6-3. Genetic regulatory network in flagellar biogenesis

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Abstract. Flagellated bacteria such as *Escherichia coli* and *Salmonella enterica* can swim in their environment to reach the most favorable environments. More than 50 genes are specifically required for flagellar formation and function. Most of them are clustered in several regions on the chromosome. They are organized into more than 15 operons, and their expression forms a highly organized cascade called the flagellar regulon. Several genes within the regulon are dedicated to its transcriptional regulation. Export of the flagellum-specific anti-sigma factor through the flagellar structure is involved in tight coupling of the flagellar gene expression to the flagellar assembly process. Interactions among the transcriptional or translational regulators, flagellar component proteins, and export chaperones contribute to the fine-tuning of the flagellar regulon. On the other hand, flagellar gene expression is extensively regulated by a large number of the global regulators and impacted by various environmental cues. Here, we summarize the structure of the flagellar regulon of *E. coli* and *S. enterica* and discuss the mechanisms of its local, global, and environmental controls. Expression control of the flagellar genes in *Bacillus subtilis* is also described.
1. Introduction

In many bacteria, flagella provide a competitive advantage by enabling them to swim toward nutrients and away from harmful environments. An individual flagellum is a complex molecular machine consisting of three distinct structural parts, a basal body, a hook, and a filament. The basal body is embedded in the cell-surface membrane and acts as a rotary motor, which is powered by the transmembrane proton-motive force. Rotation generated in the basal body is transmitted via the flexible hook to the helical filament, which acts as a propeller to push the bacterial cell [1].

The bacterial cell assembles first the basal body in the cell-surface membrane. The basal body is composed of nearly 20 protein species and acts as an anchor and a secretion conduit of the component proteins for the rest of the flagellar structure. The hook subunits are secreted through the basal body and assembled onto the tip of the axial rod structure of the basal body. Once hook assembly is completed, thousands of the filament protein, flagellin, are secreted through the basal body-hook structure and assemble into the filament onto the hook.

In many bacterial species, more than 50 genes have been shown to be required for flagellar formation and function. Many of them encode the component proteins of the flagellar structure and the flagellum-specific protein export apparatus, and some are involved in flagellar rotation, sensory reception, and signal transduction. Bacterial cells use a complex hierarchy of gene regulation to control synthesis and export of the flagellar component proteins and coordinate flagellar assembly. This hierarchical structure of the flagellar genes is called the flagellar regulon. Several genes within the flagellar regulon are dedicated to this hierarchical control.

The process of flagellar biogenesis has been studied most extensively and intensively in Salmonella enterica serovar Typhimurium, and the structure and regulation of the flagellar regulon are best understood in this organism. Since the flagellar system of Escherichia coli is essentially identical with that of Salmonella, knowledge of the Salmonella flagellar regulon can be applied equally to the E. coli flagellar regulon in most aspects. However, there are a few examples in which differences are observed between these two bacterial species especially in the global and environmental controls of the flagellar regulon. Although Bacillus subtilis possesses a similar set of the flagellar genes, their chromosomal organization and operon structure are quite different from those of Salmonella and E. coli. The regulation of the flagellar genes is less understood in B. subtilis.

In this chapter, we describe the expression control of the flagellar genes, focusing on the flagellar regulon of Salmonella and E. coli. The flagellar regulon of B. subtilis is also described, but only briefly.
2. Overall structure of the flagellar regulon

$E.\ coli$ and $Salmonella$ possess almost the same set of the flagellar genes [2, 3]. Most of these flagellar genes are highly clustered on the limited regions of the chromosome (Fig. 1A). Their chromosomal locations and operon structures are also almost identical in these two bacteria. These flagellar genes are organized into more than 15 operons [4, 5].

