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Review

Evolution of cytomotive filaments: The cytoskeleton from prokaryotes to eukaryotes

Jan Löwe*, Linda A. Amos

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK

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ABSTRACT

The basic features of the active filaments that use nucleotide hydrolysis to organise the cytoplasm are remarkably similar in the majority of all cells and are either actin-like or tubulin-like. Nearly all prokaryotic cells contain at least one form of FtsZ, the prokaryotic homologue of tubulin and some bacterial plasmids use tubulin-like TubZ for segregation. The other main family of active filaments, assembled from actin-like proteins, occurs in a wide range of bacterial species as well as in all eukaryotes. Some bacterial plasmids also use ParM, another actin-like protein. Higher-order filament structures vary from simple to complex depending on the cellular application. Equally, filament-associated proteins vary greatly between species and it is not possible currently to trace their evolution from prokaryotes to eukaryotes. This lack of similarity except in the three-dimensional structures and longitudinal interactions between the filament subunits hints that the most basic cellular function of the filaments is to act as linear motors driven by assembly dynamics and/or bending and hence we term these filament systems 'cytomotive'. The principle of cytomotive filaments seems to have been invented independently for actin- and tubulin-like proteins. Prokaryotes appear to have a third class of cytomotive filaments, typically associated with surfaces such as membranes or DNA: Walker A cytoskeletal ATPases (WACA). A possible evolutionary relationship of WACAs with eukaryotic septins is discussed.

Contents

1. Introduction

To reproduce themselves exactly, cells need mechanisms to control their shape during growth and to effect division into two daughter cells, each possessing a copy of the genetic information. Because of the dimensions of cells (whose size in turn is partly dictated by the space taken up by the DNA, from 100 nm up to tens of microns), only a very large superstructure will be able to influence and access all parts. Currently, all the cells that have been studied in detail use dynamic polymeric filaments for these purposes. For many years, a filamentous cytoskeleton was believed to be one of the defining characteristics of eukaryotic as compared with prokaryotic cells. However, researchers have gradually discovered the relatively inconspicuous but still highly active filaments that prokaryotic cells use to control their shapes and to constrict the membrane during cell division (recent reviews: Graumann, 2007; Pogliano, 2008). Now it is clear that cells possessing these filamentous proteins are so successful at reproducing themselves that

* Corresponding author. Tel.: +44 1223 252969.
E-mail address: jyl@mrc-lmb.cam.ac.uk (J. Löwe).

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natural selection has allowed them to displace life-forms that must have been able to grow and divide before the filaments we now see everywhere could evolve.

Filaments of the tubulin/FtsZ/TubZ family and actin/MreB/MreB-like/ParM family all bind nucleotide (GTP or ATP) and use unidirectional cycles of nucleotide hydrolysis to drive either dynamic instability (stochastic growth and shrinkage) or treadmilling or controlled assembly/disassembly or bending. They appear to be present in all types of cells, with the exception of the crenarchaea (Natale et al., 2000), whose division proteins are currently unknown.

In this review, we focus on the common principles of these filament systems, starting at the molecular level. It appears that the only properties conserved throughout evolution are their nucleotide hydrolysing activities and their structures, including the longitudinal contacts between the subunits that form a filament. The two basic classes of proteins, (actin-like and tubulin-like) exist in a bewildering number of uses in different cellular contexts, going hand in hand with a large number of accessory factors controlling these functions. We include, in this list of optional accessory factors, the molecular motors (kinesin, myosin) that are found in eukaryotes and use the filaments as tracks on which to travel long distances. No such molecular motors have been found in prokaryotes and the recent paper by Osawa et al. (2008) provides convincing evidence that none are required for FtsZ-driven membrane constriction.

Based on the conservation of only the most basic properties, including the longitudinal contacts and nucleotide hydrolysis during assembly, to us, the underlying key to the success of the two filament systems is their function as linear motors. Using energy stored in the nucleotide, the filaments themselves can create linear force. The force can actively push or pull objects or can be used to position objects against concentration gradients or thermal motion. Some filaments remodel membranes, possibly by actively sliding relative to each other. Therefore, we would like to propose the term '*cytomotive filaments*' for the dynamic filaments of actin and tubulin and their homologues that form the heart of the cytoskeleton, having been strongly conserved by natural selection. This will distinguish these proteins from the fibrous cytoskeletal proteins such as eukaryotic intermediate filaments (Oshima, 2007) and various coiled-coil filaments found in bacteria (Hurme et al., 1994; You et al., 1996; Ausmees et al., 2003; Yang et al., 2004; Mazouni et al., 2006), whose function is thought to be purely structural.

