Proc: Natl. Acad. Sci. USA
Vol. 74, No. 2, pp. 533–537, February 1977
Biochemistry

Identification of a protein methyltransferase as the *cheR* gene product in the bacterial sensing system

(regulation of tumbling frequency/*S*-adenosylmethionine/*Salmonella typhimurium*/chemotaxis)

WAYNE R. SPRINGER AND D. E. KOSHLAND, JR.

Department of Biochemistry, University of California, Berkeley, Calif. 94720

Contributed by Daniel E. Koshland, Jr., November 19, 1976

ABSTRACT **Methylation of membrane-bound proteins with apparent molecular weights around 65,000 does not occur in mutants of the generally nonchemotactic *cheR* class of *Salmonella typhimurium*. This was shown to be due to the lack of a protein methyltransferase in these mutants by means of an *in vitro* assay using soluble proteins, membranes, and *S*-adenosylmethionine as the methyl donor. The methylase from the wild type was purified, characterized, and shown to be of molecular weight 38,000. It is specific for proteins in *S. typhimurium* and *Escherichia coli* membranes. The methylase is not required for tumbling but appears to be essential for maintaining the appropriate rate constants and levels of the regulator of the chemotactic response.**

Chemotaxis in bacteria is a behavioral response that allows bacteria to migrate towards favorable chemicals and away from unfavorable ones. Because of the relative simplicity of bacteria, chemotaxis is a system that may provide clues as to the operation of more complex behavioral systems. Many features of the system have been elaborated (1–4), but the actual biochemical pathways have yet to be elucidated. One major clue seems to reside in the role of methionine and methylation.

In 1967 Adler and Dahl showed that methionine was essential for chemotaxis by demonstrating that methionine auxotrophs could not respond in the capillary assay (5). The findings that (*a*) regulation of tumbling frequency was the manner in which the bacteria controlled their migration (6, 7) and (*b*) methionine-starved auxotrophs did not tumble (5, 8, 9) explained the lack of chemotaxis in these bacteria. It was, however, still not clear whether methionine was required for the mechanical process of tumbling itself or for the sensing system that regulated tumbling frequency. That it was part of the regulatory process was shown by Aswad and Koshland (10), who found that a constantly tumbling methionine auxotroph continued to tumble even when it was deprived of methionine, and that the time response of its sensing system was altered relative to that of bacteria in methionine-containing medium.

The methionine requirement was next shown to be a requirement for *S*-adenosylmethionine (AdoMet) by Armstrong (8) and by Aswad and Koshland (10). Recently another major clue to the methionine effect was discovered by Adler and his coworkers (11) when they showed that a methylated protein, the methyl-accepting chemotaxis protein, MCP, of *Escherichia coli* was formed. Although the levels of methylation of this protein varied in chemotactic mutants, attempts to correlate methylation levels with chemotactic parameters did not reveal any clear pattern.

In this paper we report the isolation and characterization of an enzyme that is responsible for the methylation of proteins in *Salmonella typhimurium* membranes. This methylase is missing in the *cheR* class of generally nonchemotactic mutants and is shown to be essential for chemotactic behavior.

Abbreviations: AdoMet, *S*-adenosyl-L-methionine; MCP, methyl-accepting chemotaxis protein (11); $NaDodSO_4$, sodium dodecyl sulfate; VBC, Vogel–Bonner citrate medium (13); Che^+, wild-type phenotype for chemotaxis.

MATERIALS AND METHODS

[*methyl*-^{14}C]Methionine (55 mCi/mmol), [*methyl*-^{3}H]methionine (6 Ci/mmol) and *S*-adenosyl[*methyl*-^{14}C]methionine (59 mCi/mmol) were purchased from New England Nuclear and used at the purchased specific activities.

Generally nonchemotactic strains of *S. typhimurium* were those described earlier (12) or obtained from B. A. D. Stocker (SL 2516, SL 4041) or through mutagenesis with ICR 372 (obtained from B. N. Ames) and the selection procedure of Aswad and Koshland (12).

Methylation of whole cells with labeled methionine was essentially the procedure described by Kort *et al.* (11) except that incubations prior to addition of methionine were for eight minutes in Vogel–Bonner citrate medium (VBC) (13) containing 1% (wt/vol) glycerol and 200 μg/ml of chloramphenicol (Sigma).

