Prokaryotic cytoskeletons: protein filaments organizing small cells

James Wagstaff and Jan Löwe

Abstract | Most, if not all, bacterial and archaeal cells contain at least one protein filament system. Although these filament systems in some cases form structures that are very similar to eukaryotic cytoskeletons, the term 'prokaryotic cytoskeletons' is used to refer to many different kinds of protein filaments. Cytoskeletons achieve their functions through polymerization of protein monomers and the resulting ability to access length scales larger than the size of the monomer. Prokaryotic cytoskeletons are involved in many fundamental aspects of prokaryotic cell biology and have important roles in cell shape determination, cell division and nonchromosomal DNA segregation. Some of the filament-forming proteins have been classified into a small number of conserved protein families, for example, the almost ubiquitous tubulin and actin superfamilies. To understand what makes filaments special and how the cytoskeletons they form enable cells to perform essential functions, the structure and function of cytoskeletal molecules and their filaments have been investigated in diverse bacteria and archaea. In this Review, we bring these data together to highlight the diverse ways that linear protein polymers can be used to organize other molecules and structures in bacteria and archaea.

Endomembrane
Membrane binding internal compartments in eukaryotic cells.

Segregation
The active partitioning of a cellular component into daughter cells at division.

Superfamily
In proteins, the largest group that can be determined to have a common ancestor.

Medical Research Council Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK.

Correspondence to J.L.
jyl@mrc-lmb.cam.ac.uk

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Eukaryotic cytoskeletons are canonically defined by their constitutive protein components: actin, tubulin and intermediate filaments. These three families of filament-forming proteins are involved in diverse cellular processes, providing long-range organization of subcellular components across broad time and length scales. In particular, protein filaments are used in eukaryotes to control cell and endomembrane morphology (dynamically and for long-term maintenance of shape), as a scaffold for long-range organization of cytoplasmic processes (including as a support matrix for cytoskeleton-associated motor proteins, which remain undiscovered in prokaryotes) and for pushing and pulling other molecules in the cytoplasm (particularly for the segregation of DNA during cell division).

The existence of a prokaryotic cytoskeleton that is analogous to eukaryotic cytoskeletons was first hypothesized more than 25 years ago when a member of the tubulin superfamily, cell division protein FtsZ, was discovered in bacteria and archaea and was found to have a role in cytokinesis[1–6]. The identification of rod shape-determining protein MreB as a 'prokaryotic actin' followed ~5 years later when a role for MreB in cell shape maintenance was linked to its polymerization properties[7–9]. Many other components of the prokaryotic cytoskeleton have been discovered since then, including more homologues of eukaryotic cytoskeletal filaments[10–15] as well as entirely new classes of filaments[16–18], leading to an expanded concept of what a cytoskeleton is (BOX 1). We now know that even at their comparatively small typical cell sizes, bacteria and archaea require the function of protein filaments and their ability to act as large and often dynamic cytoskeletons in order to accomplish cellular processes at large length scales (reviewed in REF. 19). This is because prokaryotic cells are still extremely large when compared with individual proteins or even large complexes, such as ribosomes. TABLE 1 summarizes the diversity of the prokaryotic cytoskeletons that are discussed in this Review. Like eukaryotic cytoskeletons, prokaryotic cytoskeletons rely on interactions between filament-forming proteins and a vast number of other proteins to modulate or provide function; these accessory factors are not discussed extensively in this Review.

The various filaments of the prokaryotic cytoskeleton collectively demonstrate how conserved proteins and their filaments have been preserved during evolution owing to their usefulness in many different cellular processes that require long-range organization (BOX 2).

In this Review, we summarize our knowledge of the molecular biology of known filament-forming proteins in bacteria and archaea, placing them into evolutionarily or structurally related classes where possible. This classification reveals that diverse biological functions are carried out by strikingly similar filaments and also

Box 1 | **Filament-forming proteins and cytoskeletons: key terms**

Filaments: self-association into linear polymers

Many proteins self-associate to form regular linear polymers: filaments. Importantly, filaments are just one way that the self-association of proteins is used to extend the geometric capabilities of the proteome, and large protein structures are just one way to organize cells at large length scales.

In many cases, filament formation is mediated through serial head-to-tail interactions between monomers, which gives the resulting single-subunit-thick protofilament (or strand) a structural polarity (the two ends are different). Protofilaments can also be nonpolar if monomers associate head-to-head and tail-to-tail. Individual protofilaments (or strands) can be associated further to form mature functional filaments. When filaments further associate in a nonregular manner, this is typically described as a bundle, although this is a term that is used inconsistently. Filaments often associate to form regular higher-order structures, the most prominent example being ordered sheets of adjacent filaments. In many cases, filament formation *in vivo* occurs on a supporting matrix, such as a membrane, DNA or another protein filament. We have previously termed these filaments 'collaborative' (REF. 166).

Cytoskeleton: a gradually expanded term with no good definition

Defining prokaryotic cytoskeletons is an evergreen problem. Taken literally, 'cytoskeleton' implies inclusion of only those filament systems that are relatively static and perform a scaffolding function exclusively; however, the term is generally implied to also include dynamic systems that push or pull cellular components. We have previously proposed a distinct term for this second class of filaments that are dynamic: 'cytomotive' (REF. 167). The ambiguity of 'cytoskeleton' and the problem that in initial stages of research it can be unclear whether a system adheres to a given definition have led some researchers of filament systems to abandon the term altogether[168]. One recent review[169] proposed an expansive definition: "all cytoplasmic protein filaments and their super-structures that either move or scaffold (stabilize or position or recruit) materials within the cell", although even this helpful definition does not fully reflect our understanding of the term. For example, in this Review, we include a discussion of the periplasmic polymer CrvA and exclude cytoplasmic Type VI secretion systems. We believe a precise definition of the prokaryotic cytoskeleton would be of limited use but that the current usage of the term does meaningfully group diverse cell biological phenomena with filaments at their heart. More importantly, the term 'prokaryotic cytoskeleton' does provide a helpful rallying point for researchers trying to gain a global understanding of the diverse and hard-to-categorize ways in which protein filaments are used to organize small cells.

reveals the converse, that a specific biological function is often performed in different organisms by unrelated filaments (BOX 2).

Tubulins

Tubulin homologues are widely distributed in bacteria and archaea and are involved in many different cellular processes. The dynamic properties of tubulin filaments are often used for cytomotive functions (to push or pull other molecules in the cytoplasm) (TABLE 1). Tubulin superfamily members share a distinctive globular domain composed of two independently folded but closely associated subdomains that are separated by a core helix, the amino-terminal GTPase domain and the carboxyl-terminal activation domain[20] (FIG. 1). Polymerization occurs through the association of a GTP-bound GTPase domain from one monomer with the activation domain of the next monomer, forming a catalytically active GTPase site around a GTP molecule at the centre of the intersubunit interface. Many monomers associate head-to-tail in this way to form a one-subunit-thick filament, a protofilament (see BOX 1). The intrinsic nucleotide hydrolysis activity of the tubulin polymer and the reduced stability of a GDP-containing intersubunit interface make the protofilament dynamic.

Overall, we have a good understanding of the structure of the conserved tubulin protofilament architecture (FIG. 1a). This basic protofilament structure has been adopted by diverse cellular genomes and by mobile genetic elements, including plasmids and viruses, often forming higher-order filaments in these cases (FIG. 1b).

FtsZ: the organizer of bacterial cell division. Cell division protein FtsZ was the first component of a prokaryotic cytoskeleton to be identified[1–4]. FtsZ has the common tubulin bipartite globular domain at the N-terminus, which is separated by a disordered linker of variable length from a short, conserved, C-terminal region that is responsible for mediating interactions with other proteins (reviewed in REF. 21).

