522 Cell 166, July 14, 2016 © 2016 Published by Elsevier Inc. DOI http://dx.doi.org/10.1016/j.cell.2016.06.057

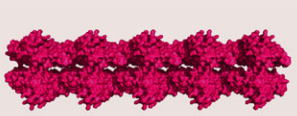
# SnapShot: The Bacterial Cytoskeleton

Gero Fink, Andrzej Szewczak-Harris, and Jan Löwe
MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK

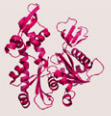
Cell

## Actin homologue MreB regulates cell morphology

ARCHITECTURE: $2a(MreB_{AXP})^N$ : membrane

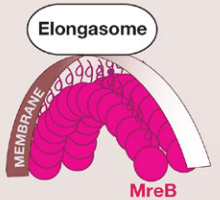


Filament structure (+ ATP)

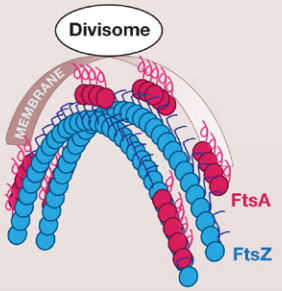


Monomer structure


- Maintains shape of many bacteria
- Forms apolar, double filaments from two antiparallel protofilaments
- Filaments are connected to the elongasome
- Filaments bind directly to the cell membrane during assembly



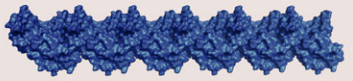

Complex outside the cell responsible for cell elongation and cell wall synthesis



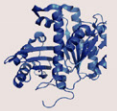

A complex responsible for cell wall remodeling

## Tubulin homologue FtsZ controls cell division

ARCHITECTURE: $FtsA_{AXP}{}^N$ : membrane $FtsZ_{GXP}{}^N$

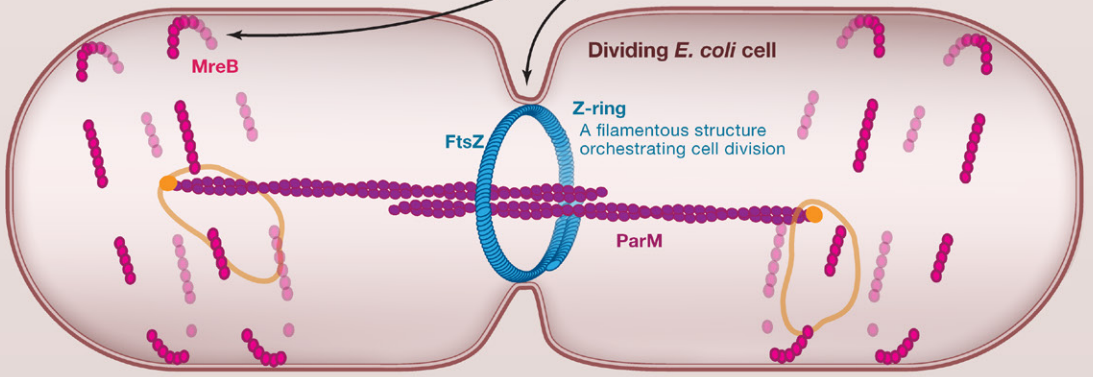


Protofilament structure (+ GTP)



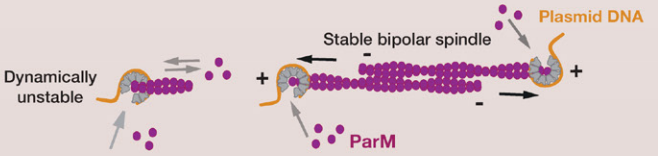
Monomer structure


- FtsZ, often together with FtsA (an actin homolog), forms the Z ring
- In presence of nucleotide FtsZ and FtsA, each form single, polar filaments assembled head-to-tail
- Filaments are cytomotive and can constrict membranes on their own
- FtsZ and FtsA act as a platform for the divisome
- FtsA acts as membrane anchor




## DNA segregation

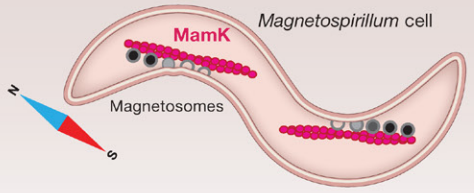
ARCHITECTURES: $2p(ParM_{AXP})^N$ and $2p(AlfA_{AXP})^N$, $4p(TubZ_{GXP})^N$ and $3p(PhuZ_{GXP})^N$




- Cytomotive filaments of actin-like ParM segregate low-copy-number plasmids between daughter cells
- ParM forms double-helical filaments that are polar and dynamically unstable
- A stable bipolar spindle consists of two annealed antiparallel ParM filaments
- Filaments of the tubulin homologue TubZ produce pulling force, but the cellular mechanism is unknown
- TubZ is a treadmilling 4-stranded filament
- AlfA and PhuZ are other filamentous DNA-transporting proteins

