Interaction of the *cheC* and *cheZ* gene products is required for chemotactic behavior in *Escherichia coli*

(reversion analysis/functional suppression/flagellar rotation/sensory transduction)

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ABSTRACT   Previous work has shown that the *cheC* gene product of *Escherichia coli* plays a key role in regulating the direction of flagellar rotation during chemotactic responses. An attempt was made to identify other stimulus transduction elements that interact with the *cheC* component by examining *cheC* revertants for functional suppressors. Approximately two-thirds of the revertants studied appeared to be due to back mutation or to second-site mutations near or within the *cheC* structural gene. The remainder of the revertants carried suppressor mutations that mapped at the *cheZ* locus. Half of these suppressors impaired chemotaxis in a *cheC*+ background and were shown by complementation analysis to be defective in *cheZ* function. These suppressors corrected *cheC* defects in an allele-specific pattern, suggesting that the *cheC* and *cheZ* proteins are in direct contact and are mutually corrective due to protein–protein interaction. Observation of swimming patterns and flagellar rotation in *cheC cheZ* mutants demonstrated that the interaction of these two gene products influences both the spontaneous frequency of flagellar reversals and the ability of the rotational machinery to respond to chemotactic stimuli. A model of this interaction and its possible role in chemotaxis are discussed.

Stimulus detection, signaling, and behavioral response are basic features of sensory transduction systems in both prokaryotes and eukaryotes. Bacterial chemotaxis is a useful model for exploring these events at the molecular level. In *Escherichia coli* chemotactic responses are initiated by specific receptors that monitor the organism's chemical environment as it swims (1). In the absence of stimuli, wild-type cells swim in a random walk pattern (2) consisting of smooth "runs" and abrupt directional changes or "tumbles", both of which are produced by rotation of the flagellar filaments (3–5): runs by counterclockwise rotation and tumbles by clockwise rotation. Upon detecting a change in attractant or repellent concentration (6), the chemoreceptors generate signals that modulate flagellar rotation to produce an appropriate locomotor response. When headed in a favorable direction, tumble probability decreases, and when headed in an unfavorable direction, tumble probability increases (2, 6).

Studies of nonchemotactic mutants have identified a number of gene products that might be components of the signaling system in *E. coli* (7). Although the functions of most chemotaxis genes are still poorly understood, the *cheC* gene appears to play a key role in the transmission of sensory information from receptors to flagella. *CheC* mutants are motile but nonchemotactic and in the absence of stimuli exhibit very little tumbling behavior (8, 9). These mutants are typically somewhat leaky and also partially dominant (9), indicating that they probably make an altered but still functional product rather than an inactive one. This product may be a component of the flagellum, because *cheC* mutants are not complemented by nonflagellate

mutants defective in *flaA* function (10). Both *cheC* and *flaA* mutants probably arise by different sorts of mutations in the same gene: null defects appear to result in a nonflagellate condition (*flaA*), whereas more subtle changes seem to permit flagellar assembly, but interfere with proper rotational behavior (*cheC*). Thus, the *cheC* (*flaA*) gene product may be an essential structural component of the flagellum that is somehow involved in determining the direction of flagellar rotation. Studies of the residual chemotactic responses in *cheC* mutants (9) and in an analogous class of *Salmonella typhimurium* mutants (11) have led to the suggestion that the *cheC* product might interact directly with the signaling system of the chemoreceptors to effect changes in rotational behavior (7, 11).

It might be possible to identify signaling functions by virtue of their ability to interact with the *cheC* product. For example, many sorts of gene interactions can result in the suppression or modification of a mutant phenotype (12). It seemed likely that *cheC* mutants, because they owe their phenotype to a seemingly minor alteration of a flagellar protein, might be suppressed by correspondingly minor changes in interacting proteins. We therefore examined a large number of chemotactic revertants of *cheC* strains to determine whether *cheC* defects could be alleviated by mutations in other chemotaxis genes. In this report, we show that many *cheC* revertants in fact carry a compensating mutation at the *cheZ* locus, and that the *cheC* and *cheZ* gene products probably interact in a direct manner. This interaction affects both the spontaneous tumbling behavior of *E. coli* and the ability of the rotational machinery to respond to chemoreceptor signals.

MATERIALS AND METHODS

E. coli K12 strains RP252 [F⁻ *his trp (am)*] and RP477 (F⁻ *thi thr leu his eda* Δ(*gal-att*λ) *strA*] and their *cheC* derivatives were used in this work. *cheC* mutations *181, 182, 183* (9), and *497* (13) were introduced into RP252 and RP477 by contransduction with the *his* locus. The *supD* marker employed in initial test crosses was derived from strain CR63 (14) and transferred into RP252 by selecting Trp⁺ transductants and then testing for the ability to support the growth of *amber* mutants of phage λ. F′ strains for complementation analysis of *che* mutants have been described (9).

All other methods, including growth media, P1 transduction, and analysis of swimming behavior and flagellar rotation, have been described (9).

RESULTS

Isolation of *cheC* revertants containing external suppressors

Four different *cheC* mutations (alleles *181, 182, 183,* and *497*) were each introduced into strain RP252, and chemotactic revertants were selected by picking "swarms" on semisolid tryptone agar (15). For the identification of revertants in which

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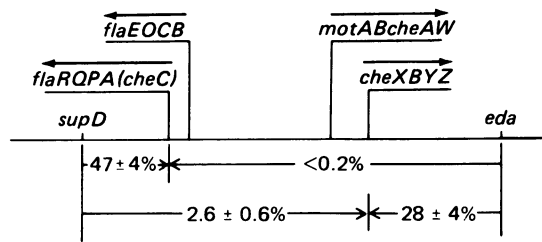
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FIG. 1. Chemotaxis and flagellar genes in *cheC* region. This segment is located between minutes 41 and 43 in the *E. coli* chromosome (16) and contains genes for chemotaxis (*che*), motility (*mot*), and flagellar assembly (*fla*), not all of which are shown. Direction of transcription and extent of operons are indicated by arrows above the genes. Location of these operons relative to the outside markers *supD* and *eda* is shown approximately to scale. Below the map are P1 cotransduction frequencies (from ref. 9) for map intervals discussed in this work. Arrows point to the unselected marker in each cross.

the *cheC* defect had been corrected by mutations outside the *cheC* structural gene, we test-crossed each revertant to map the site responsible for restoring chemotaxis. In true revertants and in those with second-site mutations in the *cheC* gene, the site of reversion should map at the *cheC* locus, whereas, in revertants that carry suppressors outside the *cheC* gene, the reversion site (suppressor locus) might not map near the *cheC* gene. As shown in Fig. 1, the *cheC* gene is approximately 50% cotransducible with the *supD* locus. Thus, any reversion sites that show a significantly different linkage to *supD* should represent external suppressors of *cheC* (hereafter designated *scc* to denote suppressors of *cheC*).

Cotransduction frequencies between the site of reversion and the *supD* locus were determined for each revertant strain by means of the test cross shown in Fig. 2. Each revertant was infected with P1 phage grown on a *supD* derivative of the original *cheC* parent strain, and the proportion of *supD* transductants that were no longer chemotactic was measured. Because the donor strain also carries the original *cheC* mutation, nonchemotactic transductants should arise by inheriting both the *supD* (selected) marker and the (unselected) donor allele corresponding to the site of reversion in any particular revertant strain. The proportion of *supD* transductants that are nonchemotactic therefore provides a measure of the distance between the *supD* marker and the reversion site.
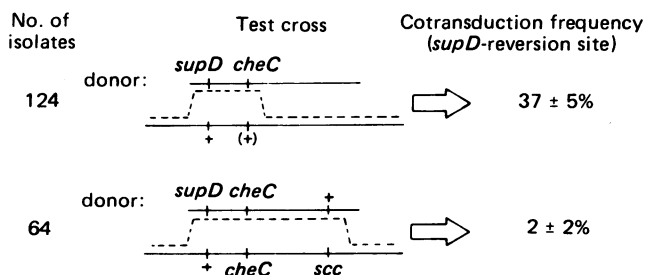

FIG. 2. Summary of test-cross results for mapping reversion sites in *cheC* revertants. Revertants of RP252 *cheC* strains were infected with P1 grown on a *supD cheC* donor, and Trp+ (i.e , *supD*) transductants were selected and tested for chemotactic ability (50 transductants from each independent revertant). The revertant strains could be divided into two discrete groups based on the frequency of nonchemotactic segregants. The crossover diagrams indicate the probable genotype of each group and the exchanges required to generate a nonchemotactic recombinant. Those revertants giving a high frequency of nonchemotactic transductants appear to have reversion sites near or within the *cheC* gene (upper diagram); those yielding a low frequency of nonchemotactic transductants carry external suppressors (*scc* mutations) some distance from the *supD* and *cheC* loci (lower diagram).

Test-cross results for a sample of 188 independent revertants indicated that at least two types of revertants were obtained (Fig. 2). Approximately two-thirds of the strains had an average cotransduction frequency of 37 ± 5%. Subsequent crosses showed that in these strains the reversion site is tightly linked to the *cheC* locus, and it seems likely that many of these revertants arose by back mutation or by secondary mutations within the *cheC* gene. Because genes that specify interacting proteins are often located near one another it is conceivable that some of these revertants actually carry suppressor mutations in nearby *fla* genes (see map in Fig. 1). This might account for the fact that cotransduction frequencies between *supD* and the reversion sites in this group of strains were generally somewhat less than would be expected if the reversion events had occurred at the *cheC* locus.

The second group of revertants exhibited cotransduction values of 2 ± 2% in the test cross (Fig. 2) and clearly contain reversion sites (i.e., *scc* mutations) some distance from the *cheC* locus, but still linked to *supD*. Several clusters of *che* genes, which are loosely linked to *supD*, are cotransducible with the *eda* locus, whereas *cheC* is not (see Fig. 1). To determine whether the *scc* mutations in this group of revertants were located near these clusters, each mutation was tested for linkage to the *eda* locus. P1 lysates prepared on each *cheC scc* (*eda*+) strain were used to transduce the *eda*+ marker into *eda cheC* recipients derived from strain RP477, and the frequency of chemotactic transductants was measured. All 64 of the *scc* donor strains tested yielded chemotactic transductants in this cross (mean cotransduction frequency of 20 ± 5%), indicating that the *scc* mutations are linked to *eda*, probably in the vicinity of the *cheX* operon (see Fig. 1).

When transferred in a similar manner into RP477 (*cheC*+), half (32/64) of the *scc* mutations produced a partial or complete defect in chemotaxis, whereas the others had little or no effect on chemotactic ability. These two groups of *scc* mutations will be referred to as type I and type II, respectively. Complementation tests (performed with F' elements carrying various *che* mutations) demonstrated that all of the type I mutations were defective in *cheZ* function. Although a definitive gene assignment for the type II mutations could not be made by complementation analysis, owing to lack of a suitable phenotype, it seems likely that these mutations are also alleles of the *cheZ* gene because type I and type II mutations have similar map positions, suppression patterns (see below), and possible modes of suppression (see below). The properties of *cheC* revertants carrying either type I or II *scc* mutations are summarized in Fig. 3 and discussed in the following sections. To simplify this discussion, we make the assumption that both groups of *scc* mutations represent alterations of *cheZ* function, and confine our attention to consideration of the CheC–CheZ interaction.

Effect of CheC–CheZ interaction on tumbling frequency

Mutants defective in *cheZ* function have very high tumbling rates (9, 17). In a *cheC*+ background, type I *scc* mutations produced very high tumbling rates comparable to those of *cheZ* mutants; type II mutations also caused above normal tumbling rates, although generally not as high as in type I strains (data not shown). In combination with a *cheC* defect, which alone causes a very low tumbling rate, both types of *scc* mutations produced various tumbling frequencies (Fig. 3 *bottom*). As a general rule, revertants with type II mutations had somewhat lower tumbling frequencies than those with type I mutations, which suggests that the opposing tumbling defects caused by *cheC* and *scc* (*cheZ*) mutations may interact in a roughly ad-
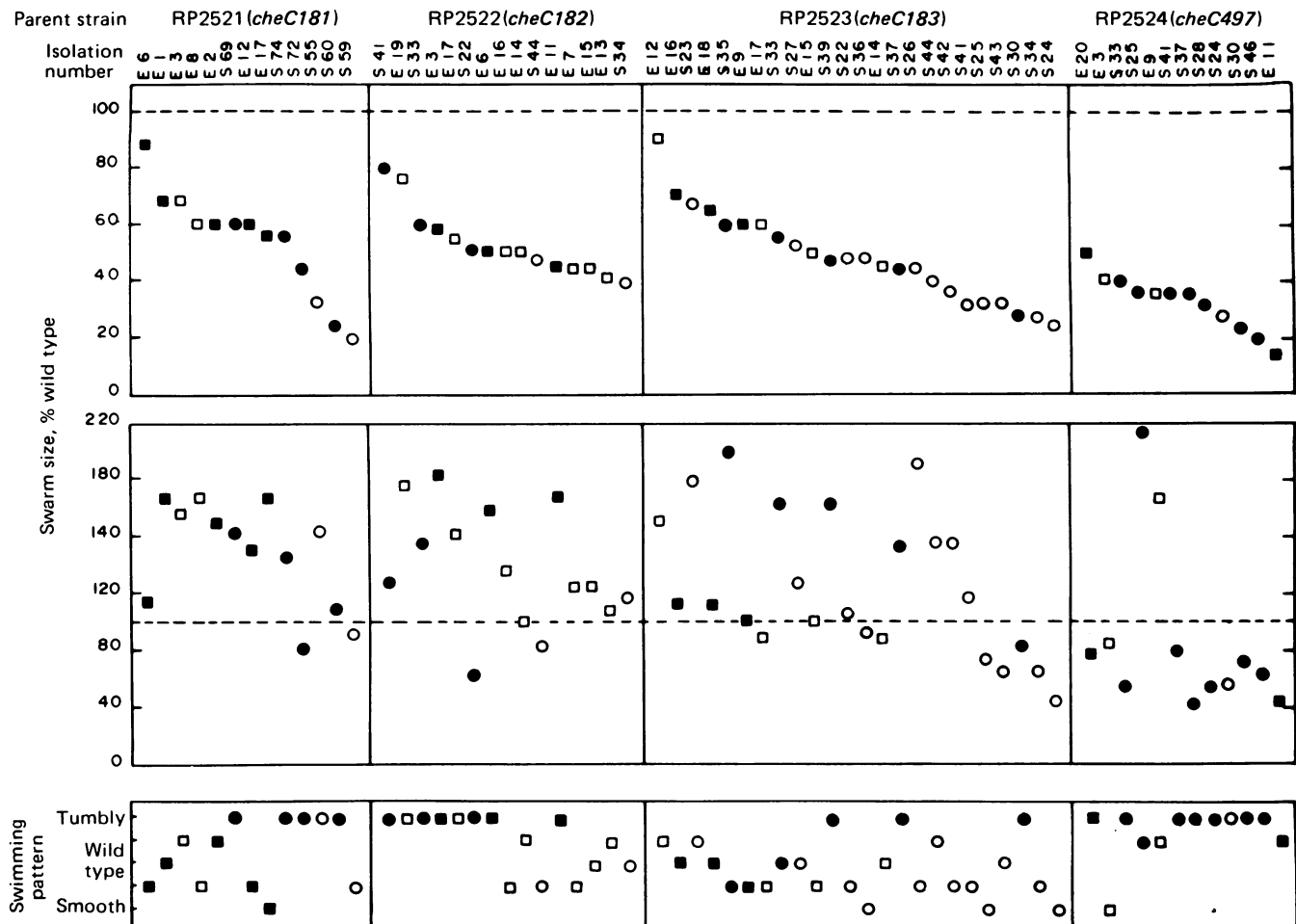
FIG. 3.   Chemotactic behavior and swimming patterns of *cheC* revertants containing *scc* mutations. Chemotactic ability was assessed by measuring swarm diameters on semisolid tryptone agar after 9 hr at 35°C (*Top*) or after 17 hr at 24°C (*Middle*). Five colonies from each revertant were measured, and a wild-type control was included on each plate for normalization purposes. Variation within each strain was negligible (the height of each data point represents over two standard deviations in every case). (*Bottom*) Swimming behavior of log phase cells grown in tryptone broth at 35°C was evaluated by direct microscopic observation at room temperature (≈24°C). Each strain was assigned to one of five categories based on the frequency of tumbling: at one extreme, smooth strains (e.g., *cheC*) showed no tumbling; at the other extreme, tumbly strains (e.g., *cheZ*) showed constant tumbling. Closed symbols denote revertants carrying type I *scc* mutations; open symbols denote revertants carrying type II *scc* mutations (see text). Spontaneous revertants are indicated by circles; ethyl methanesulfonate-induced revertants are indicated by squares.

ditive fashion. Because the ability to tumble is essential for chemotaxis, this might account for the ability of *scc* mutations to suppress the chemotaxis defect of *cheC* strains. To test this notion, we examined chemotactic ability and patterns of flagellar rotation in a series of *cheC cheZ* double mutants in which the *cheZ* mutations had been derived directly from wild type rather than as suppressors of *cheC*. As shown in Fig. 4, these double mutants had rotational patterns intermediate between those of the component single mutants, which confirms that *cheC* and *cheZ* have an additive effect on tumbling behavior. However, none of these double mutants were chemotactic (data not shown), which indicates that restoration of a fairly normal tumbling rate is not a sufficient condition for chemotaxis in *cheC cheZ* strains. Comparison of chemotactic ability and swimming patterns in *cheC* revertants also demonstrates this point (Fig. 3): some revertants with wild-type tumbling rates were less chemotactic than some with very low or very high tumbling rates. That tumbling frequency and chemotactic ability are not necessarily related in the revertants implies that, in addition to setting the spontaneous tumbling rate, the CheC-CheZ interaction may influence the ability of the tumbling machinery to respond to chemoreceptor signals.

Allele-specificity of the CheC–CheZ interaction

As mentioned above, not all *cheZ* mutations are capable of suppressing the chemotaxis defect of *cheC* mutants, which shows that a specific sort of *cheZ* alteration is required. Moreover, absence of *cheZ* product evidently does not lead to suppression, because none of the *scc* alleles appear to be nonsense mutations (which would have exhibited apparent 100% linkage to *supD*, a nonsense suppressor, in the initial test crosses). These findings indicate that the *cheC* and *cheZ* products may interact directly and that only combinations that properly "fit" one another are capable of restoring chemotaxis. Examination of suppression efficiency in different *cheC scc* strains demonstrates that this is probably the case (Fig. 5). Twenty-five *scc* mutations were transferred to various *cheC* backgrounds, and chemotactic ability was determined by measuring swarm size on semisolid tryptone agar. Many of the suppressors (e.g., *scc-5*, *scc-6*) seemed to function in all four *cheC* backgrounds, which suggests that they are able to recognize and correct some aspect of the *cheC* defect common to all four mutant strains. Other suppressors, however, were able to distinguish between these *cheC* alleles. For example, *scc-12* works very well with *C182*
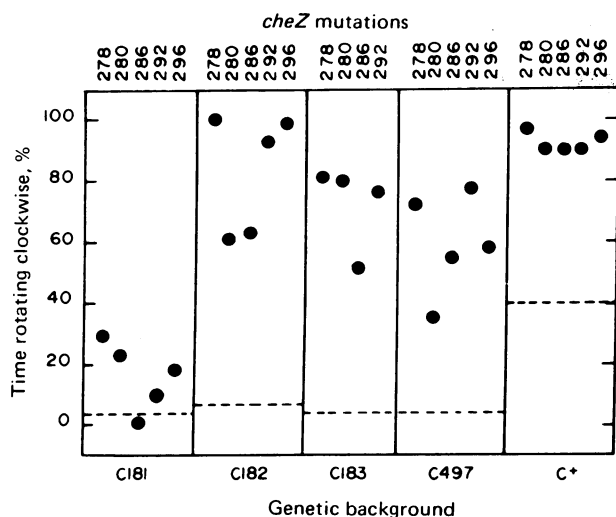
FIG. 4. Flagellar rotation patterns of *cheC cheZ* double mutants. Double mutants were constructed by introducing *cheZ* mutations into RP477 *cheC* recipients by P1 cotransduction with the *eda* locus. The *cheZ* alleles employed are described in refs. 9 and 17; alleles *286* and *292* are amber mutations. Since both the double mutants and the original recipients were nonchemotactic, doubles were identified by picking *eda*⁺ transductants at random and backcrossing each to RP477 to test for the presence of an *eda*-linked *che* mutation. Strains were grown and tethered for rotational analysis as described in ref. 9. Each rotating cell was examined for 30 sec, and the proportions of time spent rotating clockwise and counterclockwise were measured. Each data point represents the average clockwise time for at least 20 cells. Dotted lines indicate the rotational behavior of the recipient *cheC* strains and RP477 (*cheC*⁺).

and *C183*, but very poorly with *C181* and *C497*. Moreover, suppressors that behaved the same in one *cheC* background (e.g., *scc-12* and *scc-18* in *C183*) often behaved quite differently in another background (e.g., *C182*). In summary, the effect of an *scc* mutation on any particular *cheC* allele could not be predicted from its behavior in other *cheC* backgrounds, demonstrating that *scc* mutations act in an allele-specific fashion. The highly specific nature of this interaction implies that the *cheC* and *scc* (i.e., *cheZ*) gene products themselves are either transiently or permanently associated during the chemotaxis process.

Comparisons of chemotactic ability in *cheC scc* strains at 35°C and 24°C emphasize the specificity of the *cheC–cheZ* interaction (Fig. 3 *top* and *middle*). At 35°C, the temperature at which they were originally isolated, none of the suppressed revertants were as chemotactic as wild type, which demonstrates that the mutant products cannot function together as well as their wild-type counterparts. This implies that any protein interactions involved are probably less stable than in wild type. At 24°C, many of the revertant strains exhibited improved chemotactic ability, often even better than wild type. The lack of correlation between chemotactic ability at the two temperatures indicates that each combination of *cheC* and *scc* (*cheZ*) gene products responds to temperature changes in a unique way, which is consistent with the notion that these proteins are in direct contact.

DISCUSSION

In wild-type *E. coli*, spontaneous flagellar reversals occur about once per second (18), ensuring that, in spatial gradients of attractants or repellents, the organism's run length is sufficiently long to detect concentration differences before tumbling and yet short enough to prevent rotational diffusion from causing major course changes (19). Thus, changes in swimming direc-
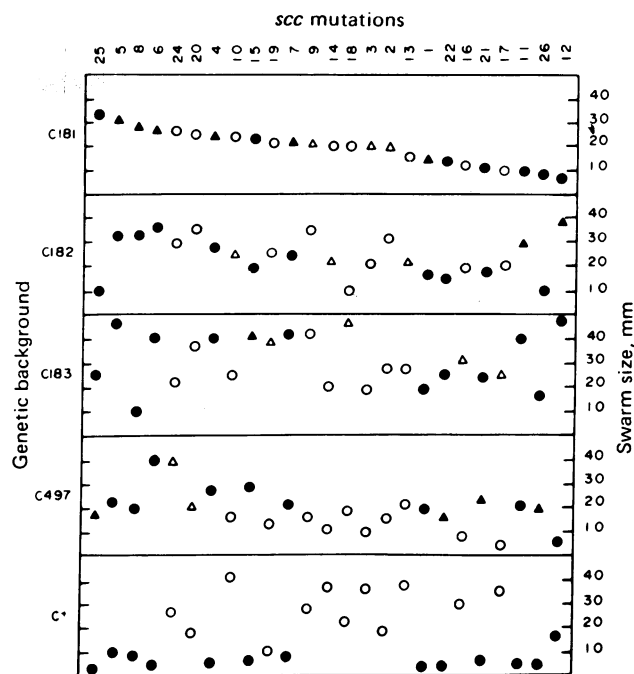

FIG. 5. Allele-specificity of *scc* mutations. Various *scc* mutations were introduced into RP477 (*cheC*⁺) and RP477 *cheC* recipients by cotransduction with the *eda* locus. Double mutants that were nonchemotactic were confirmed by backcrosses as described in Fig. 4. Chemotactic ability was assessed by measuring swarm diameters as described in Fig. 3. Each data point represents the mean swarm size for a particular *cheC scc* combination; in all cases, SD was 2 mm or less. Closed symbols denote type I *scc* mutations; open symbols denote type II *scc* mutations (see text). The *cheC* mutation from which a particular suppressor was originally derived is indicated by a triangle. The set of suppressors has been ordered with respect to efficiency of suppression in the *cheC181* background to facilitate comparisons with the other *cheC* backgrounds.

tion are brought about by tumbling, and chemotaxis can be achieved by modulating the probability of flagellar reversal in response to stimuli. Like wild type, the flagella of *cheC* and *cheZ* mutants are capable of rotating in either direction (9). However, *cheC* mutants tend to remain in the counterclockwise (run) mode, whereas *cheZ* mutants rotate predominately in the clockwise (tumble) mode. Analysis of *cheC* revertants has demonstrated that certain combinations of *cheC* and *cheZ* defects can lead to restoration of chemotactic ability even though each mutation separately produces a nonchemotactic phenotype. This interaction probably involves direct contact between the *cheC* and *cheZ* proteins and generally leads to a tumbling rate that is intermediate between the very low and very high rates produced by the two component mutations separately.

A model of the CheC–CheZ interaction is shown in Fig. 6. We assume that the *cheC* product is a flagellar component, perhaps located in the basal body or the adjacent cytoplasmic membrane, that determines the direction of flagellar rotation. The *cheZ* product, which is known to be a cytoplasmic protein (20), appears to influence the pattern of flagellar rotation by binding to the *cheC* component. We suggest that transitions between clockwise and counterclockwise rotation are accompanied by the formation or dissociation of a CheC–CheZ complex: counterclockwise rotation by CheZ binding, and clockwise rotation by CheZ release.

In the absence of chemotactic stimuli, the relative affinities of the *cheC* and *cheZ* proteins probably play a major role in establishing the spontaneous tumble rate of the cell, and the
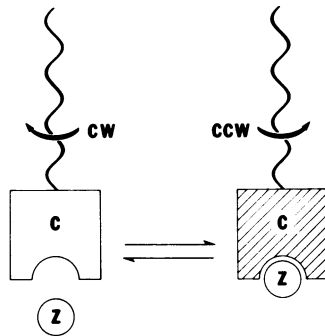
FIG. 6.   Model of CheC–CheZ interaction. The *cheC* and *cheZ* proteins may interact in a reversible manner to control tumbling behavior. Additional features of the model are discussed in the text.

properties of *cheC* and *cheZ* mutants are consistent with this picture. For example, all *cheZ* mutants, including those with nonsense mutations, have high tumbling rates, which could reflect a decreased ability to bind to the *cheC* component. On the other hand, *cheC* mutants that have low tumbling rates and are partially dominant might have an increased affinity for *cheZ* protein. In such mutants tumbling should be raised to more normal levels by reducing the ability of *cheZ* protein to bind to *cheC*, which may account for the observation that *cheC* and *cheZ* mutations have a roughly additive effect on tumbling frequency. According to this model, there should exist tumbly *cheC* mutants, which have reduced affinity for *cheZ*, and nontumbling *cheZ* mutants, which have increased affinity for *cheC*. Although mutants of the latter type have not yet been observed, we have recently obtained *cheC* mutants with very high tumbling rates, and similar mutants have also been found in *S. typhimurium* (11).

What is the role of this interaction in chemotaxis? Clearly one consequence is to set the spontaneous tumbling rate of the cell; however this is not a sufficient condition for chemotaxis because *cheC scc* strains with similar tumbling rates often had very different chemotactic abilities, whereas those with similar chemotactic behavior often had very different tumbling rates (see Fig. 3). Moreover, mutations that appear to alter the relative affinities of the *cheC* and *cheZ* proteins can restore normal tumble frequencies but still preclude chemotaxis (see Fig. 4), suggesting that one or both of these proteins must participate in other processes necessary for chemotaxis. Because *cheZ* mutants still respond to chemotactic stimuli, although with high thresholds (21), it seems unlikely that *cheZ* product is responsible for *initiating* changes in flagellar rotation during chemoreceptor signaling, but it could be involved in *facilitating* or *maintaining* such changes.

Several lines of genetic evidence indicate that the *cheZ* product may also interact with another chemotaxis protein, the *cheB* product (7, 17, 22). Mutants defective in *cheB* function

lack a protein methylesterase activity (23) that has been implicated in the process of sensory adaptation (24). It may be that *cheZ* protein, through its interaction with *cheB* product, somehow regulates the activity of the adaptation system and thereby controls the duration of chemotactic responses. For example, the *cheB* and *cheZ* proteins might form a tight complex so that when the *cheZ* portion is bound to the *cheC* component the methylesterase is unable to reach its target sites. It should be possible to test this notion by further studies of the behavior, particularly the sensory adaptation ability, of *cheC* and *cheZ* strains. By extending the sorts of genetic studies described in this report we may eventually be able to construct a detailed picture of the ways in which various elements of the chemotaxis machinery interact with one another to generate chemotactic behavior.

1.   Adler, J. (1969) *Science* **166**, 1588–1597.
2.   Berg, H. C. & Brown, D. A. (1972) *Nature (London)* **239**, 500–504.
3.   Berg, H. C. & Anderson, R. A. (1973) *Nature (London)* **245**, 380–382.
4.   Silverman, M. & Simon, M. (1974) *Nature (London)* **249**, 73–74.
5.   Larsen, S. H., Reader, R. W., Kort, E. N., Tso, W.-W. & Adler, J. (1974) *Nature (London)* **249**, 74–77.
6.   Macnab, R. W. & Koshland, D. R., Jr. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2509–2512.
7.   Parkinson, J. S. (1977) *Annu. Rev. Genetics* **11**, 397–414.
8.   Armstrong, J. B., Adler, J. & Dahl, M. M. (1967) *J. Bacteriol.* **93**, 390–398.
9.   Parkinson, J. S. (1976) *J. Bacteriol.* **126**, 758–770.
10.   Silverman, M. & Simon, M. (1973) *J. Bacteriol.* **116**, 114–122.
11.   Rubik, B. A. & Koshland, D. E., Jr. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2820–2824.
12.   Hartman, P. E. & Roth, J. R. (1973) *Adv. Genet.* **17**, 1–105.
13.   Armstrong, J. B. & Adler, J. (1969) *Genetics* **61**, 61–66.
14.   Signer, E. R., Beckwith, J. R. & Brenner, S. (1965) *J. Mol. Biol.* **14**, 153–166.
15.   Adler, J. (1966) *Science* **153**, 708–716.
16.   Bachmann, B. L., Low, K. B. & Taylor, A. L. (1976) *Bacteriol. Rev.* **40**, 116–167.
17.   Parkinson, J. S. (1978) *J. Bacteriol.* **135**, 45–53.
18.   Berg, H. C. (1974) *Nature (London)* **249**, 77–79.
19.   Brown, D. A. & Berg, H. C. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1388–1392.
20.   Ridgway, H. F., Silverman, M. & Simon, M. (1977) *J. Bacteriol.* **132**, 657–665.
21.   Parkinson, J. S. (1974) *Nature (London)* **252**, 317–319.
22.   DeFranco, A. L., Parkinson, J. S. & Koshland, D. E., Jr. (1979) *J. Bacteriol.*, in press.
23.   Stock, J. B. & Koshland, D. E., Jr. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3659–3663.
24.   Goy, M. F., Springer, M. S. & Adler, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4964–4968.