Tryptophan-Scanning Mutagenesis of MotB, an Integral Membrane Protein Essential for Flagellar Rotation in *Escherichia coli*†

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ABSTRACT: The MotB protein of *Escherichia coli* is an essential component of the flagella that functions together with the MotA protein in transmembrane proton conduction. MotB has a single hydrophobic segment that spans the membrane. In order to determine which parts of the membrane-spanning segment can tolerate the introduction of a large, hydrophobic side chain, single Trp residues were substituted into many consecutive positions in the segment and the effects on function were measured. Trp residues were tolerated at positions near the periplasmic end of the MotB segment but not at positions near the cytoplasmic end. These results are different from what was seen in a similar mutational study of MotA, in that protein Trp residues were tolerated at positions that would be clustered together on one face of each hydrophobic segment if they are α-helices [Sharp, L. L., Zhou, J., & Blair, D. F. (1995) *Proc. Natl. Acad. Sci. U.S.A.* (in press)]. Those results suggested that the membrane-spanning segments of MotA are α-helices arranged in a bundle so that each has a face directed toward the lipid. The contrasting results seen with MotB indicate that its relationship to neighboring protein segments is different. Double-Trp substitutions, one each in MotA and MotB, also were studied. Many double substitutions had strongly synergistic effects which imply that the membrane segments of these proteins interact. Together, the results suggest a hypothesis for the structure of the MotA/MotB channel in which the membrane-spanning segment of MotB is associated with those of MotA but is tilted relative to them so that its cytoplasmic end is embedded in the complex and its periplasmic end is relatively exposed to the lipid.

Many species of bacteria swim using flagella, thin helical propellers driven by rotary motors in the cell membrane [for reviews of flagellar structure and function, see Macnab (1992), Blair (1995)]. Rotation of the motors is powered by the flow of ions across the cell membrane, protons in some species (Larsen et al., 1974; Belyakova et al., 1976; Manson et al., 1977; Matsuura et al., 1977) and sodium ions in others (Hirota & Imae, 1983). The present challenge is to understand the mechanism by which proton flow is coupled to rotation. Many (ca. 50) genes are needed for the assembly and operation of flagella (Macnab, 1992), but the products of only a few have been implicated in the process of torque generation (Yamaguchi et al., 1987; Block & Berg, 1984; Blair & Berg, 1988). Among the proteins needed for torque generation are MotA and MotB, which function together to conduct protons across the cytoplasmic membrane (Blair & Berg, 1990; Stolz & Berg, 1991).

MotA has four hydrophobic segments that could traverse the membrane, whereas MotB has only one (Dean et al., 1984; Stader et al., 1986). The membrane topologies of MotA and MotB suggested by their sequences are illustrated in Figure 1. The essential features of this model for MotA and MotB topology have been confirmed experimentally (Chun & Parkinson, 1988; Blair & Berg, 1991; Zhou et al., 1995). As a step toward understanding the molecular basis of proton conduction by the MotA/MotB channel, we have undertaken a study of the secondary structures and arrangement of the five hydrophobic, membrane-spanning segments. The structure of the MotA segments was probed by introducing Trp residues in many consecutive positions in each spanner and assessing the consequences for function (Sharp et al., 1995). In all four segments, Trp residues were tolerated (did not disrupt function) at positions that would be clustered together on one face if the segments are α-helices. Those results suggest that the membrane-spanning segments of MotA are α-helices, each with a face that is exposed to the lipid and can therefore tolerate the large but moderately hydrophobic Trp side chain.

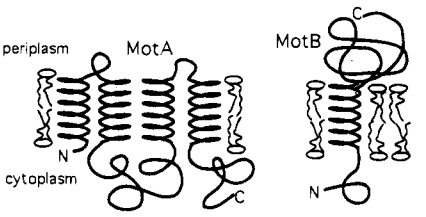

FIGURE 1: Membrane topology of the MotA and MotB proteins. Trp-scanning mutagenesis of the MotA segments indicated that they are α-helices, as pictured (Sharp et al., 1995). The amino-terminal segment of MotB is known to be in the membrane (Chun & Parkinson, 1988). It is also drawn as an α-helix because the number of hydrophobic residues (ca. 20) is appropriate for a helical membrane spanner.

