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MotY, a Component of the Sodium-Type Flagellar Motor

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Energy to power the rotation of bacterial flagella can be derived from the proton or sodium transmembrane potential. Until now, genes encoding a bacterial sodium-type flagellar motor have not been defined. A gene, *motY*, encoding one component of the sodium-type flagellar motor of *Vibrio parahaemolyticus* was cloned by complementation of a Mot$^-$ mutant strain. Sequencing revealed an open reading frame of 879 nucleotides in which a transposon conferring a motility defect mapped. Overexpression of *motY* in *Escherichia coli* allowed identification of a product 33 kDa in apparent size on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This size correlated well with the predicted molecular mass of 33,385 Da. Unlike *mot* genes identified in other bacteria, localized transposon mutagenesis suggested that the locus was not an extended region containing multiple genes required for swimming motility. Sequencing upstream and downstream of *motY* confirmed that the gene maps alone and placed it within a locus homologous to the *E. coli rnt* locus. Although data bank searches failed to reveal significant similarity to known motility components, the carboxyl terminus of MotY showed extensive homology to a number of outer membrane proteins known to interact with peptidoglycan, including OmpA and peptidoglycan-associated lipoproteins. To a limited extent, this domain could also be identified in the *Bacillus subtilis* MotB protein. This finding suggests that MotY plays the role of a stator in the sodium flagellar motor, stabilizing the force-generating unit through direct interaction with the cell wall.

Swimming bacteria are powered by tiny, reversible motors embedded in the cytoplasmic membrane (27). Energy to drive the motors is derived from the transmembrane electrochemical potential (24). For *Escherichia coli*, the torque-generating machinery is composed of two kinds of proteins: MotA, which functions as a proton channel, and MotB, which is thought to serve as a stator anchoring the channel to a stationary component of the cell wall (7, 11). Rotation of the flagellum is somehow coupled to the flow of ions through the channel in the motor. Two kinds of motors have been described, proton and sodium driven (21, 26). Until now, genes encoding the sodium motor have not been characterized.

Vibrio parahaemolyticus is a gram-negative, marine bacterium and human pathogen. It is a bacterium with two alternative cell types, the swimmer and the swarmer. Each cell type is adapted for locomotion in different environments (31). A single, sheathed polar flagellum propels the swimmer cell in liquid. Many lateral flagella move swarming bacteria over surfaces or through highly viscous layers. Although both motility systems are flagellar and are driven by reversible motors, the systems are distinct with no shared structural components (30). Moreover, the energy source for the polar flagellar motor is the sodium motive force, while the lateral flagellar motors are driven by the proton motive force (4). Two genes, *lafT* and *lafU*, encoding components of the proton-driven motor of the lateral flagella of *V. parahaemolyticus* have been described previously (33). These proteins clearly resemble MotA and MotB of *E. coli*. In this work, one gene, *motY*, encoding a component of the polar sodium-type flagellar motor is identified.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The strains and plasmids used in this work are described in Table 1. *V. parahaemolyticus* strains were propagated at 30°C in heart infusion medium (25 g of Bacto Heart Infusion [Difco] plus 20 g of NaCl per liter). Solidified medium was prepared with 2% Bacto Agar (Difco). Semisolid motility plates were made with the minimal medium of Broach et al. (10) supplemented with 0.4% galactose, 20 mM NH_4Cl, 2% NaCl, and 0.34% Bacto Agar. Minimal medium for gene replacement conjugations was the same as described above except it was made with 1.5% agar. LB, 2×YT, and NZCYM media for propagation of *E. coli* strains and lambda were prepared as described by Sambrook et al. (37). Antibiotics (from Sigma Chemical Co.) were used at the following concentrations: gentamicin, 125 μg/ml; kanamycin, 100 μg/ml; and tetracycline, 10 μg/ml. 5-Bromo-4-chloro-3-indolyl phosphate (XP; Sigma) was used at 40 μg/ml. Mini-Mu *lac* (Tet^r) strains were made tetracycline sensitive by using the medium of Bochner et al. (9) supplemented with additional salt (20 g of NaCl per liter, final concentration).

