

The evolution of the cytoskeleton

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The cytoskeleton is a system of intracellular filaments crucial for cell shape, division, and function in all three domains of life. The simple cytoskeletons of prokaryotes show surprising plasticity in composition, with none of the core filament-forming proteins conserved in all lineages. In contrast, eukaryotic cytoskeletal function has been hugely elaborated by the addition of accessory proteins and extensive gene duplication and specialization. Much of this complexity evolved before the last common ancestor of eukaryotes. The distribution of cytoskeletal filaments puts constraints on the likely prokaryotic line that made this leap of eukaryogenesis.

Introduction

The view that the cytoskeleton was a feature unique to eukaryotes was dramatically overturned about 20 years ago by the discovery that bacteria possess homologues of both tubulin (de Boer et al., 1992; RayChaudhuri and Park, 1992; Mukherjee et al., 1993) and actin (Bork et al., 1992). Since that time, a combination of bioinformatics, structural data, and advanced cell imaging has cemented the idea that both bacteria and archaea have active and dynamic cytoskeletons. However, as more information has emerged regarding the function of prokaryotic filaments and the distribution of cytoskeletal components, it has become clear that there is no simple relationship between the cytoskeletons of prokaryotes and eukaryotes. Moreover, there is considerable diversity in both composition and function between cytoskeletons in different lines of prokaryotes and eukaryotes.

Like eukaryotic actin-based microfilaments and tubulin-based microtubules, several of the filaments of the bacterial cytoskeleton are intrinsically “cytomotive” (Löwe and Amos, 2009); i.e., the filaments themselves can act as linear motors driven by the kinetics of polymerization/depolymerization. In eukaryotes, this activity has been hugely augmented by the

evolution of multiple classes of motors, as well as a menagerie of nucleators, severing agents, tip-binding factors, and (de)polymerases. Other cytoskeletal filaments appear to be more structural in function, providing resistance to external force or acting as a scaffold. Such filaments may still be dynamic in cells without being intrinsically cytomotive. The most closely studied of these are the intermediate filaments of animal cells, but a protein of bacteria, crescentin, also builds intermediate filament-like structures that function in cell shape determination.

In this review, we discuss the relationships between the major components of the bacterial, archaeal, and eukaryotic cytoskeletons. We compare the function of filaments in these three groups and also interrogate the distribution of key components across the tree of life. Finally, we examine what can be inferred with respect to the origins of cytoskeletal components and discuss the means by which the simple prokaryotic cytoskeleton might have evolved into the elaborate system of filaments, motors, and accessory proteins that is characteristic of the eukaryotic cell.

Filaments I: tubulin-related proteins

Eukaryotic microtubules are constructed from protofilaments resulting from the polymerization of heterodimers of α - and β -tubulin. Most microtubules consist of 13 protofilaments that interact laterally to form a hollow tube. Heterodimers added to the plus end of microtubules contain GTP in both subunits. Subsequent hydrolysis of GTP bound to the β subunit encourages a conformational change in the heterodimer that is resisted by the geometry of the microtubule, thus trapping energy in the lattice (Howard and Hyman, 2003). This difference in the free energy of GTP- and GDP-bound polymers is the cause of microtubule dynamic instability—whereby the presence of unhydrolyzed GTP at the plus end of microtubules promotes further polymerization, but cap loss induces rapid depolymerization (Erickson and O’Brien, 1992; Howard and Hyman, 2009).

The first evidence for a bacterial homologue of tubulin came with the finding that FtsZ, an essential cell division protein of *Escherichia coli*, bound and hydrolyzed GTP and possesses a conserved seven-residue sequence nearly identical to

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Abbreviations used in this paper: ARP, actin-related protein; DHC, dynein heavy chain; ESCRT-III, endosomal sorting complex required for transport, type III; IF, intermediate filament; LECA, last eukaryotic common ancestor; WACA, Walker A cytoskeletal ATPase.

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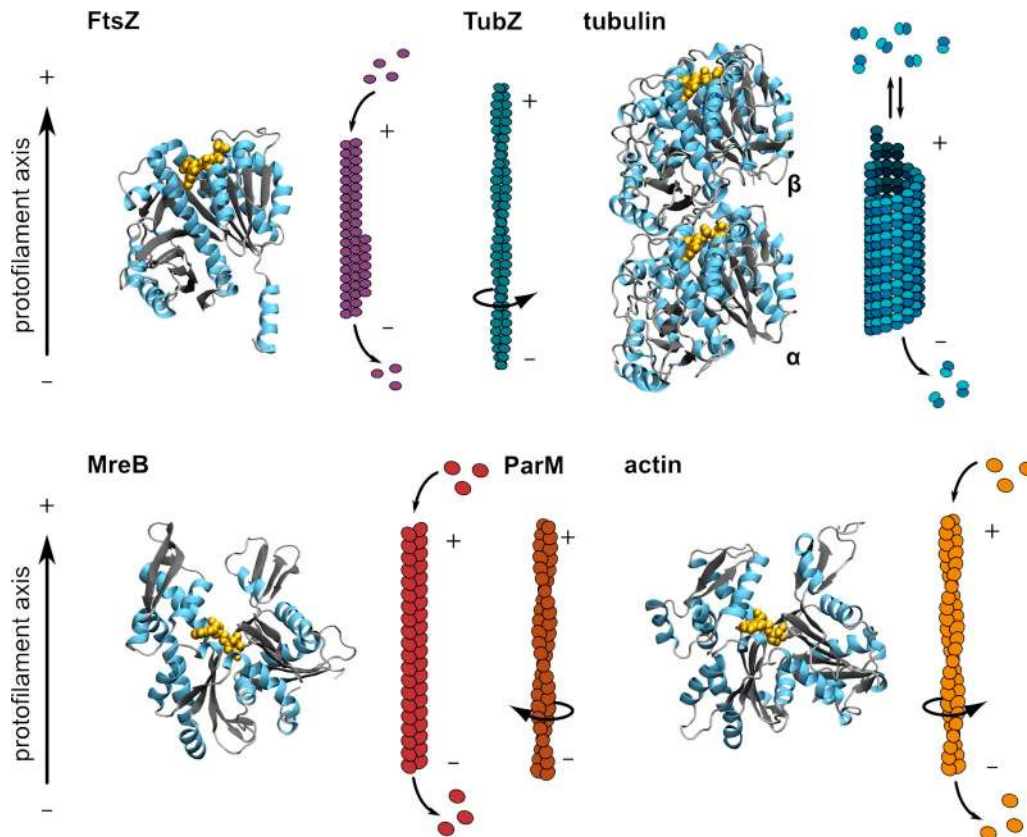


