Journal of
Molecular Microbiology
and Biotechnology

J Mol Microbiol Biotechnol 2006;11:159–166
DOI: 10.1159/000094052

Cytoskeletal Cytoplasmic Filament Ribbon of *Treponema:* A Member of an Intermediate-Like Filament Protein Family

Jacques Izard

The Forsyth Institute, Boston, Mass., USA

© Free Author Copy - for personal use only
PLEASE NOTE THAT ANY DISTRIBUTION OF THIS ARTICLE WITHOUT WRITTEN CONSENT FROM S. KARGER AG, BASEL IS A VIOLATION OF THE COPYRIGHT.
Upon request a written permission to distribute the PDF file will be granted against payment of a permission fee depending on the number of accesses required. Please contact Karger Publishers, Basel, Switzerland at permission@karger.ch

Key Words
Bacterial cytoskeleton · Cell division · Cytoplasmic filament · Spirochete

Abstract
Development of genetic systems for many bacterial genera, including *Treponema*, now allow the study of structures that are specific to certain pathogens. The cytoplasmic filament ribbon of treponemes that is involved in the cell division cycle has a unique organization. Cytoplasmic bridging proteins connect the filaments, maintaining the distance between them and providing the overall ribbon-like structure. The filaments are anchored by proteins associated with the inner membrane. Each filament is composed of a unique monomer, the cytoplasmic filament protein A (CfpA), with coiled-coils secondary structures. CfpA is part of a growing family of proteins that we propose to call bacterial intermediate-like filaments (BILF). Copyright © 2006 S. Karger AG, Basel

Evidence for the presence of a dynamic cytoskeleton in prokaryotes is growing. The cytoskeleton is a network of protein complexes, each of which has a filament as a central component. In eukaryotes, actin, tubulin and intermediate filament polymers make up the cell structure. The numerous functions of these filaments include participation in motility-associated structures, cell shape, and other cytoplasmic functions including cell division. These eukaryotic filaments may have widespread prokaryotic counterparts. New technological advances in the visualization of microscopic structures and in genetic engineering of difficult-to-grow (nutritionally fastidious) prokaryotic organisms are opening new horizons for the study of filamentous structures.

The scope of this review is to discuss advances in understanding the nature and function of the cytoplasmic filaments of *Treponema*. The structure of treponemal cytoplasmic filaments will be discussed in the context of the bacterial cytoskeleton and the emergence of a network of filamentous structures within these bacteria.

Ubiquity of the Cytoplasmic Filament Ribbon

A cytoplasmic filamentous ribbon has been described in multiple spirochete genera. *Spirochaeta*, *'Pillotina'*, *Leptonema*, *'Hollandina'*, *'Diplocalyx'* and *Treponema* bacteria have in common filamentous structures that are distinct from the periplasmic flagella [Bermudes et al., 1987, 1988; Hollande et al., 1967; Holt, 1978; Hovind-Hougen, 1979]. The filaments have been described as tubulin, macrotubules, fibers, fibrils, in attempts to link microscopic data to the available biological observations.

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2006 S. Karger AG, Basel

Accessible online at:
www.karger.com/mmb

Jacques Izard
The Forsyth Institute, 140 The Fenway
Boston, MA 02115 (USA)
Tel. +1 617 892 8279, Fax +1 617 892 8432
E-Mail jizard@forsyth.org

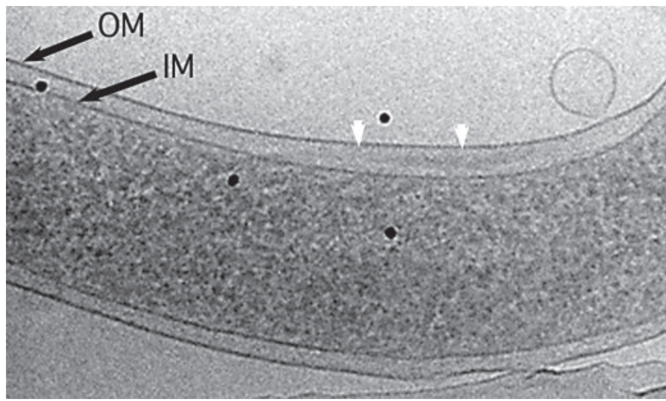

Fig. 1. Electron micrograph of a flash-frozen hydrated *T. phagedenis* Kazan 5 cell [C.E. Hsieh and J. Izard, unpubl. data]. Images were recorded using a Jeol JEM4000FX electron microscope in absence of stain or fixative. OM = Outer membrane, IM = inner membrane. White arrowheads indicate the side view of a flagellar filament bundle.

Fig. 2. Purified cytoplasmic filaments of *T. phagedenis* Kazan 5. A band of six filaments shows a twist similar to that seen in vivo [W.A. Samsonoff and J. Izard, unpubl. data]. The filaments were purified by a modified protocol of Masuda and Kawata [Izard et al., 1999; Masuda and Kawata, 1989]. They were visualized by electron microscopy after negatively staining the sample with sodium phosphotungstate as previously described [Izard et al., 1999]. Scale bar: 30 nm.