Here, we report the effects of introducing Trp residues at many consecutive positions in the single hydrophobic segment of MotB. Given the length of this segment, in MotB from *Escherichia coli* and in homologs from several other bacterial species, it is likely to traverse the membrane in α-helical conformation. The pattern of Trp substitution effects was unlike that seen with MotA, however. Substitu-

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tions were tolerated at many positions near the periplasmic end of the segment and not tolerated at the cytoplasmic end. This suggests that the cytoplasmic end of the MotB membrane spanner is constrained by interactions with neighboring protein segments, whereas the periplasmic end is less constrained or makes contacts that are not functionally important.

Because MotA and MotB both appear to be involved in proton conduction (Stolz & Berg, 1991), their membrane-spanning segments might interact. To test that proposal, we examined a large number of double-Trp substitutions, one each in MotA and MotB, to see if any mutation pairs behaved synergistically. Of 120 double mutations examined, 22 showed strongly nonadditive behavior, abolishing function completely when present together even though each was tolerated singly. We conclude that the membrane-spanning segments of MotA and MotB interact. On the basis of these results, a hypothesis is suggested for the arrangement of the membrane segments in the MotA/MotB channel, in which the cytoplasmic end of the MotB segment is surrounded by MotA segments and the periplasmic end is more exposed to the lipid phase.

EXPERIMENTAL PROCEDURES

E. coli strain RP437 is wild-type for motility. Strain RP3087 is defective in *motB*, and strain RP6894 is defective in both *motA* and *motB*. Plasmid pGM1 (Boyd et al., 1982) encodes *motB* under control of the *lacUV5* promoter and confers ampicillin resistance, and was the source of the *motB* gene used in constructing other plasmids used here. Derivatives of pACYC184, encoding chloramphenicol resistance, were used when a compatible second plasmid was needed. Plasmid pLS6, used for site-directed mutagenesis, is a derivative of pAlter-1 (Promega, Madison, WI) that contains *motB*. Plasmid pLW3 (Wilson & Macnab, 1988) encodes *motA* and a part of *motB* behind the *trp* promoter and was used in assays of growth impairment caused by overproduction of MotA together with a fragment of MotB (Wilson & Macnab, 1988; Stolz & Berg, 1991). Plasmid pDFB45 contains the same promoter and *motA* sequences as pLW3 but also encodes full-length *motB*.

Culture media and growth conditions were as described elsewhere (Sharp et al., 1995). Manipulations of DNA, plasmid preparation, and transformation were according to standard procedures (Sambrook et al., 1989). Site-directed mutagenesis used the Altered Sites procedure (Promega) with plasmid pLS6. Mutations were verified by dideoxy sequencing (Sanger et al., 1977) of double-stranded plasmid DNA using reagents from Amersham (Arlington Heights, IL). To assay the function of mutant variants of *motB*, *Mlu*I-*Nsi*I fragments containing the mutant genes were subcloned from pLS6 into plasmid pGM1 and the resulting plasmids were transformed into RP3087. Motility of the resulting strains was assayed by measurements of swarming rates in soft agar, as described previously (Sharp et al., 1995). To assay codominance of the mutations, the mutant pGM1 derivatives were transformed into the wild-type strain RP437 and swarming rates were measured and compared to a control strain harboring wild-type *motB* on plasmid pGM1.

To examine the combined effects of pairs of mutations, one each in *motA* and *motB*, the *motB* mutations that permitted function were subcloned from pGM1 into pACYC184, using *Ssp*I and *Sal*I sites in the former and *Nru*I and *Sal*I sites in the latter plasmid. The resulting plasmids encoded the mutant variants of MotB and chloramphenicol resistance and were compatible with a second set of plasmids encoding mutant variants of MotA and ampicillin resistance. The *motA* mutations were encoded by derivatives of plasmid pLW3; all were single-Trp substitutions that permitted complete or partial function in motility assays, as described by Sharp et al. (1995). The *motA* and *motB* mutant plasmids were introduced in all pairwise combinations into strain RP6894, which has a chromosomal deletion of both *motA* and *motB*, and swarming rates of the resulting strains were measured. For these assays of motility, the *trp* operon inducer indoleacrylic acid (IAA) was omitted so that MotA was produced at levels only slightly elevated relative to the wild type (the MotB fragment encoded by pLW3 is not present at detectable levels under these conditions; Stolz & Berg, 1991), and growth was unaffected.

