Proc. Natl. Acad. Sci. USA
Vol. 74, No. 8, pp. 3317–3321, August 1977
Biochemistry

Chemotaxis in *Escherichia coli*: Methylation of *che* gene products

(chemotaxis gene/recombinant DNA/sensory transduction)

MICHAEL SILVERMAN AND MELVIN SIMON

Department of Biology (B-022), University of California, San Diego, La Jolla, California 92093

Communicated by Warren L. Butler, June 6, 1977

ABSTRACT **The products of three chemotaxis-specific genes in *Escherichia coli, cheM, cheD,* and *cheZ,* are methylated. The *cheZ* gene codes for the synthesis of a 24,000 molecular weight polypeptide that appears in the cytoplasm. *cheM* codes for the synthesis of a membrane-bound polypeptide with a molecular weight of 61,000. *cheD* codes for another membrane-bound polypeptide with an apparent molecular weight of 64,000. CheM$^-$ mutants show chemotaxis toward some attractants (Tar$^-$ phenotype), while CheD$^-$ mutants respond to other attractants (Tsr$^-$ phenotype). The double mutant (CheD$^-$, CheM$^-$) does not respond to any attractant or repellent tested. Therefore, these polypeptides play a central role in chemotaxis. They collect information from two subsets of chemoreceptors and act as the last step in the chemoreceptor pathway and the first step in the general processing of signals for transmission to the flagellar rotor. It is suggested that they may be involved in both an initial process that reflects the instantaneous state of the chemoreceptors and in an integrative, adaptive process. Two other genes, *cheX* and *cheW,* are required for the methylation of the *cheD* and *cheM* gene products.**

Behavioral studies have shown that *Escherichia coli* and *Salmonella typhimurium* sense differences in the concentration of chemoattractants or chemorepellents as a function of time (1). For responsive swimming behavior, a decision-making process is required that integrates and compares sensory information over time and produces a signal that influences the direction of flagellar rotation. The *che* gene products are central to this process, while the *mot* gene products control flagella rotation, and the *fla* gene products control the assembly of the flagellar apparatus (2–4). The biochemical events involved in chemotaxis are far from clear. However, recently Kort *et al.* showed that the methylation of an inner membrane protein, MCP (methyl-accepting chemotaxis protein), of about 60,000 daltons was specifically influenced by several *che* and *fla* gene mutations and by the application of chemotactic stimuli (5). In *Salmonella,* nine *che* cistrons have been defined (6) and the product of one of these was shown to behave as a methyl transferase (7). In *E. coli,* eight specific *che* cistrons were defined and many of their gene products were identified on polyacrylamide gels (2). Fig. 1 summarizes the positions of most of the *che* genes in *E. coli.* One of these genes, *cheM,* was shown to form a product which had many of the properties of MCP (9). However, in further studies, it became clear that there are a number of methylated chemotaxis proteins and that these have different functions in integrating the signals transmitted from chemoreceptors to the flagellar apparatus.

We report here the methylation of three *che* gene products and describe experiments that suggest that they play a central role in receiving information directly from chemoreceptors and in translating this information into specific events that effect flagellar rotation.

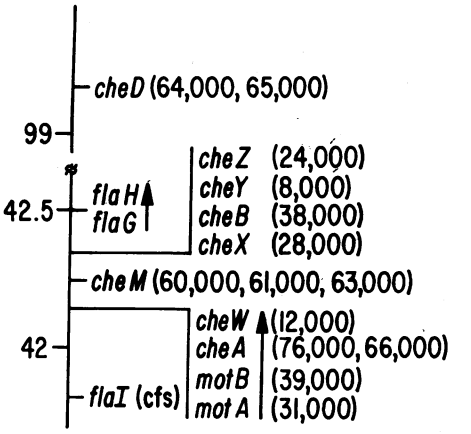


FIG. 1. Position (min) of the chemotaxis genes on the *E. coli* genome. The arrows over groups of genes indicate a cotranscribed unit. Numbers in parentheses indicate the molecular weight determined for the gene product. The *cheD* gene is located at about 99 min on the *E. coli* genetic map. The *cheC* gene is not shown. The peptide maps derived from the multiple bands associated with *cheM* were almost identical, suggesting that these polypeptides are derived from a single gene (8). The peptides derived from the 66,000 band associated with *cheA* were all contained in the pattern derived from the 76,000 band, suggesting that these two polypeptides were derived from a single gene.

