JOURNAL OF BACTERIOLOGY, Jan. 1987, p. 371–379
0021-9193/87/010371-09$02.00/0

Vol. 169, No. 1

Phenol: a Complex Chemoeffector in Bacterial Chemotaxis

YASUO IMAE,[1] KENJI OOSAWA,[1]† TAKAFUMI MIZUNO,[1]‡ MAY KIHARA,[2] AND ROBERT M. MACNAB[2]*

Institute of Molecular Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464, Japan,[1] and Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06511-8112[2]

Received 7 August 1986/Accepted 24 October 1986

Earlier observations that phenol is a repellent for *Salmonella typhimurium* but an attractant for *Escherichia coli* were confirmed. This behavioral difference was found to correlate with a difference in the effect phenol had on receptor methylation levels; it caused net demethylation in *S. typhimurium* but net methylation in *E. coli*. On the basis of mutant behavior and measurement of phenol-stimulated methylation, the attractant response of *E. coli* was shown to be mediated principally by the Tar receptor. In *S. typhimurium*, two receptors were found to be sensitive to phenol, namely, an unidentified receptor, which mediated the repellent response and showed phenol-stimulated demethylation; and the Tar receptor, which (as with *E. coli*) mediated the attractant response and showed phenol-stimulated methylation. In wild-type *S. typhimurium*, the former receptor dominated the Tar receptor, with respect to both behavior and methylation changes. However, when the amount of Tar receptor was artificially increased by the use of Tar-encoding plasmids, *S. typhimurium* cells exhibited an attractant response to phenol. No protein analogous to the phenol-specific repellent receptor was evident in *E. coli*, explaining the different behavioral responses of the two species toward phenol.

Escherichia coli and *Salmonella typhimurium* are bacterial species that closely resemble each other in many respects, including their motility systems and the expression of the systems in the behavioral response known as chemotaxis (R. M. Macnab, *in* J. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, H. E. Umbarger, and F. C. Neidhardt, ed., Escherichia coli *and* Salmonella typhimurium: *cellular and molecular biology*, in press). For the most part, these species respond to the same repertoire of stimuli. There are exceptions, including citrate, which is an attractant for *S. typhimurium* but not for *E. coli* (13, 15); maltose, which is an attractant for *E. coli* but not for *S. typhimurium* (whose Tar receptor fails to recognize maltose-binding protein [8, 22]); and phenol, which is a repellent for *S. typhimurium* but an attractant for *E. coli*. Phenol sensing is the subject of the present study.

The original discovery of the differing responses to phenol of the two bacterial species was made serendipitously by Lederberg in 1956 (19) in the course of using antisera that contained phenol added as a preservative. The repellent response of *S. typhimurium* to phenol was subsequently confirmed by Tsang et al. (40); positive and negative temporal gradients cause tumbling (repellent) responses and smooth-swimming (attractant) responses, respectively, and cells move across a stepwise spatial gradient to the side with the lower phenol concentration. The absence of a repellent response to phenol by *E. coli* and the presence of an attractant response were confirmed by capillary assay studies of Tso and Adler (41). The response is a puzzling one, since phenol cannot be used as a carbon source for *E. coli* (data not shown) and is just as toxic to that species as to *S. typhimurium*, inhibiting growth at a concentration of 10 mM (41).

Subsequently, Khan and Macnab (14) found, in apparent conflict with the findings of Lederberg (19) and of Tso and Adler (41), that phenol was a repellent for *E. coli*, causing a smooth-swimming mutant to give a prolonged tumbling response. It was not realized then that the nature of the mutant was important for the repellent response.

We investigated in more detail the phenol responses of *E. coli* and *S. typhimurium*, including the role of the chemotaxis methylation system. We conclude that the attractant response of *E. coli* is accompanied by the methylation of known receptors (principally Tar), whereas the repellent response of *S. typhimurium* is accompanied by the demethylation of another as yet unidentified receptor.

(In the remainder of this paper, we use the subscripts E and S to distinguish the genes and gene products of *E. coli* and *S. typhimurium*, respectively.)

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* and *S. typhimurium* bacterial strains and plasmids used in this study are listed in Table 1. Isolation of plasmid DNA and transformation of cells were performed by standard methods described previously (22). Transformation of *S. typhimurium* with *E. coli*-based plasmids was accomplished by first transforming *S. typhimurium* LB5000, a restriction-defective mutant (6).

Chemicals. Phenol and glycerol were obtained from Wako Pure Chemicals, Osaka, Japan, and J. T. Baker Chemical Co., Phillipsburg, N.J.; phenol was redistilled before use. Synthetic L-serine, L-aspartate, and L-leucine were obtained from ICN K and K Laboratories, Inc., Plainview, N.Y.; L-[*methyl*-^{3}H]methionine (12 Ci/mmol) was obtained from New England Nuclear Corp., Boston, Mass. Autofluor was obtained from National Diagnostics, Somerville, N.J. Chloramphenicol and tetracycline were obtained from Sigma Chemical Co., St. Louis, Mo.

Cell growth. Cells were grown at 35°C with shaking in tryptone broth (1% tryptone, 0.5% NaCl) supplemented with 0.5% glycerol or in nutrient broth (Difco Laboratories, Detroit, Mich). In the late log phase of growth, cells were harvested and washed with motility medium (10 mM potassium phosphate buffer [pH 7.0] and 0.1 mM potassium EDTA, with 10 mM sodium DL-lactate or 10 mM glycerol as

* Corresponding author.
† Present address: Division of Biology, California Institute of Technology, Pasadena, CA 91125.
‡ Present address: Yakult Central Institute for Microbiological Research, Kunitachi, Tokyo 186, Japan.