When pLW3 is induced with IAA, MotA is overexpressed and an amino-terminal fragment of MotB containing the membrane-spanning segment is also expressed at a low level (Stolz & Berg, 1991), causing the cytoplasmic membranes to become leaky to protons and reducing the growth rate by a factor of about 2 (Wilson & Macnab, 1988; Blair & Berg, 1990; Stolz & Berg, 1991). To examine the effects of the *motB* mutations on proton conduction as assayed by growth impairments, *Mlu*I-*Sca*I fragments of pLS6, containing the mutations, were ligated to an *Mlu*I-*Pvu*II fragment of pDFB45 containing *motA* and the fragment of *motB*. The resulting plasmids were identical to pLW3, encoding the same segment of MotB, except for the *motB* mutations. These were transformed into the wild-type strain RP437, and growth rates were measured in the presence or absence of IAA, as described previously (Blair & Berg, 1990).

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          31                  51
E.c.   ADFMTAMMAFFLVMWLISISS
B.s.   ADILTLLLALFIVLYASSSID
B.m.   ADLVTLILVFFILLFSMSSVD
V.p.   ADFMIALMALFLVLWVMQVVD
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FIGURE 2: Sequences of the hydrophobic segments of the MotB protein from *E. coli* and three other bacterial species. *E.c., E. coli; B.s., Bacillus subtilis; B.m., Bacillus megaterium; V.p., Vibrio parahaemolyticus*. The numbers correspond to the sequence from *E. coli*. Conserved residues are shown in boldface. The segment mutagenized in the present study is underlined in the *E. coli* sequence [from Stader et al. (1986), McCarter and Wright (1993), Mirel et al. (1992), and Hueck et al. (1994)].

RESULTS

The amino acid sequence of the hydrophobic segment of MotB from *E. coli* is shown in Figure 2, together with sequences of the corresponding part of MotB from three other bacterial species. All of the reported MotB sequences have a hydrophobic segment near their amino-termini, whose length (ca. 20 residues) is in the range expected for an α-helical membrane spanner. Using PhoA fusions and protease digestion of MotB in spheroplasts, Chun and Parkinson (1988) showed that the hydrophobic segment is inserted in the membrane, with carboxy-terminal sequences located in the periplasm.

Effect of Trp Substitutions on Function. The rationale for the Trp substitution experiment has been described (Sharp et al., 1995). Briefly, it is postulated that when Trp residues

Table 1: Properties of Mutant MotB Proteins

mutation	relative swarming rate[a] RP3087 (*motB*)	RP437 (wt)	mutation	relative swarming rate[a] RP3087 (*motB*)	RP437 (wt)
F33W	0.3	0.2	F41W	1.0	1.0
M34W	0.0	0.3	L42W	0.0	0.3
T35W	0.0	0.1	V43W	1.2	1.1
A36W	0.0	0.1	M44W	1.0	1.1
M37W	0.0	0.5	L46W	0.4	0.8
M38W	1.0	0.9	I47W	1.2	1.1
A39W	0.0	0.0	S48W	1.1	1.0
F40W	0.9	0.9			

[a] Swarming rates in soft agar were determined for the mutant MotB proteins expressed in either the motB-defective strain RP3087 or the wild-type strain RP437, as described in Experimental Procedures. Values are the average of three determinations, which differed by an average of 3% and no more than 15%. The *motB* mutations were expressed from plasmid pGM1. Rates are relative to corresponding strains expressing wild-type *motB* from pGM1.

are substituted in successive positions in a membrane-spanning segment, they should usually be tolerated at positions facing the lipid but should usually disrupt structure and function at positions that contact adjacent protein segments or point into the channel interior. To determine which positions in the hydrophobic segment of MotB can tolerate bulky substitutions, single Trp residues were introduced at 15 positions (consecutive except for position 45, already a Trp in the native protein). The mutant *motB* genes were subcloned onto plasmid pGM1 to allow expression of the MotB variants and transformed into the *motB*-defective strain RP3087. Motility was tested in a soft-agar swarming assay. The results are summarized in Table 1 and Figure 3.

