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GENETICS OF MOTILITY AND CHEMOTAXIS OF A FASCINATING GROUP OF BACTERIA: The Spirochetes

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■ **Abstract** Spirochetes are a medically important and ecologically significant group of motile bacteria with a distinct morphology. Outermost is a membrane sheath, and within this sheath is the protoplasmic cell cylinder and subterminally attached periplasmic flagella. Here we address specific and unique aspects of their motility and chemotaxis. For spirochetes, translational motility requires asymmetrical rotation of the two internally located flagellar bundles. Consequently, they have swimming modalities that are more complex than the well-studied paradigms. In addition, coordinated flagellar rotation likely involves an efficient and novel signaling mechanism. This signal would be transmitted over the length of the cell, which in some cases is over 100-fold greater than the cell diameter. Finally, many spirochetes, including *Treponema*, *Borrelia*, and *Leptospira*, are highly invasive pathogens. Motility is likely to play a major role in the disease process. This review summarizes the progress in the genetics of motility and chemotaxis of spirochetes, and points to new directions for future experimentation.

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INTRODUCTION

> *These minute filamental organisms dart through the soft medium with great rapidity, first in one direction and then in another, searching for a loose spot which they can pierce through. When encountering an impenetrable obstacle they reverse their progression and start anew. A striking sight is thus presented by these little vermicular organisms darting in all directions* (99).

As Hideyo Noguchi noted above in 1918, and as has been witnessed by biologists from Leeuwenhoek in 1681 (33) to the present, watching the movement of spirochetes is indeed fascinating. Noguchi was describing *Leptospira icterohaemorrhagie*, the then newly described bacterial species that causes the highly fatal Weil's disease. The present review focuses on the genetics of motility and chemotaxis of the spirochete group of bacteria. Spirochete motility and chemotaxis were reviewed several years ago (22, 27, 48) and again more recently within the last two years (78, 84). We hope that the reader comes away with an appreciation that this medically important and ecologically significant group of bacteria has evolved some unique and exciting features related to their motility and chemotaxis. In addition, because spirochete genetics is now in the early stages of development, we also hope that this review is timely in formulating important areas of future research.

Unique Morphology of Spirochetes

Spirochetes are a conspicuous group of motile bacteria (58). Most are helically shaped (59), but some species have a flat sinusoidal or meandering waveform (47, 50). In addition to a typical bacterial plasma membrane surrounded by a cell wall containing peptidoglycan, they have an outer lipid bilayer membrane,

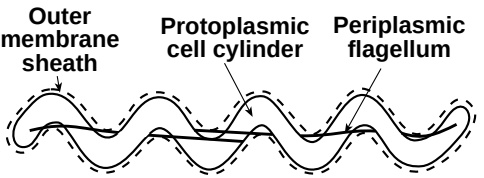

Figure 1 Schematic diagram of spirochete illustrating the outer membrane sheath, protoplasmic cell cylinder, and periplasmic flagella.

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also referred to as an outer membrane sheath (Figure 1). The space between the protoplasmic cell cylinder and the outer membrane sheath is referred to as the periplasm. The spirochetes are unique, as their periplasm contains periplasmic flagella that are similar in many respects to the external flagella of rod-shaped bacteria. Each periplasmic flagellum 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 periplasmic flagella, and whether the periplasmic flagella overlap in the center of the cell. The organisms can be quite large: For example, *Cristispira* are 0.5–3 μm wide, 30–180 μm long, and have over 100 periplasmic flagella attached at each cell end. In contrast, the Leptospiraceae (which include *Leptospira* and *Leptonema* sp.) are approximately 0.1 μm in diameter, 10–20 μm long, and have only one periplasmic flagellum at each end.

Spirochete Diseases and Ecology

Several species of spirochetes cause medically important diseases, some of which are quite prevalent and can have grave consequences. *Treponema pallidum* subspecies *pallidum* causes the dreaded sexually transmitted disease syphilis, and other closely related treponemes cause yaws, bejel, and pinta (2, 100). Worldwide, syphilis is a major disease in developing countries (44). This is of special concern, as syphilis is a recognized cofactor in the acquisition and transmission of human immunodeficiency virus (HIV) (127). *Borrelia burgdorferi* causes Lyme disease, which is the most prevalent vector-borne disease in the United States (23). *Borrelia hermsii* and other *Borrelia* species cause relapsing fever (4). Many *Leptospira* sp. cause leptospirosis (75). This potentially fatal waterborne zoonosis has many possible manifestations and occurs worldwide. *Treponema denticola* and other oral treponemes are associated with periodontal disease (32). *Brachyspira hyodysenteriae* causes swine dysentery, and *Brachyspira pilosicoli* and *Brachyspira aalborgi* are associated with human intestinal infections in developing countries and in immunocompromised individuals (93). Recently, spirochetes have been associated with chronic digital dermatitis in cattle (112).

Spirochetes are a metabolically diverse group of bacteria found in many different habitats, and several of them have yet to be cultured in the laboratory

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(19, 21). They vary, for example, with respect to oxygen requirements. *Leptospira* are obligate aerobes; *Spirochaeta* are often facultative; *Borrelia*, *Brachyspira*, and *T. pallidum* are microaerophilic; and most *Treponema* sp. are obligate anaerobes. They also vary with respect to nitrogen utilization. The free-living *Spirochaeta aurantia* and *Treponema* from termite guts utilize atmospheric nitrogen as a nutrient source. Because the diet of termites is low in nitrogen, these spirochetes may be essential to the termite for nutrient supply (81). Both free-living and commensal spirochetes are widespread in nature. Saprophytic *Leptospira*, *Spirochaeta*, and other uncultivable species are found in soil, freshwater, and saltwater (57, 91). Other spirochetes are found attached to protozoa sp. in the termite gut. These attached spirochetes enable the protozoa to be motile (22). The large uncultivable *Cristispira* resides in the crystalline style of bivalve mollusks (22, 57). Recently, spirochetes have been found associated with the hydrothermal vent polychaete annelid, *Alvinella pompejana* (20). In sum, besides residing in several different hosts, spirochetes also inhabit soil and saltwater and freshwater.

Selective Advantages of Spirochetes

There are two obvious advantages to being a spirochete. First, these organisms can swim in highly viscous, gel-like media, such as ones containing methylcellulose, that slow down or stop most externally flagellated bacteria (9, 50, 54, 67, 73). Such media include hyaluronic acid and collagen within the mammal (73), and mud or sediments encountered in the environment (22). In fact, the speed of several spirochete species actually accelerates as macroscopic viscosity increases. For example, the speed of *T. denticola* increases from less than 1 μm/sec in liquid media to 19 μm/sec in the presence of 1% methylcellulose (140 centipoise) (108). This attribute may allow *T. denticola* and other spirochetes to penetrate, invade, and adapt to specific ecological niches that exclude other bacterial species (22). Second, the periplasmic flagella reside within the periplasmic space. Because these organelles are not readily accessible to the ambient environment, they do not interact with flagellar-specific antibodies and hence circumvent the immobilization of the intact organism (13, 15, 30). Such antibodies often arise during infection (64, 103). Furthermore, their intracellular location may also offer protection from possibly disruptive extremes in the environment such as pH or salt concentration (36).

