JOURNAL OF BACTERIOLOGY, July 2000, p. 3693–3704 Vol. 182, No. 13
0021-9193/00/$04.00+0

Analysis of the Polar Flagellar Gene System of *Vibrio parahaemolyticus*

YUN-KYEONG KIM AND LINDA L. MCCARTER*

Department of Microbiology, The University of Iowa, Iowa City, Iowa 52242

Received 8 February 2000/Accepted 14 April 2000

Vibrio parahaemolyticus **has dual flagellar systems adapted for locomotion under different circumstances. A single, sheathed polar flagellum propels the swimmer cell in liquid environments. Numerous unsheathed lateral flagella move the swarmer cell over surfaces. The polar flagellum is produced continuously, whereas the synthesis of lateral flagella is induced under conditions that impede the function of the polar flagellum, e.g., in viscous environments or on surfaces. Thus, the organism possesses two large gene networks that orchestrate polar and lateral flagellar gene expression and assembly. In addition, the polar flagellum functions as a mechanosensor controlling lateral gene expression. In order to gain insight into the genetic circuitry controlling motility and surface sensing, we have sought to define the polar flagellar gene system. The hierarchy of regulation appears to be different from the polar system of *Caulobacter crescentus* or the peritrichous system of enteric bacteria but is pertinent to many *Vibrio* and *Pseudomonas* species. The gene identity and organization of 60 potential flagellar and chemotaxis genes are described. Conserved sequences are defined for two classes of polar flagellar promoters. Phenotypic and genotypic analysis of mutant strains with defects in swimming motility coupled with primer extension analysis of flagellar and chemotaxis transcription provides insight into the polar flagellar organelle, its assembly, and regulation of gene expression.**

Many bacterial species are motile by means of flagellar propulsion (reviewed in references 5, 32, and 33). Powered by a rotary motor, the flagellum acts as semirigid helical propeller, which is attached via a flexible coupling, known as the hook, to the basal body. The basal body consists of rings and rods that penetrate the membrane and peptidoglycan layers. Associating with the basal body and projecting into the cytoplasm is a structure termed the C ring, which contains the switch proteins and acts as the core, or rotating part, of the motor. Maintenance of a flagellar motility system is a sizable investment with respect to cellular economy in terms of the number of genes and the energy that must be committed to gene expression, protein synthesis, and flagellar rotation. As a result, flagellar systems are highly regulated. A hierarchy of regulation has been elucidated for peritrichously flagellated *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (26, 27, 30). This scheme of control couples gene expression to assembly of the organelle. The pyramid of expression possesses three classes, or tiers, of genes. Genes in each class must be functional in order for expression of the subsequent class to occur. Class 1 genes, *flhD* and *flhC*, encode the master transcriptional activators of class 2 flagellar gene expression. The *flhDC* operon is controlled by a σ^{70} promoter and a number of global regulatory factors (28). The majority of the class 2 flagellar genes encode components of the flagellar export system and the basal body (21). One class 2 gene encodes an alternative sigma factor devoted to recognition of flagellar genes (44). Flagellar class 3 operons are positively controlled by the flagellar σ^{28} factor and negatively regulated by FlgM, an anti-sigma factor (45). The anti-sigma factor is retained within the cell until the flagellar basal body and hook are completed (18, 29). At that time, FlgM is exported, and σ^{28} becomes free to direct expression of class 3 genes encoding flagellin subunits, hook-associated, motor, and chemotaxis signal transduction proteins. There are additional intricacies to this cascade, e.g., transcriptional classes within classes and translational modulation coupled to basal body assembly, as well as linkage between cell division and flagellar production (1, 22, 31, 48, 49). The regulatory hierarchy established for *E. coli* and *S. enterica* serovar Typhimurium serves as the paradigm for peritrichous flagellar systems of other bacteria.

The other well-characterized set of flagellar genes and scheme of flagellar control are those of *Caulobacter crescentus* (reviewed in references 46 and 65). In this organism, flagellation and cell division are strikingly coupled. On cell division, the daughter cell is motile and propelled by a polar flagellum, while the mother cell is nonmotile and stalked. DNA replication is repressed in the motile cell until later in the cell cycle when that cell differentiates to a new stalked cell. Many of the genes required for flagellar biosynthesis are homologs of *E. coli* and *S. enterica* serovar Typhimurium genes; however, the flagellar hierarchy differs between *C. crescentus* and the enteric bacteria. The flagellar genes of *C. crescentus* are organized in four levels of expression with two assembly checkpoints: completion of the MS-ring-switch export complex and completion of the basal body-hook structures. Genes at the bottom of the hierarchy are transcriptionally regulated from σ^{54} promoters. The master transcriptional regulator at the top of the hierarchy is CtrA, a member of the response regulator family of two component signal transduction systems, and this regulator controls the initiation of DNA replication, DNA methylation, cell division, and flagellar biogenesis (11).

The flagella of *V. parahaemolyticus* are of particular interest because this organism possesses two flagellar systems: a peritrichous (or lateral) one that is expressed when the bacterium is on a surface or in viscous environments and a polar system that is expressed continuously, i.e., when the bacterium is grown in liquid or on surfaces (reviewed in reference 39). Thus, under some conditions the bacterium simultaneously assembles two distinct flagellar organelles. Prior genetic analysis suggests that the gene systems are distinct and that no

* Corresponding author. Mailing address: Department of Microbiology, University of Iowa, Iowa City, IA 52242. Phone: (319) 335-9721. Fax: (319) 335-7679. E-mail: linda-mccarter@uiowa.edu.

structural or assembly components are shared; mutants isolated with defects in swarming translocation are competent for swimming motility in liquid, and swimming-defective mutants remain proficient for swarming. Energy for rotation of the two kinds of flagella derives from different sources. The sodium motive force powers rotation of the polar flagellum, and the proton motive force drives rotation of the lateral flagella. Some of the chemotaxis genes have been demonstrated to be shared by the two motility systems (53). In addition to its propulsive role in swimming, the polar flagellum is believed to act as a tactile sensor informing the bacterium of contact with surfaces. Conditions that inhibit rotation of the polar organelle induce the alternative, lateral motility system. In this work, we elucidate the genes and gene organization involved in the polar motility system. Until now the circuitry of a polar flagellar system, apart from *C. crescentus*, has not been traced. This work should provide a foundation for gaining insight into the flagellar organelle and regulation of flagellar gene expression for a number of polarly flagellated bacteria, including *Pseudomonas aeruginosa*, *Vibrio cholerae*, and other marine *Vibrio* species.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *V. parahaemolyticus* strains were cultured at 30°C. The strains used in this work are derivatives of *V. parahaemolyticus* BB22 (4). Strain LM1017 contains a mutation in the lateral flagellar hook gene and is unable to swarm (42). Strains were routinely propagated in heart infusion (HI) broth, which contained 25 g of HI broth (Difco) and 20 g of NaCl per liter. Marine broth 2216 (Difco) (28 g per liter) was filtered after autoclaving to remove precipitate. Solidified swarming medium was prepared by adding 15 g of Bacto-Agar (Difco) per liter to HI broth. Semisolid motility medium (M agar) contained 10 g of tryptone, 20 g of NaCl, and 3.25 g of agar per liter.

Genetic and molecular techniques. General DNA manipulations were adapted from the methods of Sambrook et al. (52). Transposon mutagenesis with mini-Mu *lac* (Tet^r) and the strategy for cloning the targeted gene have been described previously (58). The *V. parahaemolyticus* cosmid library was prepared by using the pLAFRII vector (40). Chromosomal DNA was prepared according to the protocol of Woo et al. (64). Southern blot analysis of restricted genomic DNA (52) was performed on Hybond-NX nylon membranes (Amersham Life Science).

Motility assays. The effect of mutations on swimming motility was assessed by examining movement in M agar. To document swimming motility, plates were inoculated with 2 μl of an overnight culture of cells normalized to an optical density at 600 nm (OD_{600}) of 2.0. Plates were incubated and photographed using a Kodak Digital Imaging System.

Immunoblot analysis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was conducted as described previously (40). Resolving gels contained 12% acrylamide. Gels were transferred to polyvinylidene fluoride membrane (Immobilon-P; Millipore Corp.) in buffer containing 12.5 mM Tris base, 96 mM Glycine, and 20% methanol for 90 min at 30 V. After blocking in TBST buffer (10 mM Tris-Cl [pH 8], 0.15 M NaCl, 0.05% Tween 20) containing 5% nonfat dry milk, blots were incubated in TBST buffer with antiflagellar antibodies. The production of antibodies to polar and lateral flagellins has been described previously (34, 42). The secondary antibody was anti-rabbit immunoglobulin conjugated to horseradish peroxidase (Amersham Life Sciences). It was incubated with the blot at a dilution of 1:20,000 in TBST for 1 h. Development of the immunoblot utilized the chemiluminescent Super Signal substrate (Pierce) according to manufacturer's instructions.

Primer extension analysis. RNA was prepared with Trizol reagent (Gibco-BRL/Life Technologies, Grand Island, N.Y.) according to the manufacturer's protocol. Broth-grown cells were harvested in late exponential phase (OD_{600} = 1.0). Plate-grown cells were harvested in cold 0.3 M sucrose after 5 to 7 h of growth. Primer extension analysis was performed as described previously (38) by use of the avian myeloblastosis virus reverse transcriptase (Promega, Madison, Wis.).

Sequence analysis. Sequence determination on both strands was performed by the DNA Core Facility of the University of Iowa. Sequence assembly and detection of potential rho-independent transcriptional terminators were accomplished by using the Genetics Computer Group (GCG) software package. Searches for homology were performed at the National Center for Biotechnology Information with the BLAST network service (2). Multiple sequence alignments were performed by using the CLUSTAL W program (62).

Nucleotide sequence accession number. The nucleotide sequences have been deposited in GenBank, and the accession numbers are U12817, AF069392, AF069391, U09005, and U06949.