Structural Organization

Spirochetal membrane organization is shown in figure 1. The cell is composed of an outer membrane, a periplasmic space where the motility-associated flagellar filaments are located, an inner membrane, the cytoplasmic cylinder [Holt, 1978; Limberger, 2004]. Within the cytoplasmic cylinder, just underneath the inner membrane, is the cytoplasmic filament ribbon [Hovind-Hougen, 1972, 1974; Hovind-Hougen et al., 1976]. Located below the periplasmic flagellar filament bundle, the ribbon's helical periodicity is equivalent to that of the cell [Eipert and Black, 1979; Hovind-Hougen, 1974; Hovind-Hougen and Birch-Andersen, 1971; Zemper and Black, 1978]. Filaments have been observed at all stages of growth and span the length of the cell [Eipert and Black, 1979; Izard et al., 1999]. After purification, a fraction of filaments remain as a ribbon [Eipert and Black, 1979] (fig. 2).

A sequence of events during cell division has been defined through the analysis of electron micrographs of negatively stained *Treponema phagedenis* cells that were stripped of their outer membranes. It is hypothesized that the filaments of the ribbon are first severed at the midsection of the cell, are associated with an anchor that secures the ribbon to the cell ends [Izard et al., 1999]. When the cell division septation ring is forming and starts to constrict, the flagellar basal bodies are positioned at the tip of the future cell ends. The flagellar filaments can be observed wrapping around the two newly formed cytoplasmic cylinders when the constriction of the ring is more pronounced [Izard et al., 1999]. After completion of cell septation, the two cytoplasmic cylinders are independent under a unique outer membrane. Their motility is under the influence of flagellar bundles at each end until the complete separation of the two cells.

Electron tomography has been used to circumvent the limitations of traditional microscopy in the ability to decipher spatial relationships between the filaments [Izard et al., 2004]. *T. phagedenis* cells were stripped of their outer membrane, negatively stained with 2% sodium phosphotungstate, a tilt series of images was recorded at a 1.5° angular interval over a range of –57.0 to 58.5° [Izard et al., 2004]. The computed tomographic reconstruction provided a three-dimensional data set. The structures within the volume were observed and analyzed using IMOD [Kremer et al., 1996] and ImageJ (rsb.info.nih.gov/ij). The data confirmed the presence of a ribbon formed of individual filaments that are continuous within the cell volume (fig. 3) [Izard et al., 2004]. Three novel features were observed: (1) filaments do not interface or touch each other; (2) bridging proteins connect filaments two by two on the cytoplasmic side of the ribbon, and (3) anchor proteins are present on the inner-membrane side of the ribbon.

The filament dimensions as well as the filament-to-filament spacing were measured. Using the cross section of the ribbon, the average inter-filament spacing was 10.4 ± 1.8 nm, while the filament width and depth were $5.0 \pm 0.5 \times 6.0 \pm 0.5$ nm (horizontal/vertical). In *T. phagedenis*, 3–10 filaments per ribbon were observed [Izard et al., 1999]. We estimated that the cytoplasmic filament ribbon covers between 3 and 18% (average 7–9%) of the inner surface of the cytoplasmic membrane [Izard et al., 2004].

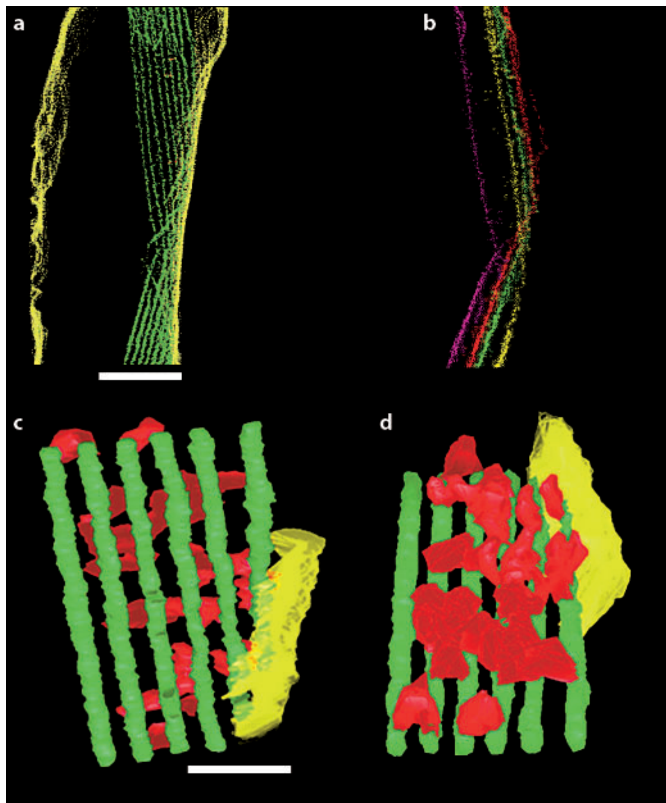

Fig. 3. Three-dimensional model of the cytoplasmic filaments of *T. phagedenis* Kazan 5 negatively stained cells after removal of the outer membrane. The model is based on tracing using the tomogram. **a** Cytoplasmic filaments along the cell within a turn of the ribbon. Note the presence of a filament most probably broken during the preparation of the sample. **b** Color coding of four pair of filaments showing their alignment in the model. The slight gap at the turn is due to the inability to trace the filaments when large amount of stain accumulates on the side of the cell. **c** View from the periplasmic space of the filaments and bridging components. **d** View from the cytoplasmic space of the filaments and bridging components. The cytoplasmic filaments are in green; the unidentified electron dense material including the accumulated stain is in yellow; the protein forming bridges between the filaments in red. Scale bars: **a**, **b** 100 nm; **c**, **d** 25 nm. Adapted from Izard et al. [2004], with the courtesy of Blackwell Publishing.

