JOURNAL OF BACTERIOLOGY, Aug. 2000, p. 4234–4240 Vol. 182, No. 15
0021-9193/00/$04.00+0

Requirements for Conversion of the Na^+-Driven Flagellar Motor of *Vibrio cholerae* to the H^+-Driven Motor of *Escherichia coli*

KHOOSHEH K. GOSINK AND CLAUDIA C. HÄSE*

Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee 38105

Received 8 March 2000/Accepted 15 May 2000

Bacterial flagella are powered by a motor that converts a transmembrane electrochemical potential of either H^+ or Na^+ into mechanical work. In *Escherichia coli*, the MotA and MotB proteins form the stator and function in proton translocation, whereas the FliG protein is located on the rotor and is involved in flagellar assembly and torque generation. The sodium-driven polar flagella of *Vibrio* species contain homologs of MotA and MotB, called PomA and PomB, and also contain two other membrane proteins called MotX and MotY, which are essential for motor rotation and that might also function in ion conduction. Deletions in *pomA*, *pomB*, *motX*, or *motY* in *Vibrio cholerae* resulted in a nonmotile phenotype, whereas deletion of *fliG* gave a nonflagellate phenotype. *fliG* genes on plasmids complemented *fliG*-null strains of the parent species but not *fliG*-null strains of the other species. FliG-null strains were complemented by chimeric FliG proteins in which the C-terminal domain came from the other species, however, implying that the C-terminal part of FliG can function in conjunction with the ion-translocating components of either species. A *V. cholerae* strain deleted of *pomA*, *pomB*, *motX*, and *motY* became weakly motile when the *E. coli motA* and *motB* genes were introduced on a plasmid. Like *E. coli*, but unlike wild-type *V. cholerae*, motility of some *V. cholerae* strains containing the hybrid motor was inhibited by the protonophore carbonyl cyanide *m*-chlorophenylhydrazone under neutral as well as alkaline conditions but not by the sodium motor-specific inhibitor phenamil. We conclude that the *E. coli* proton motor components MotA and MotB can function in place of the motor proteins of *V. cholerae* and that the hybrid motors are driven by the proton motive force.

Many bacteria swim by rotating their flagella, the filamentous organelles that function as a propeller. Flagellar rotation is carried out by a rotary motor in the cell membrane at the base of the flagellar filament. The motor complex generating torque converts ion flux to motor rotation. The source of energy for motor rotation is the electrochemical gradient of protons or, in some species, sodium ions across the cytoplasmic membrane. Extensive studies on the proton-driven motors of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium showed that the rotor part of the motor is composed of the FliG, FliM, and FliN proteins, whereas the stator complex consists of the MotA and MotB proteins (for reviews, see references 5, 6, 9, and 24). MotA and MotB interact via their transmembrane regions and function as the proton-conducting channel (8, 31, 34). Although the mechanism of the conversion of electrochemical energy into mechanical work is not completely understood at the molecular level, torque generation is believed to occur at an interface between cytoplasmic domains of the MotA-MotB complexes and the C-terminal domain of FliG (15, 20, 32).

The architecture of the sodium-type motor is much less well defined. In the sodium-driven polar flagella of *Vibrio alginolyticus* and *Vibrio parahaemolyticus*, four proteins, PomA, PomB, MotX, and MotY, have been shown to be essential for rotation and may comprise the stator (2, 26, 27, 29). PomA and PomB have sequence similarities to MotA and MotB, respectively. The rotation of sodium-driven flagella is specifically inhibited by phenamil, an amiloride analog, and mutations conferring resistance to phenamil mapped to the *pomA* and *pomB* genes, implicating both proteins in sodium transfer (16, 18). More indirectly, MotX was also implicated in Na^+ channel function (26). Recently, the *V. parahaemolyticus* FliG, FliM, and FliN proteins were demonstrated to be important in flagellum assembly (7).

Vibrio cholerae, the causative agent of the severe diarrheal disease cholera, is motile via a single polar sheathed flagellum. The life cycle of *V. cholerae* consists of a free-swimming phase outside the host and a virulent phase when colonizing the human small intestine. While motility is thought to contribute to the pathogenicity of *V. cholerae*, the relationship between motility and virulence is not yet understood (30). Interestingly, alterations in motility phenotypes were found to correlate with changes in expression of the major virulence genes (12). Recently, induced changes in the membrane sodium flux were found to affect virulence gene regulation perhaps by affecting motility, suggesting an intriguing interplay of sodium energetics, motility, and virulence in this organism (14).

The polar flagellum of *V. cholerae* was recently demonstrated to be sodium driven (14, 19), and gene homologs of *pomA*, *pomB*, *motX*, *motY*, and *fliG* are present in the genome. In the present study, we analyzed the involvement of the four putative stator proteins, PomA, PomB, MotX, and MotY, and the torque-generating FliG protein in flagellum function and assembly in *V. cholerae* and tested specific mutations in these genes for functional complementation by their *E. coli* counterparts.

MATERIALS AND METHODS

Strains, plasmids, and culture conditions. *V. cholerae* strain O395N1 was used for mutagenesis of the *pomAB*, *motX*, *motY*, and *fliG* genes (Table 1). *E. coli* strain DH5αλpir was used to maintain the suicide plasmids during cloning steps, whereas *E. coli* strain β2155 (supplemented with 0.002% diaminopimelic acid) was used as the host of the suicide plasmids for conjugation with *V. cholerae* cells. The *E. coli fliG* deletion strain DFB225 (named EcΔG in this study) was kindly provided by D. Blair (21). The plasmid vector pBAD-24 (13) was used for the cloning and expression of the various *fliG* genes, with 0.02% L-arabinose used for

* Corresponding author. Mailing address: Infectious Diseases, St. Jude Children's Research Hospital, 332 North Lauderdale St., Memphis, TN 38105. Phone: (901) 495-2865. Fax: (901) 495-3099. E-mail: claudia.hase@stjude.org.

