Published in final edited form as:

Trends Microbiol. 2010 January ; 18(1): 30. doi:10.1016/j.tim.2009.11.001.

Sense and sensibility: flagellum-mediated gene regulation

Jennifer K. Anderson, Todd G. Smith, and Timothy R. Hoover

Department of Microbiology, University of Georgia, Athens, Georgia 30602, USA

Abstract

The flagellum, a rotary engine required for motility in many bacteria, plays key roles in gene expression. It has been known for some time that flagellar substructures serve as checkpoints that coordinate flagellar gene expression with assembly. Less well understood, however, are other more global effects on gene expression. For instance, the flagellum acts as a 'wetness' sensor in *Salmonella typhimurium* and as a mechanosensor in other bacteria. Additionally, it has been implicated in a variety of bacterial processes, including biofilm formation, pathogenesis and symbiosis. Although for many of these processes it may be simply that motility is required, for other cases it seems that the flagellum plays an underappreciated role in regulating gene expression.

Roles of bacterial flagella in microbial processes

The bacterial flagellum is a complex rotary engine comprised of three basic parts: basal body, hook and filament [1] (Figure 1). The basal body anchors the flagellum in the cell envelope, and also contains the motor and a specialized type III secretion system (T3SS) for exporting flagellar proteins. The motor consists of a motile part, or rotor, that is connected to the hook via the rod, and a stationary part, or stator, which converts the potential energy of an electrochemical gradient to kinetic motion of the rotor. The hook is a flexible coupling that transfers torque from motor to the propeller-like filament

In addition to motility, flagella have roles in other microbial processes, such as adherence to host cells, host cell invasion, protein secretion, autoagglutination (i.e. clumping or self-adherence of bacterial cells) and biofilm formation [2–6]. For some of these processes a direct involvement of flagella is clear. For example, *Campylobacter jejuni* flagella are responsible for secretion of several non-flagellar proteins [7–9]. Interestingly, some obligate intracellular symbionts of insects (e.g. *Buchnera aphidicola*) have lost many of the genes needed for assembly of the flagellum and appear to use the remaining flagellar assembly gene products for export of bacterial proteins to the host [10–12].

For other processes, such as biofilm formation, it is difficult to ascertain the specific role of flagella, which can be quite different among bacteria. For example, flagella contribute to biofilm formation by acting as adhesins to promote attachment to surfaces in *Aeromonas* spp. [13], but not in *Escherichia coli* [14]. Additionally, studies with non-motile (Mot⁻) mutants that make but are unable to rotate flagella, although useful, do not distinguish if a particular trait of a mutant is due to defects in locomotion, chemotaxis or just flagellar rotation (independently of the first two processes). Indeed, recent studies suggest a role for flagellar

© 2009 Elsevier Ltd. All rights reserved.

Corresponding author: Hoover, T.R. (trhoover@uga.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

rotation in the regulation of biofilm formation. In *Bacillus subtilis*, a molecular clutch that disengages the flagellum from the rotor might facilitate the transition of free-swimming cells to biofilm-associated bacteria [15]. For *Vibrio cholerae*, it has been proposed that the attachment of the cell body and flagellum to a surface stops the flagellar motor, resulting in decreased ion flow through the motor and a transient increase in membrane potential ($\Delta\Psi$), which might initiate the transition for transient to permanent attachment in biofilm formation. This hypothesis is supported by the fact that experimental dissipation of $\Delta\Psi$ blocks transition from transient to permanent attachment [16].

Regulatory roles for the flagellum in other processes are clearer. For example, the flagellum is involved in the temporal regulation of genes encoding its own components. It monitors the status of its own assembly and communicates this information to regulatory systems that control flagellar gene expression. Thus, the flagellum displays a responsiveness (or sensibility) in its assembly. Additionally, in some bacteria, it senses environmental conditions leading to regulated expression of other genes. Here, we review recent studies on the role of the flagellum in autoregulation, as well as its role as an environmental sensor in global gene regulation. In addition, we examine a recent report that the flagellum plays a role in interspecies communication [17].

Expression of σ^{28} -dependent flagellar genes is coordinated with flagellar assembly

Flagellar assembly is a highly ordered process that requires the coordinated expression of dozens of genes. During flagellar biogenesis, the basal body is the first structure to be assembled, followed by the hook and then the filament. Flagellar genes are expressed temporally within a transcriptional hierarchy that results in their products being made as they are needed for assembly. The best characterized systems are those of the enteric bacteria *Salmonella enterica* serovar Typhimurium (*Salmonella typhimurium*) and *E. coli*. In this section we present a brief overview of flagellar gene regulation in these two bacteria and refer the reader to recent reviews for more detailed information [18–20].

The earliest flagellar genes expressed — *flhD* and *flhC* — are referred to as class I genes and encode a master regulator FlhDC that activates transcription of the class II flagellar genes [21,22]. Class II genes encode components of the basal body and hook, the alternative sigma factor FliA (or σ^{28}) and the corresponding anti-sigma factor FlgM (or anti- σ^{28}) [21–23]. Class III genes encode flagellin and other proteins needed late in flagellar biogenesis and require σ^{28} for their transcription [21,24]. FlgM binds σ^{28} and prevents expression of class III genes [25]. Upon completion of the hook–basal body (HBB) complex, FlgM is secreted from the cell via the flagellar protein export apparatus, which allows the class III genes to be expressed [26]. Thus, the flagellum has a direct role in regulating its own biosynthesis, with formation of the HBB complex serving as a key checkpoint for controlling flagellar gene expression (Figure 2a).

How does formation of the HBB complex trigger secretion of FlgM? The flagellar protein export apparatus undergoes a conformational change upon completion of the HBB complex that causes a switch in secretion of rod- and hook-type substrates to filament-type substrates. Two flagellar proteins, FlhB and FliK, are involved directly in the substrate specificity switch [27, 28]. FlhB is a membrane-bound component of the export apparatus that functions as an export switch. The protein consists of a hydrophobic N-terminal domain and a large cytoplasmic C-terminal domain (FlhB_C) [29]. FlhB_C undergoes autocleavage between the asparagine and proline residues of a specific motif (NPTH) that is conserved among FlhB proteins and T3SS FlhB homologues of plant and animal pathogens [30–34]. Amino acid substitutions within the NPTH motif prevent autocleavage and lock the export apparatus in the

rod/hook-type specificity state, indicating that autocleavage is essential for the switch in substrate specificity [35]. Recent reports on the crystal structures of the cytoplasmic domains of four FlhB T3SS homologs have shown that autocleavage does not affect protein folding but rather results in localized electrostatic and conformational changes that might influence interactions with other export apparatus components [32–34].