RESULTS

Transposon mutagenesis and isolation of strains with swimming motility defects. After mini-Mu *lac* (Tet^r) mutagenesis of strain LM1017, a transposon bank containing approximately 15,000 mutants was screened for mutants with defects in the polar motility system. Strain LM1017 contains a *lux* operon fusion to the lateral flagellar hook gene; therefore, there is no contribution from the lateral flagellar system to the motility of this strain (42). Strain LM1017 expresses the *lfgE*::*lux* fusion when grown on solidified medium and is luminescent. All of the mutants with swimming motility defects produced as much light on plates as the parental strain LM1017 produced, were unable to swarm over surfaces, and failed to synthesize lateral flagellin. Strains that appeared nonmotile or poorly motile in semisolid motility (M) agar potentially possessed defects in polar flagellar structure or assembly, motor function, or chemotaxis.

Phenotypic analysis of mutants. The majority of nonmotile mutants of *E. coli* are nonflagellated (Fla$^-$) due to the nature of feedback control built into the hierarchy of gene expression (66). Loss-of-function mutations in only two genes (*motA* and *motB*) yield the Mot phenotype, which is a flagellated but paralyzed cell. Insertion of the torque-generating components of the motor into the membrane is not required for assembly of the *E. coli* flagellar organelle, and expression of *mot* genes occurs at the final stage in the hierarchy of expression (57). The phenotypes of *V. parahaemolyticus* motility mutants differed from *E. coli*. Forty mutants were segregated into four phenotypic classes: class 1, Fla$^-$ mutants were nonmotile in semisolid M agar and in the light microscope and failed to produce flagellin in immunoblots (26%); class 2, Mot$^-$ mutants were nonmotile in M agar and in the microscope but produced flagellin antigen levels equivalent to the wild-type strain (1%); class 3, Che$^-$ mutants appeared nonmotile in M agar but motile in the light microscope and produced wild-type levels of flagellin (39%); and class 4, Mot$^\pm$ mutants showed limited radial expansion in M agar after prolonged incubation and produced detectable, but low levels of Fla antigen (34%).

The fourth class was the unexpected class. Further analysis of a subset of mutants from this class and representative mutants of the Fla$^-$ class was pursued. Two phenotypic classes of selected motility mutants were observed in M agar: (i) completely nonmotile strains with defects that resulted in no translocation (Fig. 1, plates A and B, incubated for 10 and 24 h, respectively) and (ii) strains with lesions that allowed slight translocation after extended incubation times (Fig. 1, plates C and D, incubated for 12 and 24 h, respectively). The partial motility of Mot$^\pm$ mutants in M agar was not the result of reversion or suppression giving rise to motile cells because the phenotype was stable. Observation of the poorly motile strains in the light microscope revealed a small percentage (≤0.05 to 0.5%) of motile cells in each population. Motility appeared to be the result of polar flagellar propulsion because all of the mutants retained the *lfg*::*lux* reporter, were unable to swarm, and failed to produce lateral flagellin. The polar flagellin profiles that are displayed in the immunoblots (see Fig. 2) correspond to the mutants in the nonmotile and the slightly motile sets shown in Fig. 1. The mutants were observed to synthesize various levels of flagellin antigen. The correlation of motility phenotype with flagellin antigen production is shown in Table 1.

Identification of polar flagellar genes: physical organization and predicted function. The tetracycline resistance from mini-Mu and flanking chromosomal DNA was cloned from some of the mutants of each class and used as a probe to retrieve

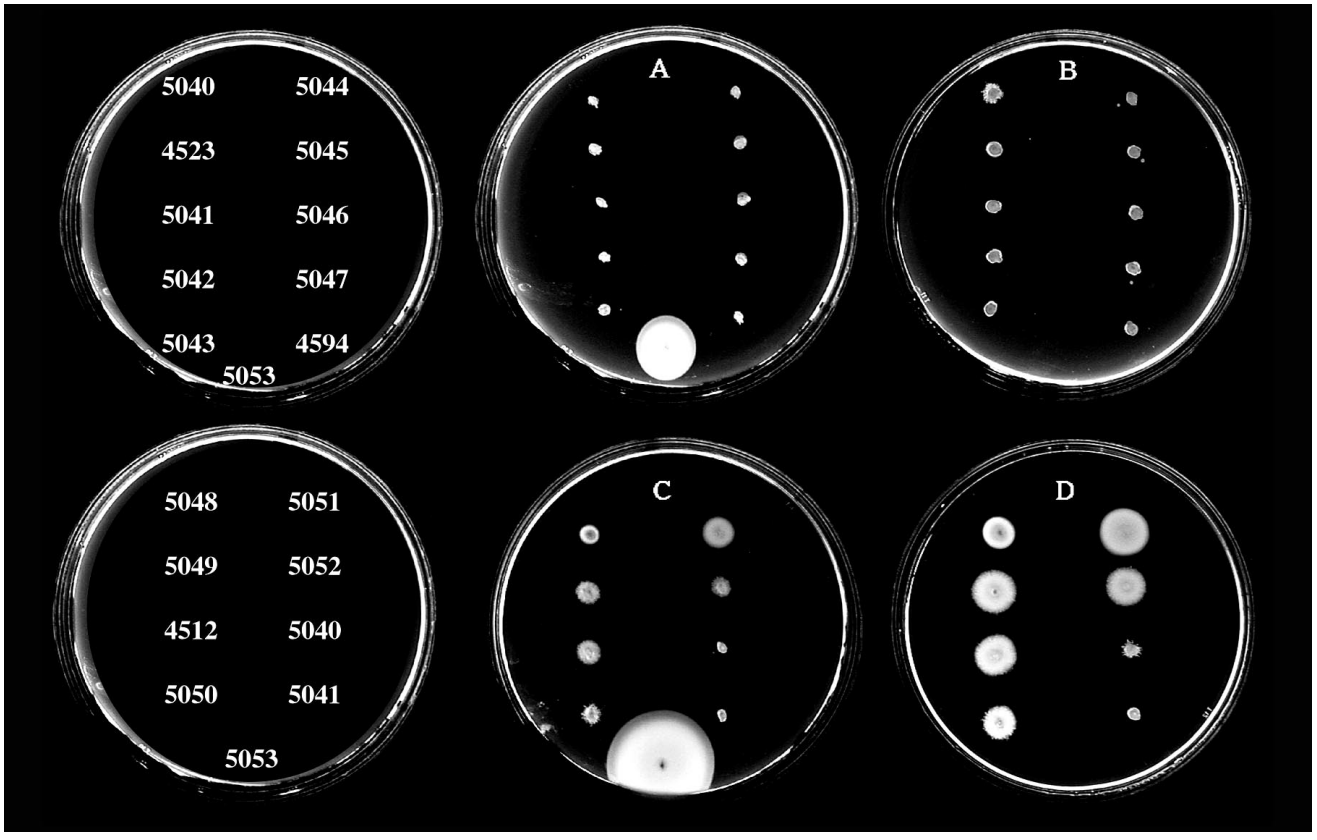

FIG. 1. Swimming motility of mini-Mu mutant strains in M agar with tetracycline. All strains were derivatives of strain LM1017. Plates A and B contain the strains indicated in the top row at the left and were incubated at 30°C for 10 and 24 h, respectively. Plates C and D contain the strains indicated in the lower row on the left and were incubated at 30°C for 12 and 24 h, respectively. Strain LM5053 was not inoculated in plates B or D. The control strain LM5053 was tetracycline-resistant and exhibited wild-type motility.

cosmids from a library of *V. parahaemolyticus* DNA. Each cosmid contained inserts of approximately 25 kb of DNA. Cosmids were used as probes for Southern blots containing restricted chromosomal DNA of the mutant strains. The cosmids revealed perturbations of the restriction pattern due to transposon insertion and allowed segregation of the mutants into linkage groups. DNA from mutants failing to show perturbations on Southern analysis was used to prepare subsequent substrates for cloning to retrieve additional loci. The initial sequence was obtained from the Mu-derived, tetracycline-resistant clones, and the nucleotide information obtained was used to continue sequencing on both strands of the cosmid clones.

Figure 3 presents a diagram of the loci obtained and the organization of the flagellar and chemotaxis genes identified. Fifty-seven potential genes encode products which are homologous to flagellar and chemotaxis proteins of other bacterial flagellar systems. In addition, there were three open reading frames (ORFs) that coded for proteins with little resemblance to flagellar sequences in the databases. The majority of the genes occurs in two regions and may be organized in large operons. Intergenic regions of less than 60 bp separate many ORFs, and some appear to be translationally coupled. The sequences contain few transcriptional termination signals (indicated by boxes in Fig. 3). The closest homologs to many of the genes are found in *V. cholerae*, *P. aeruginosa*, and *Pseudomonas putida* species. For similar genes that have been sequenced in these bacteria, the gene organization also seems highly conserved between organisms.

