Bacterial ancestry of actin and tubulin

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The structural and functional resemblance between the bacterial cell-division protein FtsZ and eukaryotic tubulin was the first indication that the eukaryotic cytoskeleton may have a prokaryotic origin. The bacterial ancestry is made even more obvious by the findings that the bacterial cell-shape-determining proteins Mreb and Mbl form large spirals inside non-spherical cells, and that MreB polymerises *in vitro* into protofilaments very similar to actin. Recent advances in research on two proteins involved in prokaryotic cytokinesis and cell shape determination that have similar properties to the key components of the eukaryotic cytoskeleton are discussed.

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Abbreviations

F-actin	filamentous actin
FtsZ	filamentous temperature-sensitive protein Z
GTP	guanosine triphosphate
Mre	murein cluster e
MSP	major sperm protein
MT	microtubule

Introduction

Despite their apparent internal simplicity, bacteria undergo division at a remarkable speed and with a high precision that requires a dynamic intracellular organisation. Until recently, the lack of a cytoskeleton, which for eukaryotes is indispensable to complete mitosis and cytokinesis successfully, has been one of the defining features of prokaryotes. Besides mitosis, the eukaryotic cytoskeleton is vital for maintenance of cell shape, and for phagocytosis, organelle movement and locomotion. At least some of these processes occur in bacteria as well, but little is known about their regulation. Recent results indicate that bacteria contain proteins that are similar to cytoskeletal elements in eukaryotic cells. In this review, we will shed light on two key components of the eukaryotic cytoskeleton that have remarkable similarities to proteins involved in prokaryotic cytokinesis and cell shape determination.

Eukaryotic cytoskeleton

In eukaryotes, cell shape and the organisation of directed movements depend on the cytoskeleton. The operation of the eukaryotic cytoskeleton is based on microtubules and filamentous actin that work together. Both tubulin and actin couple intrinsic nucleotide triphosphate hydrolysis to polymer formation [1], whereas passive structures such as intermediate filaments are dependent on accessory proteins for polymer formation [2]. Intermediate filaments are not evolutionarily conserved, hence, in this review, we shall focus on tubulin and actin and their putative homologues in prokaryotic cells.

The eukaryotic cytoskeleton is not a static structure. The polymers of the cytoskeleton are highly dynamic, allowing the cytoskeleton to rapidly re-organise. Owing to the polymerisation dynamics, the polymers have the potential to carry out mechanical work, either via treadmilling (assembly at one end and dissociation at the other end) or via dynamic instability (stochastic changes in their length) [3,4]. Both actin and tubulin require nucleotides for their polymerisation, the hydrolysis of which destabilises the polymers. This is in contrast to polymer formation of bacterial flagellin and the tobacco mosaic virus (TMV) coat protein [1]. Another eukaryotic filament-forming protein is major sperm protein (MSP). Although MSP does not show any sequence homology to actin, it replaces actin in the sperm of certain nematodes [5]. The mobility of those cells is powered by dynamic polymerisation of MSP. (The exact mechanism of MSP polymerisation *in vivo* is not known. *In vitro*, ethanol was used to induce reducible polymerisation, and experiments are underway that show that accessory proteins may be involved in the control of MSP polymerisation.) The structure elucidation of the 14 kDa *Ascaris suum* α-MSP protein showed that it is a member of the immunoglobin superfamily of proteins [6]. There is no obvious candidate for an MSP-like protein in bacteria.

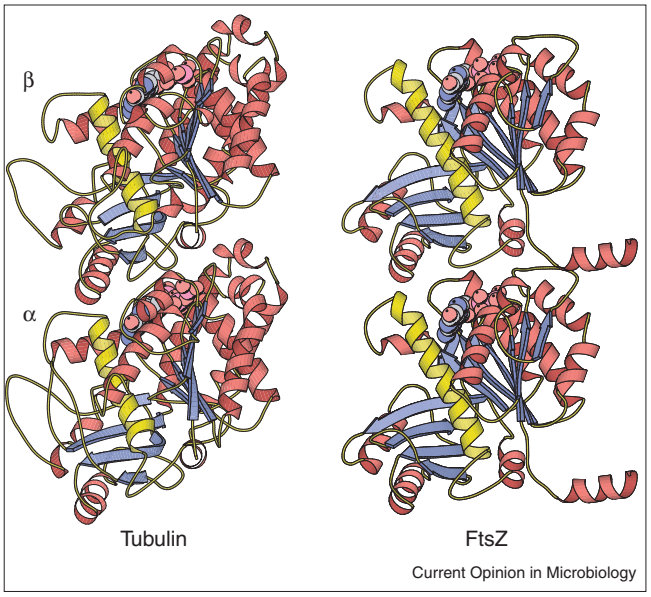
Dynamic polymerisation is only one mechanism by which actin and tubulin achieve some of their specific functions. The polar nature of microtubules and actin filaments controls the direction of motor proteins and hence enables the spatial organisation of the cell. Microtubules (MTs) are thought to be involved in long-range transport of organelles [7]. MTs serve as a track on which motor proteins, such as those of the kinesin and dynein superfamilies, carry their cargo. They form the mitotic spindle that segregates chromosomes and determine the plane of cleavage. In non-dividing cells, MTs are involved in the organisation of the cytoplasm, in positioning the nucleus and various organelles, and in the formation of flagella and cilia [8]. Filamentous actin (F-actin) forms the track for myosin motors and is used for local transport [3,9], as well as for cytokinesis and motility.