![Flagellar gene organization on the chromosome in Salmonella (A) and B. subtilis (B). Arrows indicate the extent and orientation of the flagellar operons. The number at the beginning of each arrow indicates the promoter class responsible for transcription of that operon. Chromosomal location and operon structure of the flagellar genes in $E.\ coli$ are essentially identical with those in $Salmonella$ with a few exceptions. $E.\ coli$ possesses another transducer gene, $tap$, between the $tar$ and $cheR$ genes but lacks the $tcp$ gene. $E.\ coli$ lacks the $hin-fljBA$ locus, which includes another flagellin gene, $fljB$, in $Salmonella$. The $hin$ gene, which is involved in flagellar phase variation, does not belong to the flagellar regulon. The large operon beginning with the $flgB$ gene is called the $fla/che$ operon in $B.\ subtilis$.](image-url)
A pioneering study to understand the transcriptional interaction among the flagellar operons was carried out in *E. coli* using the lacZ gene transcriptionally fused to the promoter of each flagellar operon, and a relatively complicated regulon model was proposed [6, 7]. This model shows a branching cascade in which the flagellar operons are divided into six classes. Later, a similar study was performed in *S. enterica* serovar Typhimurium and revealed a simpler regulon structure consisting of only one sequential pathway in which the flagellar operons are divided into only three classes with respect to their transcriptional hierarchy [8, 9, 10]. With the accumulation of information on the molecular details of the flagellar genes and their regulatory signals, the *E. coli* flagellar regulon is now believed to have the same structure as the *Salmonella* flagellar regulon.

Fig. 2A shows the three-tiered structure of the flagellar regulon in *Salmonella* and *E. coli* [8]. Class 1 contains only one operon, *flhDC*, which is located at the top of the cascade in the flagellar regulon. Since its function is absolutely required for the expression of the rest of the flagellar regulon, it is called also the master operon. Class 2 contains a large number of the flagellar genes, most of which are involved in formation of the basal body-hook structure. Class 3 contains the genes involved in filament assembly, flagellar rotation, and chemotaxis. The *fliC* gene encoding the filament protein flagellin, which is required in the highest amount for flagellar assembly, is included in this class. The most remarkable feature of the flagellar regulon is the coupling of sequential transcription of the flagellar operons to the assembly process of the flagellar structure, that is, all the genes involved in basal body-hook assembly belong to class 2, while those involved in filament assembly belong to class 3. Loss-of-function mutations in any one of the basal body-hook genes inhibit not only normal assembly of the basal body-hook structure but also transcription of the class 3 operons.

### 3. Local control of the flagellar regulon

Three flagellar genes, *flhD*, *flhC*, and *fliA*, are central for the hierarchical expression of the flagellar regulon in *E. coli* and *Salmonella* (Fig. 2A) [8]. The *flhD* and *flhC* genes are encoded by the master operon *flhDC*, which is the sole one belonging to class 1. Their gene products, FlhD and FlhC, assemble into an FlhD₄C₂ heterohexamer [11], which acts as an essential activator of the class 2 operons [12, 13]. The *fliA* gene belongs to class 2 and encodes a flagellum-specific sigma factor, σ²⁸, essential for class 3 gene expression [14]. Therefore, in other words, there are three distinct promoters, each of which is responsible for the expression of specific one of the three classes in the flagellar regulon. The class 1 promoter is expressed
Figure 2. Overall structure of the flagellar regulon in *Salmonella* (A) and *B. subtilis* (B). Each flagellar operon is named after the gene transcribed first in that operon. Therefore, the *fla/che* operon of *B. subtilis* is labeled *flgB* in this figure. Non-flagellar gene and proteins are written in filled boxes. The flagellar regulon of *E. coli* is believed to have the same structure as that of *Salmonella* except for the YdiV control. Details are given in the text.

independent of the functions of the class 2 and class 3 flagellar genes. However, the definition of the class 1 promoter is still elusive. In contrast, the definitions of the class 2 and class 3 promoters are clear, that is, the class 2 and class 3 promoters are absolutely dependent upon FhD4C2 and FliA, respectively.

The *flhDC* operon of *Salmonella* was shown *in vivo* to be transcribed from multiple promoters, and at least two of them were proven *in vitro* to be
transcribed by $\sigma^{70}$-RNA polymerase [15]. In contrast, only one major promoter has been reported in the flhDC operon of E. coli [16]. The flhDC operon of Salmonella is moderately repressed by its own products [17], but its molecular mechanism remains to be analyzed. The class 1 expression is believed to integrate multiple signals from environmental and physiological cues into the cellular activity of flagellar biogenesis. This is discussed further in sections 6 and 7.

