

A growing family: the expanding universe of the bacterial cytoskeleton

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Abstract

Cytoskeletal proteins are important mediators of cellular organization in both eukaryotes and bacteria. In the past, cytoskeletal studies have largely focused on three major cytoskeletal families, namely the eukaryotic actin, tubulin, and intermediate filament (IF) proteins and their bacterial homologs MreB, FtsZ, and crescentin. However, mounting evidence suggests that these proteins represent only the tip of the iceberg, as the cellular cytoskeletal network is far more complex. In bacteria, each of MreB, FtsZ, and crescentin represents only one member of large families of diverse homologs. There are also newly identified bacterial cytoskeletal proteins with no eukaryotic homologs, such as WACA proteins and bactofilins. Furthermore, there are universally conserved proteins, such as the metabolic enzyme CtpS, that assemble into filamentous structures that can be repurposed for structural cytoskeletal functions. Recent studies have also identified an increasing number of eukaryotic cytoskeletal proteins that are unrelated to actin, tubulin, and IFs, such that expanding our understanding of cytoskeletal proteins is advancing the understanding of the cell biology of all organisms. Here, we summarize the recent explosion in the identification of new members of the bacterial cytoskeleton and describe a hypothesis for the evolution of the cytoskeleton from self-assembling enzymes.

Introduction

Cellular organization is fundamental for the viability of all organisms. Eukaryotes have long been known to organize cellular components such as DNA, RNA, and protein using both membrane-bound organelles and cytoskeletal proteins. Bacteria on the other hand were classically thought to be unorganized because of their small size and lack of organelles. However, advances in our ability to visualize cellular structures and subcellular protein localizations have revealed that bacteria, like eukaryotes, are highly organized. In fact, we now appreciate that the ability to localize proteins, both spatially and temporally, is essential for the function of many fundamental bacterial processes including division, growth, and motility. For example, the reorganization of the cell wall during bacterial cell division is mediated by penicillin-binding protein 3 (PBP3) (Curtis et al., 1985), which must be directed to the nascent division site in order for division to occur (Weiss et al., 1997; Goehring & Beckwith, 2005). The

skeletal *tus*, and *Pseudomonas aeruginosa* depends on the proper localization of motility structures such as flagella and pili to the cell poles. In *P. aeruginosa*, for example, if the localization of polar pilus determinants is altered, ectopic pili are produced and cell motility is decreased (Cowles & Gitai, 2010). Subcellular protein localization plays similarly important roles in many other bacterial processes, including chromosome segregation (Gitai *et al.*, 2004, 2005), cell polarity (Gitai *et al.*, 2004), and virulence (Jain et al., 2006). More recently, it has become clear that in addition to organizing their proteins, bacterial cells coordinate the subcellular positions of other macromolecules such as DNA (Jensen *et al.*, 2002; Kahng & Shapiro, occur 2003), RNA (Russell & Keiler, 2009; Valencia-Burton). The *et al.*, 2009), and lipids (Romantsov *et al.*, 2007; Renner

localization of another penicillin-binding protein, PBP2,

along the cell wall is essential for proper cell growth and

shape determination in many rod-shaped bacteria (Figge

et al., 2004; Dye et al., 2005). The motility of several bac-

terial species such as Vibrio cholerae, Caulobacter crescen-

& Weibel, 2011). Thus, cellular organization is a central theme in the biology of eukaryotes and bacteria alike.

Cytoskeletal proteins are key mediators of cellular organization

In eukaryotes, cytoskeletal proteins have long been appreciated as key mediators of cellular organization. Cytoskeletal proteins can both interpret spatial cues and exert forces on cellular scales because of their ability to selforganize into large polymers that are on the same physical scale as that of cells. Although the definition of what constitutes a cytoskeletal protein is open for interpretation, here we classify a protein as cytoskeletal by its ability to polymerize into linear filaments and play a structural or regulatory role in promoting cellular organization. Cytoskeletal protein filaments can organize the cell by acting as tracks or scaffolds on which proteins are trafficked or assembled for precise spatial and temporal localization. Cytoskeletal filaments may also contribute mechanically to cellular organization by exerting forces on targets such as membranes or chromosomes, thereby contributing to the structural integrity of the cell itself. Finally, cytoskeletal proteins can act as scaffolds to spatially organize the activities of other proteins.

