Structural/functional homology between the bacterial and eukaryotic cytoskeletons

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Structural proteins are now known to be as necessary for controlling cell division and cell shape in prokaryotes as they are in eukaryotes. Bacterial ParM and MreB not only have atomic structures that resemble eukaryotic actin and form similar filaments, but they are also equivalent in function: the assembly of ParM drives intracellular motility and MreB maintains the shape of the cell. FtsZ resembles tubulin in structure and in its dynamic assembly, and is similarly controlled by accessory proteins. Bacterial MinD and eukaryotic dynamin appear to have similar functions in membrane control. In dividing eukaryotic organelles of bacterial origin, bacterial and eukaryotic proteins work together.

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Abbreviations

AMPPNP	5′-adenylyl-imidodiphosphate
EM	electron microscopy
GFP	green fluorescent protein
GTP	guanosine 5′-triphosphate
MAPs	microtubule associated proteins
MD	mitochondrion-dividing
PD	plastid-dividing
PH	pleckstrin homology

Introduction

Almost all eukaryotes depend on polymers of actin and tubulin to organize their cytoplasm, although the same basic elements are used in a variety of ways [1]. The ancestors of these two self-assembling dynamic filamentous proteins are believed to have evolved first in prokaryotes. The hypothesis that the bacteria cytoskeleton is related to the eukaryote cytoskeleton was first established when the bacterial Z-ring, which constricts the cell during division, was visualized using fluorescence (Figures 1a,b) and was shown to consist of FtsZ, a protein with a fold that mirrors tubulin [2] and displays similar dynamic properties [3,4•]. The relationship between eukaryotic and prokaryotic filaments became pronounced when the proteins MreB, Mbl and ParM [5,6,7••–9••] were shown to possess structures (Figures 2 and 3) and dynamic properties [7••,9••] that resemble those of actin.

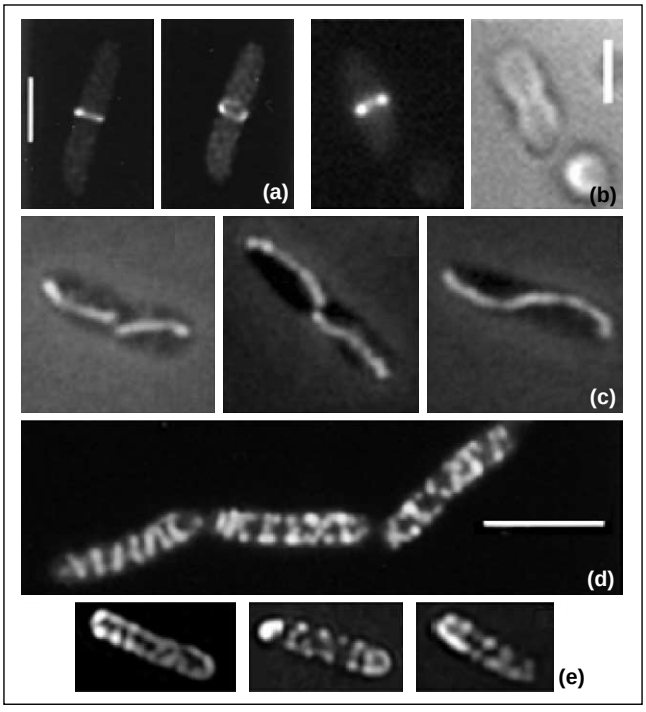
The ancestors of other eukaryotic cytoskeletal proteins are likely to be identified in prokaryotes in the near future. Owing to low sequence conservation, uncovering distant relationships between structural proteins will depend on comparisons of their macromolecular and atomic structures, as well as their functional mechanisms; FtsZ amino acid sequences show less than 20% identity to tubulin, while MreB and ParM have 15% and 12% identity to actin, respectively. Furthermore, prokaryotes seem to be more diverse than eukaryotes because the only cell division protein to be identified as common to almost all prokaryotic organisms is FtsZ; however, other common elements might have been missed because of sequence variation.

Determination of cell shape

Actin filaments and microtubules usually cooperate in controlling the shapes of eukaryotic cells [1]. Cell membranes tend to be supported by an underlying layer of one or other of these cytoskeletal filaments. Such a role for actin-like filaments seems to have originated in bacteria. Two proteins, MreB and Mbl (MreB-like), each form cables that follow a helical path close to the membrane of *Bacillus subtilis* (Figure 1d) [5,9••]. Equivalent proteins are found in most other non-spherical bacteria. Mutants lacking MreB are round instead of rod-shaped; however, some non-spherical bacteria that naturally lack actin-like proteins control their shape by extending the cell-wall only at the poles [10].