Genetic techniques. Mutagenesis with λTn*phoA* (from C. Manoil [28]) was performed by infecting a culture of *E. coli* LLM1717. This procedure and the procedures for conjugation and gene replacement in *V. parahaemolyticus* have been described elsewhere (39). All strain constructions were confirmed by Southern blot analysis of restricted genomic DNA (29, 32) on 0.45-μm Magna Charge nylon membranes (Micron Separations Inc., Westborough, Mass.).

SDS-PAGE. Samples were suspended in Laemmli sample buffer (23), and proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE [2]) and visualized by the method of Fairbanks et al. for Coomassie blue staining (13) (Brilliant Blue R; U.S. Biochemicals, Cleveland, Ohio). The resolving gel was 20 cm in length and was composed of 12.5% acrylamide. Acrylamide and SDS were from BDH (Poole, England), and the molecular weight mark-

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or description	Source or parent strain; reference
V. parahaemolyticus strains		
BB22	Wild type	R. Belas; 6
ML118	*lafX313*::*lux motX118*::mini-Mu *lac* (Tet^r)	LM1017; 30
ML141	*lafX313*::*lux motY141*::mini-Mu *lac* (Tet^r)	LM1017; 30
LM1017	*lafX313*::*lux*	BB22; 30
LM4170	*lafX313*::*lux motX118*::mini-Mu *lac* (Tet^s)	ML118; this work
LM4171	*lafX313*::*lux motY141*::mini-Mu *lac* (Tet^s)	ML141; this work
LM4288	*lafX313*::*lux* Tn*phoA* insertion 1718 near *motY*	LM1017; this work
LM4289	*lafX313*::*lux* Tn*phoA* insertion 1719 near *motY*	LM1017; this work
LM4290	*lafX313*::*lux motY1721*::Tn*phoA*	LM1017; this work
LM4296	*lafX313*::*lux* Tn*phoA* insertion 1730 near *motY*	LM1017; this work
LM4312	*lafX313*::*lux* Tn*phoA* insertion 1763 near *motY*	LM1017; this work
LM4313	*lafX313*::*lux* Tn*phoA* insertion 1764 near *motY*	LM1017; this work
E. coli strains		
CC118	*araD139* Δ(*ara-leu*)*7697* Δ*lacX74* Δ*phoA20 galE galK thi rpsE rpoB argE*(Am) *recA1*	C. Manoil; 17
JM109	*endA1 gyrA96 hsdR17* ($r_K^-m_K^+$) *mcrB*$^+$ *recA1 relA1 (130*(*lac-proAB*) F'[*traD36 proAB lacI*^{q}ZΔM15]λ$^-$	Clontech
LLM1717	CC118/pLM1717	This work
LLM1750	JM109/pLM1750	This work
LLM1751	JM109/pLM1751	This work
Plasmids		
pKK388-1	Tet^r Ap^r expression vector	Clontech
pLAFRII	Tet^r; pLAFRI with polylinker	F. Ausubel; 15
pLM1717	pLAFRII recombinant cosmid complementing strain LM4171 for motility	*V. parahaemolyticus* bank; 32
pLM1750	2.3-kb *Eco*RI fragment containing *motY* with P_{trc} promoter aligned	pLM1717 and pKK388-1
pLM1751	2.3-kb *Eco*RI fragment in reverse orientation of pLM1750	pLM1717 and pKK388-1

ers (low-molecular-weight range) were from Bio-Rad Laboratories (Hercules, Calif.).

DNA manipulation and sequencing analysis. Transformations, ligations, and other general cloning procedures were performed by the methods of Sambrook et al. (37). Sequences were determined by using a shotgun strategy: a fragment was self-ligated and then sonicated to generate random subfragments of 300 to 600 bp (5). After end repairing with T4 DNA polymerase, the subfragments were ligated into the *Sma*I site of M13mp8 (34) and sequenced by the dideoxy-chain termination procedure of Sanger et al. (38) with the Sequenase 2.0 kit from U.S. Biochemicals. The source of radioactivity was α-^{35}-S-dATP (Amersham). Two overlapping fragments from pLM1717 were prepared in this manner (1.6-kb *Hin*dIII and 2.3-kb *Eco*RI fragments). The nucleotide sequence was obtained for both strands. Occasionally synthetic oligonucleotides, prepared by Integrated DNA Technologies, Inc. (Coralville, Iowa), were used as primers on long templates to obtain additional sequence. Sequence assembly was performed with Roger Staden's sequence assembly program (version 5.0), and the Genetic Computer Group software package version 7.0 was used for sequence analysis. Searches for homology were performed at the National Center for Biotechnology Information with the BLAST network service (1). Tn*phoA* insertions in plasmid pLM1717 were sequenced directly. The oligonucleotides used as sequencing primers specific to each unique end of Tn*phoA* were 5′ CAGAGCGGCAGTCTGATCAC 3′ (left) and 5′ CCGCACGATGAAGAGCAG 3′ (right).

