B, CcfM, AglZ	Cell-shape generation, subcellular organization, cell motility
Bactofilins[b]	BacA, BacE, BacF, CcmA	Cell-shape generation, subcellular organization, cell motility

[a]Tubulin and actin homologues in bacteria and archaea form nucleotide-dependent filaments, most of which display cytomotive properties, such as dynamic instability and/or treadmilling, with the exception of MreB among the examples discussed in this Primer. [b]Bactofilins and coiled-coil-rich proteins (CCRPs) form nucleotide-independent filaments, which have not been shown to exhibit cytomotive properties and primarily play scaffolding or structural roles. Prokaryotic CCRPs include intermediate-filament-like proteins that are closely related to the eukaryotic intermediate filaments, and other coiled-coil proteins. Non-intermediate-filament-like CCRPs and bactofilins are mostly specific to bacteria and archaea.

of multi-protein machineries whose constitutive components, such as effector enzymes, specify the cellular function of a cytoskeletal protein. At the subcellular level, cytoskeletons operate at length scales associated with functions. Those that span a significant portion or the entirety of the cell length are often associated with global cell morphologies or with transport of intracellular components. Yet some cytoskeletons serve as multiple locally organizing centers in the form of patches of short filaments that collectively drive specific processes, including cell elongation and division.

In this Primer, we discuss how different cytoskeletal proteins and their binding partners shape a multitude of cellular functions in bacteria and archaea.

Cell division

To divide, cells often rely on a ring-like protein assembly as the central organizer of biochemical activities at the future division site. In most bacteria, filaments of FtsZ, a structural homologue of tubulin, cooperate with membrane anchors and regulators to form the ring-like structure, the Z-ring, that encircles the division plane underneath the cytoplasmic membrane (Figure 1A). The Z-ring acts as a platform for the assembly of a cytokinetic machinery, the divisome, that coordinates peptidoglycan cell-wall synthesis and cell-membrane invagination. During cell constriction, the Z-ring directs circumferential and processive movements of cell-wall-synthesizing enzymes within the divisome, enabling synthesis and remodelling of the cell wall as the septum moves inwards (Figure 1A). The divisome movements are driven by GTP-hydrolysis-coupled treadmilling of FtsZ filaments, cytomotive filaments that grow at one end and shrink simultaneously at the opposite end without moving individual monomers. Despite these established roles of bacterial FtsZ, recent studies have just begun to touch upon the functions of FtsZ in other domains of life, including archaea and eukaryotic plastids and mitochondria. For instance, many archaea possess two FtsZ proteins that appear to fulfill scaffolding and constrictive roles in cell division, respectively. A division of labor between functionally interdependent FtsZs could add a new layer of complexity to FtsZ-dependent cell division.

Unlike many eukaryotes that require motor proteins of the myosin family for cytokinesis, filament-associated motor proteins have not yet been identified in any bacteria or archaea. So, what provides the constrictive force for dividing prokaryotic cells? Although it was initially thought that FtsZ itself would generate at least part of the constrictive force through a variety of possible mechanisms, it has recently been proposed that in walled bacteria, FtsZ-guided cell-wall synthesis provides the constrictive force. It is important to highlight that several mechanisms are likely needed to act in concert to remodel the cell envelope. Thus, a shift from the FtsZ-centric perspective to a holistic, divisome-based view of cell division is anticipated to clarify these points.

Some bacteria and archaea have also evolved FtsZ-independent systems for cell division. For instance, some archaea, mostly belonging to the Crenarchaea, encode a CdvABC system, which is homologous to the eukaryotic ESCRT-III/Vps4 complexes that are notable for membrane remodelling and their role in eukaryotic cell division. The similarity of the CdvABC cell-division machinery to ESCRT-III suggests a probable archaeal origin of eukaryotic cell division. Although largely underexplored, CdvABC-dependent cell division is known to progress through a non-contractile, ESCRT-III-like CdvB ring, which plays a scaffolding role and licenses cell constriction by a contractile, ESCRT-III-like CdvB1–CdvB2 division ring. Furthermore, Chlamydiae, a group of bacteria lacking FtsZ, divide by co-opting polymers of actin-like MreB, more commonly utilized as a cell-shape determinant (see below). Similarly, some lineages of archaea are proposed to use yet another putative actin-based cell division system.