Figure 1. **Homology between prokaryotic and eukaryotic cytoskeletal filaments.** Despite low levels of sequence similarity, the homologous cytoskeletal proteins FtsZ/TubZ/tubulin (top) and MreB/ParM/actin (bottom) have considerable conservation of folding and also longitudinal interaction. ParM and actin form similar helical filaments, but with opposite chirality (Orlova et al., 2007). Structures of filament subunits are derived from the following Protein Data Bank accession numbers: 1W5A (FtsZ; Oliva et al., 2004), 1JFF (α/β -tubulin; Löwe et al., 2001), 1JCG (MreB; van den Ent et al., 2001), and 1YAG (actin; Vorobiev et al., 2003).

an N-terminal tubulin signature motif (de Boer et al., 1992; RayChaudhuri and Park, 1992; Mukherjee et al., 1993). Subsequent alignments showed that the similarities between FtsZ and tubulin sequences extended beyond the tubulin signature motif (Mukherjee and Lutkenhaus, 1994). Nevertheless, tubulin and FtsZ are very divergent in primary sequence, sharing only $\sim 10\%$ identity compared with $>40\%$ between most FtsZ sequences (Vaughan et al., 2004; Erickson, 2007). Despite low sequence similarity, the determination of the crystal structures of tubulin and FtsZ revealed proteins with near identical folds (Fig. 1; Löwe and Amos, 1998; Nogales et al., 1998a,b).

Unlike tubulin, FtsZ does not assemble into microtubules, but it does form a range of other structures *in vitro* using lateral interactions between protofilaments (Bramhill and Thompson, 1994; Mukherjee and Lutkenhaus, 1994; Erickson et al., 1996; Löwe and Amos, 1999, 2000). *In vivo*, FtsZ forms a dynamic “Z ring” between prospective daughter cells during cytokinesis (Fig. 2 A). This ring acts as a scaffold for the recruitment of the “divisome”, which gradually constricts to divide the cell (Adams and Errington, 2009). This system of cytokinesis was clearly an evolutionary success because FtsZ is both widely distributed and highly conserved. Its presence in most lineages of bacteria is indicative of it being an ancient protein. It has even been suggested that an over-representation of amino acids with simpler biosynthetic pathways in conserved positions of FtsZ

might be evidence that the protein predates the completion of the 20–amino acid standard code itself (Davis, 2002).

FtsZ is also encoded by many archaeal genomes, but it is not ubiquitous in prokaryotes. Most notably, FtsZ (and other tubulin homologues) are entirely absent from one of the major clades of archaea, the Crenarchaeota (Margolin, 2000; Vaughan et al., 2004)—with the possible exception of a highly divergent FtsZ-like gene in *Sulfolobus solfataricus* probably acquired by horizontal gene transfer (Makarova et al., 2010). FtsZ is also absent from at least one sequenced euryarchaeon (*Picrophilus torridus*; Fig. 3), as well as the bacterial groups Chlamydiae, Planctomycetes, and some Mycoplasmataceae (Vaughan et al., 2004; Adams and Errington, 2009). In the Crenarchaeota, FtsZ-independent division is possible because of an alternative cytokinesis machinery provided by ESCRT-III (endosomal sorting complex required for transport, type III; Lindås et al., 2008; Samson et al., 2008). ESCRT-III proteins are conserved between archaea and eukaryotes, and eukaryotic proteins of this complex form dynamic polymers that have a role in membrane scission events, including cell abscission during cytokinesis (Hurley and Hanson, 2010). In contrast to the lack of FtsZ in Crenarchaeota, the repertoire in Euryarchaeota has been duplicated to form three monophyletic families (FtsZ1, 2, and 3), with FtsZ3 being the most divergent and least widely distributed (Vaughan et al., 2004).

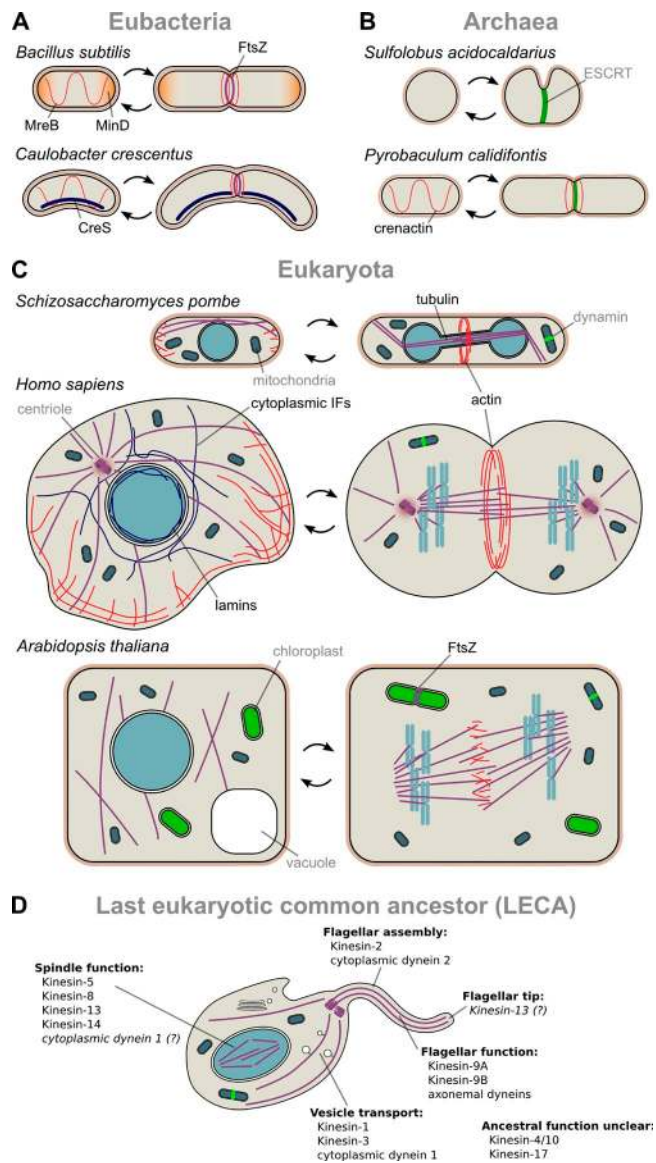


Figure 2. Bacterial, archaeal, and eukaryotic cytoskeletons. Schematic representations are shown for a small number of model organisms from each of the three domains of life (A–C), showing the organization of the cytoskeleton in dividing and nondividing cells (right and left of each pair, respectively). Homologous filaments are colored similarly. Also shown is the possible organization of the cytoskeleton in the LECA (D), highlighting the ancestral families of microtubule motors.