The polar flagellar system (Fla) is the default motility system and is produced continuously; therefore, most of the polar flagellar genes have been named analogously to homologs in other bacteria (20). Genes in the lateral flagellar system (Laf) are expressed under particular conditions and have been assigned designations that are permutations of the *fla* nomenclature. Table 2 summarizes the homology and predicted function of the gene products. By comparison with *E. coli*, a full complement of genes encoding flagellar structural components and the export apparatus has probably been elucidated; there are a few omissions and a few additions. The ORF directly downstream of *flgM* (region 1) encodes a polypeptide 141 amino acids (aa). Although it shows no homology to known flagellar gene products, we predict it may be functionally equivalent to FlgN, which is reported to act as a chaperone required for filament assembly (14), due to its size and location. Similarly, the *fliT* gene equivalent is missing, although there are two ORFs in region 2 (*flaG* and *flaI*) that encode proteins of similar size to *E. coli* FliT, which is also reported to play a chaperone-like role (14, 67). No homologs to the products of the flagellar master regulatory genes *flhD* and *flhC* exist, although alternate, potential regulatory genes, i.e., *flaK*, *flaL*, and *flaM*, occur in region 2. The predicted gene products, which resemble a number of two-component response regulators, show highest similarity to flagellar regulatory proteins of *P. aeruginosa* and *V. cholerae* (3, 25, 50). There are additional genes present in *V. parahaemolyticus* that are found in flagellar operons of other nonenteric bacteria, in particular *flhF* and

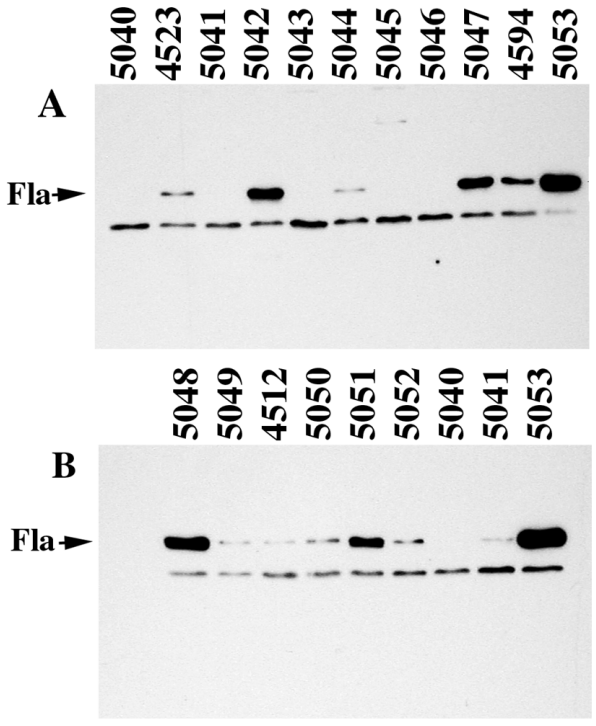

FIG. 2. Western immunoblot analysis of polar flagellin production. Blots were reacted for 2 h with antiserum (1:1,000 dilution) directed against polar flagellins (Fla). The strain numbers are indicated above the lanes. The polar flagellins are similar in molecular size and comigrate in the resolving gel system used. An antiserum-reactive, nonflagellin band serves as a control for the amount of whole cells loaded in each lane.

flhG, which resemble GTP- and ATP-binding proteins, respectively.

The complement of *che* genes and their organization are different from *E. coli*. Genes encoding the methyl-accepting chemotaxis proteins have not been found within the flagellum-chemotaxis clusters. Novel genes include *cheV* (in region 1), which encodes a hybrid CheY-CheW protein that also exists in *Bacillus subtilis* (51) and three unusual ORFs that occur within the *che* gene cluster of region 2. ORF1 encodes a protein that resembles Soj of *B. subtilis* and other ATPase proteins involved in chromosome partitioning (55); the other ORFs encode proteins that fail to resemble proteins of known function. Similar coding regions, specifically ORF1 and ORF2, have been observed in a chemotaxis locus of *P. putida* (10).

Most of the predicted *V. parahaemolyticus* polar flagellar gene products align with flagellar counterparts in other organisms throughout the length of each protein. Some proteins show divergence at the N terminus. An example is the M ring, which is the *fliF* product. Alignment begins at aa 62 of *V. parahaemolyticus* FliF with aa 33 of *E. coli* FliF. Another case occurs with the product of *flgH* (259 aa), which potentially encodes the L ring of the flagellar basal body. The first 94 aa of *V. parahaemolyticus* FlgH fail to align with known FlgH proteins, whereas the remainder of the molecule produces significant alignment with other FlgH proteins, e.g., 39% identities and 57% positives with *E. coli* FlgH using BLAST analysis. In comparison, the full lengths of *V. parahaemolyticus* FlgI and *E. coli* FlgI align completely (46% identities and 65% positives). *E. coli* FlgI forms the P ring. A few proteins show significant gaps within the alignment. One striking example is FlhF (505 aa), which contains an insertion spanning 170 aa that is not found in other homologs; this domain shows limited homology with the sodium channel I of rat using BLAST analysis (35% identities and 55% positives).

Correlation of genotype with phenotype. Sequence information coupled with restriction patterns using Southern analysis allowed assignment of lesions to specific gene intervals. The majority of the chemotaxis-defective mutants analyzed showed transposon-induced perturbations that placed the insertion defects within *che* clusters in region 1 or region 2. A minority (1%) of nonmotile *V. parahaemolyticus* mutants displayed the Mot^- phenotype, and three mutants were determined to contain mutations in novel motor genes, *motX* and *motY* (35, 36). A correlation of the genotype of the Fla^- and $Mot^\pm$ mutants examined in Fig. 1 and 2 with phenotype is presented in Table 1. Strains LM5040, LM5043, LM5045, and LM5046 produced no flagellin, and the mini-Mu insertions in these strains mapped to intervals within the *flhBA* locus, which encodes components of the flagellar export pathway. One other insertion in the *flhBA* locus was detected. The phenotype and genotype of this strain, LM5051, was puzzling until the precise mutation was cloned and sequenced. LM5051 was partially motile and produced levels of flagellin comparable to wild-type levels. Cloning and sequencing of the mini-Mu insertion of this strain revealed that the transposon was inserted into the intergenic region between *flhA* and *flhF*. Nonmotile strain LM5042 also produced as much flagellar antigen as the wild type and contained a defect in the region encoding hook-associated-like proteins (the *flgKL* interval). The phenotype of LM5042 matched other strains with insertions in *flgK* and *flgL* that were previously created by allelic exchange (37). All of the mutants in the $Mot^\pm$ class mapped in the *flgB-flgH* interval, which encodes hook and basal body components. Thus, mutants with defects in genes encoding assembly apparatus fail to produce a flagellum or synthesize flagellins, whereas mutants with defects in many of the genes encoding structural parts of the basal

TABLE 1. Phenotypes of mini-Mu insertion mutant strains

Strain name[a]	Mot phenotype[b]	Fla phenotype[c]	Polar gene defect interval[d]
LM4512	$Mot^\pm$	$Fla^\pm$	*flgFGH*
LM4523	Mot^-	$Fla^\pm$	*flgFGH*
LM4594	Mot^-	$Fla^\pm$	*flgDE*
LM5040	Mot^-	Fla^-	*flhBA*
LM5041	Mot^-	$Fla^\pm$	*flgDE*
LM5042	Mot^-	Fla^+	*flgKL*
LM5043	Mot^-	Fla^-	*flhA*
LM5044	Mot^-	$Fla^\pm$	*flgHIJK*
LM5045	Mot^-	Fla^-	*flhBA*
LM5046	Mot^-	Fla^-	*flhBA*
LM5047	Mot^-	$Fla^\pm$	*flgBC*
LM5048	Che^-	Fla^+	*cheB*
LM5049	$Mot^\pm$	$Fla^\pm$	*flgFGH*
LM5050	$Mot^\pm$	$Fla^\pm$	*flgBC*
LM5051	$Mot^\pm$	Fla^+	*flhAF*
LM5052	$Mot^\pm$	$Fla^\pm$	*flgFGH*
LM5053[e]	Mot^+	Fla^+	None

[a] All strains were derived from strain LM1017, which is defective for swarming motility as a result of a mutation in the lateral flagellar hook gene (*lfgE313*::*lux*).

[b] Mot^-, nonmotile in M agar and in light microscope; $Mot^\pm$, slight radial expansion in M agar and a low population of motile cells in light microscope; Che^-, slight radial expansion in M agar and highly motile population in light microscope.

[c] Fla^+, polar flagellar antigen levels in immunoblots equivalent to wild type; Fla^-, no Fla antigen; $Fla^\pm$, intermediate levels of Fla antigen.

[d] Mutations created by mini-Mu insertion were mapped by Southern analysis to specific restriction fragments carrying indicated genes.

[e] Wild-type phenotype for motility with random mini-Mu insertion.

