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Rhodobacter sphaeroides WS8 Expresses a Polypeptide That Is Similar to MotB of *Escherichia coli*

DEEPAN S. H. SHAH,[1] JUDITH P. ARMITAGE,[2] AND R. ELIZABETH SOCKETT[1]*

Department of Life Science, Nottingham University, University Park, Nottingham NG7 2RD,[1] and Microbiology Unit, Department of Biochemistry, Oxford University, Oxford OX1 3QU,[2] United Kingdom

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A gene which complements a paralyzed flagellar mutant of *Rhodobacter sphaeroides* was sequenced. The derived protein sequence has similarity to MotB. *R. sphaeroides* MotB lacks the C-terminal peptidoglycan-binding motif of other MotB proteins. This divergence of sequence may reflect the unusual, unidirectional, stop-start action of the *R. sphaeroides* flagellar motor.

The bacterial flagellum is a mechanoenzyme complex which acts as a rotating propeller enabling bacteria to move toward tactic stimuli. Flagellar rotation is driven by a transmembrane ion gradient, usually involving protons (28, 29) or, in some cases, sodium ions (17). Flagellar structure, function, assembly, and genetics have been the subjects of much study (reviewed in references 24, 25, and 26). Purified flagella consist of a long helical filament, a short hook, and a basal structure of two to five rings mounted on a rod (1, 11, 13, 19, 40). In addition to this core complex, there are other components which are not seen with purified flagella; one of these is the cytoplasmic switch complex. This complex includes proteins which are involved in switching rotational direction of bidirectional flagella and proteins involved in coupling the ion gradient to rotation (25, 27). The other known component is the MotA-MotB complex, which is thought to lie in the inner membrane surrounding the innermost rings of the core flagellum (9, 21, 39). The MotA-MotB complex is involved in torque generation (4, 7), with the MotA protein probably functioning as the proton-conducting component (5).

Most of the available information on flagellar structure, function, and genetics is derived from studies of *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*, and *Caulobacter crescentus*. To extend this range, we are studying flagellar motility in *Rhodobacter sphaeroides*. There are a number of features of *R. sphaeroides* that make it an interesting model for study.

R. sphaeroides is a photosynthetic bacterium which can grow aerobically or anaerobically. It is motile under a wide range of growth conditions (2). Like *C. crescentus*, *R. sphaeroides* has a single flagellum, but it is medially located rather than polar. Upon cell division, the daughter flagellum arises from the side laterally opposing that of the parent cell (2, 38). Thus, *R. sphaeroides* also provides a model for studies on membrane targeting and cell cycle dependence of flagellar assembly. The flagellum rotates unidirectionally in the clockwise direction (3) and can propel the cell at speeds of up to 100 μm/s (34). The cell moves in a series of runs and stops, being reoriented by Brownian motion during stops (3). The stopping frequency and run duration are modulated during a tactic response, resulting in a net migration toward favorable stimuli (33, 37). The mechanism by which the motor is stopped and restarted is unknown.

* Corresponding author. Phone: 1159-513234. Fax: 1159-513251. Electronic mail address: PLZRES@VAXA.CCC.Nottingham.AC.UK.

Our aim in studying the flagellar motor of *R. sphaeroides* is to compare and contrast the motor functions from unidirectionally and bidirectionally rotating flagella and to differentiate functions associated with rotation from those associated with stopping or switching. A previous study using transposon mutagenesis to identify genes involved in the *R. sphaeroides* flagellar motor resulted in the isolation and characterization of one paralyzed mutant, PARA1 (36). This mutant was found to assemble substantially intact flagella but was nonmotile. The wild-type gene which restores motility to PARA1 in *trans* was isolated on a 3-kb *Bgl*II-*Sal*I fragment (36). In this note, we present the sequence and analysis of the gene affected in PARA1.