FtsZ is localized near the membrane at future division sites in almost all bacteria and most archaeal phyla[6,22] and forms a ring-like structure known as the Z-ring, which contracts during cytokinesis. In bacteria, FtsZ is among the first molecules to arrive during the assembly of a poorly characterized macromolecular complex known as the divisome, which incorporates many of the enzymatic activities and other functional modules that are needed to carry out cytokinesis and to remodel the cell wall (reviewed in REF. 23). Although the central role of FtsZ in cell division of bacteria is well established[23], it has recently been better characterized using new light microscopy techniques[24–28]. Higher-resolution imaging of the Z-ring in live bacteria using improved pulsed labelling of newly synthesized peptidoglycan suggests a new model for FtsZ function whereby relatively short FtsZ filaments treadmill circumferentially around the division plane to drive cell wall remodelling divisomes around with them. In this model, FtsZ-driven divisome procession results in inwardly progressive synthesis of peptidoglycan around the septum, ultimately leading to scission of daughter cells. Treadmilling is a theoretically well-understood feature of multistranded filaments (seen, for example, in microtubules in eukaryotic cells), but until recently, it was unclear how a single-stranded filament such as FtsZ could treadmill[29].

This new model differs from previous hypotheses that proposed that overlapping FtsZ filaments, probably encircling the entire cell, directly drive division of the plasma membrane by maximizing overlap or through iterative bending[21,30]. Recent cryo-electron tomography (cryo-ET or electron tomography) of cells from different bacterial species early in division[31] revealed that division often initiates asymmetrically, at a single position on the division plane, and initial ingression of the cell wall is able to proceed when short FtsZ filaments are present at the point of ingression on the interior of the cell, presumably locally organizing cell wall remodelling. Although many questions remain unanswered, it now seems likely that FtsZ filaments are required mostly for organizing the cell division machinery in space and time rather than manipulating membranes directly. However, these two functions of FtsZ filaments are not mutually exclusive[24]. For example, an ancient function of FtsZ to shape membranes might have been complemented or largely replaced by its role in divisome coordination in bacteria. The

Protofilament
The one-subunit-thick filament formed by repeated linear interactions between monomers; also known as a strand.

Divisome
The set of proteins that divide a bacterial cell.

Peptidoglycan
The peptide-crosslinked sugar polymer that is the major component of bacterial cell walls.

Treadmilling
The situation in which a filament simultaneously grows and shrinks at opposite ends, leading to overall motion in the direction of growth without subunits moving.

mechanism of FtsZ function in archaea remains almost totally unexplored, although it is known that many euryarchaeal genomes encode two FtsZs (termed FtsZ1 and FtsZ2; FIG. 1c), with both involved in division[32]. Most plastids[33] and many mitochondria[34] also use FtsZs, which are closely related to bacterial FtsZs, during division.

Some magnetotactic bacteria encode an additional FtsZ-like protein, termed FtsZm, that does not have the C-terminal extension that is required for interactions with many partners[35,36]. Although the biological role of FtsZm is unclear, it apparently co-polymerizes with FtsZ and is therefore recruited to the Z-ring[37]. FtsZm filaments have also been implicated in redox-dependent regulation of magnetosome biogenesis[37].

CetZ: a divergent tubulin in some archaea. A distinct group of tubulin superfamily genes are found in some euryarchaeal genomes in addition to division-related FtsZs (reviewed in REF. 38). Their sequences diverge from both eukaryotic tubulins and bacterial and archaeal FtsZs, but they share features with each. These proteins were recently investigated in the model Haloarchaeum *Haloferax volcanii*[12], and the group was renamed CetZ after the prototypical member that was characterized in this study: tubulin-like protein CetZ1 (cell structure-related Euryarchaeota tubulin/FtsZ homologue 1). CetZ1 is required in *H. volcanii* for the differentiation of irregular plate-shaped cells into a rod shape that is essential for efficient swimming motility. CetZ1 forms dynamic cytoplasmic filaments, possibly membrane associated, that are required for cell shape determination. The mechanism by which CetZ proteins modulate cell shape is unknown, although the localization of the protein was shown to be related to membrane curvature.

TubZ: a tubulin that is used for segregation of nonchromosomal DNA in bacteria. TubZ proteins (for example, Q8KNP3) are a diverse but likely monophyletic group within the tubulin superfamily. TubZ proteins are predominantly encoded on rapidly evolving nonchromosomal DNA, for example, in bacterial plasmids, and in bacteriophage genomes (reviewed in REF. 39). TubZs include the type III systems of plasmid segregation[40,41] and the PhuZ subfamily of bacteriophage proteins (for example, B3FK34) that position viral DNA and virions within the host cell during viral assembly[42,43]. Both TubZ and PhuZ filaments act as one-dimensional molecular motors, although in opposite directions: plasmid-encoded TubZ filaments apparently pull plasmids to cell poles through depolymerization[44], whereas PhuZ filaments push bacteriophage particles to the centre of the cell using the growth of dynamically unstable filaments[45]. Although we still do not know whether or how FtsZ and CetZ protofilaments might associate to form higher-order structures in cells, in the case of TubZ, several parallel protofilaments associate to form the functional cytoplasmic filaments. TubZ from the *Bacillus thuringiensis* plasmid pBtoxis forms four-stranded helical filaments[46], whereas PhuZ from *Pseudomonas chlororaphis* phage 201ϕ2-1 forms three-stranded helical filaments[47] with an inside-out topology compared to both TubZ helices and microtubules (FIG. 1). The additional filament stability that is conferred by lateral interactions between protofilaments may be necessary for these cytomotive functions where filament integrity across large length scales (as compared to FtsZ) is required. In general, a(multiprotofilament) helical architecture ensures equal rigidity in all directions and restricts polarity to the longitudinal direction. These properties are particularly suited to filaments that extend through cytoplasmic space, such as eukaryotic actin and microtubules, and TubZs and the prokaryotic actins plasmid segregation protein ParM and magnetosome allignment protein MamK. TubZ proteins have a C-terminal extension that reaches along the protofilament to the next monomer and is crucial for robust filament formation and for wild-type filament dynamics.

Other prokaryotic tubulins. Other members of the tubulin superfamily are found in bacteria and archaea; these are identified by the highly conserved sequence motifs of the GTPase domain.

A pair of bacterial tubulins termed Btubs A and B (BtubAB) that are more similar in sequence[48] and structure[49] to eukaryotic tubulins than to FtsZs have been found in addition to FtsZ in several *Prothescobacter* spp. genomes[48]. BtubAB was found to form tubular structures *in vivo*; however, these are much smaller in diameter than microtubules[50]. Btub genes were most likely acquired in a horizontal gene transfer event from a eukaryote to a *Prosthecobacter* spp. ancestor. Recent work that characterized BtubAB filament structure and dynamic behaviour supports this hypothesis[51]. BtubAB forms four-stranded minimicrotubules *in vitro* (FIG. 1), exhibiting a seam and dynamic instability (two hallmarks of eukaryotic microtubules). The biological function of BtubAB remains unknown.

Some members of the Thaumarcheota (within the archaeal TACK superphylum) encode a eukaryotic γ-tubulin-like protein called artubulin[52]. It is unclear whether artubulins are the product of a horizontal gene transfer event or represent a vertically inherited orthologue of γ-tubulin. More convincing putative ancient tubulin-like genes were found in recently published archaeal genomes assembled from metagenomic sequence data[53].

Two groups of proteins with homology only to the GTPase domain of the tubulin superfamily have been identified in bacterial and archaeal genomes; both groups have been termed FtsZl (FtsZ-like)[54]. It has been suggested on the basis of the proximity of FtsZl genes to membrane remodelling genes in genomes that these proteins are involved in membrane remodelling[54]. It is unclear how or whether FtsZl proteins could form filaments because they lack a C-terminal domain homologous to the tubulin superfamily C-terminal (activation) domain.

Actins

Actins, like tubulins, are versatile cytoskeletal components that are able to form robust cellular scaffolds and also provide dynamic properties that can be harnessed for cytomotive functions. The ATP-binding actin-like

Magnetotactic bacteria
Bacteria that are able to sense magnetic fields using tiny intracellular magnets.

Magnetosome
Inward bulges of the inner membrane in some bacteria that contain biomineralized iron compound crystals.

Horizontal gene transfer
A process in which genetic material is transferred between genomes by any means except whole genome reproduction.

Seam
The discontinuity in helical symmetry classically observed in 13 protofilament microtubules.

Dynamic instability
Rapid and stochastic switching between filament growth and depolymerization, an emergent property of some filaments, notably microtubules.

TACK superphylum
An archaeal clade that is a sister to the clade that includes the Asgard superphylum and the archaeal ancestor of eukaryotes. TACK: Thaumarchaeota, Aigarchaeota, Crenarchaeota and Korarchaeota.