TABLE 1. Bacterial strains and plasmids used for complementation analyses

Strain or plasmid	Description	Source, parent, and/or reference
Strains		
V. cholerae		
O395N1	Wild type	
VcΔX	Δ*motX*	O395N1, this study
VcΔY	Δ*motY*	O395N1 (20)
VcΔAB	Δ*pomAB*	O395N1, this study
VcΔG	Δ*fliG*	O395N1 (20)
VcΔXYAB	Δ*motX* Δ*motY* Δ*pomAB*	O395N1, this study
VcΔXYABG	Δ*motX* Δ*motY* Δ*pomAB* Δ*fliG*	O395N1, this study
HM-1	Spontaneously hypermotile	VcΔXYABG, pMotAB, pBAD-VcG
HM-2	Spontaneously hypermotile	HM-1
HM-3	Spontaneously hypermotile	HM-2
E. coli EcΔG	(DFB225) Δ*fliG*	D. Blair (22)
Plasmids		
pACYC184	Cam^r, cloning vector	
pMotAB	(pJZ19), *E. coli fliG* in pACYC184	D. Blair (6)
pBAD-24	Amp^r, cloning vector	
pBAD-VcG	*V. cholerae fliG* in pBAD-24	This study
pBAD-EcG	*E. coli fliG* in pBAD-24	This study
pBAD-FP1	*V. cholerae-E. coli* chimeric *fliG* in pBAD-24	This study
pBAD-FP2	*E. coli-V. cholerae* chimeric *fliG* in pBAD-24	This study

induction. Plasmid pJZ19 (34) (named pMotAB in this study) carrying the *E. coli motAB* genes in pACYC184 was generously provided by D. Blair. MotAB is expressed from the *trp* promoter. Plasmid pLS25 (from D. Blair) was used as the template in a PCR to clone the *E. coli fliG* gene into pBAD-24 (pBAD-EcG). All strains were grown in Luria broth (LB) containing the appropriate antibiotics at the following concentrations: streptomycin, 100 μg/ml; ampicillin, 50 μg/ml; and chloramphenicol, 10 μg/ml. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and phenamil were purchased from Sigma.

Genetic manipulations. Mutants of *V. cholerae* were generated by homologous recombination. Preliminary sequence data for *V. cholerae* were obtained from The Institute for Genomic Research website (http://www.tigr.org). The genes and surrounding sequences were amplified by PCR using specific primers and cloned into the plasmid vector pCR2.1 (Invitrogen) or pUC19. Internal deletions were generated by using convenient restriction sites present in the genes, and the DNA was then subcloned into pWM91 (28) (generously provided by B. Wanner). An in-frame deletion in *fliG* was constructed using internal *Sal*I (filled in with T4 polymerase) and *Sna*BI sites; this results in deletion of amino acids 94 to 258. The *pomAB* deletion was constructed by using internal *Bam*HI and *Bgl*II sites; this results in an out-of-frame deletion of amino acids 63 to 243 encoded by *pomA* and amino acids 1 to 187 encoded by *pomB*. The *motX* deletion was constructed using *Hin*cII and *Pml*I sites, deleting amino acids 1 to 79. *Acc*I (filled in by T4 polymerase) and *Hin*cII sites were used to delete amino acids 34 to 295 encoded by *motY*. The mutated alleles were introduced into the chromosome of strain O395N1 following sucrose selection as described elsewhere (11). Plasmid DNA was prepared by using a Qiagen (Chatsworth, Calif.) Miniprep extraction kit and introduced into bacteria by electroporation as described by the supplier. The FP-1 chimeric construct was generated by replacement of a *Sal*I-*Eco*RI fragment of pBAD-VcG containing the C-terminal 268 bp of the *fliG* gene with a *Sal*I-*Eco*RI fragment containing the 272-bp C-terminal fragment of *E. coli fliG*. pBAD-FP2 was constructed by replacement of the *Sal*I-*Hin*dIII fragment of pBAD-EcG containing the C-terminal 272 bp of the *E. coli fliG* gene with a *Sal*I-*Hin*dIII fragment containing the 268 bp of the *V. cholerae fliG*.

Motility assays. Motility phenotypes were assessed for swarm diameter following inoculation into 0.3% soft agar. Swarm plates were inoculated with cells toothpicked from colonies. Bacterial cells were also assayed for swimming ability under a dark-field microscope after the addition of various compounds. CCCP was added at 30 μM, and phenamil was added at 50 to 100 μM. A score of +++ indicates that more than 70% of the bacteria were swimming. Swimming of the *V. cholerae* parental strain was scored ++++ to indicate the increased speed compared to *E. coli* or the hybrid *Vibrio* strain. A score of − indicates that less than 10% of the bacteria were swimming.

RESULTS

Involvement of the *V. cholerae* PomAB, MotX, MotY, and FliG proteins in flagellar function and assembly. To study the roles of the *V. cholerae pomAB*, *motX*, *motY*, and *fliG* homologous genes in flagellar function and assembly, we created strains with specific deletion mutations in these genes, a strain deleted in the four putative stator genes, as well as a strain lacking all of these genes. Analyses of the resulting strains in soft agar plates and under light microscopy showed that all mutant strains displayed nonmotile phenotypes (Fig. 1A). Electron microscopy (EM) studies of these strains revealed that all strains with deletions in the putative stator genes, including the quadruple mutant (Fig. 1B), produced apparently normal flagella. In contrast, strains with a deletion in the

