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cheA, *cheB*, and *cheC* Genes of *Escherichia coli* and Their Role in Chemotaxis

JOHN S. PARKINSON

Department of Biology, University of Utah, Salt Lake City, Utah 84112

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Motile but generally nonchemotactic (*che*) mutants of *Escherichia coli* were isolated by a simple screening method. A total of 172 independent mutants were examined, and four genes were defined on the basis of mapping and complementation studies. The role of three of these genes, *cheA*, *cheB*, and *cheC*, was investigated by determining their null phenotypes with nonsense or bacteriophage Mu-induced mutations. The *cheA* and *cheB* products were essential in producing changes of swimming direction and flagellar rotation. The *cheC* product appeared to be an essential component of the flagellum; however, specific mutational alterations of this component allowed flagellar assembly but prevented directional changes in swimming. Since some *cheB* mutants changed directions incessantly, this gene product may also serve to control the direction of flagellar rotation in response to chemoreceptor signals. Thus most or all of the common elements in the signalling process were involved in the generation and regulation of changes in the direction of flagellar rotation.

The chemotatic behavior of *Escherichia coli* and other bacteria parallels in several major respects the more complex sensory behavior of eukaryotic organisms and is therefore viewed as a useful system for investigating the genetic and biochemical bases of chemoreception and sensory transduction. Adler (2) has shown that *E. coli* possess specific chemoreceptors that detect gradients of attractant or repellent chemicals. After detection by the chemoreceptors, information about the chemical environment is then transmitted to flagella, the motor organelles, to effect changes in swimming behavior that will promote movement toward attractants or away from repellents.

In the absence of chemical stimuli, the swimming pattern of *E. coli* approximates a random walk (10). Periods of smooth, translational swimming are punctuated by brief tumbling episodes that result in random directional changes. Chemotaxis involves modulation of the spontaneous tumbling pattern in response to changes in the concentration of attractants or repellents in the environment (10, 13, 20, 31). For example, an increasing attractant concentration causes a decrease in tumble probability, which in a spatial gradient would cause net movement of the population toward the attractant. Thus chemical stimuli in the form of temporal concentration changes are detected by chemoreceptors, which in turn produce signals that modulate the tumbling machinery of the flagella. The nature of these signals, how they are transmitted and how they control tumbling, is not understood.

The pathways of information flow from receptors to flagella can be analyzed through genetic dissection of the chemotaxis machinery. Mutants that are motile but generally nonchemotactic should define common elements of the communication system. Such mutants (designated *che*) have been described in *E. coli* (6) and in *Salmonella* (7, 33; A. Tsui and B. Stocker, personal communication). This report describes the properties of 172 newly isolated *che* mutants of *E. coli*. All of these mutants exhibit aberrant tumbling behavior, either failing to tumble (smooth swimmers) or tumbling incessantly (tumbly swimmers). This implies that common elements of the communication system are components of the tumbling machinery. To better understand the role of these components in chemotaxis, the null phenotype of each *che* gene was determined. Evidence will be presented that the defective phenotypes of *che* mutants can result from specific functional alterations as well as from the loss of the normal gene product.

MATERIALS AND METHODS

Strains. The bacterial strains used in this work are all derivatives of *E. coli* K-12 (Table 1). Strains RP420, RP461, RP470, RP477, and RP4770 are essentially isogenic except for the differences indicated.

TABLE 1. *Bacterial strains*

Strain	Sex	Relevant genotype[a]	Comments
RP120	F⁻	*recA aroD his pro*	KL159 of B. Low; repository strain for F′ elements
RP252	F⁻	*his trp(am)*	Used for *che* mapping studies
RP259	F⁻	*recA his trp(am) pro*	Repository strain for F′ elements
RP420	F⁻	*thr(am) leu his met(am) gal Δ eda strA*	Used for *che* mapping studies
RP461	F⁻	*thr(am) leu his gal Δ strA*	*rec*⁺ parent for *che* isolations[b]
RP470	F⁻	*thr(am) leu his gal Δ recA strA*	*recA* parent for *che* isolations
RP477	F⁻	*thr(am) leu his gal Δ eda strA*	Repository strain for *che* mutations
RP4770	F⁻	*thr(am) leu gal Δ eda hag3110 strA*	Straight-flagella strain[c]
PK25	Hfr	*thr leu;* see Fig. 1	From P. Kahn (16) via E. Kort
KL96	Hfr	*thi rel;* see Fig. 1	From B. Low (19) via E. Kort
F-prime elements			
F14 *supU*		See Fig. 1	*supU* donor; from L. Soll (27) via I. Herskowitz
F188-2		See Fig. 1	*supD* donor[d]
F410		See Fig. 2	MSF1334 of M. Silverman (24)
F410-1		F410 *cheC497*	From M. Silverman (25)
F420		See Fig. 2	MSF1338 of M. Silverman (24)

[a] Genotype designations are given in Taylor and Trotter (30). Known nonsense mutations are designated *(am)* for amber.

[b] RP461 was derived from AW574 of S. Larsen (18) by constructing a λJam6xis1cI857 lysogen and selecting Gal⁻ colonies at 42 C.

[c] The *hag* mutation in strain W3110 that causes straight flagella (33; M. Silverman, Ph.D. thesis, Univ. of California at San Diego, 1972) was transduced into RP477 by cotransduction with *his*⁺.

[d] F188-2 was constructed in several steps by crossing KL96 to RP470 with selection for *his*⁺*[strA]*. F′ *his*⁺ were verified by crossing to RP259, and a nonmucoid derivative (F188-1) was kept. *Trp*⁺ revertants were selected and mated to RP470 for suppression tests with λ and T4 amber mutants. An F′ *his*⁺ *sup* isolate (F188-2) whose suppression spectrum was the same as *supD* was retained for use as a *supD* donor.

Reference *che* mutations used in this work were *cheA593*, *cheB590*, and *cheC497* of Armstrong and Adler (4).

Genetic markers relevant to the present work are shown in Fig. 1. The notation of amber suppressors follows the current convention (30). For example, *supD*⁻ is the suppressing allele and *supD*⁺ is the wild-type or nonsuppressing allele.

Media. Tryptone broth contains 1% tryptone (Difco) and 0.5% NaCl. Tryptone swarm plates contain tryptone broth and 0.35% agar (standard swarm plates) or 0.5% agar (miniswarm plates). Minimal salts medium (H1) has been described (3). To this medium were added carbon and energy sources (25 mM), required amino acids (1 mM), and vitamin B1 (1 μg/ml). Motility buffer (KEP) contains potassium phosphate (pH 7, 10 mM) and potassium ethylenediaminetetraacetate (0.1 mM). Minimal swarm agar contains potassium phosphate (pH 7, 10 mM), $MgSO_4$ (1 mM), $(NH_4)_2SO_4$ (1 mM), vitamin B1 (1 μg/ml), carbon and energy sources and required amino acids (0.1 to 0.5 mM), and either 0.25% agar (standard swarm plates) or 0.5% agar (miniswarm plates).

