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Putative Channel Components for the Fast-Rotating Sodium-Driven Flagellar Motor of a Marine Bacterium

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The polar flagellum of *Vibrio alginolyticus* rotates remarkably fast (up to 1,700 revolutions per second) by using a motor driven by sodium ions. Two genes, *motX* and *motY*, for the sodium-driven flagellar motor have been identified in marine bacteria, *Vibrio parahaemolyticus* and *V. alginolyticus*. They have no similarity to the genes for proton-driven motors, *motA* and *motB*, whose products constitute a proton channel. MotX was proposed to be a component of a sodium channel. Here we identified additional sodium motor genes, *pomA* and *pomB*, in *V. alginolyticus*. Unexpectedly, PomA and PomB have similarities to MotA and MotB, respectively, especially in the predicted transmembrane regions. These results suggest that PomA and PomB may be sodium-conducting channel components of the sodium-driven motor and that the motor part consists of the products of at least four genes, *pomA*, *pomB*, *motX*, and *motY*. Furthermore, swimming speed was controlled by the expression level of the *pomA* gene, suggesting that newly synthesized PomA proteins, which are components of a force-generating unit, were successively integrated into the defective motor complexes. These findings imply that Na^+-driven flagellar motors may have similar structure and function as proton-driven motors, but with some interesting differences as well, and it is possible to compare and study the coupling mechanisms of the sodium and proton ion flux for the force generation.

The bacterial flagellar motor rotates the helical flagellar filament that extends from the cell body to propel the cell. The energy source for motor rotation is the electrochemical gradient of a specific ion (either a proton or a sodium ion) across the cytoplasmic membrane (6). Thus, the flagellar motor is a molecular machine that couples ion flux to force generation. The proton-driven motors of bacterial flagella have been extensively studied in terms of structure, genetics, biochemistry, and biophysics. Five proteins, MotA, MotB, FliG, FliM, and FliN, appear to be involved in force generation of proton-driven motors. It has been proposed that FliG, FliM, and FliN make a switch complex and function as the rotor of the flagellar motor (27, 51). They form the C ring, which was newly found on the cytoplasmic face of the MS ring (13, 53). MotA and MotB, which are the membrane proteins and have four transmembrane segments and one transmembrane segment, respectively, are associated in a complex and function as a proton-conducting channel (6, 8, 47). MotB is believed to act as an anchor to the cell wall (11, 12) and to work with MotA as the stator of the flagellar motor. It has been shown that MotA interacts, but only weakly, with FliG and FliM of the switch complex (49).

Sodium-driven motors have advantages for experimental analysis, since sodium motive force can be manipulated more readily than proton motive force (18, 19, 52). Moreover, amiloride and its analogs, which specifically and strongly inhibit the polar-flagellum motors, are available and are thought to interact with a sodium channel of the flagellar motor (1, 3, 48). Some marine *Vibrio* species have two types of flagella, polar (Pof) and lateral (Laf), which are rotated by sodium- and proton-driven motors, respectively, in the same cell (2, 21). The polar flagella work better for swimming in a liquid or a low-viscosity environment (4). By laser dark-field microscopy, it has been shown that the rotation of the polar flagella is very fast in *Vibrio alginolyticus*; speeds of ca. 600 revolutions per s (rps) are observed in 50 mM NaCl at room temperature, and surprisingly, the speeds increase up to ca. 1,700 rps in 300 mM NaCl at 35°C (28, 29, 37).

The rotation of the sodium-driven motor seems to be very stable at high speed (37). When the rotation rate is reduced by amiloride, the motor still rotates smoothly though the rotation rate tends to fluctuate. On the other hand, polar flagella showed remarkably larger speed fluctuations upon addition of phenamil, an amiloride analog (38). The speed fluctuations induced by phenamil were explained by a low rate of dissociation of the inhibitor from the force-generating unit. Mutants resistant to phenamil were isolated, and the K_i value for phenamil in the resistant strain was estimated to be five times larger than that in the wild-type strain. By analysis of motor speed fluctuations, it was suggested that a phenamil-specific binding site of the motor was mutated in the resistant strain (24). The resistance mutation may map to one of the genes coding for the motor proteins.