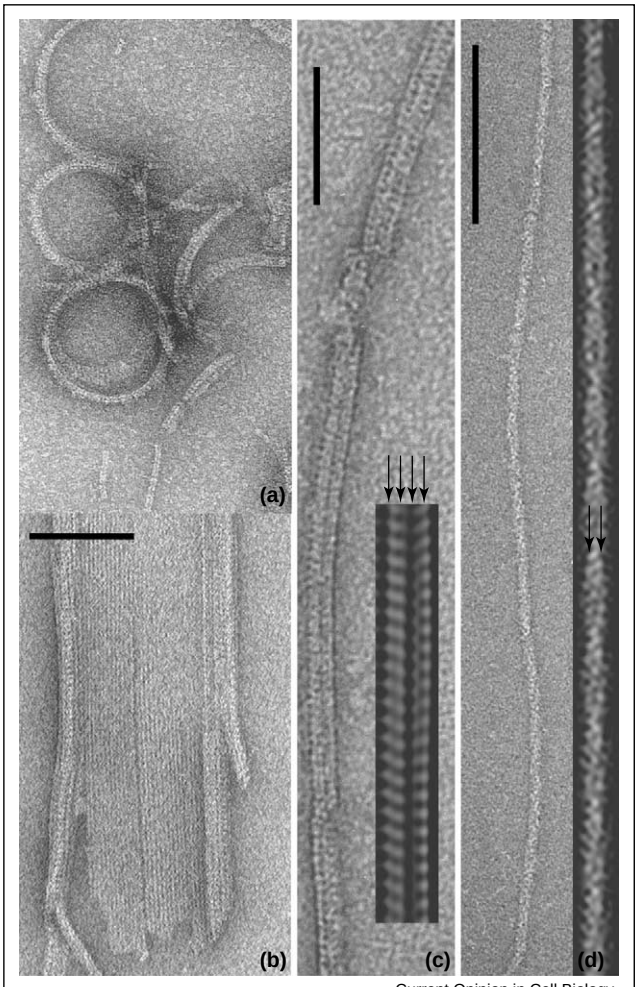
Investigation of the structure of MreB from *Thermotoga maritima* by X-ray crystallography and electron microscopy have amply confirmed that it is an actin homologue [6]. In the crystal structure (Figure 3), the four subdomains around the central nucleotide binding site in each monomer show a strong similarity to those of actin. More importantly, MreB was found to crystallize in such a way that monomers were lined up to form filaments whose longitudinal contacts confirm predicted contacts in an actin filament. The 5.1 nm longitudinal spacing of subunits in MreB filaments, compared with 5.5 nm for F-actin, was confirmed in electron micrographs of sheets and filaments that were assembled from purified protein (Figure 2) MreB appears to form two-stranded filaments (Figure 2c) similar to F-actin, except that the strands do not twist around each other. The double-stranded filaments further associate into pairs and larger bundles.

Figure 1



Light microscopy of fluorescently-labeled cytoskeletal filaments in bacteria. **(a)** Visualization of the Z-ring in *E. coli* cells expressing GFP–FtsZ; two different views of a cell undergoing constriction. Reprinted from [17], Copyright (1996) National Academy of Sciences, U.S.A. Bar 1 μm. **(b)** Brightfield and fluorescence images of an *E. coli* cell expressing a low level of GFP–FtsZ. Photobleaching experiments revealed that the Z-ring exchanges subunits rapidly with a cytoplasmic pool. Reprinted from [4•], Copyright (2002) National Academy of Sciences, U.S.A. Bar 2 μm. **(c)** Combined phase-contrast and immunofluorescence microscopy of fixed *E. coli* cells expressing wild-type levels of ParM. The fluorescent staining shows intracellular actin-like filaments. Reprinted from [7••], Copyright (2003), with permission from OUP. **(d)** Localization of GFP–Mbl (MreB-like protein) expressed in *Bacillus subtilis* shows dynamic helical bands. Immunofluorescence microscopy of endogenous Mbl in fixed cells gave similar images. Reprinted from [9••], Copyright (2003), with permission from Elsevier. Bar 4 μm. **(e)** *E. coli* cells expressing YFP (yellow fluorescent protein)-MinD. After deconvolution, these optically sectioned images of fixed cells reveal that MinD is present in helical filaments. MinE–GFP gave similar images. Reprinted from [47••], Copyright (2003) National Academy of Sciences, U.S.A.