The three canonical eukaryotic cytoskeletal proteins are actin, tubulin, and intermediate filaments (IFs). One of the primary functions of actin filaments is in the establishment and maintenance of cell shape during growth and division (Pollard & Cooper, 2009; Heng & Koh, 2010). The dynamic polymerization and depolymerization of actin filaments can push against the membrane or produce contractile forces that are useful in cell motility, adhesion, and cytokinesis (Mogilner & Keren, 2009; Pollard & Cooper, 2009). Tubulin forms dynamic filaments that are generally used for intracellular transport. For example, during mitosis, microtubules attach to the centromere through the kinetochore and pull chromosomes apart through a depolymerization-based mechanism. Tubulin filaments exhibit dynamic instability, i.e. the rapid switching from addition to subtraction of monomers in the filament (Wade, 2009). This instability is harnessed by cells in the retraction of chromosomes to opposite sides of the dividing cell to ensure faithful segregation (Kline-Smith & Walczak, 2004). Unlike actin and tubulin, IFs assembly is not nucleotide dependent. These proteins assemble into elastic fibers that are primarily used in structural roles throughout the cell (Chang et al., 2009; Iwatsuki & Suda, 2010).

More recently, a number of other filament-forming protein families such as septins, BAR domain proteins, amyloid proteins, and ESCRT-dependent proteins have been discovered in eukaryotes. For example, septin

proteins polymerize to form rings at the division plane, and septin polymerization is essential in yeast (McMurray et al., 2011). Septin rings also inhibit the diffusion of membrane proteins, helping to compartmentalize the mother and daughter cell in yeast (Takizawa et al., 2000) and the axonal and somato-dendritic compartments in neurons (Caudron & Barral, 2009). BAR domain proteins can polymerize into tightly curved structures that both sense and regulate membrane curvature (Rao & Haucke, 2011). There are many members of this family, some of which play important roles in endocytosis and vesicular trafficking (Rao & Haucke, 2011). Amyloid proteins can form prion-like filaments in certain conformations (Baxa, 2008). Amyloids were originally implicated in a range of neurodegenerative diseases and were thus largely thought to be toxic accidents of protein folding (Baxa, 2008). However, recent evidence suggests that amyloid formation can be dynamically regulated. For example, phosphorylation promotes the polymerization of CPEB, and regulated prion assembly appears to have a beneficial function in local protein synthesis during memory formation (Si et al., 2010). ESCRT complexes are involved in eukaryotic membrane and vesicle trafficking, and the ESCRT-III proteins Snf7 can form filaments (Henne et al., 2011). Recently, helical filaments that depend upon ESCRT-III were identified at the sites of cell septation at the end of mammalian cytokineses, suggesting that these filaments may help generate a constricting force (Guizetti et al., 2011). Thus, while the word cytoskeleton generally evokes discussion of actin, tubulin, and IFs, it is clear that eukaryotic cells possess an additional repertoire of filament-forming proteins whose polymerization serves a wide range of structural cytoskeletal functions.

The 'canonical' bacterial cytoskeleton

Like eukaryotic cells, bacteria also contain cytoskeletal elements that are essential for cellular organization and play structural roles in the cell. The first bacterial cytoskeleton to be characterized was the tubulin homolog FtsZ (Bi & Lutkenhaus, 1991), followed by the actin homolog MreB (Jones *et al.*, 2001) and the IF-like protein crescentin (Ausmees *et al.*, 2003). These three cytoskeletal elements remain the best-characterized bacterial cytoskeletal proteins and have been reviewed extensively elsewhere, such that we will only briefly introduce them here to provide context for the newly appreciated filaments.

FtsZ, the tubulin homolog

The FtsZ tubulin homolog polymerizes into linear protofilaments in a GTP-dependent fashion and is conserved in nearly all free-living bacteria (Erickson *et al.*, 2010). In almost all bacteria examined, FtsZ localizes to a so-called Z-ring at the site of cell division, where it serves an essential function in cell constriction. Although different bacterial species use different mechanisms to regulate the site of FtsZ assembly, most species actively preclude FtsZ polymerization at sites away from midcell, thereby using the localization of FtsZ assembly as a mechanism for determining the site of cell division (Kirkpatrick & Viollier, 2011). FtsZ sits atop a cascade of additional cell division proteins that get recruited to the division plane (the divisome). FtsZ thus serves as a scaffold that coordinates all of the events that are necessary for proper division, including cell constriction, cell wall rearrangements, and chromosome decatenation (Margolin, 2005). In addition to this scaffolding function, purified FtsZ filaments can exert a constrictive force on isolated membranes (Osawa et al., 2008), suggesting that FtsZ may help generate the motive force for cell constriction. Both biochemical and electron cryotomography studies have proposed that FtsZ filaments generate this constrictive force by initially associating with the membrane in a linear conformation and then using GTP hydrolysis to bend the filaments, thereby pulling the membrane inward (Lu & Erickson, 1999; Li et al., 2007). A recent imaging-based screen for FtsZinteracting proteins in C. crescentus identified an accessory factor, FzlA, which may assist in this curvature-based division mechanism (Goley et al., 2010).