Recently, the *motX* and *motY* genes, which seem to encode components of the force-generating units of the Na^+ motor, were isolated from *Vibrio parahaemolyticus* (30, 31). However, the predicted products have no homology to MotA and MotB, which are components of the force-generating units of H^+ motors and are thought to form a H^+ channel (6, 8, 44). On the other hand, the C-terminal domains of MotY and MotB do have sequence similarities to peptidoglycan-interacting proteins. MotX is inferred to be a component of the Na^+ channel of the motor because overproduction of MotX was lethal to *Escherichia coli* in proportion to the external Na^+ concentration, and this effect was suppressed by the addition of amiloride (31). Genes identical to the *motX* and *motY* Na^+ motor genes were also detected in *V. alginolyticus* (reference 40 and unpublished data). So, it had been thought that the force-generating units of H^+ and Na^+ motors were very different. MotX and MotY of the Na^+ motor, each of which has a

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or description[a]	Reference or source
V. alginolyticus strains		
138-2	Wild type	50
VIK4	138-2 *rif* (Rif^r Pof^+ Laf^+)	40
VIO5	VIK4 *laf* (Pof^+ Laf^-)	40
VIO586	VIO5 *pomA* (Laf^- Pom^-)	This work
V. parahaemolyticus		
LM4170	*lafX313 motX118* (Laf^- Pom^-)	31
LM4171	*lafX313 motY141* (Laf^- Pom^-)	30
E. coli strains		
DH5α	F^- λ^- *recA1 hsdR17 endA1 supE44 thi-1 relA1 gyrA96* Δ(*argF-lacZYA*)U169 ϕ80d*lacZ*ΔM15	16
XL1-Blue	*recA1 hsdR17 endA1 supE44* Δ(*lac-proAB*) {F′::Tn*10 proAB lacI*^{q}*Z*ΔM15}	Stratagene
S17-1	*recA hsdR thi pro ara* RP-4 2-Tc::Mu-Km::Tn*7* (Tp^r Sm^r)	46
Plasmids		
pSU21	*cat* (Cm^r) P_{lac} *lacZ*α	5
pSU41	*kan* (Km^r) P_{lac} *lacZ*α (MCS same as that in pSU21)	5
pIO3	pSU21 0.4-kb and 6.0-kb *Hin*dIII fragment (*motY*$^+$)	40
pYA101	pSU21 5.0-kb and 3.6-kb *Hin*dIII fragment (*pomA*)	This work
pYA201	pSU21 5.2-kb and 3.3-kb *Eco*RI fragment (*pomA*$^+$)	This work
pYA202	pSU21 3.3-kb *Eco*RI fragment (*pomA*$^+$)	This work
pYA2021	pSU21 2.7-kb *Eco*RI-*Hin*dIII fragment (*pomA*)	This work
pYA2022	pSU21 1.9-kb *Eco*RI-*Xba*I fragment (*pomA*)	This work
pYA2023	pSU21 1.6-kb *Eco*RI-*Sac*I fragment (*pomA*)	This work
pYA203	pSU21 3.3-kb *Eco*RI fragment (*pomA*$^+$) (containing the same fragment as pYA202, but in the opposite orientation)	This work
pYA2031	pSU21 2-kb *Sac*I-*Eco*RI fragment (*pomA*$^+$)	This work
pYA2032	pSU21 1.45-kb *Xba*I-*Eco*RI fragment (*pomA*$^+$)	This study
pHK2	pSU21 3.3-kb *Sac*I fragment (*pomAB*$^+$)	This work
pHK3	pSU21 1.2-kb *Hin*dIII-*Sac*I fragment *pomA pomB*$^+$)	This work
pSK1	pSU41 1.45-kb *Xba*I-*Eco*RI fragment (*pomA*$^+$)	This work
pSK1-Δ28	pSU41 with 329 bp deleted from *Xba*I site of pSK1; lack of the native promoter	This work
pMMB206	*cat* (Cm^r) IncQ *lacI*^{q}Δ*bla* $P_{tac\text{-}lac}$ *lacZ*α *rrnB*	35

[a] Cm^r, chloramphenicol resistant; Km^r, kanamycin resistant; MCS, multicloning site; P_{lac}, *lac* promoter; $P_{tac\text{-}lac}$, tandemly located *tac* and *lac* promoters; Rif^r, rifampin resistant; Sm^r, streptomycin resistant; Tp^r, trimethoprim resistant.

putative single transmembrane region, were thought to form a channel component and/or a stator component, functionally homologous to the H^+ motor components MotA and MotB. However, in this study, we isolated novel *pom* (polar flagellum motility) genes whose predicted products are structurally homologous to the H^+ motor components, MotA and MotB. This shows that the channel components of the Na^+ motor are similar to those of the H^+ motor.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The strains and plasmids used are shown in Table 1. *V. alginolyticus* and *V. parahaemolyticus* cells were cultured at 30°C in VC medium (0.5% polypeptone, 0.5% yeast extract, 0.4% K_2HPO_4, 3% NaCl, 0.2% glucose) or VPG medium (1% polypeptone, 0.4% K_2HPO_4, 3% NaCl, 0.5% glycerol). When necessary, chloramphenicol and kanamycin were added at the final concentrations of 2.5 and 100 μg/ml, respectively.

