JOURNAL OF BACTERIOLOGY, Jan. 1985, p. 96–104
0021-9193/85/010096-09$02.00/0

Vol. 161, No. 1

Genetics of Methyl-Accepting Chemotaxis Proteins in *Escherichia coli*: *cheD* Mutations Affect the Structure and Function of the Tsr Transducer

ANN M. CALLAHAN† AND JOHN S. PARKINSON*
Biology Department, University of Utah, Salt Lake City, Utah 84112

Received 9 July 1984/Accepted 19 October 1984

The *tsr* gene specifies a methyl-accepting membrane protein involved in chemotaxis to serine and several repellent compounds. We have characterized a special class of *tsr* mutations designated *cheD* which alter the signaling properties of the Tsr transducer. Unlike *tsr* null mutants, *cheD* strains are generally nonchemotactic, dominant in complementation tests, and exhibit a pronounced counterclockwise bias in flagellar rotation. Several lines of evidence showed that *cheD* mutations were alleles of the *tsr* gene. First, *cheD* mutations were mapped into the same deletion segments as conventional *tsr* mutations. Second, restriction site analysis of the transducing phage deletions used to construct the genetic map demonstrated that the endpoints of the deletion segments fell within the *tsr* coding sequence. Third, a number of the *cheD* mutants synthesized Tsr proteins with slight changes in electrophoretic mobility, consistent with alterations in Tsr primary structure. These mutant proteins were able to undergo posttranslational deamidation and methylation reactions in the same manner as wild-type Tsr protein; however, the steady-state level of Tsr methylation in *cheD* strains was very high. The methylation state of the Tar protein, another species of methyl-accepting protein in *Escherichia coli*, was also higher than normal in *cheD* strains, suggesting that the aberrant Tsr transducer in *cheD* mutants has a generalized effect on the sensory adaptation system of the cell. These properties are consistent with the notion that the Tsr protein of *cheD* mutants is locked in an excitatory signaling mode that both activates the sensory adaptation system and drowns out chemotactic signals generated by other transducer species. Further study of *cheD* mutations thus promises to reveal valuable information about the functional architecture of the Tsr protein and how this transducer controls flagellar behavior.

Many of the chemotactic responses exhibited by motile *Escherichia coli* are mediated by a class of inner membrane proteins termed methyl-accepting chemotaxis proteins (MCPs) (39). These proteins serve several important functions in the sensory transduction systems of bacteria. As the organism moves about in spatial chemical gradients, MCP molecules monitor the concentrations of chemoeffector compounds. Changes in chemoeffector concentration elicit MCP signals that alter the pattern of flagellar rotation, enabling the cell to carry out an appropriate chemotactic response. For example, an increase in attractant concentration favors counterclockwise (CCW) flagellar rotation and causes the cell to swim in a smooth path; a decrease in attractant concentration favors clockwise (CW) rotation and causes the cell to tumble and try a new swimming direction. The rapid excitatory response to a chemical stimulus is accompanied by a slower change in MCP methylation state that results in sensory adaptation and restoration of the prestimulus rotation pattern, which is characterized by frequent flagellar reversals. Thus, MCP molecules are responsible for detecting chemical stimuli and for regulating the signals that control the flagellar rotational machinery. The nature of those signals and how they are modulated by MCP molecules during stimulus transduction and sensory adaptation are still poorly understood.

E. coli contains four different MCP structural genes designated *tsr*, *tar*, *trg*, and *tap*. Each species of MCP appears to handle a different subset of sensory inputs: Tsr (MCP-I) is the chemosensor for serine (13); Tar (MCP-II) is the chemosensor for aspartate and maltose (45); Trg (MCP-III) is the chemosensor for ribose and galactose (12, 18); Tap (MCP-IV) has no known function but closely resembles other MCPs at the biochemical level (4, 37, 44). The Tsr protein is the major species of MCP in *E. coli* and comprises about one-half of the MCP molecules in the cell. Null mutants lacking *tsr* function exhibit wild-type swimming patterns under unstimulated conditions but are incapable of responding to serine and several other related amino acid attractants (30). They also have aberrant responses to a number of repellents (16, 23, 31, 42) and to temperature changes (21), suggesting that the Tsr protein is responsible either directly or indirectly for processing a variety of stimuli. However, *tsr* mutants still respond to aspartate and other chemoeffectors that are processed by other MCPs.

A group of generally nonchemotactic (*cheD*) mutants are also thought to have defects in Tsr function (26). These mutants exhibit very low tumbling frequencies as they swim and fail to show chemotactic responses to any stimuli, including those normally handled by other species of MCP. The CheD phenotype is dominant in complementation tests, implying that the mutant *cheD* product actively inhibits CW flagellar rotation. However, the evidence that *cheD* mutations represent special alleles of the *tsr* gene has thus far been largely circumstantial. First, both *cheD* and conventional *tsr* mutations have been mapped to 99 min on the *E. coli* chromosome, a region devoid of any other known chemotaxis-related genes. Second, *cheD* mutants give rise to Tsr$^-$ pseudorevertants at high frequency, indicating that the dominant inhibitory effects of the mutant *cheD* gene product could be abolished by null mutations in the *tsr* structural

* Corresponding author.
† Present address: USDA Appalachian Fruit Research Station, Kearneysville, WV 25430.

TABLE 1. *E. coli* strains[a]

Strain	Relevant markers[b]	Comments	Source or reference
RP437	*thr-1 eda-50*	Wild type for chemotaxis; standard background for introduction of *thr-* or *eda*-linked chemotaxis mutations	24
RP1267	*uvrA6* (λ *ind*$^-$)	Wild type for chemotaxis; UV programing host	34
RP4532	Δ(*tar-tap*)*5201*	Donor of *tar* deletion	This laboratory
RP5543	Δ*tsr9101*	*tsr* deletion host for growth of λtsr phage	Callahan and Parkinson, in preparation
RP5762	*uvrA6* (λ *ind*$^-$)	Wild type for chemotaxis; UV programing host	This work
RP5763	*uvrA6* (λ *ind*$^-$) Δ(*cheA-cheR*)*2216*	CheR$^-$ host strain for UV programing	This work
RP5764	*uvrA6* (λ *ind*$^-$) Δ(*tar-cheB*)*2234*	CheR$^-$ CheB$^-$ host for UV programing	This work
RP5823	*uvrA6* (λ *ind*$^-$) *cheD193*	CheD$^-$ host strain for UV programing	This work

[a] Other strains used in this work were derived from these by P1 transduction as described in the text.
[b] All of these strains carry additional mutations, particularly auxotrophic markers, that are not relevant to the work described here.

gene (26). These properties of *cheD* mutants can be accommodated by models in which either the quality or the quantity of the Tsr protein has been altered by the *cheD* mutation. For example, it is conceivable that overproduction of wild-type Tsr protein could lead to suppression of CW flagellar rotation and a general loss of chemotactic ability. Thus, *cheD* mutations might simply affect the expression of the *tsr* locus. Alternatively, *cheD* mutations might directly affect the *tsr* coding region, resulting in an aberrant transducer that somehow prevents CW flagellar rotation. In either case, a null mutation in the *tsr* gene would destroy the inhibitory gene product and lead to a Tsr$^-$ pseudorevertant, thereby accounting for the unusual reversion properties of *cheD* mutants.

In this report, we present direct evidence that *cheD* mutations affect the structure of the Tsr protein rather than its level of expression. First, we show by fine-structure deletion mapping that *cheD* mutations are located within the coding region of the *tsr* gene. Second, we show that the level of expression of the Tsr protein in *cheD* mutants is not detectably different from that of the wild type. Finally, we show that at least some *cheD* mutations affect the intrinsic electrophoretic mobility of the Tsr protein, presumably by altering its primary structure through amino acid substitution. Based on additional phenotypic and biochemical characterizations, we conclude that *cheD* mutations create an aberrant transducer protein that is locked in a CCW signaling mode. One of the interesting consequences of such a defect is that all species of MCP molecules in a *cheD* mutant have an unusually high steady-state level of methylation, owing perhaps to futile efforts of the adaptation machinery to cancel the CCW signal coming from the aberrant Tsr protein. In the accompanying paper, Kehry et al. (15) have explored the basis for these abnormal methylation levels in more detail and demonstrate that they are correlated with an inhibition of the MCP-specific methylesterase activity, which plays a key role in regulating MCP methylation state. Thus, *cheD* mutations appear to represent an interesting type of transducer defect and should be useful in attempts to understand structure-function relationships in MCP molecules.

MATERIALS AND METHODS

Strains. The bacterial strains used in this study (Table 1) were derivatives of *E. coli* K-12. All genetic and phenotypic characterizations of *cheD* mutations were performed in derivatives of strain RP437 (27). The *cheD* mutations were derived from a variety of sources and are listed in Table 2. They were transferred into RP437 by P1 cotransduction with the *thr* locus (26). The *tsr* mutations used in deletion mapping experiments were obtained by localized mutagenesis of the *tsr* locus (Callahan and Parkinson, manuscript in preparation) and also transferred to the RP437 genetic background.

The *tsr* specialized transducing phages used in this work were λfla91 (36) and λtsr70 and λtsr72 (Callahan and Parkinson, in preparation). Deletion derivatives of λtsr70 and λtsr72 were isolated by chelating agent selection (28) with EDTA (pH 8.0) as detailed previously (27). λche22Δ25-Δ5 (37) was used to label the Tar protein in UV programing experiments. Plasmid pAB100-139 (4), which carries a portion of the *tsr* gene (Fig. 1), was obtained from A. Boyd.

Growth media. Tryptone broth, plates, and swarm agar (24) were used for growth of all bacterial strains unless otherwise noted. Liquid phage stocks were prepared in NZYM medium as previously described (27). Minimal salts medium for UV programing experiments contained 50 mM Tris-hydrochloride, 25 mM Na_2PO_4, 22 mM KH_2PO_4, 20 mM NH_4Cl, 3 mM $MgCl_2$, 0.1 mM $CaCl_2$, 3 μM $FeCl_3$, and 1 mM $MgSO_4$ (MUV medium).

Flagellar rotation assays. Cells were grown at 35°C in Tryptone broth to about 5×10^8/ml, deflagellated by Waring blender treatment, and tethered to microscope slides with flagellar antiserum as previously described (46). Each rotating cell was observed for 15 s, and its rotational behavior was classified in the manner previously described (26). At least 100 rotating cells were scored for each strain under study.

Complementation tests. Complementation assays were performed on Tryptone swarm plates containing approximately 10^8 particles of λfla91, λtsr70, or λtsr72 per ml, all of which supply the wild-type Tsr function. Individual colonies of

cheD or *tsr* mutants (made lysogenic for λ wild type to prevent killing by the superinfecting phage in the plate) were transferred to the test plates and examined for formation of chemotactic rings (25).

Deletion mapping. Deletion derivatives of λtsr70 and λtsr72 were grown on strain RP5543 to preclude formation of undeleted phage by recombination with the host chromosome. The phages were crossed to *tsr* and *cheD* strains, using the high-resolution method described previously (27). In brief, the transducing phages were treated with approximately 2,000 ergs of UV irradiation per mm^2 to increase recombination frequencies and then adsorbed to the mutant host strain at a multiplicity of about one. Although cell death is minimal under these conditions, we generally employed host strains that were lysogenic for λ wild type to prevent any killing by the mapping phage. The infected cells were streaked across the surface of a Tryptone swarm plate and incubated overnight at 35°C to allow chemotactic recombinants to swarm away from the parental cells.

Plasmid pAB100Δ139 was transferred into recipient strains by $CaCl_2$ transformation (20) with selection for tetracycline-resistant transformants. Individual transformant colonies were picked to Tryptone swarm plates containing tetracycline to score for production of chemotactic recombinants arising from subsequent exchanges between the plasmid and chromosomal *tsr* regions.

In both the transducing phage and plasmid mapping experiments, the *cheD* and *tsr* strains were crossed as double mutants containing a *tar* deletion introduced from RP4532 by cotransduction with the *eda* locus. As detailed previously (37), Tsr^- Tar^- strains are generally nonchemotactic, which greatly facilitates the detection of chemotactic (in this case, tsr^+) recombinants. Moreover, this ploy avoided the possibility of mistaking Tsr^- pseudorevertants of *cheD* mutants for recombinants, because *tsr* mutations cannot restore chemotaxis to *cheD* mutants in a *tar* genetic background.

Restriction mapping. Phage DNA was extracted from CsCl-purified particles by the formamide method (8). Restriction enzymes were purchased from New England Biolabs or Bethesda Research Laboratory and used under the conditions recommended by the supplier.

UV programming. The procedures used for labeling of phage-directed proteins and their analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were minor modifications of those described previously (35, 38). Cells were grown at 37°C in MUV medium containing required growth supplements, 1% glycerol, and 0.5% maltose. At a density of 2×10^8/ml, the cells were harvested by centrifugation, suspended in 10 mM $MgCl_2$ at a density of 5×10^9 cells per ml, and then UV irradiated for approximately 2 min at a flux of 380 μW/cm^2. (The optimum dose was empirically determined for each strain.) Cells and phage were mixed at a ratio of 1:10 and incubated at 37°C in MUV medium for 40 min before addition of label. Proteins were labeled by addition of 5 to 15 μCi of [^{35}S]methionine (Amersham Corp. or New England Nuclear Corp., translation grade) and incubation for 15 min at 37°C. The samples were either prepared for electrophoresis at this point or incubated with 5×10^{-4} M serine to examine stimulus-induced changes in MCP methylation state as detailed previously (34). Sample preparation and electrophoresis procedures were as described before (38).

RESULTS

Origin and phenotypic properties of *cheD* mutants. The three *cheD* mutations described previously (*cheD191*, *cheD192*, and *cheD193*) (26) were found among a group of approximately 200 independent mutants with a generally nonchemotactic phenotype (24). The relative rarity of these mutations and their dominant behavior in complementation tests suggested that the CheD phenotype was due to a specific alteration, as opposed to complete inactivation, of a chemotaxis-related protein, most likely the *tsr* gene product. To test this assertion and in the process obtain additional *cheD* mutations, we undertook a more extensive mutational analysis of the *tsr* region, using specialized λ transducing phages (29; Callahan and Parkinson, in preparation). Mutations within the *tsr* region were induced by growth of λtsr70 in a *mutD* host (11) and subsequently identified by screening individual phages for failure to confer normal chemotactic ability on a host tester strain bearing a deletion of the *tsr* locus. A total of 111 independent mutants were isolated in this manner. Each of the phage mutations was transferred into the host chromosome by homologous recombination, and the chemotaxis behavior of the resulting strains was examined on Tryptone swarm plates. Seven of the new mutants appeared to carry *cheD* mutations and were chosen for additional testing. Two *cheD* mutations (*cheD34* and *cheD518*) from other sources were also included in this study. The phenotypes of strains containing these new mutations as well as the three original *cheD* alleles are summarized in Table 2 and discussed below.

Of the 12 *cheD* strains, 9 formed small colonies on semisolid Tryptone swarm agar, indicative of a complete, generalized defect in chemotactic ability. The other three strains formed somewhat larger colonies, two of which had sharp boundaries and resembled small Tsr^- swarms. These latter strains evidently retained some chemotactic ability, but nevertheless, all of the *cheD* mutants were partly or completely defective in aspartate chemotaxis as evidenced by the lack of a characteristic band of cells undergoing aspartate taxis at the periphery of the colonies. Under the same conditions, strains with null mutations in the *tsr* gene formed larger swarms with prominent aspartate bands (26).

TABLE 2. Phenotypic properties of *cheD* mutants

cheD allele[a]	Source or reference	T swarm phenotype[b]	Flagellar rotation[c]		
			CCW	Reversing	CW
34	R. Reader, unpublished data	Che^-	49	51	0
191		Small Tsr^-	62	38	0
192	EMS induced 27,29	Che^-	98	2	0
193		Che^-	97	3	0
302		Che^-	100	0	0
303	*mutD*-induced	Small Tsr^-	97	2	1
329	(Callahan and	Che^-	50	48	2
331	Parkinson, in	Che^-	59	40	1
352	preparation)	Che^-	86	13	1
364		Che^-	99	1	0
394		Che^-	91	9	0
518	29	Large Che^-	65	35	0

[a] Each of these mutations was transferred into strain RP437 for analysis; all were dominant in complementation tests.

[b] Swarm appearance after 12 to 16 h of incubation at 35°C. Colonies of *cheD* strains were examined after 24 h of incubation on Tryptone swarm agar at 35°C; all gave rise to Tsr^- revertants.

[c] Percentage of rotating cells.

Thus, *cheD* strains exhibit defects in chemotactic responses which are not normally dependent on the Tsr transducer. After overnight incubation (16 to 24 h) on swarm agar, all of the *cheD* strains gave rise to numerous chemotactic pseudorevertants with a Tsr^- phenotype (i.e., normal aspartate taxis, but no serine responses). The serine taxis defect of these pseudorevertant strains could be corrected by complementation with a wild type copy of the *tsr* locus provided on a λ transducing phage. In contrast, the general chemotaxis defect of the parental *cheD* mutants could not be corrected by complementation with λtsr70, λtsr72, or λfla91, although wild-type recombinants arose at normal frequencies. Thus, it appears that all of the *cheD* mutations in our study sample were dominant.

The unstimulated swimming behavior of all 12 *cheD* strains was characterized by a greatly reduced frequency of spontaneous turning or tumbling movements. The extent of the tumbling defect in *cheD* strains was quantitated by examining flagellar rotation patterns in tethered cells (Table 2). Whereas over 90% of the rotating cells in tethered wild-type or *tsr* null strains exhibited at least one reversal (corresponding to a tumbling episode) within the 15-s observation period allotted each cell (24, 26), the frequency of reversing cells in *cheD* mutants ranged from 0 to 51%. There was no consistent correlation between the residual chemotactic ability of a *cheD* strain (as judged by its colony morphology on swarm agar) and the proportion of reversing cells in tethering assays. Clearly, spontaneous tumbling frequency is not the only factor that influences chemotactic responses in *cheD* mutants; the mutants may differ to some extent with respect to other chemotaxis properties as well. Nevertheless, all of the *cheD* strains displayed similar traits in these tests; they exhibited dominance in complementation tests, underwent pseudoreversion at high frequency, and had a pronounced CCW bias in flagellar rotation.

Relationship of *cheD* mutations to the *tsr* locus. If *cheD* mutations exert their dominant effects on chemotaxis through structural alteration of the Tsr protein, they should be located within the coding portion of the *tsr* gene. Alternatively, if *cheD* mutations affect the *tsr* promoter or some other element that influences the level of Tsr expression, they should be located outside the *tsr* gene borders. To distinguish these possibilities, a genetic map of *cheD* and *tsr* mutations was constructed, using deletion derivatives of λtsr70 and λtsr72 obtained by EDTA selection. Crosses of the phage deletions to a series of 51 *tsr* point mutant tester strains served to divide the *tsr* locus into 10 deletion segments (Fig. 1) (Callahan and Parkinson, in preparation). Since all known mutations with a Tsr^- phenotype are located within this interval and belong to the same complementation group, the *tsr* coding region must span deletion segments I-X. The *cheD* mutations were mapped to deletion segments II-X, indicating that they lie within the *tsr* coding region (Fig. 1). Because we do not know the relative order of *tsr* and *cheD* alleles within any of the deletion segments, it is possible that the *cheD* mutations in segment X actually lie

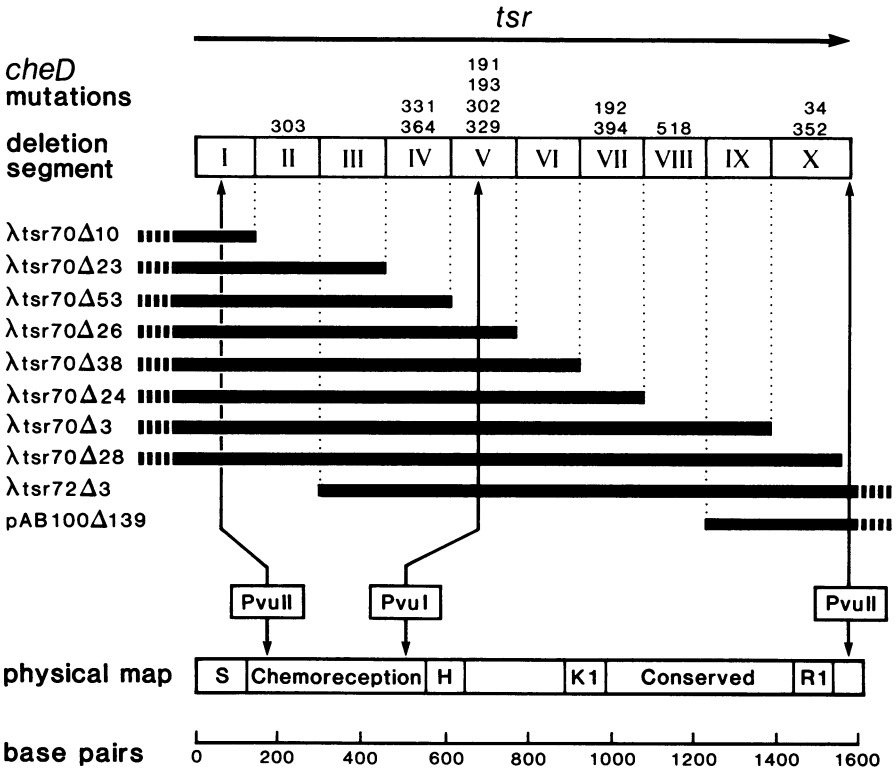

FIG. 1. Genetic and physical maps of *cheD* mutations. (Top) Genetic map of the *cheD* mutations described in the text. The *tsr* gene was divided into 10-deletion segments to establish the relative order of these *cheD* alleles. Although the deletion segments were drawn equal in size, their actual physical sizes cannot be inferred from these mapping data alone. (Bottom) Physical representation of the *tsr* gene and the functional domains of the Tsr protein which have been deduced from nucleotide sequence studies (4, 5, 19). A comparison of the genetic and physical maps was made by testing the transducing phage deletions used to prepare the genetic map for the presence of the *Pvu*I and *Pvu*II restriction sites, whose locations in the *tsr* gene are known. See the text for additional details.

TABLE 3. Properties of Tsr protein from *cheD* mutants

cheD mutation	SDS-PAGE position of unmodified form[a]	Subject to deamidation[b]	Methylation state
34	Shifted down	Yes	Higher than WT
191	Shifted up	Yes	Higher than WT
192	Not shifted	Yes	Higher than WT
193	(Shifted down)	Yes[c]	Higher than WT
302	(Shifted down)	Yes	Higher than WT
303	(Shifted down)	Yes	Higher than WT
329	Not shifted	Yes	Higher than WT
331	Shifted up	Yes	Higher than WT
352	NT	NT	
364	NT	NT	
394	Not shifted	Yes	Higher than WT
518	(Shifted down)	Yes	Higher than WT

[a] Position relative to the unmodified form of wild-type Tsr protein. Band shifts shown in parentheses were slight but appeared to be significant.

[b] All of the mutant Tsr proteins, like those of the wild type, exhibited two deamidated bands.

[c] Some of the unmodified form was still present; the majority of the deamidated material was in the lower of the two deamidated bands.

outside the *tsr* gene borders. This ambiguity was resolved by comparing the genetic map and physical map of the *tsr* coding region.

The physical organization of the *tsr* coding region has been established by nucleotide sequence studies (3) and by restriction site analyses (4; Callahan and Parkinson, in preparation). To confirm that all of the *cheD* mutations were located within the *tsr* coding region, we examined the physical relationship between deletion segments and several restriction site landmarks in the *tsr* gene. The entire *tsr* coding region is just over 1,600 base pairs and contains *Pvu*II cleavage sites at approximately nucleotides 160 and 1,590 (Fig. 1). All of the transducing phage deletions that extended through deletion segment I lacked the promoter-proximal *Pvu*II restriction site, placing that site in segment I. The other *Pvu*II site was only missing in those deletions that entered segment X from the downstream side (Fig. 1), indicating that the site must be promoter distal to segment X. This evidence demonstrates that deletion segments II-X were entirely within the *tsr* coding region. Since all of our *cheD* mutations were located in segments II-X as well, we conclude that *cheD* mutations must affect the structure of the Tsr protein rather than its level of expression. The locations of *cheD* alleles relative to functional domains in the Tsr molecule are discussed below.

Analysis of *cheD* gene products. The Tsr protein, like other MCP molecules, undergoes several types of posttranslational modification. During or shortly after synthesis, MCP molecules are irreversibly altered in a CheB-dependent reaction (32, 34) that appears to involve the specific deamidation of several glutamine residues (14). In addition, MCP molecules are reversibly modified by the formation or hydrolysis of glutamyl methyl esters (17, 43). The MCP-specific methyltransferase and methylesterase are products of the *cheR* and *cheB* genes, respectively (40, 41). Each of these alterations has a detectable effect on the migration pattern of MCP molecules in SDS-polyacrylamide gels, and it is possible to assess the modification state of MCP molecules by SDS-PAGE. We used this technique to examine the modification properties of the Tsr protein from various *cheD* mutants. In the experiments discussed below, the Tsr proteins were specifically visualized by labeling with [^{35}S]methionine after infection of UV-irradiated host strains with λtsr70 derivatives carrying *cheD* mutations. The experimental findings are summarized in Table 3; representative examples of the results are presented below.

CheD proteins with intrinsic mobility alterations. When synthesized in the absence of both CheR and CheB activities, wild-type Tsr protein was neither deamidated nor methylated and migrated as a single band in SDS-PAGE (32, 34) (Fig. 2, lane 3). The corresponding form of the mutant Tsr product made by the various *cheD* strains also migrated as a single band of approximately the same intensity as that of the wild-type control, demonstrating that the level of Tsr expression in *cheD* strains, at least under UV programming conditions, was not significantly different from that of the wild type. Moreover, all of the *cheD* strains examined made a Tsr protein of essentially normal size, suggesting that *cheD* mutations typically cause missense changes in the Tsr protein rather than more severe or null defects. However, we often observed slight alterations in the positions of these bands relative to that of unmodified wild-type Tsr protein (Table 3). Two particularly striking examples were the *cheD34* and *cheD191* proteins, in which the mobility shifts corresponded to an apparent difference of several thousand daltons (Fig. 2). The *cheD34* protein migrated faster than did the wild type, whereas the *cheD191* protein migrated slower than the wild type. Other mutant proteins exhibited less dramatic mobility shifts: four migrated slightly faster and one migrated slightly slower than did the wild type (Table 3).

It could be argued that the mobility changes of the Tsr protein observed in many of the *cheD* mutants were caused by an aberrant posttranslational modification activity rather than by an intrinsic alteration of Tsr primary structure. For example, these *cheD* strains may carry a mutation in a previously unsuspected MCP modification function. To test this possibility, we examined the Tsr proteins made in a mixed infection of λtsr70 *cheD191* and λtsr70 (*tsr*$^+$). If the *cheD191* gene product migrated differently because it was processed differently, the λtsr70 *cheD191* phage used in the programing experiments would have to carry the aberrant processing function, and mixed infection should result in aberrant modification of the wild-type Tsr product as well as that made by the *cheD191* allele. The results of such an experiment are shown in Fig. 3. The mixed infection produced no evident shift of material from the wild-type position to the *cheD191* position, demonstrating that the altered

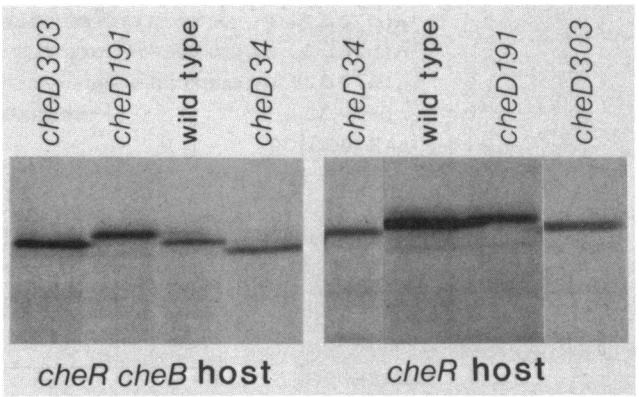

FIG. 2. Unmodified and deamidated forms of Tsr protein made by *cheD* mutants. Tsr proteins synthesized by λtsr70 (wild type) and λtsr70 derivatives carrying *cheD* mutations were labeled by UV programing (see the text). Proteins made in the *cheR cheB* host (RP5764) were in the unmodified state, whereas those made in the *cheR* host (RP5763) were deamidated but unmethylated.

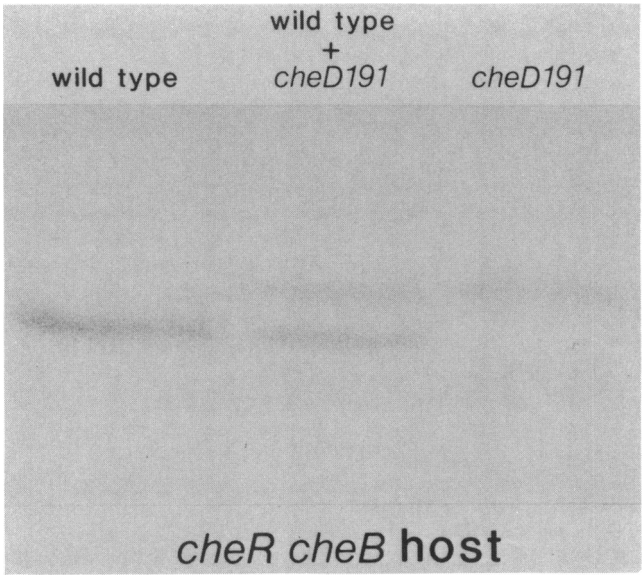

FIG. 3. Intrinsic change in SDS-PAGE mobility of the Tsr protein caused by a *cheD* mutation. Strain RP5764 was infected with λfla91 (wild type) or λtsr70 *cheD191* or with equal numbers of both phages, and their Tsr proteins were labeled by UV programing. The overall multiplicity of infection was 10 in all cases (see the text).

mobility of the *cheD191* protein was an intrinsic property of the mutant Tsr molecule. Such mobility shifts could be caused by changes in the actual size of the Tsr protein or simply by amino acid substitutions that affect migration in SDS-PAGE. In either case, this observation provided additional evidence that *cheD* mutations affected the structure of the Tsr protein.

Deamidation of Tsr proteins made by *cheD* mutants. The ability of Tsr molecules to undergo CheB-dependent deamidation was examined by comparing the SDS-PAGE patterns of proteins synthesized in a $CheR^-$ $CheB^-$ host with those made in a $CheR^-$ $CheB^+$ host. Previous studies have shown that under these conditions, wild-type Tsr molecules are shifted to two slower-migrating forms that represent the intermediate and final products of the deamidation process (32, 34). The CheD proteins were surveyed initially on short gels, which did not resolve the two deamidated forms (Fig. 2). In every case, the mutant Tsr proteins exhibited an upward shift in band position characteristic of CheB-dependent deamidation (Table 3). The intrinsic mobility changes of the *cheD34* and *cheD191* molecules were not altered by deamidation (Fig. 2). The efficiency of deamidation was estimated from the relative intensities of the intermediate and final reaction products, which were resolved by running samples on longer gels (Fig. 4). The mutant proteins typically displayed two deamidated forms of roughly equal intensities, whereas the wild-type control showed very little of the intermediate form, implying that the rate of deamidation of the CheD proteins was slower than that in the wild type. The *cheD193* results were particularly dramatic (Fig. 4). The intermediate band was considerably more intense than the fully deamidated form, and there was a significant amount of material remaining in the unmodified form. In the accompanying paper, Kehry et al. (15) demonstrate that *cheD* mutations, especially the *cheD193* allele, depress CheB-dependent methylesterase activity; presumably, the CheB-dependent deamidation activity is similarly reduced.

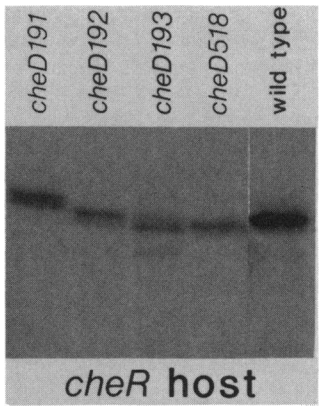

FIG. 4. Details of Tsr deamidation in *cheD* strains. λtsr70 phages carrying the various *cheD* alleles shown were used to program Tsr synthesis in RP5763. The labeled samples were run on 30-cm gels to resolve the two deamidated forms, the lower of which is an intermediate (34).

This would account for the slow deamidation rate observed in our experiments.

Methylation properties of Tsr proteins made by *cheD* mutants. The methyl-accepting ability of the Tsr protein from *cheD* mutants was examined by programming Tsr synthesis in a wild-type host strain having both CheR and CheB functions. High-resolution SDS-PAGE analysis of wild-type Tsr molecules made under these conditions reveals a complex series of bands mainly corresponding to the various possible methylation states (5, 6, 9, 10). In general, band mobility increases with the number of methyl groups on the molecule, so the relative intensity of the faster species provides a measure of the overall methylation state in the population of molecules. In this manner, we determined that the Tsr molecules made by *cheD* mutants were not only capable of accepting methyl groups (Table 3), but the average methylation state, as judged by the intensity of the fastest-migrating bands, was considerably higher than that in

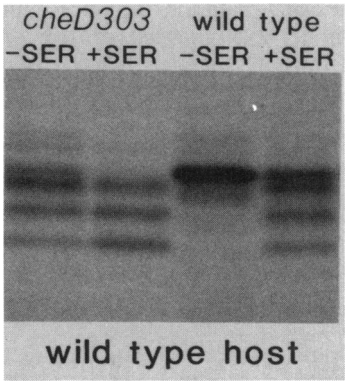

FIG. 5. Overmethylation of the Tsr protein caused by a *cheD* mutation. λtsr70 (wild type) and λtsr70 *cheD303* were used to program Tsr synthesis in strain RP5762. Each infected cell sample was divided into two portions, one of which was labeled in the absence (−SER) and one in the presence (+SER) of serine. The upper bands represent Tsr molecules with few or no methyl groups; the lower bands represent highly methylated species.

the wild type control (Fig. 5). Similar results were obtained by Kehry et al. (15), who observed even more pronounced overmethylation in *cheD* mutants. In our experiments, the methylation levels of the mutant Tsr molecules were clearly not saturated, even in the *cheD193* strain, which exhibited the highest methylation state of all the mutants examined. Application of serine stimuli caused a further increase in methylation level (Fig. 5), whereas Kehry et al. (15) observed little or no serine effect. The difference in the two sets of results most likely reflects variations in the experimental setup. Their experiments involved methyl labeling of Tsr protein in *cheD* strains, whereas ours involved backbone labeling of CheD proteins made by superinfecting phage in a wild-type cell. In addition, we used somewhat higher serine levels to stimulate the cells. Nevertheless, in both cases the findings were consistent with an increase in the relative efficiency of the methyltransferase reaction, presumably due to inhibition of methylesterase function.

We also examined the effects of *cheD* mutations, provided either on a coinfecting transducing phage or in the genetic background of the host strain, on the methylation properties of wild-type MCP molecules in UV programing experiments. The results of one such experiment are shown in Fig. 6. We found that wild-type Tsr and Tar molecules displayed an atypically high methylation site when synthesized in the presence of a *cheD* mutation. Thus, *cheD* mutations are capable of influencing the methylation of other MCP species in the cell. This finding indicates that *cheD* mutations may directly affect the relative activities of the methylation and demethylation enzymes. Alternatively, *cheD* mutations may only perturb the substrate properties of the mutant Tsr molecules, which in turn interact with other MCP species, thereby modulating their substrate properties as well.

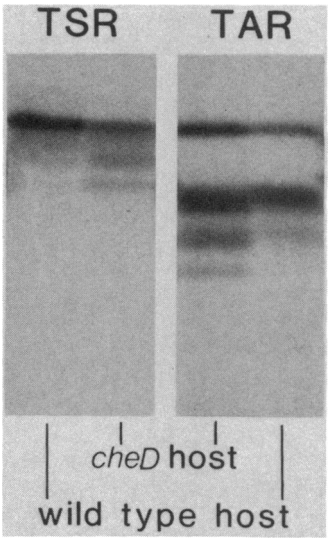

FIG. 6. Overmethylation of wild-type Tsr and Tar proteins synthesized in a *cheD* genetic background. Wild-type Tsr (λfla91) and Tar (λche22Δ25-Δ5) proteins were labeled by UV programing in either a wild-type host (RP1267) or an isogenic *cheD* host (RP5823). The uppermost band in the Tar lanes was not a form of Tar protein but rather a fusion protein generated by the *tap-cheZ* deletion in λche22Δ25-Δ5 (37).

DISCUSSION

***cheD* mutations affect the structure of the Tsr transducer.** The genetic and physical mapping studies presented in this report demonstrated that *cheD* mutations were alleles of the *tsr* gene, whose product was an MCP involved in chemotaxis to serine and several other compounds. The low frequency at which *cheD* mutants arose, their dominance in complementation tests, and the fact that they synthesized a full-size Tsr protein were all consistent with the conclusion that *cheD* mutations represent a special class of missense defects in the *tsr* gene.

A number of *cheD* mutations produced minor changes in the intrinsic mobility of the Tsr protein on SDS-PAGE, demonstrating directly that *cheD* mutations affected the Tsr structure. Although the nature of the structural changes responsible for these mobility shifts is not yet known, they need not involve a change in mass caused by gain or loss of amino acids (22). There are several ways in which relatively subtle changes in the primary structure of the Tsr protein could have profound effects on its SDS-PAGE behavior; the effects of methylation and deamidation are good examples. On the one hand, amino acid substitutions that result in a net charge change could affect the SDS-binding properties of the protein and consequently its mobility. On the other hand, an integral membrane protein such as Tsr might possess secondary structure features that persist in the presence of SDS. Amino acid replacements that perturb this structure could have a significant effect on SDS-PAGE mobility of the protein.

Functions of the Tsr transducer. The Tsr protein played a central role in mediating chemotactic responses to serine and possessed several distinct activities needed for detecting and transducing sensory information. Tsr has a serine-binding site that serves as a chemoreceptor (7, 13), enabling the protein to monitor serine levels in the environment. Upon sensing a change in serine concentration, Tsr generated or modulates a signal of unknown nature that controlled the pattern of flagellar rotation. The Tsr protein then undergoes net methylation or demethylation, which cancels the excitatory signal and brings about sensory adaptation. These various activities, chemoreception, flagellar signaling, and adaptation, which are common to all MCP species, are most easily understood in terms of two alternative MCP signaling modes: one that corresponds to CCW flagellar rotation and one that corresponds to CW rotation. Transitions between signaling modes are probably mediated by stimulus- or methylation-induced conformational changes. The relative proportion of MCP molecules in the two signaling states is determined by the swimming behavior of the cell.

Nature of the functional defect in *cheD* strains. Complete loss of Tsr function resulted in an inability to respond to serine gradients and to other stimuli handled by the Tsr transducer. The unstimulated swimming pattern of *tsr* null mutants is normal; they exhibit frequent reversals in flagellar rotation and still respond to chemotactic stimuli that are processed by other transducers. In contrast, *cheD* mutants had a severe CCW bias in flagellar rotation and were defective in chemotaxis to all compounds. Since modulation of tumbling behavior produced by CW flagellar reversals is the basis for chemotactic movements, the low frequency of CW rotation in *cheD* strains was probably responsible for their inability to carry out chemotactic responses to stimuli that are not processed by the Tsr transducer.

The CCW flagellar bias of *cheD* mutants in the absence of overt stimuli indicated that the Tsr transducer may be locked in the CCW signaling mode (26). In wild-type cells, an increase in serine concentration elicits a CCW flagellar response, and adaptation to that stimulus is accompanied by an elevation of the Tsr methylation state. In *cheD* strains, the Tsr molecules exhibited high methylation levels in the

absence of stimuli, implying that the adaptation machinery had been activated in an attempt to cancel the CCW signal from the mutant transducers. Methylation of these mutant transducer molecules appeared to be ineffective in countering their aberrant signaling properties. Thus, the CCW signal could not be canceled, and the cells behaved as though they were in a permanently excited condition. Kehry et al. have reached a similar conclusion (15).

This model of *cheD* action readily accounts for other aspects of the mutant phenotype. For example, the dominance of *cheD* mutations indicated that the mutant gene product played an active role in inhibiting a process required for chemotaxis to all compounds, presumably the generation of spontaneous tumbling episodes. The permanent CCW signal from the mutant Tsr transducer must also be directly or indirectly responsible for the high methylation state of other MCP species in *cheD* cells. Kehry et al. (15) demonstrated that these elevated methylation levels are correlated with a reduced methylesterase activity in *cheD* strains and suggest that, as part of the normal adaptation process, the activity of the CheB protein may be modulated by a diffusible signal, which in turn is regulated in some manner by the MCP transducers. However, there is reason to believe that MCP substrate properties are also important in controlling MCP methylation patterns, at least in wild-type cells, because stimuli elicit permanent methylation changes only in those transducer molecules engaged in flagellar signaling (39). Conceivably, the aberrant transducer molecules in *cheD* mutants could interact directly with other MCP species, altering their suitability as a substrate for the CheB methylesterase.

Domain structure of the Tsr transducer. Nucleotide sequence studies of MCP genes in *E. coli* (2, 3, 19) and *S. typhimurium* (33) indicate that MCP molecules may be organized into discrete structural and functional domains (Fig. 1). MCPs contain two hydrophobic regions in the amino-terminal half of the molecule which are thought to play a role in inserting and anchoring the protein in the cytoplasmic membrane (Fig. 1). The part of the molecule between the hydrophobic regions probably juts into the periplasmic space and contains the chemoreceptor domain. The primary sequence in this region varies considerably among MCPs with different receptor specificities, whereas the remainder of the molecule is rather highly conserved, presumably reflecting conservation of shared functions. The methyl-accepting sites are located in two peptides in the carboxyl half of the molecule, separated by a conserved region, which could be involved in signaling activity or in recognition by the methylation and demethylation enzymes.

Structure-function analysis of MCP molecules. The permanent CCW signaling behavior of Tsr molecules in *cheD* strains can be explained in at least three alternative, but not necessarily mutually exclusive, ways. First, the serine-binding domain at the receptor end of the molecule may have become locked in the occupied conformation as though serine were constantly present at high concentration. Even wild-type cells cannot fully adapt to high levels of serine (1). Second, the methylation region may have been altered in a way that prevents methylation changes from inducing transitions between the CCW and CW signaling modes. Third, the portion of the transducer molecule actually responsible for generating flagellar signals may be at fault. Because little is known about the nature of transducer signals and how they are controlled by stimuli and by methylation state, *cheD* mutations should be valuable in delineating those portions of the transducer molecule associated with signaling activity. The mapping data presented in this study were not sufficiently precise to locate the *cheD* mutations with respect to the functional domains discussed above (Fig. 1). However, it is clear that *cheD* mutations are not restricted to a small segment of the *tsr* coding region and therefore could involve several different types of structural defects that result in a similar alteration of signaling function. In this regard, it is interesting to note that at least three *cheD* alleles were located in the chemoreceptor portion of the Tsr molecule (Fig. 1) and could represent examples of the locked-receptor alteration. The remainder of the *cheD* mutations appear to affect the carboxyl half of the Tsr protein. Kehry et al. (15) have studied three alleles of the latter type but found no evidence that they affected the primary structure of the K1 and R1 methylated peptides. We are currently determining the nucleotide sequence changes associated with various *cheD* mutations to identify and precisely locate the amino acid substitutions involved. That work should provide valuable clues to the signaling domains of the Tsr transducer.

ACKNOWLEDGMENTS

We thank M. Kehry and F. Dahlquist for communication of results before publication.

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

LITERATURE CITED

1. **Berg, H. C., and D. A. Brown.** 1972. Chemotaxis in *Escherichia coli* analyzed by three-dimensional tracking. Nature (London) **239**:500–504.
2. **Bollinger, J., C. Park, S. Harayama, and G. L. Hazelbauer.** 1984. Structure of the Trg protein: homologies with and differences from other sensory transducers of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **81**:3287–3291.
3. **Boyd, A., K. Kendall, and M. I. Simon.** 1983. Structure of the serine chemoreceptor in *Escherichia coli*. Nature (London) **301**:623–626.
4. **Boyd, A., A. Krikos, and M. Simon.** 1981. Sensory transducers of *E. coli* are encoded by homologous genes. Cell **26**:333–343.
5. **Boyd, A., and M. I. Simon.** 1980. Stimulus-induced methylation generates multiple electrophoretic forms of methyl-accepting chemotaxis proteins in *Escherichia coli*. J. Bacteriol. **143**:809–815.
6. **Chelsky, D., and F. W. Dahlquist.** 1980. Structural studies of methyl-accepting chemotaxis proteins of *Escherichia coli*: evidence for multiple methylation sites. Proc. Natl. Acad. Sci. U.S.A. **77**:2434–2438.
7. **Clarke, S., and D. E. Koshland, Jr.** 1979. Membrane receptors for aspartate and serine in bacterial chemotaxis. J. Biol. Chem. **254**:9695–9702.
8. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. A manual for genetic engineering and advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **DeFranco, A. L., and D. E. Koshland, Jr.** 1980. Multiple methylation in the processing of sensory signals during bacterial chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **77**:2429–2433.
10. **Engstrom, P., and G. L. Hazelbauer.** 1980. Multiple methylation of methyl-accepting chemotaxis proteins during adaptation of *E. coli* to chemical stimuli. Cell **20**:165–171.
11. **Fowler, R. G., G. E. Degnan, and E. C. Cox.** 1974. Mutation specificity of a conditional *Escherichia coli* mutator, *mutD5*. Mol. Gen. Genet. **133**:179–191.
12. **Hazelbauer, G. L., P. Engstrom, and S. Harayama.** 1981. Methyl-accepting chemotaxis protein III and transducer gene *trg*. J. Bacteriol. **145**:43–49.
13. **Hedblom, M. L., and J. Adler.** 1980. Genetic and biochemical properties of *Escherichia coli* mutants with defects in serine chemotaxis. J. Bacteriol. **144**:1048–1060.

14. **Kehry, M. R., M. W. Bond, M. Hunkapiller, and F. W. Dahlquist.** 1983. Enzymatic deamidation of methyl-accepting chemotaxis proteins in *E. coli* catalyzed by the *cheB* gene product. Proc. Natl. Acad. Sci. U.S.A. **80**:3599–3603.
15. **Kehry, M. R., T. G. Doak, and F. W. Dahlquist.** 1984. Aberrant regulation of methylesterase activity in *cheD* mutants of *Escherichia coli*. J. Bacteriol. **161**:105–112.
16. **Kihara, M., and R. M. Macnab.** 1981. Cytoplasmic pH mediates pH taxis and weak-acid repellent taxis of bacteria. J. Bacteriol. **145**:1209–1221.
17. **Kleene, S. J., M. L. Toews, and J. Adler.** 1977. Isolation of glutamic acid methyl ester from an *Escherichia coli* membrane protein involved in chemotaxis. J. Biol. Chem. **252**:3214–3218.
18. **Kondoh, H., C. B. Ball, and J. Adler.** 1979. Identification of a methyl-accepting chemotaxis protein for the ribose and galactose chemoreceptors of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **76**:260–264.
19. **Krikos, A., N. Mutoh, A. Boyd, and M. Simon.** 1983. Sensory transducers of *E. coli* are composed of discrete structural and functional domains. Cell **33**:615–622.
20. **Lederberg, E. M., and S. N. Cohen.** 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. J. Bacteriol. **119**:1072–1074.
21. **Maeda, K., and Y. Imae.** 1979. Thermosensory transduction in *E. coli*: inhibition of the thermoresponse by L-serine. Proc. Natl. Acad. Sci. U.S.A. **76**:91–95.
22. **McClellan, T., G. F.-L. Ames, and K. Nikaido.** 1983. Genetic variation in proteins: comparison of one-dimensional and two-dimensional gel electrophoresis. Genetics **104**:381–390.
23. **Muskavitch, M. A., E. N. Kort, M. S. Springer, M. F. Goy, and J. Adler.** 1978. Attraction by repellents: an error in sensory information processing by bacterial mutants. Science **201**:63–65.
24. **Parkinson, J. S.** 1976. *cheA*, *cheB*, and *cheC* genes of *Escherichia coli* and their role in chemotaxis. J. Bacteriol. **126**:758–770.
25. **Parkinson, J. S.** 1978. Complementation analysis and deletion mapping of *Escherichia coli* mutants defective in chemotaxis. J. Bacteriol. **135**:45–53.
26. **Parkinson, J. S.** 1980. Novel mutations affecting a signaling component for chemotaxis of *Escherichia coli*. J. Bacteriol. **142**:953–961.
27. **Parkinson, J. S., and S. E. Houts.** 1982. Isolation and behavior of *Escherichia coli* deletion mutants lacking chemotaxis functions. J. Bacteriol. **151**:106–113.
28. **Parkinson, J. S., and R. J. Huskey.** 1971. Deletion mutants of bacteriophage lambda. I. Isolation and initial characterization. J. Mol. Biol. **56**:369–384.
29. **Parkinson, J. S., M. K. Slocum, A. M. Callahan, D. Sherris, and S. E. Houts.** 1983. Genetics of transmembrane signaling proteins in *E. coli*, p. 563–576. *In* H. Sund and C. Veeger (ed.), Mobility and recognition in cell biology. Walter de Gruyter & Co., Berlin.
30. **Reader, R. W., W. Tso, M. Springer, M. Goy, and J. Adler.** 1979. Pleiotropic aspartate taxis and serine taxis mutants of *E. coli*. J. Gen. Microbiol. **111**:363–374.
31. **Repaske, D. R., and J. Adler.** 1981. Change in intracellular pH of *Escherichia coli* mediates the chemotactic response to certain attractants and repellents. J. Bacteriol. **145**:1196–1208.
32. **Rollins, C., and F. W. Dahlquist.** 1981. The methyl-accepting chemotaxis proteins of *E. coli*: a repellent-stimulated, covalent modification distinct from methylation. Cell **25**:333–340.
33. **Russo, A., and D. E. Koshland, Jr.** 1983. Separation of signal transduction and adaptation functions of the aspartate receptor in bacterial sensing. Science **220**:1016–1020.
34. **Sherris, D., and J. S. Parkinson.** 1981. Posttranslational processing of methyl-accepting chemotaxis proteins in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:6051–6055.
35. **Silverman, M., P. Matsumura, and M. Simon.** 1976. The identification of the *mot* gene product with *Escherichia coli*-lambda hybrids. Proc. Natl. Acad. Sci. U.S.A. **73**:3126–3130.
36. **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.
37. **Slocum, M. K., and J. S. Parkinson.** 1983. Genetics of methyl-accepting chemotaxis proteins in *E. coli*: organization of the *tar* region. J. Bacteriol. **155**:565–577.
38. **Smith, R. A., and J. S. Parkinson.** 1980. Overlapping genes at the *cheA* locus of *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:5370–5374.
39. **Springer, M. S., M. F. Goy, and J. Adler.** 1979. Protein methylation in behavioural control mechanisms and in signal transduction. Nature (London) **280**:279–284.
40. **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.
41. **Stock, J. B., and D. E. Koshland, Jr.** 1978. A protein methylesterase involved in bacterial sensing. Proc. Natl. Acad. Sci. U.S.A. **75**:3659–3663.
42. **Tso, W.-W., and J. Adler.** 1974. Negative chemotaxis in *Escherichia coli*. J. Bacteriol. **118**:560–576.
43. **Van Der Werf, P., and D. E. Koshland, Jr.** 1977. Identification of a gamma-glutamyl methyl ester in a bacterial membrane protein involved in chemotaxis. J. Biol. Chem. **252**:2793–2795.
44. **Wang, E., K. Mowry, D. Clegg, and D. E. Koshland, Jr.** 1982. Tandem duplication and multiple functions of a receptor gene in bacterial chemotaxis. J. Biol. Chem. **257**:4673–4676.
45. **Wang, E. A., and D. E. Koshland, Jr.** 1980. Receptor structure in the bacterial sensing system. Proc. Natl. Acad. Sci. U.S.A. **77**:7157–7161.
46. **Yonekawa, H., H. Hayashi, and J. S. Parkinson.** 1983. Requirement of *cheB* function for sensory adaptation in *Escherichia coli*. J. Bacteriol. **156**:1228–1235.