MreB, the actin homolog

MreB is the most widely conserved bacterial actin homolog and can be found in most, although not all, nonspherical bacteria as well as in some archaeal species (Jones et al., 2001). In vitro, MreB has best been characterized from Thermatoga maritima and forms linear double filaments that polymerize in an ATP or GTP fashion (van den Ent et al., 2001; Salje et al., 2011). The MreB structure closely resembles that of actin (van den Ent et al., 2001), although MreB has an additional amphipathic helix that promotes its direct association with membranes, enabling it to generate membrane curvature in vitro (Salje et al., 2011). MreB assembly may differ between species, as the Bacillus subtilis MreB homolog has been suggested to polymerize independently of any nucleotides (Mayer & Amann, 2009). The most conspicuous MreB-associated phenotype is the loss of rod-like cell shape; in most species, perturbing MreB leads to cell rounding (Shaevitz & Gitai, 2010). MreB functions in cell shape determination by interacting with several cell wall-associated proteins such as MreC, MreD, Pbp2, RodA, RodZ, and MurG, thereby dictating the positions at which new cell wall material is incorporated (Shaevitz & Gitai, 2010). Biophysical measurements of Escherichia

coli cell stiffness suggest that MreB can also play a structural role that contributes to determining the mechanical properties of bacterial cells (Wang et al., 2010). While early studies suggested that MreB forms long continuous helical structures in vivo (Jones et al., 2001; Gitai et al., 2004), such structures have not been observed by electron cryotomography (Swulius et al., 2011), and recent livecell imaging studies suggest that MreB forms short discontinuous structures (Dominguez-Escobar et al., 2011; Garner et al., 2011; van Teefelen et al., 2011). These short MreB structures are rotated around the cell circumference by a motor activity that depends upon cell wall synthesis (Dominguez-Escobar et al., 2011; Garner et al., 2011; van Teefelen et al., 2011). Computational modeling suggests that the proper maintenance of rod-like morphology requires a dynamic interplay between MreB and cell wall synthesis, where MreB positions the initial sites of new cell wall synthesis, while coupling MreB rotation to the process of cell wall assembly ensures a uniform distribution of initiation sites (van Teefelen et al., 2011). The specifics of how MreB promotes rod-like growth and the details of the in vivo ultrastructure of MreB remain active areas of investigation. Finally, in addition to its role in cell shape determination, various species may require MreB for a number of other cellular processes including cell polarity, protein localization, chromosome dynamics, and virulence factor regulation (Shaevitz & Gitai, 2010).

CreS, the IF homolog

Crescentin (CreS) is a coiled-coil-rich *Caulobacter* protein that has a similar domain architecture to that of mammalian IFs (Ausmees *et al.*, 2003). Like IFs, crescentin forms linear filaments *in vitro* in a nucleotide-independent fashion. In *Caulobacter*, crescentin localizes to the inner cell curvature, and loss of crescentin causes the normally crescent-shaped *Caulobacter* to grow as straight rods (Ausmees *et al.*, 2003). At least in standard laboratory settings, crescentin null mutants grow and divide as well as wildtype cells and have no reported phenotypes other than cell straightening (Ausmees *et al.*, 2003). Consequently, the selective advantage of crescentin expression and *Caulobacter* curvature remains mysterious.

