Isolation and Behavior of *Escherichia coli* Deletion Mutants Lacking Chemotaxis Functions

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Six *Escherichia coli* che loci (cheA,-B,-R,-W,-Y, and -Z) are located in two adjacent operons that map at minute 42 on the chromosome. Point mutants defective in any of these six functions have aberrant swimming patterns and are generally nonchemotactic. Deletions within the two major che gene operons were isolated in order to examine epistatic interactions among these genes. We first constructed a specialized transducing phage (λche22), which carries both of the che operons and their associated promoters. Deleted λche22 derivatives were selected by chelating agent inactivation, and these derivatives were characterized by mapping them against a series of host strains with point mutations. Representative nonpolar deletions were then transferred into the *E. coli* chromosome by homologous recombination. Although the phenotype of cheR mutants (smooth swimming) was expected to be epistatic to that of cheB mutants (tumbly swimming), we found that deletion mutants lacking both of these functions exhibited frequent directional changes or tumbling episodes as they swam. An examination of larger deletions indicated that both the cheA-cheW and cheY-cheZ functions were required for the anomalous tumbling behavior observed in these mutants. Loss of the cheB function was also correlated with an inverted behavioral response to sodium acetate, a strong repellent of wild-type cells. These findings indicate that an important component of the signal transducing machinery may be altered in cheB mutants.

Chemosensitive movements in *Escherichia coli* and other motile bacteria are carried out by modulation of the pattern of flagellar rotation of the organism (15). In the absence of chemical stimuli, such cells typically exhibit frequent flagellar reversals, which enable the organism to move about in a three-dimensional random walk (4, 5, 28). Counterclockwise (CCW) rotation produces smooth swimming, whereas clockwise (CW) rotation causes abrupt turns or tumbles. Upon encountering a change in attractant or repellent concentration, the cell alters the relative probability of these two rotational modes to bias swimming movements in the preferred direction (5, 6, 16). For example, an increase in the concentration of an attractant compound tends to decrease the probability of CW rotation and thereby lengthen the swimming paths of individuals when they are headed toward an attractant source. In static chemical environments, regardless of the absolute levels of attractants and repellents, bacteria undergo a process of sensory adaptation which restores swimming movements to the random walk pattern characteristic of the unstimulated state (6, 16, 34). The production of spontaneous flagellar reversals is therefore a critical component in the chemotactic behavior of bacteria; however, the machinery responsible for these events is still poorly understood at the molecular level.

Genetic studies of *E. coli* and *Salmonella typhimurium* have identified nine loci in which mutations can cause a very low rate of spontaneous flagellar reversals (2, 3, 17-19, 30, 36). Mutants with such mutations (designated che) have aberrant swimming patterns and consequently are unable to carry out chemotactic responses to any compounds. Six of these che genes are located in two adjacent operons that map at approximately minute 42 on the *E. coli* chromosome (18, 29) (Fig. 1). Mutations at these loci always produce a characteristic CW or CCW bias in flagellar rotation (Fig. 1), suggesting that these che functions are directly involved in generating or regulating flagellar reversals (20). The other three types of che mutants known in *E. coli* (cheC, cheD, and cheV) are relatively rare and appear to represent specific defects of the flaA, *tsr*, and flaB products, respectively (19, 20, 27).

Attempts to determine the biochemical roles of the six major che genes have been hampered by the lack of suitable null defects in these functions. Most of the available che mutants
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FIG. 1. Map of the major che loci. The arrows above the operons indicate the extent and direction of transcription. Each of the che loci has a characteristic mutant pattern of flagellar rotation.

have missense mutations, which only alter a single amino acid in the mutant protein. Consequently, many che mutants may have a considerable degree of residual function. Furthermore, suppression studies have provided evidence for protein-protein interactions among the che products and between che products and other components of the chemotaxis machinery (11, 18, 20, 22). Since each che product appears to participate in more than one functional interaction, it seems unlikely that missense mutations would be able to eliminate all interactions of this sort. Some che mutants containing nonsense mutations or transposon insertions have also been isolated (17; unpublished data). Although these sorts of mutations generally lead to null defects, they also produce polar effects on the expression of downstream genes in the same operon, which influence the resultant mutant phenotype.

In this report we describe the isolation of mutants with deletions in the two major che operons shown in Fig. 1. Many of these deletion mutants had no detectable polar properties and should prove valuable in ascribing functional roles to che gene products. Behavioral properties of some representative che deletion strains are described.