METHODS

Bacterial Strains. Most of the nonchemotactic (Che$^-$) strains used in this study were isolated by Parkinson (10). The assignment of *che* gene defects in these mutants has been reported (2). Strains AW518 (Tsr$^-$; *t*axis defects to *s*erine and *r*epellents) and AW539 (Tar$^-$; *t*axis defects to *a*spartate and *r*epellents) were isolated by Adler (11). Defects in the *cheD* gene give rise to two phenotypes: (*i*) complete lack of chemotactic function, and (*ii*) lack of chemotaxis with serine and some repellents, Tsr$^-$. The null phenotype of a strain with a mutation in the *cheD* gene is Tsr$^-$ (12); therefore we have used CheD$^-$ and Tsr$^-$ interchangeably. *cheM* was previously used to refer to a region of the genome between *cheW* and *cheX* (2); CheM$^-$ referred to the absence of the *cheM* gene product. During the course of the present work, the phenotype of the CheM$^-$ strain was found to be lack of chemotaxis to aspartate, Tar$^-$ (11). Therefore, in this paper we will use the terms CheM$^-$ and Tar$^-$ interchangeably. Strain MS5033 (CheA$^-$) was used as the parent

The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: MCP, methyl-accepting chemotaxis protein; M_r, molecular weight; WT, wild type. Che and Fla refer to phenotypes related to chemotaxis (*che*) and flagella (*fla*) genes; Tsr, phenotype for *t*axis defects to *s*erine and *r*epellents (*tsr*); Tar, phenotype for *t*axis defects to *a*spartate and *r*epellents (*tar*).

for the construction of isogenic strains with *cheM* and *cheD* mutations. The construction of a CheM$^-$ strain presented particular difficulty because, initially, the gene product of the *cheM* locus was known but the phenotype of a mutation in this gene was not (2, 9). The strategy used was to introduce a *cheW cheM* deletion carried on a hybrid λ phage (λ*fla52*Δ22) into the chromosome of strain MS5033. Specifically, the introduction of the deletion required two events: (*i*) the integration of the hybrid λ*fla52*Δ22 in the homologous *cheM* region of the chromosome and (*ii*) eduction of the prophage to replace the functional *cheW cheM* locus with the *cheW cheM* deletion. The CheM$^-$ CheW$^-$ mutant was called MS5206. A CheM$^-$ strain (MS5209) was constructed by lysogenizing MS5206 with λ*fla52*Δ*1* (see ref. 4), which carried *cheW*$^+$ and a deletion of *cheM*. A Che$^+$ strain (MS5207) was constructed by infection with λ*fla52* (*cheW*$^+$ *cheM*$^+$). The CheM$^-$ CheD$^-$ mutant strain (MS5228) was constructed by phage P1-mediated transduction of the *cheD* (*tsr*) defect from strain AW518 (J. Adler) to strain MS5209 with selection for threonine-independent recombinants. An isogenic CheD$^-$ strain (MS5234) was constructed by infection of MS5228 with λ*fla52* (*cheM*$^+$) and another CheM$^-$ strain (MS5235) was derived from strain MS5228 by infection with λ*fla91* (*cheD*$^+$).

Methylation. The methylation of cellular proteins in the absence of protein synthesis was similar to the procedure devised by Kort *et al.* (5). Ten microcuries of L-[*methyl*-^{3}H] methionine with a specific activity of 14 Ci/mmol (New England Nuclear) was added and the mixture was incubated for 10 min before chemoeffector was added. Incubation was continued for another 10 min, and cells were pelleted and immediately resuspended in sodium dodecyl sulfate gel buffer.

Polyacrylamide Gel Electrophoresis. The procedures for electrophoresis on 12.5% polyacrylamide gels and preparation of fluorograms have been described previously (2–4, 13). The two-dimensional gel technique was that of O'Farrell (14). The programming of protein synthesis using hybrid λ in UV-irradiated cells and hybrid plasmids in minicells has been described (2–4, 13).