Cell-shape generation

The faithful generation and reproduction of defined cell geometries is essential for bacterial physiology (for example, during environmental colonization and motility) and for intracellular organization (as exemplified by cell polarization). Bacteria exhibit a rich diversity of cell morphologies, including rod, helical and branched shapes. The mesh-like peptidoglycan cell wall generally acts as an exoskeleton that shapes cell morphology and maintains cell integrity. Collectively, bacteria have evolved a large array of cell-shape-determining cytoskeletal proteins that

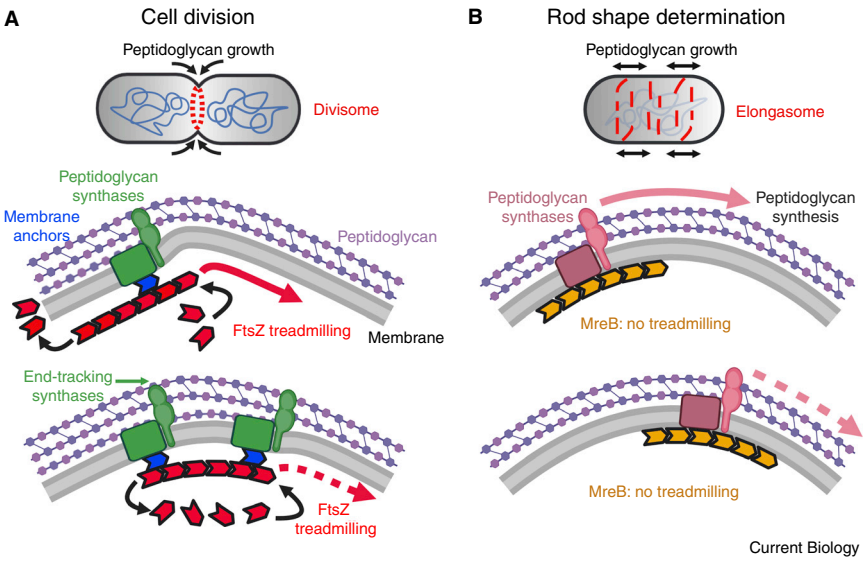

Figure 1. Cytoskeletal proteins are master organizers of cell division and cell elongation in bacteria and archaea.
The tubulin-like FtsZ and actin-like MreB form patches of short filaments that act as spatiotemporal orchestrators of biochemical activities in cell division (A) and cell elongation (B), respectively. A ring-like structure, created by FtsZ and membrane anchors, drives the assembly and circumferential motions of a multi-protein cytokinetic machinery, the divisome, at the future division site. Analogously, MreB filaments act as a platform for the formation of an evolutionarily related multi-protein complex, the elongasome, which moves around the cell width to maintain the rod cell shape during cell growth. The middle and lower panels show close-up views of the action and motions of the divisome (A) and elongasome (B). Both machineries are notable for cytoskeleton-guided peptidoglycan cell-wall synthesis and remodelling of the cell envelope. The circumferential movement of the divisome is powered by FtsZ treadmilling, in which filament polymerization and depolymerization take place at distinct ends while individual monomers stay stationary. Peptidoglycan synthases track the FtsZ filaments, allowing for the directional and processive movements of these enzymes as FtsZ filaments treadmill. Differing from the divisome, elongasome movement depends on peptidoglycan cell-wall synthesis, and its direction is guided by the intrinsic curvature of MreB filaments, which act like a membrane-curvature-sensing rudder. Dashed arrows in the lower panels indicate future moving trajectories of the respective machineries.

serve as spatiotemporal organizers of peptidoglycan remodelling.