Nucleotide sequence accession number. The DNA sequence has been assigned GenBank accession number U06949 (3,516 nucleotides).

RESULTS

Cloning of *motY* locus. Previously, the isolation of transposon mutants of *V. parahaemolyticus* with defective swimming motility phenotypes was described (30). Among a collection of 5,000 transposon mutants, 24 were defective for swimming in motility agar, and 2 of these mutants, strains ML118 and ML141, seemed to be good candidates for having motor defects. These strains were completely immotile in motility plates and under the light microscope. They produced wild-type levels of polar flagellin antigen. Their polar appendages were indistinguishable from those of the wild type when examined with the electron microscope.

To retrieve clones carrying genes complementing these defects, strains ML118 and ML141 were made tetracycline sensitive (LM4170 and LM4171, respectively). Pools of tetracycline-resistant clones from a *V. parahaemolyticus* cosmid bank were mated into both LM4170 and LM4171, with selection for tetracycline resistance and movement in motility plates. Three classes of cosmids restoring motility were isolated. One family, of which pLM1717 is representative, complemented the defect (*motY141*) in strain LM4171. Another family, which will be described elsewhere, complemented the defect (*motX118*) in strain LM4170. Cosmids in the third family restored motility to both LM4171 and LM4170. These complemented the swarming defect (*lafX313*::*lux*) present in both strains. Either swarming or swimming motility allowed the bacterium to move through semisolid motility agar.

Transposon mutagenesis of *motY* locus. When cosmid pLM1717 was used to probe Southern blots of restricted chromosomal DNA from the wild type and a variety of motility-defective strains (including *mot*, *fla*, and *che* mutants),

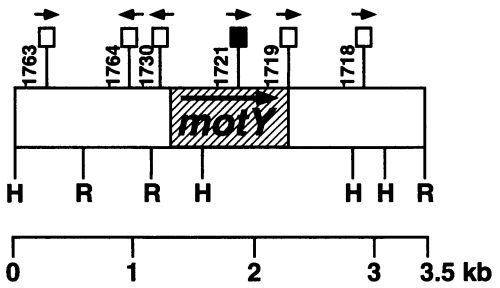

FIG. 1. Physical map of the *motY* locus. The 1.6-kb *Hin*dIII and 2.3-kb *Eco*RI fragments were isolated for sequencing. The positions of Tn*phoA* insertions used for gene replacement mutagenesis in *V. parahaemolyticus* are shown on the restriction map; the direction of transcription of *phoA* (arrows) and the only transposon conferring a motility defect when introduced into the chromosome (solid square) are indicated. Positions of the transposons were obtained by restriction mapping (RM) and/or sequencing (S): 1763 is at 0.3 (RM), 1764 is at 1.022 (S), 1730 is at 1.204 (S), 1721 is at 1.933 (S), 1719 is at 2.310 (S), and 1718 is at 2.95 (RM) kb. H, *Hin*dIII; R, *Eco*RI.

only strain LM4171 (and its parent ML141) showed rearrangements. These blots revealed that the *motY141* mutation was the result of a transposon insertion in a 2.3-kb *Eco*RI fragment. In order to characterize the *motY* locus, cosmid pLM1717 was mutagenized with the transposon Tn*phoA*, a probe for exported proteins. Curiously, of approximately 1,000 transposon insertions in pLM1717 isolated from 15 independent mutagenesis experiments, no significantly active *phoA* fusions that mapped in the 2.3-kb *Eco*RI fragment were obtained, although one transposon, number 1719, was very faintly blue on indicator plates with the chromogenic substrate XP. A collection of transposons that mapped in or near the 2.3-kb *Eco*RI fragment was used for gene replacement mutagenesis in *V. parahaemolyticus*. The transposons used to make the mutants are shown on the physical map in Fig. 1. Of six insertions mapping within 3 kb of each other, only transposon 1721 resulted in a motility-defective phenotype when introduced into the chromosome.