Figure 1 illustrates a partial restriction map of the 2.4-kb *Sph*I-*Sal*I fragment that contains the *para1* gene. The *para1* gene has been shown to be transcribed in the *Sph*I-to-*Sal*I direction (36). Complementation analysis using various subclones in the broad-host-range vector pRK415-1 (20) revealed that the region between *Bst*EII and *Sal*I was sufficient to complement PARA1 but required read-through transcription from the *tet* promoter in the vector.

The nucleotide sequence of this 1.6-kb *Bst*EII-to-*Sal*I fragment containing *para1* was determined on both strands, using subclones and internal oligonucleotide primers. The sequencing reactions were carried out with the Sequenase kit (U.S. Biochemical). Deaza-G mixes were used, and the reactions were carried out at 42°C to alleviate some of the problems associated with sequencing GC-rich DNA. Analysis of the sequence with the Genejockey program (Biosoft, Cambridge, England) revealed only one long open reading frame (ORF) which spanned the site of transposon insertion in PARA1 (between positions 996 and 997). This ORF runs from positions 139 to 1137 on the *Bst*EII-*Sal*I fragment (Fig. 2, positions 13 to 1011). The codon usage in this ORF matches the high GC bias of *R. sphaeroides*. There is a putative ribosome binding site (GAGG) (Fig. 2, bases 2 to 5) which resembles sequences for ribosome binding sites in many bacteria (15, 22, 35, 41).

In the mutant strain PARA1, the Tn*phoA* has inserted such that the *phoA* gene is in frame with the *para1* gene, as indicated by the production of a fusion protein (36). Sequence primed from the transposon toward the 3′ end of *para1* showed that the transposon had inserted very near the end of the gene, deleting only four amino acids (RSPR) from the predicted coding sequence, which ends at TGA 1009 (Fig. 2). After this stop sequence, there is a potential stem-loop in the sequence running from positions 1115 to 1151 as indicated in Fig. 2.

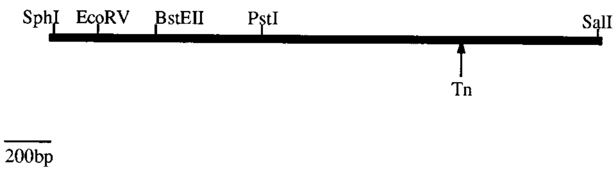

FIG. 1. Partial restriction map of the *Sph*I-*Sal*I fragment which contains the *motB* gene. The site of Tn*phoA* insertion in PARA1 is marked with an arrow.

