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MINIREVIEW

A Complex Transcription Network Controls the Early Stages of Biofilm Development by *Escherichia coli*†

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Historically, researchers have studied bacterial signaling as if it functioned as a set of isolated, linear pathways. More recent studies, however, have demonstrated that many signaling pathways interact and that these interacting pathways should be construed as an intricate network. This network integrates diverse signals, both extracellular and intracellular, to ensure that the the correct amount of the appropriate subset of genes is expressed at the proper time. Complete delineation of this complex signal transduction network and use of the network to predict the full range of cellular behaviors are major goals of systems biology.

Despite considerable progress, we remain near the beginning of this process, which thus far has been dominated by the development of enabling technologies and the compilation of gene lists. Although development and compilation will continue to be essential, the next critical step must be to organize the copious data compiled over 5 decades of pregenomics research and the massive amount of postgenomics data generated over the last decade. This minireview, in which we describe a portion of the overall network of *Escherichia coli*, is an attempt to perform part of this next step.

THE NETWORK

As the model organism for this network, we chose the enterobacterium *E. coli*. We focused specifically on the common laboratory strain K-12 in order to mine the wealth of information available for it. When appropriate, we included observations made with other *E. coli* variants (e.g., enterohemorragic *E. coli* [EHEC] or uropathogenic *E. coli*) or with the close relative *Salmonella enterica*. With easy to moderate effort, the network can be adapted to other enterobacterial relatives. However, more distantly related species may lack some of the global regulators discussed here.

As a unifying theme, we chose the early stages of biofilm development. Defined as a sessile community of bacteria encased in a matrix, a biofilm tends to develop on a surface or an interface in a series of ordered steps, designated reversible attachment, irreversible attachment, maturation-1, maturation-2, and dispersion (121). Each step requires reprogramming of gene expression that occurs in response to the changing environment (122). The reprogramming associated with the earliest steps of biofilm development can be identified easily by the distinct organelles that decorate the bacterial surface. For example, reversible attachment often involves flagella that permit individual planktonic cells to swim toward an appropriate biotic or abiotic surface. Irreversible attachment involves the loss of these flagella and the elaboration of adhesive organelles (e.g., curli or type 1 fimbriae); the type of organelle depends on the environment. Finally, production of the colanic acid capsule permits construction of the distinctive three-dimensional structure typical of mature biofilms (for a recent review of biofilm formation, see reference 149).

For the surface organelles to appear in proper order, expression of these organelles must be coordinately regulated (137). Indeed, there is evidence for regulatory relationships between flagella and fimbriae (10, 75), between flagella and capsule (80, 124, 158), and between different types of fimbriae (52, 159). The coordinate regulation of these surface organelles, whose expression responds to similar subsets of external signals, second messengers, and regulators, is the main focus of this minireview.

The total network consists of 16 regulators and the several hundred genes that they regulate. Some regulators in this network function globally. For example, CRP (162) and H-NS (13, 53) each regulate hundreds of genes. In contrast, some regulators, including LrhA (73) and HdfR (67), affect transcription of only a small number of genes. Some global regulators are members of a family of two-component signal transduction (2CST) pathways, a predominant system used by bacteria to relay environmental signals in order to elicit changes in cellular functions (for reviews of 2CST pathways, see references 36, 61, 100, and 156). Each 2CST pathway consists of a sensor and a response regulator. The sensor, often an integral cytoplasmic membrane protein, is a histidine kinase that uses ATP as its phosphodonor to autophosphorylate a conserved histidine residue (H1). Some sensors also possess phosphatase activity. In contrast, the response regulator is an aspartyl kinase that uses the phosphorylated sensor as its phosphodonor to autophosphorylate a conserved aspartyl residue (D1). Most, but not all, response regulatory domains are fused to a DNA binding domain and thus function as transcription factors. *E. coli* pos-

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sesses 29 histidine kinases and 32 response regulators (80, 86), including EnvZ/OmpR, QseC/QseB (QseCB), and CpxA/CpxR (CpxAR).

