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Restoration of motility to an *Escherichia coli fliA* flagellar mutant by a *Bacillus subtilis* σ factor

(transcription/chemotaxis/RNA polymerase/promoter)

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ABSTRACT **The activation of additional promoter sites by production of an alternative σ subunit for RNA polymerase is a common strategy for the coordinate regulation of gene expression. Many alternative σ factors control genes for specialized, and often narrowly distributed, functions. For example, most of the alternative σ factors in *Bacillus subtilis* control genes necessary for endospore formation. In contrast, the *B. subtilis* σ^D protein controls the expression of genes important for flagellar-based motility and chemotaxis, a form of locomotion very broadly distributed in the eubacteria. A homologous σ factor, σ^F, controls a similar group of motility genes in the enteric bacteria. The conservation of both promoter specificity and genetic function in these two regulons allowed us to test the ability of a *B. subtilis* σ factor to function within an *Escherichia coli* host. We demonstrate that expression of the *B. subtilis sigD* gene restores motility to an *E. coli* strain mutant in the *fliA* locus encoding the σ^F factor. This result suggests that the *B. subtilis* σ^D protein can bind to the *E. coli* core RNA polymerase to direct transcription initiation from at least four of the late operon promoters, thereby leading to the synthesis of flagellin, motor, and hook-associated proteins. Conversely, expression of σ^D protein in a normally chemotactic strain of *E. coli* (*fliA*$^+$) leads to a hyperflagellated, nonchemotactic phenotype.**

The alternative σ factor, σ^D (formerly σ^{28}), directs the *Bacillus subtilis* core RNA polymerase to the promoter site controlling transcription of flagellin (1). In addition, genetic and biochemical evidence suggests that the methyl-accepting chemotaxis proteins and at least one operon required for motility are also under σ^D-dependent control (2, 3). The similarity between known σ^D-dependent promoters and the promoter elements for flagellar genes in *Salmonella typhimurium* and *Escherichia coli* led to the proposal that a related alternative σ factor might control expression of flagellar genes in these enteric bacteria (4). Indeed, biochemical studies identified such an activity in *E. coli* extracts (σ^F), and the corresponding structural gene (*fliA*) was cloned and sequenced from *S. typhimurium* (5, 6).

In *E. coli* and *S. typhimurium*, the genes for flagellar biosynthesis (*flg, flh, fli*), motility (*mot*), and chemotaxis (*che*) are regulated by very similar transcriptional cascades under the control of the catabolite-sensitive "master operon," *flhDC* (Fig. 1) (6–10). The FlhD and FlhC gene products (class I) are required for the expression of the seven class II operons encoding components of the basal body, the hook, a flagellar-specific export apparatus, and an alternative σ factor (FliA or σ^F) (6, 8, 10). The σ^F-RNA polymerase then directs the transcription of the seven class III operons. These "late" gene products include the hook-associated proteins, flagellin, the motor proteins MotA and MotB, and the four methyl-accepting chemotaxis proteins. The late operons are transcribed from σ^F-dependent promoters similar in sequence to σ^D-dependent promoters from *B. subtilis*. In addition, many of the class II genes, including *fliA* itself, also contain similarities to the so-called σ^{28} consensus promoter sequence (4, 6, 11).

It has been established (5) that the *B. subtilis* σ^D and *E. coli* σ^F RNA polymerases have an overlapping promoter recognition specificity. Indeed, the sequence of the cloned *S. typhimurium fliA* gene predicts a protein with 36.8% amino acid identity with the *B. subtilis* σ^D protein (6). The close correspondence between these two alternative σ factors in both their promoter specificity and genetic function encouraged us to attempt to complement an *E. coli fliA* mutant with the corresponding cloned *sigD* gene from *B. subtilis*. Remarkably, the expression of σ^D protein restores motility to a *fliA* mutant strain of *E. coli*, implying that the σ^D factor can interact productively with the *E. coli* RNA polymerase core enzyme. Although motile, the resulting strain is deficient in chemotaxis. Expression of σ^D in a normally chemotactic *E. coli* strain leads to hyperflagellated cells locked in a tumbling mode.

