AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Bacteriology®

MINIREVIEW

Involvement of Two-Component Signaling on Bacterial Motility and Biofilm Development

Birgit M. Prüß

Department of Microbiological Sciences, North Dakota State University, Fargo, North Dakota, USA

ABSTRACT Two-component signaling is a specialized mechanism that bacteria use to respond to changes in their environment. Nonpathogenic strains of *Escherichia coli* K-12 harbor 30 histidine kinases and 32 response regulators, which form a network of regulation that integrates many other global regulators that do not follow the two-component signaling mechanism, as well as signals from central metabolism. The output of this network is a multitude of phenotypic changes in response to changes in the environment. Among these phenotypic changes, many two-component systems control motility and/or the formation of biofilm, sessile communities of bacteria that form on surfaces. Motility is the first reversible attachment phase of biofilm development, followed by a so-called swim or stick switch toward surface organelles that aid in the subsequent phases. In the mature biofilm, motility heterogeneity is generated by a combination of evolutionary and gene regulatory events.

KEYWORDS CheA, *Escherichia coli*, OmpR, RcsB, biofilm, fimbriae, flagella, motility, two-component signaling

Accepted manuscript posted online 22 May 2017

Citation Prüß BM. 2017. Involvement of two-component signaling on bacterial motility and biofilm development. J Bacteriol 199:e00259-17. https://doi.org/10.1128/JB.00259-17.

Editor William Margolin, McGovern Medical School

Address correspondence to Birgit.Pruess@ndsu.edu.

Bacteria colonize a wide variety of environmental niches and survive the challenges associated with them by tightly regulating gene expression. This happens in part by means of a phosphotransfer mechanism employing two-component signal transduction systems (2CSTSs) (for review articles, see references 1 to 5). The prototype 2CSTS consists of a sensor kinase and a response regulator. Most sensor kinases are membrane-bound proteins that autophosphorylate in the presence of ATP at a conserved histidine residue, from which the phosphoryl is transferred to a conserved aspartate in the response regulator (6). Nonpathogenic strains of *Escherichia coli* K-12 possess 30 histidine kinases and 32 response regulators (7), of which the majority control gene expression, primarily at the level of transcription. As specific examples of systems that follow the two-component mechanism and impact motility and biofilm, EnvZ/OmpR controls the response to changes in osmolarity (8), RscC/RcsD/RcsB (referred to as RcsCDB throughout this article) activates colanic acid synthesis (for a review, see reference 9), CheA/CheY/CheB controls the direction of flagellar motor rotation (for an early review, see reference 10), and QseC/QseB connects quorum sensing with motility, biofilm development, and virulence (11).

This minireview article focuses on the impact of 2CSTS signaling on the expression of motility and biofilm genes. Special emphasis will be on the involvement of motility in the early phases of biofilm development and the swim or stick switch that allows bacteria to transit from reversible to irreversible attachment. The concept of motility heterogeneity as one mechanism of niche adaptation in the mature biofilm will be developed. Specific examples of coupling mechanisms between motility and/or biofilm and pathogenesis are included for pathogenic *E. coli* and a small selection of other bacterial pathogens.