DNA nucleotide sequence and deduced amino acid sequence. The entire region shown in Fig. 1 was sequenced, and the nucleotide sequence corresponding to positions 1.101 through 2.400 kb is presented in Fig. 2 along with the deduced amino acid sequence for MotY. The open reading frame (ORF) starts with an ATG codon that is preceded by features resembling an *E. coli* ribosome binding site (16). Disruption of this ORF, which codes for a potential polypeptide 293 amino acids in length, by insertion of transposon 1721 resulted in a motility defect in *V. parahaemolyticus*. The point of insertion at nucleotide position 1933 (determined precisely by priming and sequencing from the ends of the transposon) placed the transposon out of frame with respect to transcription of *motY* and at amino acid 165. Immediately upstream of the *motY* gene and coded on the same strand is a short ORF (composed of 22 predominantly hydrophobic amino acids) with its own potential ribosome binding site. Further upstream are sequences that show some similarity to a σ^{28}-like promoter: TAAT n15 GCGCTTGA, compared with the consensus derived for flagellar promoters of *E. coli*, TAAA n15 GCCGATAA (18). Such a promoter sequence is consistent with the known dependence of *V. parahaemolyticus* polar flagellin gene expression on σ^{28} in *E. coli* (33). Insertion of transposon 1730 at nucleotide 2310 interrupted this putative promoter. Transposon 1730 did not confer a motility defect when introduced into the chromosome; however, if the promoter for *motY* is indeed disrupted, Tn*5* could provide weak, constitutive expression which should be a suitable level for *motY*. (Since there is only one flagellum per cell, one would expect approximately 10 motor units per cell.) Sequencing of the clone containing insertion 1719 revealed that transposition created a *phoA* fusion at the very end of *motY* at nucleotide 2310. Only four amino acids are lost from the C terminus, and the fusion protein appeared to be functional, for the insertion does not cause a motility-defective phenotype in *V. parahaemolyticus*. The weak alkaline phosphatase activity in *E. coli* observed for this fusion protein is consistent with a low level of expression of *motY* and/or requirement of other parts of the motor for incorporation into the membrane. Following the ORF are sequences resembling an *E. coli* rho-independent transcriptional terminator.

Predicted structure of MotY: identification of a potential peptidoglycan interaction domain. MotY showed no sequence similarity to *E. coli* or *V. parahaemolyticus* lateral Mot proteins or to other flagellar or chemotaxis sequences. It does possess an amino-terminal domain that could be membrane spanning, containing a stretch of 19 amino acids with 11 hydrophobic residues and no charged residues. Although MotY failed to resemble the Mot proteins of *E. coli* and *V. parahaemolyticus*, a TBLASTN search revealed similarity to a small region of the MotB protein of *Bacillus subtilis* (GenBank locus BACMOTAB; 50% identical and 73% positive over 26 amino acids). In addition, this search turned up a large number of proteins with more extensive homology to MotY than MotB of *B. subtilis*. These are proteins known to interact with the cell wall, and they belong to two families, OmpA-like proteins and peptidoglycan-associated lipoproteins. The shared domain maps to the carboxyl terminus of each protein, and the multiple sequence alignment for a representative set is shown in Fig. 3.

***motY* maps alone.** For all of the organisms examined previously, the *mot* genes have been found together in an operon and usually linked to other flagellar or chemotaxis genes. In *V. parahaemolyticus*, gene replacement mutagenesis suggested that there were no additional motor or other motility genes mapping upstream or downstream of *motY*. The DNA sequence suggested a potential flagellar promoter preceding *motY*. However, to be certain of the configuration of *motY*, the DNA sequence 1.8 kb upstream and 1.2 kb downstream of the gene was obtained. As shown in Fig. 4, the *motY* gene is located in the middle of four ORFs all directed in the opposite orientation from *motY*. Deduced protein sequences of ORFs on each side of *motY* resembled sequences found in the *E. coli* locus coding for RNase T (19). Thus, it appears that the *motY* gene is inserted into the equivalent of the *E. coli rnt* locus.