The many uses of cytomotive filaments, with or without accessory motors, are somewhat analogous to the very widespread use of motors in engineering where many different tasks are performed with the same device. In accordance with the idea that the only truly conserved function of cytomotive filaments is their longitudinal dynamic assembly, none of the large number of accessory factors that control the filaments seems to be conserved between prokaryotes and eukaryotes or even across all groups of prokaryotes (Michie and Löwe, 2006).

2. The tubulin/FtsZ/TubZ family of cytomotive filaments

This family of cytomotive filaments is almost ubiquitous in living cells. It now appears that the feature conserved during evolution of tubulin-like filaments is the longitudinal contact between adjacent 40–50 kDa protein subunits (Fig. 1A). Tubulin-like proteins consist of two conserved domains with the N-terminal domain providing nucleotide-binding and one interface of the active contact, whereas the C-terminal domain provides the other interface (Nogales et al., 1998a). After the contact is made during filament assembly, residues on the second interface directly activate the nucleotide, thus linking nucleotide hydrolysis with polymerisation. The two-domain structure and distribution of functions across the domains has led to the hypothesis that tubulin-like proteins once were two separate molecules with nucleotide-binding and hydrolysis-activation activity, respectively (Oliva et al., 2004). Generally, filament assembly (and not the nucleotide state of the subunits) is thought to cause a conformational change that in turn increases the hydrolysis rate in subunits other than the last one (Oliva et al., 2007; Huecas et al., 2008; Rice et al., 2008). This important feature and the ability to 'trap' the nucleotide in the filament, with no exchange (Romberg and Mitchison, 2004), enables the filaments to have dynamic instability (Mitchison and Kirschner, 1984), although it is currently thought FtsZ does not use this feature.

Clearly, FtsZ is an ancient protein (Erickson, 2007). Nevertheless, it is a multi-domain molecule with a sophisticated mode of activation. Almost certainly, cells were able to divide, by some unknown means, before this protein fold was perfected. The widespread occurrence of FtsZ and its homologues is proof of the superiority of this design, with its conserved three-dimensional structure and conserved longitudinal interaction around the GTP-binding pocket. Several different implementations of tubulin-like proteins exist in nature: FtsZ, TubZ, tubulins and BtubAB. We are confident that more will appear with more genome sequencing and others will also have existed, including intermediates that have gone extinct. Vaughan et al. (2004) have made a comprehensive survey of tubulin/FtsZ like sequences currently known in prokaryotic genomes, while FtsZ and eukaryotic tubulin sequences are compared by Erickson (2007).

FtsZ filaments consist of one type of subunit and they are involved in bacterial cell division, where the protein forms the Z-ring around the middle of the cell (Bi and Lutkenhaus, 1991) that, together with other proteins, brings about division of a cell into two daughter cells (Haeusser and Levin, 2008). A number of accessory proteins have been identified (review: Löwe et al., 2004), but their exact mode of action remains unclear at this moment (SulA is the only exception, (Cordell et al., 2003; Dajkovic et al., 2008)). It seems that at least part of the division process, namely the generation of a constrictive force on the membrane can be accomplished by FtsZ alone (Osawa et al., 2008), provided it has a means of linking to the membrane (normally provided by an accessory protein but Osawa et al. engineered their FtsZ to have its own membrane-binding peptide). It is thought that the nucleotide in these filaments is freely available, making it impossible for FtsZ filaments to be controlled by GTP-bound 'caps' at their ends as in microtubule dynamic instability (Romberg and Mitchison, 2004). *In vitro* under certain conditions, FtsZ shows complex dynamics (Chen and Erickson, 2005) and there is a continual turnover of GTP.