FtsZ is the most common tubulin homologue in prokaryotes (Fig. 3). However, there are at least four other tubulin-like protein families in bacteria, most of them with a restricted distribution. Several *Bacillus* plasmids encode tubulin-like proteins, which are very divergent in sequence from one another and also from FtsZ and tubulins (Larsen et al., 2007). These proteins include TubZ and RepX, which play important roles in the stability of the plasmids that encode them (Tinsley and Khan, 2006; Larsen et al., 2007). Monomeric TubZ has a structure very similar to α/β -tubulin (Ni et al., 2010), but forms right-handed helix filaments consisting of two protofilaments (Aylett et al., 2010).

An exception to the low level of sequence conservation between prokaryotic and eukaryotic cytoskeletal proteins are BtubA and B, found in the Verrucomicrobia *Prostheco*

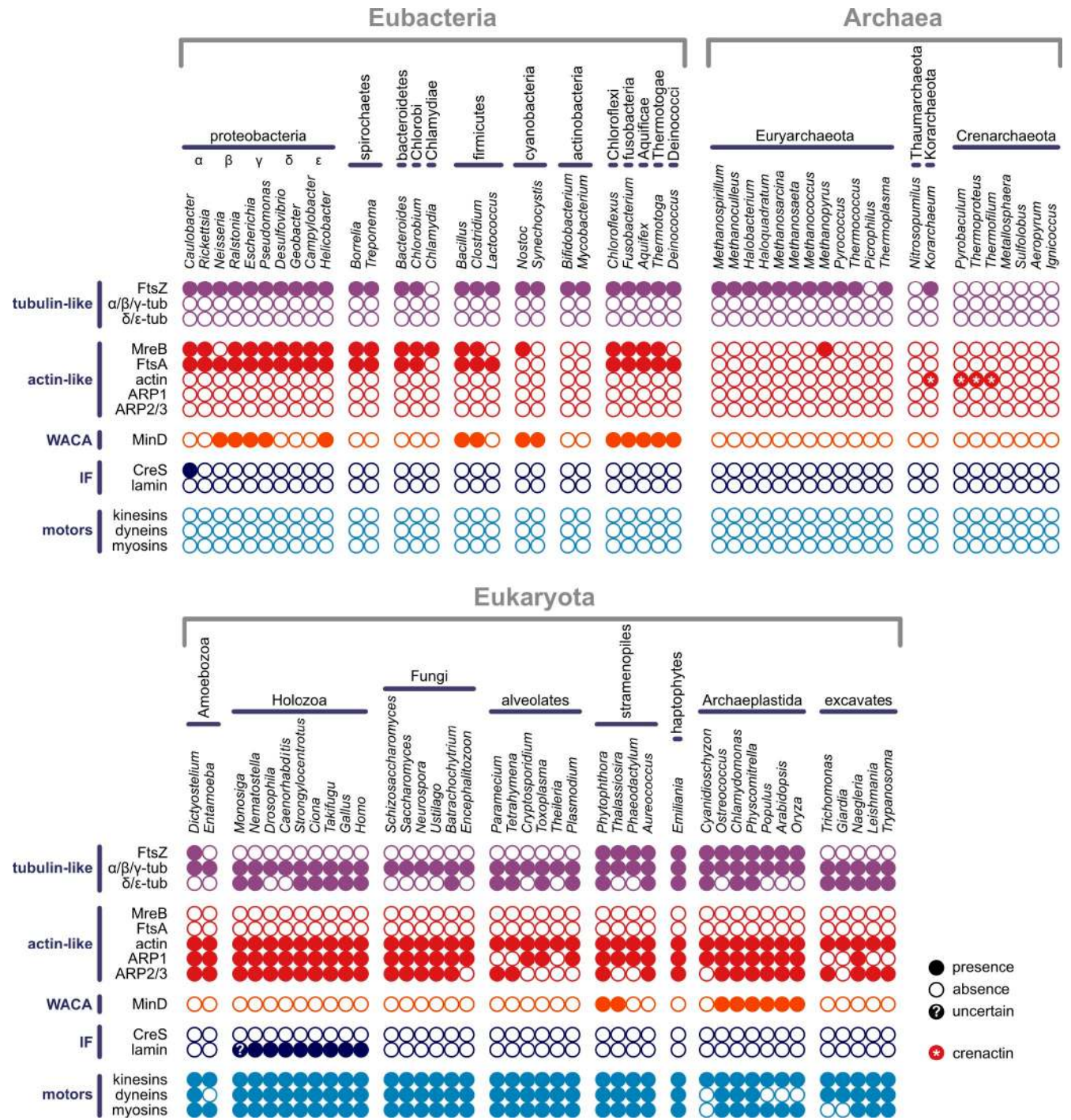
(Jenkins et al., 2002). These proteins are much more similar in sequence to eukaryotic tubulins than are FtsZ, TubZ, or RepX. This high similarity in sequence, their restriction to *Prostheco*, and phylogenetic analyses of FtsZ/tubulin all strongly suggest that BtubA/B were acquired by horizontal gene transfer from a eukaryote and are not descendants of bacterial FtsZ/TubZ (Jenkins et al., 2002; Vaughan et al., 2004; Schlieper et al., 2005; Pilhofer et al., 2007a). It is likely that BtubA is a homologue of β -tubulin and BtubB of α -tubulin (Sontag et al., 2009), but the eukaryotic lineage that acted as the donor for these proteins is not known. They retain the ability to form protofilaments (not microtubules), both in vitro and in vivo (Sontag et al., 2005; Sontag et al., 2009), and their continued expression and functional conservation suggests that they are of some benefit to *Prostheco* cells. However, they have not displaced the native tubulin-like cytoskeleton in these organisms, but rather coexist alongside conventional FtsZ (Pilhofer et al., 2007b).

Many eukaryotes possess FtsZ genes of prokaryotic origin (Fig. 3) inherited from the endosymbiotic ancestors of mitochondria and chloroplasts. These genes have subsequently moved from the organellar genomes to the nucleus, but their origins are still apparent in FtsZ phylogenies, where they group with sequences from either α -proteobacteria or cyanobacteria (Kiefel et al., 2004; Vaughan et al., 2004; the bacterial groups that gave rise to mitochondria and chloroplasts, respectively). These eukaryotic FtsZs function in the division of mitochondria and chloroplasts by forming a division ring with striking similarity to that found in bacteria (Margolin, 2005). Redundancy of this FtsZ-based division machinery with the eukaryotic dynamin fission apparatus may provide a mechanism for multiple independent losses of mitochondrial FtsZ since the eukaryotic root (Osteryoung and Nunnari, 2003; Praefcke and McMahon, 2004). In contrast, chloroplast FtsZ genes are more widely conserved (Fig. 3).

