Journal of Molecular Microbiology and Biotechnology

J Mol Microbiol Biotechnol 2006;11:221–227
DOI: 10.1159/000094056

The Flagellar Cytoskeleton of the Spirochetes

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Key Words
Spirochetes, flagellar cytoskeleton · Flagellar cytoskeleton · *Borrelia burgdorferi* · *Treponema denticola* · *Treponema phagedenis* · *Leptonema* (formerly *Leptospira*) *illini*

Abstract
The recent discoveries of prokaryotic homologs of all three major eukaryotic cytoskeletal proteins (actin, tubulin, intermediate filaments) have spurred a resurgence of activity in the field of bacterial morphology. In spirochetes, however, it has long been known that the flagellar filaments act as a cytoskeletal protein structure, contributing to their shape and conferring motility on this unique phylum of bacteria. Therefore, revisiting the spirochete cytoskeleton may lead to new paradigms for exploring general features of prokaryotic morphology. This review discusses the role that the periplasmic flagella in spirochetes play in maintaining shape and producing motility. We focus on four species of spirochetes: *Borrelia burgdorferi*, *Treponema denticola*, *Treponema phagedenis* and *Leptonema* (formerly *Leptospira*) *illini*. In spirochetes, the flagella reside in the periplasmic space. Rotation of the flagella in the above species by a flagellar motor induces changes in the cell morphology that drives motility. Mutants that do not produce flagella have a markedly different shape than wild-type cells.

Introduction

Until recently, bacterial morphology was stigmatized by the complexity of the eukaryotic cytoskeleton. The simplistic picture of an elastic bacterial wall resisting a turgor pressure [Boudaoud, 2003] was dwarfed by the interconnected actin network, microtubules, and intermediate filaments that composed the dynamic scaffolding of eukaryotic cells. The one anomaly appeared to be the spirochetes. This unique group of bacteria, with some members being highly virulent to humans, uses long helical filaments, called flagella, embedded inside their periplasmic space (the space between the inner membrane-cell wall complex (i.e. cell cylinder) and outer membrane sheath (fig. 1)) to help establish and maintain cell shape. The rotation of these flagella by molecular motors induces gyration, rotation, and dynamic deformation of the cell cylinder, which propel the bacteria through fluids. Therefore, at least one group of bacteria was known to use polymer filaments to maintain and dynamically alter their shape, i.e. possess a cytoskeleton.

However, in recent years, prokaryotic homologs to all three primary cytoskeletal proteins (tubulin, actin, and intermediate filaments) have been discovered [Gitai, 2005]. In the mid-1990s, FtsZ, a ubiquitous bacterial division protein, was proposed as a bacterial tubulin homolog

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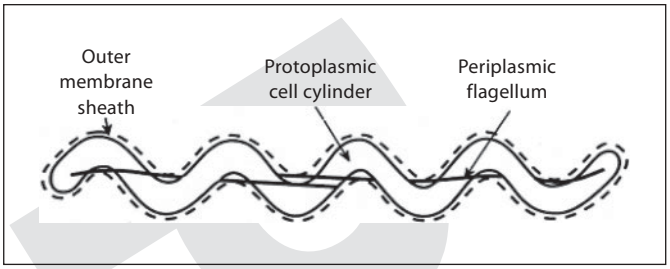

Fig. 1. Schematic diagram of a spirochete. Printed with permission from the *Annual Review of Genetics* [Charon and Goldstein, 2002].

based on sequence analysis and its ability to polymerize [Erickson, 1995; Mukherjee et al., 1993]. FtsZ forms a ring at the center of the cell during division and is necessary for formation and proper placement of the septum [Bi and Lutkenhaus, 1991]. Since these discoveries, FtsZ has also been observed to form spiral structures in vivo [Ben-Yehuda and Losick, 2002] and a single mutation in *ftsZ* has been shown to lead to morphological defects [Addinall and Lutkenhaus, 1996]. Sequence analysis of the prokaryotic protein MreB suggested an actin-like ATP-binding site [Bork et al., 1992]. Furthermore, electron microscopy and diffraction analysis of MreB polymers suggest that these polymers are closely related to single strands of F-actin [van den Ent et al., 2001]. Fluorescence microscopy has now revealed that MreB and another homologous protein, Mbl, form helical cables near the inner membrane in *Bacillus subtilis* and are both required for cell shape maintenance [Daniel and Errington, 2003; Jones et al., 2001]. Most recently, an intermediate filament homolog, crescentin, has been discovered in the curved rod-shaped bacterium *Caulobacter crescentus* [Ausmees et al., 2003]. Without crescentin, *C. crescentus* assumes straight rod morphology.