Deletions of the genes that encode all these 2CST pathways have been constructed and analyzed by microarray technology. This has been done for both gene expression analysis (97) and phenotypic characterization (163). For example, one analysis showed that the EnvZ/OmpR pathway, initially discovered as a regulator of the outer membrane porins OmpC (79) and OmpF (148), actually regulates a much larger set of genes (97). Another analysis showed that QseCB, identified as a regulator of quorum sensing in EHEC (134), also regulates flagellum biogenesis (27, 135). Other studies showed that the CpxAR system, which was first shown to sense envelope insult (31), also regulates the DNA repair gene *ung* (93), genes encoding the type IV bundle-forming pili (91), and both curli operons (59).

A more complex variant of the 2CST pathway is the multistep phosphorelay, which includes four domains instead of two domains (6, 86). Like the conventional 2CST pathway, the multistep phosphorelay proceeds from the sensor to a response regulator. A histidine phosphotransferase then transfers the phosphate from this first response regulator to a second response regulator. The second response regulator is often a transcription factor. RcsC/RcsD/RcsB (RcsCDB) is one of the few phosphorelays possessed by *E. coli* (6, 86). One of the four domains that comprise the complete phosphorelay, RcsC, contains both the sensor and the first response regulator. RcsD includes the histidine phosphotransferase, and RcsB carries the final response regulator. The signal travels from H1 to D1 on RcsC, then to H2 on RcsD, and finally to D2 on RcsB (for a review of RcsCDB signaling, see reference 80). Originally identified as a regulator of the capsule synthesis genes (*cps*) (43), RcsCDB is now known to regulate up to 5% of the *E. coli* genome (32, 44).

To construct the network, data were gleaned from the literature and/or from work performed in our laboratories. These data came primarily from functional genomics experiments, such as microarray analysis or analysis of genomic libraries of reporter gene fusions, but they also were obtained from direct interaction studies, such as electrophoretic mobility shift assays or DNase I footprint analyses.

To visualize the network, the open source TouchGraph visualization software was adapted as follows: functionality was added to distinguish multiple types of relationships and to systematically select a center of focus. TouchGraph uses the spring layout concept and allows user interactions through focus and context techniques (51). The network is presented in its entirety in the supplemental material (see Fig. S1 in the supplemental material). The remaining information in the supplemental material focuses on areas where there is intense regulation.

Here we summarize the subset of genes most closely aligned with biogenesis of the surface structures associated with the transition from motile, planktonic individuals to a sessile biofilm community (Fig. 1). First, we focus on three biofilm-associated surface organelles (flagella, curli, and type 1 fimbriae) whose biogenesis is controlled by the network. We then shift our attention to the network itself, concentrating on the three most prominent regulators, FlhD/FlhC (FlhDC), EnvZ/OmpR, and RcsCDB. A fourth biofilm-associated structure, the capsule, is mentioned in the context of RcsCDB. Finally, we discuss three small molecules. Two of these molecules, cyclic AMP (cAMP) and acetyl phosphate (acetyl~P), affect the network directly. The third, cyclic di-GMP (c-di-GMP), is included because it influences the formation of biofilms (131) in parallel with the network, although how it performs this function remains unknown.

THE REGULATED PROCESSES

The three major cellular processes regulated by the network are biogenesis of the surface organelles flagella, curli, and type I fimbriae.

Flagellum biogenesis. Flagella enable bacteria to reach favorable environments, and they have functions in adhesion, biofilm formation, and colonization (47). The environmental conditions that control the levels of expression of *flhDC* include temperature (1), osmolarity (128), pH (133), the concentrations of catabolite-repressing carbon sources (161), and a number of small molecules (65, 71, 72, 74, 89, 109, 125, 126), including acetate and propionate (104). The mechanisms by which these factors regulate *flhDC* expression are largely unknown.

In *E. coli* K-12, the transcription initiation site for *flhDC* is located 198 bp upstream of the translation start site for *flhD* (132) (Fig. 2); in EHEC, it is located (27) only 53 bp upstream of the *flhD* open reading frame (132). In *E. coli* K-12, DNA binding sites have been identified for H-NS (132), phosphorylated OmpR (128), LrhA (73), RcsB (33), and CRP (132) (Fig. 2); in EHEC, additional DNA sites have been identified for phosphorylated QseB (27).