Figure 2



Electron micrographs of negatively stained filaments. Both MreB [6] and ParM [8••] assemble into protofilaments with a subunit repeat close to that in F-actin. MreB protofilaments associate into pairs but do not twist to form helices like actin filaments; the 2-stranded filaments further associate into straight or curved bundles **(a-c)**; the various MreB polymers all assemble together in the same conditions. (b) Individual protofilaments, equivalent to those seen in atomic detail in the crystal structure of MreB, can also assemble into flat sheets. (c) A pair of double-stranded filaments; the inset is an enlarged filtered image. **(d)** ParM double protofilaments have an even more remarkable structural similarity to F-actin, as is clear from the enlarged filtered image (right). Bars 100 nm.

MreB can form curved, as well as straight, bundles *in vitro* (Figure 2a), suggesting that a cooperative conformational change can occur.

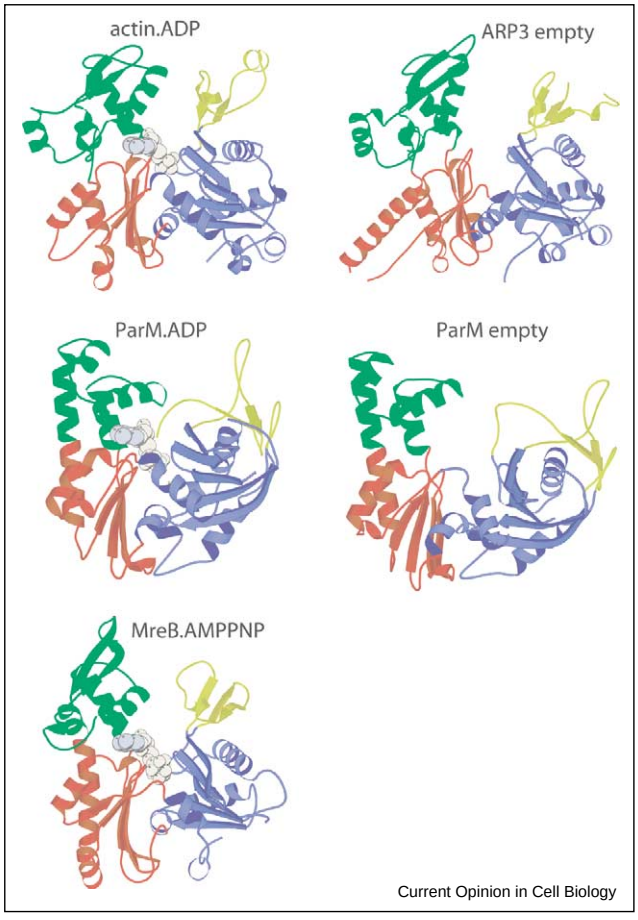
Chromosome separation

In eukaryotes, pairs of chromosomes are always moved apart by a spindle that is constructed from tubulin-containing microtubules and is driven by microtubule-associated motors. This is the most obvious feature that universally differentiates eukaryotes from prokaryotes; however, there are indications that bacteria do have simple cytoskeletal machines to assist in DNA segregation.

ParM self-assembly drives plasmid separation

The protein ParM [7••] forms highly dynamic actin-like filaments in the bacterium *Escherichia coli* and uses a self-assembly mechanism for force production and transport that is also used by eukaryotic actin. The crystal structure of the ParM monomer [8••] has a clear homology to those of actin and MreB (Figure 3). A bonus result from this study was a crystal structure of ParM without bound nucleotide. Of the eukaryotic proteins, only the