The predicted polypeptide consists of 332 amino acids and has a predicted molecular weight of 35,318 and a pI of 9.55. It is rich in arginine, proline, and histidine. Searches using the database at the European Molecular Biology Laboratory (Heidelberg, Germany) and alignments using the BESTFIT and GAP programs from the University of Wisconsin Genetics Computer Group (UWGCG) (12) showed that the Para1 polypeptide is homologous to the MotB proteins encoded by *E. coli* (19.9% identity, 40.2% similarity) and *B. subtilis* (17.2% identity, 43.9% similarity) (32, 39). Para1 showed homology to a partial sequence of MotB from *Vibrio parahaemolyticus* (31) (data not shown), but only the first 200 amino acids of *Vibrio*

```
1    /   1                                      31   /   11
TGA GGC CAG GCC ATG TCC GCA AAG CCC AAG GTC ATC CGG TTC CAG CCG CCC GTC CCC GAC
 rbs            met ser ala lys pro lys val ile arg phe gln pro pro val pro asp
61   /   21                                     91   /   31
GAC GAC GAG GGC GAG GAC TGC CCG AAA TGT CCG CCC CCC GGC GCG CCG GCA TGG CTT GCG
asp asp glu gly glu asp cys pro lys cys pro pro pro gly ala pro ala trp leu ala
121  /   41                                     151  /   51
ACC TTT GCC GAC ATC GCG ACC AAC CTC ATG GCC TTC TTC GTG CTG ATC CTG GGC TTC GCG
thr phe ala asp ile ala thr asn leu met ala phe phe val leu ile leu gly phe ala
181  /   61                                     211  /   71
AAG TTC GAC GAG CCC TCG TTC AGC AAG ATG GCG GGG GCG ATG CGG GAG ACC TTC GGC TTC
lys phe asp glu pro ser phe ser lys met ala gly ala met arg glu thr phe gly phe
241  /   81                                     271  /   91
CAT TCG ATC CGG GAT GCG ACC TCG GGC AAC ACG ATG ATC GAC TTC GGC CTG CCC ACC GCC
his ser ile arg asp ala thr ser gly asn thr met ile asp phe gly leu pro thr ala
301  /  101                                     331  /  111
GAT CCC GAC GGG GCC CAG CCG GAC GAG AAG TCG GAC ACG GGC GGG TCG GAG GAC GGC GGC
asp pro asp gly ala gln pro asp glu lys ser asp thr gly gly ser glu asp gly gly
361  /  121                                     391  /  131
GAC GCG GCA GAG CGG GTG GCC GAG GCG CTG AAG AAG GCG CTC GAG GAC GGC AAG CTG CAG
asp ala ala glu arg val ala glu ala leu lys lys ala leu glu asp gly lys leu gln
421  /  141                                     451  /  151
GTG CGC TCG GAC GAG GGC GAG GTC GTG ATC GAG CTG TCG GGC GAG GAC GGG CGG CAG CAG
val arg ser asp glu gly glu val val ile glu leu ser gly glu asp gly arg gln gln
481  /  161                                     511  /  171
GCG CAG AGC CTC GCG CGG GCT CTG GCA GAG ACC GCG GGG CTT GGT CCG CTC CCC GAG CCG
ala gln ser leu ala arg ala leu ala glu thr ala gly leu gly pro leu pro glu pro
541  /  181                                     571  /  191
CAG ACC ACG TCC AGC CGC GGC CCG AGC CGA AGG TCG GGC CGT CGG GCC CCG GAG AGG GTC
gln thr thr ser ser arg gly pro ser arg arg ser gly arg arg ala pro glu arg val
601  /  201                                     631  /  211
ACG GGC GCC CGG GCC GCC CGT TGG CGG CGA CAC GGG CGC TGC GCT GCG CCA GTC GGT GCG
thr gly ala arg ala ala arg trp arg arg his gly arg cys ala ala pro val gly ala
661  /  221                                     691  /  231
GGC CGA ACT CGA TGC GCT CCG GCT GCG CAA TGC GCT CGA CCG CGA AGT GGC CGA GGG GCT
gly arg thr arg cys ala pro ala ala gln cys ala arg pro arg ser gly arg gly ala
721  /  241                                     751  /  251
GGT GAA GGT GGA GCA GAC CGA CGG CAA GGT GTT CGT GAG CCT CGG CGC GGG GCG GAT CCT
gly glu gly gly ala asp arg arg gln gly val arg glu pro arg arg gly ala asp pro
781  /  261                                     811  /  271
TCC CTC CGG CTC CGA CGA CCT CAC GCC CGA TGC GCG CGC GGT CAT GGC CCG GAT TGC CGA
ser leu arg leu arg arg pro his ala arg cys ala arg gly his gly pro asp cys arg
841  /  281                                     871  /  291
GGC CAC GCG CAA TCC CGA ACG CAC CAT CAC CGT GAC GGG TCA TAC CGA CAA TGT CCC GTG
gly his ala gln ser arg thr his his his arg asp gly ser tyr arg gln cys pro val
901  /  301                                     931  /  311
TGG GCG GCG CCT TCC GGG ACA ATA TCG CGC TCG CCG CCG GGC GCG CCG CAA GCG TGG TGC
trp ala ala pro ser gly thr ile ser arg ser pro pro gly ala pro gln ala trp cys
961  /  321                                     991  /  331
GCG AGC TTG TCG CCT CGG GCA GCG TCG ATC CCG GAC▼CGA TCA CCG CGG TGA GCC CGC GGC
ala ser leu ser pro arg ala ala ser ile pro asp arg ser pro arg OPA
1021 /  341                                     1051 /  351
GAG TTC GAC CCG GTG GCG GAC AAT GCA ACC GAG GAA TGG CCC GGG CCC AGA ACC GCC GGA

1081 /  361                                     1111 /  371
TCG AGA TCG AGA TTT CTA CAA GGA CTG ACT TGT CGC GCC GCA TTG CGA AGC CGT TCA CGG

1141 /  381
CGA TCC GCC GCA
```