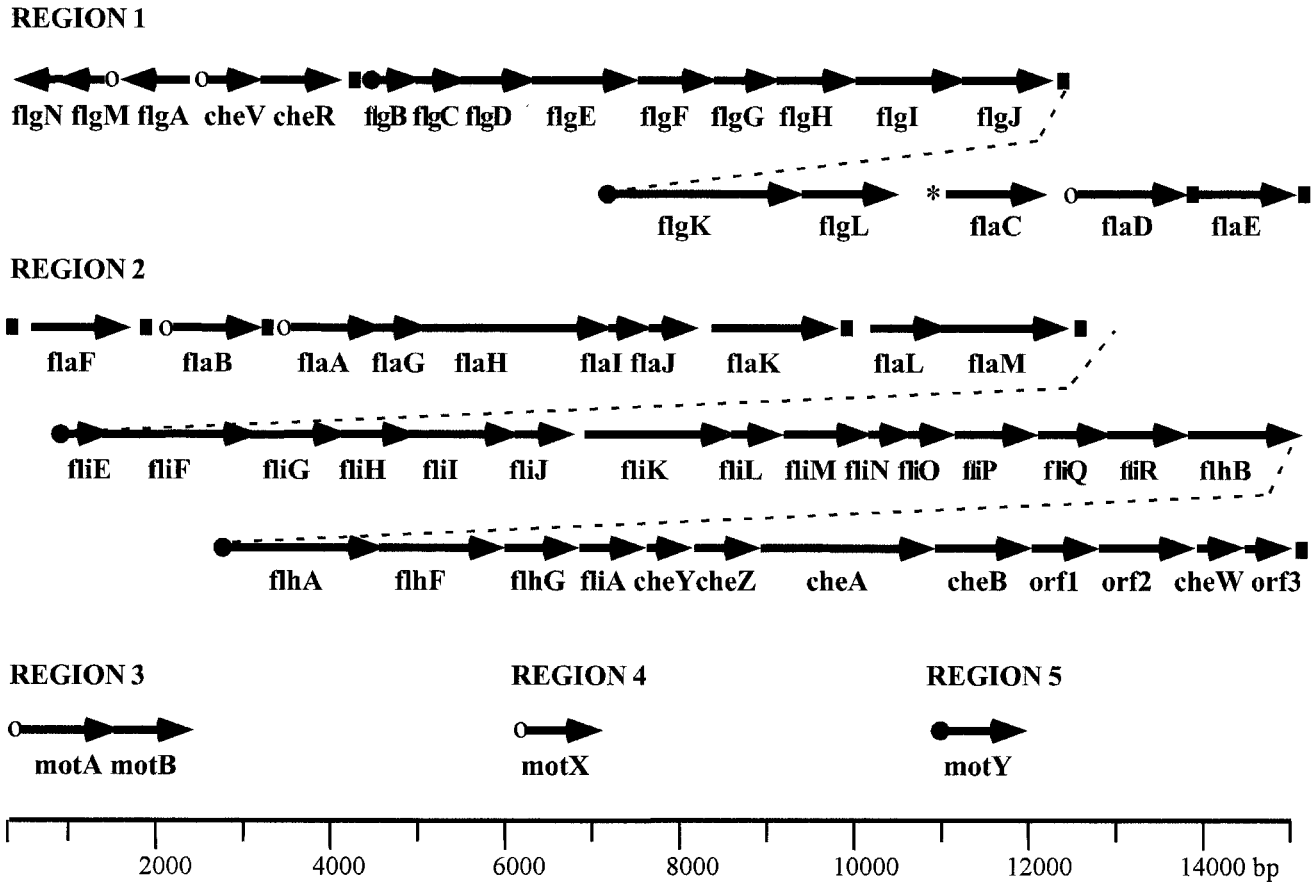

FIG. 3. Organization of polar flagellar gene system. Arrows indicate the direction of transcription and the extent of coding sequence for each gene. The filled circles indicate the σ^{54} class, and the open circles indicate the σ^{28} class of promoters that have been mapped by primer extension analysis. The promoter region for *flaC* is unusual and is indicated by the asterisk. The filled boxes indicate predicted rho-independent transcriptional terminators.

body, but not hook-associated proteins, seem to be able to occasionally synthesize a functional polar flagellum.

Six polar flagellin genes. Prior work identified genes encoding four polar flagellin subunits that were organized in tandem in two distinct loci, *flaBA* and *flaCD* (37). Further sequencing of the flagellin-encoding loci revealed two additional flagellin genes, *flaF* (located upstream of *flaB* transcription) and *flaE* (located downstream of *flaD* transcription). Thus, the present total number of genes encoding the structural subunits of the polar flagellar filament is six. A comparison of their relatedness to each other and to the lateral flagellin is shown in Table 3. Their location, homology, and genetic analysis suggest that these are polar genes; however, none of the flagellin genes appears to be essential for polar filament formation (37).

In order to gain insight into why this organism possesses such an extraordinary number of flagellins and to begin to elucidate flagellar transcriptional control, primer extension analysis was used to define promoter structure. Previous analysis suggested that the genes occurred in distinct transcriptional units. The *flaA*, *flaB*, and *flaD* genes possessed upstream sequences resembling the consensus σ^{28}-dependent flagellar promoter (TAAA n15 GCCGTTAA [17]), and flagellin production in *E. coli* was shown to be dependent on the product of *E. coli fliA*, σ^{28} (42). In contrast, *flaC* was very poorly expressed in *E. coli*, and immunodetection of FlaC flagellin required the product of an additional gene, *flaJ*, which resembles the putative chaperone FliS (60). In *E. coli*, *flaC* expression did not require σ^{28}.

Primer extension analysis in *V. parahaemolyticus* supports the idea that a polar flagellum-specific σ^{28} recognizes the promoters of *flaA*, *flaB*, and *flaD* (Fig. 4 and Table 4). Primer extension products using *flaA*-, *flaB*-, or *flaD*-specific primers were identical in reactions using RNA prepared from broth- or plate-grown cells. Upstream of the coding sequence for flagellin F are sequences similar to the promoters of *flaA*, *flaB*, and *flaD*; however, we have been unable to detect a discrete primer extension product. There appears to be some readthrough transcription originating upstream of the *flaF* gene. Primer extension analysis suggested that *flaE* is cotranscribed with the *flaD* coding region, although the intergenic region between the *flaD* and *flaE*, which is 122 bp, contains a predicted rho-independent terminator structure. A ladder of products was obtained using reverse transcriptase that had been primed with an oligonucleotide designed to hybridize to the 5′ end of the *flaE* message. The lengths of the products were calculated to extend into the *flaD*-coding region (data not shown); therefore, it appears that *flaD* and *flaE* may be coexpressed as a single transcriptional unit. Prior genetic evidence supports this hypothesis; no polar flagellin can be detected in a Δ*flaFBA* Δ*flaCD* mutant (37).

Expression of *flaC* is unique. Figure 5 shows the primer extension reactions that were initiated using an oligonucleotide specific for *flaC*. A major product was obtained (lane 1, indicated by arrow), and the upstream sequences do not resemble the upstream regions of the other σ^{28}-like flagellin promoters (Table 4). Moreover, the product was only observed in RNA

TABLE 2. Chemotaxis and polar flagellar genes of *V. parahaemolyticus*

Region (GenBank no.) and gene	Gene product homolog or predicted function
1 (U12817)	
flgN	None; potential chaperone
flgM	Anti-σ^{28} factor
flgA	Necessary for P-ring addition
cheV	Chemotaxis CheY/CheW hybrid
cheR	Chemotaxis methyltransferase
flgB	Rod
flgC	Rod
flgD	Rod
flgE	Hook
flgF	Rod
flgG	Rod
flgH	L ring
flgI	P ring
flgJ	Peptidoglycan hydrolyzing flagellar protein
flgK	HAP1
flgL	HAP3
flaC	Flagellin
flaD	Flagellin
flaE	Flagellin
3 (AF069391)	
motA	Na^+ motor component
motB	Na^+ motor component
4 (U09005), *motX*	Na^+ motor component
5 (U06949), *motY*	Na^+ motor component
2 (AF069392)	
flaF	Flagellin
flaB	Flagellin
flaA	Flagellin
flaG	Slight homology to N terminus of multiple flagellins
flaH	HAP2
flaI	None
flaJ	FliS
flaK	Two-component response regulator
flaL	Two-component sensor kinase
flaM	Two-component response regulator
fliE	Hook-basal body component
fliF	M ring
fliG	Switch component
fliH	Fla export and assembly
fliI	Fla export; ATP synthase
fliJ	Fla export and assembly
fliK	Hook length control
fliL	Flagellar protein
fliM	Switch component
fliN	Switch component
fliO	Fla export and assembly
fliP	Fla export and assembly
fliQ	Fla export and assembly
fliR	Fla export and assembly
flhB	Fla export and assembly
flhA	Fla export and assembly
flhF	Flagellar protein; also homologous to FtsY; potential GTP-binding protein
flhG	MinD and other ATP-binding proteins
fliA	RNA polymerase σ^{28} factor
cheY	Causes change in direction of flagellar rotation
cheZ	Dephosphorylates CheY
cheA	CheA kinase
cheB	Chemotaxis methylesterase
ORF1	Soj-like and other chromosome-partitioning ATPase proteins
ORF2	Unknown
cheW	Purine-binding chemotaxis protein
ORF3	Unknown

prepared from plate-grown wild-type cells and not in RNA from wild-type cells grown in broth (lane 1 versus 2). Expression of *flaC* appeared to be surface dependent. The control panel, labeled flaD, demonstrates that equivalent amounts of plate- and broth-derived RNA were used. The control reactions used the same RNA preparations and a *flaD*-specific primer (lanes 1 and 2 compared to 4 and 5, respectively). The *flaD* transcript was expressed in the wild-type strain in liquid and on surfaces. We have shown previously that plate-grown cells are starved for iron (41). To examine whether the environmental signal controlling *flaC* expression might be the result of iron starvation or some other nutrient condition, RNA was prepared from the wild-type cells that were grown in 2216 marine broth, which is growth limiting for phosphate and iron (40, 41). No *flaC*-dependent primer extension product was observed under this condition, although a *flaB*-dependent transcript could be detected (lane 7 versus lane 10). Primer extension reactions using RNA prepared from HI broth and plate cultures that were cultured in parallel to the 2216 broth culture reproduced the surface-induced *flaC* product (lanes 8 and 9 versus lanes 11 and 12). There is also a ladder of large primer extension products (most evident in lanes 7 to 9), suggesting some basal level of readthrough transcription originating with the upstream *flgK* operon.

Prior work established that the mutation in LM1017 occurs in a gene (the hook gene, *lfgE*) near the top of the lateral flagellar hierarchy and that many surface-dependent genes, including genes coding the lateral-specific flagellar σ^{28} and lateral flagellin, fail to be expressed in LM1017 (42). No *flaC*-dependent primer extension product was obtained using RNA prepared from plate-grown LM1017 (lane 3), although *flaD*-specific product could be detected (lane 3 versus lane 6). Thus, *flaC* expression appears to require an intact lateral flagellar genetic pathway.

TABLE 3. Comparison of the flagellins of *V. parahaemolyticus*

Flagellin	% Identity[a] with other *V. parahaemolyticus* flagellins						Length (aa)	Homolog[b]	% Identity to homolog[b]
	FlaA	FlaB	FlaC	FlaD	FlaE	FlaF			
FlaA	100	78	65	78	48	67	376	vcFlaB	83
FlaB		100	68	99	50	69	378	vaFlaD	85
FlaC			100	68	45	64	384	vcFlaA	78
FlaD				100	50	68	378	vaFlaD	85
FlaE					100	47	374	vcFlaD	49
FlaF						100	377	vaFlaE	78
LafA[c]	34	33	35	33	27	34	284	ppFla	41

[a] Percent identities were determined by GCG BestFit analysis.
[b] Closest homolog in another organism identified by using a BLAST search. va, vc, and pp, *V. anguillarum*, *V. cholerae*, and *P. putida*, respectively.
[c] LafA is the lateral flagellin.