Tubulin and FtsZ

MTs are hollow cylinders (25 nm wide) that normally consist of 13 parallel filaments. Each filament is a longitudinal array of heterodimers of α- and β-tubulin. Both tubulin subunits bind the nucleotide guanosine triphosphate (GTP), but only GTP in β-tubulin is hydrolysed, resulting in destabilisation of the filament. The α- and β-tubulin subunits are 50% identical to each other in sequence. The

three-dimensional structure of tubulin, determined by electron crystallography, reveals remarkable structural similarity to a bacterial cell-division protein called filamentous temperature-sensitive protein Z (FtsZ) [10,11]. Despite low sequence similarity, the three-dimensional structures of tubulin and FtsZ are extremely similar. Both tubulin and FtsZ have a Rossmann fold in their amino-terminal part, with the characteristic parallel β-sheet of six strands, and co-ordinate the nucleotide (GTP) in a corresponding manner (Figure 1). The resemblance extends to the functions of tubulin and FtsZ [12]; both proteins exhibit GTP-dependent polymerisation into filamentous structures. GTP hydrolysis regulates the dynamic behaviour of FtsZ filaments and microtubules [8,13,14]. *In vitro*, tubulin and FtsZ form tubes and sheets that consist of parallel or antiparallel filaments [15,16]. *In vivo*, the tubulin filaments are arranged in a parallel fashion, whereas the arrangement of filaments in the Z-ring remains elusive. We believe the FtsZ tubes observed *in vitro* may be the polymer *in vivo*, for reasons discussed in [17].

Figure 1



The three-dimensional structure of a FtsZ dimer, shown on the right, has a similar fold to that of a tubulin dimer, which consists of α-tubulin (bottom left) and β-tubulin (top left). The FtsZ dimer was modeled using the coordinates with Protein Data Bank (PDB) entry code 1FSZ [15]. The tubulin dimer was based on electron crystallography data [49], PDB entry code 1JFF.

Functional role of FtsZ

FtsZ is the structure-forming component of the divisome [18], a putative protein complex involved in bacterial cell division. It forms a ring at the site at which division will occur. FtsZ is highly conserved and is present in most bacteria and archaea [19,20]. The Z-ring is also found in chloroplasts ([21•,22,23•,24•,25]; see also the review by KW Osteryoung [pp 639–646] in this issue), which is expected, as these organelles originated from cyanobacteria. In contrast, FtsZ is absent from most mitochondria even though mitochondria originated from prokaryotes [26]. It is only recently that FtsZ has been detected in mitochondria from the alga *Mallomonas splendens* [27•]. (Correct distribution of mitochondria during cytokinesis of higher organisms involves dynamin, which, like FtsZ, polymerises into a ring-like structure at the site at which constriction will occur [28].) Shortly after FtsZ-ring formation, FtsA is located at midcell. FtsA is the key component in the sequential recruitment of other components of the divisome [19,20].