Construction of Hybrids λ. Hybrids of λ containing the *cheD* gene were constructed by using λgtλc as the vehicle and endonuclease *Eco*RI-generated fragments of plasmid pLC8-9 (15) as the donor of DNA carrying the *cheD* gene. The specific conditions for endonuclease restriction, ligation, transfection, and genetic testing of hybrid λ have been described (3). A hybrid λ with an insert containing the *cheD* gene (λ*fla90*) was originally isolated but was unstable, probably due to the excessively large size of the insert. Deletion mutants of this hybrid arose spontaneously, and these hybrid λ phages were either CheD$^+$ or CheD$^-$. Twenty of these were examined for their capacity to program protein synthesis, and one CheD$^+$ hybrid λ (λ*fla91*) was used extensively for strain construction.

Measurement of Chemotaxis. Chemotaxis of the strains used in this study was measured by the capillary assay of Adler (11). Adaptation times were determined by the method of Parkinson (12) except that chemotaxis buffer contained 10 mM sodium D,L-lactate and 1 mM L-methionine.

RESULTS

The product of the *flaI* gene is required for the synthesis of flagella-related proteins and FlaI$^-$ mutants do not synthesize MCP (5). A comparison of the methylated polypeptides in the wild-type and a FlaI$^-$ mutant strain is shown in Fig. 2. Three polypeptides labeled by [*methyl*-^{3}H]methionine in the absence of protein synthesis were missing in the FlaI$^-$ strain (see Fig. 2, column *a*). They were: a 64,000 molecular weight (M_r) polypeptide, a 61,000 M_r polypeptide, and a 24,000 M_r polypeptide. The 61,000 and 64,000 M_r polypeptides had approximately the molecular weight reported for the MCP described by Kort *et al.* (5). The addition of the chemoattractants serine and α-methylaspartate (Fig. 2, columns *a* and *b*) resulted in increased methylation of the 61,000 and 64,000 M_r components. The autoradiogram shown in Fig. 2 was deliberately overexposed to reveal the less intensely labeled polypeptide of 24,000 M_r. The methylation of the 24,000 M_r polypeptide was not obviously affected by the addition of attractants. For convenience, we will denote the 64,000, 61,000, and 24,000 M_r species as MCP(1), MCP(2) and MCP(3). MCP(1) and MCP(2) often display a complex pattern of banding on polyacrylamide gels. The pattern depends on the chemoeffector used to stimulate methylation and the conditions of the polyacrylamide gel electrophoresis. For example, MCP bands at molecular weights 60,000, 61,000, 63,000, 64,000, and 65,000 are often seen, with 61,000 and 64,000 being the prominent bands. The *cheM* gene has been reported to code for at least part of MCP (9). All the known *che* genes have been isolated on hybrid plasmids and λ phage, and the products of most of these genes have been determined by programming protein synthesis in mini-cells containing hybrid plasmids (13) or in UV-irradiated cells infected with hybrid λ (2–4). Therefore, a comparison was made between the methylated polypeptides and polypeptides synthesized from the cloned chemotaxis genes. This comparison is shown in Fig. 3, where [^{3}H]methyl-labeled polypeptides and [^{35}S]methionine-labeled polypeptides were resolved by the two-dimensional polyacrylamide gels of O'Farrell (14). Both MCP(1) and MCP(2) form streaks on the gels. However, superimposition of the autoradiograms revealed that MCP(1) had the same position as a polypeptide synthesized in minicells carrying a hybrid plasmid with the *cheD* gene. This [^{35}S]methionine-labeled polypeptide was shown to be the *cheD* gene product in experiments involving specific λ hybrids that carried the *cheD* gene (see below). MCP(2) had the same position on the two-dimensional gel as a polypeptide programmed by another plasmid that carried the *cheM* gene. This polypeptide had previously been identified as the *cheM* gene product (9). MCP(3) had the same position as the product of the *cheZ* gene.