Very recently, the FtsZ-tubulin family has been expanded further by the identification of two new FtsZ-like protein families in prokaryotes—named FtsZ11 and FtsZ12 (Makarova and Koonin, 2010). Both of these families are divergent from tubulin, FtsZ, or other previously identified families. One of the new families, FtsZ12 is found only in proteobacteria, but the FtsZ11 family has a much wider distribution that includes several bacterial groups and Euryarchaeota. The function of FtsZ11 and FtsZ12 is unknown. It is possible that they do not form FtsZ/tubulin-like filaments, given their predicted structural divergence from other members of the superfamily (Makarova and Koonin, 2010).

Filaments II: the actin superfamily

Actin is a ubiquitous eukaryotic filament-forming protein. Actin filaments (also called microfilaments or F-actin) consist of two protofilament polymers wound together in a right-handed helix (Fig. 1). ATP hydrolysis by actin causes a much less dramatic change in polymer stability than is seen for GTP hydrolysis in microtubules. As a result, pure actin shows little dynamic instability in vitro (Mitchison, 1992), but rather undergoes “treadmilling” through the polarized addition of ATP-bound subunits. In vivo, actin is much more dynamic than in vitro



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Figure 3. **The distribution of key components of the cytoskeleton across the tree of life.** Filled circle indicates presence of an identifiable member of a protein family in an organism; open circle indicates absence/not found. Organisms are identified by genera only and are grouped into higher taxonomic groups. *Emiliania huxleyi* has not been placed into one of the eukaryotic taxonomic groups in reflection of uncertainty as to the placement of Haptophyta. "MreB" includes MreB-like (Mbl/MreBH) sequences, which colocalize with MreB and are very similar in sequence (Carballido-López and Errington, 2003; Carballido-López et al., 2006). The archaeal sequences identified as MreB using the arCOG technique (arCOG04656; Makarova et al., 2007, 2010) have a closer affinity to Hsp70 sequences and are not included. Archaeal crenactin (*) is orthologous to the single common ancestor of eukaryotic actin and ARPs (Yutin et al., 2009; Ettema et al., 2011), but has been entered as actin for clarity. The distributions of the large number of prokaryotic actin-like proteins other than MreB and FtsA (such as AlfA, Alp6/7/8) are not included here because of current difficulties in resolution of individual families (Derman et al., 2009; Yutin et al., 2009). There are possible orthologues of MinD in Euryarchaeota (Leipe et al., 2002), but their true membership is still unclear.

due to the presence of monomer-binding factors, filament-severing agents, and capping/stabilizing agents.

Eukaryotic actin is a member of a large and diverse superfamily of ATPases that includes Hsp70 chaperones and

several classes of sugar/sugar alcohol kinases (Flaherty et al., 1991; Bork et al., 1992), as well as eukaryotic actin-related proteins (ARPs; Frankel and Mooseker, 1996; Schafer and Schroer, 1999). Also identified as members of this superfamily were

several other bacterial proteins, including MreB, FtsA, and ParM (Bork et al., 1992). Since initial identification, the superfamily of actin-like proteins in bacteria has proved to be very complex, encompassing more than 20 classes of protein (Derman et al., 2009).

The most common prokaryotic homologue of actin is MreB. As for tubulin/FtsZ, actin and MreB are very divergent in primary sequence but have similar structures (Fig. 1), based on the “actin-fold” that unites the superfamily (Kabsch and Holmes, 1995). In vitro MreB forms assemblies of two protofilaments that are similar in structure to F-actin, but lack the helical twist (van den Ent et al., 2001; Esue et al., 2005). The conserved function of bacterial MreB (and closely related proteins such as Mbl and MreBH) appears to be in maintenance of cell shape (Jones et al., 2001; Graumann, 2007; Margolin, 2009). MreB filaments form a helix below the cell membrane and influence the position of cell wall synthesis (Fig. 2 A; Jones et al., 2001; Daniel and Errington, 2003). Consistent with this function, MreB is generally conserved only among rod-shaped bacteria, but absent from spherical cocci. Because extant rod-shaped bacterial lineages are probably more ancient, it has been suggested that the coccal forms have been derived from them multiple times by the loss of MreB and associated genes (Margolin, 2009). However, the correlation between prokaryote shape and MreB is not strict: some cocci still possess MreB and there are rod-shaped bacteria that lack MreB (Margolin, 2009).

ParM (previously StbA) is a plasmid-borne bacterial actin homologue with a filament-forming role. ParM is only ~20% identical to MreB, which is comparable to the degree of conservation between actin and either ParM or MreB. Nonetheless, ParM assembles twisted polymers in vitro that are very reminiscent of F-actin (van den Ent et al., 2002), but with a left-handed rather than right-handed twist (Orlova et al., 2007). The energy of polymerization of ParM is used in the segregation of the R1 plasmid (and others containing the *parMRC* operon) by pushing newly synthesized plasmid to the cell poles (Garner et al., 2007; Salje and Löwe, 2008).

To date, unambiguous ParM homologues are restricted to a close group of γ -proteobacteria, the Enterobacteriaceae. It is likely that the annotated “ParM” genes in the genomes of Firmicutes, β -proteobacteria, and cyanobacteria represent other classes of bacterial actin-like proteins, rather than true ParM orthologues. In support of this interpretation, “ParM” encoded on the *Staphylococcus aureus* pSK41 plasmid shares only 19% identity with γ -proteobacterial ParM sequences and appears to be more structurally related to archaeal actin-like proteins (Popp et al., 2010).

Both MreB and FtsA are almost exclusively restricted to bacteria (Fig. 3). Clear examples of MreB are also found in euryarchaea of the genera *Methanopyrus*, *Methanobrevibacter*, and *Methanothermobacter* (Yutin et al., 2009). Given their limited distribution and close similarity to bacterial sequences, these are most likely the result of horizontal gene transfer from bacteria. However, it cannot be entirely ruled out that they are rare but highly conserved genes of linear descent. Several other archaeal sequences have been identified as possible MreB orthologues using the “archaeal cluster of orthologous groups” technique

(Makarova et al., 2007, 2010). These sequences have a closer affinity to Hsp70 sequences than to MreB from bacteria or *Methanopyrus* and their grouping in arCOG04656 may be an artifact of the technique. These sequences are not considered as true MreB members herein (Fig. 3).

