JOURNAL OF BACTERIOLOGY, July 1978, p. 45-53
0021-9193/78/0135-0045$02.00/0

Vol. 135, No. 1
Printed in U.S.A.

Complementation Analysis and Deletion Mapping of *Escherichia coli* Mutants Defective in Chemotaxis

JOHN S. PARKINSON
Department of Biology, University of Utah, Salt Lake City, Utah 84112

Received for publication 22 February 1978

Motile, but generally nonchemotactic (*che*) mutants of *Escherichia coli* were examined for complementation and recombination with specialized λ*fla* transducing phages. The complex complementation behavior of these mutants found previously in F-prime tests could largely be accounted for by intragenic complementation and by polarity effects. Mutants of the "*cheA*" class defined two genes, *cheA* and *cheW*, which appeared to be cotranscribed. Mutants of the "*cheB*" class defined four genes, *cheX*, *cheB*, *cheY*, and *cheZ*, which also constituted a transcriptional unit. Mutants defective in *cheA*, *cheW*, *cheX*, or *cheY* function swam smoothly, with little or no tumbling, whereas *cheB* or *cheZ* mutants exhibited very high tumbling rates. These functions are probably involved in initiating or controlling changes in flagellar rotation in response to chemotactic stimuli.

Escherichia coli swims by rotating its flagellar filaments (7, 20). In the absence of chemotactic stimuli, the bacteria move about in a random walk (8) consisting of smooth "runs," produced by counterclockwise rotation, and abrupt directional changes or "tumbles" caused by clockwise rotation (14). In the presence of a chemical gradient, chemotaxis is carried out by modulating the direction of flagellar rotation in response to changes in attractant or repellent concentration (9, 14, 15). These behavioral responses are mediated by specific chemosensors that feed information through a network of signaling elements, which in turn control flagellar movement (1).

The final stages of signaling and stimulus transduction in *E. coli* and *Salmonella typhimurium* have been defined through studies of motile, but generally nonchemotactic (*che*) mutants (5, 6, 10, 18, 26). Although *che* mutants are currently being characterized physiologically and biochemically, their genetic properties are not well understood. Complementation analyses of nearly 200 independent *che* strains indicated that there may be as few as four *che* loci in *E. coli* (4, 18). Most of the mutants, however, fell into one of two general classes, "*cheA*" or "*cheB*." Members of either class often exhibited complementation with one another, but the complexity of the patterns suggested that they might be due to intragenic complementation (18). In contrast, nine *che* genes have been reported in *S. typhimurium*, whose chemotaxis machinery is probably similar to that of *E. coli* (26).

Recently, Silverman and Simon (22) constructed hybrid λ phages that carried the "*cheA*" and "*cheB*" regions of *E. coli* and found that these regions probably contain *che* gene clusters. By comparing the complementation activities of each λ*fla* strain with the *che* proteins that it made, they were able to show that the "*cheA*" region encodes several different proteins which correspond to two complementation activities, designated *cheA* and *cheW*. The "*cheB*" region codes for four different proteins which correspond to four complementation activities, designated *cheB*, *cheX*, *cheY*, and *cheZ*.

The purpose of the present study is to examine the basis of the complex complementation behavior of "*cheA*" and "*cheB*" mutants of *E. coli*. Various λ*fla* phages were used to construct a deletion map of the *che* region and to localize mutations with anomalous complementation properties. Examination of Mu-induced mutants indicated that "*cheB*" is a cluster of four cotranscribed genes and that pleiotropic defects within the "*cheB*" group are mainly caused by polarity effects. Two other factors, intragenic complementation and gene product interaction, also contribute to the genetic complexity of these regions. These studies show, in agreement with Silverman and Simon (22), that the "*cheA*"-"*cheB*" segment contains six *che* genes. The phenotypes and epistatic relationships of these six genes are discussed.

MATERIALS AND METHODS

Strains. All bacterial strains used in this work are derivatives of *E. coli* K-12, and many have been described previously (18). Mapping and complementation studies of *che* mutations were performed in derivatives of strain RP461 [F⁻ *thi thr leu his* Δ(*gal-att*λ) *strA*]. The isolation and initial analysis of the *che* mutants used here have also been described (18). A few of the mutants were obtained from RP461; however, most were isolated from RP470 (RP461 *recA*).

Complementation tests with F-primes (see below) were performed in the RP470 background. For complementation and mapping studies with λ*fla*, *che* alleles were transduced with phage P1*kc* into a *recA*$^+$ strain, RP437 [RP461 (*gal-att*λ)$^+$ *eda*], by selecting *eda*$^+$ recombinants on H1 miminal medium (2) containing glucuronic acid as the sole carbon and energy source. The *eda*$^+$ transductants were then tested for chemotaxis on tryptone swarm agar, and Che$^-$ clones that proved to be nonlysogenic for P1 were saved for further use. Mapping of RP470 *che*::Mu strains with λfla was done by introducing the *recA*$^+$ allele from Hfr strain PK25 by conjugation as previously described (18).

The λ*fla* hybrids used in this work were obtained from M. Silverman. The genetic content of these strains is shown in Fig. 1, and their complementation activities are listed in Tables 2 and 4. Stocks were prepared on strain C600 (3) by confluent plate lysis.