Composition

The filament and its constitutive protein have been isolated only from *Treponema* strains [Izard et al., 1999; Masuda and Kawata, 1989; You et al., 1996]. You et al. [1996] were able to isolate and partially sequence the protein from *Treponema pallidum*. PCR primers were designed from the derived putative nucleotide sequence, a library was screened by hybridization, resulting in the identification of the open reading frame named *cfpA* [You et al., 1996]. Analysis of the gene in four other treponemes *(T. phagedenis, T. denticola, T. vincentii, T. pallidum* subsp. *pertenue)* showed significant similarities ranging from 69 to 100%. Although some gaps and mutations were noted among the nucleic acid sequences, a large number those mutations were conservative at the amino-acid level. BLAST and FASTA searches [Altschul et al., 1997; Pearson, 1990] revealed no significant sequence similarity to cytoplasmic filament protein A (CfpA) to other open reading frames in GenBank database. There was a conserved coiled-coils segment organization among the CfpA proteins: the first is located at the beginning of the sequence (amino acids 24–49, in *T. pallidum* CfpA), three more are grouped starting around the second half of the sequence (amino acids 290–311, 397–417, 522–540).

The cytoplasmic filament ribbon structure and the periplasmic flagellar filaments, which are separated by the inner membrane, do not influence each others' structure and organization. A potential physical link between the two structures was hypothesized by Hoving-Hougen and Birch-Andersen [1971]. In both *T. phagedenis* and *T. denticola,* neither the number nor the organization of cytoplasmic filaments was affected in the absence of flagellar filaments. In the absence of cytoplasmic filaments, single cell motility was not altered [Izard et al., 2001].

Two other proteins are also associated with the filaments, as shown by electron tomography (see above). These proteins could be seen bridging and anchoring to the inner-membrane components, but their nature is still to be discovered. Other proteins are expected to be involved in severing and in anchoring at the cell end of the filaments during the cell cycle.

Gene Regulation

In *T. denticola* the *cfpA* promoter was identified by primer extension, differed by only one nucleotide from the consensus sigma-70 promoter sequence found in *Escherichia coli* [Hawley and McClure, 1983; Izard et al., 1999]. Little is known about temporal regulation of genes in *Treponema* [Indest et al., 2000]. Transcriptome analysis of *T. pallidum* subsp. *pallidum* during an experimental rabbit infection underscored the importance of CfpA for the cell biology. Gene transcription quantitation of *cfpA* by real-time PCR grouped the gene in the top 10 most transcribed genes [Smajs et al., 2005]. These genes are critical to sustain the challenges of cell population maintenance and propagation in the host.

Toward a Cell Function

CfpA was disrupted in *T. denticola* with an erythromycin resistance cassette [Izard et al., 2001]. The resulting mutant strain did not express CfpA, consequently no filamentous ribbon was present in the cell. The phenotypic consequences affected multiple cell functions. Motility was impaired, but it appeared that the filamentation phenotype (concatenation of cytoplasmic cylinders under the same outer membrane) was most probably the source of this observation. In fact, cells with a single cytoplasmic cylinder seem to have a normal motility in dense media when compared to the wild-type strain [Izard et al., 2001]. Over 99% of the cells at any stage of growth were composed of at least two cytoplasmic cylinders under a unique outer membrane, resulting in a filamentation of the cells and a crankshaft-like movement. The independence of the cytoplasmic cylinders was confirmed by electron microscopy and by darkfield microscopy. While observing live culture under darkfield microscopy, it was possible to see the end of each cytoplasmic cylinder rotating individually inducing cell torsions. Cell shape was not altered by the absence of the cytoplasmic ribbon. The most noticeable phenotypes were a segregation defect phenotype with condensation of the chromosomal DNA [Izard et al., 2001]. Most of the cytoplasmic cylinders contained an area of condensed DNA (95%). Their distribution was random between the cytoplasmic cylinder ends (64%) and the center (31%).

The participation of the cytoplasmic filaments in the cell division process and its relation to the chromosomal DNA is unclear. Chromosome segregation, arrangement, duplication are critical activities within the cell. For chromosome duplication, the two replication forks initiate from *oriC* and meet at the opposite side in the terminus *(ter)* region [Hill, 1996; Messer and Weigel, 1996]. The *oriC* and terminus region have a defined physical location in the cell (and a defined position within the chromosome) [Niki and Hiraga, 1998; Webb et al., 1997] and between them, the chromosome regions are laid out according to the order of replication [Niki and Hiraga, 1998; Teleman et al., 1998]. Even if the model of duplication and separation of *oriC* and *ter* differs between *E. coli* and *Bacillus subtilis*, the existence of an active chromosome segregation mechanism seems unambiguous [Glaser et al., 1997; Jensen and Shapiro, 1999; Niki and Hiraga, 1998; Sharpe and Errington, 1999; Webb et al., 1998], compatible with a replisome at a fixed position in *B. subtilis* [Gordon and Wright, 1998; Lemon and Grossman, 1998]. The mechanism that spatially and temporally controls and directs the *oriC* region is unknown; however it seems likely that this mechanism involves the action of a spindle or mitotic-like apparatus and 'motor' proteins [Sharpe and Errington, 1999]. Further experimentation is required to demonstrate that the filamentous ribbon of treponemes is part of a permanent mitotic apparatus.