Several other actin superfamily members exist in bacteria—notably: MamK, which is required for magnetosome organization in *Magnetospirillum* (Komeili et al., 2006; Pradel et al., 2006); AlfA, which is involved in plasmid segregation in *Bacillus subtilis* in a manner similar to ParM (Becker et al., 2006); and Ta0583 from the euryarchaeon *Thermoplasma acidophilum* (Roeben et al., 2006; Hara et al., 2007). These proteins have either a limited phylogenetic distribution or their families have yet to be well delimited (Derman et al., 2009; Yutin et al., 2009). The possibility that Ta0583 might be the closest prokaryotic homologue to eukaryotic actin (Hara et al., 2007) has not been supported by subsequent analyses (Yutin et al., 2009; Ettema et al., 2011). However, recently an archaeal actin-like family has been described that is monophyletic with eukaryotic actin and actin-related proteins (Yutin et al., 2009; Ettema et al., 2011). This protein family, dubbed “crenactin,” has a localization in *Pyrobaculum* cells very similar to MreB in bacteria (Fig. 2 B; Ettema et al., 2011), but is more closely related to eukaryotic actin than to MreB or ParM. The possible implications of this family are discussed further below.

Filaments III: intermediate filaments

Eukaryotic intermediate filaments (IFs) are unlike microtubules and microfilaments in structure, biochemistry, and phylogenetic distribution. Unlike actin and tubulin, which are globular proteins that form polarized protofilaments, IF proteins are extended dimers that overlap to form unpolarized cables. There are many types of IF protein in vertebrates, most of which can be grouped into five classes: (1) type I (acidic) keratins; (2) type II (basic) keratins; (3) vimentin and desmin; (4) α -internexin and neurofilament proteins; and (5) lamins (Fuchs and Weber, 1994). Lamins are also present in protosomes, suggesting that all IF protein families are derived from a single lamin-like sequence in the common metazoan ancestor (Weber et al., 1989; Dodemont et al., 1994; Bovenschulte et al., 1995). Significantly, however, eukaryotic IF proteins have only been found unambiguously in animals and their relatives (Erber et al., 1998), suggesting that they are an innovation specific to this lineage and not present in the last eukaryotic common ancestor (LECA; see Fig. 3).

The bacterium *Caulobacter crescentus* encodes a protein with a predicted arrangement of coiled-coils similar to that in animal lamin A (Ausmees et al., 2003). This protein, CreS or crescentin, forms helical filaments that are necessary for the vibrioid or helical cell shapes adopted by *Caulobacter*. Although crescentin was originally described only as “IF-like”, the function and predicted secondary structure of the protein have often been interpreted as evidence that bacteria possess ancient homologues of eukaryotic IFs. However, there are several arguments against such an interpretation. First, CreS has a very restricted distribution (Fig. 3); to date, it has only been found in *Caulobacter*. Given this restricted range, if lamin A and crescentin are truly homologous, then *CreS* would most likely

represent a lateral transfer of a eukaryotic gene to *Caulobacter* rather than a true bacterial homologue. Second, identifiable homologues of eukaryotic IF proteins are restricted to metazoa (or possibly holozoa; Fig. 3) and may not have been present in the LECA—precluding vertical inheritance from prokaryotes. Third, similarities in predicted protein architecture (in this instance, coiled-coil positions) are not equivalent to fold homology. At present, it is not possible to compare eukaryotic IF proteins and crescentin for evidence of fold homology, as there are no representatives of either family for which full structures have been determined. Moreover, although orthologues of crescentin have not been identified outside of *Caulobacter*, there is evidence that CreS is a member of a larger family of bacterial proteins (Bagchi et al., 2008). All of these proteins are predicted to contain long stretches of coiled-coils, but the vast majority have no striking architectural similarity to lamin A (or other vertebrate IF proteins). Hence, the distribution of coiled-coil regions in crescentin and lamin A is more likely an example of convergence than a reflection of shared ancestry.

Filaments IV: WACA proteins

Prokaryotes have a fourth class of filament-forming proteins known as Walker A cytoskeletal ATPases (WACAs; Michie and Löwe, 2006). WACA proteins are a diverse family of ATPases (Koonin, 1993), which are themselves part of the extremely large superclass of P-loop proteins including signal recognition particle proteins, Rho/Ras GTPases and cytoskeletal motors (Leipe et al., 2002). The WACA MinD is an active ATPase (de Boer et al., 1991) that forms dynamic filaments around the cell periphery in *E. coli* and inhibits Z ring formation (Pichoff and Lutkenhaus, 2001; Shih et al., 2003). In *Bacillus subtilis*, MinD is statically associated with the cell poles (Marston et al., 1998; Marston and Errington, 1999), making it unclear if dynamic polymerization is an evolutionarily conserved feature of MinD biology. MinD sequences are found in many groups of bacteria (Fig. 3) and there are putative orthologues identified in Euryarchaeota (Leipe et al., 2002). However, the distinctions between the prokaryotic WACA families are yet to be fully resolved, so it is presently unclear if these archaeal sequences are monophyletic with bacterial MinD. Even if not strict MinD orthologues, examples of WACA proteins do appear to be present in both Euryarchaeota and Crenarchaeota (Makarova et al., 2010).

Several other bacterial WACA proteins have been described. ParA, ParF, and Soj all play roles in DNA segregation by different mechanisms (Pogliano, 2008; Löwe and Amos, 2009), demonstrating an apparent versatility of this system for segregation. It is perhaps surprising, therefore, that there are no eukaryotic WACA filaments. The fold of MinD and Soj is distantly related to that of eukaryotic septins (Cordell and Löwe, 2001; Leonard et al., 2005; Löwe and Amos, 2009), but this most likely reflects the distant relationship shared by all P-loop GTPases (Leipe et al., 2002). There are, however, MinD genes of bacterial ancestry in Viridiplantae (green algae and land plants) and some stramenopiles (Fig. 3), which play a role in plastid division (Marrison et al., 1999; Colletti et al., 2000; Kanamaru et al., 2000). It is noteworthy that all eukaryotes that possess MinD genes also encode endosymbiont-derived FtsZ.

Prokaryotic division: a common problem with many solutions

If there is an overarching theme running through the evolution of the prokaryotic cytoskeleton, it appears to be this: plasticity. Each of the major families of cytomotive filament-forming proteins consists of several paralogues, which are often as divergent from each other as they are from eukaryotic homologues. Some of the classes—such as FtsA (from the MreB/actin superfamily) or the newly identified FtsZ-like families—may not form filaments *in vivo* at all. However, among those families that do polymerize, lateral interactions in the core filament appear to be quite malleable over evolutionary time-scales without disrupting polymerization (for example, straight MreB filaments, against left-handed twisting ParM and right-handed actin).