Identification of MotY protein. The 2.3-kb *Eco*RI fragment was subcloned in both orientations into the expression vector pKK388-1, which uses the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible P_{trc} (*trp-lac* fusion) promoter. Exponentially growing cultures of strains with pLM1750 (promoter aligned with *motY*) and pLM1751 (*motY* opposed to the promoter) were diluted into fresh media with and without IPTG. Optical density at 600 nm was monitored as the cultures continued to grow, and samples were periodically harvested for analysis on protein gels. Induction with IPTG had no appreciable effect on the growth rate of the cultures; therefore, overproduction of MotY did not impair the growth rate. The deduced protein sequence has a calculated molecular mass of 33,385 Da. Induction of strain LLM1750 resulted in increased synthesis of a protein migrating at 33 kDa (Fig. 5, lanes 1 and 2). Strain LLM1751 failed to produce such a protein (lanes 3 and 4). There is a basal level of MotY production (lane 1), which is

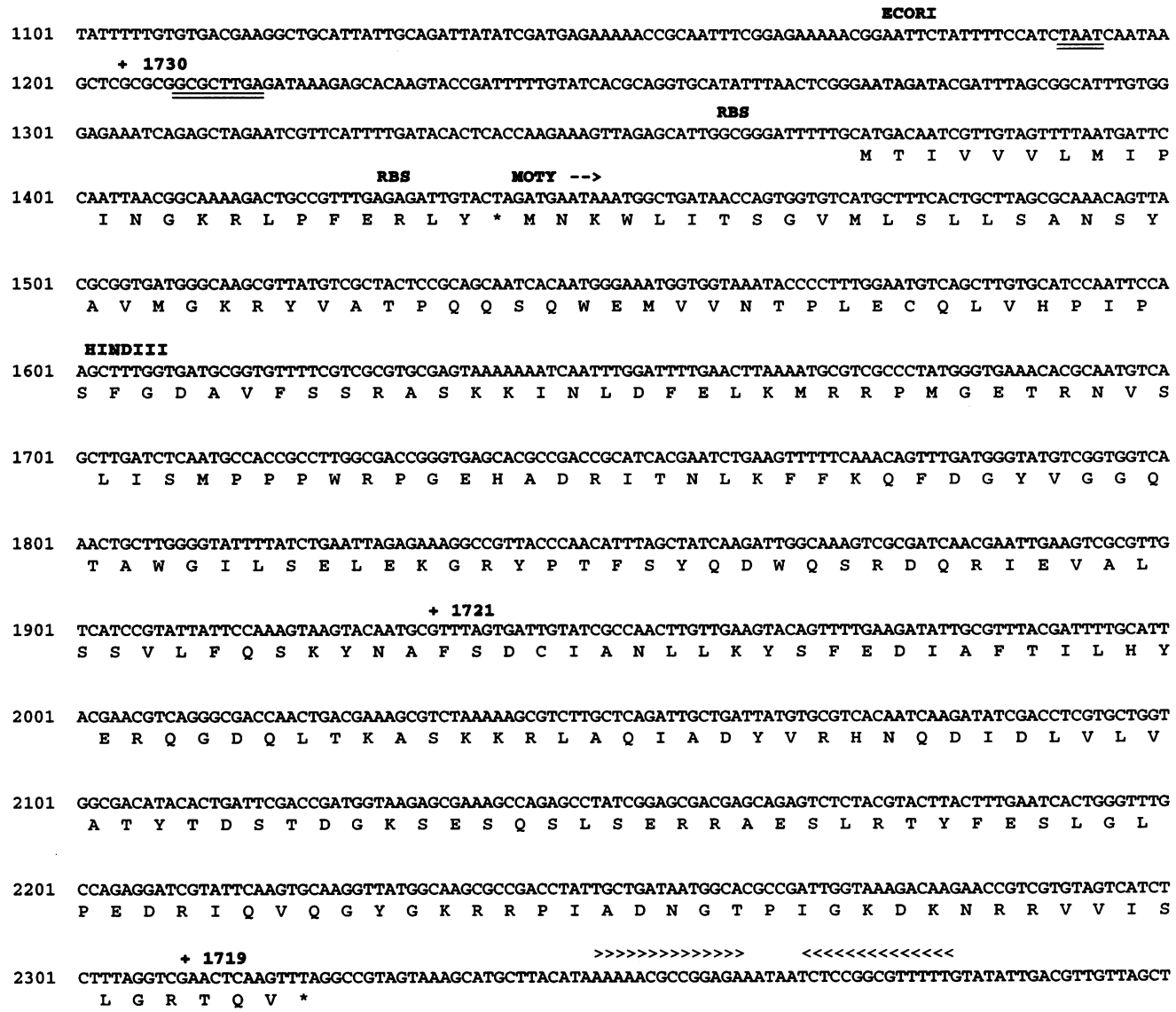

FIG. 2. DNA nucleotide sequence and deduced amino acid sequence of *motY*. The nucleotide sequence corresponds to 1.101 through 2.400 kb in Fig. 1. Selected restriction sites are shown to facilitate orientation to the physical map. The features of a potential σ^{28} promoter are double-underlined. Sites resembling the *E. coli* ribosome binding site (RBS), a structure that could serve as a transcriptional terminator (chevrons), and the point of insertion of the specific transposons indicated (plus signs) are indicated. Only transposon 1721 causes a motility-defective phenotype when introduced onto the *V. parahaemolyticus* chromosome. Note that there is a short ORF immediately preceding *motY*, with its own putative RBS.

probably due to loss in a small percentage of the population of the F factor encoding *lacI*^{q}. Synthesis of MotY is increased when the P_{trc} promoter is induced by the addition of 2 mM IPTG to the growth medium (lane 2).

DISCUSSION

Flagella are rotated by extremely small molecular motors. Electrochemical energy stored as membrane potential is converted to mechanical energy used to turn the flagellar propeller. *V. parahaemolyticus* is a bacterium with two kinds of motors utilized to power two separate flagellar systems. The polar system is employed for swimming in liquid environments, and the lateral system is suited for movement over surfaces or through viscous layers. Although the lateral system is adapted for surface translocation, it appears similar in many ways to the flagellar systems of *E. coli* and *Salmonella typhimurium*: the flagella are homopolymers composed of a single flagellin subunit, they are arranged peritrichously, and the propulsive energy is derived from the proton motive force. The polar system is more unusual: a single, sheathed polar flagellum, composed of four flagellin subunits, is powered by the sodium motive force.