Mutagenesis. Bacteria grown in tryptone broth at 35 C to about 1×10^9/ml were washed and resuspended at 2×10^9/ml in H1 buffer containing 3.5% ethyl methane sulfonate. After 1 h of incubation at room temperature, survival was greater than 90% for *rec*⁺ strains and about 1% for *recA* strains. The treated cells were washed twice with 5 volumes of H1 buffer and resuspended in H1 glucose medium at 1×10^6 viable cells/ml for overnight growth at 35 C to allow for segregation and expression of new mutations. Under these conditions, the mutation frequency at several loci that were tested increased about 1,000-fold. Mutagenesis with phage Mu-1 (29) essentially followed a described procedure (12).

Isolation of chemotaxis mutants. Approximately 150 mutagenized bacteria were mixed with 10 ml of miniswarm agar and poured into an empty petri dish. The plates were incubated at 35 C and examined for *che* mutants after 24 h (tryptone) or 36 h (minimal). Mutant candidates were transferred by sterile toothpick to standard tryptone swarm plates at 35 C for verification and then cloned twice before further characterization. Mutants were isolated from strains RP461 and RP470 and were assumed to

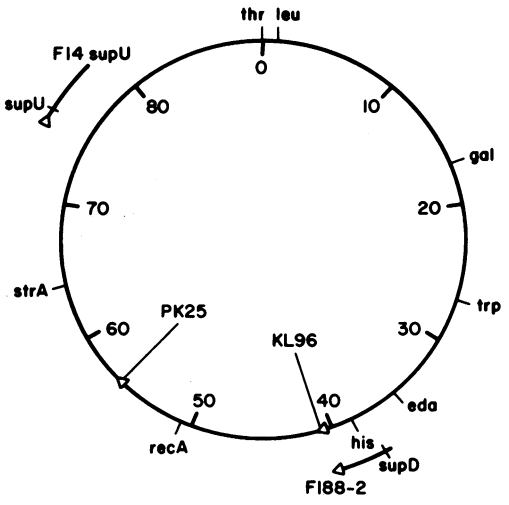

FIG. 1. *Genetic map of E. coli (30). Loci, F-primes, and Hfr origins relevant to the present study are shown.*

be independent if they came from separately mutagenized cultures. Allele numbers were assigned after completion of the complementation anlayses and swimming pattern characterizations. Numbers 101 to 172 are *cheA* mutations; 201 to 268 are *cheB* (smooth) mutations; 271 to 296 are *cheB* (tumbly) mutations; and 181 to 183 are *cheC* mutations.

Characterization of new mutants. (i) Swimming pattern and motility. A subjective evaluation of swimming behavior was made by examining log-phase cultures in the light microscope as described previously (21). Each mutant was also tested for sensitivity to the flagellotropic phage χ (23) by a described method (6).

(ii) Suppressor tests for amber mutants. Each *che* isolate was tested for suppressibility by F188-2, which carries *supD*, and by F14 *supU*. Neither of these F-primes carries any known *che* genes. F188-2 was introduced by plate mating F188-2/RP259 by RP461 and RP470 *che* strains with selection for *his*$^+$ [*pro*$^+$]. F14 *supU* was introduced by plate mating F14 *supU*/RP259 by RP461 and RP470 *che* strains with selection for Thr$^+$ [*pro*$^+$] [the *thr(am)* allele in RP461 and its derivatives is suppressible by *supU* but not by *supD*]. Recombinants were transferred to the appropriately supplemented minimal swarm plates to score chemotaxis in the presence of the amber suppressors.

(iii) Capillary assay for chemotaxis. This procedure has been described in detail by Adler (3). My assays were performed for 60 min at 32 C at a cell density of 0.01 optical density unit at 590 nm.

(iv) Analysis of flagellar rotation. Strains to be tested were cloned on tryptone plates at 35 C, and a single colony was picked into H1 glycerol medium for overnight growth at 35 C. The glycerol culture was diluted 500-fold into H1 glucose medium and grown for 5.5 to 7 h at 35 C to an optical density at 590 nm of 0.3 to 0.6. The bacteria were washed twice with equal volumes of KEP at room temperature and resuspended in KEP at an optical density of 0.25 to 0.3. A 0.1-ml sample of cells was mixed with 3 μl of antiflagellar serum (1:1,000 dilution in KEP) (a gift of S. Larsen), and 20-μl drops were placed on a clean microscope slide. Sample drying was prevented by placing a hollow circular spacer (0.5 mm thick) around the sample and sealing with a cover slip. Each rotating cell was observed for 10 to 15 s and its behavior was recorded.

(v) Temporal stimulation. Cultures were prepared and subjected to threefold increases in aspartate or serine concentration as previously described (21). The durations of the smooth swimming responses were measured by direct observation in the light microscope.

Complementation tests. Complementation for chemotaxis was measured by constructing partial diploids (F'*che1*/*che2*) and assaying chemotactic ability on tryptone swarm plates. Two important features of these tests should be mentioned. First, to prevent recombination between donor and recipient *che* alleles, tests were always performed in the *recA* strain RP470. Second, diploids for the *his-cheC* region are very mucoid (24; J. S. Parkinson, unpublished observations), and this interferes with motility. Since *E. coli* exopolysaccharide contains galactose (15), its synthesis can be abolished by *galE* mutations, hence the *gal*Δ marker in RP461 and its derivatives.

A series of F420 derivatives bearing *che* alleles was prepared and tested in the following manner. F420 was introduced into a series of RP461 *che* mutants, each partial diploid was cloned on minimal medium, and individual colonies were tested for chemotaxis on tryptone swarm plates. Che$^-$ colonies were found with a frequency of about 1% and were due either to loss of the episome or to deletion of the episomal *che*$^+$ allele or to homogenote formation by recombination of *che* alleles between endogenote and exogenote. Events of the first type were discarded by transferring each F-prime (if there was one) to a repository *recA* strain, RP120. The second possibility, deletion of F-prime material, was checked by crossing each RP120/F' strain to representative *recA che* mutants of Armstrong and Adler (4) to determine the *che* defect of the F-prime by complementation. Each episome was then crossed to *rec*$^+$ *che* mutants to look for recombinational rescue of *che*$^+$ alleles from the defective gene. A final test was performed on episomes that should have carried suppressible *che* alleles by constructing F'*che(am)*/*che recA* strains that failed to complement and in which the endogenote *che* allele was not suppressible. These strains were lysogenized with ϕ80*supF* (22) and tested for chemotaxis. Only if the F-prime carried the *che(am)* allele would these strains regain chemotaxis upon introduction of an amber suppressor.