MATERIALS AND METHODS

Bacterial strains. The strains used were all derivatives of E. coli K-12, and most were directly related to strain RP437 (F− thi thr leu his met eda rpsL), which is wild type for chemotaxis (18). Strain RP446 is an RP437 derivative carrying a Tn10 insertion in the srIC (sorbitol) gene, which is located near the recA locus. Strain RP3098 is an eda+ derivative of RP446 that carries a deletion of the entire fla-flaH region (31), spanning the two major che operons shown in Fig. 1. Strain KL1699 is an Hfr strain that transfers the recA1 and srIC+ alleles as early markers. Strain RP442 is a P2 lysogen of strain 594 (37). The point mutations used in mapping and complementation studies are listed in Table 1.

Phage strains. Φla3Δ14 (30) was obtained from M. Silverman (University of California, San Diego) and carries a portion of the main che region, as shown in Fig. 2. Transductional crosses were done with phage P1kc as previously described (17).

Media and growth conditions. Tryptone broth, swarm agar, and H1 minimal salts medium have been described previously (17). Unless specifically indicated, all experiments were performed at 35°C on tryptone medium. Motility buffer contained 0.1 mM EDTA (potassium salt) plus 10 mM potassium phosphate at either pH 7 or pH 5.5. NZYM broth contained (per liter of water) 10 g of NZ amine A (Kraft, Inc., Memphis, Tenn.), 5 g of yeast extract, 5 g of NaCl, and 2 g of MgCl2.

Growth of phage stocks. All λ phages carrying che genetic material were grown on strain RP3098 to preclude changes in the che gene content arising from recombination with the che region of the host chromosome. Stocks were prepared either as plate lysates on tryptone medium or as liquid cultures in NZYM broth by a modification of the procedure described by Blattner et al. (7). In the latter method, phage were plated onto strain RP3098, and individual plaques (including a bit of the surrounding bacterial lawn) were picked with sterile capillary tubes and transferred to NZYM broth. As a general rule, one plaque was used for every 5 to 25 ml of broth, but this factor was not a critical one. Cultures were incubated at 37°C with vigorous shaking until lysis was complete (usually 8 to 12 h). We found that lysates could be incubated for as long as 24 h with little change in phage titers, although such cultures were usually turbid from growth of resistant cells. Titers of 1 × 1010 to 5 × 1010 phage per ml were routinely obtained with this procedure.

Mapping and complementation tests. In this work we used several different mapping techniques, most of which have been described previously (18, 31). In all of these methods host tester strains were infected with λche22 deletion mutants and examined for chemotaxis on tryptone swarm agar. Positive complementation was identified as a uniform spread of growth away

<table>
<thead>
<tr>
<th>TABLE 1. Point mutations used in mapping tests</th>
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<td>Gene</td>
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<td>cheZ</td>
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a Mutations were introduced into the strains indicated by cotransduction with the eda locus; the tsrI defect was transferred by cotransduction with the thr locus.

b A total of 57 mutant cheA alleles were used.

c A total of 21 mutant cheY alleles were used.
from the site of inoculation. Wild-type recombinants formed rapidly spreading swarms that originated from discrete sites on the periphery of the colonies. We did not attempt to quantitate the number of such recombinants; we only attempted to determine whether they could be formed.

The mutant host strains used in these tests were lysogenic for wild-type λ to minimize killing upon infection by the λche22 deletion phage to be mapped. The mutant alleles of these tester strains are listed in Table 1. The motA and motB mutations produce a nonmotile phenotype; the che mutations produce a generally nonchemotactic phenotype. Both sorts of defects can be distinguished readily from the wild type on tryptone swarm plates. Mapping studies of the tar locus proved to be more difficult because the loss of the tar function alone does not preclude chemotaxis on tryptone swarm agar (M. K. Slocum and J. S. Parkinson, manuscript in preparation). To circumvent this problem, we employed polar point mutations in the tar gene which produce a Che− phenotype due to loss of the cherBYZ functions. In addition, we transferred these mutations into a tsr mutant background because tar−tsr double mutants have been shown to be generally nonchemotactic on tryptone swarm agar (13, 32). This latter step was necessitated by the finding that λche22 deletion mutants in which the cherBYZ functions were expressed through transcriptional fusion to the motA promoter gave Che+ recombinants with all tar tester mutants, even though the tar locus had been deleted. These phage mutants were evidently able to restore chemotaxis, not by forming tar+ recombinants but rather by supplying the che functions missing in the polar tar tester strains. In the presence of a tsr defect, however, only recombinants with tsr function were able to carry out chemotaxis on tryptone swarm agar.