The existence of these prokaryotic cytoskeletal proteins provides clear evolutionary links between morphological mechanisms in eukaryotes and bacteria; however, the way these proteins function in bacteria remains unclear. The more studied flagellar cytoskeleton of the spirochetes provides a useful archetype for exploring the role of polymer proteins in bacterial form and motility. This review will discuss the role that the periplasmic flagella (PFs) play in the maintenance of form and the production of motility in the spirochetes. We focus on four spirochetes in particular: *Borrelia burgdorferi*, *Treponema denticola*, *Treponema phagedenis*, and *Leptonema* (formerly *Leptospira*) *illini*. Based on the general features that are present in these systems, we suggest a biophysical or biomechanical view of the spirochete flagellar cytoskeleton that can suggest new experiments for probing the mechanical and dynamic behavior of this system.

The Flagellar Cytoskeleton

Most spirochetes are helically-shaped [Holt, 1978], but some species have a flat, sinusoidal or meandering waveform [Charon and Goldstein, 2002; Goldstein et al., 1994, 1996; Holt et al., 1994]. In addition to a typical bacterial plasma membrane surrounded by a cell wall containing peptidoglycan, they have an outer lipid bilayer membrane, also referred to as an outer membrane sheath (fig. 1). The region between the protoplasmic cell cylinder and the outer membrane sheath is referred to as the periplasm, or periplasmic space. The spirochetes have flagella that are similar in many respects to the external flagella of rod-shaped bacteria. However, the spirochetes are unique in that their flagella, referred to as PFs, are located between the protoplasmic cell cylinder and outer membrane sheath, i.e. within the periplasm. Each PF is attached subterminally to only one end of the cell cylinder and extends toward the opposite end. Spirochete species vary with respect to size, number of PFs, and whether the PFs overlap in the center of the cell. *Cristispira*, for example, are 0.5–3 μm wide, 30–180 μm long, and have over 100 PFs attached at each cell end, while the Leptospiraceae (which include *Leptospira* and *Leptonema* spp.) are approximately 0.1 μm in diameter, 10–20 μm long, and have only one PF at each end [Canale-Parola, 1984].

The PFs are the spirochetes' organelles of motility. Early on, the analysis of chemically induced and spontaneously occurring mutants and their revertants pointed towards this conclusion [Charon et al., 1992b; Li et al., 2000b], but recent targeted mutagenesis studies conclusively showed that mutations that inhibit the synthesis of PFs result in non-motility. These mutations include the following for *B. burgdorferi*: *fliG2*, *flgE*, *fliF*, *flaB* [Motaleb et al., 2000; M. Sal, C. Li, and N.W. Charon, unpubl. data]; *T. denticola: flgE, FliG, fliK* (hook assembly protein [Li et al., 1996]); *Brachyspira hyodysenteriae: fliG*, *flaB1-flaB2* double mutant [C. Li and N.W. Charon, unpubl. data], and *L. biflexa (flaB)* [Picardeau et al., 2001]. In some of the above mutations, complementation restored the wild-type phenotype [Chi et al., 2002; Sartakova et al., 2001].