The class 2 promoter is transcribed by $\sigma^{70}$-RNA polymerase in the presence of FlhD$_4$C$_2$ [11, 12, 13]. The FlhD$_4$C$_2$ complex binds to the FlhD$_4$C$_2$-binding site located upstream of the class 2 promoter. In the FlhD$_4$C$_2$ complex, the FlhC subunit bears the DNA-binding activity, while the FlhD subunit strengthens its DNA-binding specificity and stabilizes the protein-DNA complex [18]. The FlhD$_4$C$_2$-binding site shows an imperfect symmetry comprising two 17- or 18-bp inverted repeats, called the FlhD$_4$C$_2$ box, separated by an 11- or a 12-bp spacer [13, 19, 20]. The most recently proposed consensus sequence of the FlhD$_4$C$_2$-binding site is CAATCGGTAGAATAAGG $N_{11-12}$ CCTTATTCTACCGATTG [21]. The FlhD$_4$C$_2$-binding site overlaps the $\sigma^{70}$-specific -35 element, and the FlhD$_4$C$_2$ complex activates the class 2 promoter via its interaction with the $\alpha$ subunit of RNA polymerase [22].

The class 3 promoter is transcribed by $\sigma^{28}$-RNA polymerase [14]. The consensus sequence of the $\sigma^{28}$-dependent promoter is TAAAGTTT-$N_{11-20}$ GCCGATAA [20, 23]. No activator protein is required for transcription from the class 3 promoter, but the activity of $\sigma^{28}$ is negatively regulated by a specific anti-sigma factor FlgM, which is encoded by the flgM gene within the flagellar regulon [24]. FlgM is a small protein consisting of 97 amino acids, which binds to $\sigma^{28}$ and inhibits its binding to RNA polymerase core enzyme [25, 26] or dissociates the $\sigma^{28}$-RNA polymerase complex [27, 28]. FlgM is excreted out of the cell through the flagellar structure by the aid of the flagellum-specific protein export machinery upon completion of the basal body-hook structure [29, 30]. This achieves tight coupling of the class 3 gene expression to the flagellar assembly process, and completion of the basal body-hook structure is the major checkpoint in the expression control of the class 3 genes [31, 32]. Therefore, the flagellar structure itself can be considered as a transcriptional regulator, that is, it turns on class 3 gene transcription upon completion of basal body-hook assembly by sequestering the intracellular FlgM protein. The coupling of the secretion of the FlgM protein to the hook assembly state involves two flagellar proteins, FliK and FlhB, and one non-flagellar protein, RflH (called also Flk) [31, 33, 34]. These proteins constitute the export-switching machinery in the flagellum-specific
Flagellar regulon

protein export apparatus, which switches its substrate specificity from the hook-type proteins to the FlgM-type proteins upon completion of the hook structure. Therefore, this export-switching machinery determines not only the timing of class 3 gene expression but also the hook length [31, 33]. Although an increasing number of studies on the export-switching machinery have been reported from various laboratories, its molecular mechanism still remains enigmatic.

Interestingly, FlgM was reported to protect FliA from being degraded by cellular proteases within the *E. coli* cell [35], whereas FliA was shown to facilitate the secretion of FlgM through the flagellar structure in *Salmonella* [36]. These observations suggest that FliA and FlgM have a chaperone-like activity specific for each other and function as more than transcriptional regulators.

### 4. Dual-promoter control of the flagellar genes

In *Salmonella*, FlgM was shown to be expressed from both *flgAMN* and *flgMN* mRNAs, which are transcribed from class 2 and class 3 promoters, respectively (Fig. 1A) [30, 37]. The FlgM protein expressed from the class 2 transcript ensures inhibition of the expression of the class 3 operons before completion of the basal body-hook structure. Since the *flgM* gene is also expressed from the class 3 promoter, its expression is under autogenous control, which may contribute to the fine-tuning of the flagellin expression during filament assembly. FlgN was shown to enhance translation of the *flgM* gene from the class 3 transcript [38]. However, its molecular mechanism remains to be analyzed.