Media. Tryptone broth, plates, and swarm agar were used for most of the experiments and have been described (18). Minimal media for selecting recombinants contained H1 salts (2) and 0.1% of a carbon and energy source, which was glucose, unless otherwise indicated. Necessary amino acids and vitamins were added to a final concentration of 1 mM and 1 μg/ml, respectively.

F-prime complementation tests. Complementation analysis of *che* mutants with F-prime *che* testers has been described (18). In brief, RP470 *che* strains were mated to donors carrying *che* derivatives of F′420 (*his*$^+$), and *his*$^+$ F-ductants were selected and tested for chemotaxis on tryptone swarm agar. The diameters of the resulting colonies were measured after 16 to 18 h of incubation at 35°C. Because RP470 is recombination deficient, no *che*$^+$ recombinants are formed in these tests.

Complementation and mapping with λ*fla*. Three similar methods were employed. Methods I and II were employed with RP437 *che* strains made lysogenic for λ wild type to minimize killing by λ*fla*. Methods II and III were used primarily in analyses of RP470 *che*::Mu strains that had been made *recA*$^+$, but which were not lysogenic for λ.

For method I, approximately 0.01 ml of an overnight tryptone culture of the RP437 *che* (λ) recipient was spotted on a tryptone swarm plate. Approximately 0.01 ml of λ*fla* lysate at a concentration of ~1 × 10^{10}/ml was added to each spot to give a multiplicity of infection of about 10. Test plates were incubated at 30°C and scored for complementation after 14 to 18 h. Positive complementation was detected as a slow uniform spread of abortive transductants (trails) away from the origin. Faster spreading wild-type recombinants began to appear at about 16 h and were scored at 20 to 24 h.

For method II, tryptone swarm plates containing 0.4% agar and ~1 × 10^8 λ*fla* particles per ml were used in this test. In some experiments the λ*fla* strains were first irradiated with approximately 2,000 ergs/mm^2 of UV light to enhance recombination and to minimize killing. Colonies of *che* strains to be tested were transferred to the plates and scored as in method I above.

For method III, tryptone plates were seeded with approximately 0.1 ml of a stationary tryptone culture of a nonlysogenic *che* strain, and λ*fla* testers were spotted at a concentration of ~1 × 10^8/ml. The plates were incubated overnight at 30°C, and the turbid centers from the spots were picked onto tryptone swarm plates at 30°C. Test plates were scored as in method I.

Other methods. Swimming patterns were determined by inspection of tryptone cultures in a phase-contrast light microscope as detailed elsewhere (18). Tests for amber mutations utilized the *supD*, *supF*, and *supU* suppressors as previously described (18).

RESULTS

The designations "*cheA*" and "*cheB*" are used to refer to the two general classes of *che* mutants described above. Each class can be shown to correspond to several *che* genes. Two of the newly defined genes were designated *cheA* and *cheB* by Silverman and Simon (22). The same terminology will be used in this report, and it is important to realize that "*cheA*" and *cheA* or "*cheB*" and *cheB* have different meanings.

Complementation analysis of "*cheA*" mutants. Seventy-seven ethyl methane sulfonate-induced "*cheA*" mutants of independent origin were studied. In a previous report (18), these mutants were assigned to six subclasses on the basis of complementation patterns in tests with F-prime elements carrying different "*cheA*" mutations. Many of these tests were repeated in the present study. Upon examination of the old and new data, one of the original subclasses (A_1) appeared to contain two types of patterns that were similar, but not identical. Subdividing the A_1 group yields a total of seven subclasses, which are shown in Table 1.

One or more representatives from each of the "*cheA*" subclasses were tested for complementation by various λ*fla* transducing strains to determine the relationship to the *cheA* and *cheW* activities defined by Silverman and Simon (22) (Table 2). Mutants belonging to the $A_{1.2}$, A_2, A_3, and A_5 subclasses are complemented by λ*fla* phages having *cheA* activity and, therefore, are considered to be *cheA* mutants. The numerous examples of complementation within this group (Table 1) are probably due to intragenic complementation, which implies that the *cheA* product functions as a multimer.

Mutant ***113***, from the A_4 subclass, was not complemented by λ*fla57*Δ*27*, which has *cheA* activity, but was complemented by λ*fla57*Δ*21*, which has no *cheA* activity (Table 2). This pattern defines *cheW*. The *cheW* mutant used by Silverman and Simon (22) was ***110***, which also falls in the A_4 subclass (Table 1). These findings are compatible with the F-prime data, because A_4 mutants complement well with $A_{1.2}$, A_2, A_3, and A_5 mutants (Table 1).

Mutants in the A_0 subclass, although recessive, failed to complement any of the other classes (Table 1) and were corrected only by λ*fla57*, which has both *cheA* and *cheW* activity (Table 2). Similarly, $A_{1.1}$ mutants lacked *cheA* function and were partially defective in *cheW* function as well. These findings and the fact that many A_0 and $A_{1.1}$ mutants have amber nonsense mutations (Table 1) suggest that the multiple functional defects are caused by polarity. The *cheA* and *cheW* genes are known to be cotranscribed, along with the motility genes *motA* and *motB*, from the Mocha promotor (21). The direction of transcription is *motA-motB-cheA-cheW*, which means that polar mutations affecting *cheA* and *cheW* expression (but not *mot*) should map in *cheA*. This appears to be the case for the three mutants (***116***, *117*, ***173***) shown in Table 2, so the polarity argument is probably correct.