MATERIALS AND METHODS

Strains and Plasmids. *E. coli* strain JM101 (supE thi Δ[lac-proAB]F'[traD36 proAB$^+$ lac^{Iq} lacZΔM15]) was used as a host for molecular cloning experiments by using standard procedures (12). *E. coli* YK410 (F$^-$ araD139 ΔlacU139 rpsL thi pyrC46 gyrA thyA his) and the isogenic *fliA*$^-$ derivative, YK4104 (13), were hosts for the expression of σ^D protein. Cloning and sequencing of the σ^D structural gene, contained on plasmids pJH6-2 (5'-end) and pJH1-1 (3'-end), has been described (14). The plasmid pBSK$^+$ (Stratagene) formed the basis for construction of a T7 RNA polymerase-driven overexpression system with T7 RNA polymerase provided from a second compatible plasmid, pGP1-2 (Kan^R), as described (15).

Plasmid Constructions. All restriction enzymes, T4 DNA ligase, and the Klenow fragment of *E. coli* DNA polymerase I were purchased from New England Biolabs and used in accord with the manufacturer's recommendations. The intact *sigD* structural gene was assembled by simultaneous ligation of a 1400-base-pair (bp) *Hin*dIII–*Eco*RI fragment from pJH6-2, a 625-bp *Eco*RI–*Pst* I fragment from pJH1-1, and *Hin*dIII- and *Pst* I-digested pBSK$^+$ DNA to generate plasmid pYFC-1. This plasmid contains the intact *sigD* gene together with 1100 bp of upstream *B. subtilis* DNA. Digestion of plasmid pYFC-1 with *Sal* I and *Sph* I followed by treatment with Klenow fragment and religation generates plasmid pYFC-3, which contains only 36 bp of DNA separating the start codon from the T7 RNA polymerase promoter (the *Sal* I site was regenerated in pYFC-3). However, pYFC-3 contains a copy of the *lac* operator sequence and fails to

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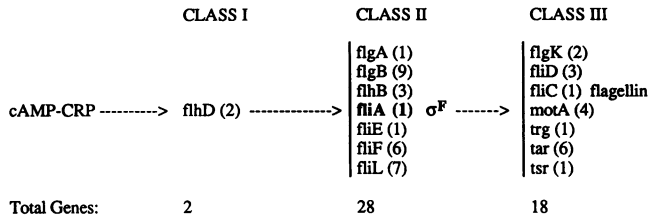

FIG. 1. Regulation of flagellar biosynthesis in *E. coli*. This diagram indicates the hierarchy of gene expression for the operons of flagellar morphogenesis and chemotaxis. Each operon is listed by the genetic designation for the first gene of the operon with the number after each gene representing the number of genes in that operon (e.g., the operon designated *flhD* contains the genes *flhD* and *flhC*). A similar cascade is operative in *S. typhimurium*. Data are adapted from refs. 7 and 8. CRP, cyclic-AMP receptor protein.

transform strain BL21/DE3, the desired host for high-level protein overexpression experiments (16). Plasmid pYFC-0 (a pBSK$^+$ derivative lacking the *lac* operator sequence) was prepared by digestion of pBSK$^+$ with *Sac* I, treatment with Klenow fragment to remove the 3′-protruding overhangs, partial digestion with *Pvu* II, and religation. The 961-bp *Kpn* I–*Pst* I fragment of pYFC-3 containing the *sigD* was ligated to plasmid pYFC-0 (digested with the same enzymes) to generate pYFC-4.