The F' *che* testers in the RP120 background were plate mated to each PR470 *che* strain with selection for *his*$^+$ [*pro*$^+$ *aroD*$^+$]. Recombinants were transferred by sterile toothpick to tryptone swarm plates containing 200 μg of streptomycin per ml to kill any

surviving donor cells. The diameter of the resulting swarms was measured after 16 to 18 h at 35 C.

Genetic methods. Plate matings were used to introduce F-prime elements carrying nonsense suppressors or *che* mutations into recipient *che* strains. An overnight culture of the donor strain in H1 glucose medium was streaked across an appropriately supplemented minimal plate. After drying, the plate was cross-streaked with recipient strains and incubated at 35 C. Recombinant growth was transferred directly to swarm plates for chemotaxis tests. No attempt was made to remove parental cells since growth of the donor was prevented by either nutritional requirements (RP259/F′ strains) or by streptomycin (RP120/F′ strains) and the recipient parent was in all cases nonchemotactic.

Liquid matings were performed at 35 C with tryptone-grown log-phase cultures mixed in a ratio of 1 male:20 females.

Transductions were performed with phage P1*kc* at an input multiplicity of 0.1 to 0.3. Transductants were selected on plates containing 10 mM sodium citrate to inhibit phage growth and checked for P1 lysogeny by spot tests with P1 (for immunity) and λ (for P1 restriction). Only nonlysogenic transductants were saved.

Construction of double mutants with straight flagella. *cheB* mutations were introduced into the straight-flagella strain RP4770 by cotransduction with the *eda* locus. In each cross, 20 *eda*$^+$ transductants were cloned on tryptone plates and then crossed to F410/RP120 in liquid matings. After 2 to 3 h of mating time at 35 C, a sample of the mating mixture was spotted on tryptone swarm plates containing streptomycin. Double mutants containing a *cheB* mutation appear Che$^-$ because the *hag* defect is corrected by F410 but the *che* defect is not. RP4770 parental types appear wild type. Transductants that were not complemented by F410 were tested further by transductional backcrosses to RP477 to confirm the presence of the *che* and *hag* alleles. These double mutants appear nonmotile on swarm agar but remain sensitive to χ phage.

RESULTS

Isolation of generally nonchemotactic mutants. Chemotactic ability can be assessed on semisolid nutrient agar swarm plates on which wild-type bacteria form large colonies by generating and then following attractant gradients leading outward from the colony origin (1). Nonchemotactic or nonmotile strains cannot follow such gradients and produce small dense colonies (6). Since nonchemotactic bacteria are still motile, their colonies, due to random swimming, are slightly larger and more diffuse than those of nonmotile bacteria (6). Thus wild-type, nonchemotactic, and nonmotile strains can be distinguished from one another on swarm agar. These differences in colony morphology were used to devise a screening method that would permit identification of rare nonchemotactic individuals in a wild-type culture. The crucial feature of this miniswarm technique is that each swarm represents a clone of bacteria descended from one individual, and therefore the size of the colony is a direct indicator of chemotaxis genotype.

Approximately 1,000 bacteria from each of 290 independently mutagenized wild-type cultures were screened on miniswarm plates for *che* mutants. About 50% of the potential *che* isolates were discarded after examination of swarm plate and swimming behavior and sensitivity to phage χ, a flagellotropic phage. A total of 172 mutants were kept for further study, yielding a *che* frequency of approximately 6×10^{-4} after mutagenesis. It is unlikely that any of these isolates are of spontaneous origin because no *che* mutants were found in control screenings of more than 10^5 unmutagenized cells. Moreover, the mutagenesis procedure used in this work produces about 1,000-fold increase in mutation frequency at several different loci in *E. coli*.

Some of the new *che* mutants were obtained from the same culture; however, subsequent analysis always turned up differences that proved such isolates were not identical. Each new *che* mutant therefore represents an independent mutational event. Twenty-five of the mutants appeared to be amber mutations since they became chemotactic when mated to donor F-prime strains carrying amber suppressors on the episome. It seems unlikely, therefore, that very many of the new mutants contain more than a single mutation affecting chemotaxis.

The swimming patterns of the new *che* mutants were aberrant; none had a wild-type pattern. Most of the isolates (146/172) were smooth swimmers and never seemed to tumble. The other mutants (26/172) were tumbly swimmers and had very high tumbling rates.

Mapping and complementation. Armstrong and Adler, in their study of *che* mutants, classified 38 isolates into three genes, *cheA*, *cheB*, and *cheC* (4), which mapped near the *uvrC* locus in the *E. coli* chromosome (5). A map of this region is shown in Fig. 2. The approximate map location of each new mutant was determined by complementation tests with two F-prime elements carrying portions of this region (see Fig. 2).

Only three of the 172 mutants were not complemented by F420, which carries the *cheA*, *cheB*, and *cheC* genes. These three mutants define a new gene, *cheD*, located near the *thr* locus, which will be the subject of a separate report (Parkinson, in preparation).

Three of the mutants corrected by F420 were also complemented by F410, a shorter episome bearing only the *his-uvrC* segment. These

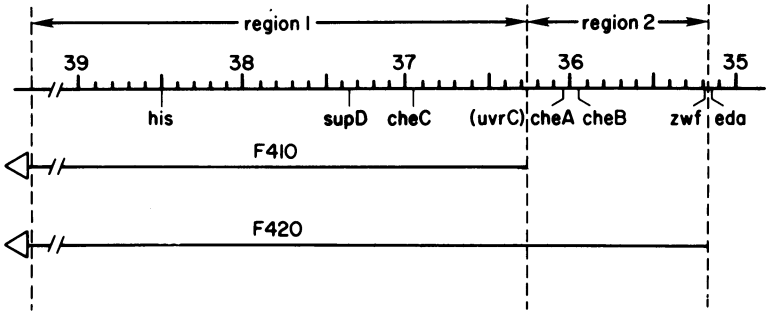

FIG. 2. *his-eda region of the E. coli genome. The his locus was assigned a position at 38.5 min, and the supD, cheC, cheA, cheB, and eda positions were determined from the transduction data of Table 2. The approximate location of the uvrC locus is based on the data of others (5, 24). The zwf locus was not mapped in the present study, but is known to be very tightly linked and to the clockwise side of eda (14). The region is subdivided by two episomes: F410, which carries the his-uvrC segment; and F420, which carries the his-zwf segment. F420 does not carry a functional eda locus.*

three mutations must lie in region 1 of Fig. 2 and the other 166 mutants must lie in region 2, between *uvrC* and *zwf*.