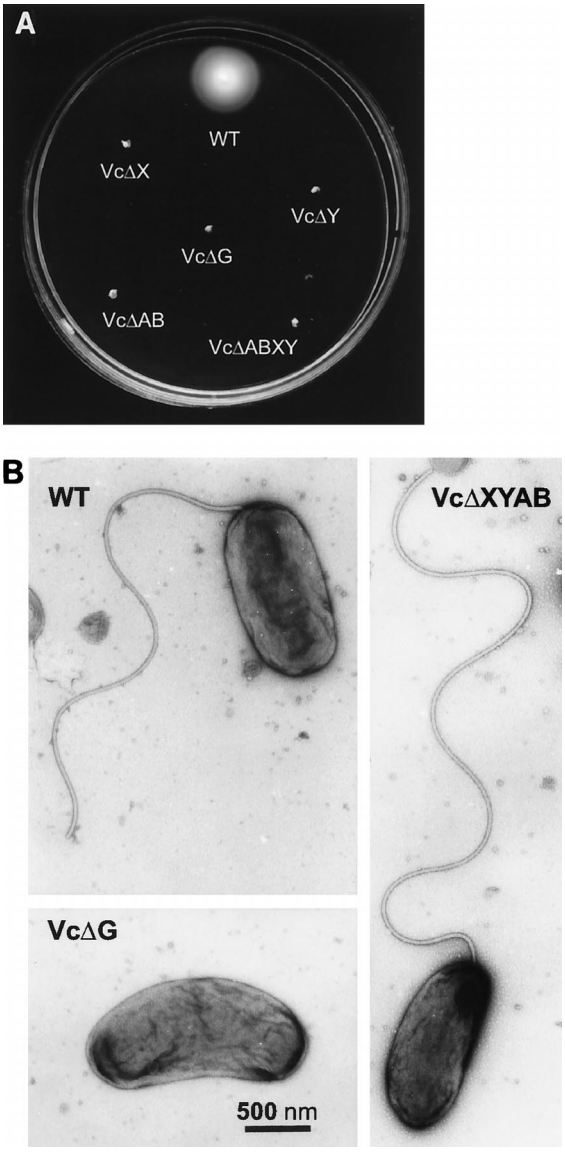

FIG. 1. Analyses of mutants for motility and flagellum production. Swarms in soft agar plates incubated for 8 h at 37°C (A) and electron micrographs (B) of the *V. cholerae* strain O395N1 (WT [wild-type]) and the *motX* (VcΔX), *motY* (VcΔY), *pomAB* (VcΔAB), and *fliG* (VcΔG) mutant derivatives as well as the *motX motY pomAB* (VcΔXYAB) quadruple mutant strain are shown.

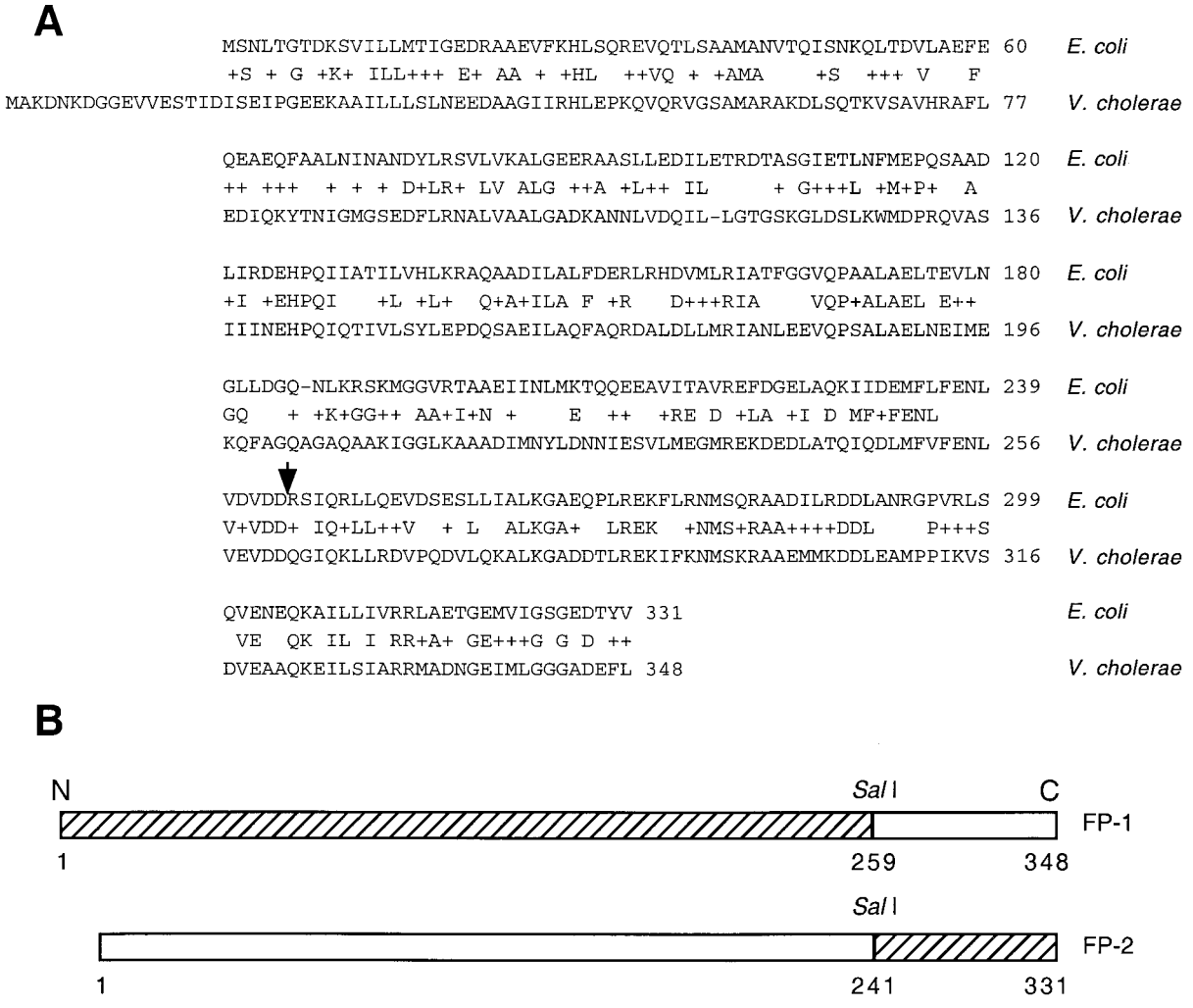

FIG. 2. Complementation of *fliG* mutants by plasmids carrying various *fliG* genes. (A) Amino acid sequence alignment of the *E. coli* and *V. cholerae* FliG proteins. The arrow indicates the position of the junction between the two domains in the fusion proteins. (B) Diagram of the chimeric FliG proteins. Hatched boxes indicate *V. cholerae* sequence, and open boxes indicate *E. coli* sequence. Numbers correspond to amino acid residues. (C) Swarming abilities in the presence or absence of arabinose (ara) of the *E. coli* (EcΔG) or *V. cholerae* (VcΔG) *fliG* deletion strains complemented by plasmids carrying the *E. coli* (pBAD-EcG), *V. cholerae* (pBAD-VcG), or chimeric (pBAD-FP1, pBAD-FP2) *fliG* genes. pBAD-24 is the parent vector and contains no flagellar genes. Plates were incubated for 8 h at 37°C.

