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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.

Chemotactic 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

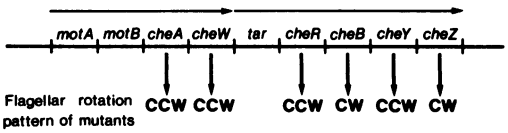

FIG. 1. Map of the major *che* loci. The arrows above the operons indicate the extent and direction of transcription. Each of the *che* loci has a characteristic mutant pattern of flagellar rotation.

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 Tn*10* insertion in the *srlC* (sorbitol) gene, which is located near the *recA* locus. Strain RP3098 is an *eda*$^+$ derivative of RP446 that carries a deletion of the entire *flaI-flaH* region (31), spanning the two major *che* operons shown in Fig. 1. Strain KL1699 is an Hfr strain that transfers the *recA1* and *srlC*$^+$ 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. λfla3Δ14 (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 $MgCl_2$.

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 1×10^{10} to 5×10^{10} 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 1. Point mutations used in mapping tests

Gene	Mutant alleles	Genetic background[a]	Reference
motA	*515*	RP446 *eda*$^+$ (λ)	1
motB	*580*	RP446 *eda*$^+$ (λ)	1
cheA	—[b]	RP446 *eda*$^+$ (λ)	18
cheW	*110, 113, 143, 144, 166, 173, 175*	RP446 *eda*$^+$ (λ)	18
tar	*226, 229, 235, 252, 253, 256*	RP437 *eda*$^+$ *thr*$^+$ (λ) *tsr-1*	18
cheR	*202, 203, 210, 213, 217, 239, 242, 243, 249, 251, 258, 260, 267, 268*	RP437 *eda*$^+$ (λ)	18
cheB	*270, 271, 272, 273, 274, 275, 276, 277, 283, 285, 287, 290, 294, 295*	RP437 *eda*$^+$ (λ)	18
cheY	—[c]	RP437 *eda*$^+$ (λ)	18
cheZ	*278, 280, 281, 286, 292, 293, 296*	RP437 *eda*$^+$ (λ)	18

[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.

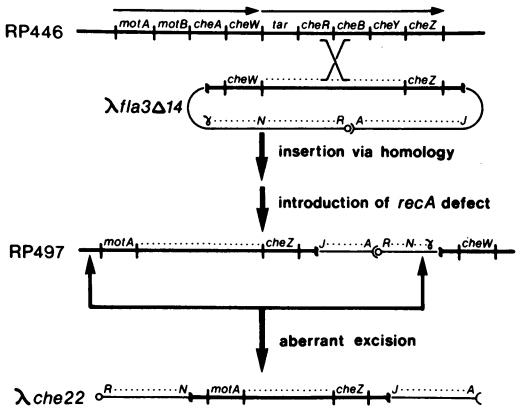

FIG. 2. Summary of the construction of λche22. See text for details.

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 *tar* 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 ($\sim 10^{10}$ 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 $\sim 5 \times 10^8$ 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 mm^2 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 10^9 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 λfla3Δ14 is *red*$^-$ but *gam*$^+$. This enabled us to select for loss of the *gam* function of the λfla3Δ14 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 λfla3Δ14 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.

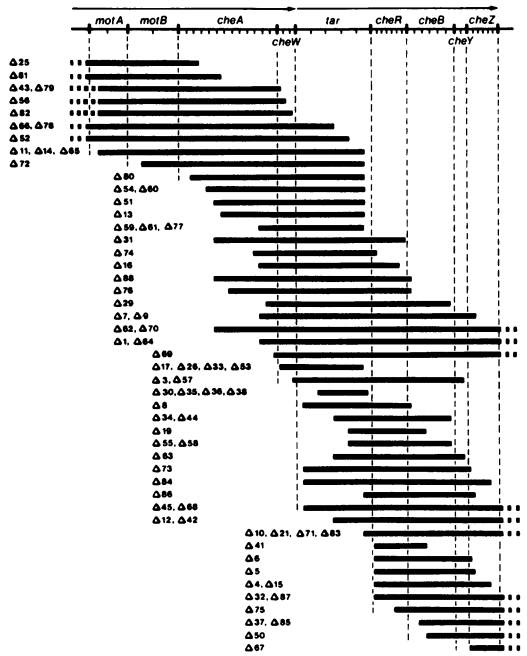

FIG. 3. Map of λche22 deletions. The positions of the deletion endpoints were determined by complementation and recombination tests against a series of host mutants. Each gene was subdivided into two or more deletion segments based on these results. The relative sizes of the genes reflect the sizes of 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 (Δ*25* and Δ*81*) 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 (Δ*52*,

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).

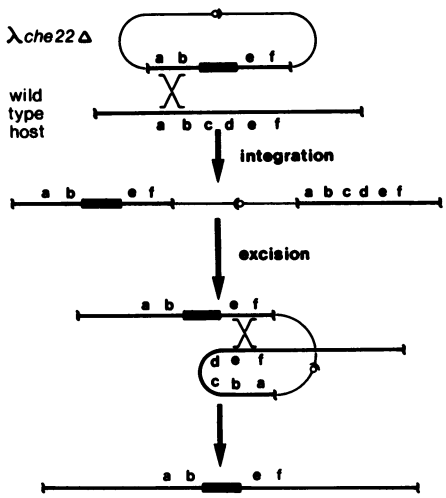

FIG. 4. Transfer of λche22 deletions to the host chromosome. See text for details.

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 P1 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 (λche22Δ3) was designated *Δ2203* when it was present in the *E. coli* chromosome. Some behavioral properties of these *E. coli* mutants are described below.

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 *Δ2216*, 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 acetate 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 2. Swimming behavior of *che* deletion strains

Strain[a]	Deletion[a]	Functions									Swimming pattern	
		motA	*motB*	*cheA*	*cheW*	*tar*	*cheR*	*cheB*	*cheY*	*cheZ*	Unstimulated[b]	Acetate stimulated[c]
RP437	WT[d]	±	±	±	±	±	±	±	±	±	Random	Tumbly (prolonged)
RP5045	*Δ2211*	−	−	−	−	−	+	+	+	+	Nonmotile	Nonmotile
RP1076	*Δ2203*	+	+	+	−	−	−	−	−	+	Smooth	Smooth
RP1091	*Δ2209*	+	+	−	−	−	−	−	−	−	Smooth	Smooth
RP2893	*Δ2206*	+	+	+	+	+	−	−	−	−	Smooth	Smooth
RP5046	*Δ2229*	+	+	−	−	−	−	−	+	+	Smooth	Smooth
RP1078	*Δ2217*	+	+	+	−	−	+	+	+	+	Smooth	Tumbly (brief)
RP2898	*Δ2260*	+	+	−	−	−	+	+	+	+	Smooth	Tumbly (brief)
RP2894	*Δ2216*	+	+	−	−	−	−	+	+	+	Smooth	Tumbly (prolonged)
RP2867	*Δ2241*	+	+	+	+	+	−	−	+	+	Tumbly	Smooth (brief)
RP2896	*Δ2234*	+	+	+	+	−	−	−	+	+	Random	Smooth (brief)

[a] Deletions were transferred from λche22 to strain RP437 as outlined in Fig. 4.

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

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

[d] 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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