A remarkable feature of spirochete motility is that cells swim faster in a high-viscosity gel-like medium than they

do in low-viscosity water-like media. Most other bacteria slow down or stop in such media [Berg and Turner, 1979; Charon and Goldstein, 2002]. Rotation of the PFs leads to deformations that propagate from the ends and rolling of the cell body. When surrounded by a gel-like environment, these circular or flat waves – depending on the spirochete species – get sufficient traction to crank the cell body through the medium, much like a screw boring through wood. If traction is not sufficient, then the cell body slips against the external medium and the cell translates more slowly. In low-viscosity, water-like media, *T. denticola* is observed to rotate without translating [Klitorinos et al., 1993; Ruby et al., 1997]. As is suggested by this argument, faster swimming by spirochetes in high-viscosity media is dependent on the gel-like properties. Merely increasing the viscosity of the medium using a non-gel-forming agent such as Ficoll does not enhance motility, which suggests that this behavior is dependent on the *viscoelasticity* of the gel. Interestingly, *Spiroplasma*, a swimming wall-less bacterium, can swim faster in non-gel-like high-viscosity media, which suggests that its swimming is driven by a different mechanism [Trachtenberg, 2004; Wolgemuth et al., 2003].

The PFs of spirochetes are known to be rather similar in structure to bacterial flagella [Limberger, 2004], with a flexible hook region in the basal body that connects to a helical flagellar filament. However, unlike other swimming bacteria where the flagellar filament is composed of a single protein, flagellin, spirochete flagellar filaments are often composed of multiple proteins. The PF core is composed of a family of FlaB proteins, which show sequence similarity to flagellin, especially in the N- and C-terminal regions [Wilson and Beveridge, 1993]. With respect to the PFs of spirochete species so far analyzed, most have a protein designated FlaA that forms a sheath, and depending on the species, possibly a partial sheath, that surrounds the FlaB inner core. Because of this unique protein sheath surrounding the filament core, their flagellar filaments are thicker than those of other bacteria, e.g., 25 nm in diameter for *B. hyodysenteriae* compared to 20 nm for *E. coli* and *S. enterica* [Li et al., 2000a; Macnab, 1996]. As we know from recent work on bacterial flagella, the bistability of the packing of flagellin monomers within the 11 protofilaments is responsible for the polymorphism on the micron scale [Samatey et al., 2001]. Whether this is true for spirochete PF filaments is not known at this time, as the microstructure of the PFs has not yet been examined.

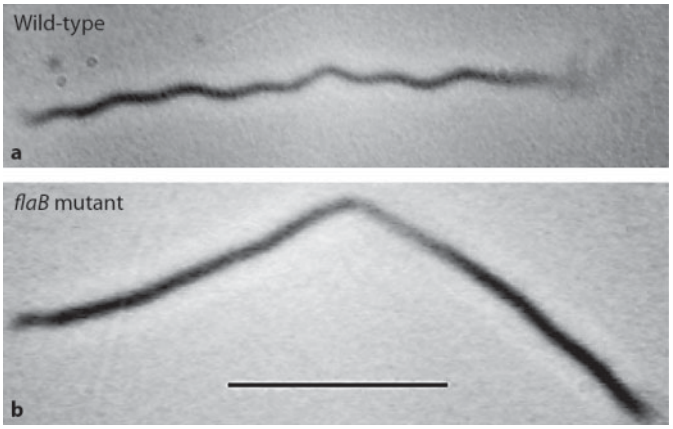

Fig. 2. *Borrelia burgdorferi*. **a** Wild-type cell with the normal plane-wave morphology. **b** Straight flagella-less *flaB* mutant, which is in the process of division. Bar represents 5 μm. Printed with permission from the *Annual Review of Genetics* [Charon and Goldstein, 2002].

Borrelia burgdorferi

B. burgdorferi are relatively large bacteria with a diameter of 0.33 μm and a length of 10–20 μm [Goldstein et al., 1996]. Wild-type cells at rest have a flat-wave morphology (having mostly planar, sinusoidal or meander-like deformations) with a wavelength of 2.83 μm and a peak-to-peak amplitude of 0.78 μm (fig. 2) [Goldstein et al., 1994, 1996]. Attached at each end are 7–11 PFs that overlap in the center of the cell [Barbour and Hayes, 1986]. These PFs are left-handed when purified and have a helix pitch of 1.48 μm and a helix diameter of 0.28 μm [Charon et al., 1992a]. In situ, their helix pitch and diameter are 2.83 and 0.45 μm, respectively. These results suggest that juxtaposition of the cell cylinder with the elastic PF influences flagellar shape. Flagella-less mutants of *B. burgdorferi* are rod-shaped (fig. 2) [Goldstein et al., 1994; Motaleb et al., 2000; Sartakova et al., 2001]. Thus, the presence of the PFs markedly influences cell shape: with the PFs, the cells are wave-like, and without them they are rod-shaped.