Table 1 | **Prokaryotic filament systems**

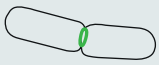
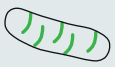
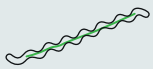
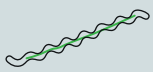
Protein	Cartoon	Bacteria or archaea	Distribution	Function	Refs
Tubulin superfamily					
FtsZ		Bacteria	Almost all bacteria	Organization of cell division processes	1–3
		Archaea	Almost all Euryarchaeota, some other archaea	Organization of cell division processes	6
FtsZm		Bacteria	Some magnetotactic bacteria	Unclear role in magnetosome function	35,36
CetZ		Archaea	Some Euryarchaeota	Control of cell shape	12
TubZ		Bacteria	Diverse bacterial plasmids, bacteriophage genomes, some chromosomal copies	Segregation of plasmids and phage DNA, others unknown	40,42
BtubAB		Bacteria	*Prosthecobacter* spp. (Verrucomicrobia)	Unknown	48
Artubulin	?	Archaea	Some Thaumarchaeota	Unknown	52
FtsZl	?	Bacteria	Diverse bacteria	Unknown, possibly membrane remodelling	54
	?	Archaea	Diverse Euryarchaeota, possibly Crenarchaeota	Unknown, possibly membrane remodelling	54
Actin superfamily					
MreB		Bacteria	Almost all noncoccoid bacteria	Organization of cell wall synthesis	7–9
	?	Archaea	Archaeal actins whose closest homologues are MreB remain unstudied	Unknown	13
FtsA		Bacteria	Many bacteria, not identified in Archaea	Cooperates with FtsZ during cell division	7,74,182
MamK		Bacteria	Magnetotactic bacteria	Alignment of magnetosomes	77,78,80
ParM-like		Bacteria	Diverse bacterial plasmids, bacteriophage genomes, some chromosomal copies	Segregation of plasmids and phage DNA, others unknown	87,88
	?	Archaea	A group of archaeal actins, including Ta0583, related to bacterial ParMs	Unknown	13,102
Crenactin	?	Archaea	Some Crenarchaeota	Putative role in cell division	13,106
Coiled coil filaments					
Crescentin		Bacteria	*Caulobacter* spp.	Required for cell curvature, modulates cell wall synthesis	10
Scy, FilP		Bacteria	Actinobacteria	Role in organization of polar growth	112,120,121
CCRPs	?	Bacteria	Diverse bacteria	Diverse cytoskeletal roles, mostly unknown	Reviewed in REF. 119
DivIVA		Bacteria	Most Gram-positive bacteria and some others	Varied roles in organization of growth at poles and division	130,133

Table 1 (cont.) | **Prokaryotic filament systems**

Protein	Cartoon	Bacteria or archaea	Distribution	Function	Refs
Coiled coil filaments (cont.)					
ESCRT		Archaea	Diverse archaea, ubiquitous in some classes of Crenarchaeota	Division in some cases, others unknown	14,142,143
CrvA		Bacteria	*Vibrio* spp.	Promotes cell curvature	16
Other filament systems					
Bactofilins	?	Bacteria	Diverse bacteria	Control of cell shape, mostly unknown	17
SepF		Bacteria	Most Gram-positive bacteria, all Cyanobacteria	Cooperates with FtsZ during cell division	151,152
	?	Archaea	FtsZ-containing archaea (Euryarchaeota and others)	Putative FtsZ membrane anchor	105,152
PopZ		Bacteria	Some Gram-negative bacteria	Cell pole marker, signalling hub	157,158
SpoIVA		Bacteria	Sporulating Firmicutes	Forms a protein coat around forespores	18,160
Periplasmic flagella		Bacteria	Spirochaetes	Forms helical cytoskeleton and produces motility	Reviewed in REF. 162
Fibril		Bacteria	*Spiroplasma* spp.	Forms cytoskeletal ribbon	Reviewed in REF. 71

BtubAB, bacterial tubulins termed Btubs A and B; CCRPs, coiled coil rich proteins; CetZ, tubulin-like protein CetZ; DivIVA, septum site-determining protein; ESCRT, endosomal sorting complexes required for transport; FilP, putative filament-forming protein; FtsA, cell division protein FtsA; FtsZ, cell division protein FtsZ; MamK, magnetosome protein MamK; MreB, rod shape-determining protein MreB; ParM, plasmid segregation protein ParM; PopZ, pole organizing protein PopZ; SepF, cell division protein SepF; SpoIVA, stage 4 sporulization protein A. ?, role is unclear.

fold (FIG. 2) is ancient and widely distributed across the tree of life in proteins that can and cannot form filaments, for example, in the nonpolymerizing proteins Hsp70 (DnaK) and hexokinase[7]. The filament-forming actins are probably monophyletic, that is, polymerization evolved only once, and all filament-forming actins have a common, filament-forming ancestor (FIG. 2c). Actin monomers associate via a conserved mode of longitudinal interaction (FIG. 2a) to form a conserved protofilament structure that has been repurposed many times throughout evolution. Actins form an astonishing variety of higher-order filament architectures that perform a wide variety of cellular functions (FIG. 2b; TABLE 1). The globular actin domain is typically composed of four subdomains (denoted either IA, IB, IIA and IIB or 1–4) that function as two pairs (I and II or 1–2 and 3–4). The adenosine nucleotide is held in a binding pocket at the centre of the molecule. The two halves of the protein rotate relative to one another upon nucleotide hydrolysis and polymerization, linking nucleotide state to polymerization properties and driving intrinsic protofilament dynamics (reviewed in REF. 55).

MreB: control of bacterial cell wall synthesis. MreB is the prototypical bacterial actin[8,9] found in almost all walled bacteria with elongated shapes (reviewed in REF. 56). MreB is essential for normal cell wall synthesis in these organisms, and it is responsible for organizing a multienzyme complex known as the elongasome[57]. Although it was controversial for a long time, a consensus is now forming about how MreB functions. MreB forms antiparallel, in register double filaments close to the plasma membrane, most likely binding it directly[58,59]. Their unusual architecture allows MreB filaments to bend in a single direction only, unlike helical eukaryotic actins. Although it was previously hypothesized that long MreB filaments organize cell wall synthesis globally by forming a cell-spanning helical structure, this view is losing support. Improved imaging techniques have led to the hypothesis that short MreB filaments move circumferentially in concert with the cell wall synthesis enzymes of the elongasome, organizing synthesis locally[60–63]. Unlike the comparable motion of FtsZ in the division plane, which is driven by filament-intrinsic dynamics (treadmilling), MreB motion is driven by cell wall synthesis[62,63].

Elongasome
The set of proteins that allow bacterial cells to grow longer.

Box 2 | **What can be achieved by filaments?**

Prokaryotic cytoskeletons and the protein filaments that form them are variations on a theme: by forming filaments, individual proteins can access larger length scales. Diverse cells have similar needs for organization at large length scales, and the filament-based solutions to any given problem can be strikingly similar in overall mechanism despite the constituent proteins being evolutionarily distant. The convergent use of filaments in four cellular processes is discussed below.

Cell division

Most cells divide through the constriction of the plasma membrane and other cell envelope components. Different filaments are used in different ways to achieve constriction. In many eukaryotic cells, a contractile ring of actin coupled to myosin motors powers cytokinesis. In most bacteria, cell division protein FtsZ filaments perpendicular to the long axis of the cell organize cell wall remodelling at division sites[23]. FtsZ rings around division sites have also been visualized in some (wall-less) Euryarchaeota[6]. In Chlamydiales bacteria, rod shape-determining protein MreB is required for division, organizing synthesis of a vestigial septum[66,67]. In Thermoproteales (phylum Crenarchaea) division-plane rings of the archaeal actin crenactin have been seen. Importantly, no filament-associated molecular motors have been identified in these organisms[13]. In the Sulfolobales (phylum Crenarchaea), a division-plane band of endosomal sorting complexes required for transport (ESCRT)-III homologue cell division protein B (CdvB) at the membrane appears to organize or power plasma membrane scission[142]. ESCRT-III based division is also commonplace in metazoa – ESCRT-III filaments deliver the final cut to the midbody[170]. Division where the plasma membrane is not divided from the outside in can also rely on filaments; for instance, assembly of the cell plate in plants has a well-understood dependence on microtubules[171].