DNA manipulations and sequencing. Routine DNA manipulations were carried out according to the standard procedures (41). Restriction endonucleases and other enzymes for DNA manipulations were purchased from Takara Shuzo (Kyoto, Japan) and New England Biolabs (Beverly, Mass.). The nucleotide sequence was determined by the dideoxy chain termination method using the Sequenase version 2.0 DNA sequencing kit (United States Biochemical Corp., Cleveland, Ohio). [α-^{32}P]dCTP (3,000 μCi/mmol; Amersham Japan, Tokyo, Japan) was used for radioactive labeling.

Electroporation. Transformation of *Vibrio* cells by electroporation was carried out as described previously (22). The cells were subjected to osmotic shock and were washed thoroughly with 20 mM $MgSO_4$. Electroporation was carried out with the Gene Pulser electroporation apparatus (Japan Bio-Rad Laboratories, Tokyo, Japan) at an electric field strength between 5.0 and 7.5 kV/cm.

Isolation of *pom* mutants. From the lateral flagellum-defective (Pof^+ Laf^-) mutants VIO5 (40), YM4 (17), and NMB201 or NMB205 (24), swarm-deficient mutants were isolated and *pom* mutants were selected from the swarm-deficient mutants as described previously (40).

***pom* gene cloning.** Plasmid pSU21 (5) and chromosomal DNA from *V. alginolyticus* VIO5 were digested with *Eco*RI or *Hin*dIII and ligated. These DNA libraries were transferred into *pom* mutant (VIO586) cells by electroporation and selected as described previously (40). Cells were incubated at 30°C on 0.3% agar–VPG plates containing chloramphenicol.

Conjugation. Plasmid transfer by conjugation was carried out according to the method of Okunishi et al. (40). Aliquots (20 μl each) of fresh overnight cultures of *E. coli* S17-1 cells carrying plasmid pMMB206 (donor) and *Vibrio* cells were mixed on a 1.25% agar–VC plate and incubated at 30°C overnight. Cells were scraped from the plate and suspended in 100 μl of VC medium. To select transconjugants, the suspension was plated on a 1.25% agar–VC plate supplemented with 2.5 μg of chloramphenicol per ml.

Measurement of swimming speed. Cell culture was diluted about 100-fold into Tris motility buffer (50 mM Tris-HCl [pH 7.5], 5 mM $MgCl_2$, 5 mM glucose, 50 mM NaCl, 250 mM KCl), and motility of the cells was observed under a dark-field microscope and recorded on videotape. Swimming speed was determined as described previously (17).

Nucleotide sequence accession number. The nucleotide sequences for the *pomA* and *pomB* genes from *V. alginolyticus* have been deposited in DDBJ under accession no. AB004068.

RESULTS

Cloning of a *pom* gene required for polar flagellar rotation. We isolated *pom* (polar flagellar motility) mutants which have paralyzed polar flagella (40). Using a *pom* mutant (VIO586) as a recipient for shotgun cloning, we cloned a *pom* gene in pYA101 and pYA201 by using *Hin*dIII and *Eco*RI, respectively. When these plasmids were introduced into VIO586 cells, the cells swarmed in the 0.3% agar–VPG plate (Fig. 1A