The whole cells were solubilized and run on 10% polyacrylamide slab gels in sodium dodecyl sulfate ($NaDodSO_4$) according to the procedure of G. F. Ames (14), stained, dried, and autoradiographed on Kodak No-Screen x-ray film.

Soluble and membrane fractions were obtained through the procedure described by G. F. Ames (14) except that the final centrifugation was performed at 48,000 × *g* and the buffer used was VBC with the sodium ammonium phosphate replaced by an equimolar amount of dibasic sodium phosphate (N^-VBC).

Cells used for this fractionation were generally grown at 37° overnight to stationary phase in nutrient broth (Difco) supplemented with thymine to 30 μg/ml.

In vitro methylations were accomplished by incubating two parts of a membrane fraction at 30 mg/ml of protein with 15 parts of soluble fraction at 5–10 mg/ml of protein and one part labeled AdoMet (0.34 mM) at 37° for 20 min. After washing, the membranes were solubilized in $NaDodSO_4$ and run on 10% polyacrylamide slab gels as above. The portion of the dried gel under each well of the slab corresponding to the molecular weights from 60,000 to 70,000, as determined using molecular weight standards, was cut from the gel, placed in a scintillation vial with 50 μl of water, and incubated for 10 min to reswell the gel. Ten milliliters of 3% Protosol in Omnifluor–toluene (New England Nuclear) was added and the vials were gently shaken overnight at 37°. After cooling, the vials were counted in a Packard Tri-Carb liquid scintillation counter. Background, as determined from a slice of gel containing no label, was subtracted from all samples. No correction was made for the efficiency of the system.

The methylase assay consists of incubating as in the *in vitro* methylation above for 20 min with one part generally being 5 μl. The reaction mixture was then diluted with 5 ml of 0.02 M

NaOH at room temperature and after 5 min the membranes were filtered onto a Millipore HAWP 0025 00 filter, the tube and filter were rinsed twice, and finally the filter chimney was rinsed once more with 5 ml of 0.02 M NaOH. The filters were dried in a 110° oven for 5 min and counted in Aquasol (New England Nuclear). The blank was buffer added in place of soluble extract.

Protein was determined by the method of Lowry *et al.* (15).

HeLa cell membranes were prepared by the procedure of Eylar and Hagopian (16) and erythrocyte ghosts by the procedure of Blumenfeld and Zvilichovsky (17).

RESULTS

Description of the *cheR* Defect. Whole cells from generally nonchemotactic mutants in the nine complementation classes described previously (18) were incubated for a brief period with [*methyl*-^{14}C]methionine and then examined for methylated proteins. The cells were harvested, solubilized in $NaDodSO_4$, and run on 10% polyacrylamide slab gels.

The autoradiograms showed missing bands only in the case of the *cheR* mutants. Two typical densitometer tracings are shown in Fig. 1. No *cheR* mutants incorporated label into proteins migrating in this region, which corresponds to molecular weights of 60,000–70,000. Label was incorporated into proteins of this region in chemotactically wild-type (Che^+) transductants from the *cheR* mutation or in spontaneous revertants (data not shown).

The method of quantitation of methylation used by Kort *et al.* (11) was followed for the various mutant classes in *S. typhimurium*. The data are shown in Table 1. The results are very similar to those in *E. coli*, with variable amounts of label in the proteins of this region. The *S. typhimurium* classes, however, showed one significant difference, i.e., the *cheR* class gave no incorporation of radioactivity at all, whereas all the *E. coli* classes showed large variations among their members.

The apparent molecular weights of the methylated proteins are 65,000 and 63,000. The reported weight for the MCP in *E. coli* is 62,000 (11).

Identification of the *cheR* Defect. The absence of a methylated band from the *cheR* mutants could be due to a lack of (*a*) the proteins that are to be methylated, (*b*) a methylase activity, or (*c*) another substrate involved in the methylation. In previous studies S-adenosylmethionine had been implicated as the methylating agent (8, 10). To select between these alternatives would be very difficult in the whole organism and therefore a cell-free assay system was developed using cell membranes, soluble proteins, and added Ado[*methyl*-^{14}C]Met. The results are shown in Table 2.

When the membranes and soluble fractions of the *cheR* mutant, ST1038, were combined and incubated with labeled AdoMet, no significant amount of label was in proteins of molecular weight 60,000–70,000. The same experiment with a Che^+ transductant of ST1038, prepared by phage P22 transduction, ST1075, or wild type gives incorporation of label. When the soluble fraction of wild type or transductant is combined with the membranes from the mutant, label is incorporated.