TABLE 1. Bacterial and plasmid strains

Strain or plasmid	Relevant genotype	Motility phenotype	References
E. coli			
RP437		Wild-type taxis	27
RP487		Wild-type taxis	28
AW405		Wild-type taxis	2
RP4080	*cheR*$_E$*217*	Defective in methylation activity and adaptation	26
AW655	*tsr*$_E$*12*	Defective in serine taxis	16
RP4368	*tsr*$_E$*-1*	Defective in serine taxis	35
RP5698	Δ*tsr*$_E$*-7028*	Defective in serine taxis	22
AW656	*tar*$_E$	Defective in aspartate taxis	16
RP4324	Δ(*tar*$_E$-*tap*$_E$)*5201*	Defective in aspartate taxis	34
AW701	*trg*$_E$	Defective in ribose and galactose taxis	16
TH403	*trg*$_E$*-1*::Tn*5*	Defective in ribose and galactose taxis	11
AW569	*tsr*$_E$*-1 tar*$_E$	Defective in serine and aspartate taxis	16, 37
MS5228	*tsr*$_E$*-1*Δ (*tar*$_E$-*tap*$_E$)*5201*	Defective in serine and aspartate taxis	33
RP4372	*tsr*$_E$*-1*Δ(*tar*$_E$-*tap*$_E$)*5201*	Defective in serine and aspartate taxis	10, 26
AW660	*tsr*$_E$*-12 tar*$_E$ *trg*$_E$*-1*	Defective in serine, aspartate, ribose, and galactose taxis	16
S. typhimurium			
ST1		Wild-type taxis	3
ST1038	*cheR*$_S$	Defective in methylation activity and adaptation	38
ST330	*tsr*$_S$[a]	Defective in serine taxis	S. Panasenko and D. E. Koshland, Jr., unpublished data
ST328	*tar*$_S$[a] Tet^r	Defective in aspartate taxis	S. Panasenko and D. E. Koshland, Jr., unpublished data
ST334	*tsr*$_S$[a] *tar*$_S$[a] Tet^r	Defective in serine and aspartate taxis	S. Panasenko and D. E. Koshland, Jr., unpublished data
LB5000	*hsdLT6 hsdSA29 hsdSB*	Restriction defective	6
Plasmids			
pNM17	p_{tac} *tar*$_E$$^+$ Ap^r	pBR322 derivative	N. Mutoh and M. Simon, unpublished data
pRK41	p_{meche} *tar*$_S$$^+$ Ap^r	pBR322 derivative	30

[a] Genotype inferred from behavioral and methylation phenotype.

a carbon source) as previously described (24, 36). When needed, 0.1 mM methionine was added to the medium.

Measurement of chemotactic responses. The response of bacteria to phenol or other chemoeffectors was measured by a temporal stimulation method. In the case of tethered cells, the changes in the rotation mode after stimulation were observed microscopically and analyzed as described previously (12). Antiserum against *S. typhimurium* flagellar filament, used for tethering the cells, was a gift of R. Kamiya, Nagoya University, Nagoya, Japan). The response of free-swimming cells was analyzed as described previously (24).

Measurement of methylation levels of receptors. Total methylation levels of receptors were measured as described previously (25) based on the method of Springer et al. (37). Methylation-banding patterns were obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (25, 36).

RESULTS

Behavioral responses of wild-type *E. coli* and *S. typhimurium* to phenol stimulation. In agreement with previous results (41), we found that phenol was an attractant for *E. coli*. Free-swimming cells of wild-type strains (AW405, RP437, or RP487) responded to 5 mM phenol with a 30 to 60 s smooth-swimming response followed by a prolonged increase in tumbling frequency and gradual adaptation to prestimulus behavior. The behavior of tethered cells is illustrated in Fig. 1a; phenol (5 mM) caused about 1 min of exclusively counterclockwise (CCW) rotation followed by mixed CCW and clockwise (CW) rotation. The cells were still chemotactically competent at this phenol concentration, judged by the fact that the attractant serine (1 mM) caused prolonged (>1 min) CCW rotation (data not shown).

Also in agreement with previous results (40), we found that wild-type *S. typhimurium* ST1 gave a repellent response to 5 mM phenol; free-swimming cells tumbled for about 1 min, adapting gradually thereafter. Tethered cell experiments also demonstrated the repellent response. The response of a tethered cell to 5 mM phenol is shown in Fig. 1b; there was a period of about 30 s of exclusively CW rotation followed by mixed CCW and CW rotation.

Behavioral response of *E. coli* mutants to phenol stimulation. We wished to know whether the attractant response of *E. coli* involved the chemotaxis methylation system. The CheR protein (38) is an enzyme that methylates several classes of sensory receptors (methyl-accepting chemotaxis proteins [17]) and thereby controls the process of adaptation to the tactic stimulus; *cheR* mutants show adaptation to non-methylation-linked stimuli, such as oxygen and phosphotransferase sugars (9) but not to methylation-linked stimuli (either attractants or repellents) (12, 28). We examined the behavioral response to phenol of an *E. coli cheR* (*cheR*$_E$) mutant, RP4080. Since the unstimulated phenotype of the mutant is exclusive CCW rotation, tethered cells were first stimulated by the repellent leucine (30 mM) to convert them to predominantly CW rotation (Fig. 1c). Subsequent addition of phenol (5 mM) gave a predominantly CCW response that

lasted indefinitely. The failure to adapt therefore indicated that the attractant response of *E. coli* to phenol was likely to be mediated by the chemotactic methylation system.

To determine which receptors were involved, *E. coli* mutants defective in the various receptor classes (Tsr_E, Tar_E, Tap_E, and Trg_E) were examined. In experiments with free-swimming cells, tsr_E and trg_E mutants gave a brief smooth-swimming response, whereas tar_E and tar_E tap_E mutants gave a prolonged tumbling response. At concentrations at which it was chemotactically effective, phenol had some deleterious effect on motility; this was more noticeable with *E. coli* than with *S. typhimurium* and could make the scoring of tumbling responses difficult. However, tar_E or tar_E tap_E mutants showing a sluggish tumbling response to phenol could be converted to slow smooth swimming by 0.1 mM serine, indicating that they were still chemotactically competent. Thus, the presence of Tar_E, but not that of Tsr_E or Tap_E, was required for the attractant response to phenol.

In tethering experiments, although not all cells responded, those that did (about 50 to 60% of the population, depending on the particular strain) provided a consistent pattern. tsr_E mutants (AW655 and RP5698) gave a CCW response, although it was less pronounced than that of the wild type; a trg_E mutant (AW701) gave a CCW response comparable to that of the wild type. tar_E mutants, in contrast, gave a CW response; cells did not display 100% CW rotation but a rapid CW-CCW oscillation that continued indefinitely. This repellent response was observed in all mutants tested (single or multiple, missense or deletion) as long as they possessed a Tar_E^- defect; strains tested included AW656, AW569, RP4324, and RP4372. The repellent response to phenol was easily demonstrated in a tsr_E tar_E mutant such as AW569 (Fig. 1e), which before stimulation showed almost no CW rotation. Such observations confirm those made by Khan and Macnab (14) with a similar strain (RP4372) and show that phenol can act as a repellent for certain mutant classes of *E. coli*. It is noteworthy that AW660, which has defects in Tsr_E, Tar_E, and Trg_E, also showed a weak tumbling response to 5 mM phenol. This would seem to indicate that only the absence of Tar_E, regardless of the presence or absence of other receptors, was sufficient to cause the repellent response of *E. coli* to phenol.