Cells swim by propagating waves along the cell from the anterior to the posterior, with beat frequencies of the fastest cells between 5 and 10 Hz [Goldstein et al., 1994]. A swimming cell usually gyrates as waves propagate along its length: the cell waveform turns about its axis counterclockwise as viewed from behind.

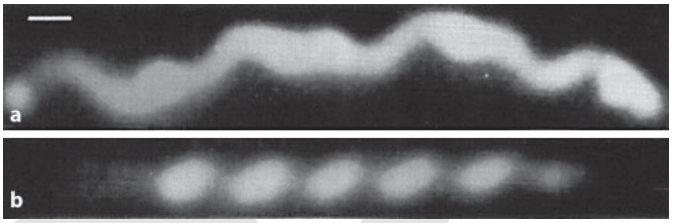

Fig. 3. a Wild-type *Treponema denticola* strain 35405 exhibiting the typical irregular morphology. **b** *flgE* flagella-less mutant exhibiting a right-handed helical morphology. The plane of focus was below the cell. Bar: 1.0 μm. Printed with permission from *Journal of Bacteriology* [Ruby et al., 1997].

Treponema

T. denticola cells are 6–16 μm in length and 0.21–0.25 μm in diameter; they have bundles, each containing two PFs, which overlap in the center of the cell [Canale-Parola, 1984; Ruby et al., 1997; Socransky et al., 1969]. Most wild-type cells of *T. denticola* have a highly irregular (twisted) morphology along their entire length (fig. 3a), combining both helical and planar regions in a non-distinct manner; however, a minority of cells is observed in a right-handed morphology (fig. 3b) [Ruby et al., 1997]. These two separate forms are relatively stable, and only rarely does one form convert to the other [Ruby et al., 1997]. If the outer membrane sheath is removed from the cell so that the flagella are no longer closely associated with the cell body, then an initially irregular cell takes on a right-handed helical form with a helical pitch only slightly different than those of the helical wild-type cells [Ruby et al., 1997]. In addition, flagella-less, nonmotile mutants also have this right-handed morphology, with helical parameters similar to those of the helical wild-type cells (fig. 3b) [Ruby et al., 1997]. These results indicate the following: (1) The right-handed helical configuration of *T. denticola* is associated with the peptidoglycan or cell wall material of the cells [Ruby et al., 1997]. (2) The motility and the irregular morphology are dependent on the presence of the PFs. (3) The PFs interact with the helically-shaped cell cylinder, and perhaps with the PFs at the other end of the cell, to dictate the irregular shape of the cells.

The PFs from *T. denticola* have been purified and their equilibrium conformation has been measured. The purified flagella are wave-like in appearance as determined by negative-stain electron microscopy and 23 nm in diameter over most of their length [Hovind Hougen, 1976; Ruby et al., 1997]. The three-dimensional shape of the purified flagella, as determined using standard methodology employing darkfield microscopy of the filaments suspended in methylcellulose, are left-handed with a helical pitch of 0.78 μm and a helical diameter of 0.26 μm [Charon et al., 1992a].

T. phagedenis, a close relative of *T. denticola*, is approximately 14–15.5 μm long and has flagellar bundles containing approximately 4–8 short PFs subterminally attached at each end [Charon et al., 1991; Limberger and Charon, 1986]. The cell body is a right-handed helix in the central part of the cell where there are no PFs. However, the ends of the cells have bent-shaped ends, which are often left-handed. Mutants that do not synthesize the PFs lack the bent ends. Furthermore, these bent ends and the PFs are approximately the same length (2.40–2.5 μm). These results indicate a close relationship between the formation of the bent-shaped ends and the presence of the PFs.

