The FtsZ protofilament and attachment of ZipA – structural constraints on the FtsZ power stroke
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Bacterial cell division protein FtsZ forms protofilaments \textit{in vitro} that can shift from a straight to a curved conformation. The inside of the curved protofilaments, which corresponds to the carboxyl terminus, should face the center of the cell as curvature increases during constriction of the Z-ring. ZipA, a membrane-tethered division protein, binds to a highly conserved short peptide on the carboxyl terminus of FtsZ. A model is proposed here for how membrane-bound ZipA can reach around the FtsZ protofilament to bind the carboxy-terminal peptide, which is facing away from the membrane.

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Abbreviations
Fts filamentous temperature sensitive
P/Q proline/asparagine

Introduction
Cell division in \textit{Escherichia coli} requires more than a dozen genes, most of which have been identified as temperature sensitive mutations. At the non-permissive temperature, division is blocked but cell growth continues for a time, producing long filaments — hence the name \textit{fts} meaning ‘filamentous temperature sensitive.’ The different genes are identified by letters, for example, \textit{ftsZ}, \textit{ftsQ}, \textit{ftsA}. The major player is \textit{ftsZ}, and more is known about it than the others (for reviews see [1–5]).

The FtsZ protein assembles into a ring at the center of the cell, which can be visualized by immunofluorescence or GFP technology. Several other Fts proteins colocalize with it, but only after FtsZ is in place. FtsZ is by far the most abundant of the division proteins, and it forms the cytoskeletal framework of the division machinery. A fascinating aspect of FtsZ is its homology to eukaryotic tubulins. This homology was first discovered in sequence similarities, and was elegantly confirmed by the solution of their atomic structures [6,7]: the folds of both proteins are virtually identical except for small segments at the amino and carboxyl termini.

Homology, as indicated by sequence similarity and the structure of the protein fold, implies a common ancestry but not necessarily a common function. For example, actin, hexokinase and the chaperonin hsp70 share a common fold, and are apparently descended from a common ancestor, but they have widely divergent functions. However, FtsZ and tubulins have a conserved function — to assemble into protofilaments. As discussed in the next section, it is important that the protofilaments of both tubulin and FtsZ are straight and without any twist. Even more remarkable is that both tubulin and FtsZ protofilaments can switch from this straight conformation to a curved one, in which there is a 22° bend between each subunit. The switch between these two conformations may provide the basis for a mechanical machine, and this machine has been conserved from bacterial FtsZ to tubulins.

The FtsZ protofilament has so far been characterized only \textit{in vitro}, but it is almost certainly the building block for the Z-ring \textit{in vivo}. I will start with a discussion of the FtsZ protofilament and the transition from the straight to the curved conformation. I will then discuss ZipA, an accessory division protein, and the remarkable conservation of a short peptide at the carboxyl terminus of FtsZ that ZipA binds to. Finally I will put together the concepts of protofilament curvature and binding of ZipA to propose a structural model for how they may be arranged at the membrane.

Why is the straight protofilament remarkable?
A protofilament is assembled from identical subunits, each with two surface patches that bind each other. If the protein subunits had the shape of cubes, one could imagine stacking them up one on top of the other, all facing in the same direction. This would produce a straight protofilament like FtsZ. However, for proteins in general the adhesive surfaces need not be 180° apart but can face in any direction. These would assemble into a helix, as illustrated in Figure 1a. There are two features to this helix: the spiral structure of the filament, and the rotation from one subunit to the next. Evolution has apparently selected against both of these features for FtsZ. First, the FtsZ protofilaments do not form a spiral, but appear to be straight. But, as shown in Figure 1b, a straight protofilament can still have a rotation from one subunit to the next. However, the FtsZ protofilaments also appear to have zero rotation from one subunit to the next. (Both of these features were deduced from the structure of two dimensional sheets of protofilaments of \textit{E. coli} FtsZ [8], and is also seen in \textit{Methanococcus} protofilaments [9]). This means that all subunits in the protofilament face in the same direction.