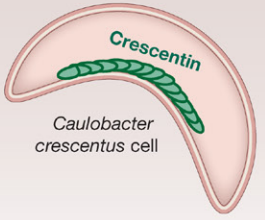
## MamK facilitates magnetotaxis

ARCHITECTURE: $2p(MamK_{AXP})^N$



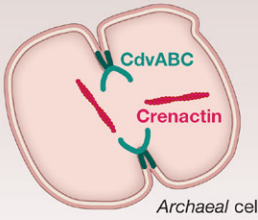

- In magnetotactic bacteria, actin-like MamK filaments align magnetic organelles
- Forms double filament from two parallel protofilaments
- Likely cytoskeletal
- Undergoes rearrangement during cell division

## Crescentin regulates cell morphology




- Bears resemblance to intermediate filament protein achitectures
- Facilitates crescent shape in *Caulobacter*
- Cytoskeletal filaments form close to cell membrane
- Architecture unknown and filaments are not dynamic

## CdvABC and Crenactin make filaments in archaea




- The CdvABC system is related to eurkaryotic ESCRT-III and required for cell division
- Crenactin forms filaments similar to actin
- The function of crenactin filaments is unknown

### Other filament systems

| Filament | Architecture | Role | Organisms |
|---|---|---|---|
| BtubAB | $4p(BTubA_{GXP}{:}BTubAB_{GXP})^N$ | ? | *Prosthecobacter* |
| CetZ | ?, tubulin-like | Cell shape | Euryarcheota |
| AlpC | ?, actin-like | Replication | Phages |
| Bactofilin | ?, beta-helical | Cell shape | Eubacteria |

### ARCHITECTURE

Number of protofilaments *parallel* or *antiparallel* $(Monomer_{nucleotide})^{polymerisation}$ : matrix,
e.g. $2a(MreB_{AXP})^N$ : membrane

See online version for legend and references.

# SnapShot: The Bacterial Cytoskeleton

Gero Fink, Andrzej Szewczak-Harris, and Jan Löwe
MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK

Cell

Most bacteria and archaea contain filamentous proteins and filament systems that are collectively known as the "bacterial cytoskeleton," though not all of them are cytoskeletal, affect cell shape, or maintain intracellular organization. The bacterial cytoskeleton contains proteins that are homologous in structure to eukaryotic actin and tubulin and also other protein classes, possibly including intermediate filaments, suggesting that the eukaryotic cytoskeleton can trace its evolutionary origins to bacterial and, more closely, to archaeal ancestors. However, the various filament systems have evolved diverse and non-convergent functions, highlighting their versatility and the conservation of underlying principles.

## Cell Division: FtsZ, FtsA

In the majority of bacterial species, cell division (cytokinesis) is dependent on the function of a contractile cytokinetic ring, the "Z-ring," which contains the filamentous assembly of FtsZ, the endogenous bacterial tubulin-homolog (Erickson et al., 2010). In some organisms, FtsZ is tethered to the cell membrane via FtsA, an actin-like protein, which can also form filaments. Both proteins are part of the "divisome"—a poorly understood macromolecular protein complex that coordinates cytokinesis in bacteria. The FtsZ ring is cytomotive, as it is able to exert force on the membrane to constrict it, while guiding the remodeling of the cell envelope, including the cell wall. FtsZ and FtsA form collaborative filaments, which means that their assembly is assisted by association with scaffolds, in this case each other's filaments and the membrane. Both FtsZ and FtsA likely form single filaments that exhibit polarity, or in other words, have distinct ends that affect the direction of the filament assembly. The polymerization of FtsZ and FtsA is nucleotide-driven as it depends on the binding of GTP and ATP, respectively, as is the case for tubulins and actins in eukaryotes. In these protein classes, filament turnover is driven by their intrinsic GTPase and ATPase activities.

## Cell Morphology: MreB, Crescentin

Maintenance of the archetypal bacterial shape—the rod, or bacillus—is dependent on the orderly elongation of the cell wall, which acts as the bacterium's stiff outer shell. The cell wall synthesis machinery that is responsible for this process is part of a protein assembly called the "elongasome." It has been suggested that the elongasome and the divisome share a common evolutionary origin. In most rod-shaped bacteria, such as *Escherichia coli* and *Bacillus subtilis*, the elongasome is organized around filaments built from the endogenous actin-homolog MreB (Jones et al., 2001; van den Ent et al., 2001). Although the intracellular organization of MreB is debated, it may assemble into short filament stretches arranged on the membrane along slightly tilted rings around the cell's circumference. Surprisingly, MreB filaments are built from two protofilaments running antiparallel; therefore, they have no polarity.

An unrelated filamentous protein called crescentin, found in *Caulobacter* sp., is responsible for the maintenance of the bacterium's characteristic curved (crescent) shape (Ausmees et al., 2003). Unlike other proteins presented here, crescentin is not a homolog of tubulin or actin, and it does not require or hydrolyze nucleotides and hence can be considered cytoskeletal. It is a predominantly coiled-coil protein, possibly related to eukaryotic intermediate filament proteins. Crescentin forms a large filament that runs along the inner curvature of the cells. The molecular structure of the crescentin filament and its subunits is unknown.