FIG. 2. Nucleotide sequence and deduced amino acid sequence of the *motB* region of the *Sph*I-*Sal*I fragment, running from the putative ribosome binding site (rbs) to a potential stem-loop (inverted repeats of stem are underlined). The MotB ORF runs from position 13 to the TGA stop at 1009, and the transposon insertion site in PARA1 is marked with an arrowhead between positions 996 and 997.

```
motbe   MKNQAHPIIVVKRRKAKSHGA--------AHGSWKIAYADFMTAMMAFFLVMWLISISSPKE
motbb   M--------ARKKKKKHEDEH--------VDESWLVPYADILTLLLALFIVLYASSSIDAAK
motbr   MSAKPKVIRFQPPVPDDDEGEDCPKCPPPGAPAWLATFADIATNLMAFFVLILGFAKFDEPS
        #              ~                   ~#     ~##~  #  ~~#~#~~~~     ~
                                          <------------M------------>

motbe   LIQIAEYFRTPL--------ATAVTGGDRISNSESPIPGGGDDYTQSQGEVNK----QPNIE
motbb   FQMLSKSFNEVF--------TGGTGVLDYSSVTPPENESDGIDEVKKEKE-EK----EKNKK
motbr   FSKMAGAMRETFGFHSIRDATSGNTMIDFGLPTADPDGAQPDEKSDTGGSEDGGDAAERVAE
        ~  ~~     ~          ~ ~      #     ~    ~    ~         ~     ~

motbe   ELKKRMEQSRLRKLRGDLDQLIE-SDPKLRALRPHLKIDLVQEGLRIQIIDSQNRPMFRTGS
motbb   EKEKAADQEELENVKSQVEKFI-----KDKKLEHQLETKMTSEGLLITIKDS---IFFDSGK
motbr   ALKKALEDGKLQVRSDEGEVVIELSGEDGRQQAQSLARALAETAGLGPLPEPQTTSSRGPSR
           #   ~~   #       ~ ~   #          ~        #     ~     ~       ~ ~~        ~

motbe   ADVEPYMRDILRAIAPVLNGIPNR-ISLSGHTDDFPYASGEKGYSNWELSADRANASRRELM
motbb   ATIRKEDVPLAKEISNLLVINPPRNIIISGHTDNMPIKNSEFQ-SNWHLSVMRAVNFMGLLI
motbr   RSGRRAPERVTGARAARWRRHGRCAAPVGAGRTRCAPAAQCARPRSGRGAGEGGADRRQGVR
              ~     ~              ~~~       ~               ~     ~     ~         ~

motbe   VGGLDSGKVLRVVGMAATMRLSDRGPDDAVNRRISLLVLNKQAEQAILHENAESQNE--PVS
motbb   ENPKLDAKVFSAKGYGEYKPVASNKTAEGRSKNRRVEVL-------ILPRGAAETNE-----
motbr   EPRRGADPSLRLRRPHARCARGHGPDCRGHAQSRTHHHRDGSYRQCPVWAAPSGTISRSPPG
              ~          ~       ~  ~                    ~    ~

motbe   ALEKPEVAPQVSVPTMPSAEPR
motbb   ---------------------K
motbr   APQAWCASLSPRAASIPDRSPR
```