Cell shape determination

For a single-celled organism, cell shape is a crucial determinant of interaction with the environment[172]. In many cases, maintenance of nonspherical cell shapes is dependent on protein filaments. For example, the role of the actin cytoskeleton in determining the shape of many animal cells is well established. In walled bacteria, cell shape is typically maintained by the properties of the rigid cell wall, although these properties are often modulated indirectly by the action of cytoskeletons on the activity of cell wall synthesis and remodelling enzymes. In many rod-shaped bacteria, dynamic MreB filaments control the synthesis of a new cell wall to maintain shape. Although poorly characterized, bactofilins in several organisms control cell morphology via modulation of cell wall properties[108]. The cytoplasmic coiled coil protein crescentin (CreS) in *Caulobacter crescentus* assembles on the inner face of the curved cell and slows cell wall synthesis relative to the outer face[115]. A periplasmic polymer, CrvA, functions similarly in *Vibrio* spp.[16] In some Euryarchaeota, the tubulin superfamily protein CetZ regulates a morphological switch between the plate shape and rod shape through unknown mechanisms[12]. In the wall-less *Spiroplasma* spp., a cytoskeletal ribbon composed largely of fibril protein is the determinant of the helical cell shape[71]. Similarly, cell-spanning periplasmic flagella in the distantly related (and walled) *Spirochaete* bacteria enforce helical morphology and drive motility[162]. In archaea, one analysis showed that the presence of actin family genes (crenactin or MreB orthologues) correlated with rod-shaped morphologies[13], although no specific mechanism for the control of cell shape by these genes is known.

DNA segregation

Segregation of chromosomes by the tubulin-based mitotic spindle is a striking example of a cytomotive filament function in eukaryotes. Segregation of chromosomes by filaments has not been observed in prokaryotes. By contrast, spindle-based segregation of nonchromosomal DNA by filaments is a broadly distributed and well-studied phenomenon. Bacterial plasmids with both actin-based (plasmid segregation protein ParM and actin-like proteins (Alps)) and tubulin-based (TubZ) spindles have been extensively characterized (reviewed in REFS 39,86). Some bacteriophages use tubulin filaments (PhuZ) to ensure the correct positioning of DNA and virions for genome packaging[45].

Organization of intracellular components

The long-range organizing potential of filaments can be harnessed to position other cytoplasmic molecules. Perhaps the most striking example of this is the magnetosome protein MamK actin family filament, which organizes membrane-bound magnetosome organelles in magnetotactic bacteria into linear arrays[78]. Pole organizing protein PopZ, which forms filaments *in vitro*, assembles into a branching filament network near cell poles in some Gram-negative bacteria that recruits a specific set of polar determinants through unstructured regions[159]. Septum site-determining protein DivIVA, a coiled coil protein, polymerizes on the membrane at the poles of some Gram-positive bacteria, where it is involved in modulating Min system behaviour via recruitment of these proteins[173,174] and potentially promoting membrane curvature[136].

Cytomotive filament
A filament that pushes or pulls other molecules in a dynamic way.

Gliding motility
A mode of bacterial self-propulsion that results in smooth motion over a surface. This is achieved by different mechanisms in different organisms.

Some bacteria have multiple copies of MreB, which can have distinct or overlapping roles[64,65]. In FtsZ-less and almost wall-less Chlamydiales, MreB and the cell wall machinery have been co-opted for the synthesis of a vestigial peptidoglycan septum that divides the cell[66,67]. In other bacteria, MreB filaments are used for functions independent of cell wall patterning, for example, as a cytoplasmic component of the gliding motility machinery of *Myxococcus* spp[68,69] (and possibly in more diverse members of the α-proteobacteria and δ-proteobacteria[70]). MreB is also associated with the cell-spanning cytoskeletal ribbon of the wall-less Mollicute *Spiroplasma* spp[71]. MreB has been implicated in the segregation of chromosomes in *Caulobacter crescentus*[72] and other species, although it has been difficult to validate how direct this role may be, given the close and complex associations between the nucleoid, cell wall and filament-forming proteins that pattern cell wall deposition. Archaeal actins whose closest homologues are MreB have been identified but remain unstudied[13].