There is also plasticity in the biological function for which filaments are used—FtsZ and MreB are involved in cell division and morphology, while their homologues TubZ and ParM play roles in plasmid segregation. The result is that prokaryotes use different systems to solve common problems. In bacteria, active DNA segregation can be achieved by at least three types of segregation machinery (Ebersbach and Gerdes, 2005; Hayes and Barilla, 2006). Type I systems use WACA proteins, such as ParA and Soj. Type II systems are based on the actin homologue ParM. Type III systems are those from *Bacillus* that use tubulin-like homologues, such as TubZ and RepX. In a striking example of apparent convergence, both ParM (type II) and TubZ (type III) form similar helical filaments that probably act to push apart plasmids (van den Ent et al., 2002; Aylett et al., 2010). However, it is type I systems that are by far the most wide spread in bacteria. It is interesting, then, that filaments based on WACA proteins are not conserved in eukaryotes, and may not be widely used in chromosome segregation in archaea (Bernander, 2000; Makarova et al., 2010). Significantly, none of the cytoskeletal protein families is ubiquitous to all prokaryotes, or even one of the two prokaryotic domains of life. The role for FtsZ in cytokinesis appears to be the function under most selective pressure for retention (Erickson, 2007), but even this protein has been lost from several lineages of bacteria and the entire crenarchaeal clade.

Eukaryogenesis: families and specialization

Despite being based on homologous filaments, the eukaryotic cytoskeleton is not simply a more extensive version of the prokaryotic one (Fig. 2 C). The complex eukaryotic cytoskeleton is actually based on a smaller set of ancestral cytomotive filaments than that of prokaryotes. With the notable exception of the prokaryote-like division machineries associated with some plasmids, only one paralogue of an MreB/crenactin family protein and one FtsZ/TubZ protein seems to have founded the eukaryotic cytoskeleton. However, this small selection has undergone several rounds of gene duplication and specialization from this ancestral set.

Heterodimers of α - and β -tubulin make up the vast majority of tubulin in eukaryotic cells. They are sufficient for the production of microtubules *in vitro*, and it is very likely that they

were the first types to evolve from the single proto-tubulin ancestor. However, they are not the only ancestral tubulins. Analyses suggest that the tubulin family encompasses at least six classes, named α , β , γ , δ , ϵ , and η , with a further two divergent types (ζ and ι) being found in some organisms (Vaughan et al., 2000; Dutcher, 2003). γ -Tubulin plays an essential role in microtubule nucleation (through the action of the conserved γ -tubulin ring complex) and is, like α - and β -, ubiquitous to all eukaryotes. In contrast, δ - and ϵ -tubulin have centriolar roles (Dutcher and Traub, 1998; Chang and Stearns, 2000; Ruiz et al., 2000) and are conserved in nearly all organisms that build centrioles/basal bodies and absent from organisms that have lost cilia/flagella (Hodges et al., 2010). At least five of the classes of tubulin (α , β , γ , δ , and ϵ) can be traced back to the last common eukaryotic common ancestor (Fig. 3). All of the tubulin types are more closely related to one another than to FtsZ/TubZ (Vaughan et al., 2000; Dutcher, 2003), implying that they all descended from a common proto-tubulin by gene duplication. Both duplication and specialization into functionally distinct classes must have occurred before the LECA.

There are striking parallels between the proto-eukaryotic evolution of tubulins and that of actin. The proteins most similar in sequence to conventional actin are the ARPs. The ARPs cover at least eight major families (Sehring et al., 2007) that are found only in eukaryotes. Four ARP families (ARP4, 5, 6/7, and 8/9) are not associated with cytoplasmic actin, but are nuclear proteins involved in chromatin remodeling (Chen and Shen, 2007; Dion et al., 2010). The remaining families—ARP1, 2, 3, and 10/11—have important roles in modification or extension of cytoplasmic actin function. ARP1 is an integral part of the dynactin complex, which links the actin- and tubulin-based cytoskeleton (Schroer, 2004). Yeast Arp10p and metazoan Arp11 (ARP10/11 family members) are also part of the dynactin complex (Eckley and Schroer, 2003; Clark and Rose, 2006). In contrast, a complex of seven subunits formed around a heterodimer of ARP2 and ARP3 is a major F-actin nucleator in most eukaryotes (Pollard and Beltzner, 2002; Goley and Welch, 2006). The only ARP that appears to form filaments is Arp1p (Schafer et al., 1994; Bingham and Schroer, 1999). These filaments are much shorter than those seen for actin, but have similar morphology.

As for tubulin, there was a single actin-like protein in the proto-eukaryote which evolved into multiple paralogous forms before the LECA. In doing so, both the actin- and tubulin-based cytoskeleton independently invented complexes containing divergent forms of filament subunits that were specialized for nucleation (ARP2/3 and γ -tubulin ring complexes). However, in the case of the actin-based cytoskeleton, this nucleating complex appears to be expendable in some circumstances because orthologues of ARP2 and ARP3 have been lost multiple times across eukaryotes (Fig. 3; note that organisms always lose both, as would be expected for a complex). Cross talk between microtubules and microfilaments via the dynactin complex has also been lost multiple times, as can be seen from the distribution of ARP1.

The history of these core eukaryotic cytoskeletal families also shows some degree of plasticity, but it is rather different from that seen in prokaryotes. The eukaryotic paralogues were,

for the most part, present in the LECA. Although rare divergent types do exist (for example ζ - and ι -tubulin), they do not have the same level of divergence from core types as is seen for divergent prokaryotic proteins. Moreover, their biological role seems more static. Divergence between the distribution of families in extant eukaryotes is largely a product of loss of function (e.g., loss of dynactin complex and ARP1; loss of centrioles and δ/ϵ -tubulin).

Cytoskeletal motors

Prokaryotes use the intrinsic cytomotive capacity of cytoskeletal filaments to do work. Eukaryotic cells also use the energy of polymerization/depolymerization (McIntosh et al., 2010). However, in eukaryotes the intrinsic dynamics of the filaments has been augmented by the addition of the cytoskeletal motors—dyneins, kinesins, and myosins—which derive energy from ATP hydrolysis to perform various cellular tasks. Each of these motors is a superfamily containing multiple classes of specialized proteins.

Kinesins and myosins share a similar fold structure, suggesting that they have common ancestry (Kull et al., 1996, 1998). This is rather surprising given that they now act exclusively on microtubules and F-actin, respectively, and presents something of a conundrum in terms of evolutionary cell biology. It is only plausible for motors to evolve after the filaments on which they act have been established. As has been discussed above, filaments made from tubulin/FtsZ and actin/MreB homologues were already well established in prokaryotes before the emergence of eukaryotes, yet both motor classes evolved only later in the proto-eukaryote. This requires that either: (1) a single “*ur*-kinesin-myosin” originally walked on both tubulin- and actin-based filaments and only later specialized into separate families; (2) a motor evolved for one type of filament and subsequently developed an ability to move on the other; or (3) both motor families evolved independently from the same family of NTPases. The large differences in structure and sequence between tubulin/FtsZ and actin/MreB filaments suggest that the last scenario is most plausible, even if less parsimonious. There is also a precedent for this because this family of P-loop NTPases also gave rise to many eukaryote-specific GTPases (including Arf, Ras/Rab, Rho/Ran, and G protein families; Leipe et al., 2002).