FliK, the hook length control protein, is also required for the switch in substrate specificity [36–38]. FliK proteins and their homologs in virulence-associated T3SS (such as *Yersinia* YscP) act as molecular rulers to control the length of the flagellar hook and T3SS needle, respectively [39–42]. The length of the hook is well controlled by FliK at about 55 nm in *S. typhimurium*, and disruption of *fliK* results in abnormally long hook structures referred to as ‘polyhooks’ [43]. FliK contains an unstable N-terminal region (FliK_N), a stably folded region near the C-terminus (FliK_C) and a partially unfolded region at the C-terminus (FliK_{CT}) [43]. FliK_C and FliK_{CT}, which interact with FlhB_C to control export specificity [38,43,44], are conserved among FliK/YscP family members and form a type III secretion substrate specificity switch (T3S4) domain [45].

The model illustrated in Figure 3 accounts for how FliK controls the length of the hook [28]. FliK is secreted by the flagellar protein export apparatus during hook assembly, with the export signal located within FliK_N [46]. Within the lumen of the nascent HBB structure the N-terminus of the unfolded FliK_N domain can bind the hook cap [40]. If the hook is very short, the T3S4 domain of FliK cannot approach FlhB_C, FliK is secreted into the surrounding medium, and hook assembly continues. When the hook length is around 55 nm, binding of the N-terminus of FliK to the hook cap stretches the unfolded FliK_N domain out fully, allowing the T3S4 domain to interact with FlhB_C and facilitating the switch in export substrate specificity.

Two distinct flagellar assembly checkpoints control expression of σ^{54} -dependent flagellar genes in *Caulobacter crescentus*

Several bacteria, including *C. crescentus*, *Helicobacter pylori*, *Campylobacter jejuni*, *V. cholerae* and *Pseudomonas aeruginosa*, employ a particular sigma factor known as σ^{54} (or RpoN) for expression of specific sets of flagellar genes [19,20]. Expression of σ^{54} -dependent flagellar genes is responsive to specific flagellar checkpoints and/or a functional flagellar protein export apparatus in some of these bacteria (Figure 2b).

In the α -proteobacterium *C. crescentus*, the expression of late flagellar genes is regulated by the σ^{54} -dependent transcriptional activator FlbD [47–49]. The activity of FlbD is controlled by FliX, which acts as both a repressor and activator of FlbD [50–52]. FliX forms a stable complex with FlbD and prevents it from activating transcription of σ^{54} -dependent flagellar genes, presumably by interfering with the ability of FlbD to bind DNA sites at target genes [53]. Upon assembly of a nascent flagellar substructure consisting of the MS-ring, motor switch and flagellar protein export apparatus, FliX responds to an unknown signal to activate FlbD, resulting in transcriptional activation of σ^{54} -dependent genes [50–52]. Following completion of the remainder of the flagellum, FliX returns to its role as a repressor of FlbD. Orthologs of *flbD* and *fliX*, which always appear to be present together, are confined to a subset of α -Proteobacteria that possess polar flagella [51]. FlbD and FliX link flagellum biosynthesis with cell division in *C. crescentus*. Evidence for this comes from the observation that class II flagellar mutants exhibit a delay in cytokinesis, but this phenotype can be suppressed by *flbD* or *fliX* alleles that allow transcription of late flagellar genes in the class II flagellar mutants [54].

Assembly of the HBB complex also serves as a checkpoint for flagellar assembly in *C. crescentus*. The two major *C. crescentus* flagellin genes, *fljK* and *fljL*, are transcribed in the

absence of the completed HBB complex but these mRNAs are not translated [55]. A regulatory protein, FlbT, inhibits translation and decreases the stability of the *fljK* transcript. The 5'-untranslated region (UTR) of the *fljK* mRNA is predicted to adopt either of two conformations: one favors translation and the other interferes with translation by obscuring the ribosome-binding site through base pairing [55]. Prior to completion of the HBB complex, FlbT is thought to bind the 5'-UTR of the *fljK* transcript to stabilize the conformation that inhibits translation, which results in decreased stability of the transcript. It is not clear how the inhibitory effect of FlbT on translation of *fljK* mRNA is alleviated following completion of the HBB complex, but other proteins might be involved. Translation of *fljK* mRNA is positively regulated by FlaF [56], which is a small protein (120 amino acid residues) in *C. crescentus*. The *flaF* gene is immediately downstream of *flbT*, a gene arrangement that is conserved in many other bacterial species. Mutant strains that lack *flaF* are able to assemble the HBB complex but are unable to translate *fljK* mRNA. A mutant strain deficient in both *flbT* and *flaF* is able to synthesize flagellin protein but is non-motile, which suggests that FlaF might play an additional role in flagellum assembly. Orthologs of *flbT* and *flaF* occur in many other members of the α -Proteobacteria, and FlbT and FlaF are proposed to control a regulatory checkpoint linking hook assembly and flagellin translation in these bacteria [56].

Expression of σ^{54} -dependent flagellar genes in ϵ -Proteobacteria is linked to the flagellar protein export apparatus

Flagellar biogenesis in the ϵ -Proteobacteria *C. jejuni* and *H. pylori* requires three distinct sigma factors. Genes required early in flagellar assembly rely on the housekeeping sigma factor (σ^{80}) for their transcription, while genes needed next in assembly require σ^{54} for their expression, and those needed at the end of flagellar biogenesis utilize σ^{28} [19,20] (Figure 2c). Disrupting any one of several genes encoding components of the flagellar protein export apparatus in *C. jejuni* or *H. pylori* interferes with expression of the σ^{54} -dependent flagellar genes [57–59]. The export apparatus of *C. jejuni* appears to modulate the activity of the FlgS/FlgR two-component system required for transcription of the RpoN regulon, perhaps by contributing to signal sensing through the sensor kinase FlgS [60]. The nature of the signal sensed by FlgS is not known, but it is possible that FlgS detects conformational changes within the export apparatus (Box 1). Because FlgS is a soluble protein, it could approach the export apparatus readily from the cytoplasm.