Immunogenicity

The cytoplasmic filament protein, CfpA, has been identified as the previously described TpN83, an antigen recognized by human serum from patients infected by *T. pallidum* subsp. *pallidum*, the agent of syphilis [Norris, 1993; You et al., 1996]. Because filament protein is cytoplasmic, it is not expected to be released unless cell death occurs and the cytoplasmic cylinder ruptures. However, that protein and its degradation products are recognized by the immune system which may be part of the autoimmune response observed in syphilitic rabbits. The suspected mechanism is the induction or generation of anti-anti-TpN83 (anti-anti-Id) antibody that would mimic the binding site of the cytoplasmic filament protein to fibronectin [Baughn, 1990; Baughn and Musher, 1992]. The binding of the cytoplasmic filament protein to fibronectin has been observed by different techniques [Baughn and Musher, 1992; J. Izard, unpubl. data]. Another *T. pallidum* antigen may also be involved in the generation of antifibronectin antibody in rabbit. There is a molecular mimicry between an immunodominant amino-acid motif on the 47-kDa lipoprotein (TpN47), a membrane protein of *T. pallidum* [Norris, 1993], and multiple repeats of analogous sequences in fibronectin [Baughn et al., 1996]. The role of each mechanism in the infectious process has not been deciphered. It is evident that CfpA and TpN47 proteins should be not included in any vaccine candidate so as to preclude immunopathologic consequences post-injection in humans.

Other Components of the Treponemal Cytoskeleton

Only two filamentous structures in treponemes have been analyzed for their protein content: the cytoplasmic filaments (CfpA) and the periplasmic flagellar filaments (FlaA, FlaB-1–3). Other filamentous structures are hypothesized to be present in all spirochetes based on genomic sequence data [Fraser et al., 1997, 1998; Ren et al., 2003; Seshadri et al., 2004] and the body of work accumulated in other model organisms. These are the septa-

tion ring and the cell shape maintenance-associated filaments.

Cell septation during bacterial cell division requires the filament formation of FtsZ [Bi and Lutkenhaus, 1991]. As the first component of the septum ring, FtsZ it is required for the targeting of all the other septation-associated proteins [Errington et al., 2003; Goehring and Beckwith, 2005; Schmidt et al., 2004]. The protein complex formed is associated with the inner membrane [Errington et al., 2003]. The coding gene, *ftsZ*, is found in all eubacteria, most archaea, in organelles of many eukaryotes [Beech et al., 2000; Bramhill, 1997]. The three-dimensional structure of FtsZ shows similarity to the tubulin fold [Lowe and Amos, 1998].

Cell shape maintenance is a critical aspect of bacterial cell biology, the filaments formed by MreB are a key factor. The *mreB* gene is present in a large range of organisms with rod- or helical-shaped cells [Daniel and Errington, 2003]. In *B. subtilis mreB* is essential [Abhayawardhane and Stewart, 1995; Jones et al., 2001]. It may be deleted in *E. coli* [Wachi et al., 1987] however, the gene is required for population viability [Kruse et al., 2005]. The filaments formed by MreB control cell length and width of the bacteria [Jones et al., 2001; Wachi et al., 1987]. MreB protein structures have been resolved and show similarity to the actin fold [Van den Ent et al., 2001]. The filament is also part of a membranous protein complex [Kruse et al., 2005]. In treponemes, the two longitudinal filaments (CfpA and MreB) coexist at all stages of cell development. Their relative positioning is still unknown.

Defining Bacterial Intermediate-Like Filaments

Eukaryotic intermediate filaments were named because of their intermediate size between actin microfilaments (7 nm) and microtubules (25 nm). The common core of the protein is composed of heptad repeats in large α-helices of the coiled-coil structure. These intermediate filament proteins are organized in five distinct classes [Herrmann and Aebi, 2004]. Their expression can be tissue-specific or ubiquitous, as with lamin B that is expressed in all nucleated cells. Other intermediate filaments in eukaryotes, including unicellular organisms, do not always match the traditional classification scheme [Karabinos et al., 2001; Perng and Quinlan, 2005; Spiliotis et al., 2005]. They have in common a coiled-coil region in the section of their peptidic sequence, form filaments. Like the other intermediate filaments they lack unity in function.

In prokaryotes, only crescentin, from *Caulobacter crescentus,* has been shown to have direct sequence similarity to an intermediate filament protein (human cytokeratin 19) [Ausmees et al., 2003]. This protein is involved in the formation of helical filaments that generate a vibrioid or helical cell shape depending on the cell length [Ausmees et al., 2003].