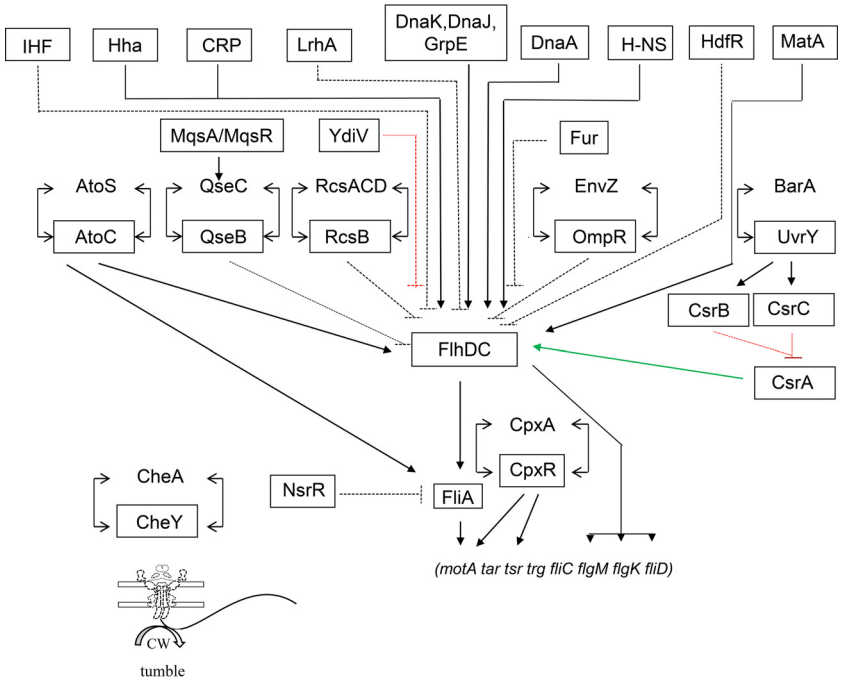

FIG 1 Global control of flagella. This figure has been modified from a previous review article (142). Positive effects on transcription are indicated by black solid lines and arrowheads; negative effects on transcription are indicated by black dashed lines with blunt ends. The green arrow indicates positive posttranscriptional regulation, and the red dashed lines with blunt ends indicate negative nontranscriptional regulation. Regulators that exert a direct effect on their targets are shown in boxes. The control of flagellar rotation by the CheA/CheY system is included in the figure. The flagellar motor complex is reprinted from reference 143 (CW, counterclockwise). All global regulators, as well as response regulators of 2CSTSs, are boxed. Sensor kinases are not boxed. (Adapted from reference 142.)

Motility and biofilm. In *E. coli*, motility requires the synthesis of flagella, which is controlled in part by a 2CSTS. A three-tier hierarchy controls transcription of some 50 flagellar genes in 14 operons and includes numerous regulatory proteins; tier I is the *flhDC* operon that encodes FlhD and FlhC (12) and is required for the transcription of all other flagellar genes. Given this central role and the high energetic cost of flagellar synthesis, it does not come as a surprise that *flhDC* undergoes complex and precise regulation in response to a myriad of environmental conditions. Regulation of the *flhDC* operon by 2CSTSs and other regulators is depicted in Fig. 1.

Motility is not just a way by which bacteria move from one location to another. It is also the first phase of biofilm development. The phasing model was first described for *Pseudomonas aeruginosa*. In this model, biofilm development starts with reversible attachment, followed by irreversible attachment, maturation, and dispersal (13). Flagella are recognized as the cell surface organelle for reversible attachment; type I fimbriae, pili, and curli were proposed to aid irreversible attachment, and the extracellular polymeric substance (EPS) facilitated maturation (for a review, see reference 14).

The phasing model for biofilm development includes the so-called swim or stick switch, where an individual bacterium can either swim (reversible attachment) or stick (irreversible attachment and maturation). Switching requires surface sensing (for a review, see reference 15), followed by a transition from the expression of flagella to the synthesis of fimbriae, pili, and/or curli and finally the production of EPS. Obviously, such a timely synthesis of cell surface organelles is dependent on precisely coordinated regulation of gene expression (16), some of which is mediated by 2CSTS signaling.

EnvZ/OmpR inversely regulates transcription of *ompC* and *ompF*, as well as flagellar genes and genes encoding type I fimbriae and curli. The EnvZ/OmpR system was first discovered as part of the response to changes in osmolarity. Such changes are detected by the sensor kinase EnvZ, leading to autophosphorylation at a

conserved His (17). This event is followed by a phosphotransfer to the response regulator OmpR at a conserved Asp (18). The resultant OmpR-P modulates the reciprocal synthesis of the outer membrane porins OmpF and OmpC (19–21). The first evidence that OmpR-P controls transcription of the *flhDC* operon came from Shin and Park (22) who found that the increase in the binding affinity for the *flhD* promoter by the phosphorylation of OmpR was approximately 10-fold. At the same time, research by Prüß and Wolfe (23) showed that acetyl phosphate, a metabolic signal that *E. coli* can use to phosphorylate OmpR (22), negatively affected *flhDC* transcription. In addition, a knockout mutation in *ompR* exhibited increased *flhDC* transcription in a growth phase-independent manner (24). An *ompR* mutant also exhibited a lack of temporal expression from the *flhD* promoter during biofilm development (25).