Unlike MreB and FtsZ, crescentin assembly does not appear to be dynamic *in vivo* (Charbon *et al.*, 2009). Crescentin can interact with other cytoskeletal elements, as its localization can be perturbed by MreB (Charbon *et al.*, 2009), and its assembly can be perturbed by a bifunctional cytoskeletal metabolic enzyme, CTP synthase (CtpS) (Ingerson-Mahar *et al.*, 2010) (CtpS is reviewed below in further detail). Although the specific mechanism by which crescentin leads to cell curvature remains unknown, it is clear that it does so by patterning cell growth (Cabeen *et al.*, 2009). One compelling hypothesis is that crescentin forms an elastic structure that is tethered near the cell poles and resists expansion during growth, generating a compressive force that biases new cell wall insertion in a gradient away from crescentin (Cabeen *et al.*, 2009).

An increasingly diverse set of tubulin, actin, and IF homologs

Recent studies have significantly expanded the catalog of potential bacterial cytoskeletal proteins, demonstrating that FtsZ, MreB, and crescentin are clearly not alone. These additional cytoskeletal proteins include both additional homologs of tubulin, actin, and IFs and novel protein families. We will first concentrate on the additional tubulin, actin, and IF homologs.

MreB is just one of a large set of bacterial actin homolog subfamilies

Many bacteria encode multiple MreB homologs in their genomes (Jones *et al.*, 2001). In some cases, these proteins appear to function together. For example, in *B. subtilis*, three MreB paralogs co-assemble into a single structure, have similar intracellular dynamics, and functionally interact to determine cell shape (Carballido-Lopez *et al.*, 2006; Defeu Soufo & Graumann, 2006). In other cases, divergent MreB isoforms have taken on other dedicated functions. For example, in *Magnetospirillum magnetotacticum*, the MreB paralog MamK forms filaments that coordinate the linear organization of magnetosome organelles (Komeili *et al.*, 2006; Katzmann *et al.*, 2010). MamK is part of a distinct branch of MreB homologs found in a variety of species, such that similar structural roles for MreB paralogs could be widespread.

Another well-studied bacterial actin homolog is ParM. ParM is an actin-like protein that is not found on bacterial chromosomes, but rather on bacterial plasmids such as R1 (Gerdes *et al.*, 2004). ParM forms filaments *in vitro* that assemble in a nucleotide-dependent fashion and exhibit dynamic instability (Garner *et al.*, 2004; Salje *et al.*, 2010). Stabilization of ParM filaments by the plasmid-associated ParR protein is required for proper plasmid partitioning, as ParM filaments extend between the two plasmids and insertional polymerization drives them to opposite cellular poles (Gerdes *et al.*, 2004). This system constitutes an extremely simple DNA segregation apparatus and has been biochemically reconstituted *in vitro* (Garner *et al.*, 2007).

ParM shares similar levels of sequence homology with both MreB and actin, such that its evolutionary origins remain unclear. A recent bioinformatic study indicated

that MreB, MamK, and ParM represent only the tip of the iceberg of bacterial actin homolog diversity (Fig. 1). Through bioinformatic mining of sequenced genomes, 35 new bacterial protein families with significant similarity to eukarvotic actin were identified (Derman et al., 2009). These new actin families bear little overall sequence similarity to eukaryotic actin or MreB, but contain five signature sequence motifs common to actin, MreB, and other actin-like proteins (Alp proteins). The vast majority of these Alp proteins are found on phage genomes, plasmids, and integrating conjugative elements, suggesting that they may function in the transmission or partitioning of DNA. A few of the Alp families, such as Alp32, are found in bacterial chromosomes. These species generally also encode an MreB family member, suggesting that much like MamK, Alps may have taken on specialized functions in different organisms.

A fluorescent fusion to one of these newly identified actin-like proteins, Alp7a, revealed that it formed linear structures reminiscent of filaments *in vivo* (Derman *et al.*, 2009). These filament structures were dynamic and exhibited subunit exchange and treadmilling, similar to the behavior of actin. Alp7a can also exhibit dynamic instability and plays a role in the segregation of plasmids in *B. subtilis* (Drew & Pogliano, 2011). The role of other Alp families is presently unknown. It is worth noting that



Fig. 1. The superfamily of bacterial actin homologs [Courtesy of Joe Pogliano; figure and caption reprinted with permission from (Shaevitz & Gitai, 2010)]. Shown is a phylogenetic tree of the bacterial actin subfamilies that have been identified to date based on sequence homology. The subfamilies that have been experimentally shown to polymerize are labeled and colored.

other bacterial proteins such as the widely conserved division protein FtsA and enzymes such as hexokinase and Hsp70 bear sequence and structural similarity to actin (Bork *et al.*, 1992), but the extent to which these proteins are cytoskeletal is still unclear.