Dyneins have a very different structure to that of myosins and kinesins. The core of the dynein complex is the dynein heavy chain (DHC), which is a member of another large and diverse superclass of NTPases, the AAA+ proteins (a family including ATPases associated with various cellular activities). DHCs contain six AAA+ domains that form an intramolecular hexameric ring (Samsó et al., 1998; King, 2000; Burgess et al., 2003), implying that DHCs originally evolved by either intragene domain duplication or fusion of genes encoding AAA+ proteins. Because all classes of DHC have the same structure, this duplication occurred before the creation of functional paralogues. The dynein superfamily is also different from kinesins and myosins in that all but one of the nine classes are associated with one specific organelle—the cilium. Only cytoplasmic dynein 1 has a conserved role outside of the cilium; the other

families being the retrograde motor of intraflagellar transport (for historic reasons known as cytoplasmic dynein 2) and seven families of dyneins built into motile axonemes.

Given their central role in the eukaryotic cell, it was surprising to find from post-genomic analyses of motor repertoires that none of the families is ubiquitous to all eukaryotes. Indeed, myosin or dynein superfamilies have been lost in their entirety from some lineages (see Fig. 3; Lawrence et al., 2001; Matsuzaki et al., 2004; Richards and Cavalier-Smith, 2005; Wickstead and Gull, 2007). To date, kinesins are encoded by all sequenced genomes, but the repertoire in each lineage can be very different (Wickstead and Gull, 2006; Wickstead et al., 2010)—for example, *Entamoeba histolytica* encodes only members of kinesin-5, -14, and -15 families (and no dynein at all), whereas the apicomplexan *Theileria parva* encodes kinesin-8 and -13.

The axoneme

One of the iconic structures of the eukaryotic cytoskeleton is the axoneme—canonically, nine microtubule doublets radially placed around a central apparatus containing two singlet microtubules (Haimo and Rosenbaum, 1981). The axoneme is the structure around which all eukaryotic cilia and flagella are formed. The evolution of flagella/cilia forms part of a review in this series by Carvalho-Santos et al. (2011) and will not be discussed at length here. However, the axoneme represents a major cytoskeletal innovation and aspects of its evolution are highly pertinent to the emergence of the cytoskeleton in the proto-eukaryote.

From its distribution in extant organisms, it is clear that the axoneme evolved before the last common ancestor of eukaryotes. Since that time, it has been independently lost from multiple eukaryotic lineages (notably, from many lineages of plants, fungi, and amoebae). These losses are closely correlated to losses of ancestral kinesin and dynein families. The axoneme evolved from the microtubules of the cytoplasm (Cavalier-Smith, 1978, 1982, 2006). It is usually inferred that the earliest axoneme-like structures were microtubule-based protrusions from the cell body with a solely sensory function, similar to the immotile cilia found on many types of differentiated animal cells (Rosenbaum and Witman, 2002; Jékely and Arendt, 2006; Satir et al., 2007). Axonemal motility, therefore, only arises later with the evolution of the specialized axonemal dynein classes. If it is assumed that cytoplasmic dynein 1 evolved before the other classes, then dynein phylogenies support this hypothesis, suggesting the evolution of intraflagellar transport (IFT) before axonemal dynein (Wilkes et al., 2008; Hartman and Smith, 2009). This rooting is also consistent with evolution of the proto-cilium as a motile organelle, but where movement was originally based on gliding driven by the IFT machinery (Mitchell, 2004, 2007). However, rooting of DHC phylogenies with the closest eukaryotic relative of dynein, midasin (Garbarino and Gibbons, 2002; Iyer et al., 2004), suggests that microtubule sliding was much closer to the origin of ciliary evolution, emerging before the specialized machinery for IFT (Wickstead and Gull, 2011). This implies that the proto-cilium evolved not from an immotile protrusion, but from a motile cytoplasmic microtubule bundle analogous to the axostyles of oxymonads

(Grimstone and Cleveland, 1965; McIntosh, 1973), presumably being assembled in an IFT-independent manner as are some axonemes today (Witman, 2003; Briggs et al., 2004).

The proto-eukaryotic revolution

One of the most surprising results of our increasing ability to probe the characteristics of the LECA has been how much of the biological complexity in extant cells can be traced back to this ancestral cell. The LECA possessed much of the complexity now seen in the replisome (Liu et al., 2009), the spliceosome (Collins and Penny, 2005), and the endocytic system (Dacks et al., 2009), as well as the machineries necessary for meiosis (Ramesh et al., 2005) and phagotrophy (Cavalier-Smith, 2002b; Yutin et al., 2009). Moreover, comparative analysis of the genome of the free-living excavate *Naegleria gruberi* identified ~4,000 protein groups that probably were present in the LECA (Fritz-Laylin et al., 2010).

This “complexity early” model of eukaryotic evolution is mirrored in the cytoskeleton (Fig. 2 D). Somewhere in the evolutionary space between prokaryotes and the LECA, single proto-tubulin and proto-actin molecules diversified into multiple specialized forms. Three classes of motors arose independently, and evolved to include at least nine classes of dynein, eleven classes of kinesin, and three classes of myosin (Richards and Cavalier-Smith, 2005; Wickstead and Gull, 2007; Wickstead et al., 2010). As well as these, the axoneme formed, with 100–200 associated proteins (Avidor-Reiss et al., 2004; Pazour et al., 2005; Broadhead et al., 2006), many of which have no prokaryotic orthologues. Between the prokaryotes and the LECA, a revolution occurred in cytoskeletal biology.

Such complexity cannot have appeared fully formed, but arose by stepwise elaborations of cell structure (and genetic repertoire). However, the large number of simpler intermediate forms that must have existed appear to have left no descendants. This is perhaps because a great many of these changes occurred in a relatively short time, with one innovation creating a favorable landscape for the evolution of the next (Cavalier-Smith, 2006). Alternatively, all descendants of these intermediate forms have been simply out-competed by the arrival of the LECA, with its mitochondrial endosymbiont, endomembrane system, and sophisticated cytoskeleton. What is clear is that since this complex LECA, the diversification into many eukaryotic lineages has often been accompanied not by the addition of further classes, but by loss of ancestral ones. Some of these losses are associated with loss of specific structures or functions (such as axonemal motility), but there appears to be a remarkable flexibility in the precise repertoire of many of these ancient families that is required for eukaryotic cell function.