fliG gene did not produce any flagella as analyzed by EM (Fig. 1B).

Complementation of *V. cholerae* and *E. coli* Δ*fliG* strains with plasmids carrying *fliG*. The *V. cholerae* predicted FliG protein has 39.5% amino acid sequence identity with the *E. coli* FliG protein (Fig. 2A). To address whether the *V. cholerae* and *E. coli* FliG proteins can functionally complement each other, we introduced plasmids with different *fliG* genes under an arabinose-inducible promoter into *V. cholerae* and *E. coli fliG* deletion strains. No restoration of the motility phenotypes as assayed in soft agar plates was observed with either the *V. cholerae* Δ*fliG* strain harboring the *E. coli fliG* gene on a plasmid or the *E. coli* Δ*fliG* strain carrying the *V. cholerae fliG* gene on a plasmid (Fig. 2C). However, the *fliG* genes did complement their parental mutations (Fig. 2C). A fusion protein consisting of the N-terminal portion of *V. cholerae* FliG fused to the C-terminal domain of *E. coli* FliG (FP-1 [Fig. 2B]) was capable of complementing the *V. cholerae*, but not the *E. coli*, *fliG* deletion strain (Fig. 2C). Similarly, the inverse *E. coli-V. cholerae* fusion protein (FP-2 [Fig. 2B]) complemented the *E. coli* but not *V. cholerae* Δ*fliG* strain (Fig. 2C). EM studies of the *V. cholerae* Δ*fliG* strain carrying different plasmids showed that none or only very few bacteria produced flagella when the *E. coli* wild-type FliG or FP-2 was expressed. In contrast, normal flagella were observed when the *V. cholerae*

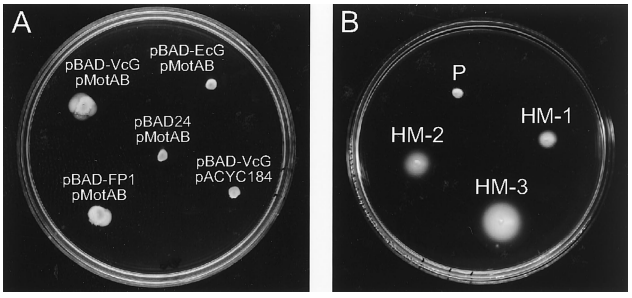

FIG. 3. Complementation of *V. cholerae* VcΔXYABG by plasmids carrying the *E. coli motAB* and different *fliG* genes. (A) Swarm circles of the quintuple deletion strain carrying the pMotAB or pACYC184 control plasmid as well as either pBAD-24, pBAD-EcG, pBAD-VcG, or pBAD-FP1. (B) Swarming behavior of the parental strain (P) and of spontaneous hypermotile derivatives (HM-1, HM-2, and HM-3) of strain VcΔXYABG carrying pMotAB and pBAD-VcG. Both soft agar plates contain arabinose. Plates were incubated overnight at 37°C.

wild-type or chimeric FP-1 protein was expressed (data not shown).

Complementation of the *V. cholerae pomAB*, *motX*, and *motY* genes by the *E. coli motAB* genes. The N-terminal domain of the *E. coli* FliG protein is believed to interact with the flagellar basal body, whereas the C-terminal domain interacts with the MotA-MotB complex (20). Thus, for functional complementation of the ion-translocating subunits of the flagella, we anticipated the need for a chimeric FliG protein. A fusion protein consisting of the N-terminal portion of *V. cholerae* FliG fused to the C-terminal domain of *E. coli* FliG was constructed (Fig. 2B). The *V. cholerae* strain carrying chromosomal deletions in the *pomAB*, *motX*, *motY*, and *fliG* genes (VcΔABXYG) was transformed with the plasmids encoding either the wild-type *V. cholerae* or *E. coli fliG* gene or the *V. cholerae-E. coli fliG* chimeric construct (FP-1) from an arabinose-inducible promoter. A second plasmid containing the *E. coli motAB* genes was then introduced. Some, although very small, swarm circles were observed in soft agar plates only in the strain carrying the *V. cholerae* wild-type or chimeric *fliG* (FP-1) gene only in the presence of arabinose (Fig. 3A). This motility was dependent on the presence of the *E. coli motAB* genes, as the control strain harboring pACYC184 produced no appreciable motility (Fig. 3A). Interestingly, spontaneous hypermotile mutants that when isolated produced larger motility circles in soft agar plates than the parental strain were readily observed (Fig. 3B). Moreover, these hypermotile strains also produced further hypermotile variants, and several such hypermotile strains when isolated demonstrated increasingly larger motility circles (Fig. 3B). Similar increasingly motile variants were also isolated from the strain with the chimeric *fliG* gene (data not shown). Normal flagellum production by the motile strains was demonstrated by EM (data not shown).

To test whether the mutations are linked to the *E. coli motAB* or *V. cholerae fliG* gene, plasmid DNA isolated from some hypermotile strains was transformed into strain VcΔABXYG, selecting for both plasmid markers. The majority of the resulting strains did not display greater motility than the original strain (data not shown). However, we were able to isolate some pMotAB plasmids that, when transformed with the original pBAD-FliG construct, resulted in increased swarming circles (Fig. 4). Three such plasmids that were independently isolated had a mutation in Tyr-61 of MotB.