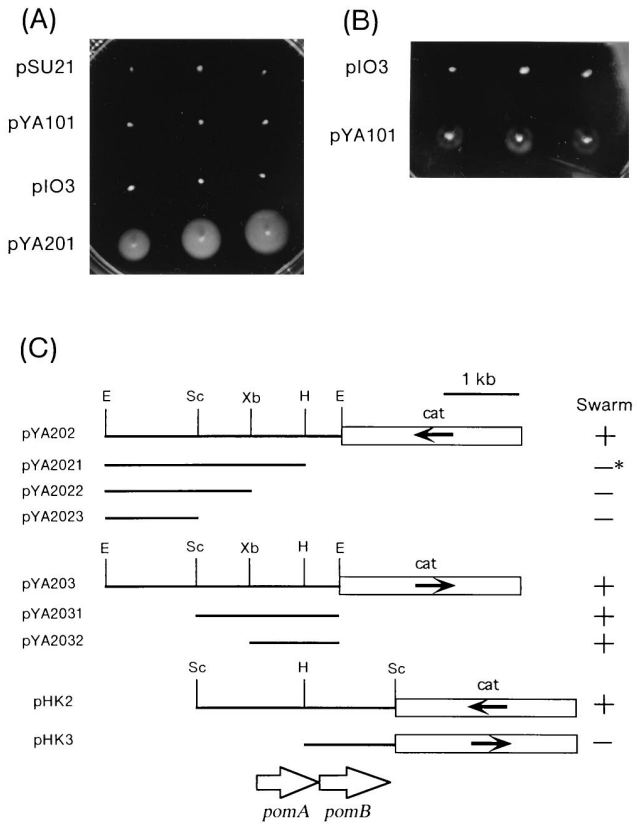

FIG. 1. Isolation of novel *pom* genes. (A and B) Swarming abilities of VIO586 cells carrying various plasmids. Fresh colonies were inoculated in 0.3% agar–VPG plates containing chloramphenicol and incubated at 30°C for 5 (A) and 9 (B) h. (C) Restriction map of plasmids. The swarm recovery of VIO586 by transformation of the plasmids in 0.3% agar–VPG plates is shown on the right side of the map. The chromosomal DNA fragments are indicated by solid lines. CAT indicates the chloramphenicol acetyltransferase gene from the vector pSU21 (5). The open arrows show the coding regions of PomA and PomB. A plasmid (pYA2021) containing a 2.7-kb *Hin*dIII-*Eco*RI fragment made small swarms after a long incubation (−*). Probably the functional *pom* gene was formed by homologous recombination between the mutant *pom* gene on the chromosome and a part of the *pom* gene on the plasmid. Restriction sites are shown as follows: E, *Eco*RI; H, *Hin*dIII; Sc, *Sac*I; Xb, *Xba*I.

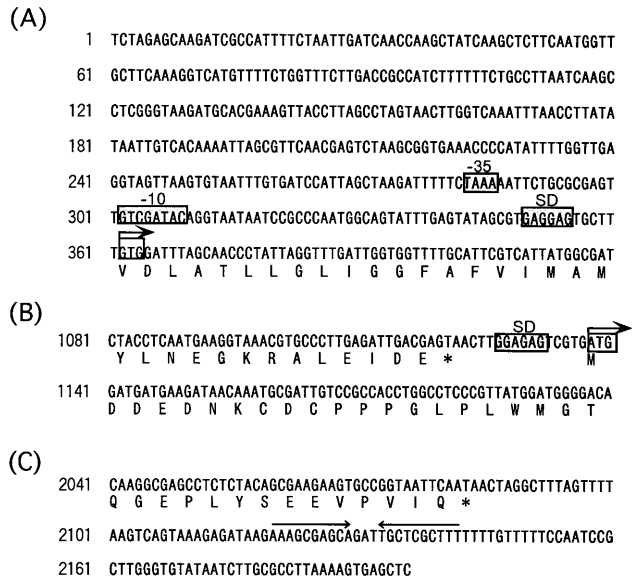

FIG. 2. The promoter region of *pomA* (A), the noncoding region between *pomA* and *pomB* (B), and the terminator region of *pomB* (C) in the 2,196-bp *Xba*I-*Sac*I fragment. The sequences enclosed by boxes indicate the predicted promoter region, Shine-Dalgarno (SD) sequences, and start codons. The complementary sequences are shown by arrows.

and 1B). Cells with pYA201 could swarm in 5 h; however, cells with pYA101 could make only small swarms in 9 h. pYA101 seemed not to contain the complete gene. Therefore, we used pYA201 for further analysis. The fragment in pYA201 did not complement *motX* and *motY* mutants of *V. parahaemolyticus* or a *motY* mutant of *V. alginolyticus* (data not shown), and the plasmid pIO3 carrying the *motY* gene of *V. alginolyticus* (40) did not complement the *pom* mutant. So, this gene must be a novel one related to the sodium-driven motor. A 3.2-kb *Eco*RI fragment, which still complemented the *pom* mutant, was subcloned, and the resulting plasmid was named pYA202. Next, we made restriction maps of pYA202 and pYA203 (which has the same fragment as pYA202 but in the opposite orientation) and constructed various deletion derivatives. Complementation assays using deletion derivative plasmids revealed that the *pom* gene was located in the 1.45-kb *Xba*I-*Eco*RI fragment containing the *Hin*dIII site (Fig. 1C). The plasmid containing the fragment is pYA2032. After sequencing the fragment (see below), we found that it also contained part of a second *pom* gene. To clone the whole gene, shotgun cloning and screening were done with *Sac*I, which cut out a ca. 3.0-kb chromosomal fragment which hybridized to the 1.45-kb *Xba*I-*Eco*RI fragment (data not shown). Plasmid pHK2, containing the ca. 3.0-kb *Sac*I fragment, complemented the mutation of VIO586.