In addition to porin and flagellar genes, OmpR also impacts the transcription of the type I fimbria genes (26). The eight genes that are required for type I fimbriae form one large operon (27), including the *fimA* gene that encodes the major structural subunit (28) and the *fimE* and *fimB* genes that encode the recombinases for phase variation (29). These two proteins invert a 314-bp switch element designated *fimS* that is located upstream of *fimA* and flanked by inverted repeats. Whereas FimB facilitates switching in both directions, FimE preferentially switches from "phase-on" to "phase-off" (30). OmpR binds to the *fimB* promoter; a mutation in *ompR* exhibited increased *fimB* transcription relative to the parent, as well as a "phase-on" orientation of the switch element, in agreement with an increased level of type I fimbria synthesis (31).

The major regulator for curli, another irreversible attachment organelle, is CsgD that controls both of the curli-encoding operons *csgAB* and *csgDEFG* (32). Transcription of *csgD* is also activated by EnvZ/OmpR (33). Altogether, EnvZ/OmpR contribute to the transition from the reversible to the irreversible attachment phase in biofilm development by inhibiting *flhDC* and increasing the synthesis of type I fimbriae and curli.

RcsCDB is a positive regulator of colanic acid synthesis and a negative regulator of flagellar and curli genes. RcsCDB was originally described as an activator of colanic acid (34), one of the extracellular polymeric substances that contribute to maturation of a developing biofilm. While RcsCDB follows the two-component signaling mechanism, it consists of more than two proteins. The hybrid kinase RcsC autophosphorylates at the conserved His. Phosphorelay ensues by transfer of the phosphoryl group to the conserved Asp in the receiver domain of the same molecule. The phosphoryl group is then transferred to the conserved His in RcsD, ultimately phosphorylating the response regulator RcsB at the conserved Asp (35–37). RcsB homodimer formation is required to activate transcription of the *wza* operon that encodes the colanic acid genes and was originally designated *cps* (38).

RcsCDB does not just contribute to the transition from reversible/irreversible attachment to the mature biofilm by activating colanic acid synthesis. It also downregulates *csgD* (39) and *flhD* (40). Regulation of *flhD* requires heterodimer formation of RcsB with the auxiliary protein RcsA, followed by binding to the conserved RcsAB box on the *flhD* promoter (40). Heterodimer formation can occur with a range of transcriptional regulators (41–43), often in a way that is unaffected by the phosphorylation state of RcsB (43, 44) and instead favored at high concentrations of the protein (45). Altogether, the RcsCDB system has a myriad of functions in *E. coli* and other members of the family *Enterobacteriaceae* (9, 46–49).

QseC/QseB connects quorum sensing with motility, biofilm, and virulence gene expression. The first function that was attributed to QseC/QseB was its involvement in the regulation of flagellar genes by quorum sensing in *E. coli* K-12 and enterohemorrhagic *E. coli* (EHEC) (11). QseB was shown to bind to the *flhD* promoter directly at high- and low-affinity sites, and transcription initiation appeared dependent on the sigma factor FliA (50). Differences between mutations in *qseB* and *qseC* pointed toward the phosphatase activity of the system to be located on the sensor kinase QseC (51). In particular, deletion of QseC but not QseB attenuated the formation of intracellular communities and virulence by uropathogenic *E. coli* (UPEC). Intriguingly, the attachment organelles type I fimbriae and curli were downregulated alongside with flagella

in the absence of QseC (51). This is different from EnvZ/OmpR and RcsCDB which regulate multiple biofilm-associated cell surface organelles in an inverse manner.

In addition to QseB, the sensor kinase QseC phosphorylates two other response regulators, QseF and KdpE, to control the Shiga toxin gene stx_2 and the LEE (locus of enterocyte effacement) pathogenicity genes, respectively (52). Site-directed mutagenesis of the QseC periplasmic sensing domain yielded four mutants that all exhibited increased levels of phosphorylation but differed in their motility, LEE, and Shiga toxin expression phenotypes (53). The mutants also differed in their abilities to phosphorylate QseB, KdpE, and QseF. It was concluded that the mutations influenced the phosphotransfer preference of QseC (53).