Prokaryotic filament diversity is expected to be greater than that found in eukaryotes. Most of the new filaments discovered in bacteria have never been directly observed in vivo by electron microscopy. Immunolabelling [Bi and Lutkenhaus, 1991] or green fluorescent protein fusions were used for visualization [Daniel and Errington, 2003; Jones et al., 2001]. These techniques do not allow a measurement of thickness, eliminating our ability to classify the filaments by size. The bacterial intermediate-like filament (BILF) definition shall include the following criteria: (1) core filaments formed by the polymerization of a monomer (as demonstrated in vivo or in vitro); (2) coiled-coil segments present within the secondary structure, and (3) located in the cytoplasm.

CfpA from *Treponema* spp. (phylogenetic class: Spirochaetes) [Izard et al., 1999], crescentin from *C. crescentus* (α-proteobacteria) [Ausmees et al., 2003], AglZ from *Myxococcus xanthus* (δ-proteobacteria) [Yang et al., 2004], TlpA from *Salmonella spp.* (γ-proteobacteria) [Hurme et al., 1994] and Scc from *Leptospira* spp. (Spirochaetes) [Mazouni et al., 2006] match the present definition. New protein members will be added with the advances in automated searches for coiled-coil structures in prokaryotic genomes [A.C. Yoshizawa, pers. commun.; Yoshizawa et al., 2004], and advances in observation technologies.

Future Directions

With the growing number of bacterial genomes sequenced (www.genomesonline.org) [Liolios et al., 2006], gene sequence diversity is becoming an issue of the past. The next challenges are the attributions of gene sequence to a peptide associated with a protein complex and its localization. The diverse filamentous ultrastructures are dynamic protein complexes. However, with rare exceptions, only the core protein is known. To understand their biological function a dual search is underway: identify the protein partners over the different cell cycles, including cell division, identify their topography in space over time. While mass spectrometry will allow the identification of the components of purified protein complexes,

fluorescence microscopy of chromophore-labeled fusion protein will facilitate the understanding of the molecular dynamics and localization of those protein complexes.

Alternative methodologies should be considered. Cryo-electron tomography techniques are improving at a steady pace. From the first tomograms of Archaea bacteria [Grimm et al., 1998] to the latest work [Izard et al., 2004; Komeili et al., 2006; Kurner et al., 2005] (fig. 3), the resolution has increased significantly, allowing the observation of cell-associated structures [Henderson, 2004]. The technology is finally at a stage where quantitative analysis of length, width and volume is possible for the comparison of structures between strains of bacteria. Mutant strains lacking ultrastructures can be observed to record quantitatively the influence of such structure on the overall cell architecture [J. Izard and C.E. Hsieh, unpubl. data]. Also, with the advance in resolution close to the theoretical limits [McEwen et al., 2002], it is possible to envision automated searches for molecular complexes within the tomograms [Bohm et al., 2000]. This type of search would allow identification of the distribution of particles like GroEL-GroES, the structure of which is already known by crystallography and cryo-electron microscopy [Ludtke et al., 2004; Svensson et al., 1994]. Once the structure of a given protein complex is known, a computational model can be constructed. An automated search of the tomogram using a template-matching method (based on tomogram characteristics: resolution, cell thickness, voxel size) can locate the complex regardless of its orientation [Bohm et al., 2000]. This technique would circumvent the present limitations of relying on visual recognition of small objects. Such an approach could also be used to detect and trace thin filaments associated with cell architecture.

Networks of specific protein interactions are present within the cytoskeleton. Known molecular machines and cellular pathways are being integrated into our knowledge of these networks. Further investigations will reveal new components and novel interconnections with previously unrelated machinery. The characteristics of these protein networks create and define hyperstructures and pseudo-compartments within the cell. These characteristics include volume occupancy, location, requirement for helper proteins to assist with function [Norris et al., 1999].

The cytoplasmic filaments of treponemes, the FtsZ filaments associated with the septation ring and the MreB shape maintenance filaments are part of membrane-bound protein complexes. None of those filaments functionality is isolated and all of them require partnership of other cell components. The molecular understanding from protein interface to system organization will facilitate the design of small molecules that inhibit protein complex function for cell biology study and drug discovery [Margalit et al., 2004; Stokes et al., 2005].

Acknowledgements

A special thank you to Ronald J. Limberger and Linda Slivienski-Gebhardt for their support. The work on the cytoplasmic filaments has been funded in part through the National Institutes of Health grant AI34354 (R.J.L.), a basic research grant from Health Research Incorporated, the John W. Hein Fellowship from The Forsyth Institute. The author would like to thank William A. Samsonoff, Chyongere E. Hsieh, Bruce F. McEwen for various aspects of the electron microscopy work, which was made possible by the NIH NCRR Biomedical Research Technology Program Grant RR01219, the Wadsworth Center's Core Facility for Electron Microscopy.

References

Abhayawardhane Y, Stewart GC: *Bacillus subtilis* possesses a second determinant with extensive sequence similarity to the *Escherichia coli mreB* morphogene. J Bacteriol 1995; 177:765–773.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389–3402.

Ausmees N, Kuhn JR, Jacobs-Wagner C: The bacterial cytoskeleton: an intermediate filament-like function in cell shape. Cell 2003; 115:705–713.