Expression of σ^{54} -dependent flagellar genes is stimulated in *H. pylori* and *C. jejuni* by deletion of *fliK*, which supports the hypothesis that the FlgS/FlgR signal transduction system is responsive to conformational changes in the export apparatus [59,61,62]. The *fliK* gene is part of the RpoN regulon in these bacteria, and so FliK might be part of a negative feedback loop that turns off expression of σ^{54} -dependent genes upon completion of the HBB complex. Disrupting *fliK* in a *H. pylori* *flhB* mutant does not restore expression of σ^{54} -dependent reporter genes [59], suggesting that the inhibition of the RpoN regulon in export apparatus mutants is not due solely to the inability to secrete FliK.

The bacterial flagellum as a sensor of wetness

In addition to communicating the status of its assembly to the transcriptional regulatory machinery, the flagellum functions as an environmental sensor in some bacteria. Recent studies revealed that the *S. typhimurium* flagellum acts as a wetness sensor to initiate swarmer cell differentiation [63]. Swarming is a specialized form of flagellum-mediated motility on moist surfaces. Swarmer cells secrete surfactants and wetting agents that assist in their movement across surfaces in coordinated groups.

Although chemotaxis is not required for the outward migration of *S. typhimurium* swarmer cells, chemotaxis (*che*) mutants fail to swarm [63]. Wang and co-workers noted that lawns of *S. typhimurium che* mutants on swarm agar were drier than wild-type cells, which prompted them to try to rescue the swarming phenotype by rehydrating the lawns with a fine mist of water [63]. Interestingly, this approach worked. DNA microarray studies revealed that only a small set of genes are down-regulated in the *che* mutants when propagated on swarm agar. These genes include class III motility genes and T3SS genes within the SPI-1 pathogenicity island required by pathogenic *Salmonella* strains for invasion of epithelial cells. The sequences of the promoters of the virulence genes do not obviously resemble those of class III motility genes, and so the molecular basis that links expression of these genes is not known. Because the class III genes are negatively regulated by FlgM, the researchers examined the ability of the *che* mutants to secrete FlgM. When grown in broth, *che* mutants exported FlgM at wild-type levels, but failed to export FlgM when grown on swarm agar. Rehydration of the *che* mutant lawn restored the ability of the mutants to export FlgM and up-regulate the class III and SPI-1 T3SS genes with the concomitant rescue of swarming [63]. The researchers proposed that when external conditions are too dry, flagellin subunits fail to polymerize at the growing end of the filament and become stuck within the filament. They hypothesize that this blocks secretion of FlgM which leads to repression of the class III genes.

Why are lawns of *che* mutants dry? The authors speculate that the flagellar filament sticks to swarm agar, and *che* mutants (which cannot change the direction of the motor) are unable to free their filaments. Consistent with this hypothesis, substitutions in FliM (a component of the motor switch) that increase the frequency of motor reversal suppress the swarming defect of a *cheY* mutant [64]. Rotation of the filament is thought to aid in external hydration by knocking lipopolysaccharide off neighboring cells which can act as a surfactant or wetting agent [63, 64].

The bacterial flagellum as a mechanosensor

Swarming has been observed for members of several other genera of bacteria, including *Aeromonas*, *Bacillus*, *Proteus*, *Serratia* and *Vibrio*. Bacteria that swarm on high agar concentrations, such as *Proteus mirabilis*, increase their number of flagella for swarming. Other bacteria, such as *Aeromonas* spp. and *Vibrio parahaemolyticus*, possess two distinct flagellar systems: a polar flagellum that is expressed constitutively and used for swimming, and an inducible lateral flagellar system used for swarming. Several environmental factors, cellular cues and regulatory proteins have been implicated in swarmer cell differentiation in these bacteria and we refer the reader to recent reviews [65,66]. We focus here on studies in *V. parahaemolyticus* and *P. mirabilis* that show that the flagellum acts as a mechanosensor that interprets changes in environmental conditions leading to swarmer cell differentiation.

P. mirabilis is a member of the family *Enterobacteriaceae* and is associated with urinary tract infections. It forms short vegetative cells when grown in liquid medium, but differentiates into elongated, highly flagellated swarmer cells (20- to 40-fold increase in both cell length and number of flagella per cell) upon contacting a solid surface [66]. Swarmer cell differentiation involves global changes in gene expression, including the increased expression of several virulence genes. The molecular signals required for swarming in *P. mirabilis* are not well understood, but inhibition of flagellar rotation is a signal for swarmer cell differentiation [66]. Evidence for this model comes from the observation that restricting the rotation of the flagella, by increasing the viscosity of the medium or tethering the flagellum with antibodies, induces swarmer cell differentiation in cells grown in liquid culture [67]. In addition, mutations in some flagellar genes result in aberrant swarmer cell elongation [67]. For example, mutations in *fliL* cause abnormal swarmer cell differentiation. FliL is an inner membrane protein within the basal body that is thought to interact with motility-enabling (Mot) proteins and/or the MS

ring [68–70]. With regard to a potential role in mechanosensing, FliL has been proposed to sense the torque applied to the basal body and motor components upon stalling of the flagellar motor in response to high viscosity environments, or it could monitor proton flux through the motor [67].

Although how inhibition of flagellar rotation is sensed by *P. mirabilis* is unclear, the signaling pathway appears to be mediated through the flagellar master regulator FlhDC. The switch from vegetative to swarmer cells is associated with a sharp transient increase in expression of *flhDC* [71], and artificial overproduction of FlhDC results in increased elongation and hyperflagellation of swarmer cells [72]. A recent report identified a novel gene, *wosA*, whose overexpression causes constitutive swarmer cell differentiation under non-inducing conditions [73]. However, *WosA* is not essential for swarmer cell differentiation as a *wosA* null mutant displays only a modest reduction in swarmer motility. Although the function of *WosA* is not known, it appears to be involved in regulating expression of *flhDC* and might be part of a signaling pathway from the flagellar motor switch [73].

Swarmer cell differentiation in *V. parahaemolyticus* similarly involves signal input from the inhibition of flagellum rotation. *V. parahaemolyticus* is a γ -Proteobacterium that is common to marine and estuarine environments. When growing planktonically, cells utilize polar flagella for swimming. When grown on a solid or highly viscous medium, *V. parahaemolyticus* forms elongated swarmer cells (5 to 20 times longer than vegetative cells) that possess large numbers of lateral flagella used for swarming motility [65]. Not only do polar and lateral flagellar (*laf*) systems in *V. parahaemolyticus* differ in their function but also in how they are powered. The polar flagellum is driven by a sodium motive force whereas lateral flagella are powered by a proton motive force [74]. Similar to *P. mirabilis*, impairing rotation of the polar flagellum — by increasing the viscosity of the medium, through sodium channel blockers or by mutations — results in an induction of the *laf* system [75–78]. The mechanism by which *V. parahaemolyticus* senses inhibition of polar flagellum rotation is not known, but the signal transduction pathway is mediated through the σ^{54} -dependent activator LafK, which controls transcription of the *laf* genes [79]. Iron-limitation and the signaling molecule cyclic di-guanosine monophosphate (cyclic di-GMP) also play crucial roles in expression of the *laf* system [80–81], but it is unclear if or how inhibition of polar flagellum rotation is integrated with these cellular signals to regulate swarmer cell differentiation. Polar flagella of some other bacteria, such as *Azospirillum lipoferum* [82], also appear to function as mechanosensors in regulating expression of lateral flagella, but this does not seem to be true for other bacteria that have dual flagellar systems (Box 2).