Region 1 mutants. The three region 1 mutants were smooth swimmers and appeared to be partially dominant in completion tests with F410 or F420. The *cheC497* mutation described by Armstrong and Adler also maps in region 1 and has similar properties (4, 5). Complementation analysis of these mutants can be carried out because their dominance is incomplete. Silverman and Simon prepared a derivative of F410 carrying the *cheC497* mutation and showed that it did not complement two of the new region 1 *che* mutants (25). This result has been confirmed and also extended to the third new *che* isolate in region 1 (data not shown). Thus the three new region 1 *che* mutants appear to be *cheC* mutations. Additional support for this conclusion is provided by the phenotypic characterizations discussed in subsequent sections.

The linkage of *cheC497* and the new *cheC* mutants to the *his*, *supD* and *eda* loci was measured in P1 transductional crosses with appropriately marked *cheC* derivatives. The *cheC* strains were first made his^+ by conjugation with Hfr PK25, and his^+ [*strA cheC*] recombinants were selected. In this way the new *cheC* mutants, which were isolated in RP470, a *recA* strain, could be made his^+ since PK25 transfers $recA^+$ as an early marker (see Fig. 1). P1 grown on the his^+ *cheC* recombinants was used to prepare *cheC* derivatives of RP252 and RP477 by cotransduction with his^+. Subsequently, another RP252 *cheC* derivative was constructed by cotransduction of *cheC* and *supD*, retaining the his^- marker. These three types of *cheC* strains, $supD^+$, eda^-, his^-, respectively, were infected with P1 grown on the appropriate $cheC^+$ donors, giving the results summarized in Table 2. All four mutants exhibited about 1% cotransduction with *his* and about 47% cotransduction with *supD*. There were no significant differences between the various *cheC* alleles. No linkage to *eda* was detected in slightly more than 2,000 transductants examined.

Region 2 mutants. Almost all of the new *che* mutants mapped in region 2 between the *uvrC* and *zwf* loci (Fig. 2). Armstrong and Adler (4, 5) have described two genes, *cheA* and *cheB*, that map in this portion of the genome. A series of F420 derivatives was prepared by introducing new region 2 mutations onto F420 and using these F-primes to complement representative *cheA* and *cheB* mutants of Armstrong and Adler. These tester F-primes were then used to complement all the new *che* mutants in region 2 (Fig. 3). Each mutant was fully complemented by either *cheA* or *cheB* testers and failed to complement at least some testers of one of these genes. Thus all the new region 2 mutants appeared to be *cheA* or *cheB* mutants; however, there were numerous instances of complementation between mutants assigned to the same gene. This is believed to be intragenic complementation for two reasons. First, such complementation was generally weak, but was never weak between mutants assigned to different genes (Fig. 3). Second, there were mutants in each gene (designated A_0 and B_0) that did not complement any other members of the same gene. These mutants could be explained as extreme polar mutations; however, they were not extensive deletions since they reverted with normal frequency. It is also important to note that apparent intragenic comple-

TABLE 2. *Linkage of che genes to the his, supD, and eda loci*

Map interval	Donor marker		Total colonies scored	Cotransduction frequency[b] (%)	Map distance[c] (min)
	Selected	Unselected[a]			
his-supD	*his*$^+$	*supD*$^-$	851	8.6	1.15
	supD$^-$	*his*$^+$	1,136	6.4	
his-cheC	*his*$^+$	*cheC*$^+$ (4)	2,360	1.1 (±0.2)	1.55
supD-cheC	*supD*$^-$	*cheC*$^+$ (4)	1,092	46.8 (±4.0)	0.44
supD-cheA	*supD*$^-$	*cheA*$^+$ (3)	468	5.1 (±0.6)	1.28
	supD$^-$	*cheA*$^-$ (3)	624	4.0 (±0.6)	
supD-cheB	*supD*$^-$	*cheB*$^+$ (4)	728	2.7 (±0.2)	1.39
	supD$^-$	*cheB*$^-$ (4)	832	2.6 (±0.6)	
eda-cheC	*eda*$^+$	*cheC*$^+$ (4)	2,072	<0.25[d]	>1.72
eda-cheA	*eda*$^+$	*cheA*$^+$ (3)	1,092	15.4 (±1.2)	0.92
eda-cheB	*eda*$^+$	*cheB*$^+$ (4)	2,028	28.3 (±3.5)	0.68

[a] The following *che* mutations were used: *cheA 102, 114, 117; cheB 202, 216* (smooth swimmers); *cheB 281, 287* (tumbly swimmers); *cheC 181, 182, 183, 497*. The number of different *che* alleles used in each type of cross is indicated by the value in parentheses.

[b] Where several alleles of the same gene were studied, the cotransduction frequency is the average of the individual values. The standard deviation of these mean values is indicated in parentheses.

[c] Distances were computed with the mapping function of Wu (34) in which map distance (in minutes) = 2[1-(cotransduction frequency)$^{1/3}$]. Where two cotransduction frequencies are given, the map distance of an interval is the average of the separate distances.

[d] No cotransductants were found. If cotransductants were distributed randomly among the sample analyzed, the probability is less than 0.01 that the actual cotransduction frequency is as great as 0.25%.

mentation could take place when one or both of the complementing mutants were amber mutations.

All the *cheA* mutants were smooth swimmers (see Fig. 3). Three of these mutations were mapped by P1 transduction by preparing appropriate derivatives in a way similar to that described for *cheC* above (Table 2). The *cheA* mutations showed approximately 5% cotransduction with *supD* and 15% with *eda*. There were no significant differences between the three mutations examined. The linkage to *his* was not measured; however, the present results and those of Armstrong and Adler (5) indicate that no cotransduction of *cheA* with *his* can be expected.

Whereas 30% of *cheA* isolates were amber, very few *cheB* strains were amber mutants (Fig. 3). The basis of this difference is not known. Another major difference between *cheA* and *cheB* mutants was in their swimming behavior. Two swimming patterns were found among the *cheB* mutants: about 75% of the isolates were smooth swimmers, but the remainder were tumbly swimmers. The tumbly *cheB* mutants showed extensive complementation with smooth *cheB* strains; however, the overall complementation pattern indicates that both types of mutants are most likely defective in the same gene (Fig. 3). Moreover, the tumbly mutants could be divided into two subclasses (designated B_7 and B_8) based on their patterns of intragenic complementation. This distinction proves to be a useful one because these mutants differed in other respects as well, which will be described in later sections.

Two smooth *cheB* mutants and one tumbly mutant of each subclass were mapped by P1 transduction (Table 2). There were no significant differences among the four alleles tested. The mutations showed about 2.5% cotransduction with *supD* and about 28% with the *eda* locus.

Phenotypic characterizations. Several representatives of each *che* type were chosen for further study. The mutations were transduced into RP477 to provide a common genetic background in which to compare the effects of the various mutant alleles. Since the *che* mutations were introduced by cotransduction with either *his*$^+$ or *eda*$^+$, the effect of these markers on the chemotactic behavior of RP477 was first investigated. As expected, neither of these loci had a detectable effect on any of the parameters under study. In the following sections, therefore, comparisons are made with RP477 for simplicity even though it differs from its *che* derivatives at two loci. The *che* mutations used are listed in Table 3.