Maintenance of a rod shape in many bacteria, including in *Escherichia coli* and *Bacillus subtilis*, requires MreB-guided cell-wall remodelling to construct the cylindrical portion of the cell during elongation (Figure 1B). In cells, discrete and submicron-sized patches of MreB filaments orient along the highest membrane curvature, perpendicular to the long axis of a rod-shaped cell. MreB filaments are intrinsically curved and hence adapt themselves to best match local membrane geometries. These patches, analogous to rudders, localize and organize peptidoglycan remodelling by recruiting peptidoglycan-metabolizing enzymes and regulatory proteins to assemble into the elongasome, a multi-protein machinery that likely shares a common evolutionary ancestor with the divisome. Differing from the FtsZ/divisome case, peptidoglycan synthesis drives circumferential motions of MreB polymers and other elongasome components around the cell width (Figure 1B). This is thought to create a local feedback between MreB localization and peptidoglycan synthesis to maintain the rod shape. Thus, global order at the cellular level emerges from the regulated coordination of biochemical activities at the molecular level.

A common way to convert the prototypical rod shape into more complex cell morphologies is to generate cell curvature. For instance, the characteristic curved-rod 'crescent' shape of the bacterium *Caulobacter crescentus* depends on an intermediate-filament-like protein, crescentin. Crescentin forms nucleotide-independent filaments that localize to the inner cell curvature where these filaments reduce peptidoglycan synthesis. This produces a spatially anisotropic pattern of cell wall growth and thus cell curvature (Figure 2A). CCRPs, including crescentin, that maintain cell curvature are widespread in curved rod-shaped bacteria (for example, CrvA and CrvB in *Vibrio cholerae*) and spiral-shaped bacteria (such as CcfM in *Magnetospirillum gryphiswaldense*). In fact, bacteria have found diverse solutions well beyond the use of CCRPs for curvature generation. For example, bactofilins represent a conserved and widely distributed family of nucleotide-independent cytoskeletal proteins with diverse functions. The bactofilin CcmA appears to be a curvature-sensing scaffold that organizes peptidoglycan-metabolizing activities in coordination with the MreB-dependent elongasome to produce the spiral shape of *Helicobacter pylori*. Despite these exciting advances, how cytoskeletal proteins curve prokaryotic cells, particularly those with complex shapes, is still poorly understood, as is the modulation of peptidoglycan synthesis by non-cytomotive cytoskeletal filaments.

Another strategy to add complexity to cell shapes is more localized morphogenesis, beyond cell curvature generation, where the action of cytoskeletal proteins is confined to specific subcellular regions. For instance, different bacteria employ evolutionarily divergent, yet functionally convergent, cytoskeletal proteins, including DivIVA, PopZ, and bactofilins that polymerize into multivalent supramolecular organizing centers at the cell poles. Polymers of DivIVA, a CCRP, localize to cell poles or growing tips presumably by sensing membrane curvature. With the aid of two intermediate-filament-like proteins (FilP and Scy), DivIVA-organized polar peptidoglycan growth is essential for branched morphologies and hyphal growth in the filamentous *Streptomyces*. Another notable example is the 'stalk', found in some Alphaproteobacteria, a thin cell appendage whose synthesis requires MreB (in *C. crescentus*) or BacA (a bactofilin in *Asticcacaulis*

biprosthecum) for the constrained peptidoglycan synthesis at the stalk base (Figure 2A). These cases highlight the power of studying evolutionarily distant species to uncover principles and novel mechanisms that underlie the function of cytoskeletons in cell morphogenesis.

DNA partitioning

The small size of most bacteria facilitates the diffusion-based, equal segregation of high-copy-number cellular components into daughter cells. Yet free diffusion is insufficient for the reliable and accurate partitioning of chromosomes and plasmids with a low copy number. Bacterial plasmids have solved this problem with specific DNA-partitioning machineries that are driven by nucleotide hydrolysis. In contrast, although molecular machineries have been discovered that enable the active transport of specific regions of bacterial chromosomes, a dedicated chromosome segregation machinery that relies on cytoskeletal proteins akin to the mitotic spindle in eukaryotes has not been identified in bacteria. Moreover, DNA partitioning in archaea remains enigmatic, though the recent discovery of eukaryote-like cell-cycle control in *Sulfolobus* suggests that this is an exciting area for future investigation. Below we illustrate general principles using two types of bacterial plasmid-partitioning systems. Both consist of a centromeric region on the plasmid, a centromere-binding protein, or 'adaptor', and a protein capable of forming cytomotive filaments.