Analysis of the coupling ion used for motility of *V. cholerae* strain VcΔABXYG, carrying the *V. cholerae fliG* and *E. coli motAB* genes on plasmids. The *E. coli* flagellar motor is known to use the translocation of protons as the energy source for rotation, whereas the polar flagella of *V. cholerae* were found to be sodium driven. By replacing the *V. cholerae pomAB*, *motX*, and *motY* genes by the *E. coli motAB* genes, we generated a functional hybrid flagellar motor and wished to investigate which coupling ion, H^+ or Na^+, was used for the observed motility. We investigated the energy requirement of several isolated hypermotile variants, both chromosomal (HM-1 and HM-2) and *motAB* dependent (HM-3) (data not shown), as the original hybrid motor strain produced these mutants so readily that a pure population of cells could not be analyzed in this strain. The protonophore CCCP, a compound known to collapse proton motive force (PMF), inhibited motility of the *E. coli* control strain and the *V. cholerae* hybrid motor strains under neutral as well as alkaline conditions (Table 2). In contrast, the *V. cholerae* control strain was inhibited at neutral pH but was insensitive to CCCP at an alkaline pH (Table 2). Interestingly, the addition of even very small amounts of CCCP (2 to 5 μM) at either pH completely inhibited motility of the *V. cholerae* hybrid motor strain, whereas much larger concentrations of CCCP (25 to 50 μM) were required to block motility of the *E. coli* strain (data not shown).

The sodium channel blocker phenamil, an amiloride analog known to specifically block the sodium-translocating portion of flagella (4), inhibited motility of the *V. cholerae* parental strain but not of the *V. cholerae* hybrid motor strains or the *E. coli*

TABLE 2. Effects of inhibitors on motility[a]

Strain	Motility					
	CCCP				Phenamil, pH 6.5	
	pH 6.5		pH 8.5			
	0	30 μM	0	30 μM	0	100 μM
EcΔG, pBAD-EcG	+++	−	+++	−	+++	+++
VcΔG, pBAD-VcG	++++	−	++++	+++	++++	−
HM-1	+++	−	+++	−	+++	+++
HM-2	+++	−	+++	−	+++	+++

[a] Motilities of the *V. cholerae* (VcΔG, pBAD-VcG) and *E. coli* (EcΔG, pBAD-EcG) control strains as well as two hypermotile derivatives of the *V. cholerae* hybrid motor strains (HM-1 and HM-2) were assayed under the microscope. Motility was scored as described in Materials and Methods.

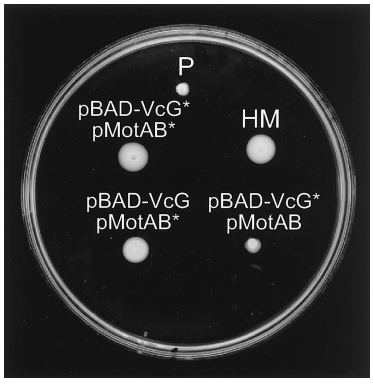

FIG. 4. Linking of the hypermotile phenotype to the pMotAB plasmid. Swarm circles in an arabinose-containing soft agar plate of *V. cholerae* strain VcΔXYABG carrying plasmids pMotAB and pBAD-VcG from different origins. Shown are the parental strain (P) and one of the spontaneous hypermotile derivatives (HM). Both plasmids, pMotAB and pBAD-VcG, isolated from the HM strain were transformed back into the host strain either together or with the original nonmutated plasmids. An asterisk indicates that the plasmid was derived from the hypermotile strain. Plates were incubated overnight at 37°C.

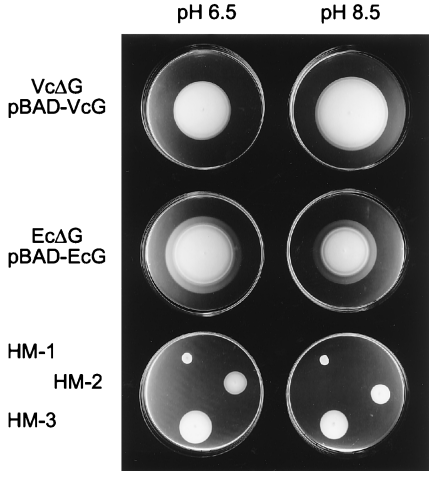

FIG. 5. Effects of different medium pHs on swarm circles. Motility of the *V. cholerae* (VcΔG, pBAD-VcG) and *E. coli* (EcΔG, pBAD-EcG) control strains as well as several spontaneous hypermotile derivatives of the *V. cholerae* hybrid motor strain (HM-1, HM-2, and HM-3) were assayed in arabinose-containing soft agar plates with a pH of 6.5 or 8.5.

control strain (Table 2). The addition of increasing concentrations of NaCl to LB increased the swimming speed of the *V. cholerae* parental strain but not of the *E. coli* or *V. cholerae* hybrid motor strain (data not shown). Similarly, increased pH resulted in increased swarm circles by the *V. cholerae* parental strain but not by the *E. coli* or *V. cholerae* hybrid motor strain (Fig. 5). Together, these results strongly suggest that this hybrid flagellar motor, like the *E. coli* but unlike the *V. cholerae* flagellar motor, uses protons as the coupling ion for rotation.

