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## Motility and Chemotaxis in *Campylobacter* and *Helicobacter*

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### Abstract

Flagellar motility of *Campylobacter jejuni* and *Helicobacter pylori* influences host colonization by promoting migration through viscous milieus such as gastrointestinal mucus. This review explores mechanisms *C. jejuni* and *H. pylori* employ to control flagellar biosynthesis and chemotactic responses. These microbes tightly control the activities of  $\sigma^{54}$  and  $\sigma^{28}$  to mediate ordered flagellar gene expression. In addition to phase-variable and posttranslational mechanisms, flagellar biosynthesis is regulated spatially and numerically so that only a certain number of organelles are placed at polar sites. To mediate chemotaxis, *C. jejuni* and *H. pylori* combine basic chemotaxis signal transduction components with several accessory proteins. *H. pylori* is unusual in that it lacks a methylation-based adaptation system and produces multiple CheV coupling proteins. Chemoreceptors in these bacteria contain nonconserved ligand binding domains, with several chemoreceptors matched to environmental signals. Together, these mechanisms allow for swimming motility that is essential for colonization.

### Keywords

flagella; *Epsilonproteobacteria*; gene expression; CheZ; FlgS; FlgR

## INTRODUCTION

*Campylobacter* and *Helicobacter* species are representative *Epsilonproteobacteria* that frequently associate with animal and human hosts. *Campylobacter jejuni* is a leading worldwide cause of diarrheal disease in humans, but it is also a harmless commensal organism of the intestinal tracts of many wild and agriculturally important animals, especially poultry (14, 79). *Helicobacter pylori* primarily infects humans, residing in the gastric mucosa in roughly half of the world's population. This infection can lead to many gastric diseases, including gastritis, gastric ulcers, and gastric carcinoma (21).

During infection of hosts, *C. jejuni* and *H. pylori* reside primarily within the thick mucus layer lining the intestinal and gastric epithelium, respectively (6, 41, 65). Flagellar motility

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is an important colonization determinant for these organisms, presumably enabling migration to and movement within mucus to reach microenvironments conducive to growth. As such, nonmotile *C. jejuni* or *H. pylori* mutants are attenuated for colonization of human or animal hosts (46, 83, 84). Like other motile bacteria, motility in *Campylobacter* and *Helicobacter* species is regulated by a chemotactic signaling system that allows the organisms to follow favorable chemical gradients in environments. Nonchemotactic ( $\text{Che}^-$ ) *C. jejuni* and *H. pylori* mutants are reduced for host colonization, indicating that recognition of proper environmental parameters via chemosensory systems is critical for in vivo growth (32, 40, 46, 111, 128). This review highlights the critical features of the flagellar and chemotactic systems of *Campylobacter* and *Helicobacter* species, with emphasis on those characterized in *C. jejuni* and *H. pylori*, that allow for the regulated construction of polar flagella and the proper chemosensory network to mediate rotational movement of the flagellar nanomachine.

## VISCOSITY-INFLUENCED FLAGELLAR MOTILITY

Owing to possible evolutionary pressures from residing in viscous mucus milieu, *Campylobacter* and *Helicobacter* species demonstrate unusually high velocities and movements in viscous substances compared with many motile bacteria. In the typically studied peritrichous, rodshaped bacteria (such as *Salmonella* species and *Escherichia coli*), highest swimming velocities occur in low to slightly viscous substances, with severe velocity reductions as viscosity increases (31, 41, 102). In contrast, *C. jejuni* and *H. pylori* are helical bacteria with exclusively polar flagella. Many of these bacterial strains demonstrate roughly twice the velocity of rod-shaped bacteria in substances of low viscosity (ranging from  $\sim 22$  to  $40 \mu\text{m s}^{-1}$ ) and can retain these velocities as the viscosity increases 40- to 80fold (31, 41, 60, 102, 122). Proportionally, the high velocity of *C. jejuni* and *H. pylori* in viscous substances is roughly equivalent to a human male of average height swimming in corn oil at  $50 \text{ m s}^{-1}$ !

The unusual motility of *Campylobacter* and *Helicobacter* species in viscous substances is hypothesized to be due to a helical cell shape and the presence of polar flagella. The flagella provide propulsive torque as well as a rotary movement of the cell body; a helical cell shape creates a corkscrew-like rotation, which may allow the bacteria to push against a rigid substance to promote motility in the viscous mucus (8, 31). However, *H. pylori* mutants with less helical cell bodies retain a high swimming velocity in viscous liquids, suggesting that helical shape may not be critical for movement through viscous substances (9, 105). An alternative idea is that the bacteria facilitate movement in mucus by altering the physical nature of the mucus immediately surrounding the organism. Through the activity of its urease, *H. pylori* increases the pH of the mucus, which lowers the viscosity to promote greater movement (13).

*C. jejuni* exhibits two different viscosity-dependent modes of motility in cecal mucosal scrapings from infected mice (65). At low viscosity, *C. jejuni* predominantly swims via rather straight vectorial movements with few direction changes (31, 102). In contrast, at high viscosity a darting motility characterized by oscillation along a relatively short and straight path is observed (102, 108). These different types of motility may be relevant in different

regions of the gastrointestinal mucus layer, as viscosity is thought to increase from the lumen to the epithelial surface. Darting motility may increase the time and number of contacts between *C. jejuni* and the epithelium, thereby augmenting adherence to or invasion of these cells (108).

## REGULATION OF FLAGELLAR GENE EXPRESSION AND BIOSYNTHESIS

### Overview of Flagellar Structure

A fully functional motility system includes the flagellum and the chemosensory system that influences flagellar rotation based on environmental conditions. Approximately 25 to 30 relatively conserved proteins compose a flagellum, with additional factors required for synthesis or secretion of these components. The collection of chemosensory components is less conserved and can involve either a few to dozens of chemoreceptors and signaling proteins in a bacterium. In all, 40 to 100 different proteins are required to form a rotating, chemoresponsive flagellum.

Based on bioinformatics, genetic screens, and functional studies, many proteins of *Campylobacter* and *Helicobacter* species are known to be required for motility, although exact roles for some have not yet been assigned. Combining these studies with information from the well-studied systems of *Salmonella* species and *E. coli* (see References 17, 73, and 77 for extensive reviews), a description of flagellar structure and biosynthesis in *Campylobacter* and *Helicobacter* species follows.

A flagellum can be divided into two broad substructures, the hook-basal body (HBB) complex and the extracellular filament (Figure 1). The HBB complex is further divided into three structures: (a) the base located in the cytoplasm and inner membrane; (b) the periplasmic rod and associated ring structures; and (c) the surface-localized hook.

The flagellar base consists of the MS ring, the flagellar type III secretion system (T3SS), the cytoplasmic C ring or switch complex, and the motor. The inner membrane MS ring is a homomultimer of FliF with a functional flagellar T3SS (composed of FlhA, FlhB, FliO, FliP, FliQ, and FliR) within the central membrane patch of the ring (Figure 1). The flagellar T3SS exports many proteins that are part of the flagellum beyond the inner membrane. The C ring, located at the cytoplasmic face of the MS ring, functions as the flagellar switch and also aids in secretion. This complex is composed of FliG, FliM, and FliN or FliY, although *Epsilonproteobacteria* contain both FliN and FliY (71; discussed below). FliG is responsible largely for the rotary component of the switch, turning the flagellum either clockwise or counterclockwise depending on input from the chemotaxis system. FliM and FliN bind the phosphorylated form of the CheY (CheY-P) chemotaxis response regulator (95). As described below, CheY-P binding to the switch results in clockwise flagellar rotation; without CheY-P bound, counterclockwise rotation occurs. FliN additionally interacts with protein complexes involved in export such as substrate-chaperone partners or with the FliH-FliI complex that assists in efficient export through the T3SS (113). FliY shares homology with FliN and may have a partially redundant function in *H. pylori* (71). Motor components of the base include MotA and MotB, which form a proton channel and the stator component

of the flagellum; these components function to link proton flow and generation of torque for flagellar rotation (77).