In vivo, the Z-ring seen by light microscopy displays strong dynamic behaviour (Anderson et al., 2004). However, it is currently unclear what mechanism the filaments use to constrict the membrane. Tomographic images of the division site in cells (Li et al., 2007) have shown isolated short filaments in contact with the membrane and the authors have suggested that GTPase-dependent bending of initially straight FtsZ protofilaments leads to a gradual cumulative constriction. However, structural data do not support the concept of nucleotide-dependent bending ((Oliva et al., 2004, 2007); see Rice et al. (2008) for a similar conclusion about tubulin). An alternative possibility to active bending by individual filaments is that pairs or small bundles of filaments interact transiently (for too short a time to be trapped in the tomographic specimens) and constrict the membrane through some form of relative sliding. Such a mechanism might explain FtsZ's high turnover of GTP.

TubZ was recently discovered and is a bacterial plasmid-borne protein (Larsen et al., 2007) that displays a highly dynamic implementation of the tubulin-like cytomotive filaments (Larsen et al.,

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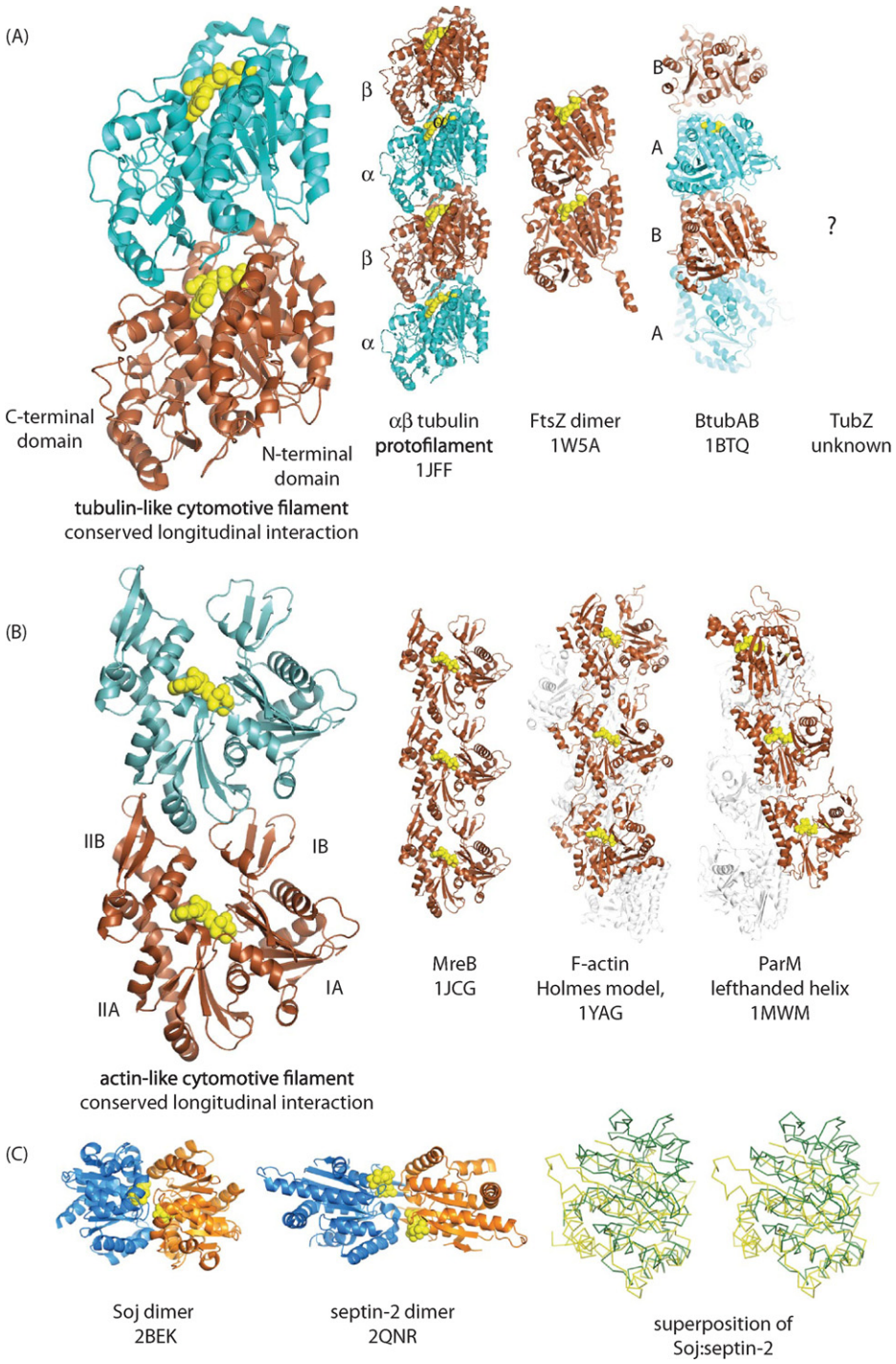