Interestingly, while all of the bacterial actin homologs studied to date form filaments, the structures and proteinprotein interaction surfaces of these filaments appear to differ dramatically. For example, eukaryotic actin forms a double helix with a right-handed pitch, while ParM forms a left-handed double helix, AlfA forms a lefthanded double helix with a different pitch angle, and MreB forms linear nonhelical protofilament pairs that directly interact with the inner membrane (Popp & Robinson, 2011; Salje *et al.*, 2011). The mechanism by which proteins with a presumed common ancestor have evolved such divergent assembly conformations remains mysterious.

Additional bacterial tubulins

Similar to the case with MreB, bioinformatic studies have identified additional bacterial tubulin homologs. One bioinformatic study revealed two additional FtsZ-like protein families present in bacteria and archaea (Makarova & Koonin, 2010). The first group, dubbed the FtsZ1 family, was found in over 120 bacterial and archaeal species. Although the function of the FtsZ1 genes remains unknown, they were often present in operons with genes that shared homology with eukaryotic genes involved in vesicle trafficking and membrane remodeling (Makarova & Koonin, 2010). Clues to the function of the second family of FtsZ proteins are even sparser.

While the functions of additional chromosomal FtsZ isoforms remain unclear, some plasmids encode tubulin homologs that may promote their proper partitioning. Supporting the general idea that many plasmids are segregated by filament-forming proteins, the tubulin homolog TubZ was found to be encoded on the *Bacillus thuringiensis* pBToxis plasmid (Larsen *et al.*, 2007). Surprisingly, TubZ forms right-handed double-helical filaments that resemble actin filaments rather than microtubules (Aylett *et al.*, 2010). Furthermore, TubZ filaments treadmill *in vivo* (Larsen *et al.*, 2007). Thus, both actin and tubulin filaments appear to exhibit remarkable plasticity in their structural and kinetic properties, potentially enabling them to evolve to accommodate a wide range of cellular tasks.

Yet another family of tubulin homologs has been identified in a limited number of *Prosthecobacter* species. These include two proteins, BtubA and BtubB, that bear far more similarity to eukaryotic alpha and beta tubulin than FtsZ or TubZ (Jenkins *et al.*, 2002). BtubA and BtubB coexist with FtsZ in some species (Pilhofer *et al.*, 2007; Yee *et al.*, 2007), and their cellular functions are not well understood. Whether these proteins represent horizontal gene transfer from a eukaryote or an evolutionary intermediate between FtsZ and tubulin remains a point of debate.

Many coiled-coil-rich proteins may form IF-like structures

Crescentin is the only bacterial protein to bear very clear sequence similarity to IFs, with four distinct coiled-coil domains and a stutter in the fourth coiled coil (Ausmees et al., 2003). However, most bacterial genomes encode for a number of coiled-coil-rich proteins (Ccrps), and increasing evidence suggests that many of these Ccrps can form filaments with some similarity to IFs. A recent study found Ccrp candidates in 21 of 26 genomes examined, and all three of the specific genes tested spontaneously self-assembled into filaments (Bagchi et al., 2008). Extending the IF analogy, one of these coiled-coil proteins, FilP, was shown to play a structural role in hyphae of Streptomyces coelicolor; strains deleted for FilP show reduced hyphal rigidity and display unusual branching morphologies (Bagchi et al., 2008). The stomach pathogen Helicobacter pylori also encodes Ccrp proteins that can assemble into extended filaments and affect cell shape (Waidner et al., 2009; Specht et al., 2011). Additional cytoskeletal coiled-coil proteins include RsmP in Corynebacterium glutamicum (Fiuza et al., 2010) and Ccrp in Bdellovibrio bacteriovorus (Fenton et al., 2010).

Additional bacterial cytoskeletal proteins unrelated to actin, tubulin, or IFs

The discovery of bacterial homologs of each of the major eukaryotic cytoskeletal families, actin, tubulin, and IFs, was rightfully hailed as a triumph for bacterial cell biology. However, it has quickly become clear that there are yet more cytoskeletal proteins in bacteria that share no sequence or structure similarity to eukaryotic cytoskeletons. For example, electron cryotomography studies of Caulobacter cells identified four families of filaments of unknown identity (Briegel et al., 2006). A genome-wide localization study performed in Caulobacter also uncovered several unexpected proteins that localized as linear structures in cells, suggesting that they may be forming filaments in vivo (Werner et al., 2009). With mounting evidence for the existence of numerous new filament-forming proteins that potentially play important roles in cellular organization, it seems likely that the

traditional 'big-three' eukaryotic homolog-centered view of the bacterial cytoskeleton needs to be updated.