Divergent Evolution of Spirochetes

Several decades ago, researchers began investigating the evolutionary origin of spirochetes. Two hypotheses were initially considered (21). First, because these organisms are so markedly diverse with respect to size, metabolism, and ecological niche, and because being a spirochete has selective advantages, their existence could be the result of convergent evolution. Alternatively, the spirochetes could have evolved divergently from a primordial proto-spirochete. To address these hypotheses, the 16S rRNA genes of several pathogenic, parasitic, and

free-living species were sequenced. The results indicate that the spirochetes are an ancient group of Bacteria, and that they evolved from one common ancestral proto-spirochete. In fact, they are one of the few phyla of Bacteria that can be identified solely from morphology (102). Recently, the entire genomes of *T. pallidum*, *B. burgdorferi*, and *T. denticola* have been or are in the process of being sequenced (39, 40; http://www.hgsc.bcm.tmc.edu/microbial). The results from this analysis also support the conclusion that these spirochetes are evolutionarily closely related.

Spirochetes as Progenitors of Cilia and Flagella

It has been speculated that spirochetes are evolutionary precusors to eukaryotic cilia and flagella (90). Several features, including the similarity of their shapes, their common use of traveling waves for movement, the presence of cytoplasmic filaments that resemble eukaryotic microtubules in some spirochete species (11), and the role of symbiotic spirochetes in the swimming of some protozoans made this an intriguing suggestion. However, several lines of evidence do not support this idea. In contrast to mitochondria and chloroplasts, DNA is not present in flagellar basal bodies (65). An apparently tubulin-like protein identified in some spirochete species has turned out to be a member of the stress family of proteins (10, 97). Further, no tubulin gene sequences were identified in the genomes of the spirochete species that have been sequenced. Instead, these genomes have homologs similar to those encoding the motility genes of other bacteria (39, 40). More importantly, eukaryotic cilia and flagella use ATP-driven dynein arms to produce active sliding between neighboring microtubules (122). In contrast, spirochetes use a proton gradient to power motility (51, 71, 95, 116) and rotate their periplasmic flagella in typical prokaryotic fashion (25, 50). Thus, the suggestion that eukaryotic cilia and flagella arose from spirochetes is less attractive than it once appeared.

Recent Progress in the Genetics of Spirochetes

The genetics of spirochetes is at a very early stage compared to that of other bacterial species. This is due to the complexity of the growth requirements of many spirochete species, their relatively long generation times, and the difficulty of formulating optimal conditions and proper vehicles for targeted mutagenesis. In fact, some medically important spirochete species, such as *T. pallidum*, have yet to be cultured; consequently, targeted mutagenesis has not been possible for these organisms. As a result, we know very little about spirochete virulence factors.

Two major breakthroughs in spirochete genetics occurred within the past decade. First, in 1992 a successful targeted mutation was obtained by electroporating DNA into *B. hyodysenteriae* (123). Subsequently, targeted mutagenesis has been achieved with *B. burgdorferi* (16, 124), *T. denticola* (80), and *Leptospira biflexa* (104). In addition, generalized transduction mediated by a bacteriophage-like particle has been shown to readily promote gene exchange in *B. hyodysenteriae* (120). *B. burgdorferi* has also been shown to contain a phage that mediates transduction (34). Second, the entire genome sequences of *T. pallidum* and *B. burgdorferi* have

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been determined, and those of *T. denticola* and *L. interrogans* are at different stages of completion (39, 40; http://www.hgsc.bcm.tmc.edu/microbial). Much useful information has been obtained from analyzing genomic sequences and in targeting genes by allelic exchange mutagenesis. The reader is referred to recent reviews describing the advances in spirochete genetics (19, 56, 100, 125).

Why Study Spirochete Motility?

One important question is, "What is the relevance of studying spirochete motility?" We address this question briefly here, with a more detailed discussion later. First, the organelles for motility are attached near each end of the cell within the periplasmic space. As discussed below, translational motility requires the rotation of the two flagellar bundles in opposite directions: This leads to swimming modalities that are quite different and more complex than the well-studied paradigms of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (referred to as *S. enterica*). Second, the coordinated flagellar rotation, flexion, and direction reversals that occur in spirochetal motility likely involve an efficient and novel signaling mechanism. The coordinating signal would be rapidly transmitted from one end of the cell to the other, which in some cases is over 100-fold greater than the cell diameter. Therefore, the analysis of spirochete motility should lead to a better understanding of the nature of such long traveling signals in both spirochetes and other large bacteria. Finally, many spirochetes, including *Treponema*, *Borrelia*, and *Leptospira* are highly invasive pathogens. Motility is likely to play a major role in the disease process, and there is some recent evidence to support this conjecture (72, 83, 84, 107, 110). Understanding both chemotaxis and motility could lead to the development of new treatments and disease prevention.

Background to Bacterial Motility

Organisms such as *E. coli* and *S. enterica* serve as model systems in helping to decipher spirochete motility and chemotaxis (7, 12, 87, 88). These species swim via the rotation of externally located helically shaped flagellar filaments (Figure 2*a*). A rotary motor at the base of each flagellum powers this rotation, which is driven by a proton gradient. The flagellum structure is complex, with a rotary motor apparatus beginning in the cytoplasm and extending through the cytoplasmic membrane, peptidoglycan cell wall layer, outer membrane, and finally the helical flagellar filament that extends into the ambient medium. In the region below the flagellum, a ring structure (C-ring complex) composed of several different proteins (FliG, FliM, FliN) is attached to another ring (MS ring encoded by *fliF*). This C-ring structure and the MS ring serve as the rotary part of the flagellar motor. MotA and MotB proteins are embedded in the cytoplasmic membrane and cell wall around the MS ring and function as the stator, or nonrotating part of the motor. The P and L rings localize at the peptidoglycan layer and outer-membrane layers, respectively, and serve as bushings. The MS, P, and L rings surround a

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central rod, which in turn is attached to the hook, an external structure approximately 55 nm long, composed of a polymer of the FlgE protein. The hook serves as a flexible coupling to the flagellar filament, a helically shaped structure that is several μm long. This filament, composed of a polymer of the FliC protein, is a hollow tubular quasi-rigid polymorphic structure that can have either left- or right-handed configurations, depending on the direction it rotates. At the distal tip of the flagellar filament is the FliD protein, which serves as the flagellar cap. This is the site where new monomers that travel through the hollow filament attach to the growing filament. Associated with the flagellar apparatus near the C-ring complex are several different proteins involved in subunit transport and flagellar assembly. External proteins of the hook-basal body complex are excreted by a Type III secretion pathway through the basal body. The only exceptions are some of the proteins that comprise the L and P rings, which are secreted by the SecA-dependent pathway. The assembly of a flagellum is quite remarkable. Indeed, the bacterium constructs the architecturally complex flagellum at a site on its membrane, and it does so by having constant feedback mechanisms governing its assembly at the growing tip (68).