(i) Chemotactic behavior. The chemotactic behavior of the *che* mutants was first evaluated qualitatively on different types of swarm

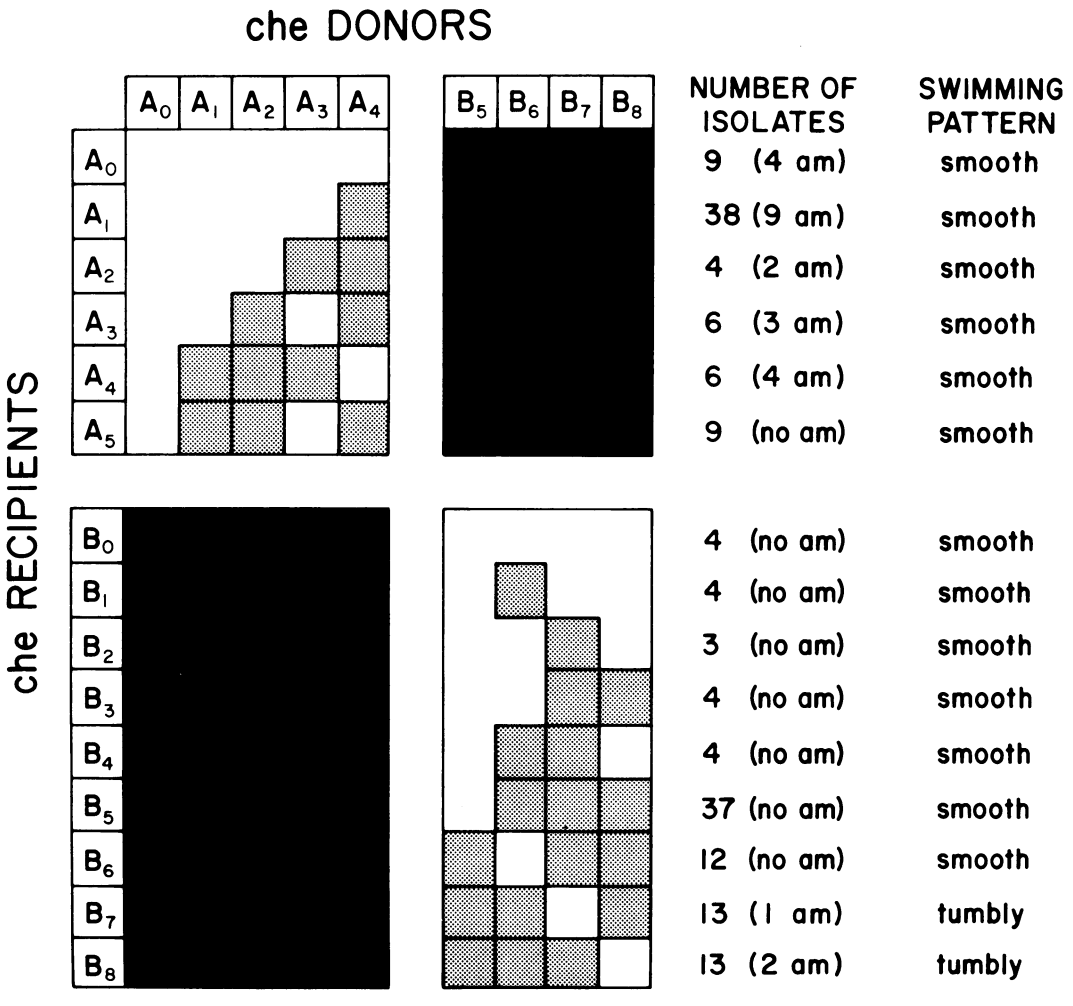

FIG. 3. *Complementation patterns of cheA and cheB mutants. The solid black areas indicate full complementation, and the shaded portions represent weak or partial complementation. The donor strains used were F420 derivatives carrying the following che alleles: A116(am), A117 (A_0); A101 (A_1); A114 (A_2); A115(am) (A_3); A113(am) (A_4); B201, B219, B220 (B_5); B202, B203 (B_6); B274, B275 (B_7); B280, B281 (B_8).*

plates. The attractants aspartate, serine, maltose, and ribose, which are detected by different chemoreceptors (2), were tested in minimal swarm agar. The *cheA* and *cheB* mutants formed small, dense colonies on all swarm plates, indicative of a generalized loss of chemotaxis. The *cheC* mutants formed slightly larger colonies that often had a discrete ring of cells at the periphery. This implies that the *cheC* mutants have a general but somewhat leaky defect in chemotaxis.

The residual chemotaxis in *cheC* mutants could be quantitated in the capillary assay described by Adler (3). The attractants aspartate, serine, and maltose were used. Mutants in *cheA* or *cheB* accumulated to the same extent in attractant-filled or buffer-filled (control) capillaries, again indicating a complete loss of chemotaxis in these mutants (data not shown). The *cheC* strains, however, exhibited responses on the order of 5 to 15% of wild type (Fig. 4). The responses were not due to a high level of revertants in *cheC* cultures because the bacteria that entered an attractant-filled capillary still behaved like typical *cheC* mutants when grown and retested. The leakiness was therefore due to phenotypic rather than genetic variability in *cheC* strains.

The responses of *cheC* mutants were not only less efficient than wild type, but the position of maximal accumulation in the mutants was shifted to somewhat lower attractant concen-

TABLE 3. *Flagellar rotation in che mutants*

Mutation (class)[a]	% of cells rotating:[b]			
	ccw only	ccw but reversing	cw but reversing	cw only
Smooth swimmers				
cheA105 (A_5)	98	2	0	0
cheA115 (A_3)	100	0	0	0
cheA121 (A_1)	96	0	0	4
cheB202 (B_6)	99	0	0	1
cheB216 (B_5)	97	0	0	3
cheB229 (B_0)	100	0	0	0
cheC181	91	9	0	0
cheC182	83	17	0	0
cheC183	89	11	0	0
cheC497	88	12	0	0
Tumbly swimmers				
cheB274 (B_7)	14	13	22	51
cheB276 (B_7)	4	10	23	63
cheB277 (B_7)	0	7	42	51
cheB287 (B_7)	5	16	26	53
cheB294 (B_7)	2.8 ± 2.2	12.5 ± 6.9	34.2 ± 7.7	50.5 ± 8.3
B_7 average[c]	5.2 ± 5.3	11.7 ± 3.4	29.4 ± 8.5	53.7 ± 5.3
cheB278 (B_8)	0	3	26	71
cheB280 (B_8)	4	6	13	77
cheB281 (B_8)	1	1	12	86
cheB286 (B_8)	7	3	9	81
cheB292 (B_8)	8	2	11	79
cheB296 (B_8)	2	4	11	83
B_8 average[c]	3.7 ± 3.3	3.2 ± 1.7	13.7 ± 6.2	79.5 ± 5.2
Controls				
Wild type[d]	9	76	15	0
hag3110[d]	5	86	9	0
hag3110 cheB216	100	0	0	0
hag3110 cheB280	2	9	10	79

[a] Mutant alleles were transduced into RP477.