DISCUSSION

It was recently reported that the single polar flagellum of *V. cholerae* is energized by the translocation of sodium ions (14, 19). The structures and functions of many proteins in the proton-driven flagella of *E. coli* and *S. enterica* serovar Typhimurium have been extensively studied (9, 24), whereas less is known about the architecture of sodium-driven flagella. The now completed sequence of the *V. cholerae* genome presents the first opportunity for extensive sequence comparisons of various proteins constituting the two types of flagellar motors. The single polar flagella of *V. alginolyticus* and *V. parahaemolyticus*, like those of *V. cholerae*, utilize an electrochemical gradient of sodium ions (sodium motive force [SMF]) as energy stock for flagellar rotation (3, 17). In these two species, four proteins, PomA, PomB, MotX, and MotY, are believed to form the sodium ion-conducting channel and the stator of the motor (2, 26, 27, 29). PomA and PomB have some sequence homology to the *E. coli* MotA and MotB proteins, which form the proton-conducting complex and stator of the *E. coli* flagella. In this study, we identified the *V. cholerae* gene homologs for *pomA*, *pomB*, *motX*, and *motY* from the genomic database and created *V. cholerae* strains with specific deletions in these genes as well as a strain deleted in all four genes. These strains showed nonmotile phenotypes but produced apparently normal flagella, indicating that these proteins, like the *E. coli* and *V. parahaemolyticus* stator proteins, are required for flagellar function but not assembly.

In *E. coli*, only three proteins, MotA, MotB, and FliG, participate closely in torque generation. Torque generation is believed to occur at the interface between cytoplasmic domains of the MotA-MotB complexes and the C-terminal domain of FliG (20, 32). Recently, the structure of the C-terminal domain of the *Thermatoga maritima* FliG protein was determined (22). A *fliG* deletion strain of *V. cholerae* did not produce flagella, suggesting that like in *E. coli* and *V. parahaemolyticus*, the *V. cholerae* FliG protein is required for flagellum assembly. Whereas the *E. coli* and *V. cholerae fliG* mutant strains were readily complemented by their homologous genes, expression of the heterologous *fliG* genes did not restore motility. Motility required chimeric FliG proteins where the N termini determined species specificity, showing that the C-terminal regions of the two FliG proteins are functionally interchangeable. Although the C-terminal domains of the two FliG proteins have high amino acid sequence homology, it is still remarkable that the *E. coli* FliG C-terminal domain can functionally interact with the sodium-translocating components (presumably PomA) of the *Vibrio* flagella and vice versa. This suggests very similar mechanisms of torque generation in the two types of motors. In contrast, the N-terminal domains of the FliG proteins apparently cannot interact properly with other flagellar proteins of the heterologous species. Similarly, the *E. coli* and *V. parahaemolyticus* FliG proteins did not functionally complement each other (7). Furthermore, a chimeric *E. coli-T. maritima* FliG protein, but not the full-length *T. maritima* FliG, restored flagellum production and motility of an *E. coli fliG* mutant strain (22).

To investigate whether the sodium-translocating components of the *V. cholerae* flagella can functionally be complemented by the *E. coli* proton-translocating proteins, a *V. cholerae* strain deleted in the *pomAB*, *motX*, and *motY* genes was transformed with the *E. coli motAB* genes. Some, although very small, swarm circles in soft agar plates were observed in this strain, with spontaneous hypermotile variants appearing readily (data not shown). As the C-terminal domain of the FliG protein is involved in torque generation by interacting with the ion channel components, we expected that the *V. cholerae-E. coli* chimeric FliG fusion protein may better interact with the MotAB proteins. A *V. cholerae* strain deleted in the four putative stator genes and the *fliG* gene carrying the *E. coli motAB* genes and expressing either the full-length *V. cholerae* or chimeric FliG proteins displayed equally small motility zones. This indicates that the C terminus of the *V. cholerae* FliG protein can interact efficiently with the *E. coli* MotAB proteins. Both strains produced spontaneous hypermotile variants, and in most strains the hypermotile phenotype was not linked to the plasmids, i.e., the *motAB* or *fliG* gene. It is hard to speculate where these non-plasmid-linked mutations might map. It is possible that these mutations result in better recognition and installation of the foreign MotAB protein by chaperone-like proteins. Alternatively, the mutations might be in systems involved in the generation of electrochemical gradients of protons or sodium ions across the membrane, thus increasing the available energy source for flagellar rotation. However, the increased swarm circles might be a result of improved chemotaxis behavior. As the FliG protein is part of the switch complex that regulates direction of flagellar rotation in response to interaction with CheY (24), perhaps the hybrid motor cannot properly interact with the chemotaxis machinery. Further experiments are required to understand the basis for the increased swarming phenotype in these mutant strains. In summary, we have created a *V. cholerae* strain that is motile by using a hybrid flagellar motor composed of the *V. cholerae* flagellar machinery interacting with the *E. coli* MotAB proteins. This hybrid motor strain may provide a useful tool to help us better understand the processes involved in flagellar

assembly and protein interactions required for flagellar function.

To investigate which coupling ion, H^+ or Na^+, was used for flagellar rotation by the hybrid motor, we used inhibitors such as CCCP and phenamil, an amiloride homolog known to specifically block the sodium-translocating portion of the flagella (4). Together with data from assays using different H^+ or Na^+ concentrations, we concluded that the hybrid motor strain, like *E. coli* but unlike *V. cholerae*, uses PMF as the driving energy source. Thus, the mechanisms of converting electrochemical energy into rotational energy in proton- or sodium-driven flagella seem to be similar and are functionally interchangeable. Furthermore, this suggests that the FliG protein does not directly interact with the ion flux across the membrane or that the sites of interaction can interact with either Na^+ or H^+. It would be interesting to create a similar but reverse hybrid flagellar motor by introducing the *V. cholerae* PomAB and MotXY proteins into a *motAB* deletion *E. coli* strain to assess their ability to function perhaps after induction of an artificial SMF. It was recently reported that the *V. parahaemolyticus motAB* genes alone did not restore motility of an *E. coli motAB* mutant strain (7).

Little is known about the residues involved in the ion selectivity of the sodium-translocating flagellar channel molecules. Recently, the *Rhodobacter spheroides* MotA protein was found to functionally complement a *pomA* mutant of *V. alginolyticus*, thus creating a hybrid motor (1). However, this motor was still using the coupling of sodium ion flux, indicating that the MotA and PomA proteins alone do not dictate the ion specificity. The ion specificity of our hybrid motor has been switched from sodium ions to protons. Apparently, the *E. coli* MotAB proteins are specialized to translocate protons but not sodium ions even in a *V. cholerae* host environment that supposedly provides a strong SMF. Even a *V. cholerae* strain deleted for only the *pomAB* genes complemented by the *E. coli* MotAB proteins seemed to use protons rather than sodium ions for flagellum rotation (data not shown), indicating that the presence of the MotXY proteins does not influence the coupling ion used by the MotAB proteins. By introducing the *E. coli motA* or *motB* gene alone into our various stator deletion *V. cholerae* strains, we should be able to address which proteins are involved in ion selectivity.