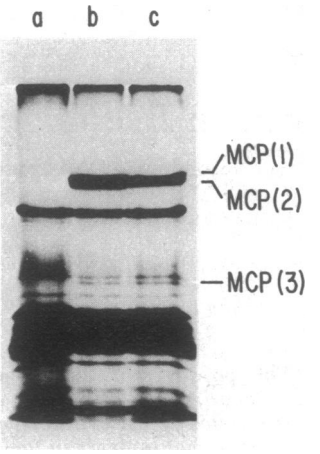


FIG. 2. Methylation of polypeptides. The separation was on a 12.5% polyacrylamide/sodium dodecyl sulfate gel. Migration was from top to bottom. Methylation with L-[*methyl*-^{3}H]methionine was in a FlaI$^-$ strain MS5026 (column *a*), and in a wild-type strain, MS5207 (columns *b* and *c*). The attractants serine and methylaspartate (1 mM) were present during the methylation shown in columns *a* and *b*.

Because the hybrid plasmid that carried the *cheD* gene

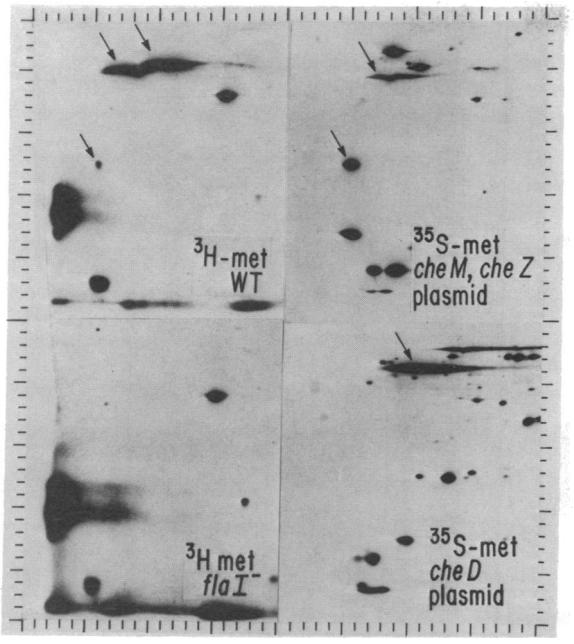


FIG. 3. Two-dimensional polyacrylamide gel electrophoresis. The fluorograms on the *left* show [^{3}H]methyl-labeled polypeptides from wild-type (WT) strain MS5207 (*upper*) and from strain MS5026 (*lower*). The fluorograms on the *right* show [^{35}S]methionine-labeled polypeptides from minicells carrying plasmid pLCl-1 (*upper*) and pLC8-9 (*lower*). The arrows show the positions of MCP(1), MCP(2), and MCP(3). The left side of each gel is the acidic end of the isoelectric focusing run. The sodium dodecyl sulfate dimension of separation in each gel is from top to bottom. Methylation was in the presence of 1 mM serine and methylaspartate.

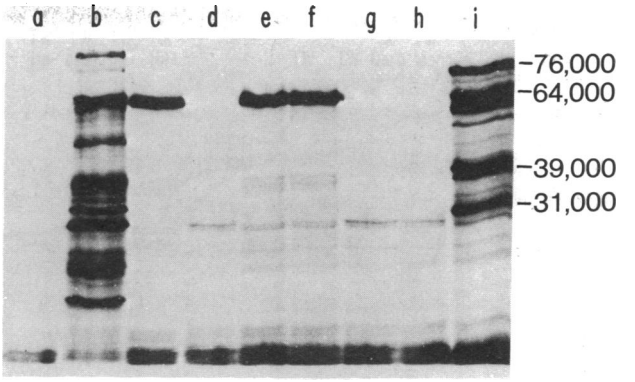


FIG. 4. Specific protein synthesis directed by hybrid λ. [^{35}S]-Methionine-labeled polypeptides were prepared as described in *Methods*, and electrophoresed on a 12.5% polyacrylamide/sodium dodecyl sulfate gel. Column *b* shows [^{35}S]methionine-labeled polypeptides from minicells carrying plasmid pLC8-9 (*cheD*$^{+}$). Synthesis of polypeptides in columns *c*, *e*, and *f* was directed by *cheD*$^{+}$ deletion mutants and that in columns *d*, *g*, and *h* was directed by *cheD*$^{-}$ λ deletion mutants. Column *a* shows polypeptides synthesized in uninfected UV-irradiated cells. Numbers at right show molecular weights for polypeptides directed by λ*fla52* (column *i*) and hybrid λ carrying the *cheD* gene.