These results show that the defective element in the *cheR* mutants is a protein methylase that is located mainly in the cytoplasm. There are small amounts of incorporation in the experiments where mutant soluble fractions, boiled soluble fractions, or VBC salts are incubated with wild-type membranes. These observations suggest that some of the methylase

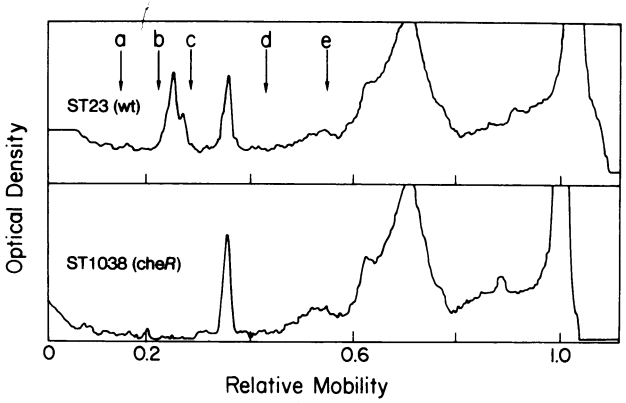

FIG. 1. Densitometer tracings of autoradiographs of polyacrylamide gel electrophoretograms of whole cells solubilized in $NaDodSO_4$. Cells were labeled with [*methyl*-^{14}C]methionine, solubilized, and run on polyacrylamide gels in $NaDodSO_4$ as described in *Materials and Methods*. The stained, dried gels were autoradiographed for 72 hr on Kodak No-Screen x-ray film, the film was developed and the autoradiogram was scanned using white light in a Transidyne RFT scanning densitometer set in the linear mode. The letters indicate the positions of marker proteins run at the same time: a, phosphorylase *a*—94,000 molecular weight; b, bovine serum albumin, 68,000; c, bovine gamma globulin heavy chain, 50,000; d, lactate dehydrogenase, 36,000; e, gamma globulin light chain, 23,500. *Upper*, ST23, chemotactically normal (wt = wild type) parent of all the generally nonchemotactic mutants. *Lower*, ST1038, *cheR* generally nonchemotactic mutant.

is not washed out in the membrane preparation. On a per cell basis, one can calculate that this amount is less than 10% of the total activity in the cell. The possibility of an overactive demethylase in the mutant was ruled out by the fact that one part ST1075 and four parts soluble fraction from ST1038 had the same activity as a one-fifth dilution of ST1075 soluble fraction.

Characterization of the Methylase. With the incorporation of label into membranes as an assay, a protein methyltransferase was purified 160-fold from the crude sonicate by means of ammonium sulfate precipitation, negative DEAE-Sephadex batch-wise ion exchange at pH 7, and Sephadex G-100 column chromatography.

In the crude extract the enzyme has an apparent molecular weight of 38,000 as judged by elution from Sephadex G-100, with a K_m for AdoMet on the order of 10 μM.

Specificity of the Methylase. When soluble fractions from the mutant ST1038 or the Che^+ transductant ST1075 are incubated with ST1038 membranes and Ado[*methyl*-^{14}C]Met, all of the label is recovered, and it is essentially entirely in the substrate, AdoMet, or in methylated protein. Deproteinized samples of ST1038 or ST1075 soluble fractions after incubation with membranes spotted onto a thin-layer chromatography plate of Adsorbasil 5 silica gel and developed in butanol/acetic acid/water showed no observable differences and no additional spots besides those resulting from unreacted AdoMet, implying that the only transfer of label under these conditions was to protein.

Since most protein methylases studied to date are somewhat nonspecific (19), the specificity of this methylase was tested by attempting to incorporate label into various proteins by means of a crude preparation of the methylase (Table 3). The proteins chosen had been shown to be methylated by other systems *in vitro* or *in vivo*. The *Salmonella* methylase shows little or no activity towards any of the soluble proteins tested. The only proteins methylated are those in the membrane of *S. typhimurium* or *E. coli*. The label was incorporated into a protein

Table 1. Methylation of proteins of molecular weights 60,000–70,000 in generally nonchemotactic mutants of *S. typhimurium*