Complementation analysis of "*cheB*" mutants. Ninety-nine ethyl methane sulfonate-induced "*cheB*" mutants of independent origin were examined in complementation studies with F-prime testers, and, in agreement with previous findings (18), nine subclasses were obtained. The majority of the mutants (85/99) fell into the four subclasses shown in Table 3. Note that each subclass shows complementation with the other three, although some combinations ($B_5 \times B_6$ and $B_7 \times B_8$) may complement rather poorly. These patterns are probably not caused, as originally believed, by intragenic complementation, because each subclass also gave a unique pattern in complementation tests with λ*fla* phages (Table 4). These data show that B_5 mutants correspond to *cheY*, those of B_6 correspond to *cheX*, those of B_7 correspond to *cheB*, and those of B_8 correspond to *cheZ*. Discussion of the remaining "*cheB*" mutants (14/99), which have pleiotropic

TABLE 1. *Complementation patterns of "cheA" mutants*

Recipient subclass	Allele and subclass of F-prime *che* testers[a]					No. of isolates	Allele no.[b]
	116, *117* A_0	*101* $A_{1.2}$	*114* A_2	*115* A_3	***113*** A_4		
A_0	0	0	0	0	0	9	***104, 107, 116,*** *117, 119, 120, 130,* ***133, 163***
$A_{1.1}$	0	0	0	0	±	18	*105, 112, 122,* ***125,*** *126, 128, 132,* ***135, 136, 140,*** *144,* ***146, 149, 151, 152, 157, 169, 173***
$A_{1.2}$	0	0	0	0	+	28	*101, 103, 106, 108, 109,* ***121,*** *124, 127, 134, 139, 141, 145, 147, 148, 153, 154, 155, 158, 159, 164, 165, 168, 170, 171, 172, 174, 176, 177*
A_2	0	0	0	±	+	3	*114, 131, 175*
A_3	0	0	±	0	+	4	***115,*** *123,* ***138,*** *167*
A_4	0	+	+	+	0	6	***110, 113, 143,*** *156,* ***161,*** *166*
A_5	0	±	±	0	+	9	*102, 111, 113, 129, 137, 142, 150, 160, 162*

[a] Tests were performed with *recA* recipients as described in the text. The diameters of the resulting swarms were compared with positive (F' *che*$^+$/*che*) and negative (*che* alone) controls for each mutant. Symbols: 0, no detectable improvement over mutant alone; ±, weak complementation, less than 50% of positive control; +, complementation, over 50% of positive control.

[b] Alleles shown in bold-face italic numerals are known amber mutations.

TABLE 2. *Mapping and complementation of "cheA" mutants with λfla phages*

che mutants		λ*fla* testers[a]					Complementation class
Subclass	Allele[b]	*57* (A^+ W^+)	*57Δ27* (A^+)	*57Δ21* (W^+)	*3Δ30* (X^+)	*3Δ11*	
A_0	***116,*** *117*	+	R	0	0	0	*AW*
$A_{1.1}$	***173***	+	±	0	0	0	*A(W)*
$A_{1.2}$	*101,* ***121***	+	+	R	R	0	*A*
A_2	*114*	+	+	R	R	0	*A*
A_3	***115,*** *175*	+	+	R	R	0	*A*
A_4	***113***	+	R	+	R	0	*W*
A_5	*102*	+	+	0	0	0	*A*

[a] Tests were performed with *rec*$^+$ recipients as detailed in the text. The complementation activities of each λ*fla* strain as defined by Silverman and Simon (22) are shown directly below the strain numbers. Symbols: +, complementation, wild-type recombinants obtained; ±, weak complementation, wild-type recombinants obtained; R, no complementation, wild-type recombinants obtained; 0, neither complementation nor recombination observed.

[b] Alleles shown in bold-face italic numerals are known amber mutations.

TABLE 3. *Complementation patterns of "cheB" mutants*

Recipient subclass	Allele and subclass of F-prime *che* testers[a]					Allele no.[b]
	201, 219, 220, B_5	*202, 203 B_6*	*274, 275 B_7*	*280, 281 B_8*	No. of isolates	
B_5	0	±/+	+	+	39	*197, 198, 201, 204, 205, 206, 207, 209, 212, 213, 219, 220, 222, 224, 225, 228, 230, 231, 232, 233, 236, 238, 240, 241, 244, 245, 246, 247, 248, 250, 253, 254, 257, 259, 261, 263, 264, 265, 266*
B_6	±/+	0	+	+	15	*202, 203, 208, 211, 217, 218, 239, 242, 243, 249, 251, 258, 260, 267, 268*
B_7	+	+	0	±/+	15	*237, 270, 271, 272, 273, 274, 275, 276, 277, 283, 285, 287, 290, **294**, 295*
B_8	+	+	±/+	0	16	*278, 279, 280, 281, 282, 284, **286**, 288, 289, 291, **292**, 293, 296, 297, **298**, 299*

[a] Tests were performed with *recA* recipients. See Table 1 for an explanation of symbols. Combinations yielding a ±/+ result are ones in which some testers or mutants gave weak complementation and others gave strong complementation.