Initial tests were performed by spotting 50 μl of an overnight cell culture onto a tryptone swarm plate and then adding 50 μl of the λche22 deletion mutant (~10^10 phage per ml) to the cell spot. Plates were incubated at 35°C and scored after 15 to 18 h. More sensitive tests were done by preparing tryptone swarm agar containing ~5 × 10^4 particles of a λche22 deletion strain per ml. These plates were inoculated with colonies of host tester mutants and incubated overnight at 35°C before scoring. In some tests of this sort, the phage were first irradiated with 2,000 ergs of UV light per mm² to enhance recombination. In the final stage of mapping we used high-resolution tests to determine the relative position of a deletion endpoint within a particular gene. In these tests 10^8 UV-irradiated phage particles were mixed with an equal number of bacteria from an overnight culture in a total volume of about 0.2 ml. After an adsorption period of 15 min at 35°C, 0.05 to 0.1 ml of the infected cell sample was spread in a line across the surface of a tryptone swarm plate. The tests were scored after overnight incubation at 35°C.

**Analysis of swimming pattern and response to acetate stimulation.** Bacteria were grown in tryptone broth to a density of 2 × 10^8 cells per ml, pelleted, and suspended at the same concentration in motility buffer at pH 7 or 5.5. The unstimulated swimming patterns of the cells were evaluated by light microscopy, as previously described (17). Acetate stimulations were performed by adding 10 μl of 0.4 M sodium acetate to 10 μl of cells on a microscope slide and immediately observing the swimming pattern. Both the acetate and the cells were suspended in pH 5.5 motility buffer for these tests. Extended observations of swimming cells were done by sealing the test chamber with a cover glass, as previously described (17).

**RESULTS**

To obtain deletions within the main che region shown in Fig. 1, we first constructed a plaque-forming λ transducing phage that carried the entire motA-cheZ segment of the *E. coli* genome. Deletion derivatives of this phage were isolated and examined for loss of chemotaxis-related functions, and then deletions of interest were transferred into the *E. coli* chromosome for further analysis. The details of these steps are described below.

**Construction of λche22.** The construction of transducing phage that carry both of the main che operons is outlined in Fig. 2. First, strain RP446, which is wild type for chemotaxis, was lysogenized with λfla3Δ14. This phage contains an insertion of *E. coli* material covering the cheW-cheZ interval and can form lysogens by using this segment of homology to integrate within the che region of the host chromosome. To prevent recombinational loss of the λfla3Δ14 prophage, the lysogen was made recA by mating to strain KL1699 and selecting for streptomycin-resistant, sorbitol-positive recombinants and subsequently testing individual colonies for sensitivity to UV light. The resulting strain (strain RP497) was induced at 42°C to yield a phage lysate. Viable phage should have arisen only through aberrant excision events because neither the host cell nor the λ prophage were able to promote generalized recombination. To obtain phage that had picked up the entire motA-cheZ segment from the host, we plated the resulting
lysate on strain RP442, which is lysogenic for phage P2. This host only plaques phage that are red- gam- (i.e., Spi-), whereas Afla3Δ14 is red- but gam+. This enabled us to select for loss of the gam function of the Afla3Δ14 parent and thereby ensured that one of the excision breakpoints had taken place within the prophage genome (Fig. 2). The Spi- selection precluded the possibility of obtaining progeny phage with duplications of *E. coli* material, because Spi- phage should only incorporate host genes from one side of the prophage (Fig. 2). Moreover, loss of the gam locus should provide space for additional host material in the phage chromosome.

A total of 600 individual Spi- plaques from 12 independent lysates were transferred to lawns of a strain RP437 derivative carrying the cheA102 allele. This mutation falls outside the *E. coli* material present in Afla3Δ14 and therefore cannot form chemotactic recombinants with this phage. Cells from the plaque centers were transferred to swarm plates to identify phage that could either complement or recombine with the cheA102 tester. We found 14 phages (not necessarily all independent) that recombined with cheA102, but only 4 of these phages were able to complement this tester. The genetic contents of these four isolates were determined by complementation tests against a series of host mutants defective in various mot and che functions to confirm that they carried the entire motA-cheZ region. The DNA contents of the phages were estimated by measuring survival rates after incubation in 10 mM EDTA (pH 7.5) for 2 min at 37°C (21). One of the phages, designated λche22, which proved to have a DNA content close to that of wild-type λ, was used to isolate deletions within the main che region.