Most flagellar proteins located beyond the inner membrane are dependent on the T3SS, the MS ring, and the switch complex for export. The flagellum is made from the inside out, beginning with the MS and switch complex, continuing with the HBB complex, and terminating with the extracellular filament. Ordered secretion of proteins to promote proper flagellar biosynthesis is an inherent property of the T3SS. FliE, FlgB, and FlgC are the first rod proteins secreted, which polymerize on the periplasmic surface of the MS ring to form a hollow tube. Additional nascently secreted flagellar proteins (such as the distal FlgF and FlgG rod proteins) are deposited into this conduit and polymerize on the tip of the sprouting flagellar structure. This pattern of secretion and polymerization on the tip extends throughout the biosynthesis of the hook and filament.

FlgI and FlgH form the P ring in the peptidoglycan and the L ring in the outer membrane, respectively. These rings form around the rod, likely providing support and passage through these cell envelope components. After completion of the rod, FlgE and minor hook proteins are secreted. FliK determines the correct number of FlgE proteins needed to form a proper hook length in *Campylobacter* and *Helicobacter* species (57, 94). As shown in *Salmonella* species (29), FliK likely functions as a temporal molecular ruler to measure the hook length as it is synthesized. FliK is secreted intermittently through the forming HBB complex in a fairly linear fashion. Simultaneous contact of the N and C termini of FliK with FlgD, which is present at the tip of the growing hook, and with FlhB of the T3SS at the flagellar base, signifies that a hook of an ideal length has formed. An autoproteolytic cleavage event then occurs in FlhB that alters the substrate specificity of secretion by the T3SS so that proteins for filament biosynthesis, such as the flagellins, are then exported.

Flagellar biosynthesis terminates with filament biosynthesis. In *C. jejuni* and *H. pylori*, the filament is composed primarily of the major flagellin, FlaA, with the minor flagellin, FlaB, less abundant in the structure (67, 69). Whereas FlaA is essential for flagellation and motility, the requirement of FlaB varies between species (52, 104, 118, 119).

### Regulatory Cascade for Flagellar Gene Expression

Expression of flagellar genes is controlled in a hierarchical manner that allows flagellar proteins to be produced in stages, facilitating their ordered secretion and proper interactions so that flagellar biosynthesis occurs correctly. This regulated mechanism of gene expression is accomplished by linking transcription of classes of flagellar genes to structural steps in flagellar biosynthesis.

In motile bacteria, flagellar genes can be organized into classes on the basis of their temporal order of expression. Class 1 genes are transcribed first and usually encode master transcriptional regulators that control expression of class 2 genes, which include those encoding HBB proteins. Genes for these canonical flagellar master regulators appear to be absent from *C. jejuni* and *H. pylori* (87, 114). Instead, *C. jejuni* and *H. pylori* class 1 genes include those encoding the flagellar T3SS, the FlgSR two-component system (TCS), the FlhF GT-Pase, and  $\sigma^{54}$  (12, 45, 82, 103, 124) (Figure 2). Unlike genes encoding master

regulators in other motile bacteria, expression of class 1 genes in *C. jejuni* and *H. pylori* appears to be constitutive.

Through genetic and biochemical analyses in *C. jejuni*, a regulatory pathway has been proposed for how class 1 proteins likely influence class 2 gene expression through  $\sigma^{54}$  (Figure 2) (5, 45, 56). Many aspects of this model appear to be valid for *H. pylori* (82, 103). In the *C. jejuni* model, two pathways either converge or work sequentially to influence activity of  $\sigma^{54}$  and, consequently, expression of class 2 genes. The first pathway involves communication between the FlgSR TCS and the flagellar T3SS (56). The FlgSR TCS is composed of the cytoplasmic FlgS histidine kinase and the FlgR response regulator, an NtrC-like transcriptional regulator. Members of the NtrC family of regulators commonly activate  $\sigma^{54}$  in bacteria (55, 56, 124). Activation of the FlgSR TCS is dependent on the flagellar T3SS components, as any T3SS mutant appears to disrupt phosphotransfer through FlgSR (56). FlgSR appears to function downstream of the T3SS on the basis of the finding that FlgR mutants that function independently of FlgS suppress the phenotypes of flagellar T3SS mutants for decreased expression of  $\sigma^{54}$ -dependent flagellar genes (56). Because many class 2 proteins are dependent on the T3SS for secretion, the T3SS ultimately controls expression of its own secretion substrates.

It is not yet known how the flagellar T3SS activates FlgSR. Mutations in the T3SS that selectively impair secretion of the FlaA flagellin but not formation of the system do not cause defects in  $\sigma^{54}$ -dependent flagellar gene expression (56). These findings suggest that T3SS formation, rather than its secretory function, activates FlgS. FlgS could possibly detect creation of a T3SS by interacting directly with one or more components of the fully formed secretory system.

The second pathway required for expression of  $\sigma^{54}$ -dependent class 2 genes involves the FlhF GTPase, but the mechanism is unclear. In a GTPase-independent manner, FlhF appears to function in a pathway that is separate from FlgSR and the flagellar T3SS (5) (Figure 2). It is not yet known whether these two pathways converge or whether the FlhF pathway functions downstream of the FlgSR-T3SS pathway to activate  $\sigma^{54}$ .

Similar to other bacterial systems, the *C. jejuni* and *H. pylori*  $\sigma^{28}$  (encoded by *fliA*) is required for expression of class 3 genes such as *flaA* and other filament genes and is negatively controlled by the antisigma factor FlgM (12, 19, 45, 53, 123, 124) (Figure 2). As in the *Salmonella* system, expression of *C. jejuni* and *H. pylori*  $\sigma^{28}$ -dependent class 3 genes is repressed until the HBB is completed because of FlgM (19, 53, 123). Repression is mediated by FlgM binding to  $\sigma^{28}$ , which prevents the sigma factor from associating with RNA polymerase. Once the HBB complex is complete in *C. jejuni* and *H. pylori*, FlgM is secreted by the flagellar T3SS, which relieves  $\sigma^{28}$  from repression and allows it to interact with RNA polymerase to express class 3 genes (92, 123) (Figure 2). FlaA and other filament proteins are produced and secreted through the HBB complex for filament biosynthesis.

Curiously, FlgM-mediated repression of  $\sigma^{28}$  activity in *C. jejuni* is temperature sensitive (123). FlgM interacts with  $\sigma^{28}$  at 37° C but not at 42° C, which allows  $\sigma^{28}$ -dependent class 3 gene expression to occur at 42° C regardless of the status of the HBB complex. It is not yet

clear whether an advantage exists in expressing class 3 filament genes at 42 °C before a HBB is complete. However,  $\sigma^{28}$ -dependent genes encoding proteins with functions not obviously involved in motility have been identified (12, 35, 90). Considering that avian species are natural hosts for *C. jejuni* and have body temperatures around 42° C, these proteins may be expressed via a  $\sigma^{28}$ -dependent, flagellum-independent mechanism to possibly assist in colonization of these hosts.

In other flagellar systems, *mot* genes encoding the stator components of the motor and genes encoding many chemosensory proteins are dependent on  $\sigma^{28}$  for expression (reviewed in Reference 18). Generally, these genes in *C. jejuni* or *H. pylori* are controlled neither by  $\sigma^{54}$  nor by  $\sigma^{28}$ , with the exception of one *H. pylori* chemotaxis gene (53, 101). Expression of these genes appears to be constitutive and outside the flagellar transcriptional cascade, although there is one identified regulator for a chemoreceptor gene (25).