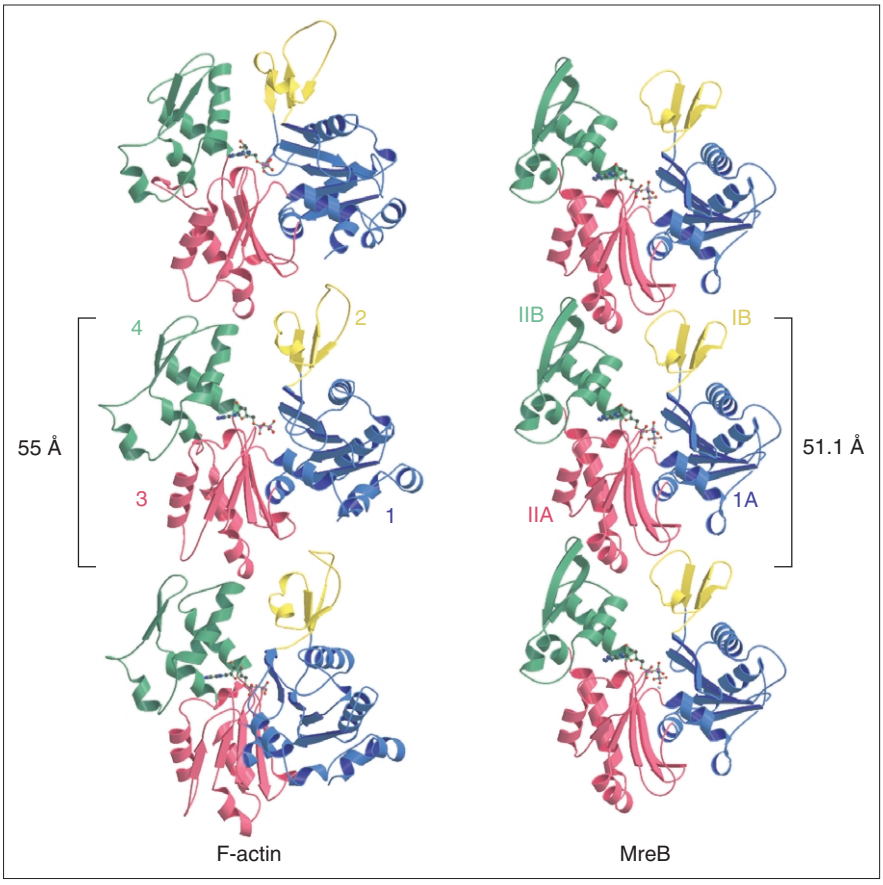
FtsZ: the bacterial ancestor of tubulin

The low sequence identity between FtsZ and tubulin (10%–18% on the amino acid level) may be a reason to argue that both proteins are the result of convergence rather than true homology. However, their three-dimensional structures are remarkably similar and both proteins exhibit a similar mechanism in their GTP-dependent polymerisation [12]. In both tubulin and FtsZ, a loop (T7) from the neighbouring subunit in a protofilament inserts into the active site and activates GTPase activity, ensuring that hydrolysis only occurs in the polymeric form. The structural and functional properties combined make it unlikely that this evolved twice. Although both tubulin and FtsZ define the division plane, the spatial organization of tubulin is more divergent than that of FtsZ. In bacteria, FtsZ actually forms the constricting ring, whereas in eukaryotes, the equatorial plane of the mitotic spindle determines where the cell divides. Only yeast and plants show an FtsZ-like ring, composed of tubulin, early in cytokinesis ([29,30]; D Brunner, personal communication). In all eukaryotes, the actual constriction is operated by bipolar myosin that constricts the actin filaments. The mechanism of constriction of the Z-ring in bacteria is poorly understood. The required force to constrict the cell could either be applied on FtsZ by motor proteins or could be intrinsic to FtsZ, the latter being dependent on a conformational change in the filament [26] or the result of treadmilling.

The actin family of proteins

The other major component of the eukaryotic cytoskeleton is filamentous actin (F-actin). F-actin is relatively thin and is composed of two strands that are twisted around each other [31]. Actin filaments are crosslinked into larger structures to obtain mechanical integrity. They are involved in cell locomotion, shape determination, phagocytosis, cytokinesis, rearrangement of surface components and the movement of organelles. The actin cytoskeleton contributes to cell locomotion in two different ways. Polymerization-driven motility accounts for phagocytosis, shape changes and ruffling of leading lamellipodia (membranous F-actin-containing sheets). Other processes of motility, including muscle contraction and cytokinesis, are based on myosin [32]. An insight into actin-based motility is provided by *Listeria monocytogenes*, an

Figure 2



The atomic structure of a MreB protofilament (shown on the right) is reminiscent of filamentous actin (on the left) – both have their subunits in the same orientation, resulting in a similar longitudinal repeat. The MreB protofilament is observed in the crystal structure [39••] (PDB entry 1JCF) and the F-actin is based on the original model [31]. Figure reprinted by permission from *Nature* (http://www.nature.com) **413**:39-44, copyright 2001 Macmillan Magazines Ltd.

intracellular pathogen that hijacks the actin machinery of the eukaryotic host cell and moves itself through the host cell by activating the host actin assembly.

