Inversion of Aerotactic Response in *Escherichia coli* Deficient in *cheB* Protein Methyltransferase

CHUAN V. DANG,¹ MITSURU NIWANO,^{1†} JUN-ICHI RYU,² AND BARRY L. TAYLOR^{1*}

Departments of Biochemistry¹ and Microbiology,² School of Medicine, Loma Linda University,
Loma Linda, California 92350

Received 26 September 1985/Accepted 6 January 1986

Mutants of *Escherichia coli* and *Salmonella typhimurium* that were deficient in protein methyltransferase activity encoded by *cheB* had an inverted response to oxygen; they were repelled by concentrations of oxygen that attract wild-type bacteria. Normal responses to oxygen and phosphotransferase substrates were observed in mutants that were deficient in protein methyltransferase (CheR) and the methyl-accepting transducing proteins (Tsr, Tar, Trg). However, the methylation-independent response to oxygen was modified by the loss of esterase activity. The inversion was apparently effected by the amidated Tsr protein present in *cheB tsr*⁺ mutants because aerotaxis was normal in *cheB tsr* strains. Chemotaxis to phosphotransferase sugars was normal in *cheB* mutants provided the extreme clockwise bias of the flagellar motors was modified to increase the probability of counterclockwise rotation.

Chemotaxis by *Salmonella typhimurium* and *Escherichia coli* toward most chemoeffector involves methyl-accepting transducing proteins that span the cytoplasmic membrane (11, 12, 21, 30, 37). Attractant, or an attractant-receptor complex if the primary receptor is a soluble periplasmic protein, binds to a transducing protein at the outer surface, probably inducing a conformational change that is propagated to the interior of the cell and signals a decrease in the probability of clockwise rotation of the flagellar motors. The transducing protein is subsequently methylated by the *cheR* product, protein methyltransferase, and the net effect is to cancel the excitation signal so that the bacteria adapt to the new concentration of attractant (5, 31). If the attractant is withdrawn or a repellent is added to the adapted cells, the transducing protein sends a signal to the flagellar motor that greatly increases the probability of clockwise rotation. The transducing protein is then demethylated by the *cheB* product, protein methyltransferase, until the signal for clockwise rotation is cancelled and the bacteria adapt (5, 33). The CheB protein is also involved in processing newly synthesized transducing proteins by glutaminy residue deamidation which yields sites for methylesterification (8, 25). Three known methyl-accepting transducing proteins are the products of the *tsr*, *tar*, and *trg* genes (6, 10, 27, 29). The *tap* gene has extensive homology to *tsr* and *tar*, but no transducing role for the Tap protein has been demonstrated (12, 28).

In addition to most chemoeffector that act on methyl-accepting transducing proteins, there are some attractants that are recognized by sensory transduction systems that are independent of methylation (19). Sugar substrates for the phosphotransferase transport system are attractants for one methylation-independent chemotactic system in which the enzyme II receptor for transport is also the receptor for chemotaxis (1). Adaptation to phosphotransferase sugars occurs normally in cells with impaired protein methylation

(19). Chemotaxis to oxygen (aerotaxis) is also normal in methylation-deficient *E. coli* and *S. typhimurium* and is mediated by changes in electron flow through the electron transport system and by concomitant changes in the proton motive force (13, 14, 26). A transducer is presumed to respond to the changes in proton motive force and to transmit a signal that alters the probability of reversal of the flagellar motors (35, 36).

Adaptation to repellents has been reported in the absence of the CheR methyltransferase in *S. typhimurium* (34). Subsequent studies of *S. typhimurium* have demonstrated the capacity for adaptation to low concentrations of serine, aspartate, and repellents in the absence of either transducer methylation or transducer demethylation (32a). Methylation-demethylation-deficient strains of *S. typhimurium* and *E. coli* do not adapt to high concentrations of attractants or repellents that interact with the Tsr or Tar proteins (32a; J. S. Parkinson, personal communication). The significance of limited adaptation to stimuli mediated by Tsr and Tar in the absence of methylation is unknown at this time. This study is concerned with adaptation to large stimuli; methylation dependent and methylation independent are used to denote the mechanisms for adaptation to large temporal stimuli. The relationship of the mechanism of methylation-independent adaptation to large temporal gradients of oxygen and phosphotransferase substrates (19) and methylation-independent adaptation to very small stimuli (32a) is not known at this time.

The methylation-dependent and methylation-independent pathways for bacterial chemotaxis converge at or before the switch that controls the direction of rotation of the flagellar motors (19). The purpose of the present study is to investigate interactions that occur between the methylation-dependent and methylation-independent pathways at sites before the convergence of the pathways.