[b] Bacteria were grown and tethered as described in the text. Each rotating cell was observed for 10 to 15 s and assigned to one of the four categories listed. At least 100 individuals were scored for each strain.

[c] The mean and standard deviation are given.

[d] Reversal rate is too high in these strains to classify them by inspection. Each cell was timed for 30 s with two stopwatches to determine the proportions of cw and ccw time. The wild-type data are based on 93 measurements; the *hag3110* data are based on 77 measurements.

trations. This suggests that the chemotactic machinery in the responding individuals is still partially defective because it becomes saturated at lower concentrations than in wild type.

The day-to-day variability in *cheC* responses was about 50% compared with 15 to 20% for wild type. Even so, the responses to different attractants did not change in relation to one another. For example, the responses of *cheC183* to serine and aspartate when compared with those of wild type were always more efficient than the response to maltose regardless of variability in the absolute responses. The response of *cheC182* was most efficient to maltose. The fact that the extent of leakiness of a mutant was not constant for the different attractants implies that the altered *cheC* product was responding better to some signals than to others.

(ii) Flagellar rotation. *E. coli* propel themselves by rotating their flagellar filaments (9, 26). Rotation can be observed by tethering cells with only one flagellum to a microscope slide by means of antibodies directed against the flagellar filament (8, 18, 26). The sense of flagellar rotation is defined as that viewed along the filament axis toward the cell body and corresponds to the direction of rotation of a tethered cell body seen from above. Tethered wild-type cells can rotate in both directions and exhibit frequent reversals in direction (8, 18, 26). Experiments with both wild-type and *che* mutants have shown that counterclockwise (ccw) rotation corresponds to smooth swimming and clockwise (cw) rotation to tumbling (18).

A series of representative *che* mutants were examined by the cell-tethering method to determine their patterns of flagellar rotation (Table 3). Each rotating cell in a preparation was clas-

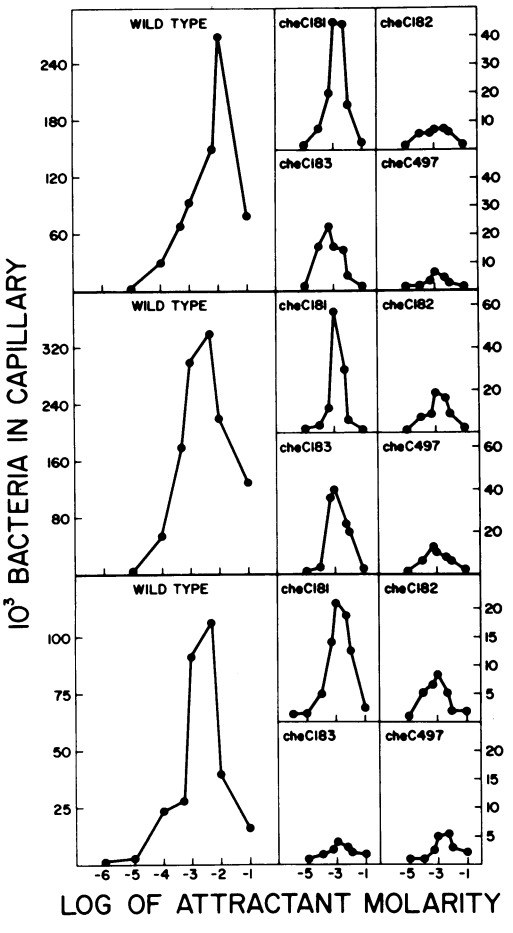

FIG. 4. *Chemotactic responses of wild-type and cheC mutants. The responses of RP477 and RP477 cheC derivatives to serine (upper panel), aspartate (middle panel), and maltose (lower panel) were measured by the capillary assay described in the text. Note that the ordinate scales differ for each attractant and for mutants and wild type.*

sified into one of four categories, depending on whether rotation was predominately ccw or cw and whether reversals occurred. The reproducibility of this classification scheme was estimated with the *cheB294* mutation. Cultures of this strain were prepared from four different colonies, tethered on different days and scored by different observers. The standard deviations for each category of rotation are shown in Table 3. The major source of variation arose in determining whether a particular cell was reversing. Since each cell was only watched for about 15 s, those with a low reversal rate could be scored as nonreversing. In any event, the patterns were quite reproducible and provided a simple method for classifying rotational behavior of the mutants.

Smooth swimmers, like wild type, rotated predominately in the ccw direction; however, the fraction of reversing individuals was much less than in wild type (Table 3). There were almost no reversing cells in *cheA* or *cheB* smooth mutants, but about 10% reversing individuals in *cheC* strains. It is likely that these reversing cells correspond to the phenotypically chemotactic individuals detected in capillary assays of *cheC* mutants. Several smooth mutants, for example, *cheA121* and *cheB216*, contained a few individuals rotating in the cw direction. These cells were probably stuck to the microscope slide by some other means and propelled in a cw direction by a free flagellum, since a *cheB216* strain with straight flagella had no cw individuals. Straight flagella would not be expected to push a cell, but they did appear to rotate normally (see controls, Table 3).

Tumbly mutants rotated predominantly in the cw direction, and a substantial proportion of the individuals exhibited reversing behavior. Many of the strains contained some cells rotating exclusively ccw. At least some of these individuals were produced by flagellar rotation in the ccw direction. Thus, a straight flagella defect did not elminate ccw cells in the *cheB280* mutant. It appears that the rotational properties of tumbly mutants were more variable than those of smooth mutants. Some flagella may even rotate exclusively in the smooth direction in tumbly mutants.

The rotational patterns of B_7 and B_8 mutants were different. As a group, the B_7 mutants had about 2.5 times as many reversing cells as the B_8 mutants (41.2 ± 7.1% compared with 16.8 ± 6.5%). These results provide evidence that the two groups of *cheB* tumbly mutants are physiologically distinct types.

(iii) Response of tumbly mutants to temporal stimulation. The tumbling behavior of wild-type *E. coli* can be altered by temporal changes in attractant or repellent concentration (13, 20, 31). For example, an increasing attractant concentration suppresses tumbling (13). A large abrupt change in attractant concentration elicits a period of smooth swimming (20) whose duration is proportional to the change in the number of chemoreceptors bound by the stimulating chemical (11, 28).