[b] Alleles shown in bold-face italic numbers are known amber mutations.

TABLE 4. *Mapping and complementation of "cheB" mutants with λfla phages*

che mutants		λ*fla* testers[a]								
Subclass	Allele	*3Δ30 (X^+)*	*3Δ28 (X^+B^+)*	*3Δ23 ($X^+B^+Y^+$)*	*3Δ1 ($X^+B^+Y^+$)*	*3Δ14 ($X^+B^+Y^+Z^+$)*	*3Δ11*	*57 ($A^+W^+X^+$)*	*57Δ27 (A^+)*	*Complementation class*
B_5	*201, 220*	0	R	+	+	+	R	0	0	*Y*
B_5	*197, 198, 219*	0	0	+	+	+	R	0	0	*Y*
B_6	*202, 203, 239, 243*	+	+	+	+	+	R	+	0	*X*
B_7	*237, 277, 287*	R	+	+	+	+	R	R	0	*B*
B_7	*274, 276*	R	+	+	+	+	R	0	0	*B*
B_7	*270, 271, 275, **294***	0	+	+	+	+	R	0	0	*B*
B_8	*278, 293*	0	0	0	R	+	R	0	0	*Z*
B_8	*280, 281, **286**, **292**, 297, 299*	0	0	0	0	+	R	0	0	*Z*

[a] Tests were performed with *rec*$^+$ recipients. Complementation activities of the λ*fla* testers are indicated directly below the strain numbers. See Table 2 for definition of symbols.

defects, is best deferred until the transcriptional organization of the "*cheB*" region has been considered.

Evidence for cotranscription of the "*cheB*" region. The genetic content, as opposed to complementation activity, of various λ*fla* phages was determined by measuring the ability of different phages to give wild-type recombinants with representative mutations from each of the six *che* groups *A*, *B*, *W*, *X*, *Y*, and *Z* (Tables 2 and 4). Those results are summarized in Fig. 1, which depicts the *E. coli* material, assumed to be a continuous segment, present in each λ*fla* strain employed. One of the phages, λ*fla3Δ11*, gave *che*$^+$ recombinants with all of the testers in *B*, *X*, *Y* and *Z*, but failed to complement any of them. This suggests that *cheXBYZ* constitutes a single transcriptional unit whose promotor is missing in λ*fla3Δ11*. It has been shown that λ*fla3Δ11* complements *flaG* and *flaH* mutants, which map to the right of *cheZ* (22) (Fig. 1), so it is unlikely that the *XBYZ* promoter lies at the *cheZ* end of the cluster. The left end point in λ*fla3Δ11* is ambiguous: it might be within the *cheX* structural gene or outside *cheX*, perhaps in the adjacent *tar* gene, which is also involved in chemotaxis (24). Experiments to resolve this point by testing λ*fla3Δ11* for recombination with *tar* mutations are still in progress. There are a number of lines of evidence which show that the *cheX* cluster is not transcribed from the Mocha promotor. For example, polar mutants in the Mocha operon complemented mutants of the *cheX* cluster; moreover, λ*fla* phages such as *3Δ14*, which complemented mutants of the *cheX* cluster, lacked the Mocha

promoter (Fig. 1). Thus, the most likely location of the *cheXBYZ* promoter is somewhere between *cheX* and *cheW*.

Insertion of phage Mu within a transcriptional unit produces extreme polar effects on the expression of genes distal to the insertion point (12). Eight Mu-induced "*cheB*" mutants were examined for possible polar effects, and the site of insertion was deletion mapped with λ*fla* strains (Table 5). Six of the eight were defective in all four complementation activities, i.e., *cheX*, *cheB*, *cheY*, and *cheZ*: three (*306*, *307*, *308*) map between *cheW* and the left end of λ*fla3Δ11*; and three (*301*, *303*, *305*) map between the left end of *3Δ11* and the right end of λ*fla57* (Fig. 1). The two remaining Mu insertions (*302*, *304*) probably map in *cheY* and eliminate *cheY* and *cheZ* function, while retaining *cheX* and *cheB* activity. These results show that the *cheXBYZ* genes constitute an operonic cluster that is transcribed from *cheX* through *cheZ*. Hereafter, this will be referred to as the *cheX* operon.

Properties of pleiotropic "*cheB*" mutants. Fourteen ethyl methane sulfonate-induced mutants gave complex complementation patterns in F-prime tests (Table 6). All of these strains were examined for complementation and recombination with λ*fla* testers to determine the basis for their pleiotropic defects (Table 7). All of the subclasses shown in Table 6, with the exception of B_3, can be accounted for by polarity effects, as the following evidence demonstrates. (i) B_0 mutants were defective in *X*, *B*, *Y*, and *Z* functions and most likely map either within or to the left of *cheX*, i.e., at the promoter-proximal end of the operon. (ii) B_2 mutants were fully defective in *X*, *Y*, and *Z* activity but appear to have some residual *cheB* function. Because these mutations also map at the head of the *cheX* operon, it is likely that they are leaky polar mutants, with a low level of *cheB* expression sufficient to allow partial complementation by *cheB* testers. (iii) B_1 mutants lacked *B*, *Y*, and *Z* function and most likely map in the *cheB* gene (Fig. 1). (iv) B_4 mutants lacked *Y* and *Z* activity and probably map in the *cheY* gene (Fig. 1).