Strain designation	Complementation class	Swimming phenotype	% wild-type methylation
ST101	*cheP*	Smooth	102
ST1002	*cheP*	Smooth	146
ST112	*cheQ*	Smooth	70
ST1001	*cheQ*	Smooth	226
ST110	*cheR*	Smooth	8
ST1038	*cheR*	Smooth	6
ST108	*cheS*	Smooth	81
ST171	*cheT*	Tumbly	71
ST203	*cheU*	Smooth	122
ST120	*cheU*	Tumbly	118
SL2516	*cheV*	Smooth	120
ST202	*cheW*	Smooth	159
ST1024	*cheW*	Smooth	154
SL4041	*cheX*	Tumbly	49

Whole cells were labeled with [*methyl*-^{3}H]methionine using the procedure of Kort *et al.* (11) with the variations noted in the *Materials and Methods*. [*methyl*-^{14}C]Methionine-labeled ST23, the parent of most of the mutants, was used to correct for differences in processing. The whole cells were solubilized and run in $NaDodSO_4$ on 10% polyacrylamide gels according to the procedures of G. F. Ames (14). Slices of the dried gels including the molecular weights 60,000–70,000, as determined by protein standards, resulting from the running of the solubilized cells were prepared and their radioactivity was measured as in *Materials and Methods*. The amount of methylation was determined as by Kort *et al.* (11). The proposed map of the generally nonchemotactic classes in *S. typhimurium* is given in Fig. 2 (18).

Table 2. Label from *S*-adenosyl[*methyl*-^{14}C]methionine incorporated into membrane proteins of molecular weight 60,000–70,000 by the *in vitro* methylation

Membranes used	Media used	cpm found in gel
ST1038	ST1038 soluble	13
ST1075	ST1075 soluble	341
ST1038	ST1075 soluble	360
ST1075	ST1038 soluble	177
ST1038	ST1075 boiled soluble	2
ST1075	ST1038 boiled soluble	62
ST1038	VBC*	5
ST1075	VBC*	44
None	ST1038 soluble	2†
None	ST1075 soluble	6†

Fifty microliters of membranes prepared from ST1038, which is *cheR*, or ST1075, which is a chemotactically wild-type transductant of ST1038, by the procedures described in *Materials and Methods* were incubated with various media under the conditions described for *in vitro* methylation. The amount of label incorporated into solubilized proteins of molecular weights 60,000–70,000 was determined as in *Materials and Methods*. Soluble fractions were prepared at concentrations of 7.5 mg/ml of protein as described, except the final centrifugation was at 300,000 × *g*. Boiled solubles were the same soluble fractions placed into a boiling-water bath for 10 min.

* Vogel–Bonner citrate medium (13)

† Radioactivity found in the soluble proteins run under the same conditions.

of molecular weight 62,000 in the *E. coli* membrane, identical to that reported for the MCP in *E. coli* (11).

To determine if membrane proteins were better substrates in general, the crude membrane fractions from HeLa cells and erythrocyte ghosts were prepared and tested, with the result that no label was incorporated as seen by this assay or radiography of gels.

The methylase described here is different from the flagellin methylase mapped to the *nml* locus because this locus is far removed from the region in which the *cheR* mutation has been mapped (18, 20). Furthermore, the molecular weights of the methylated proteins do not correspond to those reported for the *Salmonella typhimurium* flagellins (14) and membranes prepared from a mutant that produces no flagellin but does have an intact flagella basal body, SL4045 (21), have label incorporated into the same proteins as membranes prepared from wild-type cells.

Properties of the Methylase-Deficient Mutant. It was of interest to characterize further the properties of the mutant lacking the methylase protein. All of the *cheR* mutants isolated are smooth swimming. These mutants can be subjected to a sudden increase in repellents with the temporal gradient apparatus (6) and be observed to tumble for a length of time that is the same as for the recovery of wild-type bacteria. [For a 0–1.5 mM phenol jump, ST1, a Che$^+$ strain, responded for 10.3 ± 0.5 sec (SEM) and ST1038 responded for 10.2 ± 0.4 sec (SEM).] These results establish that the methylase is not essential for tumbling but rather controls the frequency of tumbling.

Under starvation for methionine, chemotactically normal bacteria became smooth swimming and no longer responded to repellents such as 17 mM acetate but remained responsive to 1.5 mM phenol even after 20 hr of starvation. A similar effect was observed for the *cheR* mutant ST1038, which is a histidine auxotroph, when it was starved for histidine. Histidine starvation has been shown not to affect chemotaxis in the capillary assay but it may reduce the responsiveness of the cell due to a general effect of deprivation, because logarithmically growing ST1038 will respond to 17 mM acetate.