The hybrid motor strain might allow us to understand the underlying mechanism for the significantly increased speed of sodium-driven flagella compared to proton-driven flagella. The *E. coli* flagellum is known to rotate at about 15,000 rpm (23), whereas *Vibrio* flagella have been reported to achieve as high as 100,000 rpm (25). Perhaps the function of the MotXY proteins is to further stabilize the motor in the membrane to allow faster rotation. Alternatively, these two proteins might form an ion channel independent of PomAB, adding to the available energy conversion. However, a *V. cholerae pomAB* deletion strain complemented with the *E. coli motAB* genes showed no significant increase in swarm circles or swimming speed compared to the similar strain that has all four stator genes deleted (data not shown). This indicates that the MotXY proteins either do not functionally interact with the *E. coli* MotAB proteins or are not involved in swimming speed. Further studies on these strains might reveal the underlying mechanism for the difference in speed between the different types of motors.

In *E. coli*, a strong PMF is generated by respiration under neutral conditions, whereas an alkaline environment results in an opposite ΔpH and a PMF is harder to maintain. Some bacteria, including several *Vibrio* species, can switch to a sodium cycle of energy, thus enabling the cells to maintain a neutral cytoplasmic pH under alkaline conditions. At neutral pH, an H^+/Na^+ antiporter converts the PMF generated by respiration into SMF, whereas at alkaline pH an enzyme complex, called NQR (NADH-quinone oxidoreductase), can generate an SMF directly linked to respiration (for reviews, see references 10 and 33). Therefore, motility of *V. cholerae* is sensitive to the ionophore CCCP, an agent widely used to collapse PMF, at neutral but not alkaline pH. Interestingly, we noticed that the sensitivity of the motility to CCCP was markedly different between the *Vibrio* hybrid motor strain and the *E. coli* control strain. Much less CCCP was required to completely prevent motility of the *V. cholerae* hybrid motor strain compared to the *E. coli* control under neutral as well as alkaline conditions. One possible explanation for this is that *V. cholerae* cells may be inherently more sensitive to CCCP, perhaps due to their membrane composition or lack of efflux systems. Alternatively, this difference in CCCP sensitivity might reflect differences in the strength of PMF production between these organisms. Perhaps at neutral pH *V. cholerae*, but not *E. coli*, converts a substantial portion of the PMF into SMF. At alkaline pH, *E. coli* might have a specific mechanism, such as induction of an electrogenic antiporter, for maintaining a PMF that is lacking in *V. cholerae*, as *Vibrio* cells usually switch to the sodium cycle of energy under these conditions. Creating hybrid motor strains might provide useful tools to investigate the differences in membrane bioenergetics between organisms.

Motility is an important virulence factor in a variety of pathogenic bacteria and, in some cases, is inversely regulated with other virulence factors (30). Motility in *V. cholerae* is known to be negatively regulated by the ToxR regulon; conversely, some motility mutants, including a *pomB* insertion mutant, showed altered expression levels of the main virulence factors (12). Inhibition of the *V. cholerae* SMF-generating NQR enzyme complex, either by mutation or addition of a specific inhibitor, resulted in increased virulence gene expression by affecting expression of the regulatory protein ToxT (14). It was proposed that the effect of loss of NQR activity on *toxT* transcription may be indirectly mediated by affecting motility. The sodium influx through the flagellum may somehow be transduced into altered transcription of *toxT*, possibly by affecting the regulatory proteins TcpP and TcpH (14). The hybrid motor strain presented in this study will help elucidate how changes in membrane sodium energetics and motility affect virulence gene expression in *V. cholerae*. We can now investigate whether the sodium influx through the flagella is sensed by an as yet uncharacterized mechanism or if the motion of the bacteria or perhaps flagellar rotation speed are signals resulting in changes of gene expression.

ACKNOWLEDGMENTS

We thank David F. Blair for providing plasmids pJZ19 and pSL25 and the *E. coli* Δ*fliG* strain DFB225 as well as for many helpful suggestions. We especially thank Eric J. Rubin and John J. Mekalanos for encouragement and many discussions. We thank Ikuro Kawagishi, Igor I. Brown, and A. Brooun for comments on the manuscript and Gillian Chesney for technical assistance. We are grateful to G. Murti for the EM analyses.

This study was supported by Cancer Center Support Grant CA 21765 and ALSAC (American Lebanese Syrian Associated Charities).

REFERENCES

1. **Asai, Y., I. Kawagishi, R. E. Sockett, and M. Homma.** 1999. Hybrid motor with H^+- and Na^+-driven components can rotate *Vibrio* polar flagella by using sodium ions. J. Bacteriol. **181:**6332–6338.
2. **Asai, Y., S. Kojima, H. Kato, N. Nishioka, I. Kawagishi, and M. Homma.** 1997. Putative channel components for the fast-rotating sodium-driven flagellar motor of a marine bacterium. J. Bacteriol. **179:**5104–5110.