## DNA Segregation: ParM, TubZ, PhuZ, AlfA

A group of cytomotive filament systems is involved in segregating genetic material, chiefly low-copy-number plasmid DNA or phages. These proteins are encoded by the segregated DNA and ensure that the plasmid's genetic information is retained during cell division. Some filaments are capable of pushing a replicated pair of plasmids to the opposite cell poles (Gerdes et al., 2010). The best-studied example involves the actin-like protein ParM, which forms polar, left-handed, double-helical filaments. The dynamically unstable ParM filaments are tethered to plasmid DNA at the growing (+) end via a helical accessory protein complex. When two such ParM filaments align antiparallel, they are stabilized and form a bipolar spindle, which can then grow freely, in both directions. This mechanism ensures the selection of productive spindles only and explains how they are able to push the plasmid DNAs apart.

Similar systems include actin-like AlfA and also tubulin-like TubZ, which is found in large plasmids from the genus *Bacillus*. TubZ forms quadruple filaments that can treadmill, like eukaryotic cytoskeletal filaments, and that pull their DNA cargo. Another example of a tubulin-like protein involved in DNA segregation is PhuZ, which forms triple, dynamically unstable filaments. PhuZ has been suggested to center the DNA of the large bacteriophages that express it. All of the described DNA-transporting cytomotive filaments contain multiple strands, are built of helical filaments, have filament polarity, and require nucleotides for assembly.

## Magnetotaxis: MamK

One especially interesting function of a bacterial filament system is found in magnetotactic bacteria such as *Magnetospirillum*. Due to the presence of crystals of magnetite contained in its magnetic membrane invaginations, "magnetosomes," the bacterium has the ability to orient itself according to the Earth's magnetic field. A row of magnetosomes runs along the cell's long axis and is aligned by a filament built of actin-like protein MamK (Komeili et al., 2006). MamK forms polar, nucleotide-driven, double-helical filaments, and it is known that MamK filaments rearrange during cell division.

## Filament Systems in Archaea: CdvABC, Crenactin

From an evolutionary perspective, archaea are closer to eukaryotes than to bacteria. Archaea of the phylum Crenarcheota contain a cell-division system homologous to eukaryotic ESCRT-III (Lindås et al., 2008; Samson et al., 2008). In this system, CdvABC proteins substitute for the cytokinetic Z-ring. Apart from ESCRT-III homologs, some Crenarcheota also contain crenactin (Ettema et al., 2011), which forms double-helical filaments that bear a strikingly close resemblance to eukaryotic F-actin. Cellular roles of crenactin have not yet been resolved, much like many other exciting aspects of archaeal biology. For example, an FtsZ homolog, CetZ, controls cell shape, instead of being involved in cytokinesis (Duggin et al., 2015).

## Glossary

Cytomotive, Nucleotide driven assembly/disassembly leading to dynamic behaviors that allow forces to be produced; Collaborative, Filaments that use a matrix (scaffold) to bind to, and/or where assembly is enhanced through cooperativity with a matrtix; Polarity, If all subunits in a filament point in the same direction, the filament has chemically different ends; Cytoskeletal, Filament systems without dynamics, often used as structural supports in cells; Dynamic Instability, Filaments grow and shrink stochastically from ends through a metastable state caused by intrinsic nucleotide hydrolysis; Treadmilling, Growth and shrinkage is restricted to distinct (+/-) ends, giving the impression that the filaments move, although only subunits move

## REFERENCES

Ausmees, N., Kuhn, J.R., and Jacobs-Wagner, C. (2003). Cell *115*, 705–713.

Duggin, I.G., Aylett, C.H., Walsh, J.C., Michie, K.A., Wang, Q., Turnbull, L., Dawson, E.M., Harry, E.J., Whitchurch, C.B., Amos, L.A., and Löwe, J. (2015). Nature *519*, 362–365.

Erickson, H.P., Anderson, D.E., and Osawa, M. (2010). Microbiol. Mol. Biol. Rev. *74*, 504–528.

Ettema, T.J., Lindås, A.C., and Bernander, R. (2011). Mol. Microbiol. *80*, 1052–1061.

Gerdes, K., Howard, M., and Szardenings, F. (2010). Cell *141*, 927–942.

Jones, L.J., Carballido-López, R., and Errington, J. (2001). Cell *104*, 913–922.

Komeili, A., Li, Z., Newman, D.K., and Jensen, G.J. (2006). Science *311*, 242–245.

Lindås, A.C., Karlsson, E.A., Lindgren, M.T., Ettema, T.J., and Bernander, R. (2008). Proc. Natl. Acad. Sci. USA *105*, 18942–18946.

Samson, R.Y., Obita, T., Freund, S.M., Williams, R.L., and Bell, S.D. (2008). Science *322*, 1710–1713.

van den Ent, F., Amos, L.A., and Löwe, J. (2001). Nature *413*, 39–44.

  DOI http://dx.doi.org/10.1016/j.cell.2016.06.057