FIG. 3. ALIEN multiple sequence alignment of MotB sequences from *E. coli*, (motbe), *B. subtilis* (motbb), and the MotB ORF shown in Fig. 2 (motbr). #, identical residues; ~, conservative substitutions. Conserved residues in *E. coli* and *B. subtilis* proposed to be involved in peptidoglycan binding (10) are underlined. The extent of the putative membrane-spanning region is marked underneath the sequence with M & arrows. *E. coli* MotB residues found by Blair and coworkers (6) to give dominant nonmotile phenotypes when mutated are marked with triangles.

sequence were available for analysis. Given these homologies, we have designated the *R. sphaeroides* gene as *motB* rather than *para1*. The amino acid sequence is presented underneath the nucleotide sequence in Fig. 2. At the N terminus of MotB there is a region of 20 amino acids which has the characteristic positively charged N terminus and hydrophobic proline-containing central region of a signal sequence, but it is not predicted to be cleaved by signal peptidase when tested by the UWGCG Sigcleave program.

The SEQNET program ALIEN (developed by Alan J. Bleasby and based on the program described in reference 16) was used to align the predicted MotB polypeptide sequence from *R. sphaeroides* with MotB sequences from *E. coli* and *B. subtilis*, and as seen in Fig. 3, the overall similarity between these three sequences was limited. Optimal pairwise alignments between *E. coli* MotB (39) and *R. sphaeroides* MotB showed more clearly whether residues implicated as essential for motor rotation in *E. coli* MotB (6) were present in *R. sphaeroides* MotB (Fig. 3). Hydropathy profiles determined by using the Genejockey program by the method of Hopp and Woods (18) are very similar for the protein encoded by the *R. sphaeroides motB* gene and the MotB protein of *E. coli*. They both have a predicted single membrane-spanning helix near the N terminus, and the rest of the protein is mainly positively charged and hydrophilic (6, 8). In agreement with this finding, an in-frame fusion of the *phoA* ORF to the C terminus of the *motB* ORF in PARA1 was previously found to produce periplasmic alkaline phosphatase (36).

The difference between the flagellar system in *R. sphaeroides* and those in *E. coli*, *V. parahaemolyticus*, and *B. subtilis* is that the *R. sphaeroides* flagellum rotates unidirectionally, and motility proceeds in a series of stops and starts; stopping the flagellum must require some sort of "brake" or "clutch" (3). In the other bacteria, flagella switch between clockwise and counterclockwise rotation with a very short pause in rotation but with no marked stops (23). This difference in the properties of the flagellar systems may explain why these proteins do not have a high degree of sequence identity. Although the similarities between *R. sphaeroides* MotB and MotB proteins from *E.*

coli, *B. subtilis*, and *V. parahaemolyticus* were not extensive, their quality was judged to be significant by using the UWGCG GAP-random program. This tested the alignments between *R. sphaeroides* MotB and 25 randomized sequences generated from the amino acid content each of the MotB proteins from the other bacteria. The degree of similarity was by far the greatest when the original MotB sequences were aligned.

The highest similarity among all four proteins lies in the region from amino acids 34 to 55 on the *R. sphaeroides* sequence (Fig. 3). This is an area which corresponds to the putative membrane-spanning helix region in the MotB sequence of *R. sphaeroides* and the other three bacterial species.

There is a short consensus region W---(F/Y)AD------A-F (shown in Fig. 3) for all three MotB proteins within this helix; it is also found in *Vibrio* MotB. Blair and coworkers (6) have identified the adjacent alanine and aspartate residues (underlined above) and the third from last residue, alanine, in this consensus sequence as being required for motor function in *E. coli* (see also Fig. 3). Mutation of the alanine (underlined above) to threonine, the aspartate to asparagine, and the other alanine to valine gave rise to a dominant nonmotile phenotype. It is interesting that these residues are absolutely conserved in MotB of all four species, including the unidirectional flagellum of *R. sphaeroides*. They are not present in the single membrane-spanning region of MotY, a MotB-like component of the *V. parahaemolyticus* sodium-driven flagellar motor (30). These conserved MotB residues seem likely be involved in important interactions between MotB and other membrane-bound proteins, or they may be involved in forming part of the proton-conducting unit along with MotA.