### Phase-Variable Control of Motility

In addition to the complex transcriptional regulatory cascade described above, flagellar gene expression and biosynthesis are also controlled by phase variation in *Campylobacter* and *Helicobacter* species. Phase variation is a random and reversible genetic process that can affect transcription or translation of a target gene. One common mechanism of phase variation involves alteration in the number of nucleotide repeats in the coding sequences or promoters of genes. These alterations may affect the level of transcription of a gene or lead to frameshifting of the coding sequence to truncate translation of the encoded protein. These mechanisms allow transcription or translation to switch reversibly between phase “on” and phase “off” states.

In *Campylobacter coli*, a phase-variable homopolymeric tract of thymines is present in *flhA*, which encodes a flagellar T3SS component (86). Alteration of this polyT tract truncates FlhA to presumably affect both  $\sigma^{28}$ - and  $\sigma^{54}$ -dependent flagellar gene expression and motility. Motility in *C. jejuni* is affected by phase variation of the FlgSR TCS (42, 43). FlgSR phase variation occurs in homopolymeric nucleotide tracts consisting of adenine or thymine residues within the coding sequences of *flgR* or *flgS*, with additional variation of heteropolymeric nucleotide tracts occurring in *flgS* (42, 43). Phase variation of these repeats affect translation of both FlgS and FlgR, providing an additional level of control of  $\sigma^{54}$ -dependent flagellar gene expression outside of the requirement of these proteins for signal transduction to activate  $\sigma^{54}$ . To date, the FlgSR TCS is unique in that both regulatory proteins are controlled by phase-variable mechanisms. In *H. pylori*, phase variation of a homopolymeric tract of cytosines in *fliP* leads to a truncated T3SS component that does not alter expression of class 3 genes but prevents flagellar biosynthesis (51).

### Posttranscriptional and Posttranslational Mechanisms Affecting Flagellar Gene Expression and Biosynthesis

Both posttranscriptional and posttranslational regulatory mechanisms influence flagellar biosynthesis in *C. jejuni* and *H. pylori*.



**HP0958.**—HP0958 is a cytoplasmic protein of *H. pylori* that binds both protein and mRNA substrates to influence transcription or translation of different flagellar components. HP0958 interacts with  $\sigma^{54}$ , FliH (a regulator of the FliI ATPase that assists in dissociating flagellar proteins from chaperones for secretion), and *flaA* mRNA (27, 88, 91, 93). Binding *flaA* mRNA promotes translation of the major flagellin, whereas interactions with  $\sigma^{54}$  likely increase stability of the sigma factor (11, 88). An interesting hypothesis has been proposed that after completion of the HBB complex, newly transcribed class 3 *flaA* mRNAs may compete with  $\sigma^{54}$  for binding to HP0958. As a result, *flaA* mRNAs are translated with a concomitant decrease in  $\sigma^{54}$  levels due to instability after release from HP0958. Thus, a reduction in  $\sigma^{54}$  levels would result in decreased class 2 gene expression (27). If true, this mechanism would provide a possible explanation for cessation of class 2 flagellar gene expression after completion of the HBB complex in *H. pylori*.

**Flagellin glycosylation influences flagellar biosynthesis.**—*Campylobacter* and *Helicobacter* flagellins must be glycosylated posttranslationally for flagellar filament biosynthesis (26, 36, 38, 54, 97). FlaA flagellins of both species and the *H. pylori* FlaB flagellin are modified by an O-linked protein glycosylation system, which adds a glycan to specific serines or threonines of the flagellins (96, 97, 112). Pseudaminic acid, legionaminic acid, and modified versions of each glycan are the major glycan additions to the *Campylobacter* flagellins (70, 76, 96, 97, 112). In contrast, flagellin glycosylation in *H. pylori* is fairly homogeneous, with only pseudaminic acid modifying the proteins (97).

Flagellin glycosylation in *Campylobacter* and *Helicobacter* spp. contributes approximately 4%–10% of the protein mass (96, 97, 112). Sixteen to 19 different serines or threonines of *C. jejuni* FlaA and 7 serines or threonines of *H. pylori* FlaA are glycosylated (97, 112). These glycosylated residues are limited to the D2 and D3 subdomains of the flagellins, which in *Salmonella* species are the surface-exposed regions of the folded filament-incorporated flagellins (97, 112, 129). Curiously, only three specific residues of *C. jejuni* FlaA need to be glycosylated for normal filament biosynthesis and motility (30).

Although the function is not entirely clear, flagellin glycosylation likely provides additional subunit-subunit interactions for stacking the flagellins during filament biosynthesis. The *Campylobacter* and *Helicobacter* flagellins lack the D1 subdomain, which in the prototypical *Salmonella* flagellin aids in flagellin subunit interactions during filament biosynthesis (4, 129). Glycosylation of the *Campylobacter* and *Helicobacter* flagellins may compensate for the lack of the D1 domain and provide structural alterations to the flagellins to promote filament biosynthesis.

## SPATIAL AND NUMERICAL CONTROL OF POLAR FLAGELLAR BIOSYNTHESIS

Unlike peritrichous bacteria, flagellar biosynthesis in polarly flagellated bacteria is spatially and numerically restricted. *Campylobacter* species commonly produce a single flagellum at one or both bacterial poles, and *H. pylori* normally produces up to six flagella at a single pole. The FliH FliG GTPase and FliH (annotated as FleN in *Pseudomonas* species) influence spatial and numerical parameters of flagellar biosynthesis in other polarly flagellated

bacteria (20, 37, 63, 81, 85). In *Vibrio cholerae flhF* mutants, *ff*<sup>54</sup>-dependent flagellar gene expression is reduced, with only a few bacteria able to synthesize a flagellum; these flagella form at lateral sites on the cell body rather than at polar sites (20, 37). *Pseudomonas flhF* mutants also produce lateral rather than polar flagella, indicating that FlhF is involved in spatial control of flagellar biosynthesis (81, 85). FlhG/FlhN of both *Vibrio* and *Pseudomonas* species regulate flagellar numbers by repressing the expression or activity of respective master transcriptional regulators that control flagellar gene expression (20, 23, 24). Increased flagellar gene expression in *flhG/flhN* mutants likely drives an increase in polar flagellar numbers. FlhG is encoded in *Campylobacter* and *Helicobacter* species but has not been well characterized in either bacterium.

The FlhF GTPases of *C. jejuni* and *H. pylori* are required for flagellar gene expression and biosynthesis, but each mechanism differs by the dependency on the GTPase activity of FlhF (5, 45, 82). As described above, *C. jejuni* FlhF is required for expression of *ff*<sup>54</sup>-dependent flagellar genes in a GTPase-independent manner. However, *C. jejuni* strains producing FlhF mutant proteins locked in a GTP-bound state (and with reduced GTP hydrolysis) generate populations of bacteria with diverse flagellar biosynthesis phenotypes, including production of multiple flagella at a single pole or flagella at lateral sites along the cell body (5). Thus, GTP hydrolysis is not required for flagellar gene expression but is required for controlling proper spatial and numerical parameters of flagellar biosynthesis.

The mechanism by which the GTPase activity of FlhF influences flagellar biosynthesis is currently unknown. One hypothesis for how FlhF may regulate spatial parameters of flagellar biosynthesis is that the protein may function to correctly position components of the flagellar base to the poles. Division of a *C. jejuni* cell with a flagellum at each pole results in daughter cells without a flagellum at the new pole. FlhF, through its GTPase activity, may specifically recognize newpoles of a daughter cells and assist in the synthesis of the MS ring, T3SS, and switch complex at the new pole. Placement of these initial components dictates where a flagella will be formed. In support of this hypothesis, FlhF is unipolarly localized in *C. jejuni*, but it is unclear if these poles are new or old poles after division (30). Continued exploration is required for understanding how the GTPase activity of FlhF is regulated and how this activity functions to precisely place a single flagellum only at poles.