Figure 3



Ribbon structures of actin-family monomers: Actin.ADP, from pdb 1J6Z; eukaryotic actin-related protein ARP3, from pdb 1K8K [11]; bacterial ParM.ADP, from pdb 1MWM; ParM empty, from pdb 1MWK [8••]; bacterial MreB.AMPPNP, from pdb 1JCG [6]. Each monomer has two similar domains with a nucleotide binding site in the cleft between them. The four subdomains (1A, 1B, 2A, 2B) in each protein are coloured blue, yellow red and green. The crystal structure of ParM with and without ADP [8••] indicates a domain movement of 25°, close to the conformational change predicted for actin from a comparison of actin.ADP with ARP3 in the empty state and from EM studies of F-actin under different conditions [67]. The protofilament in the MreB crystal structure provided the first atomic-resolution view of inter-subunit contacts in an actin-family filament [6].

actin-related protein ARP3 has been seen in the empty form [11]. In both cases, the empty cleft is ~25° wider than in the ADP-filled form of the protein, indicating a hinge-like movement between domains. Electron microscopy of assembled filaments showed them to be double-stranded and helical (Figure 2d), with a subunit arrangement and longitudinal repeat similar to F-actin [8••]. The longitudinal repeat of 4.9 nm is slightly smaller than that of MreB (5.1 nm) or actin (5.5 nm); as a result of this and the fact that there are 12.5 monomers per repeat (as opposed to 12.5–14 in F-actin), the distance between crossovers is only 30 nm.

ParM is a product of one of the genes on the R1 plasmid, which maintains its presence in the bacterial host by segregating copies of itself towards the two cell poles, before cell division. Plasmids of this type encode a centromere-like DNA sequence, called *parC* in the case of plasmid R1, and two proteins, called ParM and ParR. Duplicated plasmids are paired at their centromeric regions through direct interactions with ParR. This ParR–*parC* complex interacts with ParM and stimulates its ATPase activity. Immunofluorescence microscopy has revealed ParM-containing filamentous bundles (Figure 1c) along the longitudinal axis of *E. coli* [7••].

Separation of bacterial chromosomes might be assisted in a similar way; orthologues of some of the *par* genes have been identified on chromosomal DNA (see Update). However, a DNA replication factory at the centre of the cell [12] is thought to supply at least some of the force that drives newly duplicated DNA towards opposite cell poles. Segregation starts with the replication origins, which move further from the centre as replication continues.

In eukaryotes, several types of protein complexes enable actin assembly to drive the movement of membranous organelles or to push forward the leading edge of the cell membrane [13]. Formins [14] seem to represent the closest system to the ParR–*parC* complex, in that they promote the assembly of a simple bundle of actin filaments. In such cases, assembly-driven activity might follow the actoclampin model [15], in which each growing filament is continuously tethered to a clamp molecule via a site on the side of the endmost, ATP-bound monomer. Addition of a new monomer on to the growing end triggers a cycle of ATP-hydrolysis and advancement of the clamp molecule to the new subunit on the same filament. By contrast, complexes that incorporate the actin-family proteins ARP2/3 nucleate new forwards-pointing actin filaments as branches from existing filaments [16]. The new filaments must then make new contacts with the membrane that is being pushed, using new ARP2/3-containing complexes.

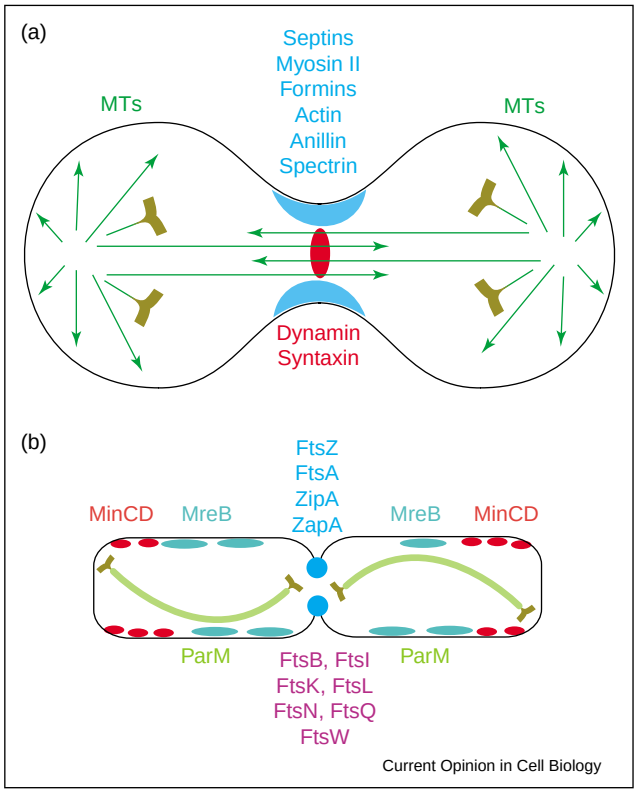
Although MreB and Mbl helical bundles are also highly dynamic *in vivo* [9••], it is not known whether their assembly can drive movement of other objects, in the manner of ParM or actin. Nor is it known whether any of the bacterial actin-like filaments can serve as tracks for motor protein-driven movement. As yet there is no evidence for linear motor proteins in prokaryotes, apart from those that move DNA [12].