DISCUSSION

The *cheR* Gene Product. The genetic analysis of the chemotactic sensing system (12, 18) of *Salmonella typhimurium* had indicated nine complementation groups as shown in Table 1 and Fig. 2. The *cheR* product has been identified in this work as a methyltransferase, and the protein is accordingly designated as the chemotaxis methyltransferase. That it is essential for chemotaxis is indicated by the failure of all attractants to produce chemotactic behavior in *cheR* mutants. *CheR* mutants completely lack the methyltransferase and Che$^+$ transductants have normal amounts of the enzyme.

The enzyme is involved in maintaining the proper level of tumbling frequency but is not essential for tumbling itself. This is shown by the fact that strong phenol gradients induce tum-

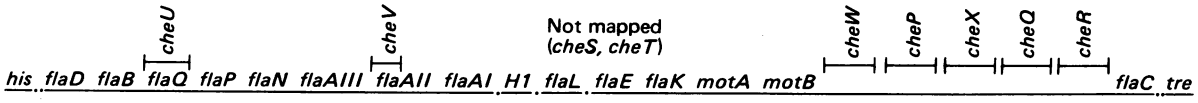

FIG. 2. Genetic map of the chemotaxis region in *Salmonella typhimurium* (cf. ref. 18).

Table 3. Specificity of the methylase

Substrate	% of MCP incorporation/ mg of protein
Bovine γ-globulin	4.5
Histones (calf thymus)	4.2
Bovine serum albumin	3.6
Arginine vasopressin	3.3
Soybean trypsin inhibitor	3.0
Bovine insulin	2.4
Lysine vasopressin	1.8
Ovalbumin	1.8
Actin	1.5
Lysozyme	1.5
Cytochrome *c*	0.8
Flagellin (*S. typhimurium*)	0.6
Ribonuclease A	0.1
Myosin	−1.9
Poly(L-lysine)	−4.4
S. typhimurium membranes	100*
E. coli membranes	103*
Erythrocyte ghosts	3.1*
HeLa membranes	−18.9*

Proteins at 2 mg/ml were incubated in place of membranes in the *in vitro* methylation. The reaction was stopped with 30% trichloroacetic acid and the mixture was heated at 90° for 15 min; the pellet was treated with 95% ethanol at 70° for 10 min and ethanol:chloroform:ether (2:1:2; vol:vol:vol) at room temperature for 10 min and finally filtered onto Whatman GF/C filters, washed, and dried. The radioactivity of the filters was measured in Aquasol (New England Nuclear). Background was determined by incubation without added protein. An estimation of the percentage of membrane proteins labeled by the methylase was determined by integration of the areas under the Coomassie-blue-stained peaks shown to be labeled by autoradiography and the area under all the peaks observed on the gel. This results in an upper limit of 3% of the total protein being substrate for the methylase.

* Given as percentage of incorporation per mg of total membrane protein.

bling in the *cheR* (methyltransferase-deficient) mutants. This conclusion extends and supports the conclusion reached by Aswad and Koshland (10), based on the responses of constantly tumbling mutants, that methionine is involved in regulation of tumble frequency.

The identification of the enzyme together with the isolation of the MCP by Adler and his coworkers (11) also establish the role of AdoMet in chemotaxis. The evidence of Armstrong (8) and our own laboratory (10) was strong in this regard but was inevitably indirect because AdoMet could have served as a precursor for other products. This work clearly places AdoMet as the substrate in the modification of the sensing system and the turnover experiments of Kort *et al.* (11) strongly suggest that a dynamic steady state is involved.

Model. It is not yet possible to describe the sensing system in detail but certain features are now more defined. The initial concept of a tumble regulator whose level is maintained by appropriate rates of formation and decomposition (6) is basically sound and additional features can now be added as shown in Fig. 3. This model assumes the following major components:

(*i*) There is a tumble regulator X that causes tumbling when its concentration falls below a threshold level and suppresses tumbling when it is above that level.