To replace the weak ribosome-binding site in pYFC-4 with a stronger ribosome-binding site we used the PCR. A 265-bp region containing the 5′ region of the *sigD* gene was amplified with *Taq* DNA polymerase by using PCR. Two oligonucleotide primers were designed to introduce new restriction sites and to provide an optimized ribosome-binding site for expression of σ^D protein. The 5′ primer was 5′-TCCGGAGCTCTAGGAGGAATACCATATGCAATCCTTGAATTATGAA-3′, and the 3′ primer was 5′-GCCCGTCTATGATTACGCCG-3′ (synthesized by the Cornell Biotechnology Center). The 5′ primer is identical to the cloned *sigD* gene from the ATG start codon to the 3′ end but introduces a *Nde* I site (underlined) overlapping the start codon and a *Sac* I site (underlined) at the 5′ end. The 3′ primer is identical to the complementary strand of the *sigD* gene just downstream of a unique *Eco*RI site. The 273-bp PCR product was purified by agarose gel electrophoresis, digested with *Sac* I and *Eco*RI, and ligated to pUC18 digested with the same enzymes to generate pYFC-7. The modified 5′-end of the *sigD* gene was excised from pYFC-7 as a 277-bp *Kpn* I–*Eco*RI fragment and ligated to pYFC-4 DNA digested with the same enzymes to generate pYFC-6. Both pYFC-6 and pYFC-4 encode functional *sigD* polypeptide, as judged by the ability to complement a *fliA* mutant strain of *E. coli*.

Electron and Phase-Contrast Microscopy. Each strain was grown in rich medium until late-logarithmic phase. A 5-μl drop of bacterial cells was observed under oil immersion with a Zeiss standard phase-contrast microscope. For electron microscopy, a grid was floated on a 15-μl drop of each strain of bacterial cells for 10 min. The grids were stained with 0.4% sodium phosphotungstate and examined on a Philips 201 electron microscope operating at an accelerating voltage of 80 kV. Electron micrographs were taken on 3.25 × 4.25 inch (1 in = 2.54 cm) negative film. For quantitation, the flagella were counted directly from the grids.

Media and Swarm Plate Assays. Cells were grown in 2× YT medium (12) with ampicillin (100 μg/ml) and/or kanamycin (40 μg/ml) added as necessary. Tryptone swarm plates contained 0.2% Bacto-agar, 1% Bacto-tryptone, 0.5% sodium chloride, 0.01% thymine, 0.01% uracil, and streptomycin at 125 μg/ml. Strains to be tested were stabbed onto swarm plates and placed at 30°C in an incubator containing several dishes of water to maintain a high relative humidity. The diameters of the swarm ring of each strain were measured by visual inspection of two or more plates.

RESULTS

Expression of *B. subtilis* σ^D in *E. coli*. The gene encoding the *B. subtilis* σ^D protein was originally isolated as two overlapping chromosomal fragments (14). To express intact σ^D protein, plasmid pYFC-4 was prepared by recombining the original fragments of the *sigD* gene in a plasmid which provides a promoter for the T7 phage RNA polymerase. The plasmid vector used for these experiments, pYFC-0, is a derivative of pBSK$^+$ with a deletion of the *lac* operator to allow transformation into *E. coli* strain BL21/DE3. This strain of *E. coli* contains a copy of the T7 RNA polymerase gene under *lac* control on a defective λ prophage (DE3) and is often intolerant of multicopy plasmids containing *lac* operator sequences (16). We subsequently prepared a derivative of pYFC-4 in which the 5′-region of the gene was replaced with a PCR-generated DNA fragment containing an optimized ribosome-binding site to enhance levels of protein expression (17). The resulting plasmid, pYFC-6, forms the basis for most experiments described here, although similar results are obtained with the parent plasmid, pYFC-4.

The ability of the σ^D protein to complement an *E. coli* strain defective in the σ^D homolog σ^F was assayed by phase-contrast microscopy, electron microscopy, and by swarm rates on soft agar plates. The *fliA* mutant strain, YK4104, and the isogenic parent strain, YK410, were transformed with plasmid pYFC-0, pYFC-4, or pYFC-6 for these experiments. A second, compatible plasmid, pGP1-2(Kan^R), was used for expression of the T7 RNA polymerase under the control of a temperature-inducible λ promoter (15).