A new gene, *motY*, required for polar motility in *V. parahaemolyticus* has been discovered. Mutants with loss of function defects in *motY* have a Mot$^-$ phenotype, i.e., although they possess apparently normal flagella these strains seem paralyzed. The deduced product of *motY* is 293 amino acids in length. Overexpression of the gene in *E. coli* allowed identification of a product 33 kDa in apparent size on SDS-PAGE.

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VPAMOTY     SLSERRAESLRTYFESL.GLPEDRIQVQGYGKRRP.IADNGTPIGK.........DKNRRVVISLGRTQV*          293
BPEOMPA     kLSERRAaSVKaYLvSk.GIdpNRIytEGkGKlnP.IAsNkTAeGR.........aRNRRVeIeIvgsr*          193
PSEOPRFF    kLSQRRADaVKqvLvkd.GVApsRItavGYGesRP.VADNATeaGR.........avNRRVeaSd*              317
SDOMPA      GLSERRAQSVvdYLiSk.GIPaDKIsarGmGesnP.VtGNtcdnvKqraalidclapDRRVeIeVkgikdvvtqpq*  350
ECOOMPA     GLSERRAQSVvdYLiSk.GIPaDKIsarGmGesnP.VtGNtcdnvKqraalidclapDRRVeIeVkGikdvvtqpq*  345
ECPAL       SLGERRANaVKmYLQGk GVsaDqISIvSYGKeKPaVlghDeaAys.........KNRRaVLvy*               173
HEAA15KLP   aLGQRRADaVKgYLaGk.GVdaGKLgtvSYGeeKPaVlghdeaAys.........KNRRaVLa*              152
PSEPORF     kLSERRANaVRdvLvNeyGVeGGRVnavGYGesRP.VADNATaeGR.........aiNRRVeaeVEaea*          349
BACMOTB     ........................sakGYGeyKP.VAsNkTAeGR.........sKNRRVevlIlprgaaetne*  260
CONSENSUS   -LSERRA-----Y-----G-----I---GYG---P----N----------------NRRV----------------
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FIG. 3. Potential peptidoglycan interaction domain. Shown is multiple sequence alignment of the carboxyl termini of membrane proteins from a variety of organisms, including OmpA proteins of *Bordetella avium* (BPEOMPA), *Pseudomonas aeruginosa* (PSEPORF), *E. coli* (ECOOMPA), and *Shigella dysenteriae* (SDOMPA); an adhesin from the outer membrane of *Pseudomonas fluorescens* (PSEOPRFF); Mot proteins of *V. parahaemolyticus* (VPAMOTY) and *B. subtilis* (BACMOTB; locus BACMOTAB); and peptidoglycan-associated lipoproteins of *E. coli* (ECPAL) and *Haemophilus influenzae* (HEAA15KLP). Proteins are designated by their GenBank accession loci. The initial alignment was created and displayed by using the PILEUP and PRETTY programs of Genetics Computer Group. Matches of identity or high-level similarity with *V. parahaemolyticus* MotY are indicated (shaded uppercase letters). The minimum plurality required to determine the consensus sequence was six identical matches with MotY. The C termini are indicated (asterisks), and the numbers at the end of each row indicate the total number of amino acids in each protein.