synthesized many polypeptides in addition to the one that had properties identical to MCP(1), a hybrid λ was constructed that contained a subset of the genes carried on the plasmid including the *cheD* gene. From this hybrid (see *Methods*) a series of deletions were obtained, and they were tested, for their ability both to complement *cheD* (*tsr*) defects in transductional tests and to program protein synthesis in UV-irradiated cells. Fig. 4 shows some of the results of this comparison. The synthesis of a 64,000 M_r polypeptide always correlated with the presence of *cheD* gene activity. Indeed, only one major polypeptide whose synthesis was under *flaI* gene control (not shown), was programmed by *cheD* hybrid λ. Thus, the *cheD* gene codes for the synthesis of a 64,000 M_r polypeptide.

Methylation with CheD^{-}, CheM^{-}, and CheZ^{-} mutant strains verified the correspondences between MCP(1), MCP(2), MCP(3), and the products of the *cheD*, *cheM*, and *cheZ* genes. Fig. 5 *A* and *B* shows a comparison between methylated polypeptides prepared in various mutants and [^{35}S]methionine-labeled standards prepared from UV-irradiated hybrid λ-infected cells that synthesized the *cheD* and *cheM* gene products. The following effects were observed: (*a*) Che^{+} cells produce MCP(1) and MCP(2); MCP(3) can be seen only in longer exposures. (*b*) MCP(1) labeling is stimulated most but not exclusively by the attractant serine (column 6). (*c*) MCP(2) is stimulated most but not exclusively by the attractant methylaspartate (column 7). (*d*) MCP(2) does not appear in the CheM^{-} deletion strains (column 10) or in a Tar^{-} strain obtained from J. S. Parkinson (not shown). (*e*) MCP(1) does not appear in the CheD^{-} strain (column 12). (*f*) MCP(1) and MCP(2) do not appear when the CheD^{-}, CheM^{-} double mutant strain is tested (column 11). In addition, mutants with defects in other *che* genes affect the methylation of MCP(1) and MCP(2). MCP(1) and MCP(2) are missing in CheX^{-} and CheW^{-} mutants (Fig. 5*B*). These genes may code for an enzyme necessary for the methylation (or demethylation) of both MCP(1) and MCP(2). In overexposed gels, MCP(3) was observed to be missing only in CheZ^{-} mutants (not shown). No other *che* gene defect has been shown to affect the methylation of the *cheZ* gene product MCP(3), but the extremely low relative intensity of MCP(3) labeling makes the resolution of this matter difficult.

The function of MCP(1), MCP(2), and MCP(3) might be inferred from the phenotypes of strains with defects in the *cheD*, *cheM*, and *cheZ* genes and in other genes that affect the methylation of their products. CheD^{-} strains have been reported to have a much diminished capacity for chemotaxis toward serine, and taxis toward some other amino acids is also impaired (11). The CheM^{-} strain constructed with a hybrid λ proved upon examination to lack taxis toward methylaspartate (a nonmetabolizable analogue of aspartate) and have diminished chemotaxis toward serine as well. J. S. Parkinson (personal communication) has mapped mutations in Tar^{-} strains (aspartate-blind mutants) and shown that they correspond to the *cheM* gene defined by deletion analysis of hybrid λ phage (2). Thus, CheM^{-} mutants have a Tar^{-} phenotype. A double mutant carrying both *cheM* (Tar^{-}) and *cheD* (Tsr^{-}) mutations was constructed; it showed no apparent chemotaxis.