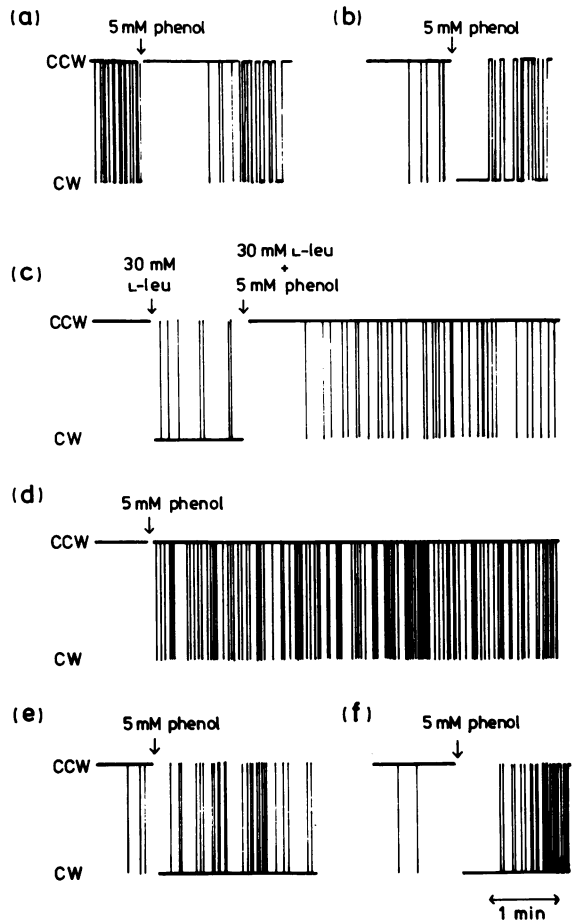

FIG. 1. Changes in rotational state of tethered cells in response to addition of phenol. At the times indicated (arrows), additions were made as shown. (a) *E. coli* AW405 (wild type), CCW response with adaptation. (b) *S. typhimurium* ST1 (wild type), CW response with adaptation. (c) *E. coli* RP4080 ($cheR_E$), CCW response with little or no adaptation. (d) *S. typhimurium* ST1038 ($cheR_S$), partial CW response with no adaptation. (e) *E. coli* AW569 (tsr_E tar_E), prolonged CW response. (f) *S. typhimurium* ST334 (tsr_S tar_S), CW response with gradual adaptation.

Behavioral response of *S. typhimurium* mutants to phenol stimulation. Analogous experiments to those described above were also performed on *S. typhimurium*. Tethered cells of an *S. typhimurium cheR* ($cheR_S$) mutant, ST1038, showed enhanced CW rotation for an indefinite time after the addition of 5 mM phenol (Fig. 1d), suggesting that the chemotaxis methylation system of *S. typhimurium* is involved in the recognition of phenol as a repellent.

Mutants of *S. typhimurium* have been isolated that are defective in either serine or aspartate taxis (22; S. Panasenko and D. E. Koshland, Jr., personal communication). It is presumed that the mutations are in the *S. typhimurium* genes homologous to tsr_E and tar_E; this presumption is supported by methylation data presented below.

Free-swimming cells of strains ST330 (tsr_S), ST328 (tar_S), and ST334 (tsr_S tar_S) all showed essentially the same response to phenol as did the wild type, namely, pronounced tumbling, suggesting that neither the serine nor aspartate receptors were responsible for the phenol response of *S. typhimurium*. The CW response of a tethered cell of ST334 is shown in Fig. 1f.

Effect of phenol on methylation levels in wild-type *E. coli* and *S. typhimurium*. Cells of wild-type *E. coli* RP487 and *S. typhimurium* ST1 were incubated with [*methyl*-^{3}H]methionine under conditions of protein synthesis inhibition and then stimulated with either phenol or glycerol (a repellent for *E. coli* that causes demethylation of several classes of receptors [24, 25]). Phenol (10 mM) caused an increase in the methylation level of wild-type *E. coli* (Fig. 2a) but a decrease in that of wild-type *S. typhimurium* (Fig. 2b). Glycerol, which is a repellent for both species (24; Y. Imae, unpublished data), caused a decrease in the methylation levels of both species. Thus, the changes in methylation level caused by a given chemical in a given species correlated with the observed behavioral response.

Effect of phenol on methylation levels in *E. coli* mutants. *E. coli* mutants with a deletion in tsr_E or tar_E permitted the methylation of the other receptor to be examined without interference. Glycerol caused a reduction in the methylation levels of both classes of mutants (Fig. 3), in agreement with previous work (25) implicating both Tar_E and Tsr_E in glycerol sensing. Phenol caused a substantial proportional increase in the methylation level of the tsr_E mutant (Fig. 3a) and a smaller proportional increase in that of the tar_E mutant

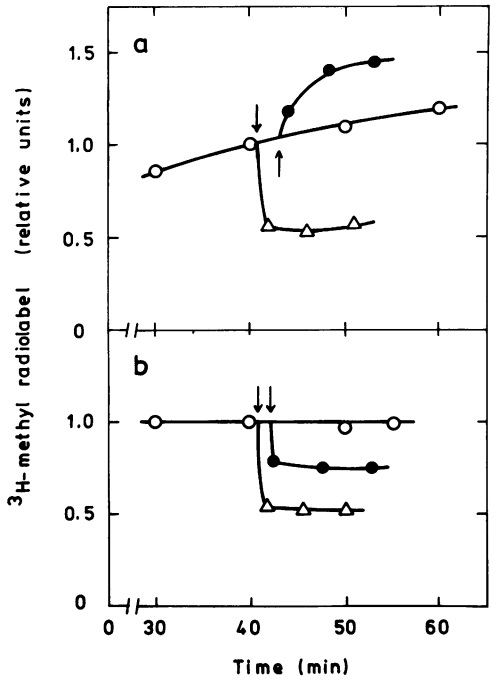

FIG. 2. Effect of phenol or glycerol on the methylation levels of receptors in wild-type *E. coli* RP487 (a) and *S. typhimurium* ST1 (b). Cells were incubated in [*methyl*-^{3}H]methionine in the presence of chloramphenicol. At the times indicated (arrows), 10 mM phenol (●), 1 M glycerol (△), or deionized water (○) was added. Samples were taken at various times, and the protein was precipitated with trichloroacetic acid and subjected to SDS-PAGE. Proteins in the 50- to 70-kilodalton range (in which the methyl-accepting receptors were found) were excised from the gels, and the radioactivity was counted. Radioactivity values are normalized to the time immediately before the additions were made.

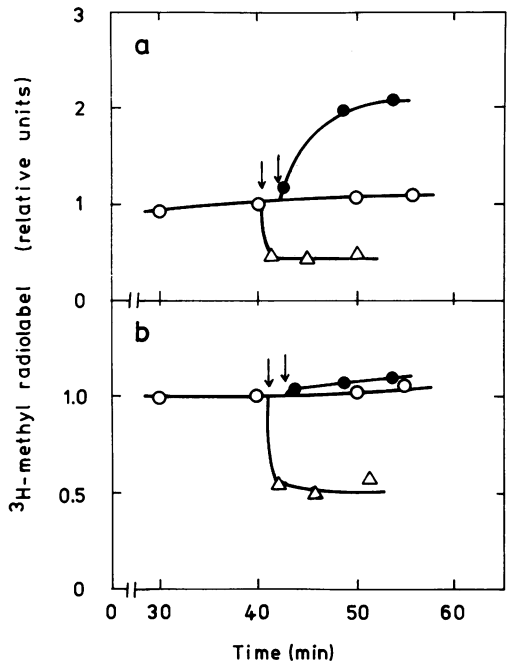

FIG. 3. Effect of phenol or glycerol on the methylation levels of receptors in *E. coli* mutants RP5698 (tsr_E) (a) and RP4324 (tar_E) (b). For other details, see legend to Fig. 2.