Nucleotide sequence of the *pom* genes. We determined the nucleotide sequence of the 2,196-bp *Xba*I-*Sac*I fragment and identified two open reading frames, which we named *pomA* and *pomB*; they encode proteins containing 253 and 315 amino acids, respectively. A consensus of ribosome binding sites was found at 12 and 11 bp upstream from the start codons of *pomA* and *pomB*, respectively (Fig. 2A and B). There are only 15 bp between the two genes (Fig. 2B). Thirty-five base pairs downstream of the stop codon of *pomB*, there is a consensus ρ-independent transcription termination site (Fig. 2C). A potential promoter sequence of TAAA(N_{15})GTCGATAC, which is very similar to the σ^{28} promoter consensus of TAAA(N_{15})GCCGATAA of *Salmonella typhimurium* and *E. coli* (32, 40), was found 67 bp upstream of the start codon (Fig. 2A). Thus, the two genes seem to constitute a single transcription unit. A plasmid, pHK3 (Fig. 1C), containing only the *pomB* gene complemented the *pom* strains NMB104, NMB152, and NMB161. This indicates that the three strains are *pomB* mutants and that PomB is actually required for polar flagellar motility.

Comparison of PomA and PomB to MotA and MotB of H^+ motor. The PomA and PomB proteins were found to be homologous to the proton-driven motor proteins, MotA and MotB, respectively, of *Rhodobacter sphaeroides*, *Bacillus subtilis*, and *E. coli* (Fig. 3). The hydropathy profiles of PomA and PomB are very similar to those of MotA and MotB of *E. coli* (data not shown), which have been shown to have four and one membrane-spanning helices, respectively. As with *motA* and *motB* in *E. coli* or *B. subtilis*, *pomA* and *pomB* are in the same operon and have a σ^{28}-type promoter sequence upstream. In *E. coli*, *cheA* and *cheW* reside downstream of the *mot* genes in the same operon. In *V. alginolyticus*, as in *R. sphaeroides*, the operon contains only the two motor genes. Upstream of *pomA*, we found genes homologous to *E. coli xseB* and *ispA*, which