Expression of σ^D Protein Restores Motility to a Strain Lacking Functional σ^F. An *E. coli* strain lacking functional σ^F, YK4104(*fliA*$^-$), is completely nonmotile when viewed by phase-contrast microscopy. In contrast, late-logarithmic cultures of strain YK4104(pYFC-6)(pGP1-2) expressing σ^D protein contain a fraction of motile cells that appear to swim straight (run) and reorient (tumble) at near normal frequencies [≈one tumble per sec at 30°C (18)]. The complemented strain, YK4104(pYFC-6)(pGP1-2), is visually distinguishable from the *fliA*$^+$ wild-type strain, YK410: the runs have a more pronounced zigzag component, and the tumbles appear less efficient in creating a net reorientation of cell direction. We hypothesize that these qualitative differences may be due to the hypoflagellated phenotype of this strain (see below).

The proportion of motile cells varies from experiment to experiment but was usually estimated to be in the range of 10–80% of the cell population. Consistent with these observations, sampling of several cultures by electron microscopy suggests that 20–50% of the total cell population is flagellated under these growth conditions (see below). Motility was observed in strain YK4104(pYFC-6) in both the presence and absence of plasmid pGP1-2, suggesting that high levels of σ^D protein are not required for genetic complementation. Motility was never observed in control experiments in which the vector plasmid, pYFC-0, was used in place of the *sigD* expression plasmid, pYFC-6.

The ability of *E. coli* cells to migrate through soft agar (swarm) in response to an induced nutrient gradient provides a sensitive assay for both motility and chemotaxis (19, 20). On rich medium, wild-type *E. coli* cells form between one and three rapidly migrating rings of cells, reflecting the successive depletion of different chemoattractants (19, 20). Strain YK410 (*fliA*$^+$) forms a rapidly migrating primary ring of cells that reaches the edge of a 100-mm Petri plate in ≈8 hr at 30°C (Fig. 2, strain A). Cells of the *fliA* mutant strain containing the vector control, YK4104(pYFC-0)(pGP1-2), grow only at the site of inoculation and fail to swarm (Fig. 2, strain F).

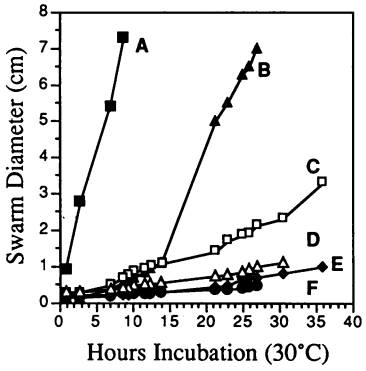

FIG. 2. Swarm rates of *E. coli* strain on soft agar medium. Strain A (■) is the wild-type YK410, strain B (▲) is the complemented *fliA*⁻ mutant strain: YK4104(pYFC-6)(pGP1-2), strain C (□) is YK4104-(pYFC-6), strain D (△) is YK410(pYFC-6), strain E (◆) is YK410(pYFC-6)(pGP1-2), and strain F (●) is the mutant strain containing the vector control: YK4104(pYFC-0)(pGP1-2).

Significantly, expression of σ^D protein in strain YK4104-(pYFC-6)(pGP1-2) restores motility, as judged by the formation of an expanding disc of cell density. The swarm rate of strain YK4104(pYFC-6)(pGP1-2) can be determined by monitoring the visible edge of cell density over time (Fig. 2, strain B). From this analysis, we estimate the swarm rate as ≈20% of that seen for the wild-type (*fliA*⁺) strain containing the vector control: YK410(pYFC-0)(pGP1-2). This rate is in the range (5–50%) observed for various *che* mutants of *E. coli* (20). From these observations we conclude that expression of σ^D (from plasmid pYFC-6) in a strain lacking functional σ^F(YK4104) converts a Fla⁻ strain to a Mot⁺ strain.