## A SHEATH ENCASES *HELICOBACTER* FLAGELLA

Each flagella of *Helicobacter* species is encased in a membranous sheath, a relatively atypical property of bacterial flagella (33, 34). The sheaths are contiguous with the outer membrane, but the mechanism of sheath biosynthesis is unknown. Mutations in flagellin or filament genes that reduce filament biosynthesis generally result in shorter sheaths equal to the length of the filament, suggesting that the sheath may form around the filament as it is synthesized (52). However, some mutants occasionally produce what were considered empty sheaths lacking internal flagella or short filaments with empty sheath extensions (52, 61). If the sheaths are indeed empty, it is unclear how a membrane would spontaneously form a sheath-like structure without an internal proteinaceous protrusion. In terms of function, one possibility is that the sheath prevents recognition by host antibodies or innate immune



receptors, a suggestion that is supported by the observation that flagellin-specific antibodies do not bind to *H. pylori* flagellins in sheaths (62). However, some nonpathogenic bacteria have sheathed flagella, indicating that the sheath likely has functions outside of promoting immune evasion or persistence in a host.

## CHEMOTAXIS

### Overview of Chemotaxis

Chemotactic signaling systems allow bacteria to follow favorable chemical gradients (117). The *E. coli* system represents the paradigm for chemotaxis, utilizing chemoreceptors to sense particular environmental cues and transmit this information to a signal transduction cascade that affects flagellar rotation (reviewed in Reference 117) (Figure 3). The core signal transduction proteins include chemoreceptors, the CheA kinase, the CheY response regulator, and the CheW coupling protein, which physically couples receptors to CheA (126). The direction of flagellar rotation is influenced by the phosphorylation status of CheY. The amount of CheY-P is regulated by ligand binding to the chemoreceptors, such that an attractant ligand squelches CheA kinase activity and non-phosphorylated CheY predominates. In this form, CheY fails to interact with the flagellar switch, the flagella rotate counterclockwise, and the bacteria swim. In the absence of attractant ligand, CheA phosphorylates CheY. CheY-P binds the flagellar switch to turn the flagellum clockwise, which promotes bacterial tumbling and direction changes.

Several chemotaxis proteins outside of the core chemotaxis set are commonly found in many but not all motile bacteria (126). The first set are phosphatases, such as CheZ and FliY, that accelerate the intrinsic dephosphorylation activity of CheY. The second set are the CheR and CheB enzymes that methylate and demethylate, respectively, chemoreceptor glutamyl residues to control adaptation responses to sustained ligand levels. The third set are the alternative coupling proteins such as CheV, which are homologs of CheW proteins with an additional receiver (REC) domain. *H. pylori* and *C. jejuni* collectively contain all of the above chemotaxis proteins and respond chemotactically to several different compounds (see sidebar, Chemotactic Signals for *H. pylori* and *C. jejuni*).

### Chemotaxis During Infections

The role of chemotaxis in infection has been dissected using mutants that are Che<sup>-</sup> due to loss of core signal transduction proteins or mutants that lack individual chemoreceptors. Generally speaking, nonmotile mutants demonstrate the most severe in vivo growth defects, followed by Che<sup>-</sup> mutants, and lastly mutants lacking a particular chemoreceptor (3, 83, 111). This trend has been most well studied in *H. pylori*; in contrast, *C. jejuni* Che<sup>-</sup> or nonmotile mutants demonstrate similar colonization defects in hosts, with chemoreceptor mutants displaying more subtle defects (16, 40, 46, 84). In *H. pylori*, motility and chemotaxis play key roles in infection establishment. For example, *H. pylori* Che<sup>-</sup> mutants have a 50% infectious dose (ID<sub>50</sub>) 250-fold higher than wild-type bacteria, and isogenic nonmotile mutants have an ID<sub>50</sub> 25,000-fold higher than wild-type strains (83, 111). In some studies, *H. pylori* Che<sup>-</sup> mutants do not colonize stomachs of hosts (32, 75). This outcome may be due to differences in infectious dose between different rodent species and strains,

such that infectious doses were not adequate to achieve colonization by Che<sup>-</sup> mutants. The colonization defects of *H. pylori* chemotaxis and motility mutants are exacerbated greatly in a competitive infection with wild-type strains; typically in these coinfections little or no mutant is recovered (83, 111).

It has been more difficult to assess whether chemotaxis plays a role in persistent infection. At a high dose, *H. pylori* Che<sup>-</sup> mutants establish colonization and persist for months similar to wild-type strains (111, 120). Che<sup>-</sup> mutants are found at roughly fivefold-lower levels than wild-type bacteria during the first month of infection, but the numbers of wild-type bacteria and Che<sup>-</sup> mutants equalize over time (111, 120). In addition, wild-type *H. pylori* can displace some Che<sup>-</sup> mutants from a preestablished infection (111). When two strains of equal colonization capacity are used in such a superinfection strategy, the first strain colonizing is not affected by the second strain (111). The finding that Che<sup>-</sup> mutants can be displaced from a persistent infection by wild-type bacteria suggests that chemotactic motility is beneficial in a persistent infection.

Chemotaxis furthermore influences *H. pylori* localization within the stomach. First, wild-type bacteria occupy both the corpus (acid-producing) and antrum regions of the stomach, whereas Che<sup>-</sup> mutants are restricted to the corpus (111). This finding suggests that chemotaxis either guides *H. pylori* to the antrum or helps it survive in this niche. Chemotaxis also promotes closer contact of *H. pylori* with the gastric epithelial cells (120). *H. pylori* Che<sup>-</sup> mutants elicit less host inflammation in mouse and gerbil models, possibly because of the observed decrease in host cell interactions as *H. pylori* adherence enhances the inflammatory response (39, 75, 120).

### ***C. jejuni* and *H. pylori* Contain Multiple Chemoreceptors**

In *H. pylori* and *C. jejuni*, chemotactic signals are sensed by chemoreceptors that exist as either integral membrane or soluble cytoplasmic proteins. Chemoreceptors are multidomain proteins, bearing a sensory domain and a signaling domain (125). The signaling domain, also called the methyl-accepting (MA) domain, is highly conserved and interacts with either the CheW or the CheV coupling proteins (Figure 3). The chemoreceptor sensory domains are highly variable, residing extracytoplasmically in transmembrane chemoreceptors, but cytoplasmically in soluble ones (125). Sensory domains can sense ligands directly or indirectly via a protein-protein interaction with a ligand-bound periplasmic binding protein. It is not possible to predict the ligand for a given receptor or whether the receptor even binds a ligand directly (125). Several chemoreceptor sensory domains are well characterized (117), although none of the chemoreceptor sensory domains of the *Epsilonproteobacteria* is characterized to date.

*H. pylori* has three integral membrane chemoreceptors, TlpA, TlpB, and TlpC, and one soluble chemoreceptor, TlpD (3, 22, 99, 120). TlpA, TlpB, and TlpD have specific responses ascribed to them. TlpA has been proposed to sense arginine (15). TlpB mediates a response to low pH in a noncanonical chemotaxis assay (22). In this assay, wild-type *H. pylori* displays arcing swimming behavior and forms a wall of bacteria in response to a drop of 0.1 N HCl introduced under a coverslip; mutants lacking *tlpB* do not display this behavior. Movement away from low pH has been noted for many bacterial species and suggested to

account for the observed localization of *H. pylori* within the stomach (98, 100). It will be exciting to see if additional pH taxis assays support these findings, as well as to determine the mechanistic details for TlpB-dependent pH sensing.

The soluble chemoreceptor TlpD mediates a tactic response to bacterial energy levels (99). Specifically, treating *H. pylori* with chemicals that disrupt electron transport and deplete cellular ATP levels causes the bacteria to swim without direction changes, a response that matches the predicted attractant response extrapolated from mutant phenotypes (72, 110). Adding back nutrients or electron donors boosted the frequency of directional switches for wild-type *H. pylori* but not for strains lacking *tlpD* or *cheA*. Further experiments showed that using 10-fold-lower concentrations of one of the electron transport disruption chemicals triggered a repellent response and prevented wild-type *H. pylori* from migrating into a compartment containing this chemical (99). These results thus suggest that TlpD senses something about cellular energy status. Future studies to examine how TlpD senses these cellular alterations, and the exact nature of what is altered, are exciting avenues for future work.