Although they are constructed from homologous proteins, the functions of prokaryotic and eukaryotic filaments are not broadly homologous. In eukaryotes, DNA segregation is ubiquitously performed by the tubulin-based cytoskeleton, whereas cytokinesis involves actin–myosin. In contrast, most prokaryotic cytokinesis is based on the tubulin homologue FtsZ, whereas actin-like, tubulin-like, or WACA proteins may be used for DNA segregation. This suggests that a switch must have occurred in cytoskeleton function in the proto-eukaryote (Löwe and

Amos, 2009). However, with several divergent forms occurring in both filament families, and increasing evidence for plasticity in prokaryotic cytoskeletal function, this was perhaps not such a dramatic transition as it might at first appear.

FtsZ and tubulin are highly conserved proteins that are constrained in sequence, yet are very divergent from one another (Erickson, 2007). Because this is the case, how did FtsZ (or a prokaryotic homologue thereof) evolve into eukaryotic tubulin? It has been suggested that eukaryotic tubulins evolved from a TubZ-like sequence (Cavalier-Smith, 2010). Such a scenario has much to recommend it. Because TubZ functions in DNA segregation, there is a plausible evolutionary transition to the development of the tubulin-based mitosis of eukaryotes. Moreover, TubZ and FtsZ coexist in bacterial cells, meaning that a tubulin-like function might evolve for a descendant of TubZ without the loss of FtsZ-based cell scission function. Alternatively, a redundant FtsZ, resulting from either gene duplication or the evolution of actin-based cytokinesis, could have provided the necessary relaxation of sequence constraints such that FtsZ might evolve into eukaryotic tubulin (Doolittle, 1995; Erickson, 2007).

Interestingly, the increase in cell size that accompanied eukaryogenesis may have been one of the factors favoring the replacement of FtsZ-based cytokinesis with an actin-based system. To date, no Z ring has been described that is much larger than $\sim 1 \mu\text{m}$ in diameter. Even very large bacteria require only these small Z rings for division because they form new cells by budding off small endospores (Angert and Losick, 1998; Robinow and Angert, 1998). This has led to the tentative suggestion that there may be a maximum diameter beyond which the Z ring cannot function efficiently (Erickson, 2007).

Eukaryotic origins

Alongside the many genes that seem to have specifically arisen during the proto-eukaryotic revolution, eukaryotic genomes contain a mixture of genes with apparent archaeal ancestry and genes of bacterial origin (Koonin, 2010). Genes of apparent bacterial origin are more numerous (Esser et al., 2004; Rivera and Lake, 2004; Makarova et al., 2005). Because all extant eukaryotes are descended from a single ancestor that already contained the mitochondrial endosymbiont, this provides a good candidate source for many of the genes of bacterial origin (although the phylogenetic affinities of these genes are actually rather mixed and not only from the α -proteobacteria; Koonin, 2010). In contrast, much of the machinery for replication, transcription, and translation appears to be fundamentally archaeal, or rather archaeal-like (Ribeiro and Golding, 1998; Rivera et al., 1998; Yutin et al., 2008), suggesting that the eukaryotic nuclear genome originated either from within Archaea or from a sister group to it.

The eukaryotic cytoskeleton more likely evolved from the archaeal-like host than a bacterial endosymbiont. If we assume that the mitochondrion was acquired by a phagocytosis-like process in a proto-eukaryote, then the close association between actin and endomembrane systems in eukaryotes would strongly suggest that a primitive actin cytoskeleton was already in existence (and therefore the actin cytoskeleton is host- and not endosymbiont-derived). Such a phagocytic origin provides a biologically plausible mechanism for acquisition of mitochondria

during eukaryogenesis (Cavalier-Smith, 2002b; Yutin et al., 2009), although the presence of prokaryotes that live in other prokaryotes demonstrates that phagocytosis is not necessarily a prerequisite for acquisition (von Dohlen et al., 2001). The origin for tubulin is less clear. However, at least initially FtsZ/TubZ filaments from the incoming α -proteobacterium would have been trapped on the “wrong side” of both the phagosome and bacterial membranes. This would make them unavailable for functions in DNA or organelle movements in the host until gene transfer to the host genome and subsequent translation in the host cytoplasm. The fact that several eukaryotic lines also still possess identifiable mitochondrial FtsZ with functions in organelle division suggests that FtsZ from the endosymbiont was constrained by a conserved function and did not evolve directly into cytoplasmic tubulin.

Phylogenomic studies variously support the origin of the archaea-like part of the eukaryotic genome from within Crenarchaeota (Cox et al., 2008; Foster et al., 2009), Euryarchaeota (Pisani et al., 2007), or basal to either clade (Yutin et al., 2008; Kelly et al., 2010). Alternatively, both Archaea and eukaryotes might be derived from an extinct “neomuran” line (Brown and Doolittle, 1997; Cavalier-Smith, 2002a). If both eukaryotic actin and tubulin are derived from an archaea-like host rather than the endosymbiont, then what does this tell us about the phylogenetic position of the proto-eukaryote? Because FtsZ (and all other identified tubulin homologues) are missing from Crenarchaeota (Fig. 3), this would exclude an origin for eukaryotes from within this group (the “eocytes”; Lake, 1988). Conversely, the recently identified archaeal protein crenactin, which is monophyletic with eukaryotic actin/ARPs and is the closest extant homologue of eukaryotic actin, is absent from Euryarchaeota (Yutin et al., 2009). Given these data, the presence of both crenactin and FtsZ in Korarchaeota, deep-branched archaea that appear to be neither Euryarchaeota nor Crenarchaeota (Barns et al., 1996; Elkins et al., 2008), is particularly interesting (see Fig. 3). Significantly, the sequenced korarchaeon also contains key components of eukaryotic RNA polymerases (Koonin et al., 2007) and histones (Bell and White, 2010). Such inferences based on distribution are by no means definitive (it is noteworthy that the sequenced korarchaeon lacks ESCRT-III [Makarova et al., 2010], which is found in eukaryotes). However, they do lend support to a root for the eukaryotes either embedded in a basal archaeal line, or as an earlier neomuran cell.

Over the last 20 years our model of the evolution of the cytoskeleton has changed greatly. The prokaryotic cytoskeleton has been shown not only to exist, but to be dynamic and diverse. Surprisingly, it has also turned out to be expendable—at least in its canonical forms. During eukaryogenesis a huge amount of complexity was built up before the LECA, but the composition in individual extant organisms shows a remarkable flexibility. As more biological data are gathered and it becomes possible to analyze sequences from a greater range of genomes, it seems likely that more surprises will emerge. For prokaryotes, it may be that some of the players have yet to join the stage.

The authors wish to thank Flavia Moreira-Leite (University of Oxford, Oxford, UK) for assistance in development of the manuscript and Stephen D. Bell (University of Oxford) for helpful critical review.

This work was supported by the Wellcome Trust.

Submitted: 11 February 2011

Accepted: 20 June 2011

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