In addition to the *flgMN* operon, three flagellar operons, *fliAZ*, *fliDST*, and *flgKL*, are expressed from both class 2 and class 3 promoters in *Salmonella* (Fig. 1A) [39, 40]. Interestingly, in the *fliAZ* operon, the *fliA* gene is translated efficiently from the class 2 transcript but not from the class 3 transcript owing to its low ribosome-binding activity [41]. This prevents the autogenous activation of the *fliA* gene, which is potentially harmful for cell physiology, since FliA overexpression results in flagellin accumulation within the cell and causes growth retardation [9]. The other genes in these four operons are likely to be translated from both class 2 and class 3 transcripts. As described in section 5, FliZ and FliT act as the transcriptional regulators specific for the class 2 promoter [10]. Dual-promoter control of these two genes may have some regulatory implication. On the other hand, FlgK, FlgL, and FliD are structural components of the flagellum, assembling at the tip of the completed hook structure prior to filament assembly [42, 43], while FlgN, FliT, and FliS are known to be secretion chaperones for FlgK.
and FlgL, FliD, and FliC, respectively [44, 45, 46, 47]. Therefore, these proteins should act at the point just after completion of the basal body-hook structure and during filament formation. This may be the reason why these genes are transcribed from both class 2 and class 3 promoters. In *E. coli*, another flagellar operon fliLMNOPQR was also reported to be transcribed from both class 2 and class 3 promoters [48]. However, its biological significance is obscure.

It should be noted that FlgN and FliT are dual-function proteins, that is, FlgN is a positive regulator of flgM translation and a secretion chaperone for FlgK and FlgL, while FliT is a negative regulator for class 2 transcription and a secretion chaperone for FliD. This suggests connections between gene regulation and protein export. This is discussed further in section 5, focusing on FliT-mediated control of the class 2 operons.

5. Anti-FlhD₄C₂ factors

The class 2 promoter is under positive and negative control by FliZ and FliT, respectively, in *Salmonella* [10]. These two regulators are both encoded within the flagellar regulon and involved in the activity control of the FlhD₄C₂ complex.

FliT was shown to act as an anti-activator, which binds to FlhD₄C₂ through interaction with the FlhC subunit and inhibits its binding to the class 2 promoter [49]. According to this mode of action, FliT is called an anti-FlhD₄C₂ factor. As described above, FliT was shown also to bind to FliD and act as its export chaperone [45]. Since FliD is exported only after completion of the basal body-hook structure [50], FliD is accumulated within the cell before completion of the basal body-hook structure and binds to FliT to inhibit its anti-FlhD₄C₂ activity. This facilitates the expression of the class 2 operons leading to enhanced assembly of the basal body-hook structure. Once the basal body-hook structure is completed, FliD is exported out of the cell and FliT, now freed from FliD, binds to FlhD₄C₂ to inhibit the expression of the class 2 operons. This may contribute to the fine-tuning of the class 2 gene expression in response to the stage of flagellar assembly [49, 51]. FliT was also shown to bind to another flagellar chaperone, FliJ [52]. This suggests a possibility that FliJ together with its cognate substrates may also affect FliT-mediated control of the class 2 gene expression.

The fliZ mutation results in marked reduction of the class 2 gene expression in low-nutrient conditions [10], though its effect is not so obvious in rich-nutrient conditions [53]. FliZ is not required for *in vitro* transcription from the class 2 promoter [12, 13], suggesting that FliZ affects indirectly the activation process of the class 2 operons. It was reported that the fliZ
mutation decreased the FlhC level [53], suggesting that FliZ is a posttranslational activator of FlhD4C2. Recently, it was shown that FliZ is a repressor of another anti-FlhD4C2 factor gene, ydiV [54].