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* Corresponding author.

† Present address: Snow-Brand Milk Products Co., Ltd., Research Institute of Life Science, 519, Shimoishibashi, Ishibashi-machi, Shimotsuga-gun, Tochigi, 329-05, Japan.

FIG. 1. Genetic map of relevant chemotaxis regions of *E. coli*. The relative positions of the endpoints of the deletions $\Delta 2241$ and $\Delta 2234$ were obtained from Yonekawa et al. (39). Strains RP1061, RP1075, and BT3001 are λ lysogenic strains. The relevant chemotaxis genes carried by λ are shown on the right of the corresponding chromosomal map. Thick lines indicate deletions, thin lines indicate chromosomal material, wavy lines indicate λ arm material, and X indicates point mutations.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains of *E. coli* and *S. typhimurium* used in this study are described in Table 1, and the genetic map of the relevant chemotaxis regions of *E. coli* is illustrated in Fig. 1. Cells were grown at 30°C in Vogel-Bonner citrate medium E (38) supplemented with 1.0% glycerol (wt/vol) or 0.7% glucose (wt/vol) and auxotrophic requirements.

Transduction. BT3001 was constructed by transducing the *tsr::Tn5* insertion from *E. coli* RP5724 into strain RP1075 with bacteriophage P1. A P1 lysate was prepared by the method of Miller (16), and transduction was performed at a multiplicity of infection of 1.0 in the presence of 10 mM CaCl_2 . The culture was incubated for 15 min at 37°C without shaking, centrifuged, suspended in 1 ml of Luria-Bertani medium (3) containing 0.1 M sodium citrate, and further incubated for 30 min at 37°C to allow phenotypic expression of the kanamycin resistance gene. Transductants were selected on nutrient agar plates containing 35 μg of kanamycin per ml.

Aerotaxis and chemotaxis assays. The temporal aerotaxis assay was performed as described elsewhere (14, 26). A bacterial suspension (5 μl) was placed on a microscope slide in a microchamber which was ventilated with nitrogen gas. Whenever the flow of nitrogen was halted, air rapidly diffused into the chamber through an open end. The cell suspension was allowed to equilibrate with nitrogen gas or air for 2 min before the assay. Tumbling frequency of the bacteria was analyzed by the method of Spudich and Koshland (32) as described previously (26). The blue-light response was used to define aerotactic responses in smooth-swimming mutants (19). After the reintroduction of air, the bacteria were exposed to blue light for 0.5 to 1.0 s; then the microscope stage was moved, and a new region of the cell suspension was exposed to blue light. This was repeated until the bacteria were observed to tumble in response to light.

Chemotaxis to a temporal gradient of chemoeffector was assayed by rapidly mixing 2 μl of attractant or repellent with 8 μl of bacterial suspension on a microscope slide. The endpoint of the chemotactic response was also estimated with the aid of blue light.

Chemicals. Kanamycin, D-mannose, D-mannitol, and α -aminoisobutyric acid were obtained from Sigma Chemical Co. (St. Louis, Mo.), and chromatographic-grade nitrogen gas (99.998%) was obtained from Big Three Industries, Houston, Tex.

RESULTS

Adaptation in methyltransferase-deficient strains. One of the goals of this study was to observe methylation-independent adaptation in a strain that is completely deficient in methylation-dependent adaptation. The *cheR* deletion mutant *E. coli* RP1061 is totally deficient in methylation-dependent adaptation and has a smooth swimming phenotype (Parkinson, personal communication). All *cheR* mutants tumble upon exposure to a pulse of intense blue light, except when the cells are responding to an increase in attractant concentration (15). When an attractant is added to the smooth-swimming *cheR* mutant, the duration of the smooth response to the attractant can be estimated by exposing the cells to frequent pulses of blue light and recording the time at which 50% of cells begin to tumble in response to blue light (19). We confirmed that *E. coli* RP1061

TABLE 1. Bacterial strains

Strain	Relevant genotype ^a	Source
<i>Salmonella typhimurium</i>		
ST1	Wild type	D. E. Koshland (2)
ST1038	<i>cheR</i>	D. E. Koshland (4)
SL4041	<i>cheB111</i>	D. E. Koshland (31)
<i>Escherichia coli</i>		
RP437	Wild type	J. S. Parkinson (20)
RP2867	$\Delta(\text{tap-cheB})2241$	J. S. Parkinson (22)
RP2896	$\Delta(\text{tar-cheB})2234$	J. S. Parkinson (22)
RP1061	$\Delta(\text{tap-cheB})2241$ ($\lambda\text{che}22\Delta 16-3\Delta 28$)	J. S. Parkinson
RP1075	$\Delta(\text{tap-cheB})2241$ ($\lambda\text{che}22\Delta 11-\Delta 37$)	J. S. Parkinson (39)
RP1119	$\Delta(\text{tar-cheB})2234$ <i>tsr-14</i>	J. S. Parkinson
RP5724	<i>tsr-501::Tn5</i>	J. S. Parkinson
RP5007	<i>cheZ293</i>	J. S. Parkinson
BT3001	$\Delta(\text{tar-cheB})2241$ ($\lambda\text{che}22\Delta 11-\Delta 37$) <i>tsr-501::Tn5</i>	This work
AW660	<i>tsr tar trg</i>	J. Adler (10)