Baughn RE: Demonstration and immunochemical characterization of natural, autologous anti-idiotypic antibodies throughout the course of experimental syphilis. Infect Immun 1990;58:766–773.

Baughn RE, Musher DM: Evidence that autologous idiotypic regulation of anti-arginine-glycine-aspartic acid autoantibodies may influence development and progression of syphilitic lesions in infected rabbits. Infect Immun 1992;60:3861–3871.

Baughn RE, Jiang A, Abraham R, Ottmers V, Musher DM: Molecular mimicry between an immunodominant amino acid motif on the 47-kDa lipoprotein of *Treponema pallidum* (Tpp47) and multiple repeats of analogous sequences in fibronectin. J Immunol 1996; 157:720–731.

Beech PL, Nheu T, Schultz T, Herbert S, Lithgow T, Gilson PR, McFadden GI: Mitochondrial FtsZ in a chromophyte alga. Science 2000; 287:1276–1279.

Bermudes D, Fracek SP Jr, Laursen RA, Margulis L, Obar R, Tzertzinis G: Tubulin-like protein from *Spirochaeta bajacaliforniensis*. Ann NY Acad Sci 1987;503:515–527.

Bermudes D, Chase D, Margulis L: Morphology as a basis for taxonomy of large spirochetes symbiotic in wood-eating cockroaches and termites: *pillotina* gen. nov., nom. rev.; *Pillotina calotermitidis* sp. nov., nom. rev.; *Diplocalyx* gen. nov., nom. rev.; *Diplocalyx calotermitidis* sp. nov., nom. rev.; *Hollandina* gen. nov., nom. rev.; *Hollandina pterotermitidis* sp. nov., nom. rev.; and *Clevelandina reticulitermitidis* gen. nov., sp. nov. Int J Syst Bacteriol 1988;38:291–302.

Bi EF, Lutkenhaus J: FtsZ ring structure associated with division in *Escherichia coli*. Nature 1991;354:161–164.

Bohm J, Frangakis AS, Hegerl R, Nickell S, Typke D, Baumeister W: Toward detecting and identifying macromolecules in a cellular context: template matching applied to electron tomograms. Proc Natl Acad Sci USA 2000;97:14245–14250.

Bramhill D: Bacterial cell division. Annu Rev Cell Dev Biol 1997;13:395–424.

Daniel RA, Errington J: Control of cell morphogenesis in bacteria: two distinct ways to make a rod-shaped cell. Cell 2003;113:767–776.

Eipert SR, Black SH: Characterization of the cytoplasmic fibrils of *Treponema refringens* (Nichols). Arch Microbiol 1979;120:205–214.

Errington J, Daniel RA, Scheffers DJ: Cytokinesis in bacteria. Microbiol Mol Biol Rev 2003; 67:52–65.

Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, White O, Ketchum KA, Dodson R, Hickey EK, et al: Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature 1997;390:580–586.

Fraser CM, Norris SJ, Weinstock GM, White O, Sutton GG, Dodson R, Gwinn M, Hickey EK, Clayton R, Ketchum KA, et al: Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. Science 1998;281:375–388.

Glaser P, Sharpe ME, Raether B, Perego M, Ohlsen K, Errington J: Dynamic, mitotic-like behavior of a bacterial protein required for accurate chromosome partitioning. Genes Dev 1997;11:1160–1168.

Goehring NW, Beckwith J: Diverse paths to midcell: assembly of the bacterial cell division machinery. Curr Biol 2005;15:R514–R526.

Gordon GS, Wright A: DNA segregation: putting chromosomes in their place. Curr Biol 1998;8:R925–R927.

Grimm R, Singh H, Rachel R, Typke D, Zillig W, Baumeister W: Electron tomography of ice-embedded prokaryotic cells. Biophys J 1998; 74:1031–1042.

Hawley DK, McClure WR: Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nucleic Acids Res 1983;11:2237–2255.

Henderson R: Realizing the potential of electron cryo-microscopy. Q Rev Biophys 2004;37: 3–13.

Herrmann H, Aebi U: Intermediate filaments: molecular structure, assembly mechanism, integration into functionally distinct intracellular Scaffolds. Annu Rev Biochem 2004; 73:749–789.

Hill T: Features of the chromosomeal terminus region; in Neidhardt FC et al (eds): *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, ed 2. Washington, ASM Press, 1996, vol 2, pp 1602–1614.

Hollande A, Gharagozlou I, Grasse P: Morphologie infrastructurale de *Pillotina calotermitidis* nov. gen.. nov. sp. Spirochaetale de l'intestin de Coloterines praecox. CR Acad Sci Hebd Seances Acad Sci D 1967;265:1309–1312.

Holt SC: Anatomy and chemistry of spirochetes. Microbiol Rev 1978;42:114–160.

Hovind-Hougen K: Further observations on the ultrastructure of *Treponema pallidum* Nichols. Acta Pathol Scand Sect B 1972;80:297–304.

Hovind-Hougen K: The ultrastructure of cultivable treponemes. I. *Treponema phagedenis*, *Treponema vincentii*, *Treponema refringens*. Acta Pathol Scand Sect B 1974;82:329–344.