The ydiV gene does not belong to the flagellar regulon, and the YdiV protein was described first as one of the EAL domain proteins that are known to be involved in metabolism of a secondary messenger molecule, cyclic di-GMP (c-di-GMP) [55]. However, YdiV is now believed to have no direct role in its turnover [56]. Instead, YdiV acts as an anti-FlhD4C2 factor [57]. Translation of the ydiV gene is enhanced in low-nutrient conditions, and the expressed YdiV protein binds to FlhD4C2 and inhibits its binding to the class 2 promoter. In contrast with FliT, which interacts with FlhC [49], YdiV interacts with the FlhD subunit [57]. This suggests that FliT and YdiV function independent of each other in the activity control of the FlhD4C2 complex.

The ydiV gene is expressed by readthrough transcription from the promoters for the nlpC and btuCED genes, which are located just upstream of the ydiV gene [54]. Of these two, the nlpC promoter is repressed by FliZ. Therefore, the fliZ mutants of Salmonella show reduced motility in low-nutrient conditions where the ydiV gene is translated efficiently. Because YdiV regulates negatively FliZ expression and vice versa, the ydiV and fliZ genes form a regulatory loop, which results in a bi-stable expression profile in switching between highly and weakly motile states in response to nutrient availability.

6. Environmental control of the flagellar regulon

Bacteria encounter a variety of environmental conditions during their life cycle, and motility and chemotaxis play a crucial role in their adaptation to environments. These external factors affect not only motility control via the chemotaxis system but also the flagellar biogenesis process via the expression control of the flagellar regulon, since bacteria invest huge amounts of energy in synthesis and operation of this large and redundant organelle. Environmental factors that have been shown to affect flagellar synthesis in E. coli include nutrient condition, high temperature, high concentration of salts, extreme pH, and so on [58]. Most of them are believed to affect flagellar synthesis via the expression control of the flhDC operon or the activity control of the FlhD4C2 complex. These controls involve perception and transduction of the external signals leading to modulation of the flagellar gene expression. However, their exact mechanisms remain largely unknown.

Among the environmental controls of flagellar synthesis, nutritional control has been well characterized at a molecular level in E. coli and
Salmonella. E. coli cells upregulate flagellar synthesis in low-nutrient conditions [59, 60]. This regulation is mediated via the transcriptional control of the flhDC operon by cAMP-Crp complex. Interestingly, however, Salmonella cells downregulate flagellar synthesis in low-nutrient conditions [57], though the flhDC operon of this bacterium is also under the positive control of cAMP-Crp [8, 17]. As described in section 5, this downregulation is mediated by an anti-FlhD4C2 factor YdiV, which is expressed in great amounts in low-nutrient conditions and inhibits transcription of the class 2 operons [57]. E. coli also possesses the ydiV gene [61], but its expression or function seems to be attenuated. These opposite effects of nutrient conditions on flagellar synthesis reflect the difference in lifestyle between these two bacterial species. Unlike E. coli, Salmonella is an intracellular pathogen [62]. After invasion into the host epithelial cells, Salmonella cells reside within a Salmonella-containing vacuole, where the nutrient level is low [63]. In this condition, flagellar synthesis is turned off by YdiV-mediated FlhD4C2 inhibition, which may offer a survival advantage to Salmonella cells, since flagellin is a major antigen against host immune system.

7. Global control of the flagellar regulon

An increasing number of global regulators have been shown to affect flagellar gene expression. The regulators that are known to be directly involved in expression control of the flagellar genes are summarized in Table 1. Like the environmental control of the flagellar gene expression, their primary target is the flhDC operon or its products in most cases. They are believed to act as mediators in specific environmental controls of the flagellar gene expression, but their relationships are largely unknown. In addition to the regulators listed in Table 1, dozens of regulators have been shown to affect flhDC transcription. Some have negative effects on flhDC transcription (CitB, GrlR, IHF, Lrp, RpoS, SdiA, and SirA in E. coli; EcNR, FimZ, PeflSrd, RpoS, SdiA, and SirA in Salmonella), while others have positive effects on flhDC transcription (ApaH, ArcA, AtoS, Fnr, GalU, H-NS, MdoA, MsqR, PgsA, Psd, and Pss in E. coli; ArcA, Fnr, Hha/YdgT, and H-NS in Salmonella). Many of these are likely to affect the flhDC expression indirectly through modulating the activity of other regulators that bind directly to the flhDC promoter region.