DYNAMICS OF SPIROCHETE MOTILITY

Essential Role of Periplasmic Flagella in Motility

The periplasmic flagella are clearly the spirochetes' organelles of motility. Early on, the analysis of chemically induced and spontaneously occurring mutants and their revertants pointed towards this conclusion (27, 78), but recent targeted mutagenesis studies were conclusive: Mutations that inhibited the synthesis of periplasmic flagella resulted in nonmotility. These mutations include the following for *B. burgdorferi*: *flgE*, *fliF*, *flaB* [filament protein, see discussion of *flaB* (below)] (95; M.A. Motaleb, M. Sal & N. W. Charon, unpublished data); *T. denticola: flgE*, *Tap1* [hook assembly protein (80, 82)]; *B. hyodysenteriae*: *fliG*, *flaB1-flaB2* double mutant (C. Li & N. W. Charon, unpublished data, see below); and *L. biflexa* (*flaB*) (104). In some cases, complementation of some mutations restores the wild-type phenotype (28, 111).

Although it has not been directly proven that the periplasmic flagella rotate within the outer membrane sheath, several lines of evidence are strongly suggestive. Protruding periplasmic flagella surrounded by outer membrane sheaths are often seen in stationary phase cells of many spirochete species, and at a high frequency throughout all growth phases in certain mutants of *Treponema phagedenis*. These protrusions have been shown to rotate (25, 50). In addition, as discussed below, many of the motions observed in swimming spirochetes are best explained by rotation of the internally located periplasmic flagella. Finally, both structural and genetic analyses indicate that the periplasmic flagella are very similar to their flagellar counterparts in other bacteria (22, 78). This obviously suggests that they function in a comparable manner.

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Skeletal Function of Periplasmic Flagella

One intriguing question is, How can spirochetes swim with their organelles for motility inside the cells? Further, How do the filaments accomplish this feat by rotating within the periplasmic space? The analysis of mutants of several spirochete species is beginning to yield answers. One consistent finding is that the periplasmic flagella of several spirochete species have both motility and skeletal functions, i.e., by skeletal function we mean that the periplasmic flagella influence the shape of the cell in the region where they reside (26, 95, 104, 109). The most remarkable example is *B. burgdorferi*, which is significantly larger than most pathogenic bacteria (10–20 μm long, 0.33 μm wide), and has 7 to 11 periplasmic flagella attached to each end of the cell. These filaments overlap in the center of the cell and form a continuous bundle in the periplasmic space (4, 47). High-voltage electron microscopy and observations of swimming cells indicate that these organisms have a flat-wave morphology (47, 50). Insertion mutations in the gene that encodes the major periplasmic flagellar filament protein FlaB (analogous to FliC of *E. coli* and *S. enterica*) result in cells that lack both periplasmic flagella and the flat-wave morphology. The resulting mutant cells are in fact rod-shaped (Figure 3) (95). The *flaB* mutation can be complemented only in *cis*; i.e., only when the plasmid containing wild-type *flaB* recombines with the chromosome does the cell regain wild-type characteristics (111). In addition, mutations in other genes that inhibit flagella synthesis such as *fliF* and *flgE* also result in cells that lack periplasmic flagella and are rod-shaped (M.A. Motaleb, M. Sal & N. W. Charon, unpublished data). Taken together, these results suggest that the periplasmic flagella affect the morphology of *B. burgdorferi* in quite a striking manner.

Model of *Borrelia burgdorferi* Motility

The skeletal function of periplasmic flagella forms the basis of motility models for several spirochete species [see (8, 26, 47, 48, 50, 78) for discussion of these models]. All these models rely on evidence that the periplasmic flagella rotate between the outer membrane sheath and cell cylinder. Recently, we described a model for *B. burgdorferi* motility (47, 50, 78; see video 1, http://www.uic.edu/orgs/blast/videos). This model states that in situ, the periplasmic flagella are juxtaposed with a highly flexible protoplasmic cell cylinder due to containment by the outer membrane sheath. The periplasmic flagella have a defined shape, which is a left-handed helix [a left-handed helix rotates counter-clockwise (CCW) going away from an observer, and a right-handed helix rotates clockwise (CW)]. Because of the close interaction of the more rigid periplasmic flagella with the flexible protoplasmic cell cylinder, the periplasmic flagella influence the shape of the entire cell. High-voltage electron microscopy has shown that the bundles of periplasmic flagella wrap CCW around the cell axis (since *B. burgdorferi* resembles a sine wave, we define the cell axis as the abscissa). In fact, the size of the bundles' left-handed helix pitch approximates that of the cell's flat wave-form. Translational motility occurs as the result of CCW rotation of the periplasmic flagella as viewed from the back of the cell. This rotation causes backward-moving waves to be propagated down the length

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of the cell. Thus, the action of the periplasmic flagella is likened to that of a worm gear in generating wave propagation. CW rotation of the cell about the body axis (i.e., the center of the protoplasmic cell cylinder—likened to the middle of a sausage) counterbalances the rotation of the periplasmic flagella. Mutants that lack the periplasmic flagella are nonmotile because they have lost the ability to propagate backward-moving waves. In addition, these mutants are rod-shaped as a result of their loss of skeletal function associated with the periplasmic flagella.

Model of *Spirochaeta aurantia* Motility

For some spirochete species, the periplasmic flagella may not have a skeletal function, and an alternative model has been proposed (6). This model is based on observations that some irregularly shaped, rigid cells translate in a low-viscosity medium with the cell rotating completely around the cell axis. For these cells to swim, the model states that the outer membrane sheath rotates or flows around the cell body in one direction, and the protoplasmic cell cylinder rotates in the opposite direction. Forward thrust is obtained by rolling of the cell cylinder in one direction and rotation of sheath in the opposite direction. This model may apply to *S. aurantia* and *Cristispira* sp., but evidence that the sheath rotates or flows in the opposite direction relative to the protoplasmic cell cylinder has not been obtained for any spirochete species.

Unique Structure and Composition of Periplasmic Flagella

The periplasmic flagella of several spirochete genera (*Leptospira*, *Treponema*, *Brachyspira*, and *Spirochaeta*) possess some features that distinguish them from the flagella of other bacteria (Figure 4) (27, 78). Because of a unique protein sheath surrounding the filament core, their flagellar filaments are thicker than those of

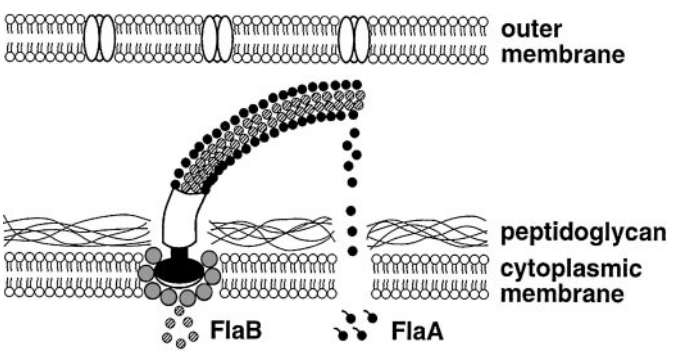

Figure 4 Structure of a spirochete periplasmic flagellum. In several spirochete species, the basal bodies lack the L and P rings found in *E. coli* and *S. enterica* (17, 60, 98). FlaA and FlaB are excreted to the periplasmic space by SecA and type III secretion systems, respectively. This complex periplasmic flagellar structure is found in *Treponema* sp., *S. aurantia*, *Leptospira* sp., and *B. hyodysenteriae* (27, 78).