(*ii*) Favorable gradients (increase in attractants, decrease in repellents) accelerate the rate of formation of the regulator v_f and its rate of decomposition v_d but the action on v_f is much

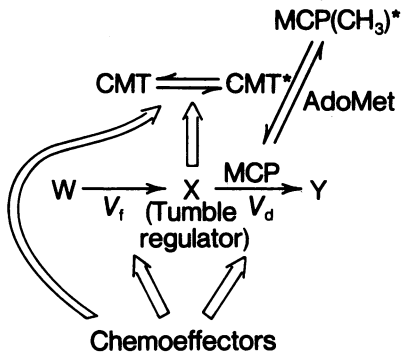

FIG. 3. A scheme for the role of a methylase in chemotaxis. CMT, chemotaxis methyltransferase; MCP, the methyl-accepting chemotactic protein (11); MCP(CH_3), methylated MCP. The form of the MCP and CMT with the asterisk is the more active form.

faster than on v_d. Unfavorable gradients have similar, but opposite effects.

(*iii*) Methylation of the protein (probably the MCP) catalyzing the decomposition of X increases the velocity of decomposition.

(*iv*) Increasing levels of attractant or X stimulates the methylase, leading to greater methylation of the MCP.

(*v*) Demethylation of the MCP occurs quite rapidly.

In this model addition of attractant stimulates an increase in the level of X because the step v_f is initially activated more readily than v_d. Increase in effector or X causes an increase in methylase activity which thereby accelerates the breakdown of X. As a result the tumble regulator returns to its steady-state value but only after a time lag during which the pool level of X has risen and tumbling is suppressed. The balance of rate constants in the various enzymatic steps can be adjusted to optimize the pool levels and the bacterial memory. This model should be considered as illustrative of a class because precisely the same time-dependence could be generated by a variety of schemes, e.g., a scheme in which the enzyme for step v_f is methylated, leading to inactivation of that enzyme. Its usefulness as a working hypothesis, however, is appreciable because it allows the explanation of a variety of observations:

(*i*) Tumbling can occur in the methylase-deficient mutants. Since the methylase helps regulate but is not itself the tumble regulator, other influences can lower the level of regulator and induce tumbling.

(*ii*) The absence of AdoMet (through methionine starvation) has the same effect as the lack of methylase. Since methylation can be stopped by either lack of enzyme or lack of AdoMet, the effect on tumbling frequency would be expected to be similar in either case.

(*iii*) ATP depletion causes smooth swimming. The ATP requirement for chemotaxis is probably a requirement for AdoMet synthesis, but differences between ATP depletion and methionine starvation have been found (10, 23). We can generate tumbling with phenol gradients in both cases, implying that neither directly affects the tumble generator but both ATP depletion and methionine starvation have similar effects on regulation of tumbling.

This scheme is a working model which is one of a number of similar types and is certainly still too simple to represent all the complexities of chemotaxis. However, it does serve to emphasize the relationships of the response regulation and as a design for experimentation. The regulation of protein activity by methylation described here may have similarities to the regulation postulated for phosphorylation by the cyclic-AMP-dependent

protein kinases (24). In both cases a molecule (X or cAMP) activates the modification enzyme, which then modifies its substrate with a resultant change in activity. Recently Diliberto and Axelrod have suggested that protein methylase II from bovine pituitary might function, through methylation of the anterior pituitary hormones, in their storage and/or transport (25). It has not yet been possible to assign specific differences in enzymatic function to methylated and unmethylated proteins. Their roles in the different species should be interesting.

Note Added in Proof. Paul Van Der Werf has now shown by isolating the glutamic acid methyl ester that the methylase modifies a glutamyl residue.

We acknowledge the valuable assistance of Kristen Sparrow and helpful discussions with Dr. Paul Van Der Werf. We also thank Dr. S. Mockrin for a gift of actin. This work is supported in part by U.S. Public Health Service Research Grant AM-09765 and Training Grant GM-00031.