**Isolation of λche22 deletions.** Deletion mutants of λche22 were selected as plaque formers on tryptone plates containing 0.8 to 1.0 mM EDTA. Under these conditions, phage particles with a DNA content greater than about 97% of wild-type λ have a plating efficiency of $10^{-4}$ or less, whereas particles with less than this amount of DNA form plaques with essentially normal efficiency (21). Approximately 100 independent λche22 deletions were selected in this manner and subsequently examined for loss of che material by complementation and recombination tests with various host mutants. The results of these tests were consistent with the results of previous work on the genetic organization of the main che region (Fig. 3). We obtained a total of 74 λche22 derivatives that had at least one deletion endpoint within the main che region. The remainder of the strains tested either had no apparent deletion of che material or had lost the entire che region and were not studied further.

**FIG. 3.** Map of λche22 deletions. The positions of the deletion endpoints were determined by complementation and recombination tests against a series of host mutants. Each gene was subdivided into two or more deletion segments based on these results. The relative sizes of the genes reflect the sizes of their protein products, as measured on sodium dodecyl sulfate-containing polyacrylamide gels (30).

The major che genes are organized in two adjacent operons with the same transcriptional orientation (Fig. 3). The motA, motB, cheA, and cheW loci constitute one transcriptional unit, whose promoter is next to the motA gene (29). Two deletions (Δ25 and Δ81) appear to lack this promoter because they retain an intact cheW locus but cannot express the cheW function. The tar, cheR, cheB, cheY, and cheZ genes comprise a second transcriptional unit, whose promoter is adjacent to the tar locus (8, 18). Approximately one-half (34 of 74) of the λche22 deletions span the cheW-tar border and therefore must lack the promoter of the tar-cheZ operon. Many of these deletion mutants were able to express one or more of the promoter-distal genes in this operon at levels sufficient for complementation of chemotactic behavior. Presumably, these functions are expressed as a result of transcriptional fusion to the motA-cheW operon. In the absence of polar effects, operon fusions of this type would be expected to produce essentially normal amounts of gene product because the motA and tar promoters appear to have similar expression activities (30). Three deletion strains (Δ52,
Δ66, and Δ78) appeared to lack both of these promoters because they were unable to complement host mutants defective in the cheB, cheY, or cheZ functions even though the deletions did not enter the coding sequences of these genes. Since these complementation tests were performed with lysogenic host strains, this finding indicates that λche22 carries no promoters (other than the promoters adjacent to the motA and tar loci) that can be used for expression of che functions under conditions where lambda gene expression is repressed.

Deletions that produce out-of-frame gene fusions can exert polar effects on the expression of cotranscribed genes. Polarity causes a reduction in the level of expression of genes that are promoter distal to the deletion, but generally has no effect on genes of the same operon that lie upstream from the deletion. A number of the deletions shown in Fig. 3 exhibited only a partial ability to complement certain host mutants and appeared to be polar because only the expression of functions downstream from the deletion was affected. Based on comparisons of swarm sizes in the complementation tests, these polar effects did not seem to be severe; however, they could still involve significant reductions in gene expression because it is not clear how critical a factor che product stoichiometry is in establishing swarm size. For this reason only deletions with no detectable polar properties were chosen for transfer to the host chromosome.

Transfer of λche22 deletions to the E. coli chromosome. The procedure used to transfer deletions to the host chromosome required two homology-dependent exchanges, one on each side of the deletion (Fig. 4). Thus, only deletions that did not span either phage-bacterium junction in λche22 were suitable for transfer. The two exchanges were obtained in stepwise fashion by first constructing lysogens containing a deleted λche22 prophage and then selecting segregants that were no longer lysogenic. Since λche22 has no site-specific integration system, stable lysogens can be formed only by recombination between homologous bacterial sequences in λche22 and the host. The resulting prophage is flanked by regions of homology and can be excised by a second recombination event. If the integrative and excisive exchanges take place on opposite sides of the deletion, the cured host cell should carry the deletion in its chromosome (Fig. 4).