FtsZ protofilaments, with each subunit facing in the same direction (Figure 1c), is ideally suited to building a division machine. As the cytokinetic ring constricts, the protofilaments need to be able to curve to accommodate the decreasing diameter. The curved conformation appears to accomplish this, by introducing a 22° bend between the
subunits (Figure 1d). If all the subunits face the same direction, this curvature could accommodate the decreasing diameter of the Z-ring during constriction. We have postulated that the transition from a straight to a curved protofilament may not just accommodate constriction, but may actually provide the force that powers constriction [3,10•].

The shift from a straight to a curved conformation is powered by GTP hydrolysis

The curved FtsZ protofilaments can form closed planar rings when stabilized on planar lipid monolayers [11] or a shallow helix that can make tubular polymers when free in solution [10•,12]. Both have a 23 nm outer diameter and a 22° bend between subunits, and they will be considered equivalent for the present discussion. Several lines of evidence suggest that the change from the straight to curved FtsZ protofilament is triggered by GTP hydrolysis [10•].

Firstly, the protofilaments may be locked into the straight conformation by assembly in the presence of the slowly hydrolyzable analog GMPCPP or EDTA, which blocks GTP hydrolysis. Secondly, assembly in the presence of GDP produces only tubular polymers. We therefore concluded that GTP at the interface favors the straight protofilament conformation, whereas GDP favors the curved conformation.

Tubulin also forms curved protofilaments, and one important structural feature can be extrapolated from experiments on tubulin to FtsZ. Numerous studies of disassembling microtubules show the rings to be curling away from the microtubule wall, which means the outside surface of the microtubule corresponds to the inner surface of the rings [11]. This surface is the location of the carboxyl terminus of tubulin. The carboxyl terminus of Methanococcus FtsZ was not resolved in the atomic structure, but most carboxy-terminal residues resolved were on this same face. It is a reasonable assumption that the unresolved carboxy-terminal peptide of FtsZ is attached to this face, which corresponds to the inside of the ring. This provides interesting structural constraints for the structural model discussed in the final section.

ZipA and its interaction with FtsZ

ZipA is an integral membrane protein that was recently discovered as an essential component for cell division in E. coli [13]. The sequence of ZipA indicated an intriguing structure. It has an amino-terminal transmembrane anchor and a cytoplasmic domain consisting of three parts: a 60 amino acid charged domain, a 100 amino acid P/Q domain (rich in proline and asparagine) and a 144 amino acid globular carboxy-terminal domain. The P/Q domain was suggested to form an extended linker between the membrane and the carboxy-terminal domain [13], and this is a crucial feature of the model I now propose.

FtsZ can assemble into the Z-ring without ZipA, but recruitment of ZipA to the Z-ring requires FtsZ [14,15]. This recruitment takes place shortly after the Z-ring is formed and is essential for cell division. It has been proposed that ZipA serves as a membrane anchor for FtsZ. It is clearly not the only anchor as the Z-ring can form in its absence, but an anchor function is still an attractive hypothesis. However, two recent studies have now addressed another possibility: ZipA might stabilize and crosslink FtsZ polymers. RayChaudhuri [16•] reported that ZipA stabilized FtsZ polymers and was especially effective in stabilizing the mutant protein FtsZ84. Polymers were stabilized even in the absence of GTP. ZipA also modified the structure of the polymers, causing protofilaments to associate laterally into bundles or sheets up to 100 nm wide. Hale et al. [17] recently confirmed the bundling activity and showed that the carboxy-terminal domain of ZipA was sufficient for bundling.

In contrast to the bundling observed by RayChaudhuri [16•] and Hale et al. [17], Liu et al. [15] found no evidence for ZipA affecting FtsZ polymerization or GTPase activity. The reasons for the discrepancy are not clear.

RayChaudhury [16•] demonstrated that ZipA stabilizes FtsZ polymers in vivo as well as in vitro. Specifically, ZipA...
was able to suppress the temperature sensitive mutation ftsZ84. Cells with a genomic copy of ftsZ84 disassemble their Z-rings within 1-5 minutes [18] when shifted from 30° to 42°C. RayChaudhury found that doubling the amount of ZipA in the cell stabilized the FtsZ84 rings at 42°C and permitted cell division. Previous studies have shown that increasing the level of FtsZ84 protein itself permitted ring formation and cell division at 42° [19]. Apparently a two-fold increase in the level of ZipA stabilized the FtsZ84 rings to about the same extent as a three- to four-fold increase in FtsZ84.