3. **Atsumi, T., L. McCarter, and Y. Imae.** 1992. Polar and lateral flagellar motors of marine Vibrio are driven by different ion-motive forces. Nature **355:**182–184.
4. **Atsumi, T., S. Sugiyama, E. J. Cragoe, Jr., and Y. Imae.** 1990. Specific inhibition of the Na^+-driven flagellar motors of alkalophilic *Bacillus* strains by the amiloride analog phenamil. J. Bacteriol. **172:**1634–1639.
5. **Berg, H. C.** 1995. Torque generation by the flagellar rotary motor. Biophys. J. **68:**163–167.
6. **Blair, D. F.** 1995. How bacteria sense and swim. Annu. Rev. Microbiol. **49:** 489–522.
7. **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.
8. **Braun, T. F., S. Poulson, J. B. Gully, J. C. Empey, S. Van Way, A. Putnam, and D. F. Blair.** 1999. Function of proline residues of MotA in torque generation by the flagellar motor of *Escherichia coli*. J. Bacteriol. **181:**3542–3551.
9. **DeRosier, D. J.** 1998. The turn of the screw: the bacterial flagellar motor. Cell **93:**17–20.
10. **Dimroth, P.** 1997. Primary sodium ion translocating enzymes. Biochim. Biophys. Acta **1318:**11–51.
11. **Donnenberg, M. S., and J. B. Kaper.** 1991. Construction of an *eae* deletion mutant of enteropathogenic *Escherichia coli* by using a positive-selection suicide vector. Infect. Immun. **59:**4310–4317.
12. **Gardel, C., and J. J. Mekalanos.** 1996. Alterations in *Vibrio cholerae* motility phenotypes correlate with changes in virulence factor expression. Infect. Immun. **64:**2246–2255.
13. **Guzman, L.-M., D. Belin, M. J. Carson, and J. Beckwith.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. J. Bacteriol. **177:**4121–4130.
14. **Häse, C. C., and J. J. Mekalanos.** 1999. Effects of changes in membrane sodium flux on virulence gene expression in *Vibrio cholerae*. Proc. Natl. Acad. Sci. USA **96:**3183–3187.
15. **Irikura, V. M., M. Kihara, S. Yamaguchi, H. Sockett, and R. M. Macnab.** 1993. *Salmonella typhimurium fliG* and *fliN* mutations causing defects in assembly, rotation, and switching of the flagellar motor. J. Bacteriol. **175:** 802–810.
16. **Jaques, S., Y.-K. Kim, and L. L. McCarter.** 1999. Mutations conferring resistance to phenamil and amiloride, inhibitors of sodium-driven motility of *Vibrio parahaemolyticus*. Proc. Natl. Acad. Sci. USA **96:**5740–5745.
17. **Kawagishi, I., Y. Maekawa, T. Atsumi, M. Homma, and Y. Imae.** 1995. Isolation of the polar and lateral flagellum-defective mutants in *Vibrio alginolyticus* and identification of their flagellar driving energy source. J. Bacteriol. **177:**5158–5160.
18. **Kojima, S., Y. Asai, T. Atsumi, I. Kawagishi, and M. Homma.** 1999. Na+-driven flagellar motor resistant to phenamil, an amiloride homolog, caused by mutations in putative channel components. J. Mol. Biol. **285:**1537–1547.
19. **Kojima, S., K. Yamamoto, I. Kawagishi, and M. Homma.** 1999. The polar flagellar motor of *Vibrio cholerae* is driven by a Na^+ motive force. J. Bacteriol. **181:**1927–1930.
20. **Lloyd, S. A., and D. F. Blair.** 1997. Charged residues of the rotor protein FliG essential for torque generation in the flagellar motor of *Escherichia coli*. J. Mol. Biol. **266:**733–744.
21. **Lloyd, S. A., H. Tang, X. Wang, S. Billings, and D. F. Blair.** 1996. Torque generation in the flagellar motor of *Escherichia coli*: evidence of a direct role for FliG but not for FliM or FliN. J. Bacteriol. **178:**223–231.
22. **Lloyd, S. A., F. G. Whitby, D. F. Blair, and C. P. Hill.** 1999. Structure of the C-terminal domain of FliG, a component of the rotor in the bacterial flagellar motor. Nature **400:**472–475.
23. **Lowe, G., M. Meister, and H. C. Berg.** 1987. Rapid rotation of flagellar bundles in swimming bacteria. Nature **325:**637–640.
24. **Macnab, R.** 1996. Flagella and motility, p. 123–146. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. 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.
25. **Magariyama, Y., S. Sugiyama, K. Muramoto, Y. Maekawa, I. Kawagishi, and Y. Imae.** 1994. Very fast flagellar rotation. Nature **371:**752.
26. **McCarter, L. L.** 1994. MotX, the channel component of the sodium-type flagellar motor. J. Bacteriol. **176:**5988–5998.
27. **McCarter, L. L.** 1994. MotY, a component of the sodium-type flagellar motor. J. Bacteriol. **176:**4219–4225.
28. **Metcalf, W. W., W. Jiang, L. L. Daniels, S. K. Kim, A. Haldimann, and B. L. Wanner.** 1996. Conditionally replicative and conjugative plasmids carrying lacZ alpha for cloning, mutagenesis, and allel replacement in bacteria. Plasmid **35:**1–13.
29. **Okunishi, I., I. Kawagishi, and M. Homma.** 1996. Cloning and characterization of *motY*, a gene coding for a component of the sodium-driven flagellar motor in *Vibrio alginolyticus*. J. Bacteriol. **178:**2409–2415.
30. **Ottemann, K. M., and J. F. Miller.** 1997. Roles for motility in bacterial-host interactions. Mol. Microbiol. **24:**1109–1117.
31. **Tang, H., T. F. Braun, and D. F. Blair.** 1996. Motility protein complexes in the bacterial flagellar motor. J. Mol. Biol. **261:**209–221.
32. **Thomas, D. R., D. G. Morgan, and D. J. DeRosier.** 1999. Rotational symmetry of the C ring and a mechanism for the flagellar rotary motor. Proc. Natl. Acad. Sci. USA **96:**10134–10139.
33. **Unemoto, T., and M. Hayashi.** 1993. Na+-translocating NADH-quinone reductase of marine and halophilic bacteria. J. Bioenerg. Biomembr. **25:** 385–391.
34. **Zhou, J., S. A. Lloyd, and D. F. Blair.** 1998. Electrostatic interactions between rotor and stator in the bacterial flagellar motor. Proc. Natl. Acad. Sci. USA **95:**6436–6441.