The nature of the polar mutation in any of these strains is not known, but none appear to be amber mutations because they could not be corrected by either the *supD*, *supF*, or *supU* nonsense suppressors (data not shown).

The B_3 mutants (Table 6), which lacked *cheX*

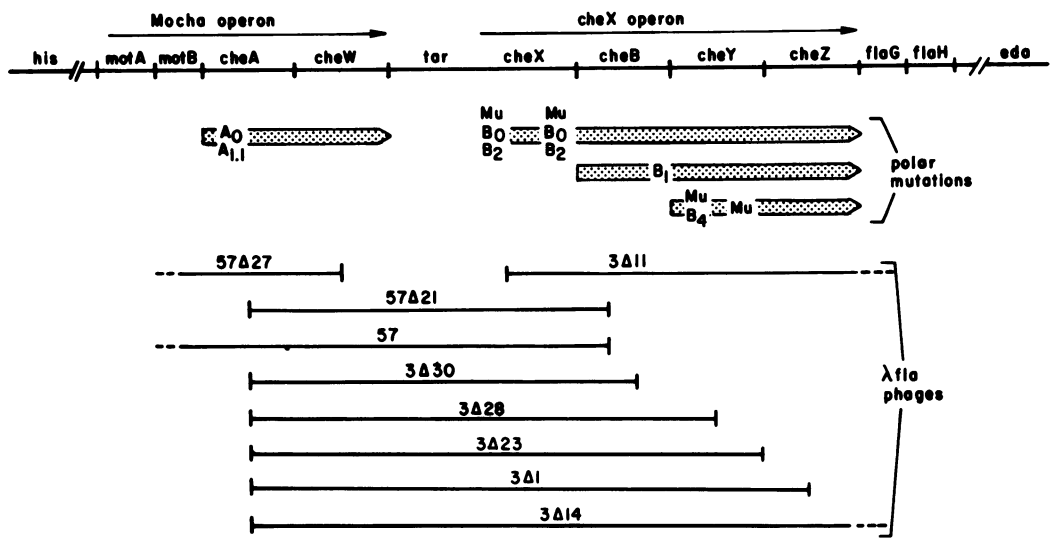

FIG. 1. *Genetic map of the "cheA"-"cheB" region in E. coli. This region is located between the his and eda loci and contains most of the known che genes, as well as genes for motility (mot) and flageller synthesis (fla). The tar gene (24), which corresponds to the cheM gene of Silverman and Simon (23), is also involved in chemotaxis and maps between cheW and cheX (23; J. S. Parkinson, unpublished data). The position of the tar-cheX boundary has not yet been established. The che genes are organized into two transcriptional units, the Mocha and cheX operons. Map locations and functional defects of polar mutations studied in this work are indicated by the stippled arrows and are based on data from Tables 2, 5, and 7. The E. coli material present in various λfla transducing phages is indicated by the solid lines at the bottom of the figure. Inclusion end points were determined by recombination and complementation tests with che point mutants (Tables 2 and 4).*

and *cheY* activity, but had *cheB* and *cheZ* activity, cannot be accounted for by polar effects, because two of the mutations (*199* and *216*) appear to map in the *cheY* gene (Table 7 and Fig. 1) and therefore could not have a polar effect on expression of *cheX*. Two other B_3 mutations (*210, 255*) map within the λ*fla3Δ11* inclusion at the beginning of the *cheX* operon. However, unlike polar *cheX* mutants, these latter strains have the swarm plate morphology and response physiology characteristic of nonpolar *cheX* mutants (J. S. Parkinson, unpublished data). For these reasons, it seems likely that B_3 mutants represent a special subset of *cheX* or *cheY* defects that cannot complement mutants in either gene. Because poor complementation between *cheX* and *cheY* strains was rather common (Table 3), the B_3 mutants could simply be extreme cases of a more general phenomenon. It seems possible that poor complementation between *cheX* and *cheY* strains is caused by some sort of interaction between the *cheX* and *cheY* gene products. For example, if the products function as a complex, certain combinations of *cheX* and *cheY* defects could lead to mutant complexes which inhibit or otherwise mask the function of the wild-type products. Such effects should be allele specific; for example, a particular *cheX* mutant may complement well with some *cheY* strains and poorly with others. A mutant-by-mutant examination of the data summarized in Table 3 strongly suggests an allele-specific pattern (data not shown). It is important to note that some *cheB* and *cheZ* combinations