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other bacteria, e.g., 25 nm in diameter for *B. hyodysenteriae* compared to 20 nm for *E. coli* and *S. enterica* (77, 87). This sheath is composed of a protein designated FlaA. Its sequence is well conserved among the spirochetes. The periplasmic flagellar core is composed of a family of FlaB proteins. FlaB proteins show sequence similarity to flagellin proteins of other bacteria, especially at the N- and C-terminal regions. There are 1 to 3 different FlaB proteins (FlaB1, FlaB2, and FlaB3), depending on the species, and a separate gene encodes each one. In addition, there is extensive sequence similarity between the FlaB proteins within a given species, and also between species of different genera. The FlaA protein is excreted into the periplasmic space by a SecA-dependent system, whereas FlaB is excreted by a Type III secretion system. The mechanism of assembly of the sheath around the core is not known, but the confining environment of the periplasmic space likely plays a critical role. The periplasmic flagellar composition of *B. burgdorferi* is somewhat different. It has only one FlaB protein, and there is considerably more FlaB than FlaA. The location of FlaA in *B. burgdorferi* is uncertain, but evidence suggests that it is closely associated with the periplasmic flagella filament (42; M.A. Motaleb & N.W. Charon, unpublished data).

Genetic analysis indicates that the interaction of FlaA with the FlaB subunits affects flagellar filament shape in a manner that has not been observed in any other flagellar system. The composition and genetics of periplasmic flagellar filament synthesis in *B. hyodysenteriae* is best understood, as its genes are more readily manipulated than other spirochetes (106, 120). Because of the extensive homology of its FlaA and FlaB proteins with those of other spirochetes, conclusions drawn from the analysis of its periplasmic flagella are likely to be to relevant to other species such as *T. pallidum* and *T. denticola*. Wild-type cells of *B. hyodysenteriae* have periplasmic flagella that are left-handed with a defined shape, as measured by helix pitch and helix diameter (77). The approximate ratios of flagellin proteins to one another are the following: FlaA : FlaB1 : FlaB2 : FlaB3 of 1 : 0.42 : 0.22 : 0.45. Thus, there is approximately one FlaA per FlaB family subunit. Targeted mutagenesis of each of the genes encoding *flaA*, *flaB1*, *flaB2*, and *flaB3* resulted in cells that were still motile and possessed periplasmic flagella that retained their left-handed configurations. However, comparing mutant and wild-type purified periplasmic flagella shapes yielded surprising results (77). Each of the *flaB* mutants had periplasmic flagella that were slightly different from the wild-type. However, the *flaA* mutant's periplasmic flagella displayed major decreases in both helix pitch and helix diameter compared to the wild type. These results suggest that FlaA impacts the shape of the periplasmic flagella. Because bacterial flagella are often quasi-rigid and undergo helical transformations, perhaps the sheath helps stabilize the FlaB helical core into one of these configurations for optimal thrust as it rotates between the outer membrane sheath and cell cylinder.

The analysis of double mutants of *B. hyodysenteriae* indicates a remarkably complex interaction between the FlaA and FlaB subunits. The location of each FlaB protein along the periplasmic flagellum is unknown, but two different models could account for their arrangement (106). One model proposes that FlaB1,

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FlaB2, and FlaB3 reside in specific regions of the flagellum in a manner analogous to the multiple flagellar subunits of *Caulobacter crescentus* (94). For example, perhaps FlaB3 is adjacent to the hook, FlaB2 is in the central region, and FlaB1 is at the distal region. However, an analysis of double mutants supported another model: The different FlaB proteins are distributed along the entire length of the periplasmic flagellum (C. Li & N.W. Charon, unpublished data). In this analysis, the periplasmic flagella from the double mutants *flaA-flaB1*, *flaA-flaB2*, and *flaA-flaB3* were left-handed. Surprisingly, however, their helix pitch and diameter were even less than those of both the wild-type and the single *flaA* mutants. These results suggest that in the absence of FlaA, the inhibition of synthesis of one FlaB protein dramatically influences the shape of the entire periplasmic flagellum. Such a result would not be expected if a given FlaB protein resided in only one domain of the periplasmic flagellum. These results also suggest that FlaA interacts with each FlaB protein to effect periplasmic flagellar shape.

The role of the different flagellin proteins in motility is unclear. Although single mutants of *flaB1*, *flaB2*, *flaB3*, or *flaA* of *B. hyodysenteriae* were motile, observations of swimming cells and swarm-agar-plate assays indicate that all these mutants swam less efficiently than the wild type (72, 77, 106). In addition, the double mutants *flaA-flaB1*, *flaA-flaB2*, and *flaA-flaB3* were also motile, but their swarming deficiency was even more pronounced than the single mutants (72; C. Li & N.W. Charon, unpublished data). The analyses of double *flaB* mutants were intriguing. Whereas the *flaB1–flaB3* mutant was still motile and had periplasmic flagella, the *flaB1-flaB2* mutant was completely nonmotile and lacked periplasmic flagella. These results indicate that the motility and structural functions of FlaB1 and FlaB2 overlap. Clearly, to fully understand the function of the multiple flagellin proteins, we need a more thorough analysis of the dynamics of motility in *B. hyodysenteriae* and the structure of wild-type and mutant periplasmic flagella.

SWIMMING AND CHEMOTAXIS OF SPIROCHETES

Complex Swim Behavior of Spirochetes

Most externally flagellated bacteria have two different swim modalities: runs and tumbles (3, 5, 7, 87). For example, in *E. coli* runs—or translational motility—occur when the flagella rotate CCW (as a frame of reference, the flagella are viewed from the end of the flagella toward its insertion into the cell). During a run, the peritrichous flagella form a bundle behind the cell and act as a propeller. When one or more of the flagella reverse direction and rotate CW, the cell tumbles (3, 87, 126). Some of the filaments leave the flagellar bundle and the cell body reorients. It then runs in a new direction when the flagella revert to the CCW direction. In some species, such as *Rhodobacter sphaeroides* and *Sinorhizobium meliloti*, the flagella stop or slow down instead of changing their direction of rotation.

The swim behavior of spirochetes is more complicated than that of other bacteria as a consequence of their complex geometry. Spirochetes have three motility modes

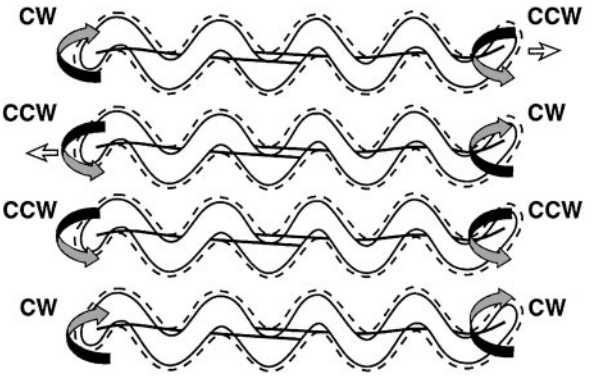

Figure 5 Swimming cells of *B. burgdorferi* as a function of direction of rotation of the periplasmic flagella. Open arrows indicate direction of swimming. Dotted lines represent the outer membrane sheath. Gray arrows indicate direction of rotation of the periplasmic flagella (*thin lines*). For simplification, only one periplasmic flagellum is shown attached at each end of the cell cylinder. In *B. burgdorferi*, there are between 7 and 11. The top two panels show translational forms, and the bottom two nontranslational forms.