The apparent change in swarm rate for strain B arises from the incomplete complementation of the motility phenotype and the nature of the swarm plate assay: only visibly turbid regions of the plate are considered to have swarming cells. The cell population (swarm) measured at late times only becomes visible after greater than 15 hr of incubation, whereas the earlier measurements reflect observations of the thicker cell density resulting from growth in the immediate vicinity of the site of inoculation. In contrast, the wild-type strain forms a discrete ring of cell density (rather than a diffuse disk), and this ring is readily visible after a relatively short incubation period.

The effects of σ^D protein on motility in a *fliA*⁺ strain background (YK410) were also surveyed. Remarkably, the expression of σ^D protein leads to a severe chemotaxis defect in strain YK410(pYFC-6)(pGP1-2), as evidenced by a very slow swarming rate on soft agar (Fig. 2, strain E). This inhibition of motility is specific to the *sigD* gene (plasmid pYFC-6 with or without plasmid pGP1-2) and is not seen in the vector control, strain YK410(pYFC-0)(pGP1-2). When observed by phase-contrast microscopy, cells expressing both the σ^D protein and the endogenous σ^F factor appear to tumble continuously.

The ability of σ^D to complement strain YK4104 for swarming does not require high levels of σ^D expression. In the experiments plotted in Fig. 2, the cells were grown at the noninducing temperature of 30°C. In addition, cells lacking plasmid pGP1-2 and, therefore, lacking the encoded T7 RNA polymerase can still swarm, although at a reduced rate relative to cells containing the complete two-plasmid system (Fig. 2, strain C). The levels of σ^D protein expressed from plasmid pYFC-6 alone, presumably from plasmid-born promoters for *E. coli* RNA polymerase, are substantially less than those from the fully induced two plasmid system, as judged by immunoblot analysis with anti-σ^D antibodies (see below).

Quantitation of Flagellar Filaments in *E. coli* Strains. Swarm plate analysis provides a sensitive assay for motile cells because these cells can swim toward nutrient-rich regions and thereby realize a distinct growth advantage (20). To determine the number and nature of the flagellated cells of each strain in the (relative) absence of any such selective pressure, we visualized cells in late-logarithmic-phase liquid cultures by electron microscopy. A histogram representing the number of flagellar filaments observed on a large sample of cells of each strain is shown in Fig. 3, and electron micrographs of representative cells are shown in Fig. 4. From this analysis, we conclude that expression of σ^D in a cell lacking functional σ^F leads to the production of a relatively small number of morphologically normal flagellar filaments in a population subset. Most of the cells remain nonflagellate, and the majority of the flagellated cells appear to have a single flagellar filament. Conversely, expression of σ^D in cells containing functional σ^F protein leads to a hyperflagellated phenotype; these cells have an average of ≈23 filaments per cell versus 10 for the wild type. In addition, strain YK410-(pYFC-6)(pGP1-2) has an altered cell morphology and flagellar filaments with a reproducibly shorter waveform, although the origins of these effects are not understood.

To directly quantitate the expression levels of σ^D protein in these various strains we performed an immunoblot analysis by using specific anti-σ^D antibodies (21). Because we do not have purified σ^D protein to provide a standard curve, we compared the levels of protein in *E. coli* and *B. subtilis* by using a *B. subtilis sigD* null mutant strain as a control (14). In these experiments, we detected no cross-reactivity with the endogenous σ^F protein in wild-type YK410 cells, although this result may reflect limitations in assay sensitivity. The level of σ^D protein expressed from the two-plasmid system (induced by a shift to 37°C) was greater than that in the logarithmic *B. subtilis* cells [previously estimated at ≈220 molecules per cell (21)], by approximately an order of magnitude (data not shown). This result would correspond to ≈0.1% of total cell protein. In the absence of plasmid pGP1-2,