(Fig. 3b), suggesting, as seemed likely from the behavioral results, that Tar_E was the receptor principally responsible for the attractant effect of phenol.

A trg_E mutant, TH403, also underwent a substantial increase in methylation level after phenol addition (data not shown), indicating that the Trg_E protein was not needed for the sensing of phenol as an attractant.

tsr_E tar_E tap_E mutants showed very low methylation levels; neither enhancement nor decrease was observed after phenol addition to such a mutant, namely, RP4372.

Effect of phenol on methylation levels in *S. typhimurium* mutants. Strains ST330 (tsr_S), ST328 (tar_S), and ST334 (tsr_S tar_S) were assayed for methylation changes after phenol or glycerol addition (Fig. 4). One major difference from *E. coli* was that the *S. typhimurium* double mutant had quite substantial methylation levels both before and after stimulation, suggesting a contribution from some other receptor. Phenol caused demethylation in all three mutants (Fig. 4) to an extent similar to that seen in the wild type (Fig. 2), indicating that impaired function with regard to serine and aspartate sensing did not interfere with phenol-induced demethylation. Glycerol-induced demethylation, in contrast, was significantly reduced when both Tsr_S and Tar_S were absent.

Effect of phenol on *E. coli* receptor methylation-banding patterns. After SDS-PAGE, the banding patterns of the Tsr_E and Tar_E proteins of *E. coli* (which reflect different multiply methylated forms of these receptors) are almost completely resolved from each other and have been described extensively (5, 7, 37). The effect of phenol on these patterns is

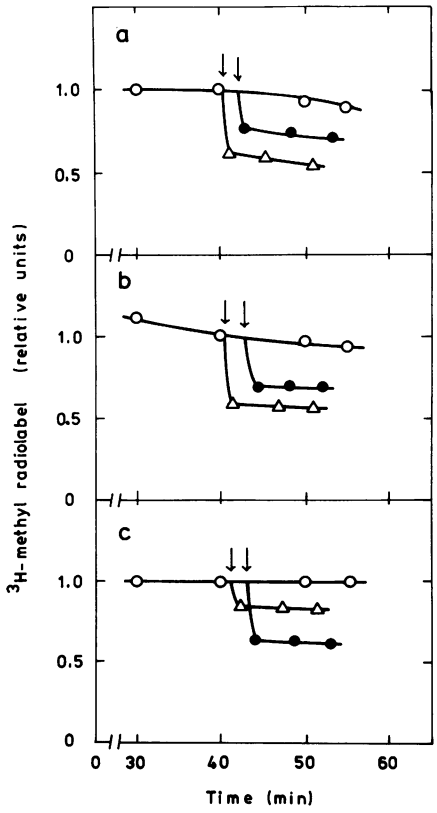

FIG. 4. Effect of phenol or glycerol on the methylation levels of receptors in *S. typhimurium* mutants ST330 (tsr_S) (a), ST328 (tar_S) (b), and ST334 (tsr_S tar_S) (c). For other details, see legend to Fig. 2.

shown in Fig. 5 for *E. coli* strains RP437 (wild type), RP4324 (tar_E tap_E), RP4368 (tsr_E), and RP4372 (tsr_E tar_E tap_E). Both Tsr_E and Tar_E showed an increase in methylation levels, with the increase being proportionally greater to Tar_E (the unstimulated methylation level of Tsr_E was greater than that of Tar_E). The increase appeared to be rather uniformly distributed over all the bands that were visible on the gel. In the case of the tsr_E tar_E tap_E mutant, no methyl label was detected in the molecular mass range of the methyl-accepting receptors (ca. 60 kilodaltons), either before or after the addition of phenol.

Characterization of *S. typhimurium* receptor methylation-banding patterns. The electrophoretic patterns of the methyl-accepting receptors of *S. typhimurium* have not been extensively described, perhaps because Tsr_S and Tar_S bands show considerable overlap. Before examining the effects of phenol, we wished to establish which bands were stimulated by serine and aspartate and, therefore, presumably derived from Tsr_S and Tar_S, respectively.

With wild-type *S. typhimurium* ST1, four major methylated bands could be recognized, as well as several minor ones (Fig. 6a). Serine caused a large increase in the intensities of bands 2 and 3; aspartate caused a large increase in band 3 only. Thus, band 2 appeared to belong predominantly to Tsr_S, and band 3 appeared to contain contributions from both Tsr_S and Tar_S. This interpretation was supported by the gel patterns from mutants (Fig. 6a). All four bands were seen in strain ST328 (tar_S); aspartate did not affect the pattern, whereas bands 2 and 3 were enhanced by serine. Band 2 was missing in strain ST330 (tsr_S); serine did not affect the pattern, whereas aspartate caused a pronounced increase in band 3.

A striking observation was that band 4, which was the most intense band in unstimulated cells of all the strains tested, did not change in intensity appreciably after the addition of serine or aspartate and was still present at a high intensity (as high as or higher than the wild type) in both tsr_S and tar_S mutant cells.

Phenol-induced methylation changes in *S. typhimurium* in the absence of Tsr_S and Tar_S function. The above results show that in *S. typhimurium* a substantial fraction of the methyl-labeled material in the molecular weight range of methyl-accepting receptors was not linked to the sensing of either serine or aspartate. Since we had already established that a tsr_S tar_S mutant gave a wild-type response to phenol and underwent phenol-induced demethylation (Fig. 4c), we were interested in which methylated bands would be affected and especially whether band 4 would be affected.

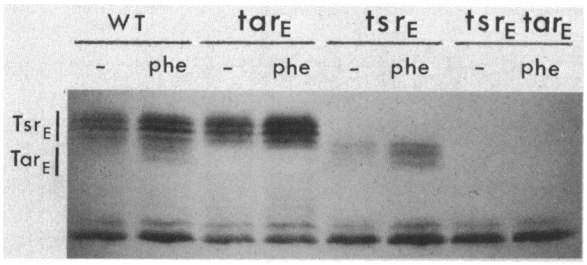

FIG. 5. Effect of phenol on the methylation of the Tsr_E and Tar_E receptors of *E. coli*. Cells were incubated with [*methyl*-^{3}H]methionine at 30°C for 40 min and then for another 15 min after the addition of motility medium (−) or 5 mM phenol (phe). The samples were then subjected to SDS-PAGE (11% polyacrylamide) and autoradiography. Strains used (in pairs of lanes from left to right) were RP437 (wild-type [WT]), RP4324 (tar_E tap_E), RP4368 (tsr_E), and RP4372 (tsr_E tar_E tap_E). (tap_E status is omitted from the headings for clarity; Tap_E made only a minor contribution to the overall methylation pattern.) The region of the gel covered by the vertical bars corresponds to proteins in the 60-kilodalton range in which the receptors were found; the upper half of that region contains mostly Tsr_E, and the lower half contains mostly Tar_E (5, 7, 37). The bands at lower molecular weight (ca. 43,000) are from elongation factor Tu and, though unrelated to chemotaxis, provide a useful indication of sample load.