Fig. 1. Cytomotive filaments. (A) Conserved longitudinal interaction of tubulin-like cytomotive filaments. The feature conserved between tubulin-like cytomotive filaments from the bacterial and eukaryotic cytoskeleton is shown on the left. The N-terminal domain binds GTP and the T7 loop, bridging the N- and C-terminal domains, is in direct contact with the nucleotide of the next subunit, switching on hydrolysis. αβ-tubulin is a tight heterodimer with GDP trapped in the middle. FtsZ assembles from identical monomers. BtubAB assembles from free A and B subunits, producing an alternating protofilament. The structure of TubZ or its protofilaments is not known. Again, the protofilament (longitudinal interaction) is similar in all cases. PDB (pdb.org) identifiers of the structures depicted are shown for reference. (B) Conserved longitudinal interaction of actin-like cytomotive filaments. The feature conserved between known actin-like cytomotive filaments is shown on the left. Two subunits interact by inserting the tip of domain IIA into a cleft formed by subdomains IIB and IB of the next subunit. MreB forms straight filaments, whereas F-actin is right-handed and double-helical. ParM forms a left-handed double helix. The protofilament (longitudinal interaction) is similar in all cases. (C) WACAs, the third class of cytomotive filaments. A possible evolutionary connection between eukaryotic septins and WACA, the putative third class of cytomotive filaments. The soj dimer, formed in the presence of ATP, is shown on the left. It is quite similar to the septin-2 dimer, formed in the presence of GDP. When comparing the two dimers, it becomes apparent that the septin dimer is more open, but generally shows the same subunit orientation and the two nucleotides 'sandwiched' in the interface. Right: stereo drawing of the superposition of the soj and septin-2 monomer, performed with SSM (RMSD 3.0 Å, Z 2.7). Septin in yellow, soj in green.

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2007; Chen and Erickson, 2008). One may envisage that the system is loosely analogous to the well-characterised ParMRC system (Kruse and Gerdes, 2005) that, in contrast, uses an actin-like cytomotive filament. TubZ probably assembles filaments to push plasmids apart in the cell for segregation. An adaptor protein, TubR, on the same plasmid might link the growing filaments and the plasmid DNA directly (Larsen et al., 2007). There is currently no structural data available and the C-terminal domain is quite diverged from FtsZ. It will be interesting to see if there are any non-plasmid genes in bacteria or archaea showing similar properties, as suggested by sequence-based genome searches (Larsen et al., 2007).

BtubAB was found in some deep-branching Verrucomicrobia (Jenkins et al., 2002) and forms longitudinal protofilaments with alternating A and B subunits (Schlieper et al., 2005; Sontag et al., 2005). Unlike tubulin heterodimers, every longitudinal interface forms a fully active GTPase. The sequences and structures of BtubAB are amazingly similar to eukaryotic αβ tubulin and this fact, combined with the finding that no other genes in these organisms are so closely related to any eukaryotic ones, prompted the hypothesis that these genes were transferred from eukaryotes by horizontal gene transfer (see section below (Schlieper et al., 2005)).

Tubulins exist in all eukaryotic organisms. αβ-tubulin consists of two different (although structurally very similar) subunits that produce a tight heterodimer that has become the (now longer) soluble building block because GDP is trapped in the middle and the dimer is stable. Tubulins have loop insertions on the sides of the subunits that enable the protofilaments to laterally associate into microtubules (Nogales et al., 1999). Microtubules are a mainstay of the eukaryotic cytoskeleton and produce an enormous amount of complexity because they are the target of hundreds of associated proteins. Erickson (2007) has argued that tubulin has a much more conserved sequence than FtsZ, very distant from any known FtsZ, because it evolved to interact with many more associated proteins, including molecular motors. The highly successful final product then supported an explosive expansion of eukaryotic species, all using essentially identical cytomotive filaments.