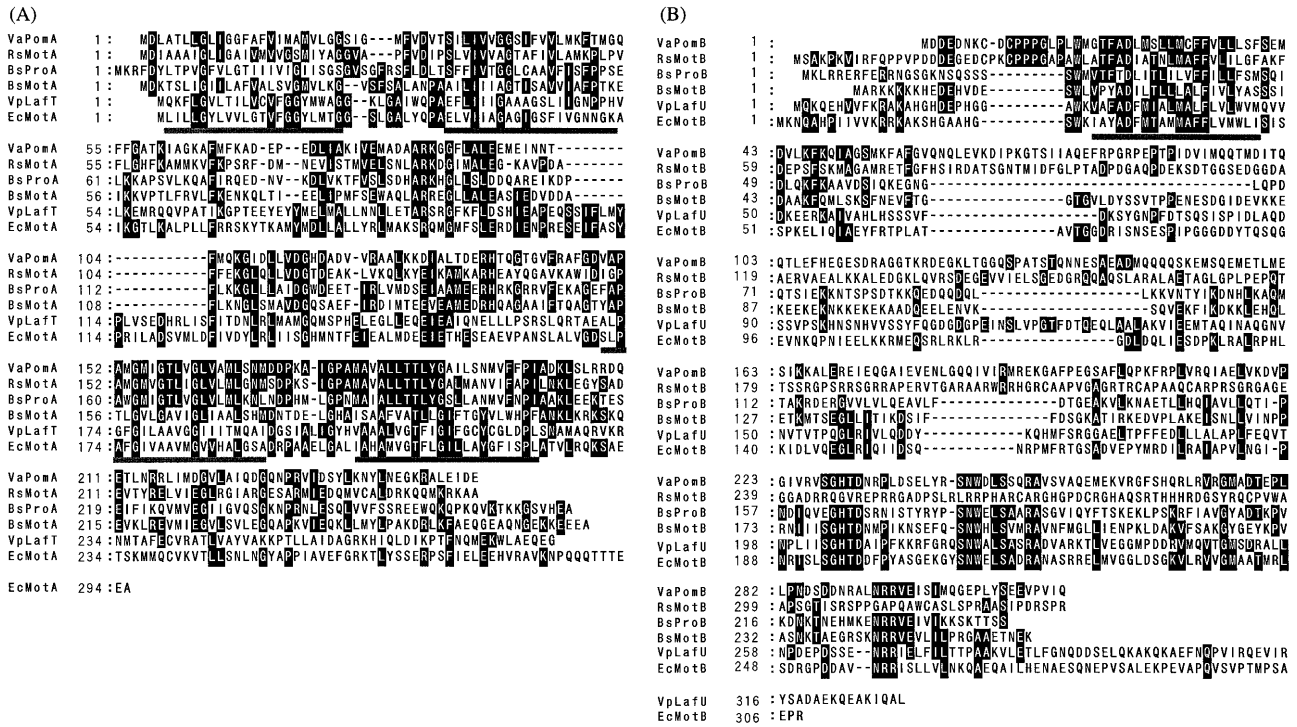

FIG. 3. Amino acid alignments of PomA (A) and PomB (B) with homologous proteins of various species. Dotted bars indicate membrane-spanning regions of *E. coli* MotA and MotB. Abbreviations: VaPomA, *V. alginolyticus* PomA; RsMotA, *R. sphaeroides* MotA; BsProA, *B. subtilis* hypothetical protein A; BsMotA, *B. subtilis* MotA; VpLafT, *V. parahaemolyticus* LafT; EcMotA, *E. coli* MotA; VaPomB, *V. alginolyticus* PomB; RsMotB, *R. sphaeroides* MotB; BsProB, *B. subtilis* hypothetical protein B; BsMotB, *B. subtilis* MotB; VpLafU, *V. parahaemolyticus* LafU; EcMotB, *E. coli* MotB. White letters in black boxes show identical residues.

encode the exoDNase small subunit and the farnesyl diphosphate synthase (14), respectively. The other sodium motor genes, *motX* and *motY*, are localized elsewhere on the chromosome (30, 31, 40).

Figure 3A shows the alignments of PomA with various MotA sequences. The four membrane-spanning regions, especially the third and fourth, are more similar than the other regions and, in MotA, are thought to form the proton channel. It has been shown that nonmotile or severe *mot* mutations are localized in the four membrane-spanning regions of MotA of *E. coli* (9). PomA is more similar to MotA of *B. subtilis* than to MotA of *E. coli*, even though the latter is phylogenetically closer to *V. alginolyticus*. PomA and *R. sphaeroides* MotA (43) are extremely similar and contain the same number of residues. It would be interesting to see whether these motor components are functionally interchangeable. For example, the channel components of proton-type and sodium-type F_0F_1 ATPases are similar, and it has been shown that the substitution of only two amino acids changes the ion specificity of F_0F_1 ATPase (20).

PomB consists of 315 amino acids, and the N-terminal and the C-terminal regions are similar to those of MotB in other species (Fig. 3B). In those C-terminal regions of *V. alginolyticus* and *E. coli*, the peptidoglycan-binding motif is highly conserved but *R. sphaeroides* does not have the motif (42). The membrane-spanning region at the N terminus of MotB is thought to complex with the transmembrane regions of MotA and form a part of the proton channel (45). The N-terminal regions of PomB and *R. sphaeroides* MotB (42) are very similar. These results suggest that PomB also has only one membrane-spanning region and interacts with the cell wall peptidoglycan via its C-terminal domain.

Controlled expression of *pomA* in *V. alginolyticus*. The *Xba*I-*Eco*RI fragment of pYA2032 was subcloned into the pSU41 vector (5). From the resultant plasmid, pSK1, various lengths of deletion were carried out with exonuclease III and mung

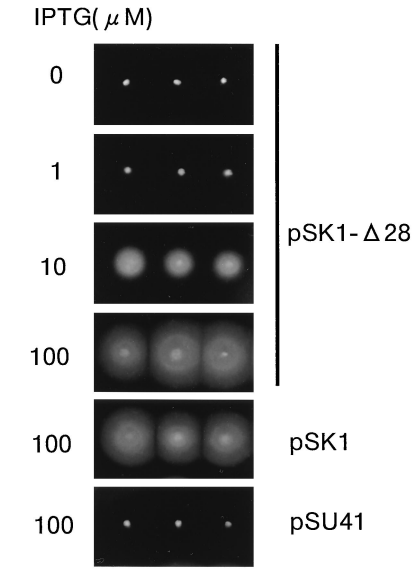

FIG. 4. Swarming profile by controlled expression of *pomA*. After pSK1-Δ28, pSK1, and pSU41 were introduced into VIO586/pMMB206, fresh overnight colonies were spotted on 0.25% agar–VC plates containing 2.5 μg of chloramphenicol per ml, 100 μg of kanamycin per ml, and various concentrations of IPTG. The plates were incubated for 5 h at 30°C.