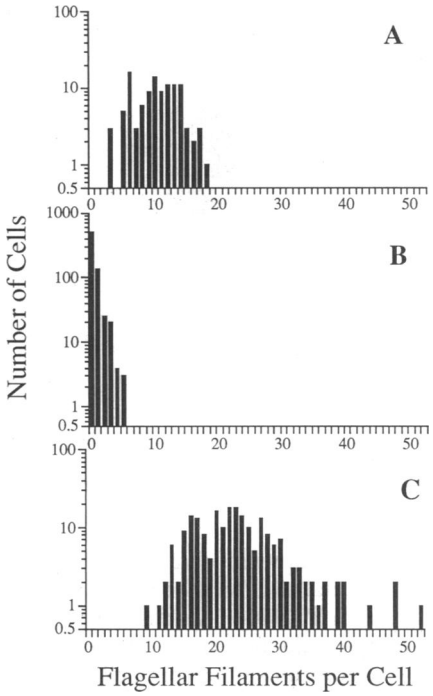

FIG. 3. Histogram of flagellar filaments observed per cell. (*A*) Wild-type *E. coli* strain YK410. (*B*) Complemented *fliA*⁻ mutant strain YK4104(pYFC-6)(pGP1-2). (*C*) Strain YK410(pYFC-6)(pGP1-2). The sample size (n), mean number of filaments (x), and range (r) for each set are as follows: *A* (n = 107, x = 10.2, and r = 3–18), for *B* (n = 688, x = 0.40, and r = 0–5), and for *C* (n = 208, x = 23.2, and r = 9–52).