Together with a great variety of cofactors, microtubules utilise any of the basic principles of cytomotive filaments (Akhmanova and Steinmetz, 2008; Gardner et al., 2008). By reducing the dynamics of the filaments, they can be made into stable tracks for molecular motors such as dyneins and kinesins. They can be actively polymerised, de-polymerised, seeded, cross-linked, severed and so on. Other tubulins (γ–ε) exist but have more specialised functions, such as seeding microtubule assembly. All apparently form the canonical longitudinal contact of tubulin-like cytomotive filaments (Nogales et al., 1998b; Rice et al., 2008).

3. The actin/MreB/MreB-like/ParM family of cytomotive filaments

The second class of cytomotive filaments consists of ~35 kDa subunits that in turn are thought to have evolved from a gene duplication of the RNAse-H fold (Artymiuk et al., 1993). The actin fold is not restricted to cytomotive filaments and several classes of enzymes and heat-shock chaperones use it as well (Kabsch and Holmes, 1995). Currently, the only reliable picture of the longitudinal contact in filaments comes from the crystal structure of bacterial MreB (van den Ent et al., 2001). In these protofilaments, the tip of subdomain IIa inserts into the cleft of the next subunit, formed by subdomains Ib and IIb (Fig. 1B). A similar contact is predicted by fitting the monomeric actin crystal structure into X-ray fibre diffraction or low-resolution EM data (Holmes et al., 1990). An important difference from the tubulin-like system is that the nucleotide in the filament is only in direct contact with one subunit and hydrolysis activation has to be more indirect. This is currently only poorly understood because of a lack of high-resolution structures in different nucleotide states. It is envisaged that activation proceeds via a conformational change that is transmitted to the nucleotide. Similar to the situation with tubulin-like cytomotive filaments several different implementations of actin-like filamentous proteins exist in nature: MreB and MreB-like, ParM and actin and they share conserved longitudinal interactions and conserved three-dimensional structures. As for tubulin and FtsZ, a variety of accessory proteins carry out functions such as assisting assembly or disassembly.

MreB is widespread in bacteria and is involved in shape determination by forming a helical superstructure under the cell membrane and localising cell wall synthesising enzymes (Jones et al., 2001). The protein forms straight protofilaments *in vitro* but the structure of filaments in cells is currently unknown (van den Ent et al., 2001). *In vitro*, the protofilaments tend to associate into ribbons. Because of biochemical difficulties with the protein, details of its properties and filament dynamics remain controversial (Esue et al., 2005; Bean and Amann, 2008). It is known, however, that the filaments are slowly dynamic in cells (Kim et al., 2006). Several bacterial species also contain genes for MreB-like proteins and they have now been shown to co-localise (Carballido-Lopez et al., 2006), opening up the possibility that they may form co-polymers, although this is highly speculative at this moment.

ParM is a bacterial plasmid-borne version of the actin-like cytomotive filaments and its main function is as a motor: the growing filaments push the low-copy-number plasmid to the extremes of the cell so they are segregated prior to cell division (Møller-Jensen et al., 2003). This partitioning system consists of only three components (ParM, adaptor protein ParR and *parC* DNA) and the system has been re-constituted *in vitro* (Garner et al., 2007). The ParM filaments are double helical, with two protofilaments winding around each other, the subunits being staggered and back-to-back (van den Ent et al., 2002; Orlova et al., 2007). This arrangement produces 'closed symmetry' so the filaments cannot grow further laterally and their helical nature is advantageous probably because it produces the same bending stiffness in all directions. Interestingly, the helix is left-handed (in contrast to F-actin, see below) (Orlova et al., 2007; Popp et al., 2008), but this is just one of the possibilities and seems important for the interaction with the adaptor protein whose geometry is dictated by the handedness of the DNA (which is fixed) (Møller-Jensen et al., 2007; Salje and Löwe, 2008). ParM filaments have dynamic instability and it is thought that the adaptor protein:DNA complex is sought and captured by ParM filaments that grow and shrink, until they are capped by complexes at both ends, when they are only allowed to grow (Garner et al., 2004). The adaptor protein ParR has 'formin-like activity', meaning it actively participates in the addition of new ParM subunits to the end. The ParR mechanism is different in detail from that of eukaryotic formins that polymerise actin and seems to have been invented independently.

