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 procaryotes 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− thr leu his eda A(gal-attλ) 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 transduction 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
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-
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.

**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.
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 direction 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
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 non tumbling 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.

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