The ParMRC system encoded by *E. coli* plasmid R1 is functionally analogous to microtubule-based spindles for chromosome segregation in eukaryotes (Figure 2B). In this system, the adaptor ParR and the plasmid centromere *parC* form ParRC complexes via multivalent interactions. Filaments of ParM, an actin homologue, exhibit ATP-hydrolysis-dependent dynamic switching between polymerization and catastrophic depolymerization. This cytomotive property of dynamic instability, first described for eukaryotic microtubules, allows ParM filaments to search for ParRC within the cell and capture it, leading to filament stabilization at one end. A bipolar spindle is formed and fully stabilized when two ParRC-bound filaments come together in an anti-parallel manner. The ParM filaments elongate where ParRC is attached such that the bipolar spindle pushes the two plasmids apart and towards opposite cell poles. Moreover, ParM belongs to a widespread family of DNA-partitioning actin-like proteins in bacteria. Among these proteins, AlfA filaments rely on AlfB-dependent filament destabilization rather than dynamic instability. This suggests that diverse regulatory mechanisms and modes of action remain to be uncovered for actin-like DNA-partitioning systems.

The TubZRC system encoded by many virulence plasmids in the genus *Bacillus* is mechanistically distinct from ParMRC. Filaments of the tubulin-like TubZ from the plasmid pBtoxis of *B. thuringiensis* have not been shown to exhibit dynamic instability. Instead, treadmilling dynamics driven by GTP hydrolysis allows for unidirectional filament growth. Rather than targeting the filament's growing end as in the case of ParMRC, centromeric TubRC complexes track the shrinking end of TubZ filaments, which hence pull on DNA. Although cytomotive TubZ filaments are required for plasmid stability, it remains unknown whether and how TubZRC segregates plasmids *in vivo*. Nevertheless, our rich understanding of TubZRC and ParMRC cytomotive mechanisms will facilitate the design of mechanical molecular devices for synthetic biology applications.

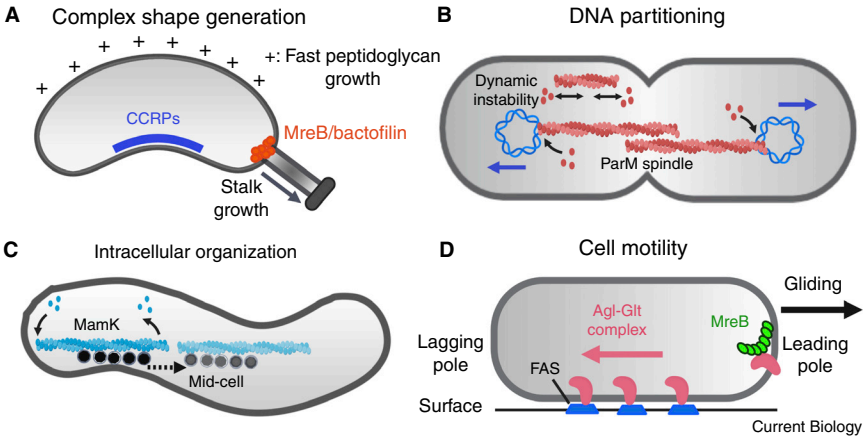

Figure 2. Representative roles of bacterial and archaeal cytoskeletons in cell morphogenesis, DNA partitioning, intracellular organization of compartments, and cell motility.
(A) Coiled coil-rich proteins (CCRPs), such as crescentin, generate a spatially uneven pattern of peptidoglycan cell-wall growth rates across the cell body, leading to cell curvature. Subcellular organizers, such as bactofilins and the actin-like MreB, drive spatially localized peptidoglycan synthesis, adding further complexity to cell morphologies. Cytoskeletal functions in (B–D) are illustrated with three different actin-like proteins in prokaryotes. (B) Freely nucleating ParM filaments dynamically switch between polymerizing and depolymerizing states, and are stabilized at one end by binding to the plasmid centromere via an adaptor, enabling the search and capture of plasmids within the cell. Two plasmid-bound ParM filaments form a fully stabilized, cell-length-spanning bipolar spindle; filament polymerization at the plasmid-bound ends then drives the partitioning of two plasmids into daughter cells. (C) MamK filaments guide the formation of linear chains of membrane-bound organelles, called magnetosomes. This process enables magnetotactic bacteria to navigate along the geomagnetic field. Cytomotive MamK filaments also facilitate the repositioning of magnetosome chains from cell poles to the future division site. (D) MreB is essential for the assembly and function of a gliding motility machinery Agl–Glt, which assembles at the leading pole and moves towards the lagging pole. The immobilization of the moving Agl–Glt machinery to external surfaces at focal adhesion sites (FASs) propels the cell forward.