Additional experimental evidence indicates that there is transcriptional regulation of *flhDC* by the chaperones DnaK, DnaJ, and GrpE (127), the nucleoid protein DnaA (88, 90), and the transcription factor HdfR (67) (see Fig. S2 in the supplemental material). Furthermore, insertion of insertion elements increases transcription of *flhDC*, presumably by uncoupling upstream binding sites for negative regulators from the core promoter (9). *flhDC* also is regulated posttranscriptionally by the carbon storage regulator CsrA (Fig. 2), which binds to *flhDC* mRNA and increases transcript stability (154). A regulatory RNA, CsrB, sequesters and represses CsrA. A complex regulatory circuit involving UvrY (also known as SirA) regulates CsrB (142). Posttranslational regulation is mediated by protease ClpX/ClpP in *S. enterica* (146, 147).

Curli biogenesis. Curli (also known as thin aggregative fimbriae) are adhesive fibers (115) that promote biofilm formation by facilitating initial cell-surface interactions and subsequent cell-cell interactions (96, 150). The environmental conditions that control curli expression include temperature, oxygen tension, starvation, osmolarity, iron, and pH (39, 106, 117, 141). Because they had not been observed under conditions that mimic the mammalian host environment (i.e., high osmolarity and high temperature) (45, 84, 95), for a long time curli were considered unable to contribute to human infections. A recent report, however, showed that cells can express curli under these conditions, if they are grown under static conditions that facilitate biofilm formation (63). At least eight regulators affect the expression of the curli genes (see Fig. S3 in the supple-