A role for the bacterial flagellum in interspecies communication

Anaerobic degradation of organic compounds involves a syntrophy (symbiosis based on nutritional cooperation) between fermentative bacteria (syntrophs) and methanogenic archaea (methanogens), resulting in the conversion of volatile fatty acids to methane [83]. This process requires the transfer of reducing equivalents, in the form of H_2 , from the syntroph to the methanogen. Because of thermodynamic constraints for some of the reactions in this process, close physical association between the symbionts is needed for efficient H_2 transfer [84]. Recent studies on the syntrophy between *Pelotomaculum thermopropionicum* (syntroph) and *Methanothermobacter thermautotrophicus* (methanogen) revealed that the filament cap protein, FliD, of *P. thermopropionicum* specifically alters expression of over 50 genes in *M. thermautotrophicus* [17]. Several of the genes that are up-regulated by FliD encode enzymes required for methanogenesis, ATP synthesis or hydrogen utilization, suggesting that *M. thermautotrophicus* perceives *P. thermopropionicum* FliD to prepare for syntrophy [17]. Thus, the *P. thermopropionicum* flagellum appears to play an important role in ensuring the proximity

of the bacterium with its methanogenic partner as well as helping synchronize the metabolism of the symbionts.

Concluding remarks and future perspectives

Discoveries on the roles of the flagellum in gene regulation and environmental sensing point to a much broader role for the flagellum in affecting gene expression in bacteria than previously appreciated. In most of the examples discussed here, the molecular basis by which the flagellum communicates with the transcriptional machinery to control gene expression is poorly understood, and many fundamental questions remain unanswered (Box 3).

Reports showing that interrupting flagellar and/or chemotaxis genes impacts bacterial pathogenesis or symbiosis are widespread in the literature. As indicated previously, one difficulty in ascribing specific roles for flagella in these processes is distinguishing between effects directly involving motility and those associated with signal transduction systems responsive to disruptions in flagellar assembly or rotation. In addition, innate immune systems can also recognize flagella, and this may affect host–microbe interactions at yet another level. As we learn more about the mechanisms used by flagella for signal transduction we will be able to discriminate between these possible scenarios more effectively and undoubtedly discover additional examples of flagellum-mediated signaling in bacteria.

Box 1

How does the flagellar protein export apparatus affect expression of σ^{54} -dependent genes in ϵ -Proteobacteria?

Any model to explain how the export apparatus affects expression of the σ^{54} (RpoN) regulon in ϵ -Proteobacteria must take into account the following observations. First, σ^{54} -dependent reporter genes are expressed at enhanced levels in *fliK* mutants of *H. pylori* and *C. jejuni*, whereas they are expressed at wild-type levels in strains that produce FlhB variants defective in autocleavage [59,60]. This suggests that FlhK and the processing of FlhB affect the export apparatus differently with regard to how the apparatus influences expression of the RpoN regulon. Second, *H. pylori* possesses a protein (FlhX) that shares homology with the C-terminal end of FlhB_C (FlhB_{CC}) and can functionally replace it [85]. Interestingly, *flhX* is required for optimal expression of σ^{54} -dependent reporter genes in a wild-type background but not in strains expressing non-cleavable FlhB variants [59]. A possible explanation for this observation is that FlhB_{CC} might be able to dissociate from the export apparatus upon autocleavage of FlhB and be replaced with FlhX, whereas FlhB_{CC} or FlhX must remain associated with the export apparatus for optimal expression of the RpoN regulon. Finally, mutations in *flhA*, which encodes a membrane component of the export apparatus, inhibit expression of the RpoN regulon in *H. pylori*, but this inhibition is alleviated by disrupting *flgM* [58]. It is not known if FlgM influences the RpoN regulon directly or indirectly. Clearly, additional studies are needed in order to clarify the role of the export apparatus in regulating the RpoN regulon in the ϵ -Proteobacteria.

Box 2

Not all bacteria with dual flagellar systems use their polar flagella for sensing surfaces

V. parahaemolyticus and other bacteria have dual flagellar systems: polar flagella are used for swimming and lateral flagella are used for swarming. While expression of lateral flagella in *V. parahaemolyticus* is linked to the function of the polar flagellum, this does not appear to be the case for expression of lateral flagella in *Rhodospirillum centum* and *Aeromonas* spp. [86,87]. However, other signal transduction systems similar to those

affecting expression of lateral flagellum genes in *V. parahaemolyticus* might be used primarily in these other bacteria. For example, expression of the *laf* system in *V. parahaemolyticus* is suppressed under high intracellular levels of cyclic di-GMP, the levels of which can be modulated by the products of *scrABC* [80]. ScrC is a cytoplasmic membrane protein that contains both GGDEF and EAL conserved protein domains which control, respectively, the formation and degradation of cyclic di-GMP. The activity of ScrC is modulated by the ScrA and ScrB proteins [88]. A recent report identified a potential cyclic di-GMP riboswitch upstream of a lateral flagellar operon in *Aeromonas salmonicida*, which suggests that *Aeromonas* spp. might have elaborated upon this signal transduction system [89]. The first gene of this operon encodes the activator LafK, which initiates the transcriptional hierarchy for lateral gene expression. In the same study, the authors identified a cyclic di-GMP riboswitch within the 5'-UTR of a large flagellar operon from *Clostridium difficile* and showed that this element together with cyclic di-GMP stimulated the premature termination of transcription of the operon in an *in vitro* transcription assay [89]. Taken together, these observations suggest that cyclic di-GMP regulates expression of the lateral flagellar genes in *A. salmonicida* by inhibiting transcription of *lafK*. This mechanism would eliminate the need for additional signal input from the polar flagellum to initiate swarmer cell differentiation and, therefore, might differ from that by which cyclic di-GMP modulates expression of lateral flagellar genes in *V. parahaemolyticus*.