A complex coupling exists between the PFs and the shape of the bent ends of *T. phagedenis* [Charon et al., 1991]. Purified PFs are left-handed, with a helix pitch of 1.26 μm and a helix diameter of 0.36 μm. The bent ends are left-handed helices with a helix pitch of 1.85 μm and a helix diameter of 0.56 μm. Thus, the shape of the bent ends differs significantly from those of the right-handed cell body, and of the isolated PFs. In addition, a certain mutant of *T. phagedenis* has a cell cylinder that is more helical than that of the wild type, and another that is rod-shaped. Both of these mutants protrude their PFs at a frequency greater than the wild-type [Charon et al., 1992a]. For example, the rod-shaped cell body mutant protrudes its PFs at greater than 90% throughout all growth phases, compared to a maximum of 25% in stationary-phase wild-type cells. These results indicate that there is a critical shape of the cell cylinder for an essential fit with the PFs to form the bent-shaped ends; otherwise, the PFs protrude. This issue of wrapping a helical filament about a cylindrical body also arises in the study of climbing vines which wrap around a cylinder and whose pitch determines the nature of point contacts [A. Goriely, pers. commun.].

Although it has not been directly proven that the PFs rotate within the outer membrane sheath, several lines of evidence are strongly suggestive. Protrusions surrounded by an outer membrane sheath are often seen in stationary-phase cells of many spirochete species, and at a high frequency throughout all growth phases in certain mutants of *T. phagedenis* [Charon et al., 1992a]. These protrusions are clearly PFs, as they have the same helix pitch and diameter of purified PFs, and are not present in mu-

tants lacking PFs. Most importantly, these protruding PFs have been shown to rotate using both darkfield and differential interference contrast light microscopy [Charon et al., 1992a; Goldstein et al., 1994]. In addition, many of the motions observed in swimming spirochetes are best explained by rotation of the internally located PFs [Charon and Goldstein, 2002; Li et al., 2000b]. Finally, both structural and genetic analyses indicate that the PFs are very similar to their flagellar counterparts in other bacteria [Canale-Parola, 1984; Li et al., 2000b]. This suggests that they function in a comparable manner.

Leptospiracae

The *Leptospiracae*, such as *L. illini*, the saprophytic *Leptospira biflexa*, and the pathogenic *Leptospira interrogans* are thin, right-handed, helically-shaped bacteria with a length of 6–20 μm and a diameter of 0.1–0.2 μm [Carleton et al., 1979; Goldstein and Charon, 1988, 1990]. At each end, they have a short single PF attached subterminally that extends toward the center of the cell; however, the flagella are not long enough to overlap at the center of the cell [Bromley and Charon, 1979]. The flagella are structurally similar to those of rod-shaped bacteria, but when observed by negative-stain electron microscopy, they coil in the form of a spring rather than being wave-like as are most bacterial flagella [Berg et al., 1978; Bromley and Charon, 1979; Holt, 1978; Nauman et al., 1969].

When the cells are at rest, fixed, or dead, the ends of the cell are hook-shaped (fig. 4a) [Berg et al., 1978; Goldstein and Charon, 1988, 1990]. Mutants that form uncoiled PFs, or lack PFs, are still helically-shaped but have ends that are straight [Bromley and Charon, 1979; Picardeau et al., 2001]. In addition, cells with their outer membrane sheath removed are still helically-shaped [Auran et al., 1972]. These results indicate that the PFs interact with the helically-shaped cell cylinder to form the hook-shaped ends.

Swimming Leptospiraceae exhibit a number of different cell shapes. In cells that are translating, the anterior end is spiral-shaped and the posterior end is hook-shaped (fig. 4c) [Berg et al., 1978; Goldstein and Charon, 1990]. Cells readily reverse directions, with the spiral end becoming hook-shaped and the hook-shaped end becoming spiral-shaped. Non-translating forms are also seen where both ends of the cell are either hook-shaped (fig. 4a) or spiral-shaped (fig. 4b) [Berg et al., 1978; Goldstein and Charon, 1990]. Several lines of evidence indicate that the spiral-shaped end is associated with counterclockwise rotation of its associated PF (the frame of reference is viewing the flagella along its length from its distal end to the insertion point on the cell cylinder), and the hook-shaped end is associated with clockwise rotation. Thus, translating cells rotate their PFs in opposite directions. Taken together, the results indicate that the direction of rotation of the PF and its interaction with the cell cylinder determines the morphology of the end [Berg et al., 1978; Charon et al., 1984; Goldstein and Charon, 1988, 1990; Li et al., 2000b].