Cytokinesis/septation

Constriction and abscission in different cell types

The division of most cells involves constriction of the membrane until there is a narrow connection between the daughter cells (Figure 4) and this is followed by a separate abscission step. One common theme in both

Figure 4



Some of the cytoskeleton proteins involved in cytokinesis. **(a)** Representation of a section through an animal cell when the actin-containing cleavage ring has contracted around the central bundle of microtubules (MTs) of the extended mitotic spindle, after the chromosomes have separated. Several proteins aside from actin are required to form the cleavage ring and produce constriction. Dynamin and syntaxin are required for membrane remodeling during the final stage of separation. **(b)** Representation of a section through a dividing bacterium. Proteins FtsA, ZipA and ZapA join the tubulin homologue FtsZ in the Z-ring, which constricts the cell membrane during division. Mutations in other Fts (filamenting temperature-sensitive) proteins prevent invagination of the outer cell wall. The actin homologue, ParM, separates copies of the plasmid on which it is encoded. Actin-like MreB is needed to give the cells the correct shape and also to segregate the chromosomes correctly. The Min proteins are needed to prevent a Z-ring from forming too close to a pole.

steps seems to be the use of filamentous GTP-binding proteins (FtsZ, septins, dynamin), although they are not closely related.

In bacteria, the Z-ring that is assembled from FtsZ initially defines the site of the division plane [17–19] and is then actively involved in the constriction mechanism. Other proteins are recruited to the ring, depending on species. In *E. coli* and *B. subtilis*, these include FtsA, another member of the actin family, and ZipA. In the early stage of constriction, either ZipA or FtsA can stabilize the Z-ring but FtsA is required for the final abscission [20,21]. Several other proteins are required to coordinate changes in the cell membrane with formation of a septum in the cell wall [18].

In fungi and animal cells, but apparently not in plants, the division plane is first defined by a ring assembled from septin filaments [22,23]. Myosin is then recruited [24] and actin bundles are assembled, organized by formins [14]. Acto-myosin sliding-filament contraction constricts the cell diameter until it is filled by the central-spindle bundle of microtubules (Figure 4).

In higher plants, the division plane is first defined by a dynamic ring of microtubules [25] but these do not cause constriction. Subsequently, other microtubules assemble at right angles to the division plane, forming a phragmoplast [26], which is similar to an extended central spindle. The final stage of cytokinesis is, perhaps, most clear in higher plants; Golgi-derived vesicles are transported along the microtubules of the phragmoplast to the plane of division, where they fuse with one another to form the incipient cell wall. Members of the dynamin family are essential components here [27•] and also in animal cells [28•]; in this situation, the role of dynamin could be either to package new membrane into tubes that can be delivered to the new cell wall and/or to remodel the newly fused membrane.

Dynamic assembly of FtsZ and its control

FtsZ and tubulin both assemble into protofilaments with a subunit spacing of 4.0–4.2 nm [29]. GTP, bound to a pocket at the top of one subunit, comes into contact with the bottom of the next subunit where a loop, known as T7, on the latter surface is responsible for triggering GTP hydrolysis [30•]. In the case of tubulin heterodimers, the T7 loop of α-tubulin is thought to hydrolyse GTP that is bound to the β-tubulin in another dimer, but the T7 loop of β-tubulin simply traps the GTP that is bound to its own α-tubulin partner. FtsZ might form homodimers as intermediate complexes during assembly [31] but GTP is, presumably, hydrolysed in every monomer. Both tubulin and FtsZ polymers become unstable after GTP hydrolysis and exhibit dynamic instability. *In vivo*, the Z-ring is even more dynamic than microtubules [4•]. The rapid turnover of GTP means that it is difficult to study the assembly of purified FtsZ *in vitro*, although some progress has been made recently [32–34]. However, the precise form of the *in vivo* polymer remains unknown. It is unlikely to closely resemble eukaryotic microtubules because lateral contacts between tubulin protofilaments are made by polypeptide loops that are missing from FtsZ [2]. It might consist of just a pair of protofilaments [29,33].