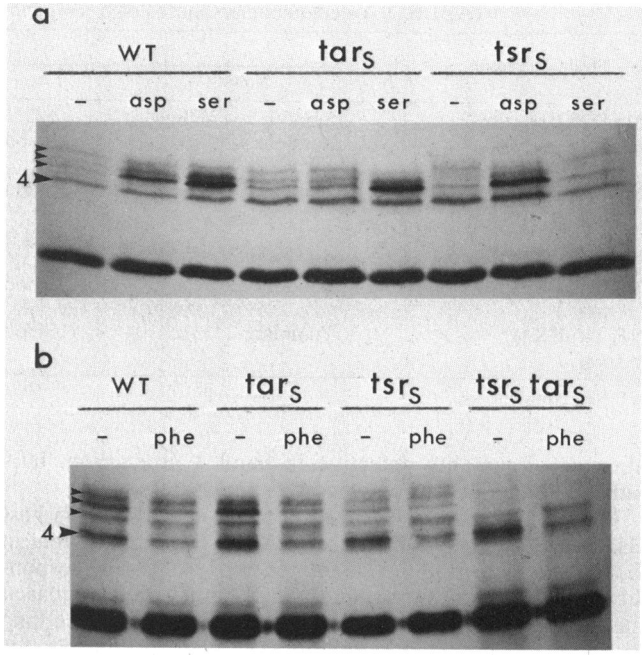

FIG. 6. Methylation-banding patterns of receptors of *S. typhimurium*. For protocol, see legend to Fig. 5. Strains used and stimuli given were as follows. (a) In sets of three lanes from left to right, with the addition of motility medium (−), 2 mM aspartate (asp), or 2 mM serine (ser), are ST1 (wild type [WT]), ST328 (tar_S), and ST330 (tsr_S). (b) In pairs of lanes from left to right, with the addition of motility medium (−) or 5 mM phenol (phe), are ST1, ST328, ST330, and ST334 (tsr_S tar_S). Within the molecular weight range of the methyl-accepting receptors are four major bands (arrows). Bands 2 and 3 were found to be serine sensitive, and band 3 was found to be aspartate sensitive. Band 4 (large arrow) was insensitive to aspartate or serine but sensitive to phenol.

Phenol caused extensive demethylation in wild-type cells (Fig. 6b); the major effect was on band 4, although the less mobile bands were also affected. Demethylation of band 4 was also observed in the tar_S and tsr_S mutants; demethylation of the less mobile bands was evident in the tar_S mutant but not in the tsr_S mutant. In the tsr_S tar_S double mutant, band 4 was by far the most heavily methylated in unstimulated cells and showed a large phenol-induced decrease. Some increase occurred around the position of band 3, suggesting that it had been generated by the demethylation of band 4.

The demethylation of the upper bands in the wild type and in the tar_S mutant might reflect some Tsr_S responsiveness to phenol as a repellent, or it might reflect the presence of less methylated, less mobile forms of the receptor responsible for band 4. Such bands were weakly evident in the tsr_S tar_S mutant, in which band 4 was especially intense (Fig. 6b) and the presence of the other major receptors could produce a shift of intensity to these less methylated forms. In the

TABLE 2. Chemotactic responses of *E. coli* and *S. typhimurium* strains with plasmid-encoded *tar* genes

Strain (plasmid)	Motility after treatment		
	None	Aspartate (1 mM)	Phenol (5 mM)
MS5228 (tsr_E tar_E)	Smooth swimming	ND[a]	Indistinct tumbling
MS5228(pNM17) (tar_E^+)	Random motility	Smooth swimming	Smooth swimming
MS5228(pRK41) (tar_S^+)	Tumbling	Smooth swimming, no adaptation	Smooth swimming, no adaptation
ST334 (tsr_S tar_S)	Smooth swimming	ND	Tumbling
ST334(pNM17) (tar_E^+)	Random motility	Smooth swimming	Weak smooth swimming
ST334(pRK41) (tar_S^+)	Tumbling	Smooth swimming, no adaptation	Smooth swimming, no adaptation

[a] ND, Not done.

absence of mutants defective in band 4 production, it is difficult to distinguish between these possibilities.

Effect of plasmid-encoded Tar_E and Tar_S proteins on host cell behavior. The results described thus far indicate that in *E. coli* the Tar_E protein was the receptor principally responsible for phenol-induced smooth swimming and increased methylation, whereas in *S. typhimurium* a methyl-accepting receptor distinct from Tar_S or Tsr_S was responsible for phenol-induced tumbling and decreased methylation. In the absence of a phenol taxis mutant, it was difficult to know whether Tar_S functioned like Tar_E with regard to phenol or whether it was totally unresponsive. Two plasmids, one (pRK41) containing the tar_S gene under its natural promoter (*meche*) and the other (pNM17) containing the tar_E gene under a foreign promoter (*tac*), were used to further explore the question of Tar function.

Observations of unstimulated and stimulated motility of free-swimming cells of *tsr tar* mutants of *E. coli* and *S. typhimurium* with and without these plasmids are summarized in Table 2. The results were qualitatively the same, regardless of whether the host was *E. coli* or *S. typhimurium* and of whether the plasmid was producing Tar_E or Tar_S. In the absence of the plasmid, unstimulated cells swam smoothly, as is characteristic of strains lacking the two major methyl-accepting receptors Tsr and Tar. Phenol addition caused tumbling, which was more pronounced in the *S. typhimurium* mutant. The presence of either the tar_E or the tar_S gene on a plasmid conferred an appreciable amount of tumbling on unstimulated cells and permitted an aspartate-induced smooth-swimming response. With the Tar_S-encoding plasmid, the response continued indefinitely, perhaps indicating a level of excitation too high for adaptation to occur. Phenol caused an indefinite smooth-swimming response in cells harboring the Tar_S-encoding plasmid. With the Tar_E-encoding plasmid, the response to phenol was less distinct. We conclude from these experiments that Tar_S recognizes phenol as an attractant, although this response is normally not manifested. The consequences of overproduction of Tsr_S for phenol-stimulated behavioral or methylation effects (see below) were not examined in this study, since the tsr_S gene has not yet been cloned.