In *E. coli* O157:H7, QseC/QseB constitutes a remarkable connection between the host stress response and bacterial environmental signaling, for which it has earned the term "adrenergic receptor" (54). When living in the intestine, *E. coli* responds to the autoinducer-3 that is produced by the gut flora and the human stress hormones epinephrine and norepinephrine (for a review, see reference 55). This trio of signals has been described as an "interkingdom chemical signaling system" (55). The signaling transduction cascade includes chemotaxis by using the serine receptor Tsr (56, 57) and activation of QseC (58). In the search for novel therapeutic targets and drugs, blocking the binding of epinephrine or norepinephrine to QseC with a proposed LED209 drug resulted in decreased QseC/QseB signaling and reduced motility and virulence (59), as well as attenuation of colitis in mice (60).

BarA/UvrY controls the expression of genes of metabolism, motility, biofilm, and virulence. The BarA/UvrY 2CSTS regulates genes important for carbon storage and responds to acetate, formate, and carboxylates (61). UvrY synthesis is enhanced by polyamines (62). The signal is transmitted by activating the transcription of genes for small regulatory RNA (sRNA) molecules. sRNAs have gained increasing attention in recent years as powerful regulators of transcription (for a recent review, see reference 63). In the case of BarA/UvrY, strong interaction was observed between UvrY-P and *csrB* and *csrC* (64). These encoded sRNAs are part of the carbon storage system. Together with the RNA-binding protein and posttranscriptional regulator CsrA, they were initially described as modulators of gene expression when bacteria transit into stationary phase (for a review, see reference 65). CsrA is a repressor of stationary-phase genes, and it also activates *flhDC* (66). The fact that the activating effect of CsrA on *flhDC* is not on the transcriptional level but on the posttranscriptional level is indicated by a green arrow in Fig. 1. The inhibitory effect of the sRNA molecules CsrB and CsrC on CsrA is one of sequestration. Multiple binding sites for sRNA on CsrA enable CsrB and CsrC to act as a CsrA sink (65). This sequestration effect is distinguished from transcriptional regulation by red lines in Fig. 1. Likewise, the negative effect of the phosphodiesterase YdiV on FlhD/FlhC is indicated by the red dashed line with a blunt end. This effect is one of posttranslation, where YdiV binds to the FlhD/FlhC complex at high concentrations to inhibit its transcriptional activity (67).

As a final 2CSTS in the context of regulation of *flhDC* transcription, AtoS/AtoC facilitates the response to acetoacetate, spermidine, and histamine via enhancing transcription of *flhD* and *fliA* (68). This 2CSTS may expand the range of nutrients that *E. coli* is able to utilize.

Many regulatory proteins act upon *flhDC* transcription beyond regulation by 2CSTSs (Fig. 1). These regulatory proteins are LrhA (69), H-NS (70), HdfR (71), integration host factor IHF (72), Hha (73), the toxin/antitoxin system MqsA/MqsR (74) through QseB, MatA (75), and the iron response regulator Fur (76). Temperature regulation of *flhDC* transcription is complemented by DnaA (77) or the action of DnaK, DnaJ, and GrpE (78). Metabolic control of the *flhDC* operon includes catabolite repression through cyclic AMP (cAMP)-cAMP receptor protein (CRP) (79). Additional sRNAs that impact *flhDC* expression in a negative way are ArcZ, OmrA, OmrB, and OxyS; McaS exerts a positive effect (80). Note that these five regulatory molecules are not included in Fig. 1.

Additional downstream regulation of flagellar genes. While it may make sense to control a complex hierarchy at the top level, fine tuning transcription can take place at other levels. In particular, the tier II gene *fliA* has emerged as a second center of regulation (Fig. 1). Specifically, the nitric oxide-sensitive repressor NsrR negatively regulates the *fliA* promoter, as well as attachment (81). A 2CSTS that acts upon the transcription of tier III genes is CpxA/CpxR. This 2CSTS responds to envelope stress, inhibits LEE gene transcription (82), and activates the synthesis pathway that leads to the production of an antimicrobial peptide that causes multidrug resistance (83). Direct binding of CpxR-P to the promoter regions of the *motA* and *tsr* operons has been shown (84) and so has transcriptional repression of *csgD* by CpxR (33, 85). This makes CpxA/CpxR another 2CSTS that inversely regulates flagellar and curli genes.