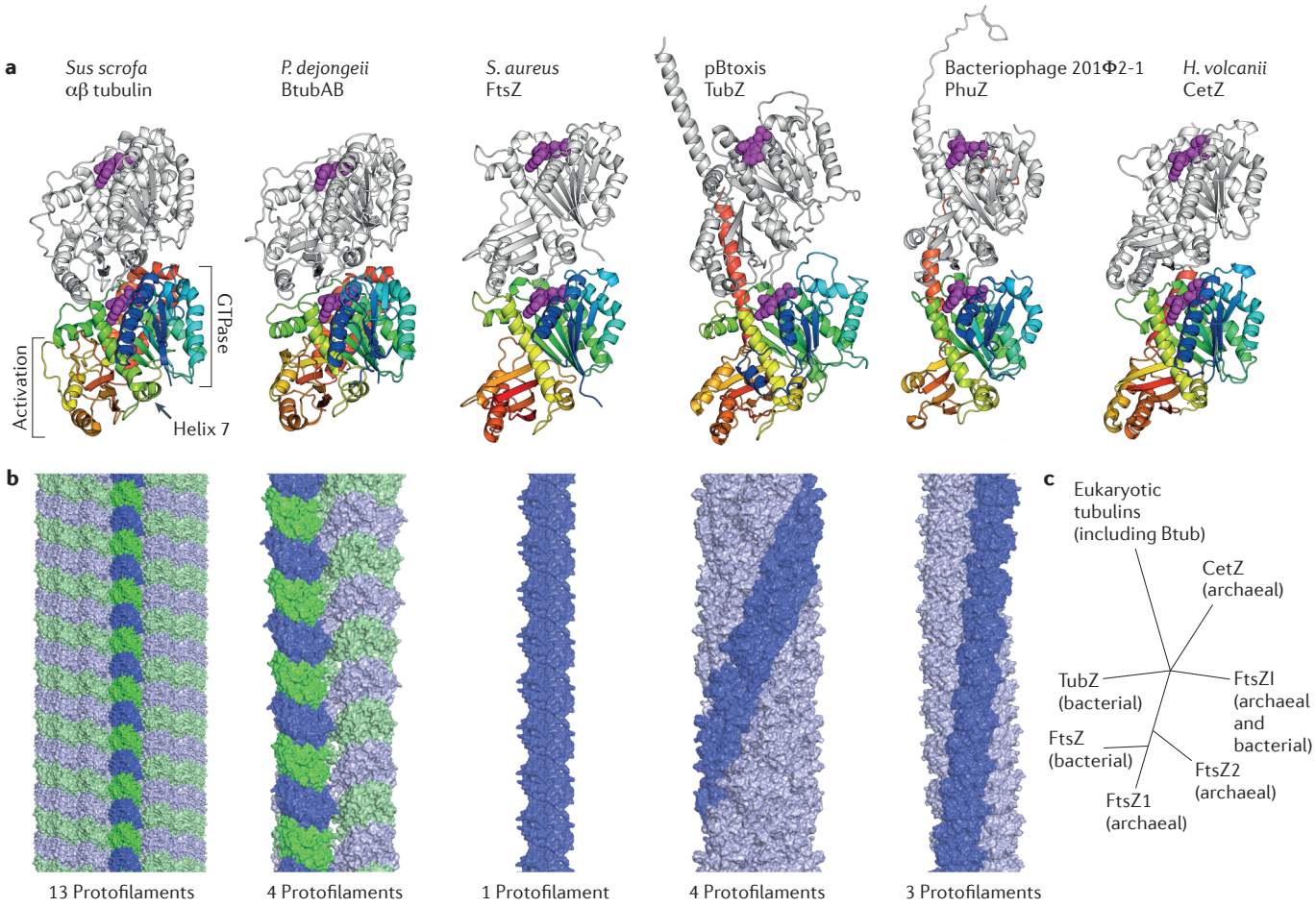


Figure 1 | **Tubulin superfamily.** Conserved monomer structures and longitudinal contacts in tubulin superfamily protofilaments. **a** | Pairs of monomers are shown in cartoon representation, with nucleotides as purple spheres. In each case, the lower subunit is coloured blue to red from the amino terminus to the carboxyl terminus, and the upper subunit is grey. **b** | Diverse tubulin superfamily filament structures derived from high-resolution X-ray or cryo-electron microscopy (cryo-EM) experiments are shown as surface representations. An individual protofilament in each filament is highlighted in darker colours. A–α subunits of heterodimers are coloured blue, and B–β are green. All protofilament interfaces are in roughly the same vertical orientation as those in part **a**. The number of protofilaments forming each filament is given below. Tubulin-like protein CetZ filament structure remains unknown. The microtubule is shown at a smaller scale. **c** | Schematic showing inferred phylogeny of the tubulin superfamily, a conservative consensus based on two sequence analyses[12,54]. The base of the tree is poorly resolved and not possible to root confidently. Protein Databank (PDB) entries for the tubulin structures in (parts **a** and **b**): αβ tubulin (PDB entry 5SYF), BtubAB (PDB entry 5O09), FtsZ (PDB entry 3VOA), TubZ (PDB entry 3J4S), PhuZ (PDB entry 3J5V), CetZ (PDB entry 4B45). BtubAB, bacterial tubulins termed Btubs A and B; FtsZ, cell division protein.

FtsA: an unusual bacterial actin that cooperates with the tubulin FtsZ. Cell division protein FtsA links FtsZ filaments to the plasma membrane and also to other components of the divisome in many bacteria, although other FtsZ membrane anchors exist (reviewed in REF. 23 and see cell division protein SepF below). FtsA is able to form filaments, and polymerization is required for wild-type function[15,73]. Although FtsA was identified as a putative member of the actin superfamily a long time ago[7], it was unclear whether it could polymerize as the amino acids that correspond to subdomain IB in actin and MreB are absent, and a large sequence insertion is present in subdomain IA. Crystal structures and negative stain electron microscopy revealed that FtsA has a recognizable but unusual actin fold that can polymerize to form actin-like protofilaments[15,74]. Domain IB was confirmed to be absent in the structure, and the insertion in IA forms an alternative subdomain (IC), which contributes to polymerization (FIG. 2).

FtsA and FtsZ co-polymerize *in vitro* to form a variety of dynamic structures[26], and they can cooperate to constrict liposomes from the inside[30,75]. Similar co-polymer structures have been observed in cryo-electron tomograms of cells overexpressing both proteins[30]; however, it is still unknown whether co-polymerization is used to regulate the divisome *in vivo*. A recent report showed that *Escherichia coli* FtsA can form small rings *in vitro*; however, the biological importance of this is unclear[76].

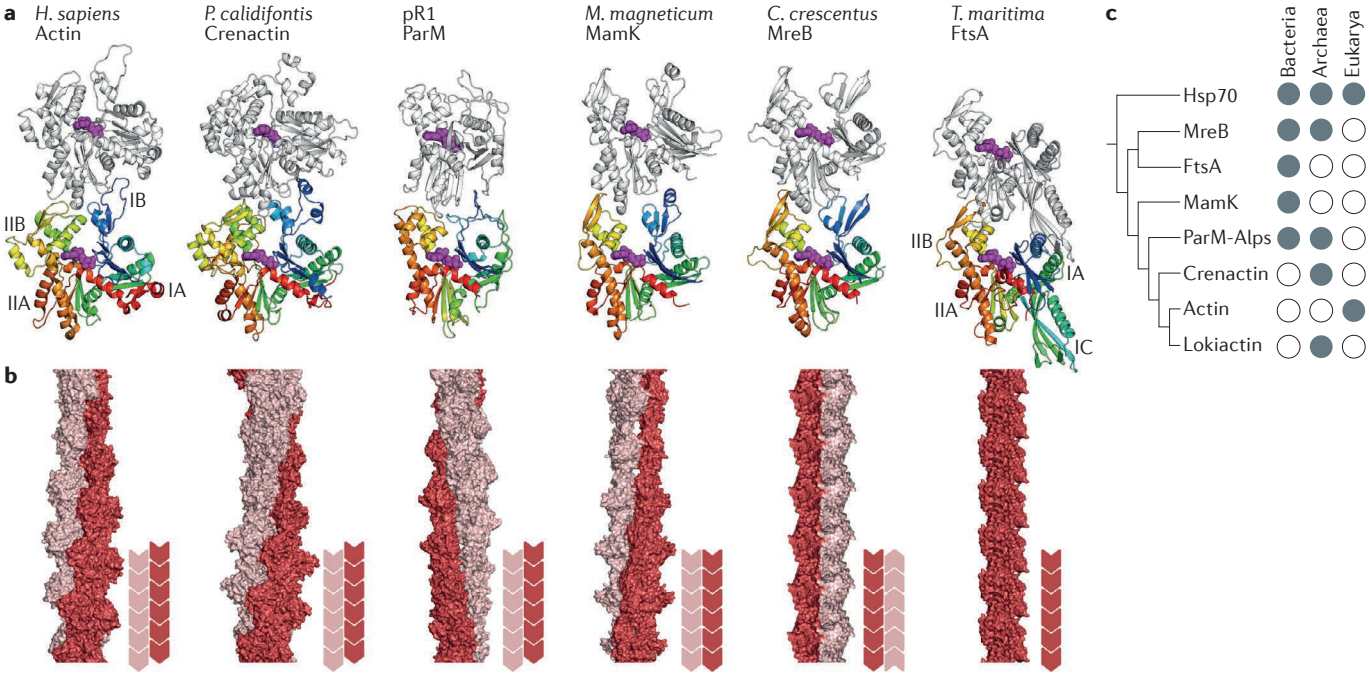


Figure 2 | **Actin superfamily.** Conserved monomer structures and longitudinal contacts in actin superfamily protofilaments. **a** | Pairs of monomers are shown in cartoon representation, with nucleotides as purple spheres. In each case, the lower subunit is coloured blue to red from the amino terminus to the carboxyl terminus, and the upper subunit is grey. Subdomains are labelled for actin and cell division protein FtsA. Overall, the protofilament contacts are very similar, but there are also notable differences, especially in subdomain IB. **b** | Diverse actin superfamily filament structures derived from high-resolution X-ray or cryo-electron microscopy experiments, shown as surface representations. Individual protofilaments are shown in a single colour. Cartoons indicate protofilament polarity (actin is shown with the pointed (plus) end towards the top of the page as in part **a**), and subunit alignment (staggered or juxtaposed). **c** | Distribution and inferred phylogeny of the actin superfamily in the Bacteria, Archaea and Eukarya domains of life. Schematic consensus phylogeny was derived from three sequence analyses[11,13,107]. Protein Databank (PDB) entries for actin structures: actin (PDB entry 5JLH), FtsA (PDB entry 5 LY4), MreB (PDB entry 5AEY), FtsA (PDB entry 5 LJV), MreB (PDB entry 4CZJ), FtsA (PDB entry 4A2B). Alps, actin-like proteins; Hsp70, heat shock protein 70; MamK, magnetosome protein; MreB, rod shape-determining protein; ParM, plasmid segregation protein.

MamK: a protein scaffold for a bacterial compass needle. MamK forms filaments in magnetotactic bacteria that are used for the alignment and segregation of magnetosomes[77,78]. MamK appears to have slightly different roles in different magnetotactic bacteria, even in the two closely related model species *Magnetospirillum gryphiswaldense MSR-1* (MSR) and *Magnetospirillum magneticum AMB-1* (AMB). In both species, MamK forms cytoplasmic filaments that connect ordered chains of magnetosomes. Deletion of *mamK* in MSR results in a dramatic disorganization of magnetosomes into short chains that do not segregate efficiently during division[79], whereas Δ*mamK* AMB magnetosomes are still found in long chains, although they are less organized and cytoplasmic filaments are no longer visible[78].

The structures of both filamentous and monomeric MamK from MSR were recently solved[80], providing mechanistic insight into filament dynamics[81]. MamK filaments are right-handed, parallel and two-stranded, with juxtaposed subunits (FIG. 2). The conformational changes during the MamK polymerization cycle are very similar to those seen for eukaryotic actin[82], ParM[83] and, to a lesser extent, MreB[58].