Box 3

Questions for future research

- Does the flagellar protein export apparatus couple flagellar assembly with gene expression in all prokaryotes?
- How does the export apparatus control the RpoN regulon in ϵ -Proteobacteria? Is this mechanism the same for σ^{54} -dependent flagellar genes in other bacteria?
- What is the role of the flagellum in mediating the activity of FlbT and post-transcriptional control of flagellin genes in *C. crescentus*?
- Are there additional mechanisms by which inhibition of σ^{28} activity by FlgM homologs is alleviated besides export of FlgM?
- How widespread is the use of the flagellum as a mechanosensor?
- How do bacteria sense inhibition of flagellum rotation and communicate this information to the transcriptional machinery?
- Do flagella have important regulatory roles in other processes such as biofilm formation or host-microbe interactions?

Acknowledgments

We thank Anna Karls and Eric Stabb for their comments on the manuscript. We also thank the reviewers for their insightful comments and the journal editor for his helpful revisions. T.G.S. was supported by fellowships from the University of Georgia Graduate School and the Atlanta Chapter of Achievement Rewards for College Scientists (ARCS) Foundation. Research on flagellar gene regulation in *H. pylori* in our lab is supported by award AI080923 to T.R.H. from the National Institutes of Health.

Glossary

Anti- σ^{28} (anti-sigma²⁸)

a protein, also referred to as FlgM, that binds the alternative sigma factor σ^{28} to inhibit its activity. In enteric bacteria, FlgM is a filament-

	type substrate of the flagellar protein export apparatus and is secreted from the cell upon completion of the basal body-hook structure.
Flagellin	protein subunit that comprises the flagellar filament.
FlhB	a membrane protein component of the flagellar protein export apparatus that undergoes autocleavage at a highly conserved site. This cleavage is linked to a conformational change in the export apparatus that results in a switch in the substrate specificity.
FliK	hook-length control protein. Once the hook reaches its mature length, FliK stimulates a conformational change in the flagellar protein export apparatus which results in a switch in substrate specificity from rod-/hook-type to filament-type substrates.
Lateral flagella	flagella used for swarming, and distributed around the surface of the cell in some bacteria that also possess polar flagella. Lateral and polar flagella are encoded by different sets of genes.
Mechanosensor	a device that receives a mechanical signal or stimulus and responds to it. In some bacteria, flagella respond to factors that affect their mechanical function, such as changes in the viscosity of the surrounding medium, and communicate this information to the transcriptional machinery of the cell.
Polar flagella	flagella used for swimming, and localized to one or both ends of a rod-shaped bacterium.
Riboswitch	a regulatory element within the 5'-untranslated region of some mRNAs. Riboswitches can assume one of two mutually exclusive conformations, depending on the binding of specific small molecules. One of the conformations allows expression of the gene, while the other prevents expression.
σ^{28} (sigma ²⁸)	an alternative sigma factor, also referred to as FliA, that is required for transcription of the flagellin genes and other late flagellar genes in enteric and other bacteria.
Swarm agar	agar medium used to induce swarmer cell differentiation.
Swarmer cell differentiation	morphological change of some bacteria from a planktonic or vegetative form that moves by swimming in liquid medium to a form in which the cells move across solid surfaces. Swarmer cell differentiation results in an increased number of flagella per cell and, in some bacteria, an alteration in how the flagella are distributed around the cell surface. Other morphological changes include a significant elongation of the swarmer cells.

References

1. Apel D, Surette MG. Bringing order to a complex molecular machine: the assembly of the bacterial flagella. *Biochim. Biophys. Acta* 2008;1778:1851–1858. [PubMed: 17719558]
2. Guerry P. Campylobacter flagella: not just for motility. *Trends Microbiol.* 2007;15:456–461. [PubMed: 17920274]
3. Mahajan A, et al. An investigation of the expression and adhesin function of H7 flagella in the interaction of *Escherichia coli* O157 : H7 with bovine intestinal epithelium. *Cell. Microbiol.* 2009;11:121–137. [PubMed: 19016776]

4. Millikan DS, Ruby EG. *Vibrio fischeri* flagellin A is essential for normal motility and for symbiotic competence during initial squid light organ colonization. *J. Bacteriol* 2004;186:4315–4325. [PubMed: 15205434]
5. Rodriguez-Navarro DN, et al. Attachment of bacteria to the roots of higher plants. *FEMS Microbiol. Lett* 2007;272:127–136. [PubMed: 17521360]
6. Roy K, et al. Enterotoxigenic *Escherichia coli* EtpA mediates adhesion between flagella and host cells. *Nature* 2009;457:594–598. [PubMed: 19060885]
7. Konkel ME, et al. Secretion of virulence proteins from *Campylobacter jejuni* is dependent on a functional flagellar export apparatus. *J. Bacteriol* 2004;186:3296–3303. [PubMed: 15150214]
8. Poly F, et al. Heterogeneity of a *Campylobacter jejuni* protein that is secreted through the flagellar filament. *Infect. Immun* 2007;75:3859–3867. [PubMed: 17517862]
9. Song YC, et al. FlaC, a protein of *Campylobacter jejuni* TGH9011 (ATCC43431) secreted through the flagellar apparatus, binds epithelial cells and influences cell invasion. *Mol. Microbiol* 2004;53:541–553. [PubMed: 15228533]
10. Maezawa K, et al. Hundreds of flagellar basal bodies cover the cell surface of the endosymbiotic bacterium *Buchnera aphidicola* sp. strain APS. *J. Bacteriol* 2006;188:6539–6543. [PubMed: 16952945]
11. Shigenobu S, et al. Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. *Nature* 2000;407:81–86. [PubMed: 10993077]
12. Toft C, Fares MA. The evolution of the flagellar assembly pathway in endosymbiotic bacterial genomes. *Mol. Biol. Evol* 2008;25:2069–2076. [PubMed: 18635679]
13. Kirov SM, et al. *Aeromonas* flagella (polar and lateral) are enterocyte adhesins that contribute to biofilm formation on surfaces. *Infect. Immun* 2004;72:1939–1945. [PubMed: 15039313]
14. Pratt LA, Kolter R. Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. *Mol. Microbiol* 1998;30:285–293. [PubMed: 9791174]
15. Blair KM, et al. A molecular clutch disables flagella in the *Bacillus subtilis* biofilm. *Science* 2008;320:1636–1638. [PubMed: 18566286]
16. Van Dellen KL, et al. Genetic analysis of *Vibrio cholerae* monolayer formation reveals a key role for $\Delta\Psi$ in the transition to permanent attachment. *J. Bacteriol* 2008;190:8185–8196. [PubMed: 18849423]
17. Shimoyama T, et al. Flagellum mediates symbiosis. *Science* 2009;323:1574. [PubMed: 19299611]
18. Chevance FF, Hughes KT. Coordinating assembly of a bacterial macromolecular machine. *Nat. Rev. Microbiol* 2008;6:455–465. [PubMed: 18483484]
19. McCarter LL. Regulation of flagella. *Curr. Opin. Microbiol* 2006;9:180–186. [PubMed: 16487743]
20. Smith TG, Hoover TR. Deciphering bacterial flagellar gene regulatory networks in the genomic era. *Adv. Appl. Microbiol* 2009;67:257–295. [PubMed: 19245942]
21. Kutsukake K, et al. Transcriptional analysis of the flagellar regulon of *Salmonella typhimurium*. *J. Bacteriol* 1990;172:741–747. [PubMed: 2404955]
22. Liu X, Matsumura P. The FlhD/FlhC complex, a transcriptional activator of the *Escherichia coli* flagellar class II operons. *J. Bacteriol* 1994;176:7345–7351. [PubMed: 7961507]
23. Pruss BM, et al. FlhD/FlhC-regulated promoters analyzed by gene array and *lacZ* gene fusions. *FEMS Microbiol. Lett* 2001;197:91–97. [PubMed: 11287152]
24. Ohnishi K, et al. Gene *fliA* encodes an alternative sigma factor specific for flagellar operons in *Salmonella typhimurium*. *Mol. Gen. Genet* 1990;221:139–147. [PubMed: 2196428]
25. Ohnishi K, et al. A novel transcriptional regulation mechanism in the flagellar regulon of *Salmonella typhimurium*: an anti-sigma factor inhibits the activity of the flagellum-specific sigma factor, σ^F . *Mol. Microbiol* 1992;6:3149–3157. [PubMed: 1453955]
26. Hughes KT, et al. Sensing structural intermediates in bacterial flagellar assembly by export of a negative regulator. *Science* 1993;262:1277–1280. [PubMed: 8235660]
27. Waters RC, et al. The FliK protein and flagellar hook-length control. *Protein Sci* 2007;16:769–780. [PubMed: 17456739]
28. Minamino T, et al. Mechanisms of type III protein export for bacterial flagellar assembly. *Mol. Biosyst* 2008;4:1105–1115. [PubMed: 18931786]