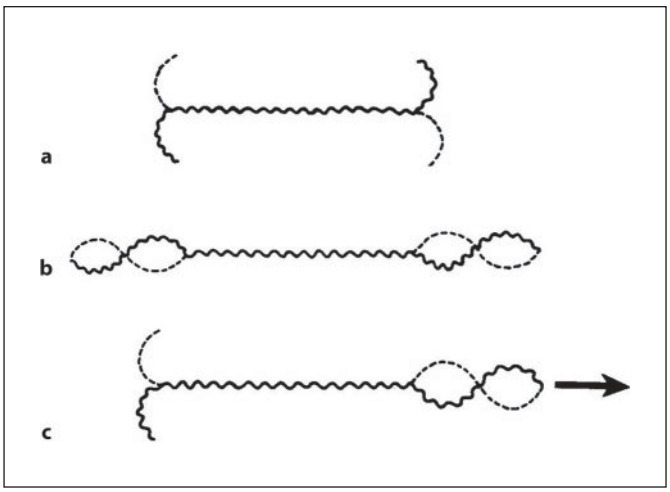

Fig. 4. Movement of *L. illini* in liquid medium. **a**, **b** Non-translational forms with either hook-hook (**a**) or spiral-spiral (**b**) ends. **c** Translating cells have one end that is hook and one end that is spiral. Cells move in the direction of the spiral end [Goldstein and Charon, 1990].

New Thoughts/Directions/Open Questions

The spirochetes constitute a remarkable biological system within which to study the interplay of elasticity, morphology, and self-propulsion. With modes of motion that are so distinct from the much more well-studied rotating helical bacterial flagella, they challenge us to understand how the dynamic coupling between two elastic components, the PF and the cell body, induces bending waves (or more complex motions like those seen in *L. illini*) which produce net propulsion. Since the resting helical or flat-wave shapes of the organism are dependent upon the presence of the PFs in the above-mentioned spirochetes, the system is clearly under tension, storing en-

ergy like a spring. How do we understand force generation in this coupled system?

Any complete characterization of the energies and forces associated with spirochete movements requires an understanding of at least three separate properties. First, the elastic moduli of the component parts must be measured, which can be determined by micromanipulation methods, such as optical trapping, which have successfully been applied to determine the bending modulus of the bacterial cell wall [Mendelson et al., 2000]. Second, a full dynamic description of the deformations of the cell body during swimming is needed, similar to the analysis that has recently been done for *Spiroplasma* [Shaevitz et al., 2005]. Third, it is necessary to understand the interaction between the deformations of the cell body and dynamics of the external fluid medium that provides thrust for the swimmer [Purcell, 1977; Goldstein et al., 2000].

Finally, it is worth noting that the fluid habitat of many spirochetes is often very viscous and possibly viscoelastic. Little is known theoretically or experimentally about the dynamics of locomotion by traveling bending waves in such an environment, but would likely shed light on fundamental aspects of low Reynolds number physics and biology.

Acknowledgments

The authors thank Alain Goriely and Mike Shelley for useful discussions. This work was supported by US Public Health Service Grants GM0072004 and AI29743 awarded to C.W.W. and N.W.C., respectively. Portions from this review were taken from the chapter, 'The beguiling motility of *Treponema*', N.W. Charon, C. Li, and S.F. Goldstein, in the upcoming book 'Molecular Biology and Pathogenesis of Treponemal Infection'. J. Raldolf and S. Lukehart, Editors. Horizon Scientific Press, Norfolk, UK.

References

Addinall SG, Lutkenhaus J: FtsZ-spirals and -arcs determine the shape of the invaginating septa in some mutants of *Escherichia coli*. Mol Microbiol 1996;22:231–237.