(Figure 5): two modes of runs (i.e., with either end leading, top two panels), and one mode of nontranslational motility (bottom two panels) (24, 37, 50, 78). During runs, the anterior bundle of periplasmic flagella rotates in one direction (e.g., CCW), and the posterior bundle rotates in the opposite direction (CW). Thus, the bundles of periplasmic flagella rotate asymmetrically. Reversals occur when both bundles change their direction of rotation. The third mode is a nontranslational interval. During this mode, the bundles rotate in the same direction: both CW or both CCW. In many species of spirochetes during this interval, the cells become quite distorted and may bend in the middle; this is referred to as a flex (37, 50). The two nontranslating forms are morphologically indistinguishable for most spirochete species except for the Leptsopiraceae (8, 24, 37, 48, 49). In the Leptospiraceae, CCW rotation of its periplasmic flagellum results in a spiral-shaped cell end, and CW rotation results in a hook-shaped cell end. Thus, the translating forms are hook-spiral and spiral-hook, and the two nontranslating forms are hook-hook and spiral-spiral. In many ways, the swim behavior of spirochetes is analogous to that of *Spirillum* species (5, 74, 92). These bacteria have a somewhat similar morphology: bundles of flagella attached at each end of a helically shaped cell but with no outer membrane sheath. In addition, there are four different morphological forms of swimming *Spirillum*, which are analogous to those of the Leptospiraceae.

One common question concerns whether both bundles of periplasmic flagella actively rotate during translation as the description above contends. As an alternative model, perhaps one bundle rotates while the other is stopped. In this model, cell reversals occur when motor rotation switches from one bundle to the other.

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One prediction of this hypothesis is that the bundles at a given end should show frequent stops. To test this hypothesis, we analyzed spirochete cells tethered to a glass surface. Testing *Treponema phagedenis* gave the most straightforward results. Its periplasmic flagella do not overlap in the center of the cell and are quite short relative to the overall cell length. Periplasmic flagella, as previously mentioned, often have a skeletal function, so they influence the shape of the cell in the domains where they reside. In the case of *T. phagedenis*, these regions are proximal to and include the two cell ends (26). The terminal regions gyrate (i.e., bend in a circular manner without necessarily rotating) as a result of the rotation of relatively short, interior, periplasmic flagella (26). Tethered cell analysis indicated that both of the terminal regions constantly gyrated and often changed direction. In some cells, one end constantly gyrated in a specific direction, and the other end switched direction quite frequently (N.W. Charon, unpublished data). These results indicate that for translation in *T. phagedenis*, both bundles of periplasmic flagella rotate. A similar conclusion based on tethered cell analyses was reached with the Leptsopiraceae and *B. burgdorferi* (24, 76).

Asymmetrical Periplasmic Flagellar Rotation in Spirochetes

Unique to both spirochete and *Spirillum* sp. is asymmetrical rotation of the flagellar bundles during translational motility. Two hypotheses may explain how these bacteria achieve asymmetry. The first hypothesis states that chemotaxis (i.e., directed movement toward an attractant or away from a repellant) plays an essential role in this asymmetry. During chemotaxis, *E. coli* and most bacteria swim by a pattern best described as a biased random walk toward a favorable medium or away from one that is toxic (3, 5, 7, 18, 87). This swim pattern is a function of a combination of runs and tumbles. Initially, an effector molecule binds directly to a membrane-bound chemoreceptor protein referred to as a methyl-accepting chemotaxis protein (MCP), or indirectly to the MCP via a periplasmic binding protein (Figure 2*b*). The MCP regulates autophosphorylation of the sensor histidine kinase CheA. Activated CheA phosphorylates the response regulator CheY forming CheY-P, which then interacts with the switch complex at the flagellar motor to increase the probability of flagellar CW rotation and reduce the probability of CCW rotation. CheA and CheY are essential elements for chemotaxis, as null mutants that encode these proteins are nonchemotactic. These mutants constantly rotate their flagella CCW and continuously run. According to this chemotaxis-based hypothesis, asymmetry in spirochetes depends on localized concentrations of CheY-P, so that during translation one cell end has a higher concentration of CheY-P than the other. This hypothesis predicts that null mutants of *cheA* will continuously rotate their periplasmic flagella in the same direction and will be nontranslating and continuously flex.

The second hypothesis states that the motors at the two ends of the cells are different. This difference results in cells whose bundles rotate asymmetrically independently of CheY-P. Thus, in the default state with no CheY-P present, motors

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at one end of the cell rotate CCW, and those at the other end rotate CW. Both hypotheses lead to specific predictions. In contrast to the first hypothesis, this hypothesis predicts that null mutants in *cheA* will continually rotate their bundles of periplasmic flagella in opposite directions and constantly run.

Targeted mutagenesis was used to determine the basis for asymmetrical periplasmic flagellar rotation (76). The genomic sequence of *B. burgdorferi* indicated the presence of two *cheA* genes, *cheA1* and *cheA2*. Both *cheA* genes were inactivated to obtain the *cheA1–cheA2* double mutant. This mutant was deficient in chemotaxis toward rabbit serum, which is an attractant for these spirochetes (116). Whereas the wild-type reversed directions quite frequently (19 reversals per minute), the double mutant constantly ran. Thus, in the absence of CheA, i.e., the default state, one bundle of periplasmic flagella rotates CCW, and the other bundle rotates CW. These results support the hypothesis that structural differences exist between the flagellar motors at the opposite ends of the cell. Similar results have been noted in *cheA* mutants of *T. denticola* (85); consequently, it is likely that other spirochetes have asymmetrical rotation of the periplasmic flagella at opposite ends of the cells in the absence of CheA.

Asymmetrical localization of specific proteins and structures has been described for several bacterial species (86, 115). For example, the stalk structure localizes at one end of the cell in *C. crescentus* at a site previously occupied by the flagellum. The ActA protein of *Listeria monocytogenes* and the IcsA protein of *Shigella flexneri* localize at the old cell poles in each of these species. Perhaps in *B. burgdorferi* and other spirochetes there is association of an unknown factor or factors with the flagellar switch complexes at one cell pole. This association could result in the periplasmic flagella at that end rotating CW rather than CCW in the default state.

Model for Asymmetrical Periplasmic Flagellar Rotation

Several candidate effectors could be involved in flagellar asymmetrical rotation. One such possible effector is FliG. Specifically, *B. burgdorferi*, *T. pallidum*, and *T. denticola* have two homologs encoding FliG (39, 40; http://www.hgsc.bcm.tmc.edu/microbial). These genes are the only known duplicate homologs of the flagellar apparatus in *B. burgdorferi* and *T. pallidum*. FliG is part of the switch complex that determines direction of flagellar rotation. Conceivably, one or both of the FliG proteins could differentially localize to motors at one cell end relative to the other. Alternatively, CheX, another well-conserved protein product in spirochetes, could also encode such an effector. Results with *B. burgdorferi* and *T. denticola* suggest that *cheX* mutants are locked in the nontranslational mode (M.A. Motaleb & N.W. Charon, unpublished data; R. Lux & W. Shi, unpublished data). These results are consistent with CheX associating with one cell end leading to periplasmic flagellar asymmetrical rotation. Finally, in *B. burgdorferi* and *T. pallidum*, several open reading frames of unknown function map within putative motility and chemotaxis gene clusters (39, 40, 43). Perhaps one or more of these genes encode proteins involved in asymmetrical flagellar rotation.