Trp substitutions were tolerated at most positions near the periplasmic end of the MotB segment but not at positions near the cytoplasmic end. On a helical-wheel projection of the segment, positions that tolerated Trp were more common on one face but were not clustered exclusively there (Figure 3). This result is in contrast to what was seen in a similar study of MotA. In the hydrophobic segments of that protein, Trp was tolerated at positions that would be grouped together on one face if the spanners are α-helices (Sharp et al., 1995). On the basis of that result, it was suggested that the membrane-spanning segments of MotA are α-helices, each with a face that is directed toward the lipid phase and can accommodate bulky substitutions. The contrasting result with MotB implies that its relationship to other protein segments is different. As stated above, the length of the hydrophobic segment indicates that it is probably an α-helix. The pattern of Trp effects suggests that the cytoplasmic end of the helix makes functionally important contacts with other protein segments, whereas the periplasmic end is less constrained, either being surrounded by lipid or making contacts that are not important for function.

Codominance of the Mutations. It is possible that the bulky Trp substitutions disrupt function by interfering with protein folding or membrane insertion rather than by altering the structure of the folded, membrane-inserted channel. As a test of whether the nonfunctional mutant MotB proteins were present in the membrane and not grossly misfolded, they were expressed in a wild-type strain (RP437) to see if they interfered with motility. If a nonfunctional MotB protein can enter the membrane and form normal associations with other flagellar components, it should impair the motility

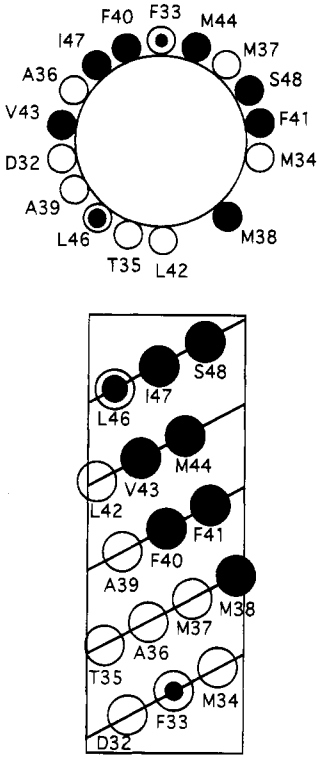

FIGURE 3: Effects of Trp substitutions in the hydrophobic segment of MotB. Top: Helical wheel projection. The mutated residues are indicated by circles, filled in proportion to the fraction of function that is retained when Trp is substituted there. Bottom: Helical net view of the same segment, with the function of the Trp-substituted variants indicated in the same way. Asp32 was not mutated in the present study but is included here in the nonfunctional category because an earlier, random mutagenesis showed that a less drastic change in this residue (to asparagine) abolishes function (Blair et al., 1991).

of the wild type to some degree (by taking the place of functional protein). Previously, most randomly generated *motB* mutations were found to impair motility in this assay. Exceptions were nonsense mutations in which a stable protein was not expressed (Blair et al., 1991). All of the MotB variants with impaired function interfered with motility when expressed in the wild type (Table 1), implying that they were present in the membrane and able to associate with some other motor component(s).

Other factors being equal, substitution of a Trp residue should be most disruptive if it replaces a small residue or causes a large change in side chain polarity. The effects of the Trp substitutions did not correlate with the changes they caused in side chain volume or polarity, however (plots not shown). The pattern of Trp substitution effects is therefore not a reflection of initial side chain sizes or polarities.