None of the genes of the main che region seems to be essential to the host, because E. coli deletion mutants, such as strain RP3098, lack this entire region and yet are perfectly viable. Therefore, bacterial strains carrying deletions within the che region were expected to have either a nonmotile phenotype or a nonchemotactic phenotype. Such mutants were obtained by inserting deleted λche22 prophages into strain RP437, which is wild type for chemotaxis, and then testing nonlysogenic segregants on swarm plates to identify ones that had acquired a chemotaxis defect. In general, four independent lysogens were constructed for each deletion to be transferred, and 40 cured derivatives of each were tested. The frequency of nonmotile or nonchemotactic segregants varied widely, not only from one deletion to another, but even for different lysogens of the same deletion phage. Putative deletion-bearing strains were then subjected to additional genetic tests to confirm that they did in fact have the expected genotype. First, linkage of the chemotaxis defect to the eda locus was examined by PI transduction. Genes in the main che region are 15 to 30% cotransducible with the eda locus, and all deletion mutants showed similar linkage values. Next, the deletions were transferred into RP437(λ +) by cotransduction with the eda locus. Finally, the resulting strains were subjected to complementation and mapping tests with a series of λche22 deletion mutants. In every case tested, the genetic properties of the E. coli mutants were identical to those of the λche22 deletions from which they were derived. Each host deletion derived from phage λ in this manner was assigned a four-digit allele number. The first two digits indicate the transducing phage used (in this case 22 for λche22), and the last two digits indicate the isolation number of that deletion. For example, the deletion carried by λche22 deletion isolate 3 (λche22A3) was designated Δ2203 when it was present in the E. coli chromosome. Some behavioral properties of these E. coli mutants are described below.

FIG. 4. Transfer of λche22 deletions to the host chromosome. See text for details.
Behavior of che deletion mutants. The swimming patterns of 10 different deletion mutants lacking one or more che functions are summarized in Table 2. One of these strains (containing Δ2211) lacked motA and motB functions and therefore was nonmotile. Seven other strains exhibited exclusively smooth swimming in the absence of chemotactic stimuli and presumably rotated their flagella only in the CCW sense. Of the two remaining strains, one (Δ2234) exhibited random motility similar to that of wild type whereas the other (Δ2241) displayed a high tumbling rate indicative of a CW bias in flagellar rotation. These last two mutants lacked both cheR and cheB functions, and their ability to tumble appeared to depend on several other che functions, because mutants with larger deletions that removed both cheR and cheB, as well as other che functions, exhibited smooth swimming behavior. For example, Δ2206 (lacking the cheRBYZ functions) and Δ2229 (lacking the cheAWRB and tar functions) did not tumble.

To determine whether any of the smooth swimmers were capable of CW flagellar rotation, each mutant was subjected to stimulation with the repellent acetate. Acetate and other fatty acid repellents can lower the internal pH of the cells and cause wild-type cells to tumble vigorously for many minutes (14, 24). Mutants lacking the cheW function (Δ2217) or the cheW and cheA functions (Δ2260) exhibited a brief but definite response to acetate stimuli. The strain carrying Δ216, which lacks cheA, cheW, and cheR functions, displayed a much longer acetate response. All three of these mutants also lack the tar function, which is not required for acute responses (14). Previous studies with less potent tumbling stimuli failed to elicit CW rotation in cheA or cheW point mutants, but our findings indicated that neither the cheA function nor the cheW function is absolutely essential for CW rotation. The enhanced CW response in the absence of the cheR function probably reflects a defect in sensory adaptation, which is known to be a cheR-dependent process (12, 23).

The other four smooth-swimming deletion strains showed no change in behavior when acetate was added. These strains may lack functions that are absolutely essential for CW flagellar rotation, but it is not possible to rule out an alternative explanation. Recent work has shown that cheB mutants have an inverted acetate response; these strains tumble before stimulation and then become smooth swimmers when acetate is added (14, 24). A similar response was observed in the two strains (Δ2234 and Δ2241) which lack both cheR and cheB functions, indicating that the inverted acetate response of cheB mutants does not require the cheR function. Since the four smooth-swimming deletion strains that showed no acetate response all lack the cheB function in addition to other che functions, it may be that the acetate response is actually inverted in these mutants and cannot be detected because they swim smoothly before stimulation. This explanation almost certainly applies to the Δ2229 strain because it closely resembles the Δ2216 strain, which does respond to acetate. The only difference between the two is that Δ2216 is cheB+, whereas Δ2229 is deleted for cheB. Studies of additional deletion types will be needed to resolve this point.