A very important recent advance was the solution of the atomic structure of the carboxy-terminal globular domain of ZipA by X-ray diffraction [20•] and NMR [21]. These and previous studies [22•,23,24] showed that the carboxy-terminal domain of ZipA binds to a short conserved peptide at the carboxyl terminus of FtsZ (see next section). The X-ray crystallographic study solved the structure of ZipA both with and without the 17-amino-acid carboxy-terminal FtsZ peptide bound to it. It therefore reveals the atomic structure of this peptide and how it binds to ZipA. The carboxy-terminal of ZipA domain is globular, with a shallow hydrophobic cleft that binds to the FtsZ peptide. The FtsZ peptide is folded into a β sheet followed by an α helix. These secondary structure elements are separated by a highly conserved proline (see below).

**Conservation of the carboxy-terminal ZipA-binding peptide**

Alignment of FtsZ sequences from different species shows a high level of conservation for the first 316 amino acids (up to VATGIG) of *E. coli*. Following this there is a segment of variable length that shows little or no conservation among different species. At the carboxyl terminus, however, there is a short segment of 15–20 amino acids that is highly conserved. This carboxy-terminal peptide is not needed for polymerization of FtsZ in vitro or for formation of the Z-ring in vivo, but it is essential for subsequent steps of cell division in *E. coli* [22•], *Staphylococcus* [24], and *Caulobacter crescentus* [25]. More specifically, this peptide is essential for the binding of two proteins, FtsA and ZipA [22•,23,24]. The most detailed functional study of this peptide was the systematic alanine scanning mutation performed by Ma and Margolin [22•], which identified several amino acids that are essential for cell division.
shows the amino acids that contact ZipA and the ones identified as essential for in vivo activity.

Several previous studies [20,22•,24,25] have noted that the carboxy-terminal peptide is conserved in many but not all prokaryotic FtsZ proteins, but I have found in a more comprehensive study that it is almost universal. I examined 57 FtsZ sequences for a recognizable match of the carboxy-terminal peptide and found only 10 that had no recognizable match.

A selection of 17 matching sequences is shown in Figure 2b. The E. coli sequence is among the best matches to the consensus. These 17 sequences were selected to show the range of species and organelles that have it, including eubacteria, chloroplasts, thermotogales and archaea, and also to show the least convincing fits (Pyrococcus horikoshii, Thermoplasma acidophilum, and Haemophilus). The most highly conserved residues are the central PxF, where the F is sometimes replaced by another hydrophobic amino acid. An extended consensus sequence, (E/D)IPxF=L, was found in more than half the species. All of the matching peptides contained the central P and at least one other match (or conservative substitution). Remarkably, there was no insert or deletion in any of the 47 sequences. The 10 sequences with no recognizable match are shown in Figure 2c, primarily to list the species. Some of these sequences may have a functionally active sequence, but they are missing the central P that was the key to recognition.

The extremely broad conservation of the carboxy-terminal FtsZ peptide leads to an important new conclusion. It is already well established that this peptide is the binding site for ZipA and FtsA. However, ZipA is found in only a small group of bacteria related to E. coli. FtsA, an actin homolog that is essential for cell division in E. coli, occurs much more broadly than FtsZ. It is found in most bacteria with completely sequenced genomes, but it does not occur in archae and is missing from Synechocystis and from Mycobacterium tuberculosis. Yet these two bacteria and many of the archaea have a conserved carboxy-terminal peptide in FtsZ that forms the binding site for ZipA and FtsA. This strongly suggests that there is an ancestral protein, which binds to the carboxy-terminal peptide, that arose early in the evolution of cell division. This ancestral protein may still be present in most bacteria, awaiting discovery. Alternatively, the ancestral protein may have been replaced by ZipA and/or FtsA in some prokaryotes and perhaps by other proteins in other species. A search for new binding partners for the carboxy-terminal peptide can be expected to discover new, perhaps primordial, cell division proteins.