Three of the four *H. pylori* chemoreceptors promote stomach colonization in wild-type animal models. *tlpA*, *tlpC*, or *tlpD* mutants demonstrate colonization defects in competition studies with wild-type strains, although they colonize mice normally when any is the sole infecting bacterium (3, 22, 120; K.M. Ottemann, unpublished data). The role of TlpB in colonization has been difficult to interpret. Two studies report that *H. pylori tlpB* mutants have no colonization defect in either mice or gerbils and actually slightly outcompete their wild-type parental strain (75, 120). One study, however, found that *H. pylori tlpB* mutants have a defect in single-strain colonization assays in transgenic IL-12-deficient mice, which are typically more permissive for colonization (22, 47). Future experiments that analyze the phenotype of Che<sup>-</sup> mutants in this model would help scientists to understand the reported *tlpB* mutant defect.

*C. jejuni* contains seven integral membrane chemoreceptors and three soluble chemoreceptors. Three of these have been paired to input signals. The first is the chemoreceptor composed of the CetA and CetB proteins (44). CetA bears the MA domain plus a transmembrane domain, and CetB is a cytoplasmic protein with similarity to a critical region of the Aer oxygen-sensing chemoreceptor. These two proteins presumably function together and trigger a response to pyruvate, a respiratory electron donor, and fumarate, a respiratory electron acceptor (28, 44). This observation coupled with homology of CetA to domains of other energy-sensing chemoreceptors suggests that this system responds to some aspect of respiratory electron flow. The chemoreceptor Tlp7 is also encoded by two genes (*cj0952c* and *cj0951c*) and senses formate (109). However, it is unclear if Cj0952c and Cj0951c function together to sense this compound. The other signal-paired chemoreceptor is the integral membrane protein Tlp1, which senses aspartate directly as a chemoattractant (40).

Several of the *C. jejuni* chemoreceptors are required for in vivo colonization. Mutant strains lacking one of two chemoreceptors, Cj0019c/DocB or Cj0262c, resulted in lower numbers of *C. jejuni* in the chick ceca, while removal of the other chemoreceptors had no effect on

colonization (46). Later work found that *Cj1506c/tlp1* mutants in a different *C. jejuni* strain had a defect in chick intestinal colonization, suggesting that the importance of chemoreceptors in colonization may vary with strains (40).

### **Core Signaling Proteins: CheA, CheW, and CheY**

Chemoreceptors communicate their ligand binding information to the flagellar motor via the chemotaxis system, utilizing the core signal transduction proteins CheA, CheW, and CheY. Discussion of each protein in *Campylobacter* and *Helicobacter* species is presented below.

***Campylobacter* and *Helicobacter* species contain CheA kinases with additional REC domains.**—CheA plays a pivotal role in chemotaxis by promoting CheY phosphorylation (Figure 3). Both *C. jejuni* and *H. pylori cheA* mutants are nonchemotactic in vitro and show defects in colonization of mice, confirming a role for CheA in chemotaxis and also for chemotaxis in infection (16, 32, 111).

CheA proteins of *C. jejuni* and *H. pylori* differ somewhat from canonical CheA proteins primarily in two ways. First, *C. jejuni* and *H. pylori* CheA proteins have the typical domains that autophosphorylate, function in phosphorelay to downstream targets, and bind CheW, but these CheA proteins lack sequence conservation in the P2 CheY binding domain (125). Despite the lack of conservation in this region, the *H. pylori* CheA P2 portion does bind CheY and even prefers it to other *H. pylori* REC-domain-containing chemotaxis proteins such as CheA or CheV (64). Across the *Epsilonproteobacteria*, the P2 region generally is not well conserved. This lack of conservation is perhaps not surprising because the P2 domain of *E. coli* CheA can be removed with minimal slowing of phosphorelay to substrates and only modest decreases in chemotaxis (49). The variability of this region suggests that the rate of phosphotransfer from CheA to CheY might differ between strains.

The second modification to *Campylobacter* and *Helicobacter* CheA proteins is the addition of a REC domain in their C termini (32, 87, 74). REC domains are found in TCS sensor proteins and are typically phosphorylated to allow phosphorylation-based control of themselves or proteins to which they are attached. Many bacteria as well as most members of the *Epsilonproteobacteria* have CheA with an added REC domain, called CheAY. Wuichet et al. (125) observed that CheA proteins that lack a well-conserved P2 domain are typically CheAY proteins. It is not yet known, however, what role this REC domain plays in the function of any CheA. Several experiments in *H. pylori* have shown that CheA can phosphorylate the CheA REC domain but CheY is the preferred substrate (50). In addition, CheY can retrophosphorylate CheA and the phosphoryl group is transferred to the CheAREC domain (50). One possible function of the CheA REC domain is to act to remove phosphoryl groups from CheY, as a so-called phosphate sink, although such a model has not been proven.

**The coupling protein CheW and response regulatory CheY.**—CheW is a coupling or scaffolding protein that links chemoreceptors with CheA to modulate the kinase activity of CheA (Figure 3). CheW is essential for chemotaxis as *C. jejuni* and *H. pylori cheW* mutants are Che<sup>-</sup> presumably because CheA is not coupled to the receptors and is not active (89, 111, 115). Consistent with their Che<sup>-</sup> nature, *H. pylori cheW* mutants have decreased

ability to colonize mice (111). Many critical residues are conserved between well-studied CheW proteins and those of *H. pylori* and *C. jejuni*, suggesting preservation of CheW function (1, 72).

CheY-P plays a key role in the chemotaxis system by binding to the flagellar switch proteins, FliM and FliN, and causing the motor to rotate in the clockwise, or nondefault, direction, which spurs direction changes (95, 117). As in model systems, *H. pylori* and *C. jejuni cheY* null mutants are Che<sup>-</sup> and colonize animals poorly (7, 32, 75, 84, 111).

*C. jejuni* and *H. pylori* CheY proteins are highly homologous to each other and maintain the conserved phosphorylation site at Asp53. As expected, *H. pylori* CheY can be phosphorylated and it interacts with FliM in a phosphorylation-dependent manner (50, 71). The crystal structure of *H. pylori* CheY-P shows that it has the basic structure of a typical REC domain, although it differs somewhat from *E. coli* CheY (64). These results suggest that *H. pylori* and *E. coli* CheY proteins may interact with their respective FliM proteins differently. This possibility was supported by the inability of *H. pylori* CheY to complement an *E. coli cheY* null mutant even though *H. pylori* CheY is phosphorylated by *E. coli* CheA (64). However, the functional consequence of a possibly different CheY-FliM interaction remains to be established.

### Accessory Chemotaxis Proteins

The function of the core chemotaxis proteins may be augmented by additional proteins that are not found in all chemotactic bacteria (126). These proteins fall into three classes: CheY-P phosphatases, adaptation-related CheR methyltransferases and CheB methylesterases, and CheV CheW-REC hybrid coupling proteins.

**CheY-P phosphatases: CheZ and FliY.**—CheY dephosphorylation is critical for efficiently controlling the direction of flagellar rotation to promote chemotaxis. Bacteria utilize several types of phosphatases for this reaction (107). *H. pylori* and *C. jejuni* produce the CheZ and FliY phosphatases which dephosphorylate CheY-P by enhancing the endogenous CheY autodephosphorylation activity (107, 130) (Figure 3). *H. pylori* and *C. jejuni* CheZ proteins are very distant from other CheZ proteins and only conserve residues corresponding to the active site and CheY-P binding regions (66, 110).