The promoter region of the flhDC operon contains binding sites for multiple global regulator proteins. As described in section 6, expression of the flhDC operon is positively regulated by the cAMP-Crp complex in E. coli and Salmonella. This complex binds to the promoter region of the flhDC operon, and the direct interaction between Crp and the α subunit of RNA
### Table 1. Global regulators directly involved in flagellar gene expression in *E. coli* and *Salmonella*.

<table>
<thead>
<tr>
<th>Affected operon</th>
<th>Effect</th>
<th>Primary target</th>
<th>Regulator <em>E. coli</em></th>
<th>Regulator <em>Salmonella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1, 2, 3</td>
<td>Positive</td>
<td><em>flhDC</em> transcription</td>
<td>Crp, QseB*, RpoN*</td>
<td>Crp</td>
</tr>
<tr>
<td>Class 2, 3</td>
<td>Positive</td>
<td><em>flhDC</em> translation</td>
<td>CsrA</td>
<td>Hfq</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td><em>FlhDC</em> maturation</td>
<td>DnaK</td>
<td></td>
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<tr>
<td></td>
<td>Negative</td>
<td><em>FlhDC</em> stability</td>
<td>ClpXP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td><em>FlhDC</em> activity</td>
<td>YdiV</td>
<td></td>
</tr>
<tr>
<td>Class 3</td>
<td>Negative</td>
<td>FliA stability</td>
<td>Lon</td>
<td></td>
</tr>
<tr>
<td>Specific operon</td>
<td>Positive</td>
<td><em>flIC</em> transcription</td>
<td>HosA*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td><em>flE, flIF</em> transcription</td>
<td>CsgD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>motA transcription</td>
<td>CpxR</td>
<td></td>
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</table>

* Regulators whose effects on flagellar gene expression have been reported only in some *E. coli* strains are indicated by asterisks: QseB, enterohemorrhagic *E. coli*; PapX and HosA, enteropathogenic *E. coli*; RpoN, *E. coli* strains carrying an insertion element IS5 upstream of the *flhDC* operon.

polymerase activates its transcription [64]. An osmotic-stress response regulator OmpR negatively regulates the *flhDC* expression in *E. coli* [16]. Consistent with this, two OmpR-binding sites are found in the *flhDC* promoter region of *E. coli*. In contrast, the *Salmonella flhDC* promoter has no OmpR-binding site and is unlikely to be under OmpR control [17].

Mutations in the *hns* gene reduce the *flhDC* expression in *E. coli* and *Salmonella* [17, 65], indicating that H-NS is a positive regulator for flagellar biogenesis. H-NS is known to silence many genes by binding to AT-rich DNA regions [66]. Positive regulation of the *E. coli flhDC* operon by H-NS was shown to be mediated by a LysR-type transcriptional repressor encoded by the *hdfR* gene, whose transcription is repressed by H-NS [67]. Recently, however, another pathway of H-NS-mediated positive regulation of the *flhDC* gene was reported [68]. In this pathway, H-NS represses the *rcsD* gene, which encodes a phosphotransmitter protein involved in phosphorylation of RcsB, and phosphorylated RcsB protein in turn represses the *flhDC* operon [69]. In *Salmonella*, H-NS was reported to be involved in inhibition of the flagellar gene expression under acidic condition [70]. However, the mechanism underlying this regulation remains unknown.

Like YdiV of *Salmonella*, some global regulators are known to affect the class 2 gene expression without affecting *flhDC* transcription (Table 1). The ATP-dependent protease ClpXP negatively regulates class 2 gene expression...
through degradation of FlhD4C2 in *Salmonella* [71]. On the other hand, the RNA-binding protein CsrA regulates positively the class 2 gene expression through enhancing translation of *flhDC* mRNA in *E. coli* [72]. The molecular chaperone DnaK regulates positively the class 2 gene expression through conversion of a premature FlhD4C2 complex into a functional form in *Salmonella* [73]. It should be noted that defects in the *dnaK* gene were also reported to reduce *flhDC* transcription in *E. coli* [74]. This suggests that the DnaK chaperone affects at least two different steps in the expression cascade of the flagellar regulon.