ParA and the Walker A Cytoskeletal ATPases (WACAs)

The best-characterized filament-forming protein families that lack eukaryotic homologs are the Walker A Cytoskeletal ATPases (WACAs). As the name implies, WACA proteins are ATPases that can form linear filaments in vitro (Lowe & Amos, 2009). WACAs form a subfamily of the large P-loop NTPase family, which include small GTPase proteins, signal recognition particle proteins, and eukaryotic cytoskeleton-associated proteins such as septins and the dynein microtubule motor (Lowe & Amos, 2009). WACA proteins are found in most bacterial species. The two most-studied WACA proteins include ParA, which mediates plasmid and chromosome segregation, and MinD, which regulates division site placement. No WACA proteins have been identified in eukaryotic nuclear genomes, although they are present in archaea and have been associated with plant and green algae plastids.

ParA forms dimers and filaments in an ATP-dependent fashion (Fig. 2) (Gerdes et al., 2010). When not bound to ATP, these proteins are monomeric; however, upon binding to ATP, ParA forms dimers that in turn polymerize and associate with additional factors such as DNA and the accessory protein ParB. ParB interaction stimulates ATP hydrolysis, promoting ParA depolymerization. In Caulobacter and V. cholerae, ParA structures appear to trap the parS origin-proximal region of one of the nascent chromosomes and pull it across the cell, with ParA depolymerization providing the putative motive force for DNA transport (Fogel & Waldor, 2005; Ptacin et al., 2010; Schofield et al., 2010; Shebelut et al., 2010). ParA homologs are also found on many plasmids where they promote plasmid segregation (Ringgaard et al., 2009). MinD also forms higher-order structures in an ATPdependent fashion (Suefuji et al., 2002; Shih et al., 2003), although the cytoskeletal nature of these proteins in vivo remains less clear. ATP-bound MinD cooperatively binds the inner membrane and recruits MinC, an inhibitor of FtsZ polymerization (Lutkenhaus, 2007). In E. coli, MinE binds ATP-bound MinD and stimulates ATP hydrolysis and dissociation from the membrane. The antagonistic interactions between MinD and MinE set up an oscillating Turing pattern that E. coli cells exploit to define the long cell axis and initiate FtsZ assembly at the cell center, away from the time-averaged highest concentration of MinD and MinC (Lutkenhaus, 2007). Species such as B. subtilis lack MinE and instead recruit MinD to the cell poles as well as the sites of recent division events

(Marston *et al.*, 1998; Gregory *et al.*, 2008). Yet, other species such as *C. crescentus* lack the entire Min system. Instead, *C. crescentus* encodes a different WACA protein, MipZ, which associates with the polar origins of replication and inhibits FtsZ assembly (Thanbichler & Shapiro, 2006). MipZ does not tightly cluster at its polar anchor but rather has a graded localization pattern (Thanbichler & Shapiro, 2006), such that it may form filaments similar to other WACA proteins.

ESCRT proteins may mediate division in archaea and some bacteria

The archaeal kingdom is comprised of two main phyla, the Crenarchaeota and the Euryarchaeota. Like most bacteria, Euryarchaeota rely on FtsZ for cell division. Crenarchaeota, however, lack FtsZ homologs but do possess filament-forming ESCRT protein homologs (Samson & Bell, 2009). In crenarchaeal *Sulfolobus* species, these ESCRT proteins localize to a ring at the division plane and perturb cell division upon overexpression (Samson *et al.*, 2008). Interestingly, some of the rare bacterial species that lack FtsZ homologs, such as *Chlamydia*, also possess ESCRT protein homologs. Thus, much as ESCRT proteins have been implicated in the final stages of mammalian cytokinesis, this filament-forming protein family might also mediate division in some archaea and bacteria.