29. Minamino T, et al. Molecular characterization of the *Salmonella typhimurium* *flhB* operon and its protein products. J. Bacteriol 1994;176:7630–7637. [PubMed: 8002587]
30. Ferris HU, et al. FlhB regulates ordered export of flagellar components via autocleavage mechanism. J. Biol. Chem 2005;280:41236–41242. [PubMed: 16246842]
31. Minamino T, Macnab RM. Domain structure of *Salmonella* FlhB, a flagellar export component responsible for substrate specificity switching. J. Bacteriol 2000;182:4906–4919. [PubMed: 10940035]
32. Deane JE, et al. Crystal structure of Spa40, the specificity switch for the *Shigella flexneri* type III secretion system. Mol. Microbiol 2008;69:267–276. [PubMed: 18485071]
33. Lountos GT, et al. Atomic resolution structure of the cytoplasmic domain of *Yersinia pestis* YscU, a regulatory switch involved in type III secretion. Protein Sci 2009;18:467–474. [PubMed: 19165725]
34. Zarivach R, et al. Structural analysis of the essential self-cleaving type III secretion proteins EscU and SpaS. Nature 2008;453:124–127. [PubMed: 18451864]
35. Fraser GM, et al. Substrate specificity of type III flagellar protein export in *Salmonella* is controlled by subdomain interactions in FlhB. Mol. Microbiol 2003;48:1043–1057. [PubMed: 12753195]
36. Kutsukake K. Hook-length control of the export-switching machinery involves a double-locked gate in *Salmonella typhimurium* flagellar morphogenesis. J. Bacteriol 1997;179:1268–1273. [PubMed: 9023211]
37. Kutsukake K, et al. Isolation and characterization of FliK-independent flagellation mutants from *Salmonella typhimurium*. J. Bacteriol 1994;176
38. Williams AW, et al. Mutations in *fliK* and *flhB* affecting flagellar hook and filament assembly in *Salmonella typhimurium*. J. Bacteriol 1996;178:2960–2970. [PubMed: 8631688]
39. Journet L, et al. The needle length of bacterial injectisomes is determined by a molecular ruler. Science 2003;302:1757–1760. [PubMed: 14657497]
40. Moriya N, et al. The type III flagellar export specificity switch is dependent on FliK ruler and a molecular clock. J. Mol. Biol 2006;359:466–477. [PubMed: 16630628]
41. Shibata S, et al. FliK regulates flagellar hook length as an internal ruler. Mol. Microbiol 2007;64:1404–1415. [PubMed: 17542929]
42. Wagner S, et al. The helical content of the YscP molecular ruler determines the length of the *Yersinia* injectisome. Mol. Microbiol 2009;71:692–701. [PubMed: 19055526]
43. Minamino T, et al. Domain organization and function of *Salmonella* FliK, a flagellar hook-length control protein. J. Mol. Biol 2004;341:491–502. [PubMed: 15276839]
44. Minamino T, et al. Two parts of the T3S4 domain of the hook-length control protein FliK are essential for the substrate specificity switching of the flagellar type III export apparatus. J. Mol. Biol 2006;362:1148–1158. [PubMed: 16949608]
45. Agrain C, et al. Characterization of a Type III secretion substrate specificity switch (T3S4) domain in YscP from *Yersinia enterocolitica*. Mol. Microbiol 2005;56:54–67. [PubMed: 15773978]
46. Minamino T, et al. FliK, the protein responsible for flagellar hook length control in *Salmonella*, is exported during hook assembly. Mol. Microbiol 1999;34:295–304. [PubMed: 10564473]
47. Ramakrishnan G, Newton A. FlbD of *Caulobacter crescentus* is a homologue of NtrC (NR_I) and activates sigma-54 dependent flagellar gene promoters. Proc. Natl. Acad. Sci. U.S.A 1990;87:2369–2373. [PubMed: 2315326]
48. Wingrove JA, et al. Spatial and temporal phosphorylation of a transcriptional activator regulates pole-specific gene expression in *Caulobacter*. Genes Dev 1993;7:1979–1992. [PubMed: 8406002]
49. Wu J, et al. FlbD, a global regulator, is required for activation of transcription from σ^{54} -dependent *fla* gene promoters. J. Bacteriol 1995;177:3241–3250. [PubMed: 7768824]
50. Mohr CD, et al. A membrane-associated protein, FliX, is required for an early step in *Caulobacter* flagellar assembly. J. Bacteriol 1998;180:2175–2185. [PubMed: 9555902]
51. Muir RE, Gober JW. Regulation of FlbD activity by flagellum assembly is accomplished through direct interaction with the trans-acting factor, FliX. Mol. Microbiol 2004;54:715–730. [PubMed: 15491362]