Hovind-Hougen K: *Leptospiraceae*, a new family to include *Leptospira* Noguchi 1917 and *Leptonema* gen. nov. Int J Syst Bacteriol 1979;29: 245–251.

Hovind-Hougen K, Birch-Andersen A: Electron microscopy of endoflagella and microtubules in *Treponema reiter*. Acta Pathol Microbiol Scand 1971;79:37–50.

Hovind-Hougen K, Birch-Andersen A, Jensen HJ: Ultrastructure of cells of *Treponema pertenue* obtained from experimentally infected hamsters. Acta Pathol Scand Sect B 1976;84:101–108.

Hurme R, Namork E, Nurmiaho-Lassila EL, Rhen M: Intermediate filament-like network formed in vitro by a bacterial coiled coil protein. J Biol Chem 1994;269:10675–10682.

Indest KJ, Ramamoorthy R, Philipp MT: Transcriptional regulation in spirochetes. J Mol Microbiol Biotechnol 2000;2:473–481.

Izard J, Samsonoff WA, Kinoshita MB, Limberger RJ: Genetic and structural analysis of the cytoplasmic filaments of wild-type and flagellar filament mutant of *Treponema phagedenis*. J Bacteriol 1999;181:6739–6746.

Izard J, Samsonoff WA, Limberger RJ: Cytoplasmic filament-deficient mutant of *Treponema denticola* has pleiotropic defects. J Bacteriol 2001;183:1078–1084.

Izard J, McEwen BF, Barnard RM, Portuese T, Samsonoff WA, Limberger RJ: Tomographic reconstruction of treponemal cytoplasmic filaments reveals novel bridging and anchoring components. Mol Microbiol 2004;51: 609–618.

Jensen RB, Shapiro L: The *Caulobacter crescentus smc* gene is required for cell cycle progression and chromosome segregation. Proc Natl Acad Sci USA 1999;96:10661–10666.

Jones LJ, Carballido-Lopez R, Errington J: Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. Cell 2001; 104:913–922.

Karabinos A, Schmidt H, Harborth J, Schnabel R, Weber K: Essential roles for four cytoplasmic intermediate filament proteins in *Caenorhabditis elegans* development. Proc Natl Acad Sci USA 2001;98:7863–7868.

Komeili A, Li Z, Newman DK, Jensen GJ: Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. Science 2006;311:242–245.

Kremer JR, Mastronarde DN, McIntosh JR: Computer visualization of three-dimensional image data using IMOD. J Struct Biol 1996; 116:71–76.

Kruse T, Bork-Jensen J, Gerdes K: The morphogenetic MreBCD proteins of *Escherichia coli* form an essential membrane-bound complex. Mol Microbiol 2005;55:78–89.

Kurner J, Frangakis AS, Baumeister W: Cryo-electron tomography reveals the cytoskeletal structure of *Spiroplasma melliferum*. Science 2005;307:436–438.

Lemon KP, Grossman AD: Localization of bacterial DNA polymerase: evidence for a factory model of replication. Science 1998;282: 1516–1519.

Limberger RJ: The periplasmic flagellum of spirochetes. J Mol Microbiol Biotechnol 2004;7: 30–40.

Liolios K, Tavernarakis N, Hugenholtz P, Kyrpides NC: The Genomes On Line Database (GOLD) v.2: a monitor of genome projects worldwide. Nucleic Acids Res 2006;34: D332–D334.

Lowe J, Amos LA: Crystal structure of the bacterial cell-division protein FtsZ. Nature 1998; 391:203–206.

Ludtke SJ, Chen DH, Song JL, Chuang DT, Chiu W: Seeing GroEL at 6 A resolution by single particle electron cryomicroscopy. Structure (Camb) 2004;12:1129–1136.

Margalit DN, Romberg L, Mets RB, Hebert AM, Mitchison TJ, Kirschner MW, RayChaudhuri D: Targeting cell division: small-molecule inhibitors of FtsZ GTPase perturb cytokinetic ring assembly and induce bacterial lethality. Proc Natl Acad Sci USA 2004;101: 11821–11826.

Masuda K, Kawata T: Isolation and characterization of cytoplasmic fibrils from treponemes. Microbiol Immunol 1989;33:619–630.

Mazouni K, Pehau-Arnaudet G, England P, Bourhy P, Saint Girons I, Picardeau M: The scc spirochetal coiled-coil protein forms helix-like filaments and binds to nucleic acids generating nucleoprotein structures. J Bacteriol 2006;188:469–476.

McEwen BF, Marko M, Hsieh CE, Manella CA: Use of frozen-hydrated axonemes to assess imaging parameters and resolution limits in cryo-electron tomography. J Struct Biol 2002;138:47–57.