TABLE 5. *Mapping and complementation analysis of che::Mu strains*

che::Mu allele	F′ testers[a]				λ*fla* testers[b]						Complementation class
	cheX	*cheB*	*cheY*	*cheZ*	*3Δ30* (X^+)	*3Δ28* (X^+B^+)	*3Δ23* ($X^+B^+Y^+$)	*3Δ14* ($X^+B^+Y^+Z^+$)	*3Δ11*	*57* (A^+W^+)	
306, 307, 308	0	0	0	0	R	R	R	+	0	R	*XBYZ*
301, 303, 305	0	0	0	0	R	R	R	+	R	R	*XBYZ*
302	+	+	0	0	0	R	R	+	R	0	*YZ*
304	+	+	0	0	0	0	R	+	R	0	*YZ*

[a] *recA* recipients were used in these tests. Allele numbers of testers are given in Table 3. Symbols are defined in Table 1.
[b] *rec*$^+$ recipients were used in these tests. Symbols are defined in Table 2.

TABLE 6. *Complementation patterns of pleiotropic "cheB" mutants*

Recipient subclass	F-prime *che* testers[a]				Complementation class	No. of isolates	Allele no.
	cheX	*cheB*	*cheY*	*cheZ*			
B_0	0	0	0	0	*XBYZ*	3	*235, 252, 256*
B_1	+	0	0	0	*BYZ*	2	*223, 227*
B_2	0	±	0	0	*X(B)YZ*	2	*226, 229*
B_3	0	+	0	+	*XY*	4	*199, 210, 216, 255*
B_4	+	+	0	0	*YZ*	3	*200, 221, 234*

[a] *recA* recipients were used in these tests. Allele numbers of the testers are given in Table 3. Symbols are defined in Table 1.

TABLE 7. *Mapping and complementation of pleiotropic "cheB" mutants with λfla phages*

che mutants		Complementation class	λ*fla* testers[a]							
Subclass	Allele		*3Δ30* (X^+)	*3Δ28* (X^+B^+)	*3Δ23* ($X^+B^+Y^+$)	*3Δ1* ($X^+B^+Y^+$)	*3Δ14* ($X^+B^+Y^+Z^+$)	*3Δ11*	*57* (A^+W^+)	*57Δ27* (A^+)
B_0	*235, 256*	*XBYZ*	R	R	R	R	+	0	R	0
B_0	*252*	*XBYZ*	R	R	R	R	+	R	R	0
B_1	*223, 227*	*BYZ*	0	R	R	R	+	R	0	0
B_2	*226*	*X(B)YZ*	R	R	R	R	+	R	R	0
B_2	*229*	*X(B)YZ*	R	R	R	R	+	0	R	0
B_3	*210, 255*	*XY*	+	+	+	+	+	R	+	0
B_3	*199, 216*	*XY*	0	R	+	+	+	R	0	0
B_4	*200, 221, 234*	*YZ*	0	R	R	R	+	R	0	0

[a] *rec*$^+$ recipients were used in these tests. Symbols are defined in Table 2.

TABLE 8. *Swimming patterns of che strains*

Functional defect	Swimming pattern
A	Smooth
W	Smooth
X	Smooth
Y	Smooth
B	Tumbly
Z	Tumbly
AW[a]	Smooth
AWB[b]	Smooth
AWZ[b]	Smooth
YZ[c]	Smooth
BYZ[c]	Smooth
XBYZ[c]	Smooth

[a] Polar mutations in *cheA*.

[b] Double mutants made by transducing a polar *cheA* mutation into a *cheB* or *cheZ* recipient.

[c] Polar mutations.

also exhibited poor complementation, which might be due to interaction of the *cheB* and *cheZ* gene products (Table 3).

Swimming patterns of the *che* strains. The swimming behavior of each mutant used in the present study was examined by direct observation in the light microscope. Two basic patterns were seen: "smooth" swimming with little or no tumbling behavior; and "tumbly" swimming with very high frequencies of tumbling and little translational movement. All of the nonpolar mutations in any particular gene produced the same swimming pattern, which implies, but does not prove, that the patterns observed were in fact the null phenotypes of each gene (Table 8). Defects in *cheA*, *cheW*, *cheX*, or *cheY* caused smooth swimming, whereas loss of *cheB* or *cheZ* function caused tumbly swimming.

The phenotypes of polar *che* mutants provide information about the epistatic relationships of the various functions (Table 8). For example, polar mutants lacking both *Y* and *Z* activity were smooth swimmers, which shows that the tumbling behavior exhibited by *cheZ* mutants is dependent on *cheY* function. In summary, in every instance where a mutation or combination of mutations involved both smooth and tumbly functions, the resulting phenotype was smooth.

DISCUSSION

The mapping and complementation studies reported here, in agreement with a previous study by Silverman and Simon (22), show that the "*cheA*" and "*cheB*" regions of *E. coli* are in fact complex loci containing six genes essential for chemotaxis. This result largely accounts for the previously noted differences between the *che* systems of *E. coli* and *S. typhimurium*. Recent complementation studies have also demonstrated an extensive correspondence between the two species with respect to organization and function of *che* genes (DeFranco, Parkinson, and Koshland, manuscript in preparation). New findings concerning the transcriptional organization of the *E. coli* genes and the nature and role of their products are discussed briefly below. Additional discussion of these topics can be found in a recent review (19).

Cotranscription of *che* genes. The "*cheA*" and "*cheB*" regions comprise two separate operons, each containing several genes. The *cheA* and *cheW* genes are part of the Mocha operon (21). A second operon consisting of the *cheX*, *cheB*, *cheY*, and *cheZ* genes was identified in the present study. Experiments in progress indicate that a fifth gene, *tar*, (Fig. 1) is also probably part of the *cheX* operon. Much of the seemingly anomalous complementation data, especially among "*cheB*" mutants, appears to be caused by genetic polarity, although in many cases the nature of the polar mutation is not yet known.

Because of polar effects and the paucity of mapping data, some *che* gene assignments reported in the literature are incorrect. For example, a number of the strains employed by Silverman and Simon (22) were polar mutants, some of which were erroneously classified on the basis of complementation patterns alone. Subsequently, these and a few other misclassified mutants have been used as reference types for various *che* defects in biochemical studies (16, 23). It is hoped that the allele numbers and gene assignments given in this report will serve to remedy some of the confusion caused by differences in gene and mutant notations used by different laboratories.

Genetic and physical sizes of *che* genes. Table 9 lists the number of independent, ethyl methane sulfonate-induced mutations, both polar and nonpolar, obtained in each *che* gene. Also listed in Table 9 are the molecular weights

TABLE 9. *Estimated genetic and physical sizes of che genes*

Gene	No. of independent mutations[a]	Target size relative to *cheA*	Mol wt of product[b]	Product size relative to *cheA*
cheA	71 (19 Am)	1.0	76,000; 66,000	1.0
cheB	17 (1 Am)	0.24	38,000	0.50
cheX	22 (0 Am)	0.31	28,000	0.37
cheZ	16 (3 Am)	0.23	24,000	0.32
cheW	6 (4 Am)	0.08	12,000	0.16
cheY	44 (0 Am)	0.62	8,000	0.11

[a] This includes both polar and nonpolar alleles. Polar mutations at the start of the *cheX* operon are assumed to be in the *cheX* gene.

[b] Data are from Silverman and Simon (22).

of *che* proteins based on data of Silverman and Simon (22). Each gene, with the exception of *cheA*, is associated with a single protein species. The *cheA* gene appears to produce two proteins which have many common peptides (16), suggesting that one may be derived from the other through posttranslational processing of some sort. The physical size estimates based on product size are calculated relative to the larger of the two *cheA* proteins on the assumption that the smaller one is produced by proteolytic cleavage. With the exception of *cheY*, there is surprisingly good agreement between the estimated physical sizes and the mutational target sizes of the *che* genes (Table 9), which implies that this collection of *che* mutants has not been influenced by mutational hot spots or by unsuspected selective factors that might have favored certain phenotypes during mutant isolation. The λ*fla* mapping studies also revealed no tendency toward clustering; however, it is possible that some highly mutable sites will show up as more fine structure data become available.

Silverman and Simon (22) found that *cheY* activity is correlated with an 8,000-molecular-weight protein that forms a rather diffuse band on sodium dodecyl sulfate-polyacrylamide gels. It is possible that this protein is not homogeneous in size (16, 22), but is instead a degraded form of a large initial *cheY* product. The disparity in genetic and physical sizes of *cheY* (Table 9) indicates that this may be the case. It will be of interest to determine the actual size of this gene, and if its product proves to be processed in some fashion, to determine whether the processing is involved in chemotaxis.

Functional role of *che* products. Control of tumbling movements is the basis of chemotaxis. All of the generally nonchemotactic mutants that have been found in *E. coli* (5, 18) and *S. typhimurium* (6, 10, 26) have aberrant tumbling rates, which shows that *che* functions are involved in the production or regulation of tumbling behavior. Because chemoreceptor mutants have normal swimming patterns, the *che* genes probably act at late steps in the transduction scheme, beyond the stimulus detection stage (1). These events are still poorly understood.

Mutations in *cheB* or *cheZ* cause excessive tumbling. In these mutants, as in the wild type, tumbling can be suppressed transiently by sufficiently large stimuli of the proper sort, for example, by an increase in attractant concentration (17). Thus, *cheB* and *cheZ* strains are able to generate both clockwise and counterclockwise rotation, but may be defective in controlling the direction of rotation in response to chemoreceptor signals. Consistent with this notion is the fact that response thresholds in tumbly mutants are usually much higher than in the wild type (17).

Mutations in *cheA*, *cheW*, or *cheY* abolish tumbling. These mutants do not respond to tumble-enhancing stimuli (14; J. S. Parkinson, unpublished data) and cannot be made to tumble by a *cheB* or *cheZ* defect. It appears, therefore, that *cheA*, *cheW*, and *cheY* functions are needed to generate clockwise rotation. Reversion studies show that they probably interact directly with components of the flagellar basal complex (J. S. Parkinson, S. R. Parker, and R. A. Smith, unpublished data).