Bactofilins form filaments and are conserved in a wide range of bacteria

Bactofilins represent a recently identified class of bacterialspecific filament-forming proteins. A screen of proteins that are upregulated in the swarmer to stalked cell transition of Caulobacter resulted in the identification of two paralogous proteins, dubbed BacA and BacB, that localize to the newly forming stalked pole where they co-localize with short filaments that can be visualized by electron cryotomography (Kuhn et al., 2010). BacA and BacB can form filaments in vitro and form linear structures when overexpressed in Caulobacter or heterologously expressed in E. coli. Co-immunoprecipitation of BacA and BacB revealed an interaction with penicillin-binding protein C (PbpC), a protein also localized to the formed stalked pole. Deletions of the bactofilins (BacA and B) in Caulobacter resulted in mislocalization of PbpC and decreased stalk length, suggesting that BacA and BacB play a role in organizing stalk biogenesis factors (Kuhn et al., 2010). Bactofilin homologs are found in most bacterial genomes. In Myxococcus xanthus, bactofilins also form filaments, but regulate motility and morphogenesis (Kuhn et al., 2010; Koch et al., 2011). Thus, the bactofilin



Fig. 2. A model of the depolymerization-based mechanism by which ParA pulls DNA (figure and caption reprinted with permission from Gerdes *et al.*, 2010) and based on Ringgaard *et al.* (2009). ParA-ATP dimers bind cooperatively to nucleoid DNA, leading to the formation of ParA filaments. Formation of filaments begins with a nucleating core from which rapid polymerization proceeds (1). Subsequently, a growing filament contacts a plasmid via ParB bound to parC centromere DNA (2). ParBs bound to *parC* on the plasmid stimulate the ATPase activity of ParA-ATP at the end of the filament (3). By this reaction, ParA-ATP is converted to its ADP form and released from the DNA, leaving a new ParA-ATP filament end accessible for interaction with the partition complex. For each depolymerization event, the plasmid can either detach (4') or remain attached to the end of the depolymerizing ParA filament (4). The moving plasmid leaves behind it a ParA-free nucleoid zone (5). Eventually, the ParA-ATP subunits released by ParB/*parC* assemble into a new filamentous structure in this zone that polymerizes toward the plasmid from the opposite side. Upon contact formation, this filament will move the plasmid in the opposite direction. In this way, a plasmid will jiggle around its position in between two other plasmids or between a plasmid and the nucleoid end. Finally, free ParA-ADP is rejuvenated to ParA-ATP and the cycle repeats (6).

proteins may play a variety of cellular roles in different bacterial species.

CtpS is a metabolic enzyme with an additional cytoskeletal function

In addition to the bacterial-specific cytoskeletons such as WACAs and bactofilins, recent studies have demonstrated that there are also previously unappreciated conserved cytoskeletal elements. A genome-wide screen of fluorescent protein fusions in *C. crescentus* identified an unusual filament-like localization for the metabolic enzyme CtpS (Werner *et al.*, 2009). Previously characterized as the primary enzyme responsible for the synthesis of CTP from UTP, ATP, and glutamine, CtpS is conserved in all organisms. In *Caulobacter*, CtpS localizes as a linear structure

along the inner curvature of crescent-shaped Caulobacter, mirroring the localization of one of the previously unidentified Caulobacter filaments observed by cryoelectron tomography (Ingerson-Mahar et al., 2010). Furthermore, Caulobacter CtpS formed filaments when expressed in heterologous systems such as fission yeast, and overexpression and depletion of the protein in Caulobacter corresponded to a lengthening and shortening of the filaments observed by EM. The E. coli CtpS homolog (a.k.a. PyrG) also formed linear structures in vivo, and purified E. coli CtpS formed filaments in vitro that could be visualized by electron microscopy (Ingerson-Mahar et al., 2010). Thus, CtpS from both Caulobacter and E. coli appears to self-assemble into linear filaments. Two additional studies demonstrated that eukaryotic CtpS homologs from yeast, flies, and rats can also form

filamentous structures (Liu, 2010; Noree *et al.*, 2010). Thus, polymerization appears to be a conserved property of this ubiquitous enzyme.

While the conserved function of CtpS polymerization may be related to regulating its enzymatic activity (Noree et al., 2010), Caulobacter cells have co-opted CtpS filaments for a cytoskeletal role in cell shape determination (Ingerson-Mahar et al., 2010). Caulobacter cells normally exhibit a characteristic crescent-shaped curvature, and the localization of CtpS to the inner cell curvature suggested that CtpS might regulate this process. Indeed, overexpressing CtpS caused Caulobacter cells to straighten, while CtpS depletion caused cells to become overly curved (Ingerson-Mahar et al., 2010). As described earlier, cell curvature in Caulobacter is also mediated by the IF homolog crescentin (Ausmees et al., 2003), which co-localizes with CtpS to the inner cell curvature. Although CtpS and crescentin can form filaments independently of one another, they associate and form a negative feedback loop. Specifically, crescentin recruits CtpS to the inner curvature, while CtpS keeps crescentin assembly in check to prevent excessive curvature (Ingerson-Mahar et al., 2010).