Tumbly mutants were subjected to threefold increases of aspartate or serine concentration to determine the duration of their responses to tumble-suppressing stimuli (Fig. 5). Of the 11 mutants tested, all but one responded to at least one of the two stimuli used; however, the responses of all the mutants were significantly less than those of the wild type. This finding indicates that tumbly mutants are defective

either in the detection of temporal stimuli or in the subsequent response to such stimuli. The B_7 mutants (with one exception) responded better to serine than aspartate, whereas the reverse was true of B_8 mutants (again with one exception).

Null phenotypes of the *che* genes. To gain information about the role of each *che* product, an attempt was made to determine the null phenotypes of the *che* genes. The null condition is defined as that in which no functional gene product is present. For the *cheA* gene, the null phenotype is probably a smooth swimming pattern with a consequent general loss of chemotaxis, because many of the *cheA* isolates were nonsense mutants. For the *cheB* gene, however, there existed two nonchemotactic phenotypes characterized by smooth or tumbly swimming. Tumbly *cheB* mutants were less common than smooth ones, suggesting that tumbly mutations represent a more specific type of defect and that smooth swimming is the more general, probably null, condition. This argument is countered by the fact that none of the smooth mutants were amber mutations, whereas three of the tumbly mutants were ambers (Fig. 3). Nevertheless, two additional lines of evidence show that the null phenotype of the *cheB* gene is smooth swimming.

Strain RP470 was lysogenized with phage Mu, and eight independent *che* isolates were obtained after miniswarm screening. Each mutant was then crossed to Hfr strain PK25, and *his*$^+$ [*strA*] recombinants were selected in plates containing 0.01 M sodium citrate to prevent nonlysogenic recombinants from being reinfected with Mu phage present in the recipient culture. The *his*$^+$ colonies were transferred to tryptone swarm plates to determine chemotaxis phenotype and then to a lawn of Mu indicator bacteria (RP461) to assay Mu release from the recombinant colonies. All the *che*$^-$ recombinants were (Mu)$^+$, and over 95% of the *che*$^+$ recombinants were (Mu)$^-$. This indicates both a very close linkage between the *che* defect and the Mu prophage(s) and the absence of additional Mu prophages at other sites in the genome. The few *che*$^+$ recombinants that still contained Mu were assumed to arise from reinfection on the selection plate and were not tested further.

The *che* defect in the eight Mu lysogens was determined by complementation tests with F-prime strains as detailed previously. Six of the mutants were smooth swimmers belonging to the B_0 complementation class (see Fig. 3); one smooth swimmer belonged to the B_2 class, and the last mutant was a tumbly swimmer of the B_8 class. None of the eight mutants had a *cheA* defect, which was rather unexpected since point mutants in *cheA* and *cheB* occur with nearly equal frequencies. It may be that *cheA* is polar on an unknown gene with a different phenotype. However, the fact that seven of the eight Mu mutants were smooth-swimming *cheB* strains supports the notion that smooth swimming is the null phenotype of this gene. The tumbly mutant may have been formed by Mu insertion at the distal end of the *cheB* gene, allowing a nearly complete product to be made.

If smooth swimming is the null phenotype of *cheB*, it should be recessive to the tumbly phenotype. This was demonstrated by constructing partial diploids of the type F'*cheB* (tumbly)/*cheB* (smooth) and observing their chemotaxis and swimming patterns (Table 4). In cases

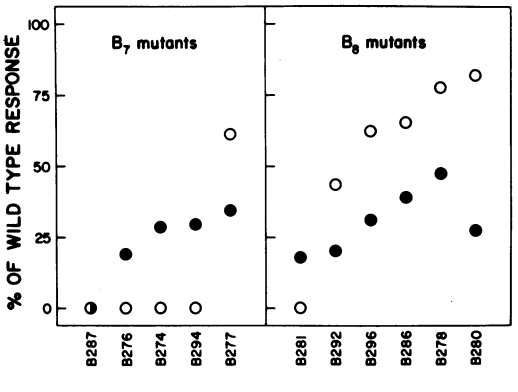

FIG. 5. *Responses of tumbly mutants to temporal stimuli. Tumbly mutants were subjected to threefold increases in serine (●) or aspartate (○) concentration as described in the text. Each response was measured five times and the average was normalized to the wild-type responses, which were 346 ± 38 s for serine and 101 ± 12 s for aspartate.*

TABLE 4. *Phenotypes of cheB heterogenotes*

Edogenote[a] (smooth swimmers)	Exogenote[a] (tumbly swimmers)			
	B274 (B_7)		B280 (B_8)	
	Taxis[b]	Swimming[c]	Taxis	Swimming
B235 (B_0)	None	Tumbly	None	Tumbly
B214 (B_1)	None	Tumbly	None	Tumbly
B208 (B_2)	Partial	Normal	None	Tumbly
B209 (B_3)	Partial	Normal	Partial	Normal
B221 (B_4)	Partial	Normal	None	Tumbly

[a] Partial diploids were constructed by introducing F420 *cheB274* and F420 *cheB280* into RP470 *(recA) cheB* strains by plate mating.

[b] Chemotaxis was evaluated on tryptone swarm plates by comparing colony sizes to those of mutant and wild-type controls. Partial chemotaxis indicates a swarm size from 25 to 75% of wild type.

[c] Swimming pattern was evaluated subjectively by examining log-phase minimal media cultures in the light microscope. Normal behavior is defined as the tumble frequency shown by wild-type control strains.

where the two mutants complemented for chemotaxis, swimming was essentially normal, but when complementation did not occur, the swimming pattern was always tumbly, not smooth. Thus tumbly swimming is dominant to smooth swimming and must not reflect a total loss of *cheB* function.

Both the rarity and the partial dominance of *cheC* mutants indicate that the *cheC* lesion is a specific one and that *cheC* mutants retain considerable function. Most mutations in the *cheC* gene probably produce a different phenotype that is overlooked when searching for generally nonchemotactic mutants. The null phenotype of *cheC* has been determined by Silverman and Simon (25), who investigated complementation between *cheC* and various flagellar genes that map in the *cheC* region. They found that *cheC* does not complement *flaA* mutants for chemotaxis although it does allow flagellar synthesis. Thus *cheC* and *flaA* are the same gene and the *che* phenotype is dominant to the *fla* defect, indicating that loss of flagella is the null phenotype of this gene.

DISCUSSION

A summary of the *che* mutants isolated in this study and their properties is presented in Table 5.