Some parameters of chemotactic ability are the initial response, i.e., the suppression of tumbles when an attractant is added, and adaptation time, i.e., the time required to resume the normal pattern of runs and tumbles after stimulation with an attractant that suppresses the tumble mode of swimming (12, 16). We tested the behavior of the CheM^{-} (Tar^{-}), the CheD^{-} (Tsr^{-}), and the double mutant strain. The time required to return to the pattern of normal swimming after stimulation by 0.1 mM serine was 5.0 min (±0.5 min) (SEM) for the wild-type strain and 11 min (±1 min) for the CheM^{-} (Tar^{-}) strain. The CheD^{-} (Tsr^{-}) strain was unresponsive to serine, i.e., tumbling was not suppressed by this attractant. The adaptation time for a 5 mM stimulus of methylasparate was 4.5 min (±0.5 min) for the wild-type strain and 6 min (±0.5 min) for the CheD^{-} (Tsr^{-}) strain. The CheM^{-} (Tar^{-}) strain was unresponsive to methylaspartate. The CheM^{-}, CheD^{-} double mutant was smooth swimming, and this mode of swimming was not influenced by serine, methylaspartate, alanine, glucose, galactose, ribose,

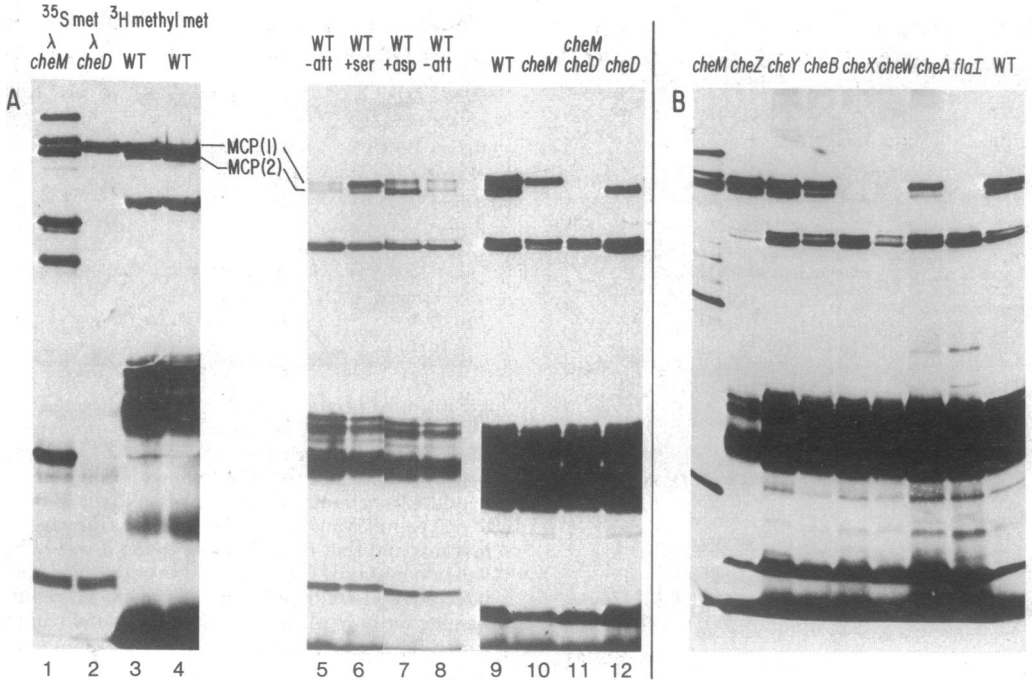


FIG. 5. (*A*) Properties of MCP(1) and MCP(2). Fluorograms were prepared from 12.5% polyacrylamide/sodium dodecyl sulfate gels that were 22 cm long. The four columns at the left show a comparison of [^{3}H]methyl-labeled polypeptides from wild-type strain MS5207 (columns 3 and 4) with [^{35}S]methionine-labeled polypeptides synthesized in UV-irradiated cells infected with hybrid λ*fla52, cheM*$^+$ (column 1) and λ*fla91, cheD*$^+$ (column 2). Methylation was in the presence of 1 mM serine and 1 mM methylaspartate. [^{3}H]Methyl-labeled polypeptides in columns 5, 6, 7, and 8 were from strain MS5207 labeled in the presence (+) or absence (−) of the attractants (att) serine (1 mM) or methylaspartate (1 mM). Columns 9, 10, 11, and 12 show the separation of [^{3}H]methyl-labeled polypeptides synthesized in the presence of 1 mM serine and methylaspartate. Polypeptides were methylated in the wild-type strain MS5207 (column 9), in the CheM$^-$ strain MS5235 (column 10), in the CheM$^-$, CheD$^-$ strain MS5228 (column 11), and in the CheD$^-$ strain MS5234 (column 12).