Just as microtubule stability is controlled by microtubule associated proteins (MAPs), assembly of FtsZ is promoted *in vivo* by proteins such as FtsA, ZipA [19–21] and ZapA [35]. ZipA, which has a long unfolded P/Q rich domain [36], perhaps best resembles typical MAPs, which

are unstructured in the absence of tubulin. MinC appears to inhibit the assembly of unwanted Z-rings (discussed in the next section) by binding to FtsZ polymer (and displacing FtsA [31]). The structure of the MinC dimer is compatible with a model in which it binds to the sides of two adjacent subunits in a protofilament [37], possibly inhibiting a lateral association of protofilaments that might be required to form the Z-ring [38]. Alternatively, MinC might cause disassembly by inducing curvature in FtsZ protofilaments. There is a precedent for this second mechanism, in the way that stathmin interacts with two tubulin dimers [39]. However, there is no evidence that MinC sequesters FtsZ in the way that stathmin sequesters tubulin. When *E. coli* or *B. subtilis* are under stress, FtsZ is sequestered by a protein called SulA, which prevents GTP hydrolysis by binding to the T7 loop [40•], or YneA [41], which may perform a similar role.

The Min systems in bacteria

A protein complex, called MinCD, is thought to be responsible for inhibiting FtsZ from assembling prematurely on to sites that will ultimately be at the centres of the two future daughter cells. MinD binds cooperatively to membranes in the presence of ATP and recruits MinC, which is required for the regulation of Z-ring assembly [31,37]. In *B. subtilis*, a protein called DivIVA recruits MinCD to each polar region [42], leaving only the site at the centre of the parent cell free to assemble a Z-ring but, in *E. coli*, MinCD is not localized in this way. Instead, a soluble protein, MinE, prevents MinCD from inhibiting Z-ring assembly at the central site [43•,44••,45,46•]. The activity of MinE sets up a surprising oscillation of all the Min proteins. Originating from the cell centre, a ring of MinE progresses towards one pole, disassembling MinCD as it moves. The depolymerized MinC and MinD are then free to assemble near to the other pole. On reaching the first pole, MinE is released to work on MinCD polymers that are formed in the second half of the cell. Each movement, from one half of the cell to the other, takes 30–50 s. Meanwhile, the whole cell gradually increases in length. Recent images with improved resolution [47••] have shown that MinCD does not coat the whole cell membrane near one pole but forms a long winding filament (Figure 1e); its relationship to the MreB filaments, if any, is still unclear.

MinD resembles dynamin

The similarities between dynamin and MinD might be purely coincidental, but it is interesting to compare them because they could share a common mechanism. The N-terminal domain of dynamin is a regular GTPase, with a strong similarity to Ras [48]. GTP regulates dimerization, as well as interactions with membranes and with a variety of actin-binding proteins [27•,28•]. The other domains have important roles in these interactions [49]. A 3D reconstruction of a dynamin polymer surrounding a membrane tubule has been calculated from electron microscope images [50]. It shows a helical arrangement of dimeric GTPase domains, which stand out from the membrane on 'legs', consisting of the smaller, regulatory domains. Contact with the membrane is via PH (pleckstrin homology) domains at the ends of the 'legs'.

The structure of MinD has been solved in the empty state as well as when bound to the nonhydrolysable ATP analogue AMPPNP (5′-adenylyl-imidodiphosphate) [51–53]. It belongs to a group of ATPases that are related to the P-loop GTPases [54]. The role of ATP is to modulate the interaction of the protein with itself and its partners, MinC, MinE and membranes [43•,44••,45,46•]. *In vitro*, MinD is known to dimerize with ATP before assembling directly on to the membrane to form a fairly tight helical polymer [43•,44••,45]. This polymer is superficially reminiscent of the outer wall of the dynamin helix, but presumably has no 'legs' distancing it from the membrane inside. Interaction with the membrane is via a short C-terminal segment [55,56], which is much smaller than the multidomain region of dynamin that follows the GTPase domain.