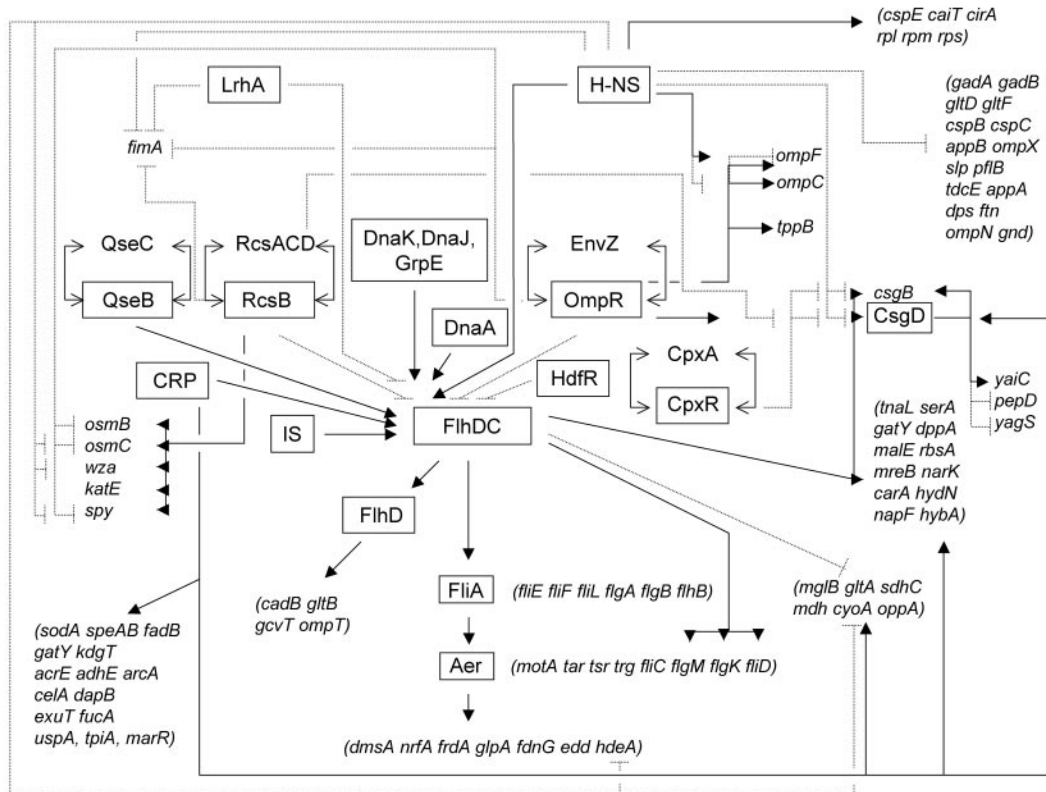

FIG. 1. Global network of transcriptional regulation in *E. coli*. Positive regulatory effects are indicated by solid lines and arrowheads. Negative regulatory effects are indicated by dotted lines with blunt ends. Microarray data were obtained for EnvZ/OmpR (97), RcsCDB (32, 44, 97), LrhA (73), H-NS (53), CRP (162), CsgD (19), FlhD/FlhC (110, 111), FlhD (110, 112), and Aer (110). Further regulation of *flhD* expression has been documented, as follows: QseCB (134, 135), CRP (132), H-NS (14), DnaK, DnaJ, and GrpE (127), DnaA (88, 90), HdfR (67), and insertion element insertion (9). The expression of *csgD* and *csgB* is further regulated by EnvZ/OmpR (106), CpxAR (59, 106), and H-NS (59). FliA has been described as an alternative sigma factor specific for the flagellar genes (78) and mediates the regulation of *aer* expression by FlhD/FlhC (B. Prüß and P. Matsumura, unpublished).

mental material), which cluster in two divergent operons, *csgDEFG* and *csgBA* (45). These regulators include three two-component systems, EnvZ/OmpR (106), RcsCDB (32), and CpxAR (59, 106), and four other regulators, CRP (162), H-NS (7, 53, 95), MlrA (20), and FlhDC (110). Most of these regulators act upon the *csgD* operon (40, 106), which encodes a transcriptional regulator of *csgB*. CsgD also regulates *yaiC*, *yagS*, *pepD* (19), and *glyA* (25). In addition to CsgD, *csgB* expression requires σ^S, an effect enhanced by the small protein Crl (18).

The best-investigated regulation of *csgD* expression involves the interplay between the negative regulator CpxAR and the positive regulator EnvZ/OmpR (59, 106). The phosphorylated forms of both CpxR and OmpR were shown to bind to overlapping DNA sites immediately upstream of the *csgD* promoter (106). CpxR bound cooperatively to six sites within the *csgD* promoter (59). Binding of CpxR and OmpR was not competitive, as both regulators could bind simultaneously. Considering that the expression and the phosphorylation state of CpxR both increased upon a shift to high osmolarity, it was postulated that induction of CpxAR mediates *csgD* repression at high osmolarity, whereas EnvZ/OmpR mediates *csgD* activation at low osmolarity (59).

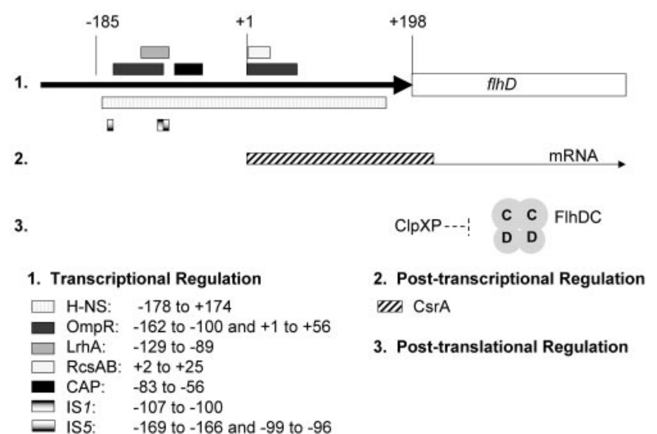

FIG. 2. Regulation of flagella and motility in *E. coli*. Environmental control of the flagellar system is mediated by regulation of the *flhDC* promoter. The translational start site was determined by Soutourina et al. (132). Footprinting data have been obtained for H-NS (132), phosphorylated OmpR (128), LrhA (73), RcsAB (33), and cAMP-CRP (132). Insertion sites for the IS*1* and IS*5* elements are indicated (9). The figure is modified from a previous study (8); posttranscriptional control by CsrA (154) and posttranslational control by ClpXP (146, 147) have been added.