As a final 2CSTS to be discussed, CheA/CheY affects the direction of rotation of the flagellar motor (Fig. 1). This 2CSTS functions with a second response regulator, CheB, as well as many other chemotaxis proteins to control bacterial motility toward an attractant or away from a repellant by modulating the direction of flagellar rotation (for a recent review on chemotaxis, see reference 86). Since CheA lacks the classical transmembrane domain, it associates with a distinct set of transmembrane proteins known as methyl-accepting chemotaxis proteins (for a recent review, see reference 87) and an adaptor protein CheW (88–91). The response regulator CheY is a single-domain molecule, whose structure has been determined in its unphosphorylated (92) and phosphonated (93) form, in complex with CheA (94, 95), and bound to its FliM target (96). Phosphorylation of CheY enables its binding to FliM at the flagellar motor (96–98), where it causes clockwise rotation of the flagella, resulting in cell tumble and change in the direction of motility (99, 100).

An additional regulatory mechanism that may not involve the control of gene expression and is not included in Fig. 1 is the interaction of H-NS with the flagellar rotor protein FliG. Direct interaction of H-NS with FliG was first seen by using a yeast two-hybrid system (101). Later, it was shown that this interaction increased the flagellar rotational speed and caused hypermotility (102). More-recent research, however, indicates that the H-NS effect on motility happens via an indirect and complex route (103).

What does metabolism have to do with it all? Some response regulators can receive signals not only from their cognate kinase but also from central metabolism. An example of such a metabolic intermediate is acetyl phosphate which stands between acetyl coenzyme A (acetyl-CoA) and acetate (104) and accumulates when the flux of carbon through glycolysis is high (105). Acetyl phosphate can phosphorylate OmpR (22) and other response regulators (106–108). This constitutes a mechanism of activation that links central metabolism with signal transduction (109). In one specific example of an *in vivo* effect of acetyl phosphate, a depletion of serine from a mixed amino acid growth medium by *E. coli* K-12 was linked to a reduction in *flhDC* transcription, presumably through the production of acetyl phosphate and increased levels of OmpR-P (110). Serine depletion simultaneously resulted in an increase in motility and a decrease in the cell division rate (110). With respect to biofilm development, acetyl phosphate (22, 23) acts at the swim or stick switch. In cases of low acetyl phosphate levels, OmpR-P is low, transcription from the *flhD* promoter is high, and bacteria are highly motile. This scenario resembles reversible attachment. In contrast, under conditions of high acetyl phosphate levels, OmpR-P is high, *flhDC* transcription is low, and motility is low (22, 23), but type I fimbriae are expressed (109). This scenario resembles irreversible attachment. The metabolic control of the swim or stick switch is in agreement with a model that was developed a few years after the phasing model and described the formation of biofilm as an output of the entire metabolic and gene regulatory network of the bacteria (111).

Interestingly, RcsB (112, 113) and CheY (114) can also be acetylated, using either acetyl-CoA or acetyl phosphate as donor of the acetyl group (115, 116). In the case of *E. coli* CheY, acetylation happens at two specific lysine residues, K91 and K109 (117). Acetylated CheY (CheY-ace) binds only to the low-affinity CheY-binding sites of FliM at

the flagellar rotor. The cooperative binding of CheY-P to these sites and the synergistic effect of acetylation and phosphorylation on CheY activation suggest that binding of CheY-ace increases the affinity for CheY-P (O. Afanzar and M. Eisenbach, personal communication). For a recent review on protein acetylation, see reference 118.

A second messenger molecule that does not involve 2CSTS signaling is cyclic di-GMP (c-di-GMP). This molecule helps facilitate the swim or stick switch (119) by inhibiting flagellar synthesis and activating the synthesis of curli and cellulose (for reviews, see references 120 and 121). Cellular levels of c-di-GMP result from the interplay of two groups of enzymes, diguanylate cyclases (122) and phosphodiesterases (123). In the case of the flagellar system, the diguanylate cyclase YegE is under the control of the stationary-phase sigma factor σ^S and increases the level of c-di-GMP (124). The phosphodiesterase YhjH is under the control of the flagellar sigma factor FliA and decreases c-di-GMP levels. c-di-GMP itself is an inhibitor of flagellar synthesis and an activator of curli through their master regulator CsgD (124). Activation of cellulose synthase involves the diguanylate cyclase YaiC and the phosphodiesterase YoaD (125).