Other flagellar and chemotaxis promoters. To establish a basis for the polar flagellar regulatory hierarchy, the transcriptional start sites for a number of other promoters were determined. The basic strategy targeted genes that possessed significant upstream, intergenic, or noncoding sequence (usually >50 bp). Figure 6 shows the primer extension products for *motA*, *motX*, and *motY*. Table 4 shows the tabulation of the upstream sequence information for all of the transcriptional start sites that have been determined. Both *motA* and *motX* possess upstream sequences that resemble the σ^{28}-dependent promoters, whereas the sequences upstream of *motY* appear to resemble a potential σ^{54}-dependent promoter (TGGCAC n5 TTGC, containing an invariant −24 GG motif and a conserved −12 GC motif [56]). All *mot* transcripts were expressed in broth- and plate-grown cells. Table 4 also shows the transcriptional start site and upstream sequences for six other genes. The promoters for *cheV* and *flgM* appear to be σ^{28} dependent, and *fliE*, *flgB*, *flgK*, and *flhA* appear to be σ^{54} dependent. The evidence suggests that *flgM* is also transcribed from an upstream promoter because of the presence of faint ladders of abortive primer extension products that extend to the top of the sequencing gel. There is one other relatively large intergenic gap (175 bp) in region 2, which occurs between *fliJ* and *fliK*. Using *fliK*-derived primers, a ladder of prominent primer extension products was observed on sequencing gels. This evidence suggests that *fliK* is transcribed from upstream sequences as part of an operon. Primer extension has not proved suitable for transcriptional analysis of *flaK* and *flaL* because prominent ladders of extension products are obtained. We hypothesize that some of the products may be the result of multiple species of RNA (i.e., multiple promoter control) and some may be caused by premature termination due to RNA secondary structure (i.e., a potential rho-independent terminator is found between *flaK* and *flaL*).

DISCUSSION

The compilation of the repertoire of polar flagellar and chemotaxis genes of *V. parahaemolyticus* represents a wealth of useful information pertinent to flagellar assembly, sheath for-

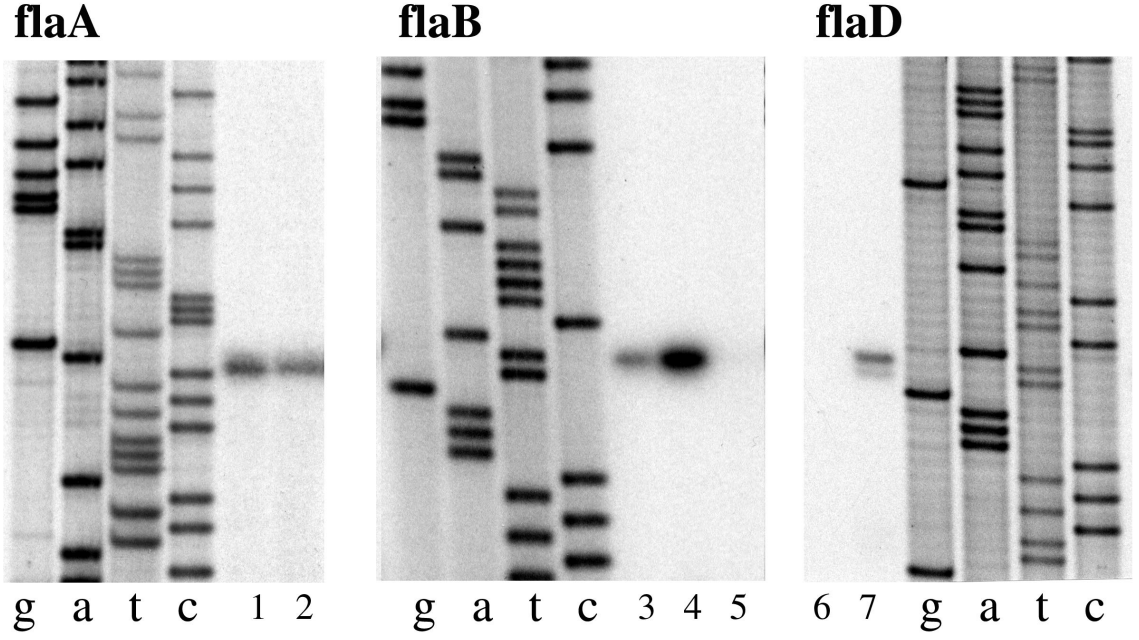

FIG. 4. Primer extension analysis of flagellin gene transcription. RNA was prepared from the wild-type strain BB22 after growth in HI broth (B) or on HI plates (P). The amount of RNA in each lane was as follows: 1, 2 μg (B); 2, 2 μg (P); 3, 2 μg (B); 4, 5 μg (P); 5, minus RNA; 6, minus RNA; and 7, 2 μg (B). Lanes g, a, t, and c correspond to the dideoxy nucleotide used in the sequencing reactions. Sequence and primer extension products were generated with *flaA*-, *flaB*-, or *flaD*-specific primers. The primers were flaA (5′-GCTGTGCGGTCATCGCAGAAACG-3′), flaB (5′-GTGTTTAATTCACTGCCATG-3′), and flaD (5′-CGGTCATCGCTGATACGTTAGTG-3′).

TABLE 4. Flagellar promoter structure

Gene	Promoter regions[a]
Potential σ^{28}-dependent promoters	
flaA	CTAAAG gatatgcatacgtc GCCGTTAA agggact G
flaB	CTAAAG aaatcaggttgagc GCCGTTAA taaaagt A
flaD	CTAAAG cttctgaatttggt GTCGTTAA tagaagt AA
motX	CTAAAG cttagctgcagatt GCCGATAA gtttatc A
motA[b]	CTAAAA aaatctgttctagtt GTCGATAC aagtaat A
cheV	CTAAAc atactgagcaaaat GCCGATAG acttagc G
flgM[b]	tTAAAG ttatcgtttggttg GTCGATAG tctggat A
Consensus	CTAAAG n14 G(C/T)CG(A/T)TAA n7 +1
Potential σ^{54}-dependent promoters	
motY	TGGC gggattt TTGC atgacaatcgtt G
flgB (P1)[c]	TGGC ttgctta TTGC agtctaaaacgtc A
flgB (P2)	TGGC acgctaa TTGC tatttagttatt A
flgK	TGGC acatctt TTGC tttcacttgtcta G
flhA[b]	aGGC gaaatgg TcGC gtataaacatt A
fliE	TGGC acataaa TTGC tgtgtcaatattt A
Consensus	TGGC n7 TTGC n11-13 +1
Other promoters, *flaC*	tttagcaagtaatttttacggtcagtgcttatccaa A

[a] Defined by primer extension analysis. Underlined, capitalized nucleotide designates +1 with respect to transcription. Uppercase letters indicate residues conserved among polar flagellar promoters or with respect to consensus promoters.

[b] Faint ladders of primer extension products suggest that the gene may also be transcribed as part of a larger transcriptional unit initiating from the promoter of an upstream gene.

[c] Two primer extension products, labeled P1 and P2, resulted for the *flgB* promoter. P1 and P2 are separated by 62 bp.

mation, polar placement, and perhaps even the connection of flagellation with the cell cycle. In addition, because the polar flagellum of *V. parahaemolyticus* appears to act a mechanosensor (34), an understanding of polar flagellar structure and regulation is critical for gaining insight into the mechanism of surface sensing and swarmer cell development.

Flagellar assembly. Flagella are assembled via a type III export pathway. No consensus flagellar export signal has been defined, although a number of models have been proposed, and it seems likely that classes of sequentially exported proteins exist (8). Since *V. parahaemolyticus* can simultaneously assemble the lateral and polar flagella, it is an ideal system to study type III secretion determinants and the specificity of export. The sequence divergence observed between the N terminus of many (but not all) of the predicted polar *V. parahaemolyticus* structural proteins, as well as for the predicted chaperone-like molecules and FlgM, and components of the lateral *V. parahaemolyticus* system and flagellar systems of other bacteria may not only allow discernment of classes of export substrates but will also provide a system for testing potential signals.

Sheath formation and the basal body complex. Little is known about the formation or function of flagellar sheaths, which are found in many bacteria, including marine *Vibrio* species, *V. cholerae*, *Bdellovibrio bacteriovorus*, and *Helicobacter pylori* (reviewed in reference 59). These sheaths appear to be extensions of the cell outer membrane, although their composition suggests that the sheath forms a distinct, stable membrane domain. Moreover, some evidence suggests that the polar basal body structure differs from peritrichously inserted basal bodies. Two models for the basal organelle of polar flagella have been derived from electron microscopy studies of *V. cholerae* (sheathed), *Campylobacter fetus* (unsheathed), *Bdellovibrio bacteriovorus* (sheathed), and *Wolinella succinogenes* (unsheathed) (12, 13, 61). Regardless of whether the flagellum is sheathed or unsheathed, all of the studies report the existence in the basal body complex of a large convex disk situated below the outer membrane. We have also seen this disk with *V. parahaemolyticus* (unpublished observation). One model suggests that the disk is the P-ring equivalent (acting as the bushing associated with peptidoglycan) and that the L ring (lipopolysaccharide associated) is not present. The second model places the large disk between the P and L rings. Thus, the identity of the genes encoding basal body parts of a polar flagellum is of interest. We have found a locus encoding the *V. parahaemolyticus* genes for basal body and hook components. Genes for both the L and the P ring exist, providing genetic support for the second model. Whereas the *V. parahaemolyticus* P ring displays homology with other P rings over the full length of the protein, the N terminus of the *V. parahaemolyticus* L ring (FlgH) is divergent. The L ring protein of *S. enterica* serovar Typhimurium has been shown to be a lipoprotein and postulated to anchor the basal body in the outer membrane (54). Perhaps the nature of this protein is key to understanding differences between sheathed and unsheathed flagella. Such a possibility awaits further analysis, particularly biochemical elucidation of the N terminus of *V. parahaemolyticus* FlgH.

Polar flagellar placement. One pair of genes not found in *E. coli* includes *flhF* and *flhG* (region 2). The *flhF* gene was first discovered in *B. subtilis*, where it was demonstrated to be required for motility (7). A nonpolar, null mutation in *flhF* produced nonmotile cells lacking flagella. FlhF shows homology to FtsY, which is a GTP-binding protein involved in the signal recognition particle targeting pathway. Intriguingly, the *V. parahaemolyticus* polar homolog contains an insertion of ~170 aa that is not found in other homologs. The inserted domain shows homology to a eukaryotic sodium channel. It is tempting to speculate that the insertion is unique for sodium-type flagella because all of the FlhF sequences deposited in GenBank are derived from organisms that possess proton-driven flagella.