Point mutants that abolish CtpS enzymatic activity but do not abolish its ability to polymerize retain the ability to regulate *Caulobacter* cell shape (Ingerson-Mahar *et al.*, 2010). Thus, morphogenesis depends on CtpS polymers independently of their ability to synthesize CTP. Furthermore, while *E. coli* cells lack crescentin and are not curved, *E. coli* CtpS can fully complement both the metabolic and cell shape functions of *Caulobacter* CtpS (Ingerson-Mahar *et al.*, 2010). Thus, *Caulobacter* cells appear to have co-opted the generally conserved property of CtpS polymerization for a cell-specific structural role in cell shape determination.

Cytoskeletal proteins may have evolved from metabolic enzymes

Several reviews have discussed the evolution of modern cytoskeletal proteins from their common cytoskeletal ancestors. But how did cytoskeletal proteins evolve in the first place? *E. coli* CtpS can complement both the metabolic and cytoskeletal functions of *Caulobacter* CtpS (Ingerson-Mahar *et al.*, 2010). Thus, CtpS likely evolved filament formation before it was adapted to serve a cyto-skeletal role in *Caulobacter*. This method of co-opting filaments whose polymerization was selected for a different function such as enzymatic regulation may be a common theme in the evolution of cytoskeletal proteins. Interestingly, CtpS is not the only metabolic protein that is known to form filaments. For example, acetyl-CoA carboxylase, glutamate dehydrogenase, and arginosuccinase all form polymers *in vitro* (Gregolin *et al.*, 1966; Kleinschmidt

et al., 1969; Haschemeyer & De Harven, 1974). Furthermore, glutamate synthase, guanosine diphosphatemannose pyrophosphorylase, and the eIF2/2B translation factors all form filaments in yeast (Noree et al., 2010), although whether these proteins play structural or cytoskeletal roles remains unknown. The crystallin proteins that make up the lens of animal eyes represent another example of metabolic enzymes whose assembly has been repurposed for a structural function (Zigler & Rao, 1991). Actin and MreB both contain domains that are structurally similar to sugar kinases such as hexokinase (Bork et al., 1992), suggesting that the actin ancestor, like CtpS, may have evolved from a filament-forming metabolic enzyme. If filament formation is a widely distributed characteristic of metabolic enzymes, then metabolic enzymes may have generally served as the raw material for the evolution of cytoskeletal proteins (see model proposed in Fig. 3).

The expanding bacterial cytoskeletal universe

Only a short while ago, bacteria were thought of as unorganized bags of enzymes. Now bacteria are known to be highly organized, and homologs of all the major eukaryotic cytoskeletal proteins have been found. Understanding the components of the bacterial cytoskeleton is critical to understanding many fundamental bacterial processes. Despite the importance of the bacterial cytoskeleton, our understanding of the components of the bacterial cytoskeleton remains incomplete, as is our understanding of the regulation and function of each of the growing number of cytoskeletal proteins. The number and diversity of localized proteins and cellular components suggests that the cytoskeleton is much more complex than



Fig. 3. A model of cytoskeletal evolution with modern examples of each step (figure and caption reprinted with permission from (Barry & Gitai, 2011). The first event in this process is the evolution of self-assembly as an enzymatic regulatory mechanism. Polymerizing filaments can then be repurposed as dual-role enzymes with both enzymatic and structural functions (such as membrane association, shown here). Eventually, dual-role enzymes may have diverged into cytoskeletal filaments that lost catalytic function (bottom) or active enzymes whose regulation by higher-order assembly was replaced by other regulatory mechanisms (top).

currently appreciated. The identification of CtpS, bactofilins, WACAs, and hosts of tubulin, actin, and IF-like proteins suggests that most cytoskeletal elements evolved before the divergence of bacteria and eukaryotes. This raises the exciting possibility that identification of novel cytoskeletal proteins in bacteria, like CtpS, may enrich our understanding of the composition and evolution of the eukaryotic cytoskeleton as well.

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