Biochemical analysis showed that the putative *H. pylori* CheZ (HP0170, CheZ<sub>HP</sub>) employs the same mechanism as *E. coli* CheZ to de-phosphorylate CheY-P, including both a similar active site and a C-terminal binding region (66). CheZ<sub>HP</sub> unexpectedly has an expanded substrate range and is able to dephosphorylate CheV2 and the REC domain of CheA. This CheZ is the first to have substrates identified beyond CheY, which suggests it can affect phosphate flow at multiple levels (66).

*H. pylori* and *C. jejuni* also produce the FliY phosphatase (71, 107). FliY is a component of the flagellar switch, along with FliM, FliG, and FliN. Bacteria typically encode only three flagellar switch proteins (FliM, FliG, and either FliY or FliN), but a common feature of *Epsilonproteobacteria* is that they encode all four, as discussed below (71). The well-studied *B. sub-tilis* FliY contains a CheY-P binding sequence and has phosphatase activity toward

CheY-P (106). Neither *C. jejuni* nor *H. pylori* FliY contains an apparent CheY-P binding domain, but the FliY proteins harbor the active site consensus sequence of the CheC phosphatase family ((D/S-X<sub>3</sub>-E-X<sub>2</sub>-N-X<sub>22</sub>-P) (71, 80; P. Lertsethtakarn & K.M. Ottemann, unpublished data). It is unknown whether these proteins have phosphatase activity. Analysis of *fliY* mutants for in vivo phosphatase activity is complicated by the fact that *H. pylori fliY* mutants are nonmotile, therefore preventing analysis of chemotaxis phenotypes (71).

**Methylation adaptation system proteins: CheB and CheR.**—Chemotaxis systems respond to a wide range of chemical concentrations that extend from nanomolar to millimolar amounts because of a process known as adaptation (10). Chemotaxis adaptation is typically performed by the CheB methyl-esterase and the CheR methyltransferase. Another adaptation mechanism involves CheV, hybrids of CheW, and a REC domain (discussed below). Most of the *Epsilonproteobacteria* (other than *H. pylori*) encode CheB, CheR, and CheV; *H. pylori* lacks CheB and CheR and instead has three CheV proteins (Figure 3).

CheB and CheR are functionally antagonistic, demethylating and methylating chemoreceptors as part of the adaptation process. CheR in *Epsilonproteobacteria* contains the canonical CheR domain and thus is predicted to function similarly to other studied CheR proteins (125). CheB proteins typically contain both a CheB methyl-esterase domain and a REC domain that contributes phosphorylation-mediated control of the demethylation activity. CheB in *C. jejuni* and *Helicobacter* species, however, lacks the REC domain, suggesting it uses a different regulatory mechanism (74, 59). Recent studies have shown that these proteins function as expected to methylate/demethylate at least DocC and Tlp1, but apparently not CetA (59).

**CheV coupling proteins.**—CheV has been best studied in *B. subtilis*, where it has a dual function as a CheW-like coupling protein and in mediating adaptation to attractants (1). As noted above, *H. pylori* has three CheV proteins, CheV1, CheV2, and CheV3, whereas other *Epsilonproteobacteria* have only one protein, simply annotated as CheV, and it is most closely related to *H. pylori* CheV3 (Figure 3). All three CheV proteins of *H. pylori* have roles in chemotaxis but their exact functions have not been determined (72, 89). *H. pylori cheV1* mutants have the most severe chemotaxis phenotype, although they are not as impaired as Che<sup>-</sup> strains, whereas mutants lacking *cheV2* or *cheV3* show only very modest chemotaxis defects. *cheV1* and *cheV2* mutant strains display predominantly smoothswimming behavior akin to *cheW*, *cheY*, or *cheA* mutants; *cheV3* mutants have opposite hyperswitching behavior (72). *C. jejuni cheV* mutants have a chemotaxis defect similar to that of a *cheW* mutant, suggesting CheV plays a role equal to that of CheW in this microbe (40).

CheV phosphorylation is required for adaptation in *B. subtilis*, but the phosphorylation status of *Campylobacter* and *Helicobacter* CheV proteins is not yet clear (1). *H. pylori* CheV proteins catalyze the removal of phosphate from CheA, but stable phosphorylated CheV proteins have not been detected, suggesting possibly that CheV-P has a very short half-life (50, 66). Alternative methods using smallmolecule phosphodonors suggest that CheV proteins are poorly phosphorylated, so the phosphorylation status of these proteins in *Epsilonproteobacteria* is not yet clear (66, 89).



It is not yet known in general whether particular chemoreceptors prefer CheV or CheW for CheA coupling (1). The *C. jejuni* chemoreceptor Tlp1/Cj1506 interacted more strongly with CheV than with CheW, supporting the importance of CheV in this system and possibly suggesting some chemoreceptors preferentially interact with CheV versus CheW (40).

### Flagellar Switch Proteins: FliG, FliM, FliN, and FliY

The signal from the chemoreceptor complex is relayed to the flagellar motor via the switch complex that consists of FliG, FliM, and FliN (77). As noted above, *C. jejuni*, *H. pylori*, and all other *Epsilonproteobacteria* encode an additional switch protein, FliY, which is homologous to FliN at its C terminus (71). Mutation of any one of these genes in *H. pylori* results in nonmotile strains. *H. pylori* *fliG* or *fliM* mutants are aflagellated, whereas *fliN* or *fliY* mutants retain partial flagellation (2, 71). *fliN* and *fliY* appear to be redundant in *H. pylori*, as double *fliN/fliY* mutants are aflagellated (71). Although the role of each protein is not yet fully known in *C. jejuni* or *H. pylori*, it seems that all four switch proteins are essential for full flagellation and motility (71).

In the *E. coli* system, CheY-P binds to FliM and FliN to control rotational direction of the flagellar rotor FliG (95, 117). *H. pylori* CheY-P seems to interact similarly with FliM, but interactions with FliN have not yet been tested (71). One spontaneous *fliM* mutation has been analyzed, with results supporting the finding that *H. pylori* FliM functions similarly to that of *E. coli* (71).

## CONCLUDING REMARKS

Understanding mechanisms of flagellar gene expression, flagellar biosynthesis, and chemotactic responses in *Campylobacter* and *Helicobacter* species is still in the relatively early stages of scientific exploration. However, within this relatively short time frame, significant advances have been made in these species to identify key mechanisms that have so far shown noteworthy significant deviations from paradigmatic models of *E. coli* and *Salmonella* species. Thus, continued exploration into regulatory mechanisms of flagellar gene transcription and biosynthesis and the chemotactic signaling systems of *C. jejuni* and *H. pylori* will undoubtedly open new paradigms of how bacteria achieve a functional, chemotactic rotary nanomachine to promote swimming behavior necessary for infectious processes.

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### CHEMOTACTIC SIGNALS FOR *H. PYLORI* AND *C. JEJUNI*

*H. pylori* and *C. jejuni* respond chemotactically to several compounds. Recent findings suggest that assays that work well for analyzing chemotaxis in *E. coli* are not conducive to other bacteria (58, 68). Specifically, the chemical-in-plug assay gives falsepositive responses for both *H. pylori* and *C. jejuni* (58, 68). In addition, *H. pylori* is not motile in buffers that lack complex nutrients (K.M. Ottemann, unpublished data), although several studies utilize such solutions. With these caveats in mind, reported attractants and repellents are noted below.

*H. pylori* responds to urea (15, 78, 121), bicarbonate (15, 78), arginine and several other amino acids (15, 121), and cholesterol (127) as attractants. *H. pylori* is repelled by low pH (22) and NiCl<sub>2</sub> (15); alterations in proton motive force trigger both attractant and repellent responses (99).