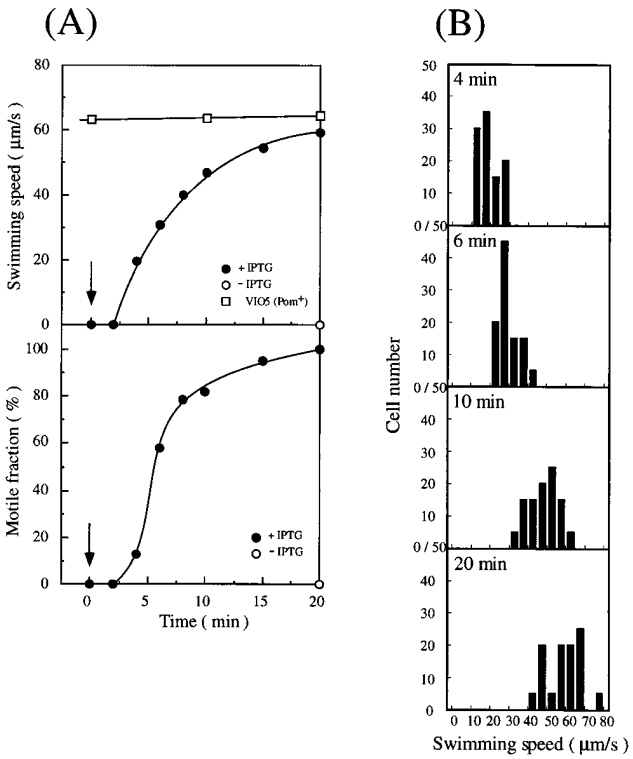

FIG. 5. Motility recovery by controlled expression of *pomA*. (A) For motility assay, VIO586/pMMB206/pSK1-Δ28 was grown in VPG medium containing 50 mM NaCl and 250 mM KCl at 30°C. At late logarithmic phase, 100 μM IPTG was added to the medium (indicated by arrows). At various times, swimming speeds of motile cells were measured. The average swimming speed was obtained by measuring at least 20 cells. The motile fraction was determined by counting the number of motile cells among the total cells in one video image and averaging at least three images in one condition. (B) The distributions of swimming speeds in panel A at 4, 6, 10, and 20 min are shown as the fractions of the total counted cells.

bean nuclease, and *pomA* was placed under the control of a *lac* promoter-repressor system that had been established previously in *V. alginolyticus* (40) (Fig. 4). The putative σ^{28} promoter sequence was deleted in plasmids pSK1-Δ28 and pSK1-Δ32 (with deletion endpoints at 329 and 346 bp, respectively, in Fig. 2A). The motility of VIO586/pMMB206 cells carrying either of the plasmids was clearly dependent on the concentration of IPTG (isopropyl-β-D-thiogalactopyranoside). Figure 5A shows the restoration of the swimming speed and motile fraction of VIO586/pMMB206/pSK1-Δ28 cells after the induction of *pomA* expression by IPTG. At 4 min, cells began to swim slowly, and the swimming speed gradually increased, reaching wild-type level at 20 min. The motile fraction showed almost the same profile as the swimming speed. After induction, the peak of the distribution progressively shifted from low swimming speed to high (Fig. 5B). The number of polar flagella is usually only one in a *Vibrio* cell, and the swimming speed of the cell reflects the motor property. These results suggest that newly synthesized PomA proteins were successively integrated into the defective motor complexes as shown in the H^+-driven motor of *E. coli* (7).

DISCUSSION

Until now, two sodium-driven motor genes, *motX* and *motY*, have been identified in *V. parahaemolyticus* and *V. alginolyticus*. Their products are both predicted to have one transmembrane