Some magnetotactic bacteria, including AMB, also encode a MamK homologue, termed MamK-like, which assists in magnetosome alignment and also forms filaments, alone and with MamK[84,85].

ParM and Alps: actin-based spindles for efficient DNA segregation in bacteria. In addition to the three well-studied chromosomally encoded bacterial actins described above, there are a large number of diverse plasmid-encoded and bacteriophage-encoded actins[11] (reviewed in REF. 86). Although the abundance and diversity of this group of bacterial actin-like proteins (Alps) were not recognized until recently[11], one member of the group has been the subject of study for many years: the ParM protein, prototypically found on the *E. coli* R1 plasmid[87]. The R1 Par locus was the founding member of the type II plasmid segregation systems, which confer stability on their host plasmids by actively segregating plasmid copies into daughter cells at division. Type II system segregation depends on actin-like ParM, a DNA-binding protein ParR and a centromeric DNA region *par*C[88]. The molecular details of ParMRC plasmid segregation were resolved recently[89], building

on decades of genetic, biochemical and structural data (reviewed in REF. 90). Briefly, after plasmid replication, several copies of ParR assemble on two sister *par*C regions. Each ParRC complex recruits a left-handed, staggered ParM filament that is parallel and double helical via the 'barbed end' (by analogy with eukaryotic actins). Antiparallel, ParRC-bound ParM filaments associate (forming a four-protofilament bundle via the pointed ends)[83] to form a bipolar spindle that pushes ParRC complexes and plasmids apart through the incorporation of ParM subunits at the ParRC-bound barbed ends. There is evidence that ParMs comprise a *bona fide* family within the Alps, having conserved properties despite relatively low sequence conservation[91]; nevertheless, some Alps outside the putative ParM family, with apparently divergent properties, have also been named ParM[92].

Aside from ParMs, only one other plasmid-encoded Alp has been extensively characterized: actin-like segregation protein AlfA, found on the *Bacillus subtilis* pBET31 plasmid. Like ParM, AlfA forms filaments and functions as part of a type II segregation system (DNA-binding segregation protein AlfB acts as the DNA adaptor and the centromeric region is known as *par*N), although the mechanism of segregation may differ in some details[93–96]. On the basis of sequence, AlfA appears to be missing subdomain IIB, which is part of the canonical longitudinal filament interface, and therefore, a high-resolution filament structure is needed to understand how polymerization is possible.

A number of other Alps have been partially characterized. These include the bacteriophage-encoded AlpC[97], two bacteriophage-encoded Alps from *Bacillus pumilus*[98], Alp12A from a *Clostridium tetani* plasmid[99], Alp7A from a *B. subtilis* plasmid[100] and a divergent ParM from *Staphylococcus aureus* multidrug resistance plasmid pSK41 (REFS 92,101). These investigations suggest that there is a substantial diversity in the filament properties and functions to be found among the Alps. One archaeal actin, Ta0583 from *Thermoplasma acidophilum*, is probably the result of a horizontal gene transfer of a bacterial Alp into an archaeaum[13,102].

Crenactin: evidence that eukaryotic actin architecture is ancient. Crenactins are found in members of the Thermoproteales class of the phylum Crenarchaeota within the TACK superphylum[13] (reviewed in REF. 103). The eukaryote lineage is rooted close to the TACK clade in the tree of life[104]. Thermoproteales have no known filament or membrane remodelling system, except for crenactin[105], and immunofluorescence microscopy data suggest that the protein has a cytoskeletal role[13]. It was recently shown that the structure of the crenactin filament is almost identical to that of filamentous eukaryotic actin[106] and forms a right-handed double helical filament that is staggered and parallel, with a hydrophobic plug between the two strands — a hallmark of eukaryotic actin (FIG. 2).

Recent metagenomics studies have revealed the existence of archaea in the Asgard superphylum with actin genes that are more similar to eukaryotic actin than crenactin, as well as what appear to be actin-related proteins (Arps) and gelsolin-like domain homologues[53,107]. Future investigation of these molecules could lead to a greater understanding of the origins of phagocytosis, an event in the evolution of the eukaryotic lineage that is presumed to predate eukaryogenic endosymbiosis.

Coiled coil filaments

Apart from actins and tubulins, the most widely distributed cytoskeletal components are proteins containing the coiled coil structural motif, which is formed through the parallel or antiparallel association of α-helices twisting around each other like the strands of a rope. Higher-order interactions, which generate filaments, are mediated by the coiled coil motif. Coiled coil cytoskeletal proteins share functional properties that are derived from their common structure rather than from a conserved amino acid sequence (reviewed in REF. 108). Coiled coil cytoskeletons typically function as minimally dynamic scaffolds in the cell, performing structural roles or promoting specific subcellular localizations of other molecules or both.

Eukaryotic intermediate filaments are the prototypical example of a cytoskeletal component that assembles through coiled coil interactions. The domain architecture of intermediate filaments is well defined and partially understood structurally (reviewed in REFS 109,110) (FIG. 3a): a central all-helical rod domain is capped by poorly ordered head and tail domains. Rod domains (like all coiled coils) can be identified with reasonable confidence from the amino acid sequence alone owing to the strict heptad-based periodicity within coiled helices, which ensures that compatible residues bridge adjacent helices[111]. Intermediate filament monomers dimerize in parallel to form coiled coils before further associating to form nonpolar filaments. Intermediate filaments are a well-defined class of coiled coil filaments — but, importantly, there are other ways of using coiled coils to build cytoskeletons (many are catalogued in REF. 112).

Whereas actins and tubulins form filaments that are dynamic owing to their intrinsic nucleotide hydrolase activity, intermediate filaments have no such activity and require additional factors to facilitate remodelling of cytoskeletal structures, often through post-translational modification[113]. Prokaryotic coiled coil filaments that do exhibit dynamic activity presumably have analogous factors, although in most cases, these have not been identified. So far, observed coiled coil dynamics have not extended to a function that is considered cytomotive at the molecular level, so these filaments are strictly cytoskeletal.

Crescentin: a determinant of cell shape. Crescentin (CreS) is a filament-forming coiled coil protein that is found in abundance close to the plasma membrane in the crescent-shaped bacterium *Caulobacter crescentus*, on the concave cytoplasmic face[10]. The sequence and *in vitro* assembly characteristics of crescentin are highly reminiscent of eukaryotic intermediate filaments;

Coiled coil
The structural motif formed by intertwined α-helices.