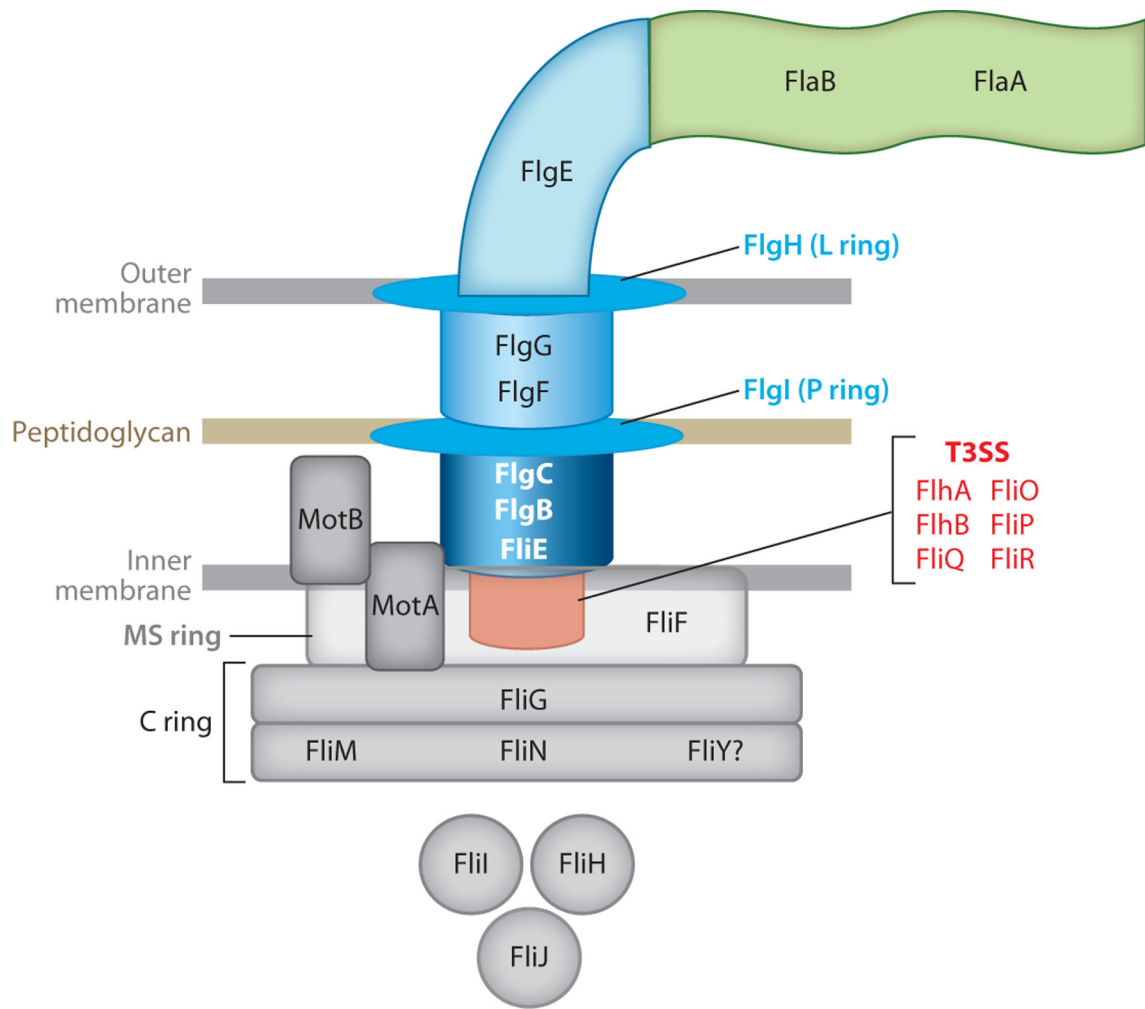
*C. jejuni* was initially reported to be attracted to multiple amino acids, carbohydrates, and organic acids (48). Several of these responses, however, were subsequently found to occur in nonchemotactic mutants and thus represent false-positive responses (58). Several compounds have withstood additional chemotaxis assays, however, with fucose, pyruvate, fumarate, aspartate, and formate being attractants for *C. jejuni* (40, 44, 48, 109, 116).

### SUMMARY POINTS

1. Relative to many other motile bacteria, *C. jejuni* and *H. pylori* are capable of a characteristically high velocity of flagellar motility in substances of moderately high viscosity.
2. A transcriptional regulatory cascade involving the flagellar T3SS, the FlgSR TCS, the FlhF GTPase, and  $\sigma_{54}$  is essential for expression of many genes required to form the flagellar HBB complex in *C. jejuni* and *H. pylori*.
3. Highly regulated mechanisms exist to tightly control the spatial and numerical parameters of flagellar biosynthesis in the polarly flagellated *Campylobacter* and *Helicobacter* species.
4. *C. jejuni* and *H. pylori* contain the basic core chemotaxis components—chemoreceptors, CheA, CheW, and CheY—that all microbes have. These appear to function similarly to orthologs in model systems, although CheA in particular has some atypical modifications.
5. The chemotaxis system of *H. pylori* is unusual in that it contains no methylation-based adaptation systems and produces multiple copies of the CheV coupling/adaptation protein.
6. Chemoreceptors of *C. jejuni* and *H. pylori* contain nonconserved ligand binding domains, and only Tlp1 of *C. jejuni* has been shown to bind a particular ligand directly.

### FUTURE ISSUES

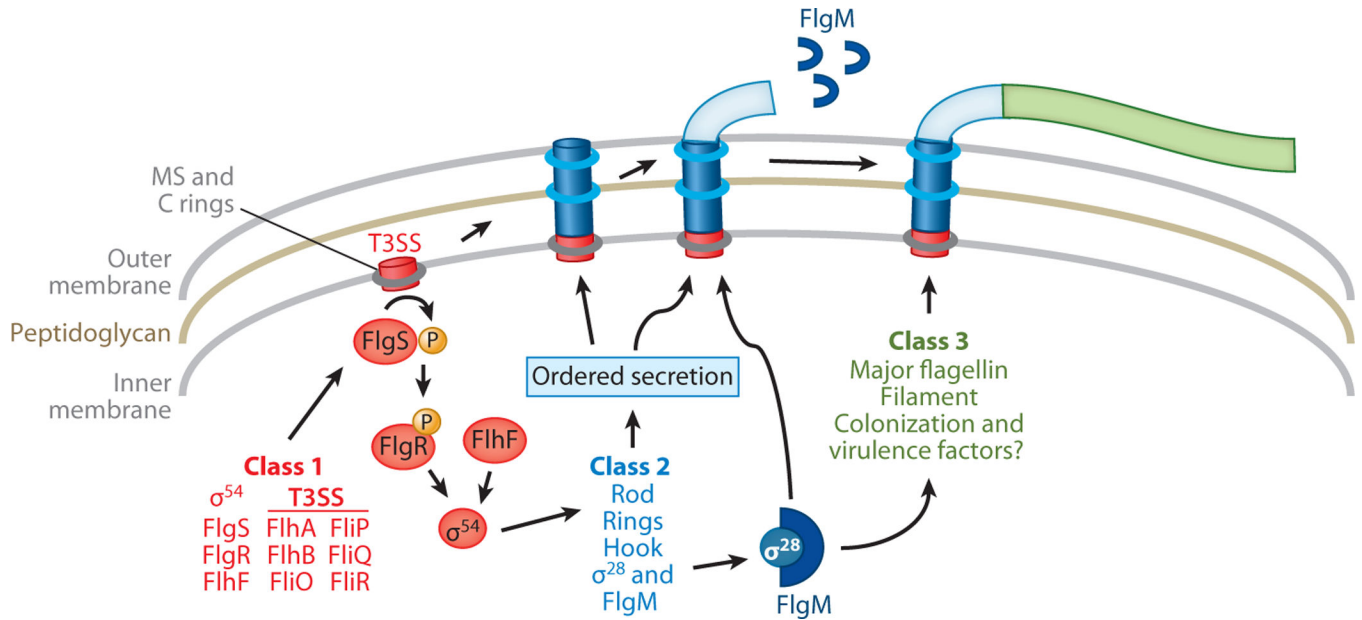
1. How do the flagellar T3SS and the FlgS sensor kinase communicate to initiate phosphotransfer to FlgR for full activation of  $\sigma_{54}$ -dependent flagellar gene expression?
2. How does the GTPase activity of FlhF contribute to regulating the spatial and numerical parameters of flagellar biosynthesis?
3. Are other factors such as FlhG involved in influencing parameters of polar flagellar biosynthesis?
4. How does the flagellar sheath form around each filament in *H. pylori*?
5. What ligands are sensed by each chemoreceptor and are these direct or indirect?
6. Does *H. pylori* adapt to sustained ligand, and if so, are the CheV proteins involved?
7. Do motility and/or chemotaxis serve functions in vivo beyond nutrient acquisition, such as immune avoidance or transmission?