A global regulator that seems to affect specifically the class 3 gene expression was also reported (Table 1). Lon, an ATP-dependent protease involved in degradation of various cellular proteins, was reported to be the main protease responsible for degradation of FliA (σ28) in *E. coli* [35]. This suggests that Lon regulates the class 3 gene expression in this bacterium. However, in *Salmonella*, the *lon* mutation has no significant effect on flagellar gene expression [41].

A few global regulators are known to affect only one specific flagellar operon (Table 1). For example, a phosphorylated form of the envelope-stress response regulator CpxR binds to the promoter region of the *E. coli* motAB-cheAW operon and represses its transcription [75]. This suggests a functional link between the envelope stress-induced processes and the flagellum-specific energy conversion or sensory transduction process.

Interestingly, some global regulators are known to regulate motility by direct interaction with the flagellar rotor protein, FliG. In addition to the positive regulation of *flhDC* transcription, H-NS is directly involved in motility control by binding to FliG to modulate rotation speed in *E. coli* [76, 77]. The secondary messenger molecule c-di-GMP, which is involved in regulation of many cellular processes including biofilm formation [78], is also involved in motility control with the aid of a c-di-GMP-binding protein YcgR, which binds to FliG to slow down flagellar rotation [79, 80, 81]. Since H-NS and c-di-GMP are both known to be mediators of environmental cues, these controls allow bacteria to modulate flagellar motor output and swimming velocity in response to environmental conditions.

### 8. Cellular processes regulated by flagellar regulators

Flagellar regulators are also known to regulate many cellular processes unrelated to flagellar formation and function. The flagellar master regulator, FlhD4C2, or FlhD alone has been shown to regulate a number of non-flagellar genes in *E. coli* [82, 83, 84, 85]. They include genes involved in anaerobic respiration and metabolic pathway. On the basis of the observation that some
*E. coli* flhD mutants showed an altered cell division phenotype [86], FlhD has long been believed to regulate cell division. Recently, however, it was shown that this phenotype is not due to the *flhD* mutation [87]. Therefore, further study is needed to answer this issue, and it is still premature to conclude that FlhD₄C₂ or FlhD is a global regulator.

The flagellum-specific sigma factor, FliA (σ₂₈), is also involved in expression of several non-flagellar genes in *E. coli* [23, 84, 85, 88, 89]. One of these genes, *yjhH*, encodes an EAL domain protein possessing the phosphodiesterase activity responsible for c-di-GMP turnover [56, 90]. Since this nucleotide regulates many cellular processes, FliA can also affect cell physiology in many aspects.

The *fliZ* mutation has been shown to impact virulence-related phenotypes in *Salmonella*. The *fliZ* mutation decreases the transcriptional expression of *Salmonella* pathogenicity island (SPI) 1 genes [91] through affecting the activity of their positive regulator HilD [92, 93]. The *fliZ* mutation increases type 1 fimbrial gene expression through affecting posttranscriptionally the activity of the positive regulator, FimZ [94, 95]. These two effects were observed in rich-nutrient conditions, suggesting that these controls are not mediated by YdiV. In *E. coli*, FliZ was shown to affect curli expression by interfering with RpoS (σ⁵) [90]. However, the precise mechanisms underlying these FliZ-mediated controls are largely unknown.