Effects of plasmid-encoded Tar_E proteins on host cell methylation. The effects of aspartate and phenol on methylation levels in the various constructions are shown in Fig. 7. In all cases, aspartate-induced stimulation of methylation was observed when a Tar-encoding plasmid was present, the effect being especially strong with Tar_S. Phenol also caused a substantial increase in the methylation level in *E. coli* or *S. typhimurium* cells harboring plasmids producing Tar of either species, in agreement with the behavioral results (Table 2).

Methylation-banding patterns in strains harboring Tar-encoding plasmids. The overproduction of Tar from a plasmid resulted in phenol-stimulated methylation (see above). We wished to see whether the receptor responsible for phenol-stimulated demethylation was still operative under these conditions, in which case two opposite effects (methylation increase and decrease) might be discernible in the banding patterns. The results are shown in Fig. 8.

Strain ST334 harboring the Tar_S-encoding plasmid showed a quite light banding pattern that consisted predominantly of bands of low mobility. Aspartate gave a large increase in methylation labeling and a shift to higher mobilities; phenol

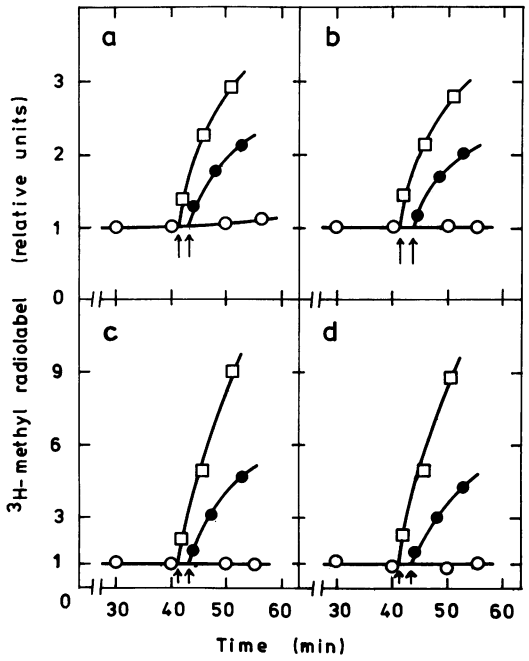

FIG. 7. Effect of phenol or aspartate on the methylation level of Tar_E or Tar_S receptor synthesized under the direction of plasmid-encoded genes in *E. coli* or *S. typhimurium tsr tar* mutant hosts. The host-plasmid combinations are *E. coli* MS5228 with pNM17(tar_E^+) (a), *S. typhimurium* ST334 with pNM17 (tar_E^+) (b), *E. coli* MS5228 with pRK41 (tar_S^+) (c), and *S. typhimurium* ST334 with pRK41 (tar_S^+) (d). At the times indicated (arrows), 10 mM phenol (●), 10 mM aspartate (□), or deionized water (○) was added. For other details, see legend to Fig. 2.

caused a smaller but still substantial increase. No radiolabel was detected at the position of band 4 either in stimulated or unstimulated cells, suggesting that the overproduction of Tar_S in some way altered the methylation of the other receptors.

When ST334 harboring the Tar_E-encoding plasmid was examined, the region of the gel corresponding to Tar_E was quite evident, even in the absence of stimulation, as was band 4 from the host receptor system (recall that Tar_E had no species at that mobility). Aspartate caused the expected stimulation of methylation of Tar_E bands. So, too, did phenol, but it also caused some decrease in the intensity of band 4. Thus, as long as both the Tar_E receptor and the phenol-specific receptor of *S. typhimurium* could be detected, so could the opposite consequences of phenol addition.

The levels of Tar methylation and of host transducer methylation as evidenced by band 4 were much less with the Tar_S-encoding plasmid than with the Tar_E-encoding plasmid. With the former, which contains the natural promoter for tar_S, overproduction should primarily be a consequence of plasmid copy number, giving perhaps a 30-fold increase over normal cellular levels. Although *tac* is a strong promoter, in the construction used (pNM17) it is not well coupled to the tar_E gene, and thus expression is only moderate (M. Simon, personal communication). We do not know whether the observed differences in methylation are a direct consequence of high levels of Tar_S protein or of the presence of multiple copies of the tar_S promoter.

DISCUSSION

Phenol (carbolic acid) historically has been a widely used disinfectant which at moderately high concentrations is toxic to bacteria. That it would be sensed as a repellent by *S. typhimurium* is, therefore, not surprising. It is, however, surprising that *E. coli* senses phenol as an attractant. This is one of the few differences between the tactic behaviors of the two closely related species.

We attempted to elucidate the molecular basis for this difference. We conclude that phenol as a chemoeffector is complex, operating in both species as an attractant via the Tar receptor (and perhaps to a slight degree via the Tsr receptor) and additionally operating in *S. typhimurium* as a repellent via an unidentified receptor.

The Tar receptor recognizes phenol as an attractant. We showed that the Tar_E protein of *E. coli* recognized phenol as an attractant. Consistent with this, phenol caused enhanced methylation of Tar_E bands. Phenol-induced methylation of Tar_E (and to a lesser degree Tsr_E) was the only detectable effect, indicating that Tar_E was the principal receptor for phenol and was responsible for the attractant response of *E. coli*. Given the repellent response of *S. typhimurium* to phenol, we suspected at first that Tar_S might have different properties with respect to phenol reception. We found, however, that this was not the case. At least under conditions of overproduction, Tar_S, like Tar_E, recognized phenol as an attractant and was methylated in consequence. It did so whether it was resident in its natural host, *S. typhimurium*, or had been artificially placed in *E. coli*. Thus Tar_E and Tar_S were qualitatively similar with respect to phenol sensing.

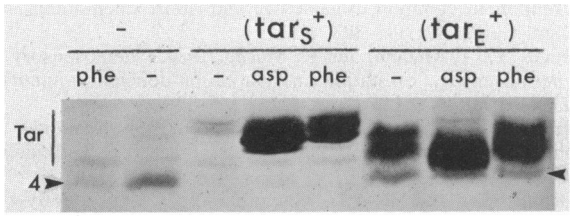

FIG. 8. Effect of phenol or aspartate on the methylation-banding patterns of Tar_S and Tar_E receptor synthesized under the direction of plasmid-encoded genes in an *S. typhimurium* host, ST334 (tsr_S tar_S). The two left lanes contain the host alone after the addition of 10 mM phenol (phe) or motility medium (−). The next three lanes contain the host with pRK41 (tar_S^+) after the addition of motility medium, 10 mM aspartate (asp), or 10 mM phenol. The three right lanes contain the host with pNM17 (tar_E^+) after the addition of motility medium, 10 mM aspartate, or 10 mM phenol. The position of Tar from both species is indicated, as is the position of band 4 (arrow).