Except for a short stretch of 26 amino acids, the deduced protein sequence failed to resemble any previously identified protein involved in flagellar motility.

The location of this gene also seems unusual. In general, genes involved in flagellar motility are clustered on the chromosome (20). In particular, the *mot* genes of *E. coli*, *S. typhimurium*, *B. subtilis*, and *V. parahaemolyticus* (lateral flagellar system) have been found in operons linked to other flagellar and/or chemotaxis genes (3, 12, 33). This does not hold true for the polar flagellar system of *V. parahaemolyticus*: *motY* maps alone. There is no second, counterpart motor gene analogous to that found in the *motAB* operon organization of other bacteria, and there are no linked motility genes. In fact, it occurs within a region that shows an extremely high degree of homology to the *E. coli rnt* locus. There is, however, a very small ORF of 22 amino acids immediately preceding *motY*, interposed between *motY* and a potential σ^{28}-like promoter. The significance of this small potential coding region is not yet known.

In *E. coli*, two cytoplasmic membrane proteins are required for torque generation. MotA and MotB couple movement of protons across the cytoplasmic membrane to rotation of flagella. The mechanism of mechanochemical coupling is not fully understood. MotA has been shown to be a proton conductor (7). Each of the deduced sequences of MotA proteins from *E. coli*, *B. subtilis*, and *V. parahaemolyticus* (lateral) predicts four potential membrane-spanning domains (12, 33, 35). MotB proteins have a single putative membrane-spanning domain near their amino terminus. The remainder of each molecule is believed to reside in the periplasm. The topology of MotB protein has been interpreted to suggest that MotB anchors the motor to the cell wall either directly or through interaction with some other stationary component of the motor (11). *V. parahaemolyticus* MotY does not look like a channel-forming protein. The deduced protein sequence of MotY possesses one, predominantly hydrophobic domain that could be membrane spanning. This domain, which is located near the amino terminus, is not as hydrophobic as the putative membrane-spanning domains of other MotB proteins, i.e., it displays a peak in a Kyte-Doolittle plot (22) at a value of approximately 2 whereas the others have scores closer to or greater than 3. Also, the hydrophobic domain is not preceded, as MotB proteins generally are, by a hydrophilic amino terminus. Unlike other MotB proteins and flagellar proteins in general, which are devoid of cysteine, MotY contains two cysteine residues.

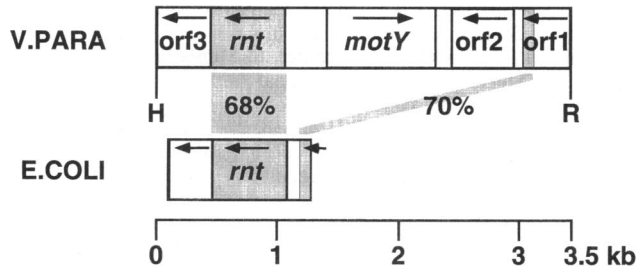

FIG. 4. Homology of regions flanking *motY* to the *E. coli rnt* locus. The *motY* gene is located in the center of four ORFs that are encoded on the strand opposite of *motY*; they are located at positions 1 to 420, 449 to 1093, 2535 to 2969, and 3113 to >3514, while *motY* is at positions 1442 to 2323. Arrows indicate the direction of transcription. The ORF at positions 425 to 1099 is 68% identical and 80% similar by Genetics Computer Group BestFit analysis to the *E. coli rnt* gene which encodes RNase T. There is a short ORF of 29 amino acids in the sequence preceding *E. coli rnt* which is 70% identical and 77% similar to the 3′ end of ORF1, which is on the opposite side of *motY* from the *rnt* gene. H, *Hin*dIII; R, *Eco*RI.