1. Adler, J. (1975) "Chemotaxis in bacteria," *Annu. Rev. Biochem.* **44**, 341–356.
2. Berg, H. C. (1975) "Chemotaxis in bacteria," *Annu. Rev. Biophys. Bioeng.* **4**, 119–136.
3. Parkinson, J. S. (1975) "Genetics of chemotactic behavior in bacteria," *Cell* **4**, 183–188.
4. Koshland, D. E., Jr. (1974) "Chemotaxis as a model for sensory systems," *FEBS Lett.* **40** (Suppl), S3–S9.
5. Adler, J. & Dahl, M. (1967) "A method for measuring the motility of bacteria and for comparing random and non-random motility," *J. Gen. Microbiol.* **46**, 161–173.
6. Macnab, R. & Koshland, D. E., Jr. (1972) "The gradient sensing mechanism in bacterial chemotaxis," *Proc. Natl. Acad. Sci. USA* **69**, 2509–2512.
7. Berg, H. & Brown, D. (1972) "Chemotaxis in *Escherichia coli* analysed by three-dimensional tracking," *Nature* **239**, 500–504.
8. Armstrong, J. B. (1972) "An S-adenosylmethionine requirement for chemotaxis in *Escherichia coli*," *Can. J. Microbiol.* **18**, 1695–1701.
9. Aswad, D. & Koshland, D. E., Jr. (1974) "Role of methionine in bacterial chemotaxis," *J. Bacteriol.* **118**, 640–645.
10. Aswad, D. & Koshland, D. E., Jr. (1975) "Evidence for an S-adenosylmethionine requirement in the chemotactic behavior of *Salmonella typhimurium*," *J. Mol. Biol.* **97**, 207–223.
11. Kort, E. N., Goy, M. F., Larsen, S. H. & Adler, J. (1975) "Methylation of a membrane protein involved in bacterial chemotaxis," *Proc. Natl. Acad. Sci. USA* **72**, 3939–3943.
12. Aswad, D. & Koshland, D. E., Jr. (1975) "Isolation, characterization and complementation of *Salmonella typhimurium* chemotaxis mutants," *J. Mol. Biol.* **97**, 225–235.
13. Vogel, H. & Bonner, D. (1956) "Acetylornithinase of *Escherichia coli:* partial purification and some properties," *J. Biol. Chem.* **218**, 97–106.
14. Ames, G. F. (1974) "Resolution of bacterial proteins by polyacrylamide gel electrophoresis on slabs," *J. Biol. Chem.* **249**, 634–644.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) "Protein measurement with the Folin phenol reagent," *J. Biol. Chem.* **193**, 265–275.
16. Eylar, E. H. & Hagopian, A. (1971) "Isolation of plasma membranes from mammalian cells," in *Methods in Enzymology*, ed. Jakoby, W. B. (Academic Press, New York), Vol. 22, pp. 123–130.
17. Blumenfeld, O. O. & Zvilichovsky, B. (1972) "Isolation of glycoproteins from red cell membranes using pyridine," in *Methods in Enzymology*, ed. Ginsburg, V. (Academic Press, New York), Vol. 28, pp. 245–252.
18. Koshland, D. E., Jr., Warrick, H., Taylor, B. & Spudich, J. (1976) "The control of flagellar rotation in bacterial behavior," in *Cell Motility*, eds. Goldman, R., Pollard, T. & Rosenbaum, J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 57–69.
19. Paik, W. K. & Kim, S. (1975) "Protein methylation: chemical, enzymological, and biological significance," in *Advances in Enzymology*, ed. Meister, A. (Interscience, New York), Vol. 42, pp. 227–286.
20. Sanderson, K. E. (1972) "The linkage map of *Salmonella typhimurium*," *Bacteriol. Rev.* **36**, 558–586.
21. Stocker, B. A. D. & Yamaguchi, S. (1973) "A new *fla* gene in *Salmonella typhimurium*—*fla*R—and its mutant phenotype—superhooks," *Arch. Mikrobiol.* **90**, 107–120.
22. Springer, M. S., Kort, E. N., Larsen, S. H., Ordal, G. W., Reader, R. W. & Adler, J. (1975) "Role of methionine in bacterial chemotaxis: Requirement for tumbling and involvement in information processing," *Proc. Natl. Acad. Sci. USA* **72**, 4640–4644.
23. Larsen, S. H., Adler, J., Gargus, J. J. & Hogg, R. W. (1974) "Chemomechanical coupling without ATP: The source of energy for motility and chemotaxis in bacteria," *Proc. Natl. Acad. Sci. USA* **71**, 1239–1243.
24. Rubin, C. S. & Rosen, O. M. (1975) "Protein phosphorylation," *Annu. Rev. Biochem.* **44**, 831–887.
25. Diliberto, E. J., Jr. & Axelrod, J. (1974) "Characterization and substrate specificity of a protein carboxymethylase in the pituitary gland," *Proc. Natl. Acad. Sci. USA* **71**, 1701–1704.