<table>
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<th>Deletion*</th>
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<th>motB</th>
<th>cheA</th>
<th>cheW</th>
<th>tar</th>
<th>cheR</th>
<th>cheB</th>
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<th>cheZ</th>
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* Deletions were transferred from λche22 to strain RP437 as outlined in Fig. 4.

* Unstimulated swimming patterns were evaluated in motility buffer at both pH 7 and pH 5.5; behavior was the same under both conditions.

* Brief responses to acetate stimulation lasted for less than 1 min; prolonged responses lasted for more than 5 min.

* WT, Wild type.
DISCUSSION

The methods which we employed to isolate che deletion mutants should be applicable in other systems, especially in cases where the phenotype of a particular deletion mutant cannot be predicted at the outset. The major drawback inherent in the technique is an inability to obtain small deletions (<1 to 2 kilobases long) due to the limited sensitivity of the chelating agent inactivation method for selecting phage deletions. Nearly all of the mutants isolated thus far have proven to lack more than one chemotaxis function. Such mutants are quite useful for exploring epistatic relationships among linked genes, but for most genetic and biochemical studies single-gene deletions would be preferable. We have been able to "reconstruct" single-gene mutants by using specialized transducing phage derivatives to supply all but one of the functions missing in extended deletion mutants (unpublished data). One particularly useful feature of the reconstruction approach is that, by using the appropriate phage and host deletions, strains lacking any desired combination of functions can be made readily. Thus, it is feasible to examine phenotypic interactions among any group of tightly linked nonessential genes where it is otherwise quite difficult to construct or recognize multiple mutants.

Aside from the inherent bias against the isolation of small deletions in our study, the deletions which we did obtain appear to have a fairly representative spectrum of endpoints, with one striking exception; 26 of the 74 deletions tested had one endpoint at the junction between the tar and cheR loci (Fig. 3). These deletions failed to recombine with all of the point mutations in either the tar or cheR gene, but left the other gene functionally intact. These results imply the existence of a large genetically silent region located between the tar and cheR loci. Recent genetic and physical analyses of the tar operon have in fact provided evidence for another chemotaxis-related gene in this region (8; Slocum and Parkinson, manuscript in preparation). Most of our "anomalous" deletions have now been shown to end at different positions within this new locus, which is comparable in size to the tar gene. Although the function of this new gene is not yet clear, it does not appear to influence the behavioral properties described in this report.

The preliminary deletion mutant studies described here reveal several interesting new properties of che functions. First, it appears that the inverse acetate response previously observed in cheR mutants is not dependent on many, if any, of the other che functions. It is conceivable that in the absence of the cheB function some component of the signaling or response machinery (for example, the flagellar motor or one of the methyl-accepting chemotaxis proteins [MCPs]) is present in an altered form that is responsible for mediating the inverse acetate response.

Second, the swimming pattern of deletion mutants lacking both the cheR and cheB functions (e.g., Δ2234 and Δ2241) is not consistent with the known roles of these two genes in chemotaxis. The cheR gene product is thought to be a methyltransferase that attaches methyl groups to several MCPs in the cell membrane (33). The cheB product is believed to be a methylesterase that removes MCP methyl groups (35). Together, these two functions are responsible for regulating the methylation state of the MCPs, which in turn seems to play an important role in modulating the reversal behavior of the flagella. Mutants defective in the cheR function have very low MCP methylation states and swim smoothly (12, 23), whereas cheB mutants have high MCP methylation states and tumble incessantly (9, 17). These findings imply that the MCP methylation state is instrumental in establishing the swimming pattern of a cell. Since cheR function is needed to obtain MCP methylation, ΔcheR ΔcheB mutants should also fail to methylate and should exhibit smooth swimming like cheR mutants. Instead, we observed that such strains tumble readily. In fact, Δ2241 causes very tumbly behavior characteristic of cheB mutants. We concluded that other factors associated with the loss of the cheB function are also important in controlling the spontaneous pattern of flagellar rotation.

Recent biochemical studies of MCP molecules synthesized in ΔcheR ΔcheB strains have demonstrated the existence of a previously unsuspected cheB-dependent chemical modification of these membrane proteins (25, 26). It seems likely that this modification could account for the anomalous swimming pattern of ΔcheR ΔcheB mutants and perhaps for the inverse acetate response as well. Additional behavioral studies of cheR and cheB deletion mutants will be described in a subsequent report.

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LITERATURE CITED


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