Type I fimbria biogenesis. Type I fimbriae mediate adherence to mannose-containing receptors and promote bacterial attachment to and/or invasion of host cells during urinary tract infections (29, 82). The structural genes (*fimA* to *fimH*) are located in a single large operon (81) that is driven by a single promoter located upstream of *fimA* (123). Expression data indicate that a strong terminator is located immediately after *fimA* (123). Expression of the *fim* operon is controlled primarily by an invertible 314-bp switch element that is located upstream of *fimA* and is flanked by inverted repeats. The inversion, called phase variation, is mediated by two recombinases, FimE and FimB (66). The genes encoding these recombinases are located upstream of the switch element and are transcribed in the same direction as the *fim* operon. Generally, FimB can promote inversion in both directions. FimE, in contrast, promotes only the switch from phase-ON to phase-OFF (34).

Phase variation is subject to tight environmental control, which is mediated by at least six global regulators (see Fig. S4 in the supplemental material). For example, the leucine response protein LrpA mediates the response to amino acids (e.g., alanine, isoleucine, leucine, and valine). LrpA binds directly to the switch, affecting *fimB*- and *fimE*-promoted switching (35). Similarly, IHF affects switching by both recombinases (15), while H-NS affects only the *fimB*-mediated inversion (92). In microarray experiments, LrpA (56) and H-NS (53) had an overall positive effect on the levels of expression of the *fim* genes. Interestingly, LrhA had a positive effect on the level of expression of *fimE* and a negative effect on the level of expression of the *fim* operon (16). This was likely due to the strong bias for phase switching from the phase-ON to the phase-OFF orientation of FimE. Other microarray studies showed that EnvZ/OmpR had a negative effect on the levels of expression of the *fim* operon (97).

THE REGULATORS

Within the network, three regulators (FlhDC, EnvZ/OmpR, and RcsCDB) affect expression of the majority of the genes, primarily the genes involved in the biogenesis of flagella, curli, and type I fimbriae.

FlhDC. Initially described as the master regulator of flagellum biogenesis in *E. coli* and *S. enterica* (68–70, 129, 130), FlhDC also regulates nonflagellar genes (110, 111) (see Fig. S5 in the supplemental material). Encoded by the *flhDC* operon (11), FlhDC sits atop a transcriptional hierarchy of flagellar genes (for reviews of flagellar hierarchy, see references 2, 24, 69, and 108). The FlhDC complex binds the upstream regions of three flagellar operons (*fliA*, *fliL*, and *flhB*) (77) and activates their transcription from σ^{70}-dependent promoters. FlhDC also activates transcription directly from a subset of promoters that depend upon σ^{28}, the product of *fliA*. The remaining σ^{28}-dependent promoters are under indirect control of FlhDC through its activation of *fliA* (50, 78, 94).

Twenty-nine nonflagellar FlhDC-dependent operons in *E. coli* were revealed by microarray analysis (110). Approximately one-half of these operons function in respiration. Transcription of the operons that encode aerobic respiratory pathways was inhibited, while transcription of the operons that encode anaerobic pathways was enhanced. This enhancement, as well as that of the Entner-Doudoroff pathway, was mediated by the oxygen sensor and chemoreceptor Aer (110). In addition, FlhDC enhanced transcription of the two curli operons, *csgB* and *csgD*. Finally, it modulated transcription of a number of genes encoding transporters and enzymes involved in amino acid metabolism.

In addition to this experimental evidence, bioinformatic analysis suggests that there are additional FlhDC targets. A consensus sequence for putative FlhDC binding sites was developed and used to identify putative targets (136). The promoter regions of four of these genes (b1904, b2446, wzz_{fepE}, and *gltI*) showed both binding and regulation by FlhDC. In addition, a FliA consensus sequence was proposed and used to identify several putative FliA targets (99). Two of these targets (*ygbK* and *ppdAB*) also were dependent on FlhDC, as determined with promoter-*lacZ* fusions.