Motility and biofilm, a paradox or not? There is an apparent paradox included in the previous paragraphs. On one hand, the proposal of flagella as the cell surface organelle for reversible attachment is consistent with the idea of motility being an advantage for bacteria forming a biofilm. However, the concept of the swim or stick switch makes motility and biofilm appear like two mutually exclusive processes. The question arises whether motility is an advantage or a disadvantage when bacteria form a biofilm.

The solution to this apparent paradox may lie in the distinction between an advantage for the individual cell (swim or stick) and an advantage for the population (reversible attachment as a phase). What constitutes an advantage for an individual bacterium at a specific time and/or location may differ from what is beneficial for the entire population. To permit such niche adaptation, bacteria have evolved diverse mechanisms that generate motility heterogeneity within their population. *E. coli* couples evolutionary events with control of gene expression by multiple 2CSTSs. The evolutionary event is a selection for mutations in the *flhDC* operon. Among the many possible types of mutations, IS elements in particular are known to lead to changes in gene expression and phenotypes (for a review, see reference 126). The first such study regarding *E. coli* motility was by Barker and coworkers (127), who found an insertion of IS*5* at bp −99 to −96 from the *flhDC* transcriptional start in an originally poorly motile version of *E. coli* MG1655 Fnr$^-$ that now exhibited a 2.7-fold increase in the rate of migration on motility plates. Interestingly, Wang and Wood (128) identified the same insertion (IS*5* at −99 to −96) in the *flhD* promoter of strain BW25311 that increased motility and biofilm amounts. Lee and Park found IS elements up to 315 bp from the *flhDC* transcriptional start (71), and IS elements identified by Fahrner and Berg were identified up to bp −476 (129). These mutations are summarized in Fig. 2A and have the following characteristics in common. (i) They were selected from a previously nonmotile *E. coli* K-12 strain. (ii) They carried the IS element 96 or more bp upstream of the *flhDC* transcriptional start. (iii) They were recovered under conditions where motility was an advantage.

A fifth study on IS elements in the *flhD* promoter by Horne and coworkers (130) reported the isolation of nonmotile derivatives from biofilm of the originally highly motile MC1000 strain. The locations of these mutations are included in Fig. 2A and are all further downstream than the mutations that increased motility. The types of mutations include small deletions, point mutations causing frameshifts and truncations, and insertions of IS elements. Many of these mutations are in the open reading frames for *flhD* or *flhC*, others are in the ribosome-binding site or the −10/−35 sites for the RNA polymerase. The conclusion from this study was that mutations in the *flhDC* operon can occur in many places and that the location of the mutation determines whether the resulting *E. coli* is more or less motile than the original strain. The authors of this article (130) believe that mutagenesis of the *flhDC* operon may be a mechanism