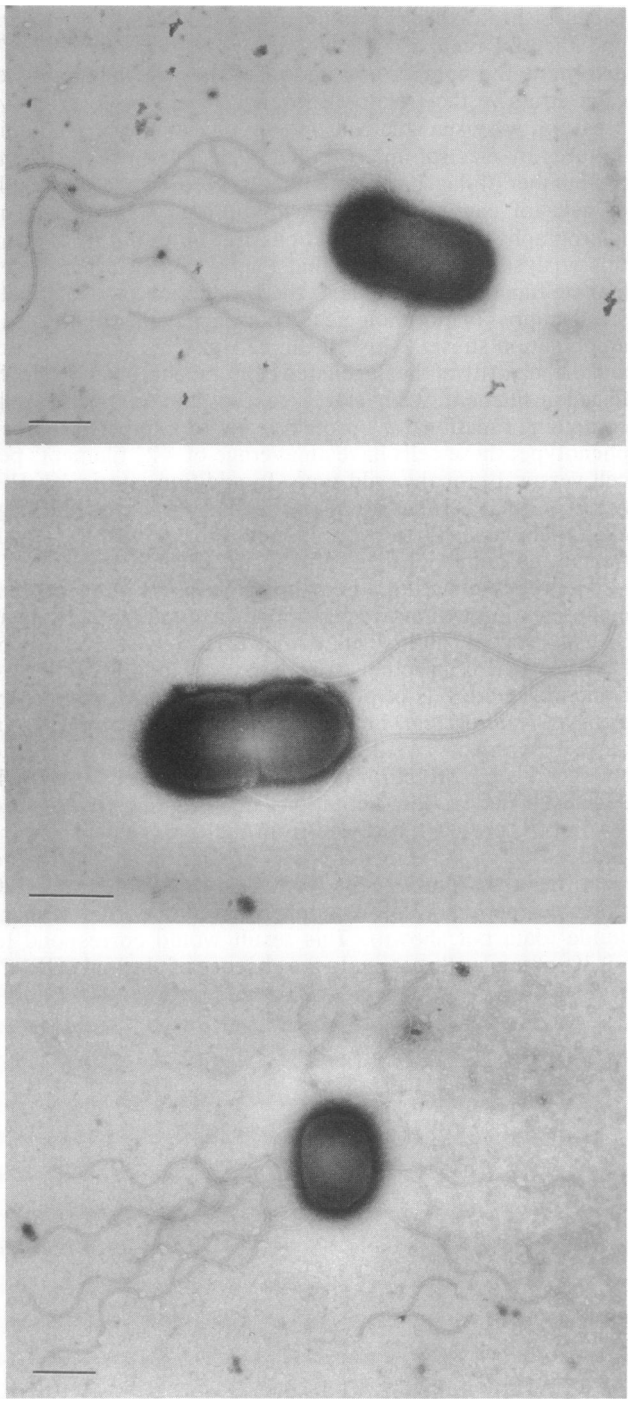

FIG. 4. Electron micrographs of *E. coli* strains. (*Top*) Wild-type strain YK410. (*Middle*) Complemented *fliA* mutant strain YK4104-(pYFC-6)(pGP1-2). (*Bottom*) Strain YK410(pYFC-6)(pGP1-2). (Bars = 1 μm.)

the levels of σ^D protein were lower than those in *B. subtilis*. There was no detectable difference in the level of σ^D protein expressed in strain YK4104 (*fliA* mutant) or YK410 (wild-type parent). It should be noted that a *B. subtilis* strain expressing <20 molecules of σ^D per cell is still fully motile, although slightly deficient in chemotaxis as judged by swarm rate analysis (2).

DISCUSSION

Our results suggest that the *B. subtilis* σ^D protein can function in *E. coli* to direct the core RNA polymerase to transcribe genes essential for flagellar-based motility and thereby complement the Mot$^-$ phenotype of a *fliA* mutant strain. In contrast, expression of the *B. subtilis* σ^D protein in a normally chemotactic strain of *E. coli* leads to a severe chemotaxis defect, altered cell morphology, and an enhanced synthesis of flagellar filaments. The ability of the *B. subtilis* σ^D protein to restore motility to a strain of *E. coli* defective in σ^F underscores the high degree of evolutionary conservation observed in eubacterial "motility regulons." The recent characterization of flagellin genes (and their promoter regions) from a number of phylogenetically diverse eubacteria lends support to the hypothesis that many motile bacteria use a homolog of the *B. subtilis* σ^D protein to direct flagellar gene expression (10). Our results support the hypothesis that the motility regulon is ancient: the appearance of alternative σ factors as a regulatory strategy predates the divergence of *B. subtilis* and the enterobacteriaciae.

A model for gene regulation in the motility regulon of *E. coli* is presented in Fig. 1. Epistasis experiments have established that expression of genes at each level of the regulon requires the function of all gene products at higher levels in the regulatory cascade (6, 7, 9). This result suggests that the cell is sensing the morphological completion of the basal body-hook structure (encoded by class II genes) before σ^F-dependent activation of the class III genes encoding flagellin (*fliC*), the hook-associated proteins (*flgKL, fliD*), the motor components (*motAB*), and the *che* gene products. Cells defective in σ^F fail to synthesize flagellar filaments (Fla$^-$) and are, therefore, nonmotile (Mot$^-$) and nonchemotactic (Che$^-$). Our results indicate that expression in *E. coli* of the *B. subtilis* σ^D protein can compensate for the absence of functional σ^F by allowing expression of genes required for flagellar synthesis and motility.