Actin filaments (F-actin) are ubiquitous in eukaryotic cells and are structurally related to ParM filaments, though twisted to have opposite handedness; the protofilaments wind around each other as right-handed rather than left-handed helices. They follow the common principle of cytomotive filaments, with a conserved longitudinal contact between subunits along the protofilaments. The exact atomic structure of the filament is still not known because of the difficulty of crystallising a slow-rising helix but has been approximated using fibre diffraction and electron microscopy. A single-start left-handed helix follows a set of lateral contacts in F-actin that are closely similar to subunit contacts along a single-start right-handed helix in a ParM filament.

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As for eukaryotic tubulin, the number of actin-associated accessory factors is vast, allowing a wide range of dynamic behaviour (Staiger and Blanchoin, 2006; Carlier and Pantaloni, 2007). For example, the leading edge of a spreading or motile cell is driven by F-actin polymerisation in an analogous fashion to ParM's plasmid-separating activity. In different situations, F-actin can be helped to polymerise or de-polymerise, be severed, branched and walked on by molecular motor proteins such as myosins. Interestingly, eukaryotes have also developed ways to interconnect transport along the actin and tubulin cytomotive filaments (Basu and Chang, 2007; Gross et al., 2007).

4. An apparent 'switch of function' between the cytomotive filaments of prokaryotes and eukaryotes?

Actin-like and tubulin-like proteins may appear to have simply exchanged roles during the evolution of eukaryotes from prokaryotes, since cytokinesis (cell division) depends upon constriction by an FtsZ ring in bacteria but by an actin ring in eukaryotic cells (Eggert et al., 2006); on the other hand, chromosomes are separated by microtubules in eukaryotes but have been reported to be moved apart by actin-like filaments in *Caulobacter* (Gitai et al., 2005) (though MreB is probably not involved in chromosome segregation in other bacteria (Hu et al., 2007; Karczmarek et al., 2007)). However, the activity of a dynamic filament can change fairly easily from "treadmilling" to "search and capture" or from pulling to pushing; such behaviour can be switched by single amino acid changes in the filament protein sequence or by the activities of accessory proteins. The choice of what is pushed or pulled (DNA, membrane etc.) can also be changed. For example, both tubulin-like TubZ and actin-like ParM cytomotive filaments are involved in plasmid segregation (see above). Actin and MreB both give shape to cells, although in very different ways. FtsZ's function is to divide cells and this seems different from the role of actin in eukaryotic cytokinesis, for which myosin is also needed.

It seems possible that all such roles could have been carried out by several versions of a single class of filaments but evolution has produced two dissimilar alternative nucleotide-binding/splitting protein structures (and possibly a third, see below) that have survived evolution. Conversely, the structural similarity between different members of each group is unlikely to be the result of convergent evolution.

5. MinD/ParA/ParF/Soj (WACAs) in prokaryotes and septins in eukaryotes

Over the past few years it has become apparent that there is potentially a third class of cytomotive filaments, structurally unrelated to actin-like or tubulin-like: Walker A cytoskeletal ATPases (WACA) (review: Michie and Löwe, 2006). No information is currently available describing the nature of the assembled filaments, apart from crystal structures of monomers, dimers and filament bundles seen in negative stain electron micrographs, and hence it is difficult to guess what the conserved longitudinal interaction might consists of. Most likely, all of these proteins form sandwich dimers (Fig. 1C, left), depending on ATP binding (Leonard et al., 2005). It is thought that dimerisation makes the proteins polymerise. Some WACAs seem to form filaments by themselves (Barilla et al., 2005; Bouet et al., 2007), others seem to need a surface to attach to: MinD attaches via an amphiphatic helix to the membrane (Hu et al., 2002), Soj and related proteins bind to DNA while polymerising (Leonard et al., 2005). Importantly, filament formation does not seem enough to stimulate ATP hydrolysis. All the proteins mentioned have accessory proteins that stimulate hydrolysis *in trans* via a normally unstructured peptide (note that it has been proposed that tubulin-like cytomotive filaments started off like this during evolution (Oliva et al., 2004)). These proteins are very widely distributed amongst bacteria and archaea and are present in plastids and mitochondria. Exact homologues seem to be lacking in eukaryotic genomes but they may be related to eukaryotic septins. Septins have a related fold and form similar sandwich dimers and polymerise (Sirajuddin et al., 2007) (Fig. 1C) but, because of a lack of information about WACA filaments, it is currently impossible to compare them with septins at the polymer level. The tentative similarity of WACAs with eukaryotic septins is especially attractive because of the cellular roles of septins in cytokinesis (Byers and Goetsch, 1976; Versele and Thorner, 2005).