Why these bacteria should have evolved a receptor that signals the cell to migrate toward a toxic chemical is unclear. It is, however, important to remember that Tar is the receptor for aspartate, a powerful attractant, and that this is probably its primary role. We think it likely that the properties of Tar with respect to phenol are a fortuitous and undesirable consequence of the properties it has evolved with respect to the sensing of aspartate and other effectors.

***S. typhimurium* has a phenol-specific methyl-accepting receptor.** What then was the cause of the opposite effect of phenol for *E. coli* and *S. typhimurium*? Inversion of responses has been observed in other situations when the receptor that normally handles the relevant stimulus is defective or missing (21, 23), and it has been suggested that the mechanism may involve compensating effects on other methyl-accepting receptors. We might have been inclined to propose a similar type of effect, involving the amounts and interactions of known receptors, had we not obtained biochemical evidence for a methyl-accepting receptor in *S. typhimurium* that is specifically responsible for the repellent response to phenol in that species. The evidence for this receptor may be summarized as follows. In the electrophoretic banding pattern of methyl-accepting receptors of *S. typhimurium*, a major band (Fig. 6 and 8, band 4) of a methylated protein was observed not only in the wild-type strain but also in mutants defective in serine taxis or aspartate taxis or both. It was unaffected by the addition of serine or aspartate, but it was the major band affected by phenol addition, showing a substantial decrease in methyl label. No equivalent band was seen in *E. coli*.

In wild-type *S. typhimurium*, the phenol-induced methylation of Tar_S produced at normal levels apparently was overwhelmed by the demethylation of phenol-specific bands, notably band 4; this correlated with phenol-induced tumbling. When the amount of Tar_S was increased by the use of Tar_S-encoding plasmids, the cells showed a net increase in methylation after phenol addition; this correlated with phenol-induced smooth swimming under these conditions. Thus, *S. typhimurium* has at least two different phenol receptors, and the behavioral response (attractant or repellent) is determined by the relative amounts that are present in the cell.

Is it certain that band 4 and the other phenol-demethylated bands correspond to a new class of receptor, or might they be heretofore uncharacterized features of known receptors? We still have no direct evidence, but the available data support the hypothesis of a new receptor class. The phenol-specific repellent receptor is unlikely to be Tar_S or Tsr_S for the following reasons. (i) Wild-type cells as well as mutants defective in serine or aspartate taxis exhibited band 4 as a major molecular species that was unresponsive to serine or

aspartate but highly responsive to phenol. In studies of methylation-banding patterns (e.g., 5, 7, 37), a stimulus such as serine causes a redistribution among all of the forms of the corresponding receptor; there is no evidence for insensitive bands such as band 4. (ii) The serine taxis and aspartate taxis mutants showed a normal behavioral response to phenol. Although we cannot say with certainty that these mutants are generally defective in their respective receptor functions, previous studies (e.g., 29) indicate that these are more common than ligand-specific mutants. The major loss of methylation label in unstimulated cells (Fig. 6) also argues in favor of a general defect, as does the reduced responsiveness to glycerol (Fig. 4). If indeed these mutants are generally defective, Tsr_S and Tar_S cannot be responsible for the repellent effects, behavioral and biochemical, of phenol.

It is also unlikely that the phenol-repellent receptor corresponds to a protein homologous to Tap_E; *S. typhimurium* does not have a homologous gene in the genomic location where tap_E is found in *E. coli* (18) and is not responsive to the established effectors of Tap_E, namely, dipeptides (20). Russo and Koshland (31) have identified a protein in *S. typhimurium*, Tip_S (taxis involved protein), which has homology to known methyl-accepting receptors; they showed, however, that the phenol response was not mediated by this protein. The remaining candidate is Trg_S, which has not yet been identified but is presumed to exist, since *S. typhimurium* (39), like *E. coli* (1, 4, 16), is responsive to ribose and galactose; however, the tumbling response of a tsr_S tar_S double mutant to phenol was not suppressed by either ribose or galactose, suggesting that Trg_S may not be involved in phenol sensing.

We therefore tentatively suggest that the phenol-repellent receptor in *S. typhimurium* is distinct from all known ones. However, to establish this beyond doubt will require the identification of the receptor gene. Isolation of *S. typhimurium* mutants that either are phenol blind or sense phenol as an attractant would be a useful approach to achieving this goal.

We should emphasize that by a specific phenol-repellent receptor, we do not necessarily mean a phenol-binding protein. Indeed, the cumulative evidence concerning repellents suggests that, in contrast to attractants, they are likely to operate by perturbing either the state of the membrane or physiological parameters, such as cytoplasmic pH, and that the specificity arises from the sensitivity of different receptors to these perturbations.

The repellent response of tar_E mutants. In the absence of Tar_E, *E. coli* cells showed a weak tumbling response to phenol, an observation made originally by Khan and Macnab (14) and confirmed in the present study. This does not seem to be a case of inversion of the sort first noted by Muskavitch et al. (23), which resulted from the properties of, for example, Tsr_E in the absence of Tar_E, since tsr_E tar_E tap_E mutants responded to phenol as a repellent, yet their methylation levels were too low to be detected either with or without phenol stimulation. We suspect that the response may result from a general perturbation of the energy state of the cell, possibly related to the mechanism of aerotaxis (32); at concentrations at which phenol elicited a behavioral response, there was often a noticeable reduction in swimming speed.

Conclusions. This study demonstrates that the role of phenol as a chemoeffector is rather complex. It is a repellent for *S. typhimurium* and an attractant for *E. coli*, but it has cryptic effects for both species that are only evident in certain mutants or with unusual levels of receptor production. Of these responses, we suspect that only the repellent response of wild-type *S. typhimurium* has evolved directly as a consequence of the environmental pressure of phenol or related compounds.

ACKNOWLEDGMENTS

We thank J. Adler, L. R. Bullas, G. L. Hazelbauer, D. E. Koshland, Jr., J. S. Parkinson, and M. Simon for providing strains and plasmids.

This work was supported by a grant-in-aid from the Ministry of Education, Science, and Culture of Japan (to Y.I.) and by Public Health Service grant AI12202 from the National Institutes of Health and a fellowship from the Japan Society for the Promotion of Science (to R.M.M.).