The ability of σ^D protein to compensate for the absence of functional σ^F could be due to direct transcription of the necessary class III operon promoters, to indirect activation of these promoters by overexpression of the defective σ^F factor in the *fliA* mutant strain, or to both. The *fliA* gene has an upstream σ^{28}-like promoter element, and the nature of the mutation in the *fliA* mutant strain is not well defined. We favor a direct activation of the late operon promoters by the heterologous σ factor for several reasons. (*i*) It is known that the purified *B. subtilis* σ^D RNA polymerase and the purified *E. coli* σ^F RNA polymerase have an overlapping promoter recognition specificity. For example, both RNA polymerases initiate transcription *in vitro* at the same nucleotides from the *tar, tsr*, and *fliL* promoters (5). (*ii*) The σ^D and σ^F factors normally occur at very low levels (5, 6, 21); an estimated 220 molecules per cell of the *B. subtilis* protein are present during the period of peak expression at late-logarithmic phase. Even the presence of as few as 20 or so molecules of σ^D allows near normal motility in *B. subtilis* (2). Therefore, the defect in the *fliA* mutant strain is probably not primarily a quantitative deficit that can be overcome by producing more protein. (*iii*) It seems unlikely that the strong phenotypic consequence of expressing the σ^D protein in a wild-type strain containing σ^F is due entirely to overexpression of the wild-type σ^F polypeptide. The *fliA* gene of *S. typhimurium* was originally cloned by the ability of a multicopy plasmid carrying *fliA* to restore motility to mutant *E. coli* strain, suggesting that this factor can also function over a range of protein concentrations (6).

If the phenotypic effects we observe were due to direct activation of class III promoters by the heterologous σ factor, this would require, at a minimum, activation of the promoters for the operons initiating with genes *fliC, flgK, fliD*, and *motA*. The activation of the other three late operons, encoding the four methyl-accepting chemotaxis proteins and four of the six general chemotaxis gene products, has not been assayed directly. The Che$^-$ phenotype of cells expressing σ^D

protein could be from a failure of the heterologous σ factor to activate transcription of one or more of these three operons or to expression of Che proteins in improper stoichiometries. It is known, for example, that overexpression of either CheY or CheZ can lead to a Che$^-$ phenotype (22). Alternatively, the relative paucity of flagellar filaments and consequent inefficiency in running and tumbling could account for the reduced swarm rate.

The ability of σ^D to compensate functionally for the lack of functional σ^F suggests that these two regulatory proteins are structurally conserved. The *fliA* gene has been cloned from *S. typhimurium*, and the predicted σ^F protein is 36.8% identical in amino acid sequence with σ^D (6). This alignment includes four out of five identities in positions predicted to be involved in promoter recognition based on genetic studies of other σ factors (23). Moreover, the consensus sequences of flagellar promoters from *E. coli* and known σ^D-dependent promoters from *B. subtilis* are virtually indistinguishable (4). There is precedence from *in vitro* studies for the observation that a heterologous σ factor can function while bound to the *E. coli* core enzyme. A number of *B. subtilis* σ factors, including the major σ factor, σ^{43} (24–27), σ^G (28), and phage-encoded σ factors (24, 29), are known to function with the *E. coli* core subunits to direct transcription initiation *in vitro*. In fact, even the eukaryotic general transcription factor RAP30/74 retains the ability to recognize and bind to *E. coli* core enzyme, and, conversely, *E. coli* σ^{70} retains the ability to bind specifically to mammalian RNA polymerase II (30). However, demonstration of functional complementation *in vivo* has not been reported in any of these systems.

Expression of the *B. subtilis* σ^D protein has striking phenotypic consequences in both a normally nonflagellate, *fliA* mutant strain, and in the wild-type parent strain. Expression of one or a few flagellar filaments under σ^D direction in strain YK4104 (lacking functional σ^F) restores motility and allows swarms to form on soft agar plates. Microscopic observations suggest, however, that the motile cells of this strain are phenotypically abnormal. While "smooth swimming," the motile cells appear to zig-zag more than the wild-type strain, and while "tumbling", they reorient less efficiently. These phenotypes may reflect the presence of only one or a few flagellar filaments on the cell surface. Similarly, the hyperflagellated strain containing both σ^D and σ^F (YK410 with plasmids pGP1-2 and pYFC-6) tumbles incessantly and appears morphologically abnormal.

Swarm plate analysis provides an exquisitely sensitive technique for monitoring both motility and chemotaxis and simultaneously imposes a selective advantage for motile cells. Because even low levels of σ factor expression allow functional complementation of the motility defect in *fliA* mutant cells, this result suggests a general strategy for the cloning by complementation of motility σ factors.

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