Auran NE, Johnson RC, Ritzi DM: Isolation of the outer sheath of *Leptospira* and its immunogenic properties in hamsters. Infect Immunol 1972;6:968–975.

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

Barbour AG, Hayes SF: Biology of *Borrelia* species. Microbiol Rev 1986;50:381–400.

Ben-Yehuda S, Losick R: Asymmetric cell division in *Bacillus subtilis* involves a spiral-like intermediate of the cytokinetic protein FtsZ. Cell 2002;109:257–266.

Berg HC, Turner L: Movement of microorganisms in viscous environments. Nature 1979; 278:349–351.

Berg HC, Bromley DB, Charon NW: Leptospiral motility. Symp Soc Gen Microbiol, 1978, vol 28, pp 285–294.

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

Bork P, Sander C, Valencia A: An ATPase domain common to prokaryotic cell cycle proteins, sugar kinases, actin, hsp70 heat shock proteins. Proc Natl Acad Sci USA 1992;89: 7290–7294.

Boudaoud A: Growth of walled cells: from shells to vesicles. Phys Rev Lett 2003;91:018104.

Bromley DB, Charon NW: Axial filament involvement in the motility of *Leptospira interrogans*. J Bacteriol 1979;137:1406–1412.

Canale-Parola E: The spirochetes; in Krieg NR, Holt JG (eds): Bergey's Manual of Systematic Biology. Baltimore, Williams & Wilkins, 1984.

Carleton O, Charon NW, Allender P, O'Brien S: Helix handedness of *Leptospira interrogans* as determined by scanning electron microscopy. J Bacteriol 1979;137:1413–1416.

Charon NW, Goldstein SF: Genetics of motility and chemotaxis of a fascinating group of bacteria: the spirochetes. Annu Rev Genet 2002;36:47–73.

Charon NW, Daughtry GR, McCuskey RS, Franz GN: Microcinematographic analysis of tethered *Leptospira illini*. J Bacteriol 1984;160: 1067–1073.

Charon NW, Goldstein SF, Curci K, Limberger RJ: The bent-end morphology of *Treponema phagedenis* is associated with short, left-handed, periplasmic flagella. J Bacteriol 1991;173:4820–4826.

Charon NW, Goldstein SF, Block SM, Curci K, Ruby JD: Morphology and dynamics of protruding spirochete periplasmic flagella. J Bacteriol 1992a;174:832–840.

Charon NW, Greenberg EP, Koopman MBH, Limberger RJ: Spirochete chemotaxis, motility, the structure of the spirochetal periplasmic flagella. Res Microbiol 1992b;143:597–603.

Chi B, Limberger RJ, Kuramitsu HK: Complementation of a *Treponema denticola flgE* mutant with a novel coumermycin A1-resistant *T. denticola* shuttle vector system. Infect Immun 2002;70:2233–2237.

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

Erickson HP: FtsZ, a prokaryotic homolog of tubulin? Cell 1995;80:367–370.

Gitai Z: The new bacterial cell biology: moving parts and subcellular architecture. Cell 2005; 120:577–586.

Goldstein SF, Charon NW: Motility of the spirochete *Leptospira*. Cell Motil Cytoskel 1988;9: 101–110.

Goldstein SF, Charon NW: Multiple-exposure photographic analysis of a motile spirochete. Proc Natl Acad Sci USA 1990;87:4895–4899.

Goldstein SF, Charon NW, Kreiling JA: *Borrelia burgdorferi* swims with a planar waveform similar to that of eukaryotic flagella. Proc Natl Acad Sci USA 1994;91:3433–3437.

Goldstein SF, Buttle KF, Charon NW: Structural analysis of the *Leptospiracae* and *Borrelia burgdorferi* by high-voltage electron microscopy. J Bacteriol 1996;178:6539–6545.

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

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST: The spirochetes; in Bergey's Manual of Determinative Bacteriology. Baltimore, Williams & Wilkins, 1994, pp 27–31.

Hovind Hougen K: Determination by means of electron microscopy of morphological criteria of value for classification of some spirochetes, in particular treponemes. Acta Pathol Microbiol Scand Sect B 1976;255(suppl):1–41.