Synergistic Effects of MotA and MotB Mutations. Several kinds of evidence suggest that MotA and MotB interact. (1) Overexpressed MotB is unstable unless excess MotA also is present (Wilson & Macnab, 1990). (2) Transmembrane proton conduction as assayed by growth impairments requires both MotA and the amino-terminal, membrane-spanning part of MotB (Stolz & Berg, 1991). (3) Certain mutations in MotB can be phenotypically suppressed by mutations in MotA (Garza et al., 1995). Thus, the inability of the cytoplasmic end of the MotB segment to tolerate bulky substitutions might be due to contacts with surrounding segments of MotA.

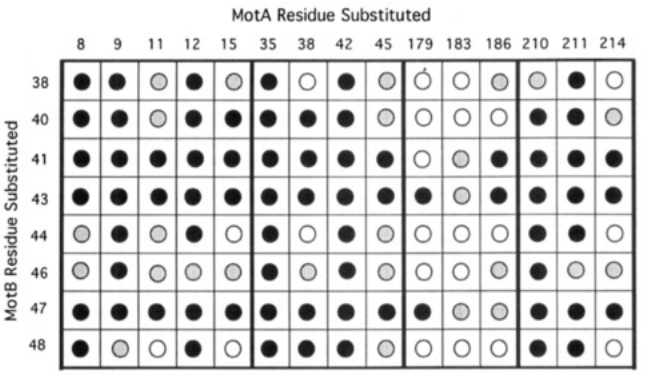

FIGURE 4: Synergistic effects of Trp substitutions in MotA and MotB. Each row corresponds to a different Trp substitution in MotB that by itself permits significant function; each column corresponds to a different Trp substitution in MotA that by itself permits significant function. Heavy vertical bars separate the four different membrane-spanning helices of MotA. Each circle represents a double mutant with one Trp substitution in MotA and one in MotB. The shading of the circles reflects the function of the double mutants. Filled circles indicate double mutants that function just as well as the more impaired of the two single mutants; shaded circles indicate double mutants that are significantly more impaired than either single mutant; open circles indicate double mutants that are completely nonmotile, thus exhibiting strong synergism.

If the membrane-spanning parts of MotA and MotB are in contact as suggested, then certain mutations in them might behave synergistically. Specifically, certain pairs of Trp substitutions in MotA and MotB could disrupt function, even though each substitution alone is tolerated. To test that proposal, MotA and MotB molecules containing single-Trp substitutions were tested for their ability to function together in various pairwise combinations. All of the Trp substitutions tested allowed significant function when present alone in an otherwise wild-type background. [The phenotypes of these and other *motA* mutations are described in detail in Sharp et al. (1995).] To test many combinations rapidly, the *motB* mutations were subcloned onto a different, compatible plasmid and transformed into a *motAB* double-deletion strain, together with plasmids harboring the *motA* mutations. Motility was tested in a soft agar swarming assay as before. The results for 120 different pairwise combinations of *motA* with *motB* mutations are summarized in Figure 4. Many pairs of mutations were identified that, although tolerated individually, abolished or significantly impaired function when present together. The strong synergistic action of the mutations suggests that the membrane-spanning parts of MotA and MotB interact and that this interaction is essential for function.

The synergistic effects of the mutation pairs could be due to direct interactions between close residues of MotA and MotB or might be mediated by more global effects on the structure or stability of the complex. Some of the *motA* mutations in hydrophobic segments 3 and 4 abolished function in combination with several, widely separated *motB* mutations (Figure 4). This was especially so for Trp substitutions at positions 179 and 183 in MotA; among the *motA* mutations studied here, these also showed the largest effects when present alone (Sharp et al., 1995). It does not appear possible for all of these residue pairs to be in close proximity, so these *motA* mutations must have some global effects on structure or stability.

Some of the mutations in hydrophobic segments 1 and 2 of MotA showed synergistic behavior that was more specific, each abolishing function in combination with only one or two of the *motB* mutations. These more specific effects might identify pairs of residues that are near each other. Data reported previously (Sharp et al., 1995) and discussed below suggest that segments 1 and 2 of MotA are those most likely to interact with MotB. In segments 1 and 2 of MotA, positions that gave strong synergistic effects were 11 and 15 (segment 1) and 38 (segment 2).