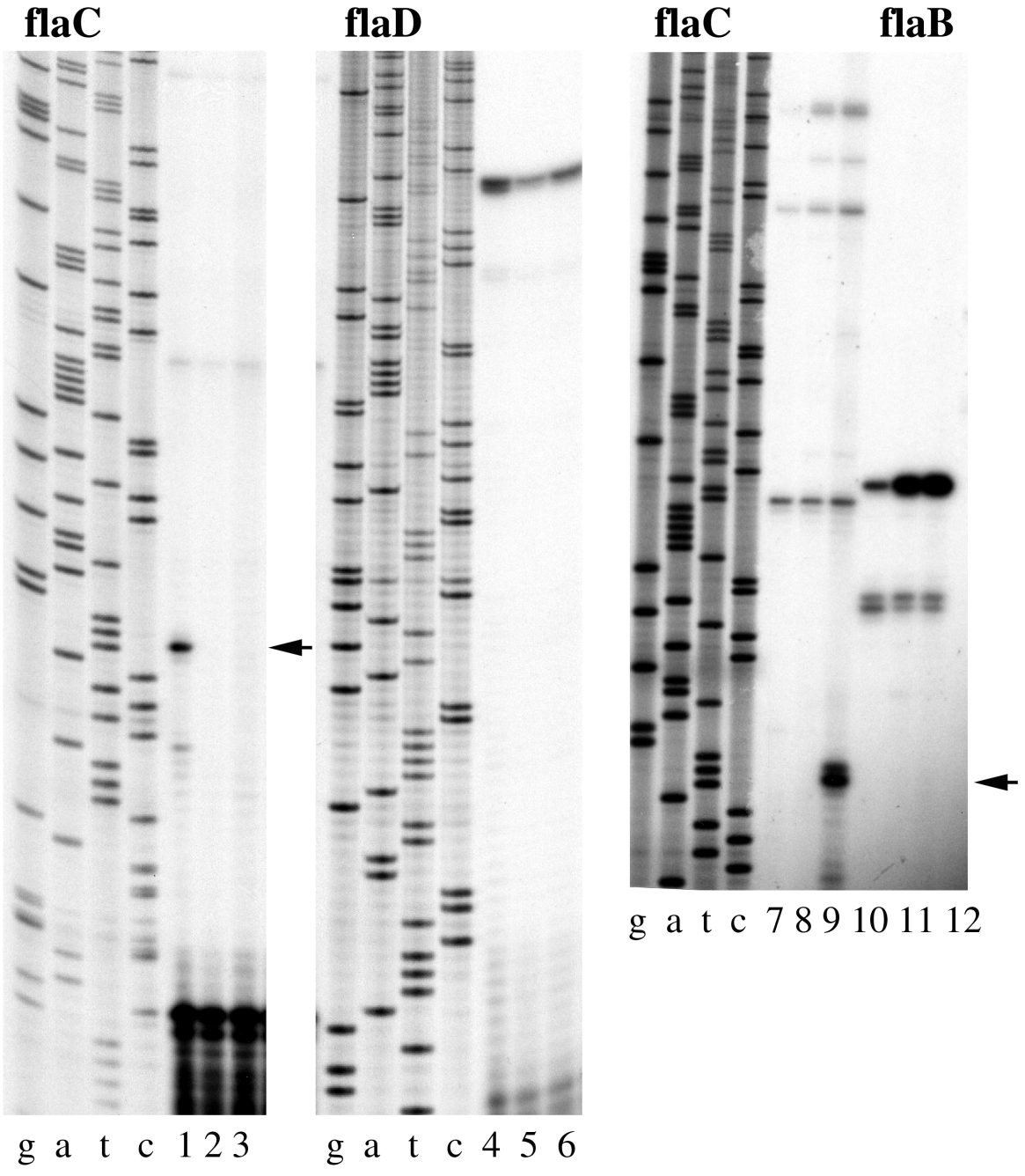

FIG. 5. Primer extension analysis of *flaC* transcription. The arrows indicate the surface-dependent *flaC* primer extension product. RNA was prepared from the wild-type strain BB22 or LM1017 after growth in HI broth (B), on HI plates (P), or in 2216 marine broth (2216B). Approximately 2 μg of total RNA was used in each primer extension reaction. Reactions 1 to 3 and 7 to 9 were primed with a *flaC*-specific oligonucleotide. Reactions 4 to 6 were primed with a *flaD*-specific oligonucleotide. Reactions 10 to 12 were primed with a *flaB*-specific oligonucleotide. Lanes: 1, BB22 (P); 2, BB22 (B); 3, LM1017 (P); 4, BB22 (P); 5, BB22 (B); 6, LM1017 (P); 7, BB22 (2216B); 8, BB22 (B); 9, BB22 (P); 10, BB22 (2216B); 11, BB22 (B); and 12, BB22 (P). Lanes g, a, t, and c correspond to the dideoxy nucleotide used in the sequencing reactions. Sequence and primer extension products were generated with *flaC*-, *flaD*-, or *flaB*-specific primers. The *flaC*-specific primer was 5′-CTGTTACAGCCATTTTGCTCTCC-3′.

Located 15 bp downstream of *V. parahaemolyticus flhF* is *flhG*, which encodes a protein that shows homology to MinD, a membrane ATPase involved in septum site determination. Perhaps FlhF and FlhG work as a pair to determine site selection of flagellar insertion. The FlhF-FlhG pair is found in a number of polarly flagellated bacteria. In *P. aeruginosa*, a mutation in *flhG* was recently shown to increase the number of polar flagella (9). It should be noted that the gene encoding σ^{28} is immediately downstream of *flhG*; in fact, translation appears to be coupled for the coding regions of *flhG* and *fliA* overlap by 10 bp.

Chemotaxis. Mutations in two distinct loci produced chemotaxis-defective strains. Possessing different kinds of upstream controlling elements, the two *che* clusters appear to occur in different classes of the hierarchy. One locus in region 2 encodes most of the major cytoplasmic chemotaxis proteins, i.e.,

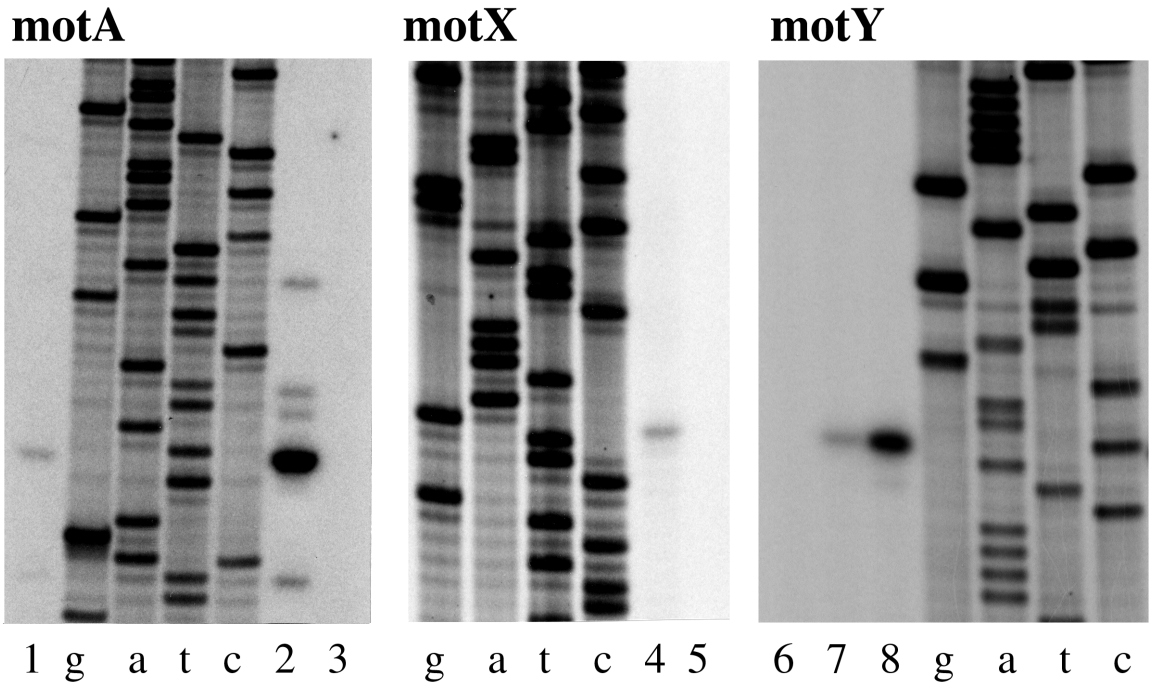

FIG. 6. Primer extension analysis of *mot* gene transcription. RNA was prepared from the wild-type strain BB22 after growth in HI broth (B) or on HI plates (P). The amount of RNA in each lane was as follows: 1, 2 μg (P); 2, 12 μg (P); 3, minus RNA; 4, 2 μg (P); 5, minus RNA; 6, minus RNA; 7, 2 μg (B); and 8, 5 μg (P). Lanes g, a, t, and c correspond to the dideoxy nucleotide used in the sequencing reactions. Sequence and primer extension products were generated with *motA*-, *motX*-, or *motY*-specific primers. The oligonucleotide primers were motA (5′-CCACCGATCAAACCTATTAGGGTTGC-3′), motX (5′-CAGTAACAGTGAAGCAGCCA CTG-3′), and motY (5′-GTTATCAGCCATTTATTCATC-3′).

CheY, CheZ, CheA, CheB, and CheW. It seems likely that these genes are under the control of σ^{54} since they are very tightly linked to each other in a potential operon initiating with *flhA*. The second cluster, which occurs in region 1, encodes CheB and a hybrid CheY-CheW protein, similar to CheV of *B. subtilis* (51). Transcription of *cheV* clearly initiates at σ^{28}-type promoter sequences. Although we know that *che* mutations in region 2 affect polar and lateral motility (53) and that *che* lesions in region 1 perturb polar motility, the roles that region 1 *che* genes play in modulating lateral motility remain to be determined. Perhaps the region 1 *che* genes are dedicated to the polar system.