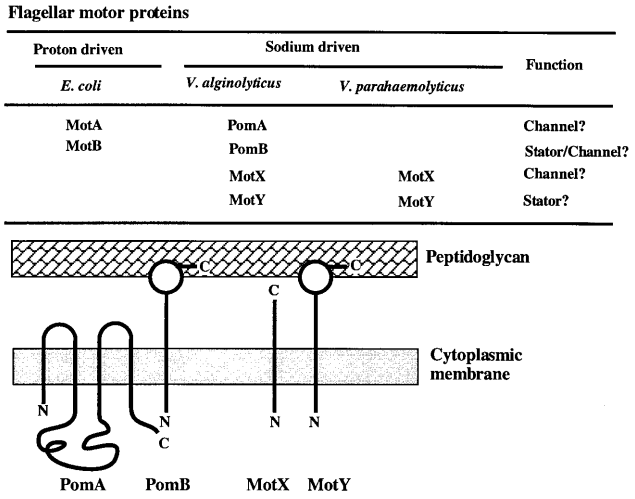

FIG. 6. Motor genes of membrane components for the proton-driven- and sodium-driven-type flagella and the predicted membrane topology of the four *pom* gene products.

region. It is believed that MotX is a sodium channel component and that MotY connects the motor with peptidoglycan, because its C-terminal region is significantly similar to that of OmpA in *E. coli*. In this study, we cloned two novel sodium-driven motor genes, *pomA* and *pomB*, which are homologous to the *motA* and *motB* genes of proton-driven motors, respectively. So, the sodium-driven motor consists of the products of four genes, *pomA*, *pomB*, *motX*, and *motY* (Fig. 6). The genes, *lafT* and *lafU*, of the lateral flagellar motor which is proton driven have been isolated, and the predicted products are shown to be homologous to the *motA* and *motB* products of *E. coli*, respectively (34).

By inducing the *E. coli motA* and *motB* genes in tethered *motA* and *motB* mutant cells, respectively, restoration of torque took place in a series of equally spaced steps (7, 10). On the other hand, the rotation rate decreased stepwise in the tethered alkalophilic *Bacillus* cells by a photoactivated amiloride analog which covalently binds to the Na^+-interaction site of a channel (36). These results suggest that the motor has multiple force-generating units and that each force-generating unit functions independently. Moreover, the torque generated by a motor is the sum of the torque generated by each unit. It has been estimated that the H^+-driven motor of *E. coli* and the Na^+-driven motor of alkalophilic *Bacillus* have 8 and 5 to 9 force-generating units, respectively. In our induction system for *pomA*, the swimming speed and motile fraction of the *pomA* mutant cells were restored to wild-type levels in 20 min, suggesting that newly synthesized PomA proteins were successively integrated into the defective motor complexes. We have shown that the swimming ability of the *motY* mutant increased with the *motY* induction (40). The independent force-generating unit probably functions as described above in the Na^+-driven motor of *V. alginolyticus*. We plan to analyze the rotation speed at low numbers of the force-generating units by laser dark-field microscopy (38) and to investigate the stability or the stepping of the speed.

From the homology to the proton motor components and their properties (15, 39, 44, 45, 54), we expect that the four transmembrane regions of PomA and the one transmembrane region of PomB also form a complex and function as a sodium channel. An Asp residue in the transmembrane region of *E. coli* MotB has been proposed to act as the donor in H^+ transfer

to a recipient near the cytoplasmic side of the protein (45). The Asp is conserved in the PomB transmembrane region and may be the donor in Na^+ transfer to a neighboring protein. It is also possible that a sodium channel is formed from the four *pom* products, PomA, PomB, MotX, and MotY, because it has been suggested that MotX is the channel component of the motor and interacts with MotY (30). It is not ruled out that MotX and MotY might form a different sodium channel.

In the proton-driven motor, MotB is thought to function as a stator by interaction with peptidoglycan. PomB presumably functions in a similar way. In addition to PomB, the sodium-driven-type motor apparently needs another component, MotY, which has a peptidoglycan binding motif but no further similarity to MotB. These two possible stators, PomB and MotY, are required for polar motility of *V. alginolyticus.*

Why does the sodium-driven motor need two stators? And why does it need four motor components, whereas the proton-driven motor needs only two? *Vibrio* has two types of flagella, polar and lateral. The polar flagellum is thought to function as a viscosity sensor (33) that regulates expression of lateral flagellar genes. Recently, we have shown that *Vibrio* cells sense a decrease in the rotation rate of (or the sodium influx through) the polar flagellar motor (23). Therefore, we speculate that MotX and MotY might sense the rotation rate or the sodium flux. Alternatively, they may be needed for the very fast rotation of the sodium-driven motor. It has been reported that its speed is ca. 600 rps in 50 mM NaCl at room temperature and increases up to ca. 1,700 rps in 300 mM NaCl at 35°C (28, 29, 37), whereas the speed of the proton-driven motor is 200 to 300 rps at most in *S. typhimurium* or *E. coli* (25, 26).

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