^a Relevant genotypes of lysogenic strains are shown in Fig. 1.

did not adapt to 20 μ M serine (Table 2). However, the *cheR* deletion mutant adapted to temporal gradients of oxygen and mannose after 49.9 and 70.2 s, respectively (Table 2). This is longer than the 19.7 and 31.0 s that elapsed before wild-type *E. coli* (RP437) adapted to oxygen and mannose, respectively. But it has been previously observed that smooth swimming responses are usually longer in smooth-swimming strains such as *cheR* mutants. The observed (methylation-independent) adaptation to oxygen and mannose is in contrast to the absence of (methylation-dependent) adaptation to 20 μ M serine in the *cheR* deletion mutant and demonstrates that the *cheR* gene product, protein methyltransferase, has little, if any, direct interaction with the methylation-independent pathways that were studied.

Effect of *cheB* mutation on aerotaxis. *E. coli* and *S. typhimurium* *cheB* mutants have an inverted response to acetate, that is, they are attracted to acetate, whereas the wild-type strains are repelled by acetate (9, 23). The deletion of *tar*, *tap*, and *cheR* as well as *cheB* (*E. coli* RP2896) does not prevent the inverse response to acetate (22) or to benzoate (data not shown). A similar inversion of aerotaxis was observed in *cheB* mutants.

Aerotaxis was measured by a temporal assay. A drop of culture was placed in a flow chamber on the stage of a dark-field microscope. The chamber was ventilated with nitrogen gas to deplete the cells of oxygen, and then air was allowed to diffuse into the chamber, and the behavioral response of the bacteria was observed through the microscope (14). Wild-type bacteria increased their tumbling frequency when oxygen was depleted. After adapting to anaerobiosis, the bacteria suppressed tumbling for 10 to 15 s when oxygen was restored. The *cheB* deletion mutant (*E. coli* RP1075) gave an inverted response to oxygen (Table 2). The cells showed a marked decrease in tumbling frequency after the oxygen was removed by flushing the flow chamber with nitrogen gas. The bacteria adapted to anaerobiosis, and tumbling frequency returned to approximately the pre-stimulation frequency. The tumbling frequency increased immediately after air was reintroduced. A similar inverted response was observed in a *cheB* point mutant of *S. typhimurium* (SL4041) (data not shown). The inversion of aerotaxis in a *cheB* mutant was not affected by the additional deletion of *tap* and *cheR* (*E. coli* RP2867) or *tar*, *tap*, and *cheR* (*E. coli* RP2896) (Fig. 2A).

The inverted aerotaxis response in *cheB* mutants was

TABLE 2. Response to attractants by *E. coli* mutants deficient in *che* functions^a

Strain (relevant phenotype)	Response (s) to:		
	Oxygen	Mannose	Serine
RP437 (wild type)	19.7 $\pm$ 3.7	31.0 $\pm$ 6.7	19.3 $\pm$ 3.8
RP1061 (<i>CheR</i>)	49.9 $\pm$ 9.7	70.2 $\pm$ 19.3	>60 min
RP1075 (<i>CheB</i>)	Inverse	NRD ^b	>60 min
RP2867 (<i>Tap CheRB</i>)	Inverse	NRD	>60 min
RP2896 (<i>Tar Tap CheRB</i>)	Inverse	NRD	>60 min
RP1119 (<i>Tsr Tar Tap CheRB</i>)	27.5 $\pm$ 3.5	37.6 $\pm$ 5.4	NRD
BT3001 (<i>Tsr CheB</i>)	23.0 $\pm$ 2.0	44.7 $\pm$ 10.3	NRD
RP5007 (<i>CheZ</i>)	30.0 $\pm$ 4.8	43.3 $\pm$ 6.1	

^a Cells were grown in Vogel-Bonner citrate medium E (38) with glycerol as the carbon source. Chemotactic responses were measured in the growth medium. The concentrations of the attractants were: oxygen, 0.25 mM; D-mannose, 10 mM; L-serine, 20 μ M. A mean $\pm$ standard deviation was obtained from seven determinations in each case.