Additional ORFs. Three additional ORFs were found within the region 2 *che* locus. Two encode potential proteins of unknown function and the third encodes a protein that resembles Soj of *B. subtilis* and other chromosome-partitioning ATPases. In *B. subtilis*, Soj plays a role in cell cycle progression by coupling chromosome segregation to development (55). It seems curious that a Soj-like protein exists within a flagellum-chemotaxis operon and that this particular arrangement is found in other bacteria, e.g., *P. aeruginosa* and *P. putida*. Perhaps these novel ORFs will provide the key for a similar linkage between cell division and flagellation or development.

The polar flagellar hierarchy. Analysis of motility mutant phenotypes provides some insight into the hierarchy of *V. parahaemolyticus* polar flagellar gene control and assembly. We have previously shown that mutants with defects in any of the four polar motor genes produce flagella, whereas mutants with defects in the switch genes do not (6). Switch genes are known to be required for flagellar assembly, rotation, and chemotaxis (66). The switch genes are found in region 2 along with other genes known to participate in the flagellar assembly and export pathway. Mutants with defects in the *flhBA* interval, which encodes components of the export apparatus, displayed the same phenotype as switch mutants, i.e., they were nonmotile, nonflagellated, and unable to synthesize flagellin. Most of these genes in region 2 are tightly linked. Precedence for large motility operons has been established in other bacteria, e.g., *Borrelia burgdorferi* (15). Primer extension analysis identified a potential σ^{54}-dependent promoter preceding *fliE*. We postulate genes in the *fliE-flhB* region constitute a large flagellar operon. Downstream of *flhB* and preceding *flhA*, there is a relatively large intergenic region (230 bp). Primer extension analysis identified a promoter region, and these sequences also resembled the canonical σ^{54}-dependent promoter.

Much of region 1 contains hook, hook-associated, and basal body genes, which also appear to be under control of σ^{54}-like promoters preceding *flgB* and *flgK*. Mutants with defects in the hook and basal body genes yielded unexpected phenotypes. Slow radial expansion could be detected after prolonged incubation of motility plates, and some flagellin antigen was produced. We hypothesize some lateral flagellar structural parts may be able to partially substitute for loss of some polar structural components; however, substitution does not seem to be highly effective because only a few cells in each population appeared motile in the microscope. Possibly, cross-functionality of polar and lateral parts is very poor, or lateral proteins are limiting because of the genetic background of strain LM1017. Region 1 also contains the gene encoding *flgM*, the anti-σ^{28} factor. In *S. enterica* serovar Typhimurium, *flgM* is controlled by two promoters (16); this appears to be the case in *V. parahaemolyticus* as well. Transcription initiates immediately upstream of *flgM* near σ^{28}-like sequences. Faint primer extension ladders suggest that the gene may also be cotranscribed with the upstream gene, *flgA*.

σ^{54}-dependent regulation of flagellar genes is consistent with

observations in other organisms. Flagellation in *V. alginolyticus*, *V. cholerae*, *V. anguillarum*, and *P. aeruginosa* has been shown to require the *rpoN* gene, which encodes σ^{54} (23, 25, 47, 63). Moreover, genes encoding σ^{54}-type regulators exist in *V. parahaemolyticus*, i.e., *flaK* and *flaM*, as well as in the above-mentioned organisms (3, 25, 50, 60). FlaL resembles a two-component sensor; FlaK and FlaM resemble two-component response regulators that show homology to each other except in their C-terminal, putative DNA-binding domains. Their precise regulatory roles remain to be defined, and they may play unique roles with respect to signal input and/or output in different organisms. For example, *flaK* contributes to, but is not required for, motility in *V. parahaemolyticus* (60), whereas it appears to be essential for motility in *V. cholerae* and *P. aeruginosa* (3, 25).

To summarize, one level of polar flagellar gene transcription appears to be controlled in a σ^{54}-dependent manner. We define the consensus promoter structure for this class of flagellar genes to be TGGC n7 TTGC n11 +1. Some of the genes in the σ^{54}-type class are dedicated to assembly of the hook-basal body structure. Additionally, one finds the motor gene *motY*, hook-associated proteins 1 and 3, chemotaxis genes, and *fliA*, encoding σ^{28}. In turn, this alternative sigma factor appears to be specific for the other large subset of flagellar promoters. We define the promoter structure for this class to be CTAAAG n14 G(C/T)CG(A/T)TAA n7 +1, which compares favorably with the recently revised structure of the σ^{28}-dependent flagellar promoters of *E. coli* and *S. enterica* serovar Typhimurium (TAAAGTTT n11 GCCGATAA) (19). The genes under this level of control encode additional motor parts (MotA, MotB, and MotX), chemotaxis proteins, the distal capping protein HAP2, FlgM, putative flagellar chaperones, and five flagellins.

Summary. Thus, there are common themes in polar flagellar gene organization and regulation, but there also appear to be unique variations. The differences may reflect the lifestyle of each organism. For example, the organization of the multiple polar flagellin genes in *V. parahaemolyticus* is similar to loci in *V. anguillarum* and *V. cholerae* (24, 43). In these organisms, only one specific flagellin is required for motility, and its expression is under σ^{54}-dependent control, whereas the other flagellin genes are dispensable and require σ^{28}. The critical flagellin gene of *V. anguillarum* and *V. cholerae* is most equivalent with respect to gene location and the predicted protein sequence to *V. parahaemolyticus flaC*. The *flaC* gene is also under different environmental control from the other *V. parahaemolyticus* polar flagellins, which have σ^{28}-dependent promoters. However, this gene is not essential for motility, and its regulation is not directed by σ^{54}. The promoter structure of the *flaC* flagellin-encoding gene is unusual, and expression is controlled in a surface-dependent manner. What this means with respect to polar flagellar function and regulation and in the context of growth on surfaces and swarmer cell differentiation remains to be investigated.

ACKNOWLEDGMENTS

We thank Deborah Noack for pioneering our primer extension studies, Jodi Enos-Berlage, Sandford Jaques, and Bonnie Stewart for helpful discussions, and the DNA Core of the University of Iowa for excellent support.

This work was supported by Public Health Service grant GM43196 from the National Institutes of Health.

REFERENCES

1. **Aizawa, S.-I., and T. Kubori.** 1998. Bacterial flagellation and cell division. Genes Cells **3:**625–634.
2. **Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
3. **Arora, S. W., B. W. Ritchings, E. C. Almira, S. Lory, and R. Ramphal.** 1997. A transcriptional activator, FleQ, regulates mucin adhesion and flagellar gene expression in *Pseudomonas aeruginosa* in a cascade manner. J. Bacteriol. **179:**5574–5581.
4. **Belas, R., M. Simon, and M. Silverman.** 1986. Regulation of lateral flagella gene transcription in *Vibrio parahaemolyticus*. J. Bacteriol. **167:**210–218.
5. **Blair, D. F.** 1995. How bacteria sense and swim. Annu. Rev. Microbiol. **49:** 489–522.
6. **Boles, B. R., and L. L. McCarter.** 2000. Insertional inactivation of genes encoding components of the sodium-type flagellar motor and switch of *Vibrio parahaemolyticus*. J. Bacteriol. **182:**1035–1045.
7. **Carpenter, P. B., D. W. Hanlon, and G. W. Ordal.** 1992. *flhF*, a *Bacillus subtilis* flagellar gene that encodes a putative GTP-binding protein. Mol. Microbiol. **6:**2705–2713.
8. **Chilcott, G. S., and K. T. Hughes.** 1998. The type III secretion determinants of the flagellar anti-transcription factor, FlgM, extend from the amino-terminus into the anti-sigma 28 domain. Mol. Microbiol. **30:**1029–1040.
9. **Dasgupta, N., S. K. Arora, and R. Ramphal.** 2000. *fleN*, a gene that regulates flagellar number in *Pseudomonas aeruginosa*. J. Bacteriol. **182:**357–364.
10. **Ditty, J. L., A. C. Grimm, and C. S. Harwood.** 1998. Identification of a chemotaxis gene region from *Pseudomonas putida*. FEMS Microbiol. Lett. **159:**267–273.
11. **Domian, I. J., A. Reisenauer, and L. Shapiro.** 1999. Feedback control of a master bacterial cell-cycle regulator. Proc. Natl. Acad. Sci. USA **96:**6648–6653.
12. **Engelhardt, H., S. C. Schuster, and E. Baeuerlein.** 1993. An archimedian spiral: the basal disk of the *Wolinella* flagellar motor. Science **262:**1046–1048.
13. **Ferris, F. G., T. J. Beveridge, M. L. Marceau-Day, and A. D. Larson.** 1984. Structure and cell envelope associations of flagellar basal complexes of *Vibrio cholerae* and *Campylobacter fetus*. Can. J. Microbiol. **30:**322–333.
14. **Fraser, G. M., J. C. Bennett, and C. Hughes.** 1999. Substrate-specific binding of hook-associated proteins by FlgN and FliT, putative chaperones for flagellum assembly. Mol. Microbiol. **32:**569–580.
15. **Ge, Y., and N. W. Charon.** 1997. Identification of a large motility operon in *Borrelia burgdorferi* by semi-random PCR chromosome walking. Gene **189:** 195–201.
16. **Gillen, K. L., and K. T. Hughes.** 1993. Transcription from two promoters and autoregulation contribute to the control of expression of the *Salmonella typhimurium* flagellar regulatory gene *flgM*. J. Bacteriol. **175:**7006–7015.
17. **Helmann, J. D.** 1991. Alternative sigma factors and the regulation of flagellar gene expression. Mol. Microbiol. **5:**2875–2882.
18. **Hughes, K. T., K. L. Gillen, M. J. Semon, and J. E. Karlinsey.** 1993. Sensing structural intermediates in bacterial flagellar assembly by export of a negative regulator. Science **262:**277–1280.
19. **Ide, N., T. Ikebe, and K. Kutsukake.** 1999. Reevaluation of the promoter structure of the class 3 flagellar operons of *Escherichia coli* and *Salmonella*. Genes Genet. Syst. **74:**113–116.
20. **Iino, T., Y. Komeda, K. Kutsukake, R. M. Macnab, P. Matsumura, J. S. Parkinson, M. I. Simon, and S. Yamaguchi.** 1988. New unified nomenclature for the flagellar genes of *Escherichia coli* and *Salmonella typhimurium*. Microbiol. Rev. **52:**533–535.
21. **Ikebe, T., S. Iyoda, and K. Kutsukake.** 1999. Promoter analysis of the class 2 flagellar operons of *Salmonella*. Genes Genet. Syst. **74:**179–183.
22. **Karlinskey, J. E., H.-C. T. Tsui, M. E. Winkler, and K. T. Hughes.** 1998. Flk couples *flgM* translation to flagellar ring assembly in *Salmonella typhimurium*. J. Bacteriol. **180:**5384–5397.
23. **Kawagishi, I., M. Nakada, N. Nishioka, and M. Homma.** 1997. Cloning of a *Vibrio alginolyticus rpoN* gene that is required for polar flagellar formation. J. Bacteriol. **179:**6851–6854.
24. **Klose, K. E., and J. J. Mekalanos.** 1998. Differential regulation of multiple flagellins in *Vibrio cholerae*. J. Bacteriol. **180:**303–316.
25. **Klose, K. E., and J. J. Mekalanos.** 1998. Distinct roles of an alternative sigma factor during both free-swimming and colonizing phases of the *Vibrio cholerae* pathogenic cycle. Mol. Microbiol. **23:**501–520.
26. **Komeda, Y.** 1982. Fusions of flagellar operons to lactose genes on a Mu *lac* bacteriophage. J. Bacteriol. **150:**16–26.
27. **Komeda, Y.** 1986. Transcriptional control of flagellar genes in *Escherichia coli* K-12. J. Bacteriol. **168:**1315–1318.
28. **Kutsukake, K.** 1997. Autogenous and global control of the flagellar master operon, *flhDC*, in *Salmonella typhimurium*. Mol. Gen. Genet. **24:**440–448.
29. **Kutsukake, K., and T. Iino.** 1994. Role of the FliA-FlgM regulatory system on the transcriptional control of the flagellar regulon and flagellar formation in *Salmonella typhimurium*. J. Bacteriol. **176:**3598–3605.
30. **Kutsukake, K., Y. Ohya, and T. Iino.** 1990. Transcriptional analysis of the flagellar regulon of *Salmonella typhimurium*. J. Bacteriol. **172:**741–747.
31. **Liu, X., and P. Matsumura.** 1996. Differential regulation of multiple overlapping promoters in flagellar class II operons in *Escherichia coli*. Mol. Microbiol. **21:**613–620.
32. **Macnab, R. M.** 1996. Flagella and motility, p. 123–146. *In* F. C. Neidhardt, R. Curtiss III, C. A. Gross, J. L. Ingraham, E. C. C. Lin, K. B. Low, Jr., B. Magasanik, W. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger

(ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.
33. **Macnab, R. M.** 1999. The bacterial flagellum: reversible rotary propellor and type III export apparatus. J. Bacteriol. **181:**7149–7153.
34. **McCarter, L., M. Hilmen, and M. Silverman.** 1988. Flagellar dynamometer controls swarmer cell differentiation of *V. parahaemolyticus*. Cell **54:**345–351.
35. **McCarter, L. L.** 1994. MotY, a component of the sodium-type flagellar motor. J. Bacteriol. **176:**4219–4225.
36. **McCarter, L. L.** 1994. MotX, a channel component of the sodium-type flagellar motor. J. Bacteriol. **176:**5988–5998.
37. **McCarter, L. L.** 1995. Genetic and molecular characterization of the polar flagellum of *Vibrio parahaemolyticus*. J. Bacteriol. **177:**1595–1609.
38. **McCarter, L. L.** 1999. OpaR, a homolog of *Vibrio harveyi* LuxR, controls opacity of *Vibrio parahaemolyticus*. J. Bacteriol. **180:**3166–3173.
39. **McCarter, L. L.** 1999. The multiple identities of *Vibrio parahaemolyticus*. J. Mol. Microbiol. Biotechnol. **1:**51–57.
40. **McCarter, L. L., and M. Silverman.** 1987. Phosphate regulation of gene expression in *Vibrio parahaemolyticus*. J. Bacteriol. **169:**3441–3449.
41. **McCarter, L. L., and M. Silverman.** 1989. Iron regulation of swarmer cell differentiation of *Vibrio parahaemolyticus*. J. Bacteriol. **171:**731–736.
42. **McCarter, L. L., and M. E. Wright.** 1993. Identification of genes encoding components of the swarmer cell flagellar motor and propeller and a sigma factor controlling differentiation of *Vibrio parahaemolyticus*. J. Bacteriol. **175:** 3361–3371.
43. **McGee, K., P. Horstedt, and D. L. Milton.** 1996. Identification and characterization of additional flagellin genes from *Vibrio anguillarum*. J. Bacteriol. **178:**5188–5198.
44. **Ohnishi, I., K. Kutsukake, H. Suzuki, and T. Iino.** 1990. Gene *fliA* encodes an alternative sigma factor specific for flagellar operons in *Salmonella typhimurium*. Mol. Gen. Genet. **221:**1139–1147.
45. **Ohnishi, I., K. Kutsukake, H. Suzuki, and T. Iino.** 1992. A novel transcriptional regulatory mechanism in the flagellar regulon of *Salmonella typhimurium*: an anti-sigma factor inhibits the activity of the flagellum-specific sigma factor σF. Mol. Microbiol. **6:**3149–3157.
46. **Ohta, M., and A. Newton.** 1996. Signal transduction in the cell cycle regulation of *Caulobacter* differentiation. Trends Microbiol. **8:**326–332.
47. **O'Toole, R., D. L. Milton, P. Horstedt, and H. Wolf-Watz.** 1997. RpoN of the fish pathogen *Vibrio (Listonella) anguillarum* is essential for flagellum production and virulence by the water-borne but not intraperitoneal route of inoculation. Microbiology **43:**3849–3859.
48. **Pruss, B. M., and P. Matsumura.** 1996. A regulator of the flagellar regulon of *Escherichia coli*, *flhD*, also affects cell division. J. Bacteriol. **178:**668–674.
49. **Pruss, B. M., and P. Matsumura.** 1997. Cell cycle regulation of flagellar genes. J. Bacteriol. **179:**5602–5604.
50. **Ritchings, B. W., E. C. Almira, S. Lory, and R. Ramphal.** 1995. Cloning and phenotypic characterization of *fleS* and *fleR*, new response regulators of *Pseudomonas aeruginosa* which regulate motility and adhesion to mucin. Infect. Immun. **63:**4868–4876.
51. **Rosario, M. M. L., K. L. Fredrick, G. Ordal, and J. Helmann.** 1994. Chemotaxis in *Bacillus subtilis* requires either of two functionally redundant CheW homologs. J. Bacteriol. **176:**2736–2739.
52. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
53. **Sar, N., L. McCarter, M. Simon, and M. Silverman.** 1990. Chemotactic control of the two flagellar systems of *Vibrio parahaemolyticus*. J. Bacteriol. **172:**334–341.
54. **Schoenhals, G. J., and R. M. Macnab.** 1996. Physiological and biochemical analyses of FlgH, a lipoprotein forming the outer membrane L ring of the flagellar basal body of *Salmonella typhimurium*. J. Bacteriol. **178:**4200–4207.
55. **Sharpe, M. E., and J. Errington.** 1996. The *Bacillus subtilis soj-spo0J* locus is required for a centromere-like function involved in prespore chromosome partitioning. Mol. Microbiol. **21:**501–509.
56. **Shingler, V.** 1996. Signal sensing by σ^{54}-dependent regulators: derepression as a control mechanism. Mol. Microbiol. **19:**409–416.
57. **Silverman, M., P. Matsumura, and M. Simon.** 1976. The identification of the *mot* gene product with *Escherichia coli*-lambda hybrids. Proc. Natl. Acad. Sci. USA **73:**3126.
58. **Silverman, M., R. Showalter, and L. McCarter.** 1991. Genetic analysis in *Vibrio*. Methods Enzymol. **204:**515–536.
59. **Sjoblad, R. D., C. W. Emala, and R. N. Doetsch.** 1982. Bacterial sheaths: structures in search of function. Cell Motility **3:**93–103.
60. **Stewart, B. J., and L. L. McCarter.** 1996. *Vibrio parahaemolyticus* FlaJ, a homologue of FliS, is required for production of a flagellin. Mol. Microbiol. **20:**137–149.
61. **Thomashow, L. S., and S. C. Rittenberg.** 1985. Waveform analysis and structure of flagella and basal complexes from *Bdellovibrio bacteriovorus* 109J. J. Bacteriol. **163:**1038–1046.
62. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
63. **Totten, P. A., J. C. Lara, and S. Lory.** 1990. The *rpoN* gene product of *Pseudomonas aeruginosa* is required for expression of diverse genes, including the flagellin gene. J. Bacteriol. **172:**389–396.
64. **Woo, T. H. S., A. F. Cheng, and J. M. Ling.** 1992. An application of a simple method for the preparation of bacterial DNA. BioTechniques **13:**696–697.
65. **Wu, J., and A. Newton.** 1997. Regulation of the *Caulobacter* flagellar gene hierarchy: not just for motility. Mol. Microbiol. **24:**233–239.
66. **Yamaguchi, S., H. Fujita, A. Ishihara, S.-I. Aizawa, and R. M. Macnab.** 1986. Subdivision of flagellar genes of *Salmonella typhimurium* into regions responsible for assembly, rotation, and switching. J. Bacteriol. **166:**187–193.
67. **Yokoseki, T., T. Iino, and K. Kutsukake.** 1996. Negative regulation by *fliD*, *fliS*, and *fliT* of the export of the flagellum-specific anti-sigma factor, FlgM, in *Salmonella typhimurium*. J. Bacteriol. **178:**899–901.