One possibility is that the MinCD polymer that is assembled *in vivo* (Figure 1) contains a tubule of fresh membrane that becomes incorporated into the cell membrane when released by MinE; such a mechanism would help ensure an even insertion of new lipid along the length of the cell. This would be similar to the proposed role for dynamin in organizing membrane insertion during the abscission of eukaryotic cells [27•,28].

Division of chloroplasts and mitochondria

FtsZ, MinD and MinE are found in eukaryotes — in chloroplasts (derived from cyanobacteria) [57] and in some primitive mitochondria (derived from α-proteobacteria) — and they serve important functions in organelle division [58,59•]. FtsZ is presumably required for marking the division site and/or constricting the inner membrane. Eukaryotic proteins, such as actin, organelle-specific dynamins [60••61••,62] and septins [63•], are also necessary for organelle division. Thus, the division mechanism is a hybrid of prokaryotic constriction and eukaryotic abscission. Most mitochondria lack FtsZ and, apparently, use only eukaryotic division mechanisms.

Electron microscopy of chloroplast structure [64•] shows electron-dense plastid-dividing (PD) rings, on the cytoplasmic face of the outer membrane and on the stromal face of the inner membrane. Mitochondria have a similar structure, known as the mitochondrion-dividing (MD) ring [65]. The chloroplast FtsZ ring localizes on the stromal side of the inner PD ring. Both the Z-ring and then the PD rings appear before division starts but the Z-ring disappears before the final abscission takes place. An attempt to isolate the PD rings in detergent [64•] yielded insoluble 5 nm filaments of unknown composition (but

the absence of septins from plants might be worth reinvestigating [63•]). A chloroplast-specific dynamin associates with the chloroplast outer membrane at a late stage of division [60••,61••] and it might be of significance that this is when the outer membrane needs to increase in area, even more than the inner membrane.

The activity of the Z-ring has been more clearly tracked in chloroplasts than in any bacterium, although we cannot be certain that the constriction mechanism, in conjunction with that of eukaryotic mechanisms, is truly representative of that in bacteria.

Conclusions

Cytoskeletal proteins that are related in both structure and function are currently being identified in bacteria and eukaryotic cells. The actin-like and tubulin-like proteins in bacteria are evolutionarily related to their eukaryotic counterparts, having similar functions and 3D structures. Bacteria also have accessory proteins that control FtsZ assembly in the way that MAPs control tubulin assembly, although there is no evidence that any are evolutionary precursors of MAPs. As yet, no associated proteins for bacterial actin have been found; the search for them will be an important objective in the near future. Researchers will also continue to look for prokaryotic motor proteins that might use FtsZ or bacterial actin filaments as tracks. The role of dynamins in the division of chloroplasts and mitochondria, and their role in eukaryotic cytokinesis, is another exciting area for further research. Finally, the full role of MinD and its accessory proteins in bacteria and chloroplasts demands declarative investigation.

Update

Recent immunofluorescence studies of helical MreB filaments in *E. coli* [66] have demonstrated that chromosomes fail to segregate properly in the round cells formed when MreB is missing or mutated. These results suggest that there is a bacterial chromosome segregation mechanism that is homologous to active plasmid separation [7••].

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The *E. coli* Z-ring was visualized with green fluorescent protein-labeled FtsZ. After photobleaching, the fluorescence recovered with a half-time of 30 s. The rapid turnover correlates well with the high GTPase activity of FtsZ *in vitro*. A mutant FtsZ, whose activity is much reduced, showed a corresponding reduction in turnover. Thus, the assembly dynamics of FtsZ, like those of tubulin, are regulated by GTP hydrolysis. Interestingly, the mutant cells divided with a normal cell-cycle time.

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Unlike most mitochondria, chloroplasts have retained FtsZ for division. This paper (and [61••,62•]) show that a requirement for a dynamin-related protein, with no obvious counterparts in prokaryotes, is common to both types of endosymbiotic organelle. ARC5 is related to a group of dynamin-like proteins that are unique to plants. A GFP–ARC5 fusion protein localizes to a ring at the chloroplast division site and ARC5 mutations cause enlarged, dumbbell-shaped chloroplasts. Import and protease protection assays indicate that the ARC5 ring is positioned on the outer surface of the chloroplast.

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Analysis of synchronized chloroplasts showed the time at which a dynamin-like protein is recruited to chloroplasts, forming a ring on the cytosolic side of the division site in the late stage of division. The ring constricts until division is complete and then disappears.

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