LITERATURE CITED

1. **Adler, J., G. L. Hazelbauer, and M. M. Dahl.** 1973. Chemotaxis toward sugars in *Escherichia coli*. J. Bacteriol. **115**:824–847.
2. **Armstrong, J. B., J. Adler, and M. M. Dahl.** 1967. Nonchemotactic mutants of *Escherichia coli*. J. Bacteriol. **93**:390–398.
3. **Aswad, D., and D. E. Koshland, Jr.** 1975. Isolation, characterization and complementation of *Salmonella typhimurium* chemotaxis mutants. J. Mol. Biol. **97**:225–235.
4. **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. USA **81**:3287–3291.
5. **Boyd, A., and M. I. Simon.** 1980. Multiple electrophoretic forms of methyl-accepting chemotaxis proteins generated by stimulus-elicited methylation in *Escherichia coli*. J. Bacteriol. **143**: 809–815.
6. **Bullas, L. R., and J.-I. Ryu.** 1983. *Salmonella typhimurium* LT2 strains which are r^- m^+ for all three chromosomally located systems of DNA restriction and modification. J. Bacteriol. **156**:471–474.
7. **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. USA **77**:2434–2438.
8. **Dahl, M. K., and M. D. Manson.** 1985. Interspecific reconstitution of maltose transport and chemotaxis in *Escherichia coli* with maltose-binding protein from various enteric bacteria. J. Bacteriol. **164**:1057–1063.
9. **Dang, C. V., M. Niwano, J.-I. Ryu, and B. L. Taylor.** 1986. Inversion of aerotactic response in *Escherichia coli* deficient in *cheB* protein methylesterase. J. Bacteriol. **166**:275–280.
10. **DeFranco, A. L., and D. E. Koshland, Jr.** 1981. Molecular cloning of chemotaxis genes and overproduction of gene products in the bacterial sensing system. J. Bacteriol. **147**:390–400.
11. **Harayama, S., E. T. Palva, and G. L. Hazelbauer.** 1979. Transposon-insertion mutants of *Escherichia coli* K12 defective in a component common to galactose and ribose chemotaxis. Mol. Gen. Genet. **171**:193–203.
12. **Imae, Y., T. Mizuno, and K. Maeda.** 1984. Chemosensory and thermosensory excitation in adaptation-deficient mutants of *Escherichia coli*. J. Bacteriol. **159**:368–374.
13. **Ingolia, T. D., and D. E. Koshland, Jr.** 1979. Response to a metal ion-citrate complex in bacterial sensing. J. Bacteriol. **140**:798–804.
14. **Khan, S., and R. M. Macnab.** 1980. The steady-state counterclockwise/clockwise ratio of bacterial flagellar motors is regulated by protonmotive force. J. Mol. Biol. **138**:563–597.
15. **Kihara, M., and R. M. Macnab.** 1979. Chemotaxis of *Salmonella typhimurium* toward citrate. J. Bacteriol. **140**:297–300.
16. **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. USA **76**:260–264.
17. **Kort, E. N., M. F. Goy, S. H. Larsen, and J. Adler.** 1975. Methylation of a membrane protein involved in bacterial chemotaxis. Proc. Natl. Acad. Sci. USA **72**:3939–3943.

18. **Krikos, A., N. Mutoh, A. Boyd, and M. I. Simon.** 1983. Sensory transducers of *E. coli* are composed of discrete structural and functional domains. Cell **33**:615–622.
19. **Lederberg, J.** 1956. Linear inheritance in transductional clones. Genetics **41**:845–871.
20. **Manson, M. D., V. Blank, G. Brade, and C. F. Higgins.** 1986. Peptide chemotaxis in *E. coli* involves the Tap signal transducer and the dipeptide permease. Nature (London) **321**:253–256.
21. **Mizuno, T., and Y. Imae.** 1984. Conditional inversion of the thermoresponse in *Escherichia coli*. J. Bacteriol. **159**:360–367.
22. **Mizuno, T., N. Mutoh, S. M. Panasenko, and Y. Imae.** 1986. Acquisition of maltose chemotaxis in *Salmonella typhimurium* by the introduction of the *Escherichia coli* chemosensory transducer gene. J. Bacteriol. **165**:890–895.
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. **Oosawa, K., and Y. Imae.** 1983. Glycerol and ethylene glycol: members of a new class of repellents of *Escherichia coli* chemotaxis. J. Bacteriol. **154**:104–112.
25. **Oosawa, K., and Y. Imae.** 1984. Demethylation of methyl-accepting chemotaxis proteins in *Escherichia coli* induced by the repellents glycerol and ethylene glycol. J. Bacteriol. **157**:576–581.
26. **Parkinson, J. S.** 1978. Complementation analysis and deletion mapping of *Escherichia coli* mutants defective in chemotaxis. J. Bacteriol. **135**:45–53.
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 P. T. Revello.** 1978. Sensory adaptation mutants of *E. coli*. Cell **15**:1221–1230.
29. **Reader, R. W., W.-W. Tso, M. S. Springer, M. F. Goy, and J. Adler.** 1979. Pleiotropic aspartate taxis and serine taxis mutants of *Escherichia coli*. J. Gen. Microbiol. **111**:363–374.
30. **Russo, A. F., and D. E. Koshland, Jr.** 1983. Separation of signal transduction and adaptation functions of the aspartate receptor in bacterial sensing. Science **220**:1016–1020.
31. **Russo, A. F., and D. E. Koshland, Jr.** 1986. Identification of the *tip*-encoded receptor in bacterial sensing. J. Bacteriol. **165**:276–282.
32. **Shioi, J., and B. L. Taylor.** 1984. Oxygen taxis and proton motive force in *Salmonella typhimurium*. J. Biol. Chem. **259**:10983–10988.
33. **Silverman, M., and M. Simon.** 1977. Chemotaxis in *Escherichia coli*: methylation of *che* gene products. Proc. Natl. Acad. Sci. USA **74**:3317–3321.
34. **Slocum, M. K., and J. S. Parkinson.** 1983. Genetics of methyl-accepting chemotaxis proteins in *Escherichia coli*: organization of the *tar* region. J. Bacteriol. **155**:565–577.
35. **Slocum, M. K., and J. S. Parkinson.** 1985. Genetics of methyl-accepting chemotaxis proteins in *Escherichia coli*: null phenotypes of the *tar* and *tap* genes. J. Bacteriol. **163**:586–594.
36. **Slonczewski, J. L., R. M. Macnab, J. R. Alger, and A. M. Castle.** 1982. Effects of pH and repellent tactic stimuli on protein methylation levels in *Escherichia coli*. J. Bacteriol. **152**:384–399.
37. **Springer, M. S., M. F. Goy, and J. Adler.** 1977. Sensory transduction in *Escherichia coli*: two complementary pathways of information processing that involve methylated proteins. Proc. Natl. Acad. Sci. USA **74**:3312–3316.
38. **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. USA **74**:533–537.
39. **Strange, P. G., and D. E. Koshland, Jr.** 1976. Receptor interactions in a signalling system: competition between ribose receptor and galactose receptor in the chemotaxis response. Proc. Natl. Acad. Sci. USA **73**:762–766.
40. **Tsang, N., R. Macnab, and D. E. Koshland, Jr.** 1973. Common mechanism for repellents and attractants in bacterial chemotaxis. Science **181**:60–63.
41. **Tso, W.-W., and J. Adler.** 1974. Negative chemotaxis in *Escherichia coli*. J. Bacteriol. **118**:560–576.