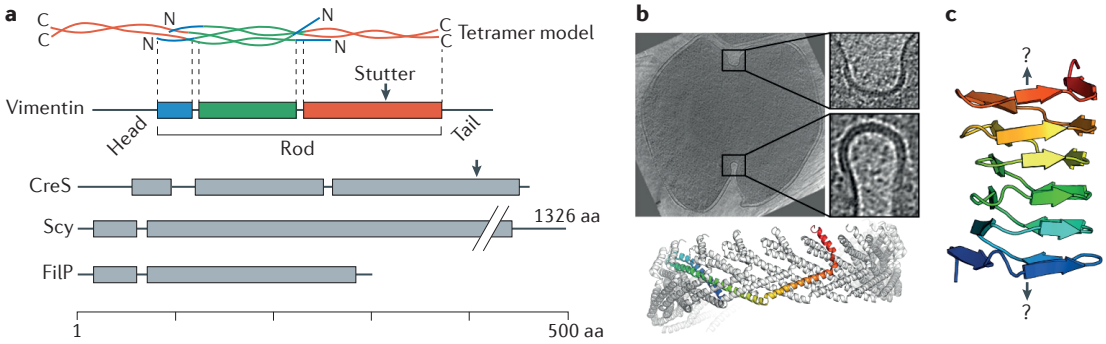


Figure 3 | **Towards a structural understanding of diverse prokaryotic cytoskeletal filaments. a** | Schematic domain architecture of some prokaryotic coiled coil proteins, which may be part of an intermediate filament-like family. Human vimentin is shown as an example of intermediate filament sequence properties and proposed intermediate filament architecture. The schematic vimentin tetramer model is derived from a previously described atomic model[110]. It illustrates how both dimerization and higher-order associations are mediated by the coiled coil rod region. Sequence schematics of vimentin, crescentin (CreS), Scy and putative filament-forming protein (FilP) show verified (vimentin) or predicted (CreS[10], FilP and Scy[112]) coiled coil regions as boxes. Archaeal endosomal sorting complexes required for transport (ESCRT) systems. **b** | Cryo-electron tomogram of a dividing *Sulfulobus solfataricus* cell, showing the cytokinetic protein belt thought to be composed of ESCRT-III homologues and associated proteins (top). Scale bar, 200 nm. Eukaryotic ESCRT-III helical assembly[181] illustrates coiled coil-like filament formation by this family of proteins (bottom) (from Protein Databank (PDB) entry 3JC1). **c** | Solid-state NMR (ssNMR) structure of a bactofilin monomer (PDB entry 2N3D). So far, it has not been possible to deduce the arrangement of monomers within a bactofilin filament (for example, head-to-tail or head-to-head). Part **b** is republished with permission of American Society for Cell Biology, from Electron cryotomography of ESCRT assemblies and dividing Sulfolobus cells suggests that spiraling filaments are involved in membrane scission. Dobro, M. J. *et al. Mol. Biol. Cell* **24**, 2319–2327 (2013); permission conveyed through Copyright Clearance Center, Inc. (REF. 143).

crescentin has predicted disordered head and tail regions and a coiled rod domain, and even a distinctive break in periodicity known as the stutter[10,114] (FIG. 3a). CreS is required for *C. crescentus* cell curvature and is sufficient to generate curved cells when ectopically expressed in *E. coli*[115]. Control of cell curvature is probably through the modulation of cell wall synthesis by mechanical strain that is applied by elastic CreS assemblies[115]; however, the molecular details of this remain unclear. MreB has been suggested to form part of a link between CreS and the cell wall[116]. One confounding factor is that CreS-induced curvature is also dependent on the metabolic enzyme cytidine triphosphate synthase (CtpS), which is filament-forming in *C. crescentus* and in almost all organisms studied, including *Homo sapiens*[117,118]. CtpS polymerization is thought to be a conserved mechanism for regulating CtpS activity.

Scy and FilP: is there a broader class of intermediate filament-like proteins? *Scy* and putative filament-forming protein (FilP) (along with septum site-determining protein (DivIVA); reviewed in REF. 119) are required for normal development of hyphae in the filamentous bacteria *Streptomyces* spp., localizing to and specifying the growing tip and future branching sites. FilP is also found in many rod-shaped Actinobacteria[120,121], which also exhibit polar growth. Like CreS, their sequences and *in vitro* assembly properties show basic similarities to intermediate filament proteins, although the arrangement of coiled coil regions and repeat architecture in the rod domain is different from those of both CreS and intermediate filaments[112] (FIG. 3a). CreS, FilP and Scy are the most-studied examples of a large group of bacterial proteins that could arguably be considered intermediate filament-like[112,119], although discriminating a meaningful intermediate filament-like group from a broader coiled coil rich class (see below) is not straightforward and is probably not feasible using sequence data alone.

Bacterial coiled coil rich proteins: a cytoskeletal motif, not a cytoskeletal family. We adopt the term bacterial 'coiled coil rich proteins' (CCRP) to discuss proteins for which a better classification is unavailable[119,122], that is, proteins that either do not belong to a well-characterized family (for example, DivIVA) or do not exhibit unambiguous intermediate filament-like properties (as for those above). Very few of the CCRPs that have been identified from sequence[112] have been studied in depth.

Filament-forming CCRPs in *Helicobacter* spp. are important for maintaining the distinctive (and virulence-associated) helical shape of these pathogens[122,123]. *Leptospira* spp. (spiral-shaped Spirochaetes) contain DNA-binding CCRPs, which may be involved in organization of the nucleoid into a cell-spanning rod[124,125]. In *Myxococcus* spp., normal gliding motility is dependent on filament-forming CCRPs adventurous-gliding motility protein Z (AglZ)[126] and response regulator FrzS[127], which are involved in linking transmembrane gliding motility machines to cytosolic components with MreB and regulating exopolysaccharide secretion at the cell pole[128], respectively. A CCRP has been identified in the related bacterial predator *Bdellovibrio bacteriovorus*, which appears to have a limited role in

maintaining cell shape integrity[129]. It is unclear if the diverse functions of CCRPs are united by common molecular mechanisms beyond polymerization, partly because *in vitro* studies of coiled coil proteins are technically challenging.

DivIVA: a coiled coil determinant of cell polarity and division site selection. DivIVA is distributed widely among Gram-positive bacteria and localizes to cell poles, hyphal branching sites and future division sites, where it has diverse roles, including modulating activity of the Min system[130,131] (reviewed in REFS 108,119,132). DivIVA binds to the membrane directly and polymerizes to form higher-order structures with intrinsic curvature[133,134], probably thereby localizing to high membrane curvatures[135,136]. In *Streptomyces* spp. and Mycobacteria, DivIVA activity is regulated by site-specific phosphorylation[137–139]. In the MreB-less rod-shaped actinobacterium *Corynebacterium* spp., DivIVA is not phosphorylated but functions synergistically with another coiled coil protein, rod-shaped morphology protein (RsmP), which is phosphorylated at specific sites[140]. No direct effect of phosphorylation on polymerization has yet been identified in either case, although modulation of phosphorylation *in vivo* has marked phenotypes in both cases.

ESCRT-III relatives in archaea: membrane scission by a polymer of coiled coils. Homologues of the eukaryotic endosomal sorting complexes required for transport (ESCRT) system have been identified in diverse archaea[14] (reviewed in REFS 105,141). ESCRT systems use multiple proteins to perform regulated scission of membranes, including during archaeal cell division[142], as visualized by cryo-ET of the FtsZ-less Crenarchaeon *Sulfolobus solfataricus*[143] (FIG. 3b). In eukaryotes, coiled coil ESCRT-III proteins polymerize at membranes leading to membrane scission; however, the molecular mechanism of this process remains incompletely understood[144]. Depolymerization and recycling of ESCRT-III subunits are achieved through the action of a hexameric AAA+ ATPase, the prototypical example being vacuolar protein sorting-associated protein 4 (Vps4) from *Saccharomyces cerevisiae*. Homologues of ESCRT-III–Vps4 pairs have been identified in many archaeal genomes, the best-studied example being cell division protein CdvB (ESCRT-III) and cell division protein CdvC (Vps4) in *Sulfolobus* spp. In eukaryotes, ESCRT-III recruitment is dependent on additional factors (ESCRT-0, ESCRT-I and ESCRT-II), archaeal homologues that have been identified in genomes that were assembled from metagenomic sequences thought to represent lineages that are sister groups to the eukaryotic lineage[53,107]. By contrast, in *Sulfolobus* spp., an unrelated protein, cell division protein CdvA, is required for recruitment of CdvB to the plasma membrane[145].

CrvA: a periplasmic cytoskeleton. A periplasmic filament-forming protein in *Vibrio cholerae*, CrvA, has recently been identified and characterized[16]. CrvA localizes to the plasma membrane at the inner face of cell curvature, where it slows peptidoglycan synthesis, so as to establish or reinforce the virulence-associated vibrioid cell morphology. CrvA filament formation is dependent on the presence of a predicted coiled coil domain, although the molecular mechanism by which CrvA modulates peptidoglycan patterning remains unclear. Improved live cell peptidoglycan labelling and imaging methods, such as those developed during the study of CrvA[16], should be helpful in many cases to help elucidate the mechanisms by which bacterial cytoskeletons modulate cell wall patterning (BOX 3).

Other prokaryotic cytoskeletons

Many protein filaments with cytoskeletal functions are neither actins nor tubulins or coiled coils. This reflects the relative ease of evolving self-assembly (or the difficulty in avoiding it)[146]: the three groups above are notable for their wide distributions, not for polymerization *per se*.

Bactofilins are a poorly understood family of bacterial filament-forming proteins, yet they are highly conserved, broadly distributed within bacteria and abundant in cells[17,108]. They have a cytoskeletal role in several organisms, often through the modulation of cell wall remodelling[17,147–149]. Bactofilin monomers from *Myxococcuss xanthus* form a right-handed β-helix, as shown by a recent solid state NMR study[150] (FIG. 3c). Filaments and sheets have been observed *in vitro* and *in vivo*, but how monomers assemble to form these remains unclear.