^b NRD, No response detected. A response to mannose was not induced by preincubation in medium containing mannose.

FIG. 2. Response to oxygen by *E. coli cheB* mutants. (A) RP2896 (*tar tap cheRB*); (B) RP1119 (*tsr tar tap cheRB*). Aerotaxis was measured by the temporal assay described in the text. Washed cells were suspended in Vogel-Bonner medium E (38) with 0.7% glucose (wt/vol) as the carbon source. Nitrogen and air were introduced to the flow chamber at the times indicated by the arrows.

dependent on the presence of the *tsr* gene. *E. coli* RP1119 [*tsr* Δ (*tar tap cheRB*)] had a normal positive response to oxygen (Fig. 2B). That is, an increase in oxygen concentration resulted in an interval of greatly decreased tumbling frequency. A similar result was obtained with *E. coli* BT3001, which had a point mutation in *tsr* and a deletion in *cheB* (Table 2). Although *tsr* was required for the *cheB* inversion of behavior, *tsr* is not essential for aerotaxis (14). Aerotaxis was normal in a *tsr cheB*⁺ mutant (*E. coli* RP5724) and in a *tsr tar trg cheB*⁺ strain (*E. coli* AW660) (19).

Strains with *cheB* deletions were also examined for response to substrates for the phosphotransferase system. No response was observed to 10 mM mannose (Table 2) or to a mixture of 10 mM each mannose, glucose, and *N*-acetylglucosamine (data not shown) in RP1075 (*cheB*), RP2867 (*tap cheRB*), or RP2896 (*tar tap cheRB*), mutants which have an extreme clockwise bias of the flagellar motor. A response to mannose was observed in *tsr cheB* and *tsr tar tap cheRB* strains which had a less extreme clockwise bias of the flagellar motors. This suggested that the extreme clockwise bias of the *cheB* mutants masked the response to phosphotransferase substrates. To confirm the hypothesis that the phosphotransferase chemotaxis was normal also in the *cheB* mutants with more clockwise bias, the response of RP1075 (*cheB*) to mannose and mannitol was measured in the presence of 20 mM α -aminoisobutyric acid. Imae and co-workers (7) have demonstrated that the steady-state tumbling frequency in a *cheB* mutant is decreased by preincubation with serine and aspartate. We achieved a smaller decrease in tumbling frequency by preincubation of RP1075

TABLE 3. Response to phosphotransferase attractants by an *E. coli cheB* mutant in the presence of α -aminoisobutyric acid^a

Strain (relevant phenotype)	Growth with:	Response (s) to 10 μ M:	
		Mannose	Mannitol
RP1075 (<i>CheB</i>)	Mannose	22.0 $\pm$ 3.2	16.0 $\pm$ 2.6
	Mannitol	20.5 $\pm$ 3.1	24.5 $\pm$ 4.2
RP437 (<i>Che</i> ⁺)	Mannose	24.3 $\pm$ 3.3	18.0 $\pm$ 2.2
	Mannitol	22.0 $\pm$ 3.7	25.5 $\pm$ 4.2

^a Bacteria were grown in Vogel-Bonner citrate medium E (38) with 0.4% mannose or mannitol, washed three times in chemotaxis buffer, and resuspended in chemotaxis buffer. The cells were then preincubated with 20 mM α -aminoisobutyric acid for a minimum of 20 min before measurement of the chemotactic responses by temporal assay. The mean $\pm$ standard deviation was calculated from four determinations in each case.

with α -aminoisobutyric acid. It was then possible to observe a normal response to both mannose and mannitol (Table 3), indicating that the *cheB* mutation did not directly affect phosphotransferase chemotaxis. In both RP1075 (*cheB*) and RP437 (*che*⁺), maximal responses to mannitol required growth of the cells in mannitol-containing medium. The response to mannitol in cells grown on mannose was surprising and was due to contamination of the mannose (R. Tribhuwan and B. Taylor, unpublished observation).

The responses of *cheB* mutants to mannose and mannitol were verified by quantitating the additivity of responses to phosphotransferase sugars and serine. The *cheB* mutant (RP1075) did not respond to 4 μ M serine but did respond and adapt to 10 μ M serine. The mutant did not adapt to serine at concentrations of 20 μ M or higher. Responses to serine and mannose or mannitol were additive (Table 4). Similar responses were obtained with *tar tap cheRB* (RP2896) and *tap cheRB* (RP2867) mutants. The observed adaptation of *cheRB* *E. coli* deletion mutants to serine confirms the finding of Stock et al. (32a) for *S. typhