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Novel Mutations Affecting a Signaling Component for Chemotaxis of *Escherichia coli*

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The genetic relationship between *tsr* and *cheD* mutations, which affect chemotactic ability and map at approximately 99 min on the *Escherichia coli* chromosome, was investigated. Mutants defective in *tsr* function typically exhibited wild-type swimming patterns, but were unable to carry out chemotactic responses to a number of attractant and repellent chemicals. In contrast, *cheD* mutants swam smoothly, with few spontaneous directional changes, and were generally nonchemotactic. In complementation tests, *cheD* mutations, unlike *tsr*, proved to be dominant to wild type, suggesting that the *cheD* defect might be due to an active inhibitor of chemotaxis. Mutations that inactivated the putative inhibitor were obtained by selecting for restoration of chemotactic ability or for loss of *cheD* dominance. The resultant double mutants were shown to carry the original *cheD* mutation and a second tightly linked mutation, some of which exhibited nonsense or temperature-sensitive phenotypes, implying that they had occurred in a structural gene for a protein. All such double mutants behaved like typical *tsr* mutants in all other respects, including complementation pattern, swimming behavior, and chemotactic ability. These findings implied that either overproduction of *tsr* product or synthesis of an aberrant *tsr* product was responsible for the chemotaxis defect of *cheD* strains. Such mutants should be useful in analyzing the role of the *tsr* product in chemotactic responses.

Chemotactic bacteria, such as *Escherichia coli*, possess specific chemoreceptors for detecting changes in their chemical environment (1). To elicit appropriate swimming responses, sensory information from the chemoreceptors must be communicated to the flagellar motor apparatus. In *E. coli*, three signaling components appear to play a major role in transmitting sensory data from the chemoreceptors to the flagella (9, 13, 22, 25). Each signaler is responsible for collecting data from a number of different receptor species and for sending some sort of integrated signal to the flagella to control swimming behavior. Mutants defective in any one of these signaling functions retain the ability to respond to stimuli that are processed by either of the other two pathways (8).

The structural genes for these three signaling components are designated *tsr*, *tar*, and *trg*. In addition, genetic studies have identified at least eight genes in *E. coli*, and a similar number in *Salmonella typhimurium*, that can mutate to produce a general loss of chemotactic ability without destroying motility (Che^- phenotype) (20). Several of these *che* functions appear to be involved in regulating the duration of chemotactic responses as part of the cell's sensory adaptation system (7, 21, 23, 27); however, the role of most *che* genes is still poorly understood. Due to their generally nonchemotactic behavior, it has usually been assumed that *che* mutants are defective in a late stage of signal transmission that is responsible for processing inputs from the *tsr*, *tar*, and *trg* signalers.

This report describes the properties of a novel class of *che* mutations that appear to affect any early step in the signaling process. These mutations define the *cheD* locus, which maps at approximately 99 min on the *E. coli* chromosome, unlike all other *che* mutations which are located in the region of 41 to 43 min (20). The only other chemotaxis mutations that map near 99 min are those found in *tsr* mutants (E. N. Kort, M. S. thesis, University of Wisconsin, Madison, 1970), which lack one of the major signal pathways. The results of the present study demonstrate that *cheD* and *tsr* mutations are somehow related even though they produce very different chemotaxis defects. It seems likely that both types of mutations affect the same gene product, but in different ways: *tsr* lesions probably inactivate the *tsr* signaling component, whereas *cheD* mutations either cause overproduction of *tsr* product or alter the *tsr* signaler in such a way that it inhibits all chemotactic responses. Various explanations of *cheD* mutant behavior are discussed in relation to our current understanding of sensory transduction events in bacterial

chemotaxis. Further studies of these mutants promise to provide unique insight about the role of the *tsr* component in the signaling scheme.

MATERIALS AND METHODS

Bacterial strains. Strains used are listed in Table 1; all are derivatives of *E. coli* K-12.

Media. Tryptone broth, minimal media, dilution buffer (TMG), and motility buffer (KEP) have been described (19). Tryptone swarm plates contained 0.35% agar (Difco Laboratories, Detroit, Mich.); minimal swarm plates contained 0.25% agar and one or more attractant compounds at a final concentration of approximately 0.1 mM.

General methods. All experiments were conducted at 35°C unless otherwise noted. Conditions for P1 transductions and Hfr crosses were essentially as described previously (19). F′ transfers were done by plate matings using one or more auxotrophic markers to counterselect the donor strain (19). In testing for the presence of an amber mutation, F′ *supU* was transferred to RP470, RP477, or their derivatives by selecting for suppression of the *thr*(Am) marker in these recipients. Capillary assays were performed according to the method of Adler (2). Patterns of swimming and flagellar rotation were assessed by published methods (19, 21).

Construction of F′ strains for complementation studies. F′ elements covering the *cheD-tsr* region were isolated from a derivative of HfrH by the general method described by Low (17). First, *cheD* and *tsr* mutations were introduced into HP246 by cotransduction with the *serB* marker. These Hfr strains were then mated to RP944, selecting for Thr$^+$ Leu$^+$ recombinants. Strains carrying F′ elements were identified by subsequent mating tests to other *recA* recipients.

TABLE 1. *Bacterial strains*

Strain	Relevant genotype	Source or reference
RP470	F$^-$ *thi thr*(Am) *leu his* Δ(*gal-att*λ) *recA rpsL*	(19)
RP477	F$^-$ *thi thr*(Am) *leu his* Δ(*gal-att*λ) *eda rpsL*	(19)
RP479	RP477 *thr*$^+$ *serB*	This laboratory
RP482	RP477 *thr*$^+$ *dnaC*(Ts)	This laboratory
RP4159	RP470 *cheD191*	Ethyl methane sulfonate mutagenesis of RP470 (19)
RP4160	RP470 *cheD192*	
RP4161	RP470 *cheD193*	
RP944	F$^-$ *thi thr leu pro arg his recA rpsL*	AB2463 (11)
RP4442	F$^-$ *thi thr leu his rpsL tsr-14*	R. Reader (22)
AW518	F$^-$ *thi thr leu his rpsL tsr-1*	J. Adler (10)
RP4790	RP477 *thr*$^+$ *tsr-14*	P1 transduction, this work
RP4791	RP477 *thr*$^+$ *cheD191*	
RP4792	RP477 *thr*$^+$ *cheD192*	
RP4793	RP477 *thr*$^+$ *cheD193*	
RP4794	RP477 *thr*$^+$ *tsr-1*	
HP146	Hfr(P045) [O-*recA-thyA-his*]	PK25 (12)
HP246	Hfr(P01) *serB* [O-*serB-thr-leu*]	R. Reader
FP5142	F′ *supU/recA his*(Am) *trp*(Am) *ilv arg met*	(24)

RESULTS

Map position of *cheD* and *tsr* mutations. Three independent *cheD* mutants, RP4159 (*cheD191*), RP4160 (*cheD192*), and RP4161 (*cheD193*), isolated in a previous study (19), were used in this work. To map these *cheD* mutations, the strains were first made *recA*$^+$ by mating to the Hfr strain, HP146. The *rec*$^+$ derivatives were then crossed to several other Hfr strains to determine the approximate position of the *cheD* locus. These Hfr crosses demonstrated that the *cheD* mutations were tightly linked to the *thr* locus at 0/100 min on the *E. coli* map. More accurate mapping crosses were then performed by P1 transduction, and the linkage of *cheD* alleles to the *dnaC*, *serB*, and *thr* markers was measured. The results (Fig. 1) indicate that *cheD* mutations are located between the *dnaC* and *serB* loci at approximately 99 min.

Two canonical *tsr* strains, AW518 (*tsr-1*) and

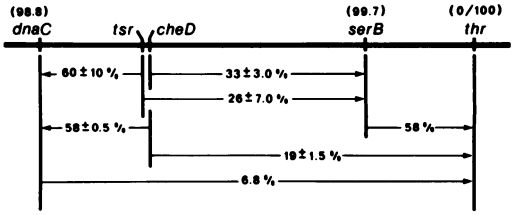

FIG. 1. *Map position of cheD and tsr mutations. Two tsr and three cheD alleles were located relative to the dnaC and serB markers by two-factor transductional crosses. Values below the map give cotransduction frequencies (and standard deviations) for each interval measured. At least 250 transductants were scored for each cross; arrows point toward the selected marker. Relative distances are shown approximately to scale based on the mapping function of Wu (30). Position of the serB and dnaC markers (shown in minutes above the map) was calculated relative to the thr locus.*

RP4442 (*tsr-14*), were also studied. The mutations responsible for the chemotaxis defects in these strains had been shown in previous work (Kort, M.S. thesis, 1970; J. S. Parkinson, unpublished data) to be about 20% cotransducible with and counterclockwise to the *thr* locus, which should place them very close to the *cheD* locus. Additional crosses to *dnaC* and *serB* recipients demonstrated that the *tsr* and *cheD* loci are probably tightly linked to one another (see Fig. 1).

In the course of these crosses, it was noticed that a small fraction of the transductants from crosses involving AW518 had acquired a nonchemotactic rather than a Tsr^+ or Tsr^- phenotype, suggesting that AW518 might be a double mutant of some sort (see bottom line of Table 4). The nature of the chemotaxis mutations in this strain is considered in a later section.

Comparison of *cheD* and *tsr* mutant phenotypes. The *cheD* and *tsr* mutations described above were transferred to strain RP477 by cotransduction with the *thr* locus in order to construct an essentially isogenic series of mutant strains for phenotypic analyses.

The chemotactic behavior of *cheD* and *tsr* mutants could be readily distinguished on tryptone swarm agar, which contains two amino acid attractants, serine and aspartic acid (Fig. 2). During growth of the bacteria these compounds are consumed, thereby establishing steep gradients which cause chemotactic cells to swarm away from the site of inoculation. Wild-type strains form two concentric rings of migrating cells, the outer due to serine taxis and the inner due to aspartate taxis. *tsr* mutants are attracted to aspartate, but are defective in serine taxis, and therefore lack the outer ring. Both rings are absent in *cheD* strains, indicating a defect in both serine and aspartate taxis. Additional swarm plate tests (not shown) demonstrated that *cheD* mutants were incapable of chemotaxis toward any of the attractants tested, including glucose, ribose, maltose, and fructose. Thus *cheD* strains exhibit swarm plate behavior characteristic of generally nonchemotactic mutants.

The chemotaxis defect in *cheD* strains was also assessed in capillary assays, which provide a quantitative measure of chemotactic ability (Fig. 3). RP4793 (*cheD193*) exhibited no detectable response to serine or aspartate; RP4792 (*cheD192*) exhibited a very slight response to aspartate, but essentially none to serine. RP4791 (*cheD191*) exhibited a significant response to both compounds, which is rather surprising in light of the swarm plate findings. Evidently, the defect in RP4791 is leaky, but for some reason this leakiness is difficult to detect in swarm plate tests. Note, however, that the *cheD191* swarm shown in Fig. 2 is slightly larger and more regular in appearance than swarms of *cheD192* or *cheD193*, which indicates some chemotactic ability even under these conditions.

The swimming patterns of *cheD* and *tsr* strains are consistent with their chemotactic ability. Like wild type, *tsr* strains exhibit frequent directional changes ("tumbles") while

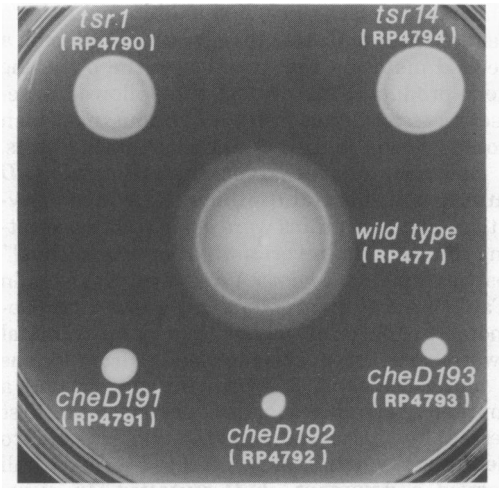

FIG. 2. *Appearance of tsr and cheD strains on tryptone swarm agar. A single colony of each strain shown was transferred to a tryptone swarm plate and incubated at 35°C for 8 h.*

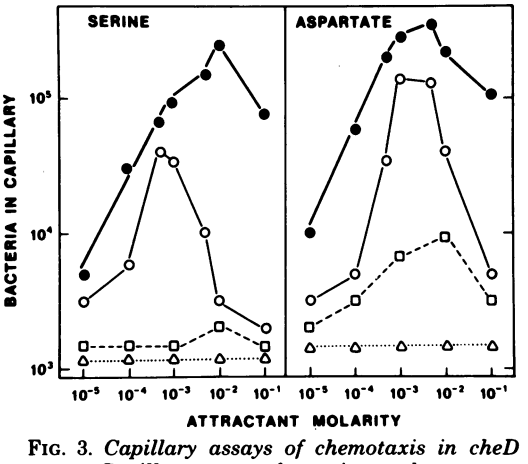

FIG. 3. *Capillary assays of chemotaxis in cheD mutants. Capillary assays for serine and aspartate were performed as described in the text. Symbols: ●, RP477 (wild type); ○, RP4791 (cheD191); □, RP4792 (cheD192); △, RP4793 (cheD193). Note that the number of bacteria inside the capillary is plotted on a logarithmic scale to permit all responses to be shown on the same figure.*

swimming. The ability to tumble is essential for chemotaxis because attractant and repellent stimuli elicit responses by modulating tumble probability (4, 18). Like most other classes of *che* mutants, *cheD* strains exhibit little or no spontaneous tumbling activity and are therefore incapable of biasing tumble frequency in response to chemical stimuli (data not shown). These differences in the swimming behavior of *cheD* and *tsr* strains were also measured in a quantitative manner by examining the patterns of flagellar rotation in the two types of mutants (Fig. 4). Swimming movements are produced by counterclockwise rotation of the flagellar filaments, whereas tumbling episodes are initiated by clockwise reversals (16). In both *tsr* and wild-type strains, nearly 100% of the rotating cells (i.e., flagella) exhibited frequent reversals, although most of the reversing cells spent the majority of their time in counterclockwise rotation (Fig. 4). RP4792 and RP4793, which exhibited essentially no response to serine or aspartate in capillary tests, also exhibited few or no flagellar reversals; nearly all of the cells rotated exclusively counterclockwise (Fig. 4). RP4791, carrying the *cheD191* allele, was rather chemotactic in capillary assays and also exhibited a significant proportion (~40%) of reversing cells (Fig. 4). Thus spontaneous flagellar reversals and residual chemotactic ability are closely correlated in *cheD* strains. It seems likely that the inability of *cheD* mutants to carry out chemotactic responses is a direct consequence of their inability to initiate tumbling movements while swimming; however, it is not yet certain that the tumbling behavior of *cheD* mutants is a direct rather than indirect effect of the *cheD* defect.

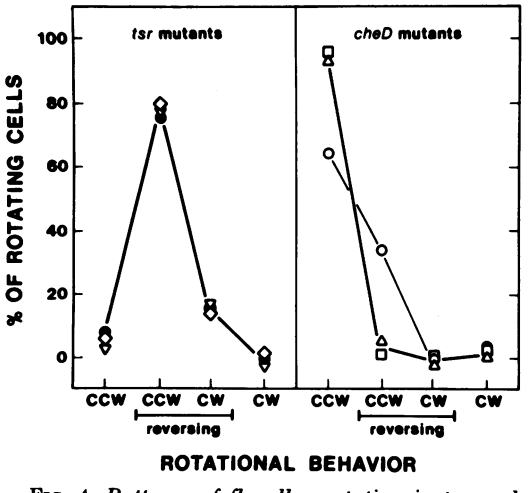

FIG. 4. *Patterns of flagellar rotation in tsr and cheD mutants. Bacteria were grown and tethered for microscope observation as described previously (19). At least 100 rotating cells of each strain were examined for 15 s each and classified as either exclusively counterclockwise (CCW), predominately CCW but reversing, predominately clockwise (CW) but reversing, or exclusively CW. Symbols: ●, RP477 (wild type); ▽, RP4790 (tsr-14); ◇, RP4794 (tsr-1); ○, RP4791 (cheD191); □, RP4792 (cheD192); △, RP4793 (cheD193).*

Complementation analysis of *tsr* and *cheD* mutants. The functional relationship between *cheD* and *tsr* defects was examined in complementation tests using F′ elements to construct partial diploids for the *cheD-tsr* region. Episomes carrying either wild-type or mutant alleles at the *cheD* and *tsr* loci were constructed as described in Materials and Methods and transferred into wild type, *cheD*, and *tsr* recipients. The chemotactic ability of the resulting merodiploids was assessed on tryptone swarm agar (Table 2). Both *tsr* recipients were complemented by F′180 (*tsr*$^+$), but not by F′181 (*tsr-1*), showing that these two *tsr* mutations are recessive to wild type and belong to the same complementation group. However, any merodiploid containing a *cheD* allele was generally nonchemotactic (Table 2), indicating that *cheD* defects are dominant to wild type. This was true regardless of whether the *cheD* mutation was carried on the episome or in the chromosome. The dominance of *cheD* mutations precluded standard complementation analysis and necessitated more indirect methods for analyzing *cheD* mutants. Since the dominance could be due to overproduction of a normal gene product or to formation of an aberrant product, either of which might interfere with the operation of the chemotaxis machinery, it should be possible to render *cheD* mutants recessive by introducing a second mutation that inactivates the offending gene product. This strategy was followed to select recessive derivatives of *cheD* mutants which could then be studied in complementation tests.

Selection of recessive derivatives of *cheD* mutants in partial diploids. Recessive derivatives of *cheD* strains were generated by selecting phenotypic revertants from *cheD*/*cheD*$^+$ partial diploids. The *recA* merozygotes listed in Table 3 were constructed, and derivatives chemotactic to serine were selected on minimal swarm agar. Phenotypic revertants from strains 1 and 2 of Table 3, which initially carried a mutant *cheD* allele on the episome, were first tested by mating to a *cheD*$^+$ *recA* tester to determine whether any of the F-primes still carried a dominant *cheD* mutation. In every such revertant tested, phenotypic reversion appeared to have taken place on the episome (Table 3). In many of these strains, this reversion also appeared to be associated with loss of other

TABLE 2. *Swarm plate phenotypes of cheD and tsr partial diploids*

Recipient strain	Mutant allele	F' donor			
		F' 180 Wild type	F' 181 *tsr-1*	F' 182 *cheD192*	F' 183 *cheD193*
RP477	Wild type	Wild type	Wild type	Che	Che
RP4794	*tsr-1*	Wild type	Tsr	Che	Che
RP4790	*tsr-14*	Wild type	Tsr	Che	Che
RP4791	*cheD191*	Che	Che	Che	Che
RP4792	*cheD192*	Che	Che	Che	Che
RP4793	*cheD193*	Che	Che	Che	Che

TABLE 3. *Location of reversion site in phenotypic revertants of cheD/cheD⁺ strains*

Partial diploid	No. of independent phenotypic revertants with reversion site located on[a]:	
	Exogenote	Endogenote
1. F'182/RP470 (*cheD192/cheD⁺*)	177	0
2. F'183/RP470 (*cheD193/cheD⁺*)	174	0
3. F'180/RP4160 (*cheD⁺/cheD192*)	0	136
4. F'180/RP4161 (*cheD⁺/cheD193*)	0	112

[a] Revertants from strains 1 and 2 were mated to a *his⁺* derivative of RP470; revertants from strain 3 were mated to a *his⁺* derivative of RP4160; revertants from strain 4 were mated to a *his⁺* derivative of RP4161. All recombinants were tested for chemotactic ability on minimal serine swarm agar.

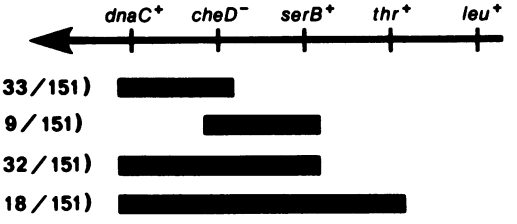

FIG. 5. *Patterns of marker loss associated with cheD reversion in partial diploids. Revertants of strains 1 and 2, Table 4, were mated to his⁺ derivatives of RP477, RP479, and RP482 to test for retention of the thr⁺, serB⁺, and dnaC⁺ markers originally present on the cheD episome. Of 151 episomes tested, 82 proved to have lost one or more markers adjacent to the cheD locus.*

episomal markers (e.g., *dnaC* and *serB*) flanking the *cheD* locus. It seems likely that the selection for loss of *cheD* dominance yielded deleted episomes that had lost the *cheD* region. The patterns of deleted markers summarized in Fig. 5 are consistent with this view.

Phenotypic revertants from strains 3 and 4 (see Table 3), which initially carried a wild-type episome, were mated to *recA cheD* recipients to determine whether any of the wild-type episomes had undergone a change that could abolish *cheD* dominance. In every case the episomes remained recessive upon transfer to a *cheD* tester (Table 3), demonstrating that in these revertants the mutational event must have occurred in the endogenote, rather than on the F'. In summary, then, it was possible to select phenotypic revertants from *cheD/cheD⁺* strains, and in every case the reversion event occurred *cis* to the mutant *cheD* allele, which suggests that inactivation of the *cheD* locus itself or of some gene under its direct control is the most common route of reversion in such partial diploids.

To study the properties of these recessive *cheD* derivatives, revertants from strains 3 and 4 (Table 3), which should carry chromosomal mutations that eliminate *cheD* dominance, were cured of their episomes by acridine orange treatment. Ten revertants of each strain were tested and all appeared to be readily cured by acridine orange treatment, implying that the reversion event had not led to a recessive lethal condition. The chemotactic behavior of the cured strains was tested on tryptone swarm agar. All strains exhibited a typical Tsr⁻ rather than Che⁻ phenotype (data not shown), suggesting that loss of *tsr* function can alleviate *cheD* dominance. This would be the case if *cheD* mutants produced an excess of *tsr* product, which somehow interfered with chemotaxis, or if *cheD* mutations actually occurred in the *tsr* gene and somehow converted *tsr* product into an inhibitor of chemotaxis. Both of these models make a number of similar predictions that have been tested and confirmed, as discussed below.

(i) If inactivation of *tsr* function can eliminate *cheD* dominance, then on tryptone swarm agar *cheD* mutants should readily revert to a Tsr⁻ phenotype. *cheD* mutants reverted at high frequency to a characteristic Tsr⁻ behavior (Fig. 6). A number of these pseudorevertants have been examined in complementation tests, and

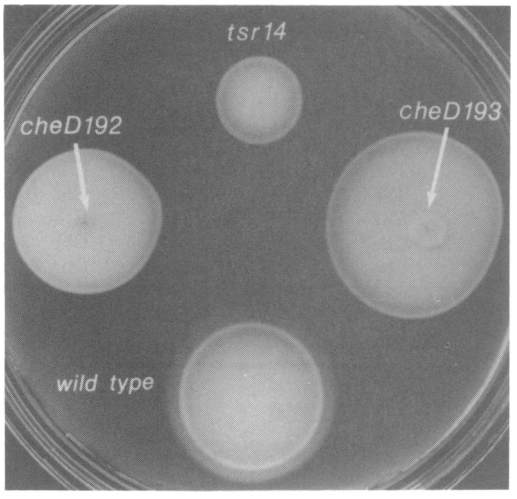

FIG. 6. *Phenotypic reversion of cheD mutants on tryptone swarm agar. Colonies of the indicated cheD strains were transferred to tryptone swarm plates and incubated at 35°C for 24 h. The wild-type and tsr control strains were inoculated at 15 h to facilitate comparison with the emerging cheD revertant swarms.*

all appear to share a common functional defect with the *tsr-1* tester mutant (data not shown). The swimming pattern and chemotactic responses of these strains are also identical to those of canonical *tsr* mutants.

(ii) The *cheD* mutants used in this work were induced by ethyl methane sulfonate and are therefore likely to have base substitutions that could probably revert to wild type. However, this type of reversion event should be much less frequent than pseudoreversion to a Tsr^- phenotype, because the latter can occur by many different sorts of mutational lesions that result in loss of *tsr* function. The relative target sizes for the two types of reversion are one or a few base pairs on the one hand and virtually the entire *tsr* coding sequence on the other. In fact, apparent wild-type *cheD* revertants do arise on tryptone swarm agar, but they constitute only 0.2 to 1% of all *cheD* revertants, as predicted (data not shown).

(iii) Pseudorevertants of *cheD* strains should be double mutants that contain the original *cheD* mutation and a second mutation, presumably in the *tsr* gene, that confers a Tsr^- phenotype. These two mutations should be tightly linked because *cheD* and *tsr* mutations map very close to one another. Five independent pseudorevertants of strain RP4792 were backcrossed to $cheD^+$ recipients by P1 transduction in an attempt to recover the original *cheD* mutation (Table 4). In all five cases, *cheD* transductants

TABLE 4. *Recovery of cheD mutations from cheD pseudorevertants*

Donor strain	Nonchemotactic recombinants among selected transductants[a]	
	$serB^+$ (recipient = RP479)	$dnaC^+$ (recipient = RP482)
RP4792 (*cheD192*)	256/728 (35)	469/816 (57)
RP4792 RV-1	12/780 (1.5)	10/832 (1.2)
RP4792 RV-2	3/520 (0.6)	9/520 (1.7)
RP4792 RV-3	10/495 (2.0)	10/572 (1.8)
RP4792 RV-4	1/509 (0.2)	3/676 (0.4)
RP4792 RV-5	8/362 (2.2)	6/520 (1.2)
AW518 (*tsr*)	13/558 (2.3)	17/832 (2.0)

[a] Transductant colonies obtained on minimal medium (RP479) or at 42°C (RP482) were transferred to tryptone swarm plates to assess chemotactic ability. All nonchemotactic clones resembled typical *cheD* mutants in their swarm plate morphology and reversion pattern. Numbers in parentheses are percentages.

could be obtained, although with very low frequency, demonstrating that the pseudorevertants carried a mutation closely linked to *cheD* that modified the *cheD* phenotype.

As mentioned previously, donor lysates prepared on AW518, the prototypical *tsr* mutant isolated by Hazelbauer et al. (10), yield a low frequency of nonchemotactic transductants in backcrosses to *dnaC* or *serB* recipients (Table 4, bottom line). These nonchemotactic segregants were tested for reversion properties, swimming pattern, flagellar rotation, and complementation behavior and resembled typical *cheD* mutants in all respects (data not shown). It seems likely, therefore, that AW518 is a double mutant containing a cryptic *cheD* mutation and a *tsr* mutation responsible for the Tsr^- behavior of the strain. This does not appear to be an essential property of *tsr* mutants, because the other *tsr* strain used in this work is not a double mutant. It may be that AW518 originated as a *cheD* mutant which then underwent pseudoreversion to yield a Tsr^- strain. This explanation would be consistent with the manner in which AW518 was isolated: Hazelbauer et al. (10) cycled cells that remained near the site of inoculation on tryptone swarm plates; after many such cycles, individual colonies were then tested for chemotactic ability. Since *tsr* mutants are chemotactic, there is no reason to expect they would be enriched at the origin of a swarm, whereas nonchemotactic mutants, including *cheD* strains, would be enriched for in this procedure.

(iv) The second mutation in *cheD* pseudorevertants should affect a protein-coding sequence, presumably the *tsr* structural gene. An attempt was made to detect either nonsense (amber) or missense (temperature-sensitive) mutations in

cheD pseudorevertants (Fig. 7). Revertants were selected at 35°C in a nonsuppressing background and then tested for chemotaxis under varying conditions. Strains that were nonchemotactic at 24°C probably carried temperature-sensitive mutations in the *tsr* gene, enabling the *cheD* phenotype to be expressed at the permissive temperature. Similarly, strains that were nonchemotactic in the presence of an amber suppressor probably contained amber mutations in the *tsr* gene. Both types of pseudorevertants were obtained (Fig. 7), proving that the reversion events can occur in the structural gene for a protein.

DISCUSSION

Mutants with *tsr* defects swim in a normal fashion (4) and carry out chemotactic responses to all but a limited number of attractants and repellents (10, 22, 28). Mutants with *cheD* defects exhibit very little tumbling activity as they swim and are generally nonchemotactic. The mutations responsible for *tsr* and *cheD* defects map very close together at about 99 min on the *E. coli* map, a region which is devoid of any other known chemotaxis functions (3, 20). The genetic studies described in this report demonstrate that *tsr* and *cheD* mutants are functionally related. The evidence to substantiate this conclusion derives from the observation that *cheD* defects are dominant, suggesting that *cheD* mutants manufacture an agent that can inhibit tumbling or some other essential aspect of chemotaxis. This putative inhibitor appears to be some form of the *tsr* product because *cheD* mutants readily regain chemotactic ability by acquiring a mutation that inactivates *tsr* function. Two alternative models could account for this behavior.

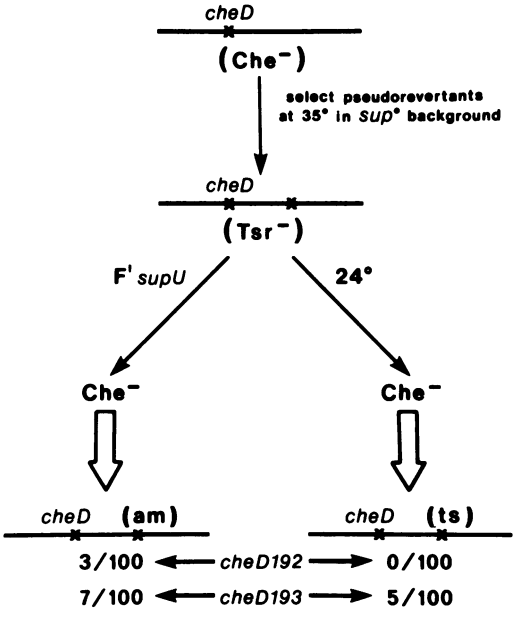

FIG. 7. *Scheme for detecting cheD pseudorevertants with amber or temperature-sensitive mutations. Independent pseudorevertants were selected from RP4160 (cheD192) and RP4161 (cheD193) on tryptone swarm agar at 35°C. Revertants were then tested for chemotaxis at 24°C or in the presence of the supU amber suppressor.*

Overproduction model. *cheD* mutations might be *cis*-acting control defects such as promoter or operator mutations that result in overproduction of the *tsr* product. The excessive levels of *tsr* product within the cell could cause, either directly or indirectly, a loss of tumbling ability, thereby preventing chemotaxis.

Aberrant product model. *cheD* mutations might result in the synthesis of an aberrant gene product, most likely a protein, that somehow interferes with the cell's ability to tumble. In the simplest version of this model, *cheD* mutations would represent a specific alteration in the *tsr* structural gene itself.

Both of these models are consistent with the genetic evidence presented in this report. Moreover, both models correctly predict that *cheD* mutants should be fairly rare, on the one hand because control elements typically present small targets for mutation, and on the other hand because a rather specific sort of structural gene alteration is called for. In studies of over 200 independent *che* mutants of *E. coli*, only three *cheD* strains have been found (19). In *S. typhimurium* no such mutants have yet been found among a rather large collection of *che* strains (5, 29).

It is also interesting to note that *cheD*-like defects associated with the *tar* or *trg* signalers have not been observed. There is some reason to believe that the *tsr* signaler may be unique in this respect. For example, serine, a strong attractant handled by the *tsr* pathway, partially inhibits chemotactic responses to attractant stimuli that are processed by other pathways (1). The serine effect is mediated by the *tsr* product and seems to be due to a decreased tumbling frequency in the presence of static concentrations of serine (4). Moreover, even in the absence of serine, attractant responses are better in *tsr* mutants than in wild type (10, 22, 25). Thus the *tsr* product seems predisposed to domineering other signalers or the tumbling machinery and might, through overproduction or structural alteration, be able to completely inhibit spontaneous tumbling activity even in the absence of receptor inputs, as *cheD* mutants seem to do.

The *tsr* and *tar* products are known to be methyl-accepting proteins located in the cytoplasmic membrane (24, 26). This is probably true of the *trg* product as well, but in this case the evidence is still mainly circumstantial (13). These three signalers are capable of modulating flagellar rotation in response to both external and internal cues. Chemical stimuli trigger changes in swimming pattern through the signalers, perhaps by direct interaction of occupied chemoreceptors with the appropriate signaler species at the outer surface of the cytoplasmic membrane (15). The adaptation system within the cell then terminates the response by the addition or removal of methyl groups on the signaler molecules (6). These changes in methylation level somehow cancel the signal elicited by chemical stimuli in order to restore the response machinery to a prestimulus condition so that new stimuli can be perceived and acted upon.

In order for the *tsr* product to become "locked" in a tumble-inhibiting mode, it would most likely have to be immune to the effects of the adaptation system. For example, if the *tsr* product could not accept methyl groups or inhibited the activity of the methylation system, it would probably send a permanent tumble inhibitory signal to the flagella. Methylation studies of *cheD* strains indicate that the level of methylation of *tsr* protein is indeed much lower than normal (26). However, methylation of *tar* protein is also greatly reduced (14, 26), implying either a direct effect of *tsr* product on *tar* product or an indirect effect in which the activity of the adaptation system is reduced, perhaps by inhibiting the methyltransferase or methylesterase enzymes.

A priori, it is difficult to imagine how an excess of *tsr* protein could produce the phenotype associated with *cheD* mutants. Attempts to produce the *cheD* defect by overloading the cell with *tsr* product synthesized from specialized λ transducing phage have been unsuccessful (Parkinson, unpublished observations). Although the overproduction model cannot be excluded until direct measurements of gene expression in *cheD* strains are made, it seems more likely that *cheD* behavior is caused by an aberrant form of the *tsr* protein. It is conceivable that some mutations in the *tsr* structural gene could create a signaler that is "locked" in a tumble-inhibiting state. To account for the influence of *cheD* mutations on methylation of other signaler species, it is necessary to propose an effect on the adaptation system or on the signalers themselves. This effect could be responsible for inhibition of tumbling behavior as well. One approach to understanding the basis of the *cheD* phenotype is to identify the chemotaxis components that serve as the "targets" of the putative *cheD* inhibitor. Such studies (to be reported elsewhere) indicate that alterations in certain elements of the signaling-adaptation system can alleviate *cheD* inhibition of tumbling and chemotaxis. Thus it may be possible to exploit *cheD* mutants to provide information about the role of the *tsr* product in particular and about the signaling process in general.

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