Curved protofilaments and ZipA binding

As suggested above, it is likely that the center of the curved protofilament faces the center of the cell, away from the membrane. This would be true whether the curved conformation is providing the force driving constriction or whether the curvature is simply accommodating constriction. The inside of the ring corresponds to the outside of the microtubule, which is where the carboxyl terminus of tubulin is found. If the carboxyl terminus of FtsZ is on this face, it would require the membrane-tethered ZipA to reach around the protofilament to bind to the carboxy-terminal peptide. Fortunately the
needed flexibility for this conformation appears to be built into the structures of FtsZ and ZipA.

Returning to the location of the carboxy-terminal peptide of FtsZ, the crystal structure from *Methanococcus* shows that the amino acids corresponding to *E. coli* G316, the last conserved amino acid of the major, amino-terminal domain, is right in the middle of the outside face, the one identified above that will face toward the center of the ring. Unfortunately this *Methanococcus* FtsZ is one of the ten without the conserved carboxy-terminal peptide, so we do not have structural information on its location in the *E. coli* protein. For *E. coli* FtsZ the carboxy-terminal peptide is likely to be exposed as a flexible extension — this would seem to be essential for it to bind into the groove of ZipA. It seems likely that at least part of the non-conserved linker sequence will also be extended and flexible.

This extension of the carboxy-terminal peptide should provide some of the flexibility needed for ZipA to bind to the carboxy-terminal peptide, but the P/Q domain of ZipA may provide a long, flexible link that permits the carboxy-terminal domain of ZipA to extend out from the membrane and reach around the FtsZ protofilament. The 300 amino acid P/Q domain is predicted to be largely unstructured and extended in solution [13]. This could provide a flexible link of 45 nm (calculated using the 0.15 nm amino acid of an α helix) to 100 nm (calculated using 0.35 nm amino acid of a β strand or fully extended polypeptide chain) between the membrane tether and the carboxy-terminal binding domain.

**Conclusions**

The FtsZ protofilament undergoes a transition from a straight to a curved conformation. This transition should accommodate the decrease in diameter of the bacterium during constriction and may even provide the force powering constriction. The carboxy-terminal peptide of FtsZ, which is the binding site for FtsA and ZipA, is probably located on the inner surface of the curved protofilament, facing away from the membrane. A model is presented in which the P/Q domain of ZipA provides a 45–100 nm flexible linker between its transmembrane anchor and its carboxy-terminal domain. This linker permits the carboxy-terminal domain of ZipA to reach around the protofilament and bind the carboxy-terminal peptide of FtsZ. Finally, the carboxy-terminal peptide of FtsZ is conserved in archaea and species of bacteria that have no FtsA or ZipA, suggesting the existence of undiscovered cell division proteins that bind it.

**Update**

The curved conformation of FtsZ protofilaments has previously been shown only for the *E. coli* protein. A new study now shows tubular FtsZ from *Methanococcus*, confirming the expectation that the curved conformation may be universal [26]. Two recent advances concern FtsA. Self association of FtsA has been investigated by yeast two-hybrid technology and also shown to be important for its role in cell division [27]. A most important advance is the X-ray structure of *Thermotoga* FtsA [28]. The structure confirms its homology to actin but reveals a unique domain as an insert. The binding sites for FtsZ and for FtsA self association are not yet known, but the atomic structure should provide the framework for important new experiments.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

** of outstanding interest


The role of GTP/GDP in determining the conformation of FtsZ is determined in this paper, and the authors propose that the conformational change may provide the force for constriction of the Z-ring.

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The authors describe a crystal structure that shows the structure of the carboxy-terminal domain of ZipA, with the carboxy-terminal peptide of FtsZ bound in a groove.


Alanine scanning mutagenesis of the carboxy-terminal peptide of FtsZ was performed to identify the key amino acids needed for its function in cell division. These would include interactions with FtsA and ZipA.