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Chemotaxis in Spirochetes: CheA, CheX, CheY, and Membrane Potential

The fact that spirochetes have three swim modes (two running and one nontranslating interval), whereas most other studied bacteria have two modes (running and tumbling), adds to the challenge of understanding their chemotactic response on a molecular level. Even the well-studied chemotaxis systems of *E. coli*, *S. enterica*, *Bacillus subtilis*, and *R. sphaeroides* seem to grow in complexity as more is known about them (1a, 3, 18, 119)! In spirochetes, the relationship between chemotaxis and reversals in direction is quite intriguing, but very little is known about their regulation.

Swim reversal in spirochetes is quite fast. In the Leptospiraceae, *S. aurantia*, *B. burgdorferi*, and even the very large *Cristispira* it is less than 300 msec (24, 37) (N.W. Charon, E.P. Greenberg, unpublished data). As previously mentioned, a reversal occurs when the sense of rotation of the periplasmic flagella bundles at each end rapidly changes. One hypothesis to explain rapid reversals is that the relative concentration of a signal transducer such as CheY increases at one end of the cell due to contact with an attractant. Along these lines, the MCPs in *S. aurantia* and other bacterial species are polarly localized, and we would expect that those of other spirochete species are similar (45, 86). According to this hypothesis, increased concentration of the signal transducer at one end of the cell causes the periplasmic flagella near that terminus to change direction. The signal transducer also diffuses to the other end of the cell to effect a change of direction at that end. This hypothesis is quite unlikely. The above-mentioned bacteria are relatively large—at least 10 μm for the Leptospiraceae and *B. burgdorferi*, and 30–180 μm for the *Cristispira*. It would take several seconds for a compound the size of CheY to diffuse from one end of the cell to the other—much longer than the time a cell takes to reverse (38, 114). However, analysis of targeted mutants in *cheA* and *cheY* genes of *B. burgdorferi* and *T. denticola* indicate that CheY is indeed involved in chemotaxis (76, 85; R. Lux & W. Shi, unpublished data; M.A. Motaleb & N.W. Charon, unpublished data). Thus, while diffusion of CheY cannot explain rapid cell reversals, this cytoplasmic protein is indeed involved in spirochete chemotaxis.

What are the possible models that could account for rapid cell reversals in spirochetes? In *S. aurantia*, a membrane potential has been found to mediate the chemotactic response (52, 53). Although a membrane potential is not implicated in chemotaxis of *E. coli* and *S. enterica* (18, 89, 117), recent results suggest its involvement in other bacterial species (1). Because spirochetes (and *Spirillum* sp.) are considerably longer than many other bacteria, a change in membrane potential could conceivably allow for communication between cell ends to coordinate periplasmic flagellar rotation (5, 37, 117). However, it is not known if cells with their membrane potential clamped still undergo rapid reversals. Moreover, no one has yet stimulated one end of a spirochete with an attractant, and monitored the motion at the other end, as was done for long filamentous cells of *E. coli* (114).

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Such an experiment would clearly resolve whether there is rapid communication between one end of the cell with the other.

The work on *S. aurantia* over a decade ago is significant in analyzing the behavior of individual cells undergoing chemotaxis (37, 38). When these organisms are initially incubated with an attractant, the reversal and flexing frequencies are immediately suppressed, and their run durations are increased. This increase in run duration indicates that spirochetes respond to a temporal gradient. Because flexing and reversals are suppressed, apparently there is a mechanism that coordinates flagellar rotation at each of the cell's ends. These results are consistent with more recent results with *B. burgdorferi* and *T. denticola*, which indicate that *cheA*, *cheX*, *cheW*, and *cheY* not only are involved in chemotaxis, but also influence reversal frequency (76, 85; C. Li, M.A. Motaleb, R. Bakker & N.W. Charon, unpublished data; R. Lux & W. Shi, unpublished data).

With *S. aurantia*, after an initial stimulation with an attractant, the flexing and reversal frequencies eventually approach those of the prestimulated state and indicate that adaptation occurs. In bacteria such as *E. coli*, adaptation is dependent on MCP methylation (Figure 2*b*) (3, 7, 18). This increase in methylation of MCPs has also been observed with *S. aurantia* when cells undergo chemotaxis (70). Recently, MCPs in *T. denticola* and *T. pallidum* have also been shown to undergo methylation, and insertion mutagenesis in *T. denticola* has verified their involvement in chemotaxis (55, 69, 79). Thus, in spirochetes the involvement of MCPs in both the excitation phase and adaptation appears similar to those found in other bacteria.

Models of Spirochete Chemotaxis

In considering what is known about spirochete chemotaxis at this time, two possible models are apparent. Both models are drawn from certain experimental findings from *S. aurantia*, *B. burgdorferi*, and *T. denticola*, and are based on the paradigm of *E. coli* and *S. enterica*. We assume that conclusions drawn from one spirochete species apply to the others. It is also assumed that coordination of the rotation of the periplasmic flagella at both ends of the cell is necessary for chemotaxis, and that this coordination is mediated by attractants binding to polarly located MCP's. We propose CheX, and possibly other proteins such as one of the FliG proteins, localize to motors at one end of the cell. This localization results in asymmetrical rotation of the periplasmic flagella in the default state, i.e., one bundle rotates CCW, the other CW in the absence of CheY-P. As discussed below, this asymmetry imposes real complexity for chemotaxis models of spirochetes.

The first model, the "one-head model," states: Under an isotropic condition, cells run, flex, and reverse at a steady state. These frequencies are a function of the internal CheY-P concentration inside the cell, which is analogous to the frequencies of tumbles and runs found in other bacteria in isotropic conditions. When a chemoattractant binds to a MCP at a cell end (end 1), a signal is transmitted to the CheA bound to that chemoreceptor. CheA autophosphosphorylation is suppressed at that end of the cell with the concomitant decrease in localized concentration

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of CheY-P. This decrease in CheY-P suppresses CW rotation of the periplasmic flagella at that cell end with a resultant increase in the frequency of CCW rotation. Concomitantly, either the decrease in CheY-P concentration at that end of the cell, or more likely the bound attractant to the MCP, generates a second signal to the cytoplasmic membrane, which causes a depolarization of the membrane that is rapidly transmitted to the other cell end (end 2). At this end, the MCPs undergo a conformational change due to the depolarization, with a resultant decrease in autophosphorylation of bound CheA almost simultaneously with end 1 and a resultant localized decrease in CheY-P concentration. The decrease in CheY-P at this end leads to suppression of CCW rotation of the periplasmic flagella, an increase in CW rotation, and a concomitant decrease in frequencies of flexes and reversals—the cell runs. Adaptation occurs by mechanisms found in other bacteria, with an increase in flexes and reversals as the MCPs undergo methylation. One consequence of this one-headed model is that the cell swims toward a higher concentration of an attractant with only one end leading, specifically, the end (end 1) that rotates its periplasmic flagella CCW in low CheY-P concentration.