(*B*) Methylation of MCP(1) and MCP(2) in Che$^-$ mutants. The conditions for methylation were the same as for *A* (columns 9, 10, 11, and 12) except that methylations were performed in (starting with the second column): strain MS5129 (CheZ$^-$), strain MS5115 (CheY$^-$), strain MS5114 (CheB$^-$), strain MS5124 (CheX$^-$), strain MS5104 (CheW$^-$), strain MS5033 (CheA$^-$), strain MS5026 (FlaI$^-$), and wild-type strain MS5207 (Che$^+$). The first column, labeled *cheM*, contains [^{35}S]methionine-labeled polypeptides synthesized in UV-irradiated cells infected with hybrid λ*fla52, cheM*$^+$.

mannitol, maltose, or the repellents leucine and acetate. The single mutants retain the capacities to show the initial response and to adapt, with somewhat extended adaptation times, if the appropriate signal is provided. However, the double mutant does not respond to any of the signals provided.

DISCUSSION

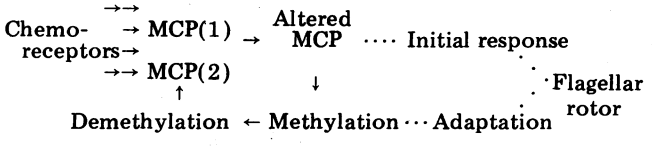
The identification of the *che* genes responsible for the synthesis of the methyl-accepting proteins allows a clearer formulation of the roles of these proteins in the responses of *E. coli*. MCP(1) is the product of the *cheD* gene, MCP(2) is the product of the *cheM* gene, and MCP(3) is the product of the *cheZ* gene. Very little is known about the methylation of the *cheZ* gene product. Unlike the *cheD* and *cheM* products, it is a cytoplasmic component rather than an integral membrane protein (H. Ridgway, M. Silverman, and M. Simon, unpublished data).

The methylation of MCP(1) is stimulated by serine, in contrast to MCP(2), whose methylation is primarily stimulated by aspartate. The influence of chemoeffectors on methylation is complex because there is some overlap in specificities. Thus serine, which is specific for MCP(1), also causes some methylation of MCP(2). CheM$^-$ mutants have the Tar$^-$ (defective for aspartate taxis) phenotype and CheD$^-$ mutants have the Tsr$^-$ (defective for serine taxis) phenotype. They are also defective in their taxis to a group of other attractants (11). In observations of individual cells that were exposed to attractant, the CheM$^-$ (Tar$^-$) mutant showed no initial response to aspartate (i.e., suppression of tumbles) and the CheD$^-$ (Tsr$^-$) mutant showed no initial response to serine. The CheM$^-$, CheD$^-$ double mutant was defective in chemotaxis to all attractants and repellents with which it was tested, including a variety of sugars and repellents. It displayed only the smooth mode of swimming and the addition of serine or aspartate or the repellents leucine and acetate produced no effect on the movement of these cells. We conclude, therefore, that the transduction of sensory information from most, if not all, chemoreceptors requires one or both of the *cheM* (*tar*) and *cheD* (*tsr*) gene products. Consistent with this conclusion is the finding that mutants in the *cheX* and *cheW* genes, which eliminate or result in low levels of MCP(1) and MCP(2) methylation, are generally nonchemotactic to all chemoattractants. Thus, the *cheM* and *cheD* gene products together play a central role in the processing of signals from receptors. The receptors themselves, or other proteins that interact with the receptors, report the binding of effectors to either the *cheM* (*tar*) or the *cheD* (*tsr*) gene products. The *cheM* product is specific for one set of receptors, while the *cheD* product responds to another set, and there is some overlap among the receptors that feed signals into MCP(1) and MCP(2).