MinD is part of the MinCDE system for septum placement in bacteria or, more precisely, for inactivation of the poles (old septa) (Lutkenhaus, 2007). MinD is the WACA and MinE the activator. MinD binds to and polymerises on membranes via an amphipathic helix (Hu et al., 2002). The interplay of MinD and MinE on the membrane produces self-organising waves that are thought to increase the concentration of the MinC inhibitor at the poles of the cell, where it interferes with FtsZ polymerisation directly (Raskin and de Boer, 1999). The moving waves of MinCD have been reproduced *in vitro* on flat membranes recently (Loose et al., 2008).

ParA is a very common bacterial plasmid-borne WACA that acts in concert with ParB, that in turn binds a specific DNA sequence *parS*. Clearly, this arrangement is somewhat similar to the ParMRC system and these have been named type I (ParA) and type II (ParMRC, actin-like) plasmid partitioning systems (Gerdes et al., 2000). How exactly ParA affects partitioning is not known, but does involve the formation of polymers (Ebersbach and Gerdes, 2004; Pratto et al., 2008).

Soj is the chromosomal version of ParA, present in quite a few bacteria. Soj is known to oscillate rapidly in concentration from one side of the nucleoid (area of condensed DNA in the cell) to the other (Marston and Errington, 1999), helped by its ATPase activator Spo0J. Like the rest of the WACAs, it forms an ATP-dependent dimer. Soj binds un-specifically to DNA to form protein:DNA filaments (Leonard et al., 2005; Hester and Lutkenhaus, 2007).

ParF is another plasmid-borne WACA and acts together with ParG, which is related to ParR, the adaptor from ParMRC (see above) (Barilla and Hayes, 2003). These permutations of similar proteins reinforce the idea that the cytomotive filaments use common principles. ParF forms ATP-dependent filaments in the absence of surfaces (Barilla et al., 2005).

MipZ and *PpfA* are also WACAs. Very little is known about these proteins apart from the sequence similarity and their involvement in cellular positioning (Thanbichler and Shapiro, 2006; Thompson et al., 2006).

6. Horizontal gene transfer of cytomotive filament systems

Throughout the above discussion, we have assumed that evolution proceeds via lineages – each gene is given by the parent organism(s) to the offspring. We would argue that this is what mostly happened. There currently exist two good examples relating to cytomotive filaments, however, that seem to break that rule: BtubAB in Verrucomicrobia and actin/profilin in a cyanobacterium. BtubAB, as mentioned above, is a *Prosthecobacter* tubulin-like cytomotive filament that is much more closely related to eukaryotic tubulin than to FtsZ and no other gene in the genome of *Prosthecobacter* shares that property (Pilhofer et al., 2007). We concluded, therefore, that BtubAB has been recently transferred by horizontal gene transfer (Schlieper et al., 2005). This is non-trivial because tubulin requires a number of co-factors for folding in eukaryotic cells and these are not present in *Prosthecobacter* species. *Prosthecobacter* in addition contains FtsZ, presumably for cell division

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(Pilhofer et al., 2007). It has recently been reported that *Microcystis aeruginosa* has acquired an actin/profilin pair from a eukaryote, because, again, no other gene in the genome is so closely related to eukaryotic genes (Guljamow et al., 2007).

In light of this, it cannot be excluded that the plasmid-borne versions of cytomotive filaments (ParM and TubZ) have been transferred from eukaryotes to bacteria, although it seems unlikely because the plasmids inhabit only bacteria.