In the other regions of MotB, there are a few isolated amino acid residues conserved in all four species but no pockets of extensive sequence identity among all of the species (Fig. 3). This finding suggests that these regions may be involved in the species-specific properties of MotB or that their specific sequences are not critical for MotB to function. Only one other functionally important residue (6) outside of the proposed membrane-spanning region, arginine 222 of *E. coli*, is conserved in *R. sphaeroides* (Arg-244) (Fig. 3).

Recently it has been proposed that MotB binds at or near MotA at its N terminus and that its periplasmic C terminus binds to the peptidoglycan wall of the cell (6, 8, 10). This model does not involve MotB in the generation of MS ring rotation, merely in provision of a structurally stable periplasmic environment in which the motor can rotate. This theory fits with the lack of substantial homology in the periplasmic region of MotB in all four species. It has been reported that MotB in *E. coli* has peptidoglycan-binding motifs that are also found in outer membrane proteins which are known to bind peptidoglycan (10). However, the amino acids thought to be responsible for peptidoglycan binding are conserved in *B. subtilis* but not in *R. sphaeroides* (Fig. 3, underlined). It is surprising that these amino acids should be conserved between a gram-positive and a gram-negative bacterium and not between two gram-negative bacteria, given the considerable cell wall structure differences between gram-positive and gram-negative bacteria. There are two possible alternatives to explain this lack of a peptidoglycan-binding region: (i) *R. sphaeroides* MotB may bind to a different component of the cell wall and therefore needs a different sequence; or (ii) there may be an additional protein which carries out the peptidoglycan-binding function, leaving MotB to have an alternative function. In light of the unidirectionality and stop-start action of the *R. sphaeroides* flagellum, MotB in this bacterium may act as a brake to stop the rotating flagellum. It could make contact with the MS ring or even with the rod. This would fit with the lack of overall homology between *R. sphaeroides* MotB and MotB from other species. There is much greater sequence identity between *E. coli* and *B. subtilis* (27.7% identity, 51.6% similarity) than either has with *R. sphaeroides*, which is consistent with the functional similarity (i.e., bidirectional, switching flagella) of flagellar rotation in these two species.

There is an unusual motif at the C terminus of MotB in *R. sphaeroides* involving a histidine residue at intervals of seven amino acids within an alpha-helical conformation (encoded from bases 802 to 865; Fig. 2). The sequence of this motif is HARCARGHGPDCRGHAQSRTHH.

Modeling studies predict that the histidine residues would lie on the same side of an alpha helix (data not shown); however, the proline residue near the center of the motif would disrupt a helix, giving a potential pincer-like structure. This motif has not been found in other proteins (including other MotB proteins), although there is a much longer series of histidine heptad repeated motifs in the heavy chain of dynein, which is the force-generating protein of eukaryotic cilia and flagella (14). In dynein, however, the repeats are almost perfect (HVIQYSIHVIQYSIHVIQYSTH, etc.) and very extensive, unlike those of MotB. The two proteins are also very different in size. Therefore, any similarity in function is unlikely. Dynein heavy chains make cross-bridges transmitting force between adjacent microtubules in linear motors. The role of MotB in the *R. sphaeroides* flagellar motor is uncertain. It may be involved in force generation or transmission, in which case it is interesting that it possesses a histidine repeat. However, as mentioned above, it may be involved in binding to an alternative component of the outer membrane, in which case the histidine repeat could provide a binding site. Alternatively, the histidine repeat could be involved in the stopping of the flagellum by providing a point of contact with the rotor and acting as a brake.

Further mutagenesis experiments are under way to determine the significance of the His repeat region and other amino acid residues of MotB. This should bring us nearer to an understanding of the role of MotB in flagellar rotation.

Nucleotide sequence accession number. The DNA sequence reported in this paper has been deposited in the EMBL database (accession number X85136).

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