Isolation of *che* mutants. Generally nonchemotactic bacterial strains (*che* mutants) have now been obtained in several different ways. In the initial study of *E. coli* mutants, Armstrong et al. (6) isolated *che* strains by repeatedly cycling bacteria that remained at the origin on semisolid media. They examined 38 independent mutants, all of which were smooth swimmers, and defined three genes, *cheA*, *cheB*, and *cheC* (4). In the present study, *che* mutants were obtained by screening individual clones for chemotaxis defects on semisolid media. A total of 172 new independent mutants, representing both smooth and tumbly swimmers, were examined. All but three of these mutants could be assigned to the *cheA*, *cheB*, and *cheC* genes. The three new isolates define a fourth gene, *cheD* (Parkinson, in preparation).

None of the *E. coli* mutants appear to have viability defects; however, there could be chemotaxis genes in *E. coli* that are also essential for viability. Such genes would not be detected by swarm plate methods. It is unlikely, however, that there are many more than four nonessential *che* genes in *E. coli*. Only two such genes, *cheA* and *cheB*, produce a generally nonchemotactic phenotype in the null state. There are so many independent isolates in each gene that additional genes of this type are quite unlikely. The other two *E. coli* genes, *cheC* and *cheD*, must undergo specific mutational alterations to yield a generally nonchemotactic phenotype. Since only three isolates of each type were found in the present study, it is possible that similar genes remain to be found.

Aswad and Koshland (7) have described a different method of obtaining *che* mutants in *Salmonella typhimurium*. In their technique, bacteria are introduced into preformed attractant gradients. Mutants that do not respond to the attractant gradient are obtained by recycling cells that swim down the gradient. This type of selection can be carried out rapidly under nongrowth conditions and may prove valuable in attempting to isolate conditionally lethal chemotaxis mutants. In its present form, however, the preformed gradient technique does not yield a representative spectrum of *che* mutants because there is considerable bias in favor of smooth-swimming behavior (7). Thus tumbly mutants are not enriched to the same extent as smooth swimmers. This sort of bias has not been seen in swarm plate selections. As a final step in their selection, Aswad and Koshland (7) screened for chemotaxis defects on semisolid media so that in effect they obtained the same types of *che* mutants as the *E. coli* studies using only semisolid media. In spite of this similarity in selection methods, however, the *Salmonella che* system may be more complex. Aswad and Koshland found six classes of *che* isolates in their sample of 57 nonindependent mutants (7). Since three of their mutant classes contain a total of five mutants, many additional classes may exist, suggesting that the *Salmonella che* genes are perhaps more complex than their counterparts in *E. coli*.

Nature and role of *E. coli che* genes. The complementation properties of the *cheA* and *cheB* genes indicate that the functional form of each of these gene products is a multimeric protein. This would account for the extensive intragenic complementation found in these genes. To account for complementation between amber mutants and (presumably) missense mutants in the same gene, it is necessary to further assume that a fragment of the monomeric subunit produced by the nonsense mutation is sufficient to restore function when incorporated into a mixed multimer. Finally, to account for intragenic complementation among amber mutants, there must exist translation reinitiation sites within the gene that permit some nonsense mutants to synthesize polypeptides from the distal portion of the gene. Each of these phenomena has been well documented

TABLE 5. *Properties of E. coli che mutants*

Complementation group	Number of isolates	Swimming pattern	Chemotaxis defect	Null phenotype	
				Swimming pattern	Chemotaxis defect
cheA	72 (22 amber)	Smooth	General	Smooth	General
cheB	68 (no amber)	Smooth	General	Smooth	General
	26 (3 amber)	Tumbly	General but with partial responses to temporal stimuli		
cheC	3 (no amber)	Smooth	General but leaky	Nonmotile	No flagella

in the β-galactosidase complementation system (32).

Null mutants were used to define the major role of each *che* gene in the chemotactic process. Although the null condition is best established with deletions, other strategies were used in this work. For example, all *cheA* mutants have a smooth-swimming phenotype and arise with a much higher frequency than *cheC* or *cheD* isolates that are not null mutants. Many of the *cheA* strains are amber mutants as well. Thus it is likely that *cheA* mutants exhibit the null phenotype. Since tumbling is required for chemotaxis, failure to tumble would be expected to cause a general loss of chemotactic ability. Therefore, the primary defect of *cheA* mutants is probably in the tumbling process, indicating that the *cheA* product is an essential component of the tumble-generating machinery of the cell.

The null phenotype of the *cheB* gene is smooth swimming. This conclusion was reached in two ways: by isolating Mu phage-induced *cheB* mutants and by examining the phenotype of *cheB* (smooth)/*cheB* (tumbly) partial diploids. The existence of both smooth and tumbly *cheB* mutants suggests that the *cheB* product has a dual function. Like the *cheA* product, it is required for tumbling. In addition, the *cheB* product appears to play a role in regulating tumble frequency. Since tumbly *cheB* mutants respond with varying efficiency to different stimuli, their high tumbling rate may be due to a reduced ability to perceive tumble-modulating signals formed by the chemoreceptors. Different signals are perceived with different efficiencies in these mutants, suggesting that chemoreceptor signals are received by the *cheB* component, which in turn control the tumbling frequency. This explanation assumes that tumbling is under negative or inhibitory control and that tumbly mutants are largely uncoupled from the control system (10, 21). Even if this view is essentially correct, it still fails to account for the paradoxical behavior of B_7 tumbly mutants. On the one hand, B_7 mutants respond to stimuli less well than B_8 mutants, implying that B_7 mutants are less efficiently coupled to the tumble-modulating system. On the other hand, B_7 mutants are less defective in their pattern of flagellar rotation than are B_8 mutants, which exhibit fewer reversals and more cw rotation. These contradictory properties can be accounted for in several ways. For example, B_7 mutants could be defective in tumble production as well as in signal perception. In this way, their pattern of flagellar rotation would reflect both a control problem and a partial defect in tumbling, whose end result is a rotational pattern with less clockwise tendency than a strictly tumbly mutant. Another possibility is that the tumbling behavior of B_7 mutants has become locked at a certain level that cannot be altered by chemoreceptor signals. In other words, the *cheB* component of B_7 mutants may act as though a particular signal level were present regardless of the actual level.

Loss of *flaA* product through null mutation results in the inability to synthesize or assemble components of the flagellum (25). Other mutations in this gene produce *cheC* mutants with flagella that rotate only in the ccw (smooth) direction. The *cheC* mutants are presumably making a structurally altered *flaA* product that permits flagellar assembly but disallows cw rotation. This product may be an essential component of the flagellar basal complex.

Very little is known about the biochemical role of any chemotaxis genes. The *cheA* and *cheB* products are involved in controlling the direction of flagellar rotation and may prove to be closely associated with the basal structure of the flagellum. Methionine auxotrophs of *E. coli* cease tumbling when starved for methionine, and it has recently been shown that *cheA*, *cheB*, and other chemotaxis mutants influence

the methylation of a protein located in the cytoplasmic membrane (17). Study of the methionine effect in chemotaxis affords perhaps the best biochemical approach at present to understanding the function of the *che* genes.

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