### 9. Structure and expression of the flagellar regulon in *B. subtilis*

Most of the flagellar genes of *B. subtilis* are homologous to those of *Salmonella* and *E. coli*. However, their chromosomal organization and regulon structure are quite different from those of *Salmonella* and *E. coli* (Fig. 1B, Fig. 2B). A total of 31 flagellar genes constitute a single operon called the *fla/che* operon, which is transcribed by RNA polymerase containing the vegetative sigma factor, σ₄, homologous to σ⁷₀ of *E. coli* and *Salmonella* [96]. This operon contains the genes for construction of the basal body-hook structure as well as a gene for σ⁵ homologous to σ₂₈ of *Salmonella* and *E. coli*. Therefore, this operon corresponds to class 2 of *E. coli* and *Salmonella*, though it contains also the *che* genes, which belong to class 3 in *E. coli* and *Salmonella*. Importantly, *B. subtilis* lacks genes corresponding to *flhD, flhC, fliZ, and ydiV* of *Salmonella* and *E. coli*, suggesting that the *fla/che* operon of *B. subtilis* is regulated by a mechanism different from that of the class 2 operons of *Salmonella* and *E. coli*. In addition to σ₄-RNA polymerase, σ⁵-RNA polymerase was shown to
contribute to transcription of the \( \text{fla/che} \) operon [97]. \( \sigma^D \)-RNA polymerase directs expression of several operons responsible for filament formation and motor function [98, 99, 100]. Therefore, these operons correspond to class 3 of *Salmonella* and *E. coli*. *B. subtilis* also possesses the \( \text{flgM} \) gene, which is expressed under the control of \( \sigma^D \). Like in *Salmonella*, FlgM binds to \( \sigma^D \) and inhibits expression of the \( \sigma^D \)-dependent genes [101]. However, FlgM secretion through the completed basal body-hook structure has not been demonstrated in *B. subtilis*.

A response regulator, DegU, is believed to play a crucial role in the checkpoint control of the *B. subtilis* flagellar regulon in response to the state of basal body assembly [102, 103]. It was inferred that the activity of the cognate sensor kinase, DegS, is regulated by assembly state of the basal body structure. The unphosphorylated form of DegU activates the \( \text{fla/che} \) operon, while its phosphorylated form activates the \( \text{flgM} \) operon. Two protein factors, SwrA and SwrB, are also involved in expression control of the flagellar regulon. SwrA is a cytoplasmic protein encoded outside the flagellar regulon and regulates the \( \text{fla/che} \) operon positively, while SwrB is a membrane protein encoded by the \( \text{fla/che} \) operon and positively regulates the \( \sigma^D \)-dependent operons. However, the molecular mechanism underlying this checkpoint control remains unclear.

Recently, another checkpoint mechanism following hook completion was reported in *B. subtilis* [104]. FliW is known to be a chaperone for flagellin (Hag) in this bacterium [105]. Upon hook completion, Hag is excreted out of the cells, which releases FliW from the Hag-FliW complex. FliW then binds to CsrA, which acts as a translational repressor of the \( \text{hag} \) gene in this bacterium [106]. This binding relieves CsrA-mediated inhibition of Hag synthesis and facilitates filament assembly.

The *B. subtilis* flagellar genes are also subject to nutritional control [107]. The \( \text{hag} \) gene is repressed in early exponential growth and activated as nutrients become low. A GTP-binding protein, CodY, is involved in this regulation [108, 109]. This protein monitors the nutritional state of the cell by sensing the intracellular GTP level, and its GTP-bound form represses the \( \text{hag} \) gene by binding to its promoter region under rich-nutrient conditions.

### 10. Concluding remarks

Flagella provide a competitive advantage to bacterial cells by enabling them to reach most favorable environments. On the other hand, their synthesis and functioning are very expensive for the bacterial cells, and they induce a strong immune response in the host organism. Therefore, the expression of flagellar genes must be highly regulated by physiological and
environmental conditions. The internal structure and local control of the flagellar regulon have been well established owing to extensive and intensive studies in *Salmonella*. However, the mechanisms underlying its environmental and global controls are still poorly understood with a few exceptions.

The cell consists of multiple regulatory networks, among which a considerable body of information is exchanged to coordinate their expression. The studies on bacterial flagellar systems, in which multiple internal and external signals are integrated, provide an excellent model for understanding complex and integrated regulatory networks. Now is the time to know in detail the interactions of the flagellar regulon with other regulatory networks and understand their significance in cell physiology.

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