Growth Impairment. When MotA is overexpressed together with a fragment from the amino-terminus of MotB, growth is impaired (Wilson & Macnab, 1988; Stolz & Berg, 1991) because the cytoplasmic membrane becomes more permeable to protons (Blair & Berg, 1990). This effect does not require other components of the flagellum; thus, the ability of the channel to conduct protons across the membrane can be assayed independently of other activities that might be essential for motility, such as proper delivery of the protons to other components in the motor. To see if the Trp substitutions in MotB impeded transmembrane proton conduction, we tested their ability to impair growth in this overexpression assay. All of the *motB* mutations that abolished motility also abolished the growth impairment (data not shown).

DISCUSSION

Pattern of Trp Substitution Effects. The present study of MotB is the second time we have used systematic introduction of Trp residues to probe the structure of an integral membrane protein. In a study of the four hydrophobic segments of MotA, Trp substitutions had effects that varied periodically along the segments in the way expected if the segments are α-helices, each with a side that faces the lipid and can tolerate introduction of the bulky Trp side chain. The results obtained here with the hydrophobic segment of MotB are quite different and do not by themselves point to a particular secondary structure. The number of hydrophobic residues in the segment (ca. 20) suggests that it is an α-helix. Recent studies have shown that β-sheets might be more common in membrane-spanning domains than has been supposed, however [e.g., Hucho et al. (1994)], so this alternative must also be considered. It is known that all of MotB beyond residue 70 is in the periplasm (Chun & Parkinson, 1988). If the hydrophobic segment crossed the membrane twice, as it would be likely to do if folded into β-strands, then the amino-terminal domain would also be in the periplasm. The amino-terminal part of MotB is highly positively charged (ca. 10 positive charges in 30 residues; Stader et al., 1986), a signature of sequences that remain in the cytoplasm (Andersson & von Heijne, 1994). This alternative therefore appears unlikely.

Bulky substitutions were tolerated at many positions near the periplasmic end of the MotB segment but not at positions near the cytoplasmic end. This pattern suggests that the arrangement of the MotB segment relative to its neighbors is different from that of the MotA segments. A simple way to account for the pattern of substitution effects is to postulate that the MotB segment is associated with the segments of MotA but is tilted relative to them, with its cytoplasmic end deeply embedded in the complex. Substitutions near the cytoplasmic end could then interfere with channel function, while substitutions at the other, exposed end might have relatively little effect. This hypothesis is illustrated in Figure 5 (left).

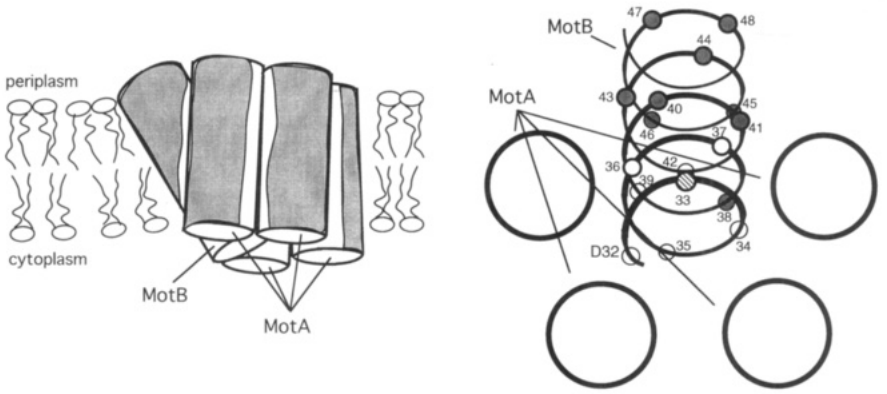

FIGURE 5: Hypothesis for the structure of the MotA/MotB channel. Only the membrane-spanning parts are shown; MotA has a large cytoplasmic domain and MotB a large periplasmic domain that are not pictured. Left: Overall arrangement of the membrane-spanning segments. In this hypothesis, the MotB segment is tilted relative to those of MotA, with its cytoplasmic end embedded in the complex. Right: View from the cytoplasmic end of the channel, showing the angular orientation of the MotB membrane-spanning segment that best accounts for the Trp substitution effects. The MotB segment, postulated to be a helix because of the number of hydrophobic residues it contains, is oriented to direct positions 33, 41, and 43 toward the outside and position 42 toward the inside, as discussed in the text. Residue positions are indicated by circles, which are shaded to reflect the degree of function retained when Trp is substituted there (cf. Table 1).