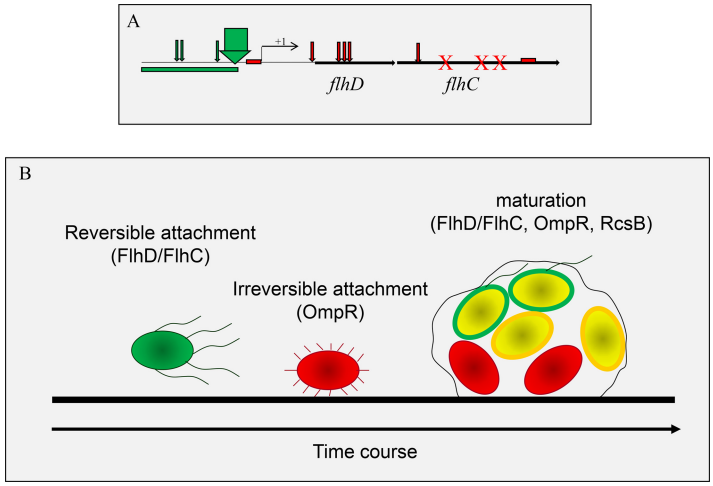

FIG 2 Generation of motility heterogeneity by *E. coli*. (A) Demonstration of the mechanism by which *E. coli* generates motility heterogeneity by means of mutating the *flhDC* operon. The big green arrow depicts the IS*5* at positions −99 to −96 (127, 128). Other IS elements that rendered *E. coli* hypermotile and were found by Lee and Park (71) are indicated by slim green arrows. The green bar indicates the region from positions −476 to −100 where Fahrner and Berg (129) found IS elements. The mutations found by Horne and coworkers (130) are indicated by red symbols. The red boxes indicate small deletions, the X letters depict point mutations, and the red arrows show insertion sites of IS elements. The mutations found by Horne et al. (130) rendered *E. coli* nonmotile. (B) Map of temporal and spatial expression patterns onto biofilm development. Transcription from the *flhD* promoter is indicated in green, *ompR* transcription is indicated in red, and *rcsB* transcription is indicated in yellow. For reversible and irreversible attachment, cell surface organelles are drawn in a slightly darker shade of the base color. For the mature biofilm, bacteria on the outermost edge of the biofilm are multicolored based on the spatial transcription data for *flhD* and *rcsB*. Note that the spatial distribution of transcription was determined across the biofilm and not across individual bacteria.

by which *E. coli* generates motility heterogeneity within their biofilm and that this heterogeneity constitutes a selective advantage for the total population of bacteria that form the biofilm.

This hypothesis is supported by a study by Samanta and coworkers, who performed temporal and spatial expression studies with the *flhD* promoter from the *E. coli* K-12 strain AJW678 that did not contain an IS element (25). The temporal transcription pattern of *flhD* was highest at 12 h and lowest at 35 h and increased again toward 51 h. The temporal profile for *ompR* was the inverse of that for *flhD*, and transcription of *rcsB* increased steadily throughout biofilm growth. Temporal expression patterns were used to map gene expression to biofilm phases, where *flhD* was an indicator of reversible attachment, *ompR* represented irreversible attachment, and *rcsB* was indicative of maturation (Fig. 2B). In the mature biofilm, *flhDC* transcription was highest at the outermost edge of the biofilm and lowest near the attachment surface. The spatial transcription of *ompR* was highest at the attachment surface and decreased with increasing distance from the surface, whereas transcription of *rcsB* increased toward the edge of the biofilm (131). Temporal and spatial patterns of *flhD* expression were abolished in *ompR* and *rcsB* mutants (25).

This minireview will conclude with two examples where motility heterogeneity has implications outside biofilm development. In one study, the authors deliberately generated heterogeneity at the level of phenotype and gene expression by exposing *E. coli* to several antibiotics, allowing them to perform a process they termed "mid-term adaptation"; among the genes that were described as "transcriptome-level signatures" were genes of the motility apparatus, the *cps* operon, and *ompR* (132). As a second example from *Salmonella enterica*, motility heterogeneity aids virulence. The *fliZ* gene encodes a posttranslational activator of FlhD/FlhC (133). The *fliT* (134) and *ydiV* (135) genes are tier III flagellar genes encoding anti-FlhD/FlhC factors. The competitive action of YdiV and FliZ generates subpopulations of motile *fliC*-ON and nonmotile *fliC*-OFF bacteria within the population (136). YdiV and FliZ constitute a nutrient-tunable

bistable switch that allows bacteria to express different phenotypes under identical growth conditions (137). It promotes a selective advantage during the infection process because it permits a division of labor, where motile cells are pathogenic and nonmotile cells serve as reservoir to feed the infection (138).

Conclusion and outlook. In summary, 2CSTSs provide the bacteria with a complex network of regulation that controls motility, biofilm development, and pathogenesis and is responsive to a large variety of signals from the environment. An intriguing unanswered question in this context is why motility and biofilm are so often reciprocally regulated by the same 2CSTS but sometimes appear coregulated. The answer to this question may feed into the growing recognition that the molecular response to environmental signals can take place at the level of a whole bacterial community, as well as at a single-cell level. The phenotypic heterogeneity that results from the latter is one mechanism by which bacteria can perform niche adaptation. Among the future aspects of this type of research is an investigation of phenotypic heterogeneity in natural communities, including symbiotic (139) and pathogenic (140) relationships with hosts and multispecies environmental communities (141). Such research takes the well-established approach of determining taxonomic relationships between multiple species of the same community ("who is there?") to the level of functionality ("what are they doing?"). In this sense, studying heterogeneity of phenotypic traits within populations removes a current limit to the predictive power of the taxonomic approach (141).

ACKNOWLEDGMENTS

B.M.P. is funded by the North Dakota Agricultural Experiment Station and Hatch project 1009442 through USDA/NIFA.

I thank Preeti Sule (Texas A&M University) for helpful discussions and Shelley Horne and Meredith Schroeder (NDSU) for critically reading the manuscript.

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