52. Muir RE, et al. The *Caulobacter crescentus* flagellar gene, *fliX*, encodes a novel trans-acting factor that couples flagellar assembly to transcription. *Mol. Microbiol* 2001;39:1623–1637. [PubMed: 11260478]
53. Dutton RJ, et al. Linking structural assembly to gene expression: a novel mechanism for regulating the activity of a sigma54 transcription factor. *Mol. Microbiol* 2005;58:743–757. [PubMed: 16238624]
54. Muir RE, et al. The trans-acting flagellar regulatory proteins, FliX and FlbD, play a central role in linking flagellar biogenesis and cytokinesis in *Caulobacter crescentus*. *Microbiology* 2005;151:3699–3711. [PubMed: 16272391]
55. Anderson PE, Gober JW. FlbT, the post-transcriptional regulator of flagellin synthesis in *Caulobacter crescentus*, interacts with the 5' untranslated region of flagellin mRNA. *Mol. Microbiol* 2000;38:41–52. [PubMed: 11029689]
56. Llewellyn M, et al. The conserved *flaF* gene has a critical role in coupling flagellin translation and assembly in *Caulobacter crescentus*. *Mol Microbiol* 2005;57:1127–1142. [PubMed: 16091049]
57. Hendrixson DR, DiRita VJ. Transcription of sigma54-dependent but not sigma28-dependent flagellar genes in *Campylobacter jejuni* is associated with formation of the flagellum secretory apparatus. *Mol. Microbiol* 2003;50:687–702. [PubMed: 14617189]
58. Niehus E, et al. Genome-wide analysis of transcriptional hierarchy and feedback regulation in the flagellar system of *Helicobacter pylori*. *Mol. Microbiol* 2004;52:947–961. [PubMed: 15130117]
59. Smith TG, et al. *Helicobacter pylori* FlhB processing-deficient variants affect flagellar assembly but not flagellar gene expression. *Microbiology* 2009;155:1170–1180. [PubMed: 19332819]
60. Joslin SN, Hendrixson DR. Activation of the *Campylobacter jejuni* FlgSR two-component system is linked to the flagellar export apparatus. *J. Bacteriol* 2009;191:2656–2667. [PubMed: 19201799]
61. Kamal N, et al. Deletion of a previously uncharacterized flagellar-hook-length control gene *fliK* modulates the sigma54-dependent regulon in *Campylobacter jejuni*. *Microbiology* 2007;153:3099–3111. [PubMed: 17768253]
62. Ryan KA, et al. *Helicobacter pylori* flagellar hook-filament transition is controlled by a FliK functional homolog encoded by the gene HP0906. *J. Bacteriol* 2005;187:5742–5750. [PubMed: 16077121]
63. Wang Q, et al. Sensing wetness: a new role for the bacterial flagellum. *EMBO J* 2005;24:2034–2042. [PubMed: 15889148]
64. Mariconda S, et al. A mechanical role for the chemotaxis system in swarming motility. *Mol. Microbiol* 2006;60:1590–1602. [PubMed: 16796690]
65. Merino S, et al. Bacterial lateral flagella: an inducible flagella system. *FEMS Microbiol. Lett* 2006;263:127–135. [PubMed: 16978346]
66. Rather PN. Swarmer cell differentiation in *Proteus mirabilis*. *Environ. Microbiol* 2005;7:1065–1073.
67. Belas R, Suvanasuthi R. The ability of *Proteus mirabilis* to sense surfaces and regulate virulence gene expression involves FliL, a flagellar basal body protein. *J. Bacteriol* 2005;187:6789–6803. [PubMed: 16166542]
68. Schoenhals GJ, Macnab RM. FliL is a membrane-associated component of the flagellar basal body of *Salmonella*. *Microbiology* 1999;145:1769–1775. [PubMed: 10439416]
69. Jenal U, et al. *Caulobacter* flagellar function, but not assembly, requires FliL, a non-polarly localized membrane protein present in all cell types. *J. Mol. Biol* 1994;243:227–244. [PubMed: 7932752]
70. Attmannspacher U, et al. FliL is essential for swarming: motor rotation in absence of FliL fractures the flagellar rod in swarmer cells of *Salmonella enterica*. *Mol. Microbiol* 2008;68:328–341. [PubMed: 18284590]
71. Dufour A, et al. Novel genes that upregulate the *Proteus mirabilis* *flhDC* master operon controlling flagellar biogenesis and swarming. *Mol. Microbiol* 1998;29:741–751. [PubMed: 9723914]
72. Furness RB, et al. Negative feedback from a *Proteus* class II flagellum export defect to the *flhDC* master operon controlling cell division and flagellum assembly. *J. Bacteriol* 1997;179:5585–5588. [PubMed: 9287017]
73. Hatt JK, Rather PN. Characterization of a novel gene, *wosA*, regulating FlhDC expression in *Proteus mirabilis*. *J. Bacteriol* 2008;190:1946–1955. [PubMed: 18192389]