**Figure 1.**

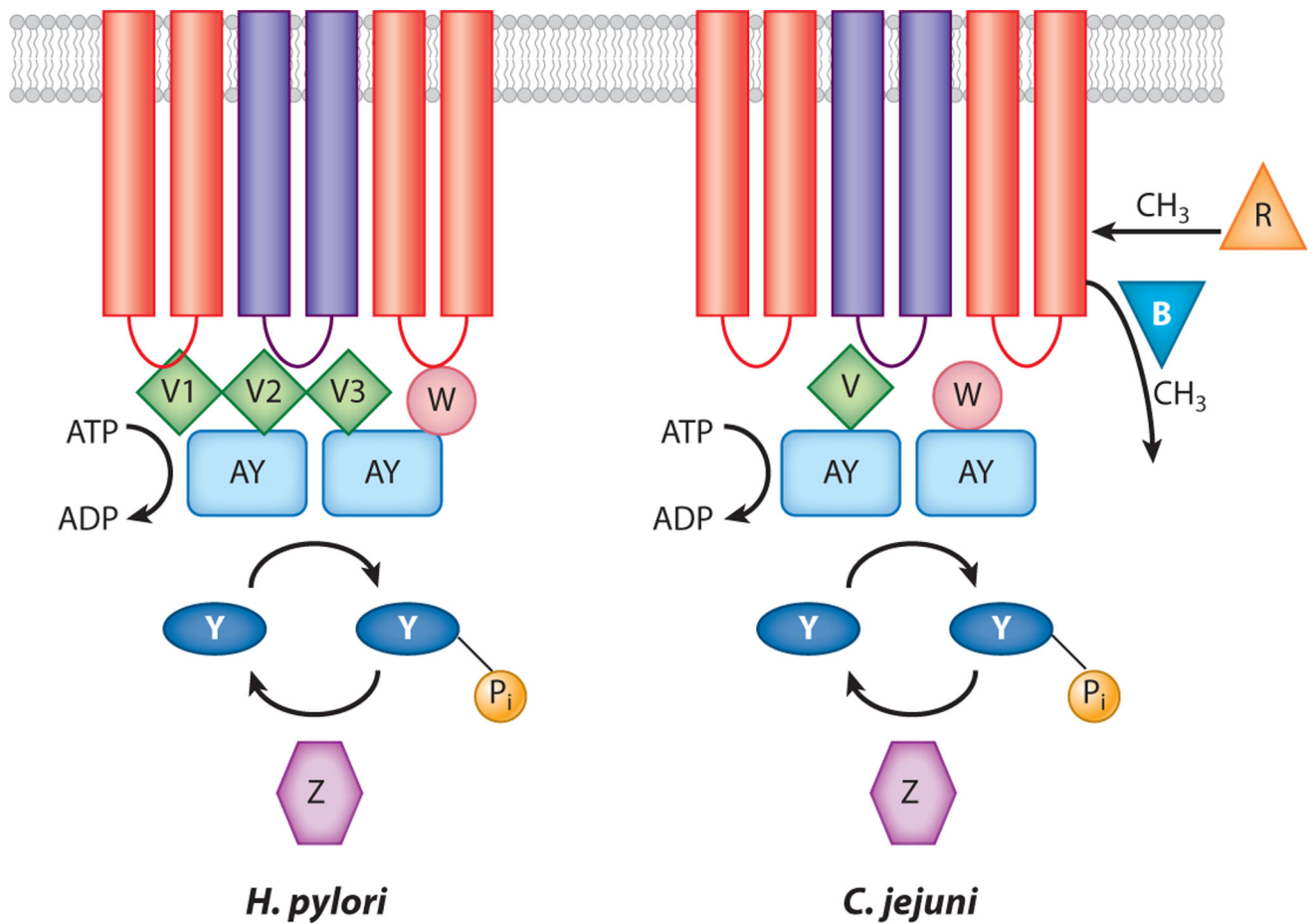
Structure of the flagellar organelle of *Campylobacter jejuni* and *Helicobacter pylori*. The major components common to the flagella of *C. jejuni* and *H. pylori* are shown. Other relatively less-characterized proteins specific to each bacterium are not shown for simplicity. Flagellar components are color-coded based on the classification of the respective genes in the transcriptional regulatory cascade for the organisms: red (class 1), blue (class 2), and green (class 3). An exception includes *flaB*, which is a  $\sigma^{54}$ -dependent class 2 gene. Proteins in gray include those encoded by genes whose transcription has not been analyzed or is known to be outside the flagellar transcriptional hierarchy. Other class 1 proteins that are required for expression of  $\sigma^{54}$ -dependent class 2 genes but are not part of the flagellar structure are not shown. Abbreviation: T3SS, type III secretion system.





**Figure 2.**

The flagellar transcriptional regulatory cascade for *Campylobacter jejuni* and *Helicobacter pylori*. The flagellar genes and respective proteins included in the flagellar transcriptional cascade are shown. These factors are color-coded on the basis of their classification in the transcriptional regulatory cascade: red (class 1 genes), blue (class 2 genes), and green (class 3 genes). *fliA* (encoding  $\sigma^{28}$ ) and *flgM* are included as class 2 genes in *C. jejuni*, but these genes are dependent only partially on  $\sigma^{54}$  for expression. Formation of the flagellar T3SS (which is composed of FlhA, FlhB, FliO, FliP, FliQ, and FliR) has been proposed to create a signal detected by the FlgS sensor kinase, resulting in autophosphorylation of the protein. Phosphotransfer to the FlgR response regulator activates the protein, allowing for interactions with and stimulation of  $\sigma^{54}$ . The FlhF GTPase is likely involved in a separate pathway that converges with or functions downstream of the FlgSR-T3SS pathway to fully activate expression of  $\sigma^{54}$ -dependent class 2 genes. The T3SS facilitates the ordered secretion of the class 2 rod, ring, and hook proteins to complete the HBB complex. Secretion of the antisigma factor FlgM through the HBB complex relieves repression of  $\sigma^{28}$  activity, resulting in expression of class 3 genes. The class 3 genes include *flaA*, which encodes the major flagellin, and those for other minor filament proteins. Other class 3 genes of *C. jejuni* appear to encode proteins not involved in motility and, instead, may be involved in virulence or colonization of *C. jejuni*. Abbreviations: HBB, hook-basal body; T3SS, type III secretion system.



**Figure 3.** Chemotaxis signal transduction systems of *Campylobacter jejuni* and *Helicobacter pylori*. Transmembrane chemoreceptors are shown as purple and red bars interacting with the coupling proteins CheW (W) or CheV (V). *H. pylori* contains three CheV proteins, termed CheV1 (V1), CheV2 (V2), and CheV3 (V3). The coupling proteins in turn interact with CheA, also called CheAY (AY), which catalyzes a kinase reaction using ATP as the phosphodonor. The phosphoryl group is passed to CheY (Y) and removed by CheZ (Z). *C. jejuni* contains CheR (R) and CheB (B), which methylate and demethylate, respectively, the chemoreceptors in other microbes. *H. pylori* and *C. jejuni* also contain cytoplasmic chemoreceptors that are not shown but are presumed to participate in the same protein-protein interactions.