Actin is a 43 kDa bilobed protein that binds ATP in a cleft between its two domains. The crystal structure of actin (Figure 2) has been solved, in complex with different proteins, to prevent actin polymerization [33–36], and recently on its own [37]. The actin family of proteins contains two domains (I and II), each of which can be divided into two subdomains (A and B), as shown in Figure 2. The larger two subdomains have a common fold, the RnaseH fold, which comprises a mixed β-sheet of five strands surrounded by α-helices. This fold is conserved within the actin superfamily of proteins (ASHKA), which includes Heat shock protein 70 (Hsp70) [38], the bacterial proteins Mreb [39••], FtsA [40•] and StbA, and sugar kinases [32,41]. The presence of those common folds in proteins with entirely different functions was the reason to postulate that these proteins could be the result of divergent evolution from a common ancestor [41]. According to this hypothesis, a single domain protein would have been duplicated and diverged into different proteins through the evolution of additional structural features. Hsp70 has an additional substrate-binding domain of more than 250 residues that is not related to the actin family of proteins. The sugar kinases have a characteristic motif comprising part of their active site that distinguishes them from the actin-like and Hsp70-like proteins. The three-dimensional structure of bacterial cell-division protein FtsA is closely related to actin and Hsp70 but, compared with actin or any other member of the superfamily, FtsA has one of its smaller subdomains located at the opposite side of the molecule [40•]. This subdomain is flexible and most likely has a functional role in cytokinesis. It is intriguing to find two proteins essential for bacterial cytokinesis (FtsA and FtsZ) that have structural resemblance to two proteins pivotal for eukaryotic cytokinesis (actin and tubulin, respectively). Nevertheless, FtsA does not form actin-like filaments *in vitro*, as was tested under various conditions [39••].

MreB: the bacterial ancestor of actin

The actin superfamily of proteins has two more putative members found in bacteria: StbA, which is involved in plasmid segregation, and MreB, which is part of the cell-shape determination system in prokaryotes [41]. The *mreB* gene is located within the *mre* (murein cluster e) operon that is associated with cell-shape determination, but not with synthesis of the cell envelope [42–44]. A first

indication that MreB is involved in the formation of intracellular structures was reported recently [45••]. Immunofluorescence experiments, using antibodies against MreB and MreB-like (Mbl) proteins, revealed large spirals in *Bacillus subtilis* that encircle the cytoplasm just under the cell membrane [45••]. The spirals that were observed with the light microscope suggested that MreB forms filamentous structures in bacteria, similar to the actin cytoskeleton of eukaryotes. Recently, it has been demonstrated that purified MreB from *Thermotoga maritima* forms polymers *in vitro* [39••]. Electron microscopy revealed that these polymers consist of protofilaments (each protofilament being a string of monomers), and that the spacing between the MreB subunits along the filament (51 Å) is reminiscent of the spacing between the subunits in filamentous actin (55 Å) (Figure 2). Also, the three-dimensional structure of MreB and actin are very similar [39••]. There is, however, one interesting difference between MreB and actin. F-actin consists of two protofilaments that gently twist around each other, whereas the pairs of MreB protofilaments are straight. The propensity of MreB to form straight polymers is reflected in the crystals, in which the MreB subunits are arranged into protofilaments. This provided, for the first time, a detailed look at the interface of an actin-like protofilament at atomic resolution.

Conclusions

In eukaryotic cells, motor proteins that operate on the cytoskeletal network facilitate active transport of cargo [46]. The small size and apparent lack of compartments in bacterial cells enables free diffusion of proteins in milliseconds [47]. However, it has been shown that many proteins as well as chromosomes have a specific subcellular localisation [48]. The internal organisation implies an anchoring structure that prevents free diffusion of cellular components. An efficient internal organisation is also required for complex processes such as cytokinesis and chromosome segregation that must resemble a mechanism almost as sophisticated as the eukaryotic mitotic spindle. A first indication of a cytoskeletal protein in prokaryotes is the resemblance between tubulin and FtsZ. These proteins are structurally related and both have a pivotal role in defining the division plane. FtsZ interacts with FtsA, which is a member of the actin family. However, FtsA has diverged from a putative actin ancestor and developed a unique structural feature. Like actin, it has an essential role in cytokinesis. MreB is another member of the actin superfamily of proteins and forms large helical structures inside the *B. subtilis* cell [45••]. The role of MreB in determining cell shape has an obvious parallel with the actin cortex underlying the plasma membrane. Furthermore, MreB has been shown to have a similar three-dimensional structure as actin and polymerises *in vitro* into polymers that resemble F-actin in many respects [39••]. Similar to the situation with FtsZ and tubulin, MreB and actin share the same longitudinal arrangement of their subunits but seem to have evolved into different polymers based on different lateral interactions of protofilaments. The recently obtained results suggest that MreB/Mbl proteins are the prokaryotic actin homologues.

Together, MreB/Mbl and FtsZ strengthen the hypothesis that the eukaryotic cytoskeleton has a prokaryotic origin and that more complex processes in bacteria such as cell division and chromosome segregation will have more similarities to processes in eukaryotes than previously thought.

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