The hypothesized channel structure is quite asymmetric. This asymmetry might be expected given the function of the MotA/MotB channels, which are components in the flagella and occupy a peripheral position relative to other parts (Khan et al., 1988). In a number of models for the mechanism of torque generation [e.g., Meister et al. (1989), Blair (1990)], the MotA/MotB channels function to deliver protons to flagellar components on the rotor, near the cytoplasmic surface of the membrane. An asymmetric channel might reflect the need for lateral proton delivery to those components. Alternatively, the inward tilt of one segment might form a constriction at the cytoplasmic end of the channel that controls its conductance, possibly conferring specificity for protons.

The pattern of Trp effects in MotB does not argue for or against an α-helical structure, but if that conformation is assumed, the results can be used to guess the most probable angular orientation of the MotB segment relative to its neighbors. Residues 41−43 are near the middle of the segment, where positions that tolerate Trp are interspersed with positions that do not. Trp is tolerated in positions 41 and 43 but not at position 42, suggesting that the segment is oriented with residue 42 directed toward the center, as illustrated in Figure 5 (right). This arrangement can also account for the fact that Trp is tolerated, albeit imperfectly, at position 33 very near the cytoplasmic end. If position 42 pointed inward, then position 33 would point toward the lipid.

Synergistic Effects of Trp Substitutions. A remarkably large fraction of the Trp substitutions in MotA or MotB showed nonadditive behavior, being tolerated alone but completely abolishing function in combination with a second substitution in the other protein. Evidently, many of the Trp substitutions have a strong cryptic phenotype, causing strain or crowding in the structure which manifests itself in an increased sensitivity to further perturbations. Even side chains facing the lipid are not expected to be entirely unconstrained but will contact neighboring side chains to some extent. Thus, a bulky substitution in the lipid-facing part of a spanner could cause some movement in nearby side chains, and there should be a limit to the total increase in side chain volume that can be accommodated. In many cases, the MotA/MotB complex can tolerate one but not two indole side chains.

The Role of Asp32 of MotB. The hypothesized inward tilt of the MotB segment might, as suggested above, be a requirement for lateral proton delivery to a recipient near the cytoplasmic surface of the membrane. Near the cytoplasmic end of the MotB segment, and positioned in the hypothesized structure where its side chain could extend into the channel, is an aspartic acid residue (Asp32 in *E. coli*) that is conserved in all of the reported MotB sequences. This residue is essential; a mutation that changed it to Asn abolished function (Blair et al., 1991). We suggest that Asp32 might serve as the donor in proton transfer to a neighboring protein on the cytoplasmic side of the membrane. Alternatively, if the inward tilt of MotB forms a constriction needed to make the channel specific for protons, the Asp residue would be a good candidate for the group that confers proton specificity.

The present study extends the previous results with MotA, giving insight into the arrangement of membrane-spanning segments in the MotA/MotB proton channel and providing evidence for an interaction between the hydrophobic segments of MotA and MotB. The data do not yet allow the position of each MotA segment in the complex to be specified; thus the four MotA segments are not identified by name in Figure 5. Some data reported previously suggest that segments 1 and 2 of MotA are those most likely to flank the MotB segment (Sharp et al., 1995). Nonfunctional MotA variants with Trp substitutions in helices 3 and 4 were strongly dominant (impaired motility when expressed in the wild type), whereas those substituted in helices 1 and 2 were less so. In helix 2 in particular there are positions where Trp substitutions strongly disrupt function and allow a stable protein to accumulate in the membrane but are nevertheless totally recessive. Both the present results and previous studies (Chun & Parkinson, 1988; Blair et al., 1991; Garza et al., 1995) suggest that MotB is the point of attachment for MotA in the flagellar motor, so the dominance of the MotA variants should depend upon their ability to bind to MotB. The assays of dominance would then suggest that segments 1 and 2 of MotA interact most strongly with MotB. Consistent with this hypothesis is the observation of a few, specific synergistic effects of mutations in those segments of MotA in combination with certain mutations in MotB (Figure 4). We are presently carrying out experiments to

elucidate the arrangement of membrane-spanning segments in the channel.