For the bacterial cytomotive filaments themselves, one piece of evidence makes horizontal gene transfer from eukaryotes to bacteria unlikely: chloroplasts and mitochondria still contain good homologues of the bacterial cytomotive filaments – as would have the precursors of these endosymbionts when they were acquired by the first eukaryotic cells. It seems that horizontal transfer of deeply integrated cellular systems, such as cytomotive filaments, is rare.

7. Is FtsZ replaced by dynamin in endosymbionts?

The prokaryotic cell division proteins FtsZ, MinD and MinE are still found in chloroplasts (Glynn et al., 2007), which originated as symbiotic cyanobacteria, and in some primitive mitochondria (Beech et al., 2000), which are derived from α-proteobacteria. These proteins serve important functions in organelle division. FtsZ is believed to be required for marking the division site and/or for constricting the inner membrane. MinD and MinE are needed to position the Z-ring at the midpoint. However, most mitochondria have lost their FtsZ and it has been suggested that they use dynamin in place of FtsZ to divide (Arimura and Tsutsumi, 2002). Similarly, a chloroplast-specific dynamin associates with the outer membrane at a late stage of chloroplast division (Gao et al., 2003). However, prokaryotic dynamins have recently been discovered in a wide range of species that also have FtsZ, suggesting that FtsZ and dynamins have independent roles (Low and Löwe, 2006).

The best characterised role for dynamin in eukaryotes is in pinching off small vesicles from the cell membrane to take up substances on the outside surface. However, this is the reverse of what is required during organelle or cell division, when a large area of extra membrane must be inserted to hold the same total volume of cytoplasm in two compartments in place of one. The role of dynamins in organelle or cell division may be to add membrane rather than remove it. Mitochondria may have lost the need for FtsZ because electron-dense "mitochondrion-dividing" (MD) rings define the division site and control the way that membrane is inserted, while constriction may be achieved by a ring of actin filaments, as in eukaryotic cell division. Thus, the likely answer to the above question is that FtsZ has not been replaced by dynamin.

Nevertheless, the final stages of separation are not well understood, for any of these systems. It may be worth pointing out that at the end of eukaryotic cytokinesis a structure termed the midbody ring is formed whose dimensions are suggestively similar to the diameter of a typical bacterial cell of 1 μm (Buck and Tisdale, 1962). Maybe there is a conserved mechanism for finally breaking the membrane connection between dividing eukaryotic cells (Barr and Gruneberg, 2007), prokaryotes and endosymbiotic organelles.

8. Summary and perspective

At the heart of the cytoskeleton, we have identified three classes of cytomotive filaments that originated in prokaryotes and acquired new functions in eukaryotes. As well as actin-like and tubulin-like proteins, which have been discussed previously, we propose here for the first time that the prokaryotic WACA filaments may be related to the enigmatic family of septins in eukaryotes. We suggest that in each of these three classes of cytomotive filaments, the only fully conserved properties are (i) the structural fold of the molecule, (ii) their activity as GTPases or ATPases, and (iii) the longitudinal contacts between subunits.

We believe it is possible to explain the bewildering complexity that has evolved in cytoskeletons, starting with these basic filaments only. The filaments were originally selected by nature for their dynamic properties and were later adapted to be more skeleton-like and thus able to support movements generated by other complexes. In the case of tubulin, a special structure had to be invented (the microtubule) and the filaments made less dynamic (with MAPs); thus the supportive function suggested by the name 'cytoskeleton' for the conspicuous networks found in large eukaryotic cells, together with MAPs and molecular motor proteins, arrived quite late in evolution. It seems safe to assume that myosin evolved from kinesin, so that in evolutionary terms both of these families of molecular motors can be regarded as 'microtubule-associated'. Thus, as Bermudes et al. and others have argued (Bermudes et al., 1994), the crucial changes between prokaryotic cytomotive filaments and eukaryotic cytoskeletons seem to depend on the emergence of the microtubule and its motors. Between the cytomotive filaments of eukaryotes today and the original prokaryotic ones there must have been a 'combinatorial explosion' leading to a large number of filament-interacting proteins that reduced the mutability of the filament-forming proteins and essentially locked them in their current state. As yet, it has not been possible to identify any surviving intermediates.

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