The second is a "two-headed" model that states that both ends of the spirochete are capable of leading a cell up a chemical gradient. How can end 2 lead the cell up a gradient if its default state is CW rotation of the periplasmic flagella? Perhaps there is asymmetry also with the MCPs, or factors that can interact with the MCPs. The model is the same as above, but in this case, when an attractant first binds to end 2, it causes CheA at that end to increase instead of decrease CheY-P concentration at that end. This results in suppression of CW rotation, and an increase in CCW rotation at end 2. The change in membrane potential as described above results in an increase in autophosphorylation at end 1, an increase in CheY-P concentration, and CW rotation of the periplasmic flagella at that end. The cell then runs with end 2 leading the cell. It should be pointed out that in *B. subtilis*, the model of *E. coli* and *S. enterica* is reversed. In *B. subtilis*, CheY-P concentration increases during chemotaxis, CW flagellar rotation is suppressed, and the probability of CCW rotation is increased (1a, 3, 14). Thus, one could speculate that, as a very basic model for spirochetes, one end behaves like *B. subtilis*, and the other end like *E. coli*. The above models are speculative, but it is hoped that they point out the difficulty in applying current knowledge of chemotaxis to spirochetes: Their geometry and asymmetry impose real constraints on possible mechanisms.

REGULATION OF MOTILITY AND CHEMOTAXIS GENE EXPRESSION IN SPIROCHETES

Organization of Motility and Chemotaxis Genes

Complete sequencing of the genomes of *B. burgdorferi* and *T. pallidum* has yielded important information about motility and chemotaxis (39, 40). Sequence homology, transcriptional and Western blot analyses, and targeted mutagenesis are beginning to yield an overall picture. From these analyses, and preliminary results

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with respect to the *T. denticola* genome (http://www.hgsc.bcm.tmc.edu/microbial), we find that all the genes necessary for flagella-based motility are present in *T. pallidum*, *B. burgdorferi*, and *T. denticola*. Both *T. pallidum* and *B. burgdorferi* have only one copy of the genes that encode the basal body and switch complex. The only exception is *fliG*; both organisms have duplications of this gene. In *B. burgdorferi* and *T. pallidum*, the genes encoding the L and P rings are absent. These results are consistent with electron microscopic observations of the basal body from these spirochetes (59, 61). In addition, the three species share many similarities in their organization of motility and chemotaxis gene clusters.

For a given spirochete, individual chemotaxis and motility genes have the highest homology among other spirochete species compared to other bacteria (78; C. Li & N.W. Charon, unpublished data). Many of the motility and gene clusters in *B. burgdorferi*, and a few in *T. pallidum*, have been shown to function as operons (76, 78). *B. burgdorferi* and *T. pallidum* each have several genes of unknown function that map within the motility and chemotaxis gene clusters. Because of their location, these genes are likely to be involved in either chemotaxis or motility. *T. pallidum* has only one copy of most of the sensory transduction genes (*cheA*, *cheX*, *cheD*, *cheY*, *cheB*, and *cheR*), but it has two copies of *cheW*. In contrast, *B. burgdorferi* has several copies of these genes (2 *cheA*, 3 *cheW*, 3 *cheY*, 2 *cheB*, and 2 *cheR*). Some of the genes were shown to be involved in chemotaxis by targeted mutagenesis in *B. burgdorferi* (*cheA2*, *cheW3*, *cheY1*, *cheY3*, *cheX*) and *T. denticola* (*cheA*, *cheX*, *dmcB*, *dmcA*) (69, 76, 79, 83; C. Li, M.A. Motaleb & N.W. Charon, unpublished data; R. Lux & W. Shi, unpublished data). One of the operons in *B. burgdorferi* (*flaA*, *cheA2*, *cheW3*, *cheX*, *cheY3* operon) is well conserved among the spirochete species (76). Several of the genes in the other chemotaxis operon of *B. burgdorferi* (*cheW2*, *orf566*, *cheA1*, *cheB2*, *orf569*, *cheY2*) are not found in *T. pallidum* and *T. denticola* and are most similar to genes found in other bacteria. This *cheW2* operon is likely the result of recent horizontal gene transfer from a Proteobacteria species (I. Zhulin, unpublished data). Its function is unknown, but the habitats for *B. burgdorferi* are both ticks and mammals, whereas the habitat for *T. pallidum* and *T. denticola* is exclusively mammalian. Perhaps the *cheW2* operon functions primarily in the tick, whereas the other functions in the mammal.

Control of Chemotaxis and Motility Gene Expression

Unique aspects of motility and chemotaxis gene expression in spirochetes are beginning to emerge. In most bacteria studied, there is cascade control of motility gene expression (29, 87). For example, in *E. coli* and *S. enterica* expression of *flhC* and *flhD* (class 1) initiate transcription of several motility genes (class 2). Two of the class 2 genes play a critical role in regulating class 3 gene transcription. Specifically, *fliA* encodes σ^{28}, which binds to RNA polymerase and promotes transcription of class 3 genes such as FliC. The other regulator gene is *flgM*, which encodes the anti-σ^{28} factor FlgM. FlgM is excreted into the medium when the hook basal body is complete. This excretion insures that the initiation of class 3

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gene transcription is timed to the assembly of the initial flagellar structure. *T. pallidum*, *S. aurantia*, *T. denticola*, and *B. hyodysenteriae* have many motility promoter sequences homologous to σ^{28} recognition sequences (27, 78). Thus, it is likely that flagellar gene regulation is similar in these spirochete species to that found in other bacteria. In contrast, all of the chemotaxis and motility gene promoters in *B. burgdorferi* are initiated by the housekeeping transcription factor σ^{70} (43, 76, 78). No motility-specific sigma factor recognition sequence is evident in genomic analysis. To our knowledge, *B. burgdorferi* is the only bacterial species that lacks transcriptional cascade control of motility gene expression by alternative sigma factors. One hypothesis is that motility and chemotaxis are so vital for the survival of *B. burgdorferi* in both the tick and mammal that many of the genes are constitutively expressed. In support of this hypothesis, *B. burgdorferi* expresses *flaB* message and produces periplasmic flagella in both these hosts (31, 46). Alternatively, perhaps *B. burgdorferi*, and possibly other spirochete species (82), primarily rely on a translational control system to regulate motility and chemotaxis gene expression. Preliminary results with insertion mutants in *flaA*, *flaB*, *flgE*, and *fliF* of *B. burgdorferi* support this possibility (M.A. Motaleb, M. Sal & N.W. Charon, unpublished data). For example, mutants in *flaB* of *B. burgdorferi* still synthesize similar amounts of *flaA* message as the wild type, but fail to encode small amounts of FlaA protein.

There are many unknown factors with respect to motility and chemotaxis gene regulation in spirochetes. For example, the periplasmic flagella are inserted into the cell cylinder subterminally at each end. Observations of dividing cells indicate that before the time of division, new periplasmic flagella emerge in both daughter cells proximal to the division plane (59, 63). However, the factors that promote synchronization and localization of basal body synthesis with cell division are unknown. Furthermore, little is known about the factors that control periplasmic flagella length and number, although we know that periplasmic flagella length is tightly controlled in some species (26). Finally, DNA microarray analysis for *B. burgdorferi* indicates that many of the transcripts encoding chemotaxis and motility genes are differentially regulated in the mammal host compared to the tick (105). Genes besides those involved in motility and chemotaxis have been known for some time to be differentially regulated as a function of host (113). Both the environmental factors within the two hosts and the molecular details within the spirochete that promote this differential regulation of gene expression are just beginning to be elucidated (62, 105).