SepF was originally identified as a component of the divisome in *B. subtilis*[151]. Subsequently, SepF was shown to bind membranes, recruit FtsZ to the membrane and form curved filaments[152]. Structural analyses suggest thatSepF filaments are nonpolar polymers of head-to-head SepF dimers. SepF is found in many Gram-positive bacteria and Cyanobacteria, where it complements and in some cases replaces FtsA function as a FtsZ membrane anchor[151,153–156]. SepF has also been found in all FtsZ-containing archaea so far, and therefore, it seems a good candidate for an archaeal FtsZ anchor — indeed, no other known bacterial anchors have widespread archaeal homologues[105].

Pole organizing protein PopZ is a proline-rich protein that forms filaments that associate to form an irregular network at cell poles in *C. crescentus* and other Gram-negative bacteria[157,158]. The network appears to function as a molecular signalling hub, with intrinsically disordered regions of PopZ responsible for recruiting at least 11 different proteins[159]. PopZ has inferred helical regions, but these are not predicted to form coiled coils.

Stage 4 sporulization protein (SpoIVA) is a Walker-A type ATPase that forms a filamentous coat around *B. subtilis* forespores[160] and assembles into filaments in an ATP hydrolysis-dependent manner *in vitro*[18]. There is substantial uncertainty as to whether other Walker-A type ATPases (for example, septum site-determining protein MinD and chromosome partioning proteins Soj, SopA and ParF, all previously classified as Walker A cytoskeletal ATPases[161]) form functional filaments *in vivo*.

Forespores
Precursors of spores, a 'cell in a cell'.

Box 3 | **Tools for studying prokaryotic cytoskeletons**

Our understanding of the molecular biology of prokaryotic filament systems has been advanced through diverse experimental approaches. However, a small range of techniques and technological improvements has been crucial for providing mechanistic insights in recent years.

Single particle (helical) cryo-EM

Accurate atomic models of protein filaments are necessary for a complete mechanistic understanding of cytoskeleton function. Single particle cryo-electron microscopy (cryo-EM) allows structure determination of specimens, including filaments, in a frozen hydrated state analogous to a solution state. By contrast, X-ray crystallography rarely permits structure determination of (helical) protein filaments in a native-like state. Several prokaryotic filament structures have been solved recently using cryo-EM[46,47,51,80,89,106], with increasingly high resolutions made possible largely because of developments in electron detectors and reconstruction algorithms[175], including those for helical reconstruction[176]. These structures have yielded insights into subunit conformational cycles[29,80], lateral interactions between protofilaments[51,89,106] and the mechanistic conservation within filament system families[80,106]. Single particle methods are powerful for determination of high-resolution helical structures but are currently limited in their ability to interrogate low-abundance populations, which are often of great interest, for instance, helical discontinuities and filament ends. Additionally, nonhelical filament specimens (for example, cell division protein FtsZ and rod shape-determining protein MreB) remain a challenge for investigation using three-dimensional cryo-EM.

***In vitro* reconstitution and single molecule imaging**

Reconstitution of filament systems *in vitro* provides a powerful approach for understanding filament behaviour and dynamics. Reconstituted systems can be visualized in a native-like state either by use of high-resolution light microscopy methods (especially total internal reflection fluorescence microscopy (TIRF)) and fluorescently labelled proteins or through cryo-EM. Important insights have been gained by reconstituting filament systems, for example, FtsZ–FtsA-driven membrane constriction[26,30,75], spatial regulation of the Z-ring by the Min system[177], MreB membrane association[58,59], plasmid segregation protein ParM[178] and TubZ[44] plasmid segregation systems, archaeal endosomal sorting complexes required for transport (ESCRT) system properties[145] and visualization of microtubule hallmarks in the dynamic properties of a bacterial four-stranded minimicrotubule (bacterial tubulins termed Btubs A and B (BtubAB)[44].

Cryo-electron tomography of cells

Cryo-electron tomography (cryo-ET or electro cryotomography) of prokaryotic cells allows subcellular structures to be visualized in a native state *in vivo* at macromolecular resolution (~4 nm), whereas subtomogram averaging produces ensemble *in vivo* structures at subnanometre resolution and beyond[179]. Cytoplasmic filaments are typically visible in tomograms[169], and cryo-ET is now an essential tool for understanding prokaryotic cytoskeletons in their cellular context. This context, which often leads to new hypotheses about function, has been provided for a number of systems, for example, MamK[78], FtsZ[30,31], ParM[89] and archaeal ESCRT systems[143]. Cryo-ET remains a relatively low-throughput method and is still limited by the lack of a practical genetic label for unambiguous molecular identification (demanding elaborate alternative approaches[30]). Nevertheless, the ability to place atomic resolution information (through hybrid methods) in the context of cells will continue to be a driving force for prokaryotic cell biology.

Fluorescence microscopy

Imaging fluorescently tagged proteins in live and fixed cells has been a core technique for the study of prokaryotic cytoskeletons for two decades[180], and it is the only method to directly observe filament dynamics *in vivo*. In recent years, improvements in microscope design, imaging methodology, reagents and software have culminated in the ability to perform more complex experiments and obtain better images with reduced acquisition times, increased contrast and improved resolutions, including beyond the diffraction limit with super-resolution methods. These developments enabled the visualization of peptidoglycan synthesis and FtsZ filament movements in live cells, revealing the nature of the association between the two processes with unprecedented clarity[24]. Similar approaches were crucial for our current understanding of MreB function[62]. Future applications of super-resolution microscopy to study other filament systems will be important for understanding their dynamics. However, light microscopy is limited for the study of protein filaments due to the resolving power (even with current super-resolution methods) being worse than the dimensions of many functional filaments and the necessity of potentially disruptive fluorescent tags for live imaging.

Spirochaetes (including the Lyme disease pathogen *Borrelia burgdorferi*) achieve an unusual mode of motility using periplasmic flagella that are anchored at either end of the inner membrane compartment and produce backward-moving waves that propel the bacterium forwards (reviewed in REF. 162). The flagella are in some cases responsible for generating the helical (or flat wave) cell morphology as well as motility and so have a cytoskeletal function. Some Spirochaetes (Treponemes) have an additional, cytoplasmic filament system that also appears to generate helical morphology[163,164].

The cell-spanning cytoskeletal ribbons of *Spiroplasma* spp. (with which MreB associates) are composed largely of unique fibril proteins, which are crucial for maintaining cell shape and in some cases needed for motility (reviewed in REF. 71).

Several unidentified cytoskeletal elements have been observed *in vivo* through electron microscopy methods (for example, REF. 165). These could be unrecognized examples of proteins that have been mentioned above, but they almost certainly include as yet undiscovered filament-forming proteins.

Subtomogram averaging
The process of aligning subvolumes extracted from tomograms to improve signal:noise ratio and obtain high-resolution structural information.

Conclusions and outlook

Over the past 25 years, it has emerged that cytoskeletons are important and often essential cellular components of bacteria and archaea. The protein filaments that form these cytoskeletons are useful because they organize cells at length scales larger than the size of their constituent monomers.

A recent surprise has been the discovery of protein filaments that organize processes using a limited number of monomers (a few tens) in dynamic ways. It appears that bacterial FtsZ and MreB, the founding members of the prokaryotic cytoskeleton, both function mainly through short filaments that organize peptidoglycan remodelling locally. Such advances in knowledge mean we are reaching a point where our understanding of the biology of the major filament systems in well-studied bacteria is becoming comprehensive. By contrast, our basic understanding of the biology of filament systems in archaea remains poor. The study of these molecules, particularly *in vivo*, will be crucial for improving our understanding of archaeal cell biology and of how ancestral filament systems functioned.

For both archaeal and bacterial filaments, our understanding of the molecular mechanisms by which these dynamic systems function is limited by a shortfall in *in vivo* and *in vitro* structural studies; the only example where we have an almost complete molecular explanation of function is the relatively simple ParM plasmid segregation system. We await with excitement the additions to that list that will come in the next 25 years of prokaryotic cytoskeleton research.

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