74. Atsumi T, et al. Polar and lateral flagellar motors of marine *Vibrio* are driven by different ion-motive forces. *Nature* 1992;355:182–184. [PubMed: 1309599]
75. Belas R, et al. Regulation of lateral flagella gene transcription in *Vibrio parahaemolyticus*. *J. Bacteriol* 1986;167:210–218. [PubMed: 3013835]
76. Kawagishi I, et al. The sodium-driven polar flagellar motor of marine *Vibrio* as the mechanosensor that regulates lateral flagellar expression. *Mol. Microbiol* 1996;20:693–699. [PubMed: 8793868]
77. McCarter L, et al. Flagellar dynamometer controls swarmer cell differentiation of *V. parahaemolyticus*. *Cell* 1988;54:345–351. [PubMed: 3396074]
78. Boles BR, McCarter LL. Insertional inactivation of genes encoding components of the sodium-type flagellar motor and switch of *Vibrio parahaemolyticus*. *J. Bacteriol* 2000;182:1035–1045. [PubMed: 10648530]
79. Stewart BJ, McCarter LL. Lateral flagellar gene system of *Vibrio parahaemolyticus*. *J. Bacteriol* 2003;185:4508–4518. [PubMed: 12867460]
80. Ferreira RB, et al. *Vibrio parahaemolyticus* ScrC modulates cyclic dimeric GMP regulation of gene expression relevant to growth on surfaces. *J. Bacteriol* 2008;190:851–860. [PubMed: 17993539]
81. McCarter L, Silverman M. Iron regulation of swarmer cell differentiation of *Vibrio parahaemolyticus*. *J. Bacteriol* 1989;171:731–736. [PubMed: 2914871]
82. Alexandre G, et al. A phase variant of *Azospirillum lipoferum* lacks a polar flagellum and constitutively expresses mechanosensing lateral flagella. *Appl. Environ. Microbiol* 1999;65:4701–4704. [PubMed: 10508112]
83. Schink B. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol. Mol. Biol. Rev* 1997;61:262–280. [PubMed: 9184013]
84. Ishii S, et al. Coaggregation facilitates interspecies hydrogen transfer between *Pelotomaculum thermopropionicum* and *Methanothermobacter thermautotrophicus*. *Appl. Environ. Microbiol* 2005;71:7838–7845. [PubMed: 16332758]
85. Wand ME, et al. *Helicobacter pylori* FlhB function: the FlhB C-terminal homologue HP1575 acts as a "spare part" to permit flagellar export when the HP0770 FlhBCC domain is deleted. *J. Bacteriol* 2006;188:7531–7541. [PubMed: 17050924]
86. Canals R, et al. Analysis of the lateral flagellar gene system of *Aeromonas hydrophila* AH-3. *J. Bacteriol* 2006;188:852–862. [PubMed: 16428388]
87. Jiang ZY, et al. Isolation of *Rhodospirillum centenum* mutants defective in phototactic colony motility by transposon mutagenesis. *J. Bacteriol* 1998;180:1248–1255. [PubMed: 9495765]
88. Kim YK, McCarter LL. ScrG, a GGDEF-EAL protein, participates in regulating swarming and sticking in *Vibrio parahaemolyticus*. *J. Bacteriol* 2007;189:4094–4107. [PubMed: 17400744]
89. Sudarsan N, et al. Riboswitches in eubacteria sense the second messenger cyclic di-GMP. *Science* 2008;321:411–413. [PubMed: 18635805]

Figure 1.

The bacterial flagellum and associated flagellar protein export apparatus. **(a)** The diagram depicts a typical cell wall with flagellum of a gram-negative bacterium. The basal body (red) consists of the flagellar protein export apparatus, motor, and membrane spanning rods and rings. The hook (blue) forms a flexible linker and the filament (green) acts as the propeller. **(b)** Diagram indicating the membrane-bound components (FliO, FliP, FliQ, FlhA, FlhB, FliR) and cytoplasmic components (FliH, FliI, FliJ) of the flagellar protein export apparatus. The export apparatus transports substrates across the cytoplasmic membrane into the lumen of the nascent flagellum for assembly. The scissors indicate the relative site of FlhB autocleavage between the two cytoplasmic subdomains of the protein. The arrangement of the membrane components relative to each other is not known.

(a) *S. typhimurium* **(b) *C. crescentus*** **(c) *H. pylori*, *C. jejuni*** **Figure 2.**

Three model systems for flagellar gene regulation. The diagrams depict the major checkpoints in flagellar gene regulation for selected bacteria. Components of the basal body, hook and filament are depicted in red, blue and green colours, respectively. **(a)** In *S. typhimurium*, transcription of the class II flagellar genes is dependent on the major form of RNA polymerase holoenzyme (σ^{70} -holoenzyme) and is activated by the master regulator FlhDC. The major checkpoint for flagellar assembly is completion of the HBB complex, which is linked to secretion of anti- σ^{28} factor, FlgM, via the flagellar protein export apparatus. Secretion of FlgM alleviates its inhibitory effect on expression of the class III (σ^{28} -dependent) genes, including those required for formation of the filament. **(b)** The master regulator in *C. crescentus*, CtrA, activates transcription of class II genes together with the major form of RNA polymerase holoenzyme (σ^{73} -holoenzyme). Class II genes encode components of an intermediate structure containing elements from both the export apparatus and the basal body, and formation of this intermediate activates the FliD/FliX system which in turn stimulates transcription of class III (σ^{54} -dependent) genes. Completion of the HBB complex is required to alleviate the inhibitory effect of FliB on translation of the class IV flagellar genes, which include the flagellin genes *fljK* and *fljL* (products of these genes shown in green). Like the class III genes, the class IV genes are dependent on σ^{54} for their transcription. **(c)** In *H. pylori* and *C. jejuni* a master regulator has not yet been identified. Early (class II) flagellar genes are dependent on the

primary sigma factor, σ^{80} , for their expression. These early flagellar genes are referred to as class I genes in the literature, but we designate them here as class II genes to be consistent with other bacterial systems. Completion of an export apparatus-basal body intermediate is thought to activate the FlgS/FlgR two-component system, which is required for transcription of σ^{54} -dependent flagellar genes that encode components of the basal body, hook and a minor flagellin. Transcription of the late (class IV) genes is dependent on σ^{28} . *H. pylori* FlgM inhibits transcription of σ^{28} -dependent genes, and its export is presumed but has not been demonstrated. FlgM does not appear to inhibit transcription of σ^{28} -dependent genes in *C. jejuni* strain 81–176 [41].

Figure 3.

Mechanism for control of substrate specificity switching by the hook length control protein FliK. **(i)** Early in flagellar assembly the flagellar protein export apparatus is in a conformation that exports rod- and hook-type substrates. **(ii)** FliK is occasionally exported during hook assembly. The unfolded FliK_N domain (purple) within the lumen of the nascent HBB structure temporarily anchors its N-terminus to the hook cap (orange). If the hook has not reached its mature length of about 55 nm, FliK is secreted into the surrounding medium. **(iii)** When the hook reaches its mature length, the stretched conformation of FliK_N and part of FliK_C (magenta) measure the length of the rod and hook, and the T3S4 domain of FliK is able to interact with FlhB_C (blue). Interactions between FlhB_C and the T3S4 domain result in a switch to filament-type protein export. Abbreviations: OM, outer membrane; PG, peptidoglycan layer; CM, cytoplasmic membrane.