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.

Klitorinos A, Noble P, Siboo R, Chan ECS: Viscosity-dependent locomotion of oral spirochetes. Oral Microbiol Immunol 1993;8: 242–244.

Li C, Motaleb MA, Sal M, Goldstein SF, Charon NW: Gene inactivation in the oral spirochete *Treponema denticola*: construction of a *flgE* mutant. J Bacteriol 1996;178:3664–3667.

Li C, Corum L, Morgan D, Rosey EL, Stanton TB, Charon NW: The spirochete FlaA periplasmic flagellar sheath protein impacts flagellar helicity. J Bacteriol 2000a;182:6698–6706.

Li C, Motaleb MA, Sal M, Goldstein SF, Charon NW: Spirochete periplasmic flagella and motility. J Mol Microbiol Biotechnol 2000b; 2:345–354.

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

Limberger RJ, Charon NW: *Treponema phagedenis* has at least two proteins residing together on its periplasmic flagella. J Bacteriol 1986; 166:105–112.

Macnab RM: Flagella and motility; in Neidhardt FC, Curtiss R III, Ingraham JL, et al (eds): Cellular and Molecular Biology. Washington, ASM Press, 1996, pp 123–145.

Mendelson NH, Sarlls JE, Wolgemuth CW, Goldstein RE: Chiral self-propulsion of growing bacterial macrofibers on a solid surface. Phys Rev Lett 2000;84:1627–1630.

Motaleb MA, Corum L, Bono JL, Elias AF, Rosa P, Samuels DS, Charon NW: *Borrelia burgdorferi* periplasmic flagella have both skeletal and motility functions. Proc Natl Acad Sci USA 2000;97:10899–10904.

Mukherjee A, Dai K, Lutkenhaus J: *Escherichia coli* cell division protein FtsZ is a guanine nucleotide binding protein. Proc Natl Acad Sci USA 1993;90:1053–1057.

Nauman RK, Holt SC, Cox CD: Purification, ultrastructure, composition of axial filaments from *Leptospira*. J Bacteriol 1969;98:264–280.

Picardeau M, Brenot A, Saint I: First evidence for gene replacement in *Leptospira* spp. inactivation of *L. biflexa flaB* results in non-motile mutants deficient in endoflagella. Mol Microbiol 2001;40:189–199.

Purcell EM: Life at low Reynolds number. Am J Phys 1977;45:3–11.

Ruby JD, Li H, Kuramitsu H, Norris SJ, Goldstein SF, Buttle KF, Charon NW: Relationship of *Treponema denticola* periplasmic flagella to irregular cell morphology. J Bacteriol 1997;179:1628–1635.

Samatey FA, Imada K, Nagashima S, Vonderviszt F, Kumasaka T, Yamamoto M, Nambda K: Structure of the bacterial flagellar protofilament and implications for a switch for supercoiling. Nature 2001;410:331–337.

Sartakova ML, Dobrikova EY, Motaleb MA, Godfrey HP, Charon NW, Cabello FC: Complementation of a nonmotile *flaB* mutant of *Borrelia burgdorferi* by chromosomal integration of a plasmid containing a wild-type *flaB* allele. J Bacteriol 2001;183:6558–6564.

Shaevitz JW, Lee JY, Fletcher DA: Spiroplasma swim by a processive change in body helicity. Cell 2005;122:941–945.

Socransky S, Listgarten M, Hubersak C, Bial JJ, Morton HE: Morphological and biochemical differentiation of three types of small oral spirochetes. J Bacteriol 1969;98:878–882.

Trachtenberg S: Shaping and moving a *Spiroplasma*. J Mol Microbiol Biotechnol 2004;7: 78–87.

Van den Ent F, Amos L, Lowe J: Prokaryotic origin of the actin cytoskeleton. Nature 2001; 413:39–44.

Wilson DR, Beveridge TJ: Bacterial flagellar filaments and their component flagellins. Can J Microbiol 1993;39:451–472.

Wolgemuth CW, Igoshin O, Oster G: The motility of mollicutes. Biophys J 2003;85:828–842.