Messer W, Weigel C: Initiation of chromosome replication; in Neidhart FC (ed): *Escherichia coli* and *Salmonella*, ed 2. Washington, ASM Press, 1996, vol 2, pp 1579–1601.
Niki H, Hiraga S: Polar localization of the replication origin and terminus in *Escherichia coli* nucleoids during chromosome partitioning. Genes Dev 1998;12:1036–1045.
Norris SJ: Polypeptides of *Treponema pallidum*: progress toward understanding their structural, functional, immunologic roles. Microbiol Rev 1993;57:750–779.
Norris V, Alexandre S, Bouligand Y, Cellier D, Demarty M, Grehan G, Gouesbet G, Guespin J, Insinna E, Le Sceller L, et al: Hypothesis: hyperstructures regulate bacterial structure and the cell cycle. Biochimie 1999; 81:915–920.
Pearson WR: Rapid and sensitive sequence comparison with FASTP and FASTA. Methods Enzymol 1990;183:63–98.
Perng MD, Quinlan RA: Seeing is believing! The optical properties of the eye lens are dependent upon a functional intermediate filament cytoskeleton. Exp Cell Res 2005;305: 1–9.
Ren SX, Fu G, Jiang XG, Zeng R, Miao YG, Xu H, Zhang YX, Xiong H, Lu G, Lu LF et al: Unique physiological and pathogenic features of *Leptospira interrogans* revealed by whole-genome sequencing. Nature 2003;422:888–893.
Schmidt KL, Peterson ND, Kustusch RJ, Wissel MC, Graham B, Phillips GJ, Weiss DS: A predicted ABC transporter, FtsEX, is needed for cell division in *Escherichia coli*. J Bacteriol 2004;186:785–793.
Seshadri R, Myers GS, Tettelin H, Eisen JA, Heidelberg JF, Dodson RJ, Davidsen TM, DeBoy RT, Fouts DE, Haft DH et al: Comparison of the genome of the oral pathogen *Treponema denticola* with other spirochete genomes. Proc Natl Acad Sci USA 2004;101:5646–5651.
Sharpe ME, Errington J: Upheaval in the bacterial nucleoid. An active chromosome segregation mechanism. Trends Genet 1999;15: 70–74.
Smajs D, McKevitt M, Howell JK, Norris SJ, Cai WW, Palzkill T, Weinstock GM: Transcriptome of *Treponema pallidum*: gene expression profile during experimental rabbit infection. J Bacteriol 2005:187:1866–1874.
Spiliotis ET, Kinoshita M, Nelson WJ: A mitotic septin scaffold required for mammalian chromosome congression and segregation. Science 2005;307:1781–1785.
Stokes NR, Sievers J, Barker S, Bennett JM, Brown DR, Collins I, Errington VM, Foulger D, Hall M, Halsey R et al: Novel inhibitors of bacterial cytokinesis identified by a cell-based antibiotic screening assay. J Biol Chem 2005;280:39709–39715.
Svensson LA, Surin BP, Dixon NE, Spangfort MD: The symmetry of *Escherichia coli* cpn60 (GroEL) determined by X-ray crystallography. J Mol Biol 1994;235:47–52.
Teleman AA, Graumann PL, Lin DC, Grossman AD, Losick R: Chromosome arrangement within a bacterium. Curr Biol 1998;8:1102–1109.
Van den Ent F, Amos LA, Lowe J: Prokaryotic origin of the actin cytoskeleton. Nature 2001; 413:39–44.
Wachi M, Doi M, Tamaki S, Park W, Nakajima-Iijima S, Matsuhashi M: Mutant isolation and molecular cloning of *mre* genes, which determine cell shape, sensitivity to mecillinam, amount of penicillin-binding proteins in *Escherichia coli*. J Bacteriol 1987;169: 4935–4940.
Webb CD, Teleman A, Gordon S, Straight A, Belmont A, Lin DC, Grossman AD, Wright A, Losick R: Bipolar localization of the replication origin regions of chromosomes in vegetative and sporulating cells of *B. subtilis*. Cell 1997;88:667–674.
Webb CD, Graumann PL, Kahana JA, Teleman AA, Silver PA, Losick R: Use of time-lapse microscopy to visualize rapid movement of the replication origin region of the chromosome during the cell cycle in *Bacillus subtilis*. Mol Microbiol 1998;28:883–892.
Yang R, Bartle S, Otto R, Stassinopoulos A, Rogers M, Plamann L, Hartzell P: AglZ is a filament-forming coiled-coil protein required for adventurous gliding motility of *Myxococcus xanthus*. J Bacteriol 2004;186:6168–6178.
Yoshizawa AC, Okuda S, Itoh M, Katayama T, Kawashima S, Kanehisa M: Prediction and comparison of coiled-coil proteins in multiple genomes. http://www.jsbi.org/journal/IBSB04/IBSB04P031.pdf., 2004.
You Y, Elmore S, Colton LL, Mackenzie C, Stoops JK, Weinstock GM, Norris SJ: Characterization of the cytoplasmic filament protein gene (*cfpA*) of *Treponema pallidum* subsp. *pallidum*. J Bacteriol 1996;178:3177–3187.
Zemper ED, Black SH: Morphology of freeze-etched *Treponema refringens* (Nichols). Arch Microbiol 1978;117:227–238.

© Free Author Copy - for personal use only
PLEASE NOTE THAT ANY DISTRIBUTION OF THIS ARTICLE WITHOUT WRITTEN CONSENT FROM S. KARGER AG, BASEL IS A VIOLATION OF THE COPYRIGHT.
Upon request a written permission to distribute the PDF file will be granted against payment of a permission fee depending on the number of accesses required. Please contact Karger Publishers, Basel, Switzerland at permission@karger.ch