SPIROCHETE MOTILITY AND CHEMOTAXIS AS VIRULENCE FACTORS

Deciphering the roles of motility and chemotaxis for spirochetes in the disease process is at its early stages (4, 84). Many spirochete researchers have long believed that motility and chemotaxis are major virulence factors. This belief is supported

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by the fact that 5–6% of the genomes of *T. pallidum* and *B. burgdorferi* are putative chemotaxis and motility genes. In addition, several spirochete species are known to be highly invasive to host tissues. This is particularly true for *T. pallidum*, *Leptospira*, the relapsing fever *Borrelia*, *B. burgdorferi*, and the spirochetes associated with digital dermatitis (4, 66, 96, 100, 121). For *B. burgdorferi* in the tick, motility may be essential for the spirochetes to migrate from the intestinal gut to the salivary glands to initiate a new infection. In addition, chemotaxis may play an essential role for the spirochetes to migrate through the skin and localize at the tick bite during feeding. These hypotheses are only speculation, as critical genetic studies have not yet been done. Interestingly, a gene that shows homology to a quorum sensor (*luxS*, gene BB0377 of *B. burgdorferi*) has been identified in the genome of *B. burgdorferi*. Quorum sensing, the ability for bacteria to sense cell density and regulate gene expression, is a key step in the regulation of virulence genes and biofilm formation in other bacterial species (41). In addition, motility plays an important role in biofilm formation in several of these species (101). Spirochetes achieve high densities when attached to host cells and to tissues (4, 59, 118) so it is likely that these organisms are capable of biofilm formation. Thus, the relationship of quorum sensing, motility, and biofilm formation is an intriguing area for future research on spirochetes.

One major concern is that because virulence is often unstable, sorting out whether a given gene is involved in the disease process is complicated. This is particularly true of *B. burgdorferi*, as virulence is associated with plasmids that are often lost on passage (35). The first in vivo studies relating targeted motility mutants to virulence were done with mutants in *flaA*, *flaB1*, and *flaAflaB1* of *B. hyodysenteriae*. The mutants were deficient in colonization and were less virulent than the wild type in the mouse model of swine dysentery (72, 107). Most recently, mutants in *flgE*, *cheA*, and in the MCPs *dmcA* and *dmcB* of *T. denticola* were found to be deficient in the penetration of gingival epithelial cell layers (83, 85). These results suggest that both motility and chemotaxis are involved in tissue penetration. In sum, virulence studies using targeted mutants are at the early stages in spirochetes. Now that the genetic tools have been and are being developed, it should be quite exciting in the next several years to relate specific gene function, including those genes involved with chemotaxis and motility, to virulence.

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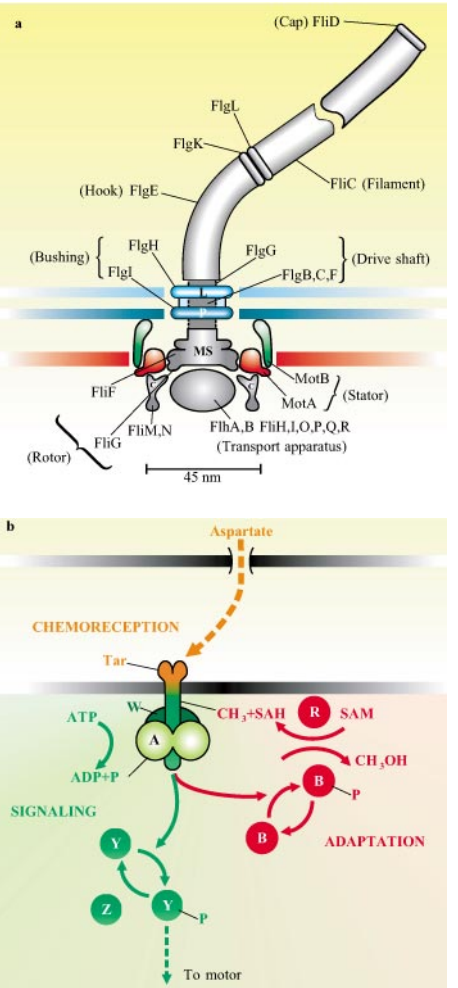

Figure 2 (*a*) Schematic bacterial motor and drive train illustrating the many protein components of the flagellar apparatus of *E. coli* and *S. enterica* serovar Typhimurium, and (*b*) their chemotaxis machinery. The chemotaxis diagram shows some of the components required for chemotaxis toward the amino acid aspartate. Information flows from the outside of the cell (*top*) by way of porins, the periplasmic space, and the cytoplasmic membrane, to the inside of the cell (*bottom*), and then to the flagellar motors (not shown). Dashed arrows indicate physical displacement of chemicals by diffusion. Solid arrows indicate chemical modifications of proteins—phosphorylation or methylation. The cytoplasmic components, all Che proteins (CheW, CheA, CheR, CheB, CheY, CheZ), are identified by their fourth letter only. The receptor complex consists of the MCP Tar, W, and A, with Tar spanning the cytoplasmic membrane. Chemoreception is depicted in orange, signaling in green (for "go"), adaptation in red (for "stop"). SAM is S-adenosylmethionine, the methyl donor. From Motile Behavior of Bacteria, Howard C. Berg, *Physics Today*, 2000, 53:24–29, by permission from the American Institute of Physics.

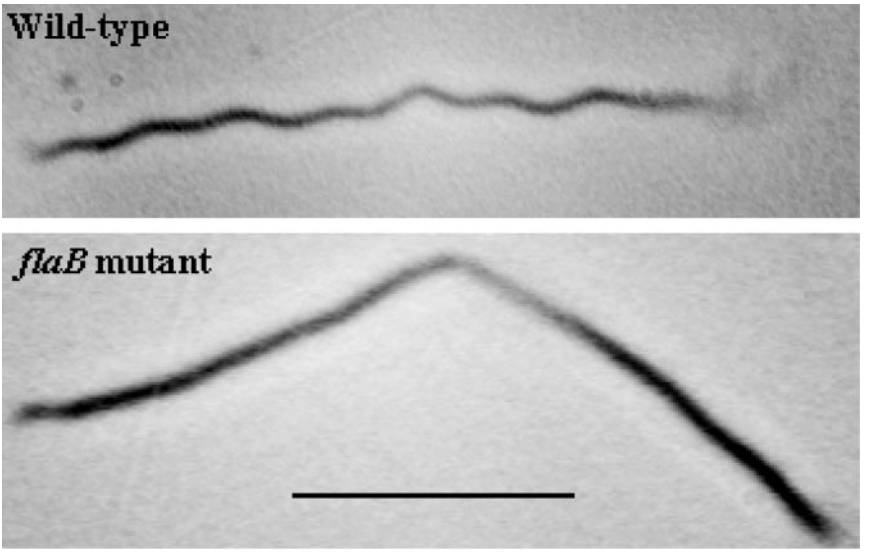

Figure 3 Morphology of *B. burgdorferi* wild-type and *flaB* mutant as observed by phase microscopy. The *flaB* mutant is in the process of division. Bar represents 5 μm.

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