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REFERENCES

Andersson, H., & von Heijne, G. (1994) *EMBO J. 13*, 2267−2272.
Belyakova, T. N., Glagolev, A. N., & Skulachev, V. P. (1976). *Biochemistry 41*, 1206−1210 (translated from *Biokhimiya 41*, 1478−1483).
Blair, D. F. (1990) *Semin. Cell Biol. 1*, 75−85.
Blair, D. F. (1995) *Annu. Rev. Microbiol.* (in press).
Blair, D. F., & Berg, H. C. (1988) *Science 242*, 1678−1681.
Blair, D. F., & Berg, H. C. (1990) *Cell 60*, 439−449.
Blair, D. F., & Berg, H. C. (1991) *J. Mol. Biol. 221*, 1433−1442.
Blair, D. F., Kim, D.-Y., & Berg, H. C. (1991) *J. Bacteriol. 179*, 4049−4055.
Block, S. M., & Berg, H. C. (1984) *Nature (London) 309*, 470−472.
Boyd, A., Mandel, V., & Simon, M. I. (1982) *Symp. Soc. Exp. Biol. 35*, 123−137.
Chun, S. Y., & Parkinson, J. S. (1988) *Science 239*, 276−278.
Dean, G. E., Macnab, R. M., Stader, J., Matsumura, P., & Burke, C. (1984) *J. Bacteriol. 159*, 991−999.
Garza, A. G., Harris-Haller, L. W., Stoebner, R. A., & Manson, M. D. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 1970−1974.
Hirota, N., & Imae, Y. (1983) *J. Biol. Chem. 258*, 10577−10581.
Hucho, F., Görne-Tschelnokow, U., & Strecker, A. (1994) *Trends Biochem. Sci. 19*, 383−387.
Hueck, C., Kraus, A., & Hillen, W. (1994) *Gene 143*, 147−148.
Khan, S., Dapice, M., & Reese, T. S. (1988) *J. Mol. Biol. 202*, 575−584.
Larsen, S. H., Adler, J., Gargus, J. J., & Hogg, R. W. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 1239−1243.
Macnab, R. (1992) *Annu. Rev. Genet. 26*, 129−156.
Manson, M. D., Tedesco, P., Berg, H. C., Harold, F. M., & van der Drift, C. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3060−3064.
Matsuura, S., Shioi, J., & Imae, Y. (1977) *FEBS Lett. 82*, 187−190.
McCarter, L. L., & Wright, M. E. (1993) *J. Bacteriol. 175*, 3361−3371.
Meister, M., Caplan, S. R., & Berg, H. C. (1989) *Biophys. J. 55*, 905−914.
Mirel, D. B., Lustre, V. M., & Chamberlin, M. J. (1992) *J. Bacteriol. 174*, 4197−4204.
Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989) in *Molecular cloning, a Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463−5467.
Sharp, L. L., Zhou, J., & Blair, D. B. (1995) *Proc. Natl. Acad. Sci. U.S.A.* (in press).
Stader, J., Matsumura, P., Vacante, D., Dean, G. E., & Macnab, R. M. (1986) *J. Bacteriol. 166*, 244−252.
Stolz, B., & Berg, H. C. (1991) *J. Bacteriol. 173*, 7033−7037.
Wilson, L. M., & Macnab, R. M. (1990) *J. Bacteriol. 172*, 3932−3939.
Wilson, M. L., & Macnab, R. M. (1988) *J. Bacteriol. 170*, 588−597.
Yamaguchi, S., Fujita, H., Ishihara, A., Aizawa, S.-I., & Macnab, R. M. (1987) *J. Bacteriol. 166*, 187−193.
Zhou, J., Fazzio, R., & Blair, D. F. (1995) *J. Mol. Biol.* (in press).

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