The behavior of *cheX* strains is unique. These mutants respond to many kinds of stimuli with fairly normal thresholds; however, unlike the wild type, the responses are not transient, but instead persist for very long times, suggesting a defect in sensory adaptation (J. S. Parkinson, unpublished data). Recent biochemical studies have shown that methylation and demethylation of certain membrane proteins are involved in the adaptation process (11, 13) and that *cheX* mutants are defective in methylation activity (11, 13, 23). Studies of the corresponding gene in *S. typhimurium* (*cheR*) suggest that it may specify a methyltransferase enzyme (25).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM-19559 from the National Institute of General Medical Sciences.

I thank M. Silverman for providing the λ*fla* strains used in this work.

LITERATURE CITED

1. **Adler, J.** 1969. Chemoreceptors in bacteria. Science **166**:1588–1597.
2. **Adler, J.** 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. J. Gen. Microbiol. **74**:77–91.
3. **Appleyard, R. K.** 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *E. coli* K12. Genetics **39**:440–452.
4. **Armstrong, J. B., and J. Adler.** 1969. Complementation of nonchemotactic mutants of *Escherichia coli*. Genetics **61**:61–66.
5. **Armstrong, J. B., J. Adler, and M. M. Dahl.** 1967. Nonchemotactic mutants of *Escherichia coli*. J. Bacteriol. **93**:390–398.
6. **Aswad, D., and D. E. Koshland, Jr.** 1975. Isolation, characterization and complementation of *Salmonella typhimurium* chemotaxis mutants. J. Mol. Biol. **97**:225–235.
7. **Berg, H. C., and R. A. Anderson.** 1973. Bacteria swim by rotating their flagellar filaments. Nature (London) **245**:380–382.
8. **Berg, H. C., and D. A. Brown.** 1972. Chemotaxis in *Escherichia coli* analyzed by three-dimensional tracking. Nature (London) **239**:500–504.
9. **Brown, D. A., and H. C. Berg.** 1974. Temporal stimulation of chemotaxis in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **71**:1388–1392.
10. **Collins, A. L. T., and B. Stocker.** 1976. *Salmonella*

typhimurium mutants generally defective in chemotaxis. J. Bacteriol. **128**:754–765.

11. **Goy, M. F., M. S. Springer, and J. Adler.** 1977. Sensory transduction in *Escherichia coli*: role of a protein methylation reaction in sensory adaptation. Proc. Natl. Acad. Sci. U.S.A. **74**:4964–4968.
12. **Jordan, E., H. Saedler, and P. Starlinger.** 1968. 0° and strong polar mutations in the *gal* operon are insertions. Mol. Gen. Genet. **102**:353–363.
13. **Kort, E. N., M. F. Goy, S. H. Larsen, and J. Adler.** 1975. Methylation of a protein involved in bacterial chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **72**:3939–3943.
14. **Larsen, S. H., R. W. Reader, E. N. Kort, W.-W. Tso, and J. Adler.** 1974. Change in direction of flagellar rotation is the basis of the chemotactic response in *Escherichia coli*. Nature (London) **249**:74–77.
15. **Macnab, R. W., and D. E. Koshland, Jr.** 1972. The gradient-sensing mechanism in bacterial chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **69**:2509–2512.
16. **Matsumura, P., M. Silverman and M. Simon.** 1977. Synthesis of *mot* and *che* gene products of *Escherichia coli* programmed by hybrid ColE1 plasmids in minicells. J. Bacteriol. **132**:996–1002.
17. **Parkinson, J. S.** 1974. Data processing by the chemotaxis machinery of *Escherichia coli*. Nature (London) **252**:317–319.
18. **Parkinson, J. S.** 1976. *cheA*, *cheB*, and *cheC* genes of *Escherichia coli* and their role in chemotaxis. J. Bacteriol. **126**:758–770.
19. **Parkinson, J. S.** 1977. Behavioral genetics in bacteria. Annu. Rev. Genet. **11**:397–414.
20. **Silverman, M., and M. Simon.** 1974. Flagellar rotation and the mechanism of bacterial motility. Nature (London) **249**:73–74.
21. **Silverman, M., and M. Simon.** 1976. Operon controlling motility and chemotaxis in *E. coli*. Nature (London) **264**:577–579.
22. **Silverman, M., and M. Simon.** 1977. Identification of polypeptides necessary for chemotaxis in *Escherichia coli*. J. Bacteriol. **130**:1317–1325.
23. **Silverman, M., and M. Simon.** 1977. Chemotaxis in *Escherichia coli*: methylation of *che* gene products. Proc. Natl. Acad. Sci. U.S.A. **74**:3317–3321.
24. **Springer, M. S., M. F. Goy, and J. Adler.** 1977. Sensory transduction in *Escherichia coli*: two complementary pathways of information processing that involve methylated proteins. Proc. Natl. Acad. Sci. U.S.A. **74**: 3312–3316.
25. **Springer, W. R., and D. E. Koshland, Jr.** 1977. Identification of a protein methyltransferase as the *cheR* gene product in the bacterial sensing system. Proc. Natl. Acad. Sci. U.S.A. **74**:533–537.
26. **Warrick, H. M., B. L. Taylor, and D. E. Koshland, Jr.** 1977. Chemotactic mechanism of *Salmonella typhimurium*: preliminary mapping and characterization of mutants. J. Bacteriol. **130**:223–231.