Intracellular organization of compartments

Compartmentalization was once assumed to be unique to eukaryotic cells. This view has been challenged by the realization that bacteria compartmentalize specific cellular processes in many membrane-bound (for example, thylakoids for photosynthesis) or nonmembrane-bound (for example, carboxysomes for

carbon fixation) organelles. Despite the well-established roles of cytoskeletons in the function of eukaryotic organelles, it has only recently been appreciated that cytoskeletal proteins are required for the positioning, motility, maintenance, and partitioning of compartments in bacteria.

An actin-like protein, MamK, forms cytomotive filaments that organize magnetic compartments in magnetotactic bacteria such as *M. gryphiswaldense* (Figure 2C). These bacteria align to, and navigate along, the geomagnetic field by relying on linear chains of magnetosomes, membrane-bound organelles that enclose magnetic nanocrystals. MamK filaments serve as a scaffold to support the assembly of magnetosome chains. Moreover, ATPase-activity-dependent treadmilling of MamK filaments enables repositioning of magnetosome chains from cell poles to the future cell division site and equal partitioning of magnetosome chains into daughter cells. Recent data suggest that MamK cooperates with scaffold proteins to form a collaborative cytoskeleton necessary for the long-range organization of magnetosome chains.

The multifunctional phage-encoded tubulin PhuZ maintains a nucleus-like compartment in infected bacteria. Many phages of *Pseudomonas* species sequester replicating viral DNAs within a protein-bounded compartment during infection. At an early stage of infection, dynamically unstable PhuZ filaments form a bipolar spindle to position the phage nucleus-like compartment at mid-cell, facilitating viral genome replication and transcription. During late infection, treadmilling PhuZ filaments power active transport of viral capsids from the cell membrane to the surface of the nucleus-like compartment for DNA packaging. It is not clear how the different properties of PhuZ filaments, particularly dynamic instability and treadmilling, are regulated. However, PhuZ represents the first example of a non-eukaryotic cytoskeletal system that shares both cytomotive properties with eukaryotic tubulins.

Cell motility

Motility provides bacteria with multiple physiological advantages. Cytoskeletal proteins have central roles in organizing motility machineries that rely on membrane-spanning motors. In rod-shaped *Myxococcus xanthus*, a multi-protein Agl–Glt machinery, assembled at the leading cell pole, moves towards the lagging pole, where it disassembles. The attachment of Agl–Glt complexes to external surfaces at focal adhesion sites propels the cell forward (Figure 2D). MreB filaments function as an organizing center for the positioning and assembly of the gliding motility machinery by recruiting MglA, a GTPase that regulates the assembly state of Agl–Glt complexes. MreB filaments are also essential for AglZ (a filament-forming CCRP) to assemble into an array of clusters with regular spatial intervals, which mark focal adhesion sites in cells. Although detailed mechanisms of all these processes remain to be defined, it is clear that the function of MreB in cell motility is independent of its role in the elongasome. Thus, a recurring theme is the functional repurposing of the same (or same kind of) prokaryotic cytoskeletal proteins in a context-dependent manner to achieve functional diversity. As an additional example supporting this notion, bactofilins (BacE and BacF) are required for flagellar assembly and motility in *B. subtilis*, thus revealing a different bactofilin function than the aforementioned roles in cell morphogenesis and polar organization.