Envz/OmpR. EnvZ/OmpR, a two-component signal transduction pathway originally shown to regulate expression of the outer membrane porins OmpF and OmpC (79, 148), also controls expression of more than 100 nonporin genes (97) (see Fig. S6 in the supplemental material). EnvZ/OmpR regulates transcription of *ompF* and *ompC* inversely; at low osmolarity, it activates *ompF*, and at high osmolarity it represses *ompF* while activating *ompC* (4). To activate transcription, OmpR binds three tandem sites upstream of and proximal to the −35 hexamers of both *ompC* (C1 to C3) and *ompF* (F1 to F3) (55, 79, 87, 148). To repress *ompF* transcription, OmpR binds a fourth distal site (F4) (55, 98). Occupancy of this distal site is believed to facilitate formation of a DNA loop between OmpR bound at F4 and OmpR bound to one or more of the proximal binding sites (F1 to F3). The binding of OmpR to C1 to C3 and to F1 to F4 seems to be independent of the degree of OmpR phosphorylation (48). Rather, the binding appears to be mediated by an osmolarity-induced conformational change (83).

The non-porin-associated functions of OmpR include regulation of the permease encoded by *tppB* in *S. enterica* (41) and *E. coli* (42), the maltose regulator encoded by *malT* (23), and the murein regulator encoded by *bolA* (160). A recent microarray study (97) identified 125 OmpR-dependent genes. The phenotypes exhibited by an *ompR-envZ* mutant include increased resistance to several antibiotics (attributed to the defect in porin synthesis) and increased use of several hexoses as carbon sources (allose, fructose, mannitol, *N*-acetyl-D-glucosamine, and glucose) (163).

Other cellular processes affected by OmpR include the biogenesis of curli (59), type I fimbriae (97), and flagella (128). DNase I footprinting demonstrated that there is direct binding of OmpR to the *flhDC* promoter at two discrete regions (128). This arrangement resembles that present at *ompF*; thus, a repression loop similar to that predicted for *ompF* might be responsible for repression of *flhDC* transcription. In contrast to *ompF* repression, regulation of *flhDC* depends on the phosphorylation state of OmpR. Phosphorylated OmpR bound the *flhDC* promoter with 10-fold-higher affinity than unphosphorylated OmpR bound the *flhDC* promoter (128). Electrophoretic mobility shift assays have demonstrated that there is binding of phosphorylated OmpR to the *csgD* promoter, which drives expression of one of the two curli operons (59, 106).

RcsCDB. The RcsCDB phosphorelay, discovered as a regulator of capsule synthesis (43), is responsible for the regulation of up to 5% of the *E. coli* genome (32, 44, 97) (see Fig. S7 in

the supplemental material). Many of the target genes encode parts of surface appendages (e.g., flagella and curli), components of the cellular multistress response (e.g., *osmB*, *osmY*, and *osmC*), or proteins involved in cell division (*ftsAZ*) (22, 30, 32, 33, 140). RcsB can bind either as a homodimer to the RcsB box (e.g., at *ftsAZ* and *osmC* [22, 30, 140]) or as a heterodimer in a complex with the auxiliary protein RcsA (e.g., at *cps* [60, 139, 152, 153]). RcsA is related to the response regulators, except for the lack of the conserved aspartate site that is required for phosphorylation (139). The RcsAB box resembles the RcsB box. The differences in the consensus sequences are indicative of the presence of RcsA in the heterodimer, and it was hypothesized that the conformation of RcsB might be modulated upon interaction with RcsA, resulting in recognition of different DNA targets (107).

Like EnvZ/OmpR, the RcsCDB phosphorelay regulates the biogenesis of flagella, curli, and type 1 fimbriae. It may also regulate an uncharacterized fimbrial locus (*sfm*). Regulation of the flagellar system by RcsCDB was shown first in *Proteus mirabilis* (12) and later in *E. coli* (33, 143). The 2CST regulator RcsB binds directly to the *flhDC* promoter to inhibit its transcription. This regulation may also involve RcsA (33), but only when an excess of it is present (C. E. Fredericks and A. J. Wolfe, unpublished). A recent study provided evidence that RcsCDB activates *fim* expression (Fredericks, and Wolfe, unpublished), while a microarray analysis indicated that RcsCDB negatively regulates both the biogenesis of curli and the expression of *fimZ* (32). In *S. enterica*, the 2CST regulator FimZ activates *fim*, while it represses *flhDC* (28). In *E. coli*, however, FimZ probably does not regulate the *fim* locus but rather regulates the *sfm* (*s*almonella-like *fim*briae) locus in which it resides (http://genolist.pasteur.fr/Colibri/, http://ca.expasy.org/sprot/). If this is true, then RcsCDB regulates *fim* and *sfm* inversely, increasing *fim* expression while decreasing expression of *sfm*. Whether FimZ regulates *flhDC* in *E. coli* remains unknown.