At its carboxyl terminus, MotY possesses a domain approximately 60 amino acids in length that shows remarkable similarity to a number of outer membrane proteins known to show close association with the murine layer, including OmpA-like proteins and peptidoglycan-associated lipoproteins. OmpA has been chemically cross-linked to peptidoglycan, and the C terminus of the molecule is believed to be positioned in the periplasm (14, 36). The C-terminal portion of *E. coli* peptidoglycan-associated lipoprotein has been shown to be involved in the interaction of the protein with the peptidoglycan (25). MotY shares with these proteins a C-terminal domain that seems to represent a conserved conformation important for binding to peptidoglycan. The MotB protein of *B. subtilis* has features in common with a smaller portion of this domain (26 amino acids). If the *E. coli* MotB sequence were included in the multiple sequence alignment in Fig. 3, a match with the consensus would be observed at NRR, which occurs at amino acids 257 to 259 in the 308-amino-acid *E. coli* MotB protein. Positively charged arginine residues have been postulated to be involved in noncovalent linkage of peptidoglycan-associated lipoproteins with peptidoglycan (25). Interestingly, the argi-

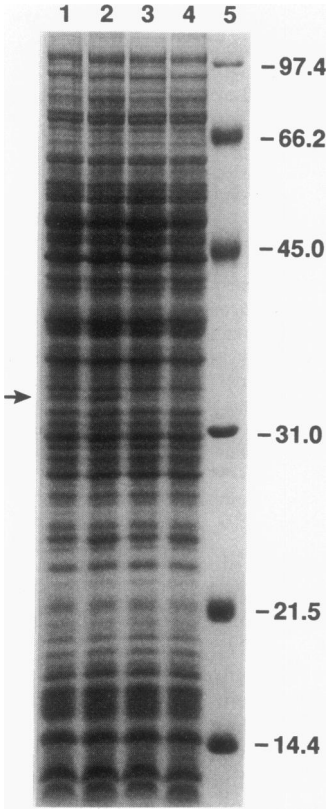

FIG. 5. Identification of MotY visualized by SDS-PAGE with Coomassie blue staining. The 2.3-kb *Eco*RI fragment containing the *motY* gene was cloned into an IPTG-inducible expression vector. Exponentially growing cultures of strains with pLM1750 (promoter aligned with *motY*) and pLM1751 (reverse orientation) were diluted into fresh media with and without 2 mM IPTG, and samples were harvested when the cultures reached early stationary phase. Lanes: 1 and 2, LLM1750 without and with IPTG, respectively; 3 and 4, LLM1751 without and with IPTG, respectively; 5, molecular mass standards (indicated at the right, in kilodaltons). MotY migrates at 33 kDa (arrow).

nine at position 258 has been mutated in *E. coli* and is essential for MotB function (8). Unfortunately, sequence information for the C terminus of the *V. parahaemolyticus* lateral MotB homolog, LafU, has not yet been obtained. To summarize, *V. parahaemolyticus* MotY shows striking homology with the peptidoglycan-associated proteins, *B. subtilis* MotB shows less similarity, and *E. coli* MotB shows very little recognizable similarity.

Because of the marked conservation of a domain known to be important for the interaction of a number of proteins with the cell wall, I postulate that the interaction of the carboxyl-terminal domain of MotY with peptidoglycan is direct and assign stator function in the sodium-type motor to MotY. Although the presumed function of MotY is analogous to that proposed for the proton-type MotB proteins, the molecule has little resemblance to these proteins. This may reflect different constraints on the molecules due to interactions with the other parts of the sodium- versus proton-driven motor or differences in evolutionary origin of the genes. How MotY interacts with the other components of the sodium-type torque-generating unit, what these other components are, and how they function to convert the chemical energy of the transmembrane sodium potential into mechanical energy remain to be determined.

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