Perspectives

Despite the considerable progress that has been made in identifying and characterizing multiple cytoskeletal systems in bacteria and archaea, the field is arguably still young. The extreme diversity and formidable complexity of prokaryotes present significant challenges ahead. A data-driven approach combining metagenomic sequencing, genetic perturbations, and high-content, high-resolution *in vivo* imaging will further accelerate the discovery of novel cytoskeletal protein candidates, particularly in the nearly unexplored archaea, many of which have so far proven refractory to laboratory cultivation. As the definition of 'cytoskeleton' is constantly challenged by newly identified proteins, it is necessary to understand the term as being broad. Moreover, unlike the well-understood ParMRC system, our mechanistic comprehension of most prokaryotic cytoskeletal systems is far from complete. A major outstanding issue is how the physiochemical properties of these systems and their interplay with binding partners are regulated for the faithful execution of their cellular functions *in vivo*. Addressing these challenges will bring us closer to controlling and building subcellular organization across spatial and temporal scales.

FURTHER READING

Chaikeeratisak, V., Khanna, K., Nguyen, K.T., Sugie, J., Egan, M.E., Erb, M.L., Vavilina, A., Nonejuie, P., Nieweglowska, E., Pogliano, K., *et al.* (2019). Viral capsid trafficking along treadmilling tubulin filaments in bacteria. Cell *177*, 1771–1780.

Erickson, H.P. (2017). The discovery of the prokaryotic cytoskeleton: 25th anniversary. Mol. Biol. Cell *28*, 357–358.

Ghosal, D., and Löwe, J. (2015). Collaborative protein filaments. EMBO J. *34*, 2312–2320.

Grant, C.R., Wan, J., and Komeili, A. (2018). Organelle formation in bacteria and archaea. Annu. Rev. Cell Dev. Biol. *34*, 217–238.

Liao, Y., Ithurbide, S., de Silva, R.T., Erdmann, S., and Duggin, I.G. (2018). Archaeal cell biology: diverse functions of tubulin-like cytoskeletal proteins at the cell envelope. Emerg. Top. Life Sci. *2*, 547–559.

Lin, L., and Thanbichler, M. (2013). Nucleotide-independent cytoskeletal scaffolds in bacteria. Cytoskeleton *70*, 409–423.

Löwe, J., and Amos, L.A., eds. (2017). Prokaryotic Cytoskeletons (Cham: Springer International Publishing).

McQuillen, R., and Xiao, J. (2020). Insights into the structure, function, and dynamics of the bacterial cytokinetic FtsZ-ring. Annu. Rev. Biophys. *49*, 309–341.

Oikonomou, C.M., Chang, Y.-W., and Jensen, G.J. (2016). A new view into prokaryotic cell biology from electron cryotomography. Nat. Rev. Microbiol. *14*, 205–220.

Schumacher, D., and Sogaard-Andersen, L. (2017). Regulation of cell polarity in motility and cell division in *Myxococcus xanthus*. Annu. Rev. Microbiol. *71*, 61–78.

Shi, H., Bratton, B.P., Gitai, Z., and Huang, K.C. (2018). How to build a bacterial cell: MreB as the foreman of *E. coli* construction. Cell *172*, 1294–1305.

Surovtsev, I.V., and Jacobs-Wagner, C. (2018). Subcellular organization: a critical feature of bacterial cell replication. Cell *172*, 1271–1293.

Tarrason Risa, G., Hurtig, F., Bray, S., Hafner, A.E., Harker-Kirschneck, L., Faull, P., Davis, C., Papatziamou, D., Mutavchiev, D.R., Fan, C., *et al.* (2020). The proteasome controls ESCRT-III-mediated cell division in an archaeon. Science *369*, eaaz2532.

Wagstaff, J., and Löwe, J. (2018). Prokaryotic cytoskeletons: protein filaments organizing small cells. Nat. Rev. Microbiol. *16*, 187–201.

Yang, D.C., Blair, K.M., and Salama, N.R. (2016). Staying in shape: the impact of cell shape on bacterial survival in diverse environments. Microbiol. Mol. Biol. Rev. *80*, 187–203.

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK.
*E-mail: jyl@mrc-lmb.cam.ac.uk