Taken together, this evidence provides strong support for the hypothesis that the RcsCDB phosphorelay plays an important role in adapting the bacterial cell surface to growth on a solid surface (32) and, thus, a critical role in the development of biofilms (L. Ferrieres and D. Clarke, personal communication).

SMALL MOLECULES

In *E. coli*, signal transduction pathways either can produce small molecules as second messengers or can be influenced by small molecules. Below, we discuss the impact of three of these molecules, cAMP, acetyl~P, and c-di-GMP, on our network.

Cyclic AMP. The product of a signal transduction pathway that consists of the phosphoenolpyruvate:carbohydrate phosphotransferase system and adenylate cyclase, cAMP is a second messenger that reports on the nutritional status of the external environment. When levels of catabolite-repressing carbon sources decrease, cAMP levels increase (105). The cAMP then docks with CRP to activate the transcription of genes required for the metabolism of secondary carbon sources and other cellular processes (for a review, see reference 46), including the biogenesis of flagella and curli.

Acetyl phosphate. The intermediate of the phosphotransacetylase-acetate kinase pathway (21, 118), acetyl~P, has a larger ΔG^0 of hydrolysis than ATP (76). Thus, acetyl~P stores more energy than ATP stores and, indeed, donates its phosphoryl group to ADP to generate ATP. This tendency to donate phosphoryl groups also forms the basis for its proposed impact on 2CST pathways (85, 151).

There is much evidence which supports the hypothesis that acetyl~P can interact with 2CST pathways. In vitro, many response regulators autophosphorylate using acetyl~P as the phosphoryl donor. Numerous in vivo studies have shown that there is a strong correlation between the status of the acetyl~P pool and activation of some 2CST targets, implicating acetyl~P in the activation of a subset of response regulators (for a review, see reference 157). One of these studies demonstrated that acetyl~P can influence the in vivo expression of almost 100 genes (158), verifying that acetyl~P correlates with decreased expression of genes involved in flagellum biogenesis (113) and showing that it correlates with increased expression of genes involved in type 1 fimbria assembly (*fim*), the biosynthesis of capsule (*cps*), and the response to multiple stresses (e.g., *osmB*, *osmY*, and *osmC*) (158). These results can be explained, in part, by the following observations: (i) acetyl~P can donate its phosphoryl group to both OmpR (62) and RcsB (F. Bernhard, personal communication), (ii) the Rcs phosphorelay controls the biosynthesis of capsule (138) and many of the stress-associated genes (30, 32), (iii) both OmpR and the Rcs phosphorelay regulate the biogenesis of flagella, curli, and type 1 fimbriae (32, 33, 97, 109, 128), and (iv) acetyl~P acts upon capsule biosynthesis and flagellum biogenesis via the RcsCDB phosphorelay (Fredericks, and Wolfe, unpublished).

Cyclic di-GMP. The second messenger, c-di-GMP, also regulates the transition from motile, planktonic cells to a sessile biofilm. Like acetyl~P, it inhibits flagellum biogenesis while enhancing capsule biosynthesis (for recent reviews, see references 58, 116, and 131). However, in contrast to cAMP and acetyl~P, which influence this transition by controlling transcription initiation, c-di-GMP tends to act posttranslationally (3, 49, 54, 64, 103).

c-di-GMP is synthesized by diguanylate cyclases (DGCs) and is degraded by phosphodiesterases (PDEs). DGC activity has been associated with the highly conserved GGDEF domain (101, 102, 120, 131, 144), while PDE activity has been associated with the highly conserved EAL domain (17, 26, 145). GGDEF and EAL domains are ubiquitous in bacteria (133). On the basis of sheer abundance, they represent a major family of signaling pathways (37). Many bacterial species possess multiple proteins with GGDEF and/or EAL domains. For example, *Pseudomonas aeruginosa* has 33 such proteins, *Vibrio cholerae* possesses 41, and *E. coli* has 36 (58, 116, 131). This abundance suggests that there is a network of pathways that either integrates multiple signals into a single second messenger or instead permits synthesis of the second messenger in response to diverse signals (38). More likely, pathways work in relative isolation due to localization or the existence of microenvironments (54, 101, 131).

Processes influenced by c-di-GMP also are abundant, but most of them result in phenotypic changes that are related to the transition between motile, planktonic individuals and a sessile biofilm (131). The mechanisms used by c-di-GMP to

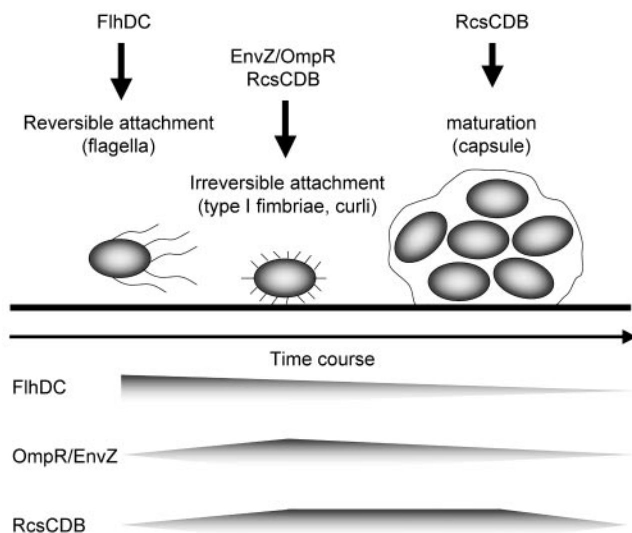

FIG. 3. Regulation of biofilm formation by FlhDC, OmpR/EnvZ, and RcsCDB. Observations from the network are projected on a time course of the early stages of biofilm formation. Biofilm formation starts with reversible attachment, proceeds to irreversible attachment, and progresses to three-dimensional construction of the mature biofilm, until the biofilm disassociates. The action points of the three major regulators in our network are indicated by vertical arrows. These points are based on the cellular processes that are affected by the regulators. A time course of expression of the regulators is hypothesized and is indicated by horizontal arrows, paralleling the time course of biofilm formation.

influence behavior remain obscure, although this molecule likely works by direct interaction with its targets (37). Such is the case with the biosynthesis of cellulose, an extracellular polysaccharide, in *Acetobacter xylinum* and *S. enterica*. In these organisms, multiple DGCs and PDEs regulate the intracellular concentration of c-di-GMP, which binds directly to a cellulose synthesis complex that includes BscA, which consists of the newly discovered c-di-GMP-binding domain PilZ attached to a glycosyltransferase (5). The result is an activated complex capable of synthesizing cellulose, which is required for the formation of biofilms and the development of rugose colonies (57, 114, 119, 131, 155). A second example is YcgR, which consists of a PilZ domain attached to a domain whose function is unknown (5). YcgR, along with the EAL domain protein YhjH, has been implicated in the ability of *E. coli* flagella to rotate (67) The mechanism is not understood, nor are the mechanisms that underly the regulation of other targets of c-di-GMP understood.

CONCLUDING REMARKS

In summary, here we describe the regulation by three global regulators of three cellular processes involved in early biofilm development. FlhDC is a positive regulator of flagella and curli, OmpR is a negative regulator of flagella and type I fimbriae and a positive regulator of curli, and RcsCDB is a negative regulator of flagella and curli and a positive regulator of type 1 fimbriae and capsule (Fig. 3). The differential use of these three global regulators to integrate diverse signals, second messengers, and metabolites likely provides much of the basis for the ability of cells to coordinate surface organelle biogenesis so that they can build a proper biofilm. Additional global regulators (e.g., CRP and H-NS) and more specific regulators (e.g., HdfR and CsgD) could provide an opportunity to further calibrate the process.

We see this minireview as a semiglobal approach to relate information about the entire network to a specific biological question. While the ultimate goal of systems biology is to decipher the entire regulatory network of the cell, here we focused on one part of that network, the sector that controls major cellular processes involved in early biofilm development. We envision this network as just one system in which multiple environmental signals feed into numerous global regulators to regulate diverse cellular processes involved in a complex behavior. We anticipate that there are other systems.

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