In addition to their role in collecting signals from receptors, MCP(1) and MCP(2) must transmit the signal to the next stage in the pathway of sensory transduction. We suggest that, in response to stimulation from specific chemoreceptors, they undergo a rapid change, e.g., a change in shape or the release of ions. This change has two effects: (*i*) it results in the induction of the initial response, i.e., the suppression of tumbles and (*ii*)

and it makes MCP available for methylation. The kinetics of methylation of MCP have been shown to correspond to the adaptation process (16). Thus, MCP(1) and MCP(2), after receiving information from chemoreceptors, could allow a bifurcation of the input information: the initial change in MCP would act through the membrane to suppress the tumbling activity of the flagellar apparatus, or it might act through the cytoplasm by releasing specific ions or stimulating the methylation of the *cheZ* gene product. In addition, the methylation of MCP(1) and MCP(2) would be correlated with the extent of suppression of tumbles and the restoration of the system to its initial state (i.e., adaptation). The frequency of reversal of flagellar rotation would be governed by the subsequent interaction of the initial change and the degree of methylation.

This model is summarized in the following scheme:



Delbrück and Reichardt (17) proposed a model to explain the adaptive changes in growth of *Phycomyces* in response to light. They argued that there was a bifurcation in the flow of sensory information such that one process recorded an instantaneous measure of sensory input while another integrated the input over a greater time period. Comparison of the information in the two processes would signal the rate of change of sensory input with time and would account for adaptation. A variety of mechanisms involving "two-process" schemes have been proposed to explain chemotaxis (1, 18). The general effect on chemotaxis of the absence of MCP(1) and MCP(2) as well as the apparent specificity of these proteins for certain chemoreceptors indicates that they collect information from chemoreceptors and participate in its transmission to the flagellar rotor. We suggest that information transmission involves the participation of these proteins in two processes, the initial response and adaptation. The elucidation of the specific changes induced in MCP(1) and MCP(2) and the mechanism of methylation as well as the role of MCP(3) will require an extensive analysis of the biochemistry of the *che* gene products.

We thank Drs. Julius Adler and J. S. Parkinson for providing bacterial strains, and we thank J. S. Parkinson for the communication of unpublished results. We thank Drs. Springer *et al.* for sending us their manuscript (19) before publication. This work was supported by grants from the National Science Foundation and from the U.S. Public Health Service.

1. Macnab, R. M. & Koshland, D. E., Jr. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 2509–2512.
2. Silverman, M. & Simon, M. (1977) *J. Bacteriol.* **130,** 1317–1325.
3. Silverman, M., Matsumura, P. & Simon, M. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3126–3130.
4. Silverman, M. & Simon, M. (1976) *Nature* **264,** 577–580.
5. Kort, E. N., Goy, M. F., Larsen, S. H. & Adler, J. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3939–3943.
6. Warrick, H. M., Taylor, B. L. & Koshland, D. E., Jr. (1977) *J Bacteriol.* **130,** 223–231.
7. Springer, W. R. & Koshland, D. E., Jr. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 533–537.
8. Matsumura, P., Silverman, M. & Simon, M. (1977) *J. Bacteriol.*, in press.
9. Silverman, M., Matsumura, P., Hilmen, M. & Simon, M. (1977) *J. Bacteriol.* **130,** 877–887.
10. Parkinson, J. S. (1976) *J. Bacteriol.* **126,** 758–770.
11. Mesibov, R. & Adler, J. (1972) *J. Bacteriol.* **112,** 315–326.
12. Parkinson, J. S. (1974) *Nature* **252,** 316–319.
13. Matsumura, P., Silverman, M. & Simon, M. (1977) *Nature* **265,** 758–760.
14. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4016.
15. Clarke, L. & Carbon, J. (1976) *Cell* **9,** 91–99.
16. Springer, M. S., Goy, M. F. & Adler, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 183–187.
17. Delbrück, M. & Reichardt, W. (1956) in *Cellular Mechanisms in Differentiation and Growth,* ed. Rudnick, E. (Princeton University Press, Princeton, NJ), pp. 3–44.
18. Berg, H. C. & Tedesco, P. M. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3235–3239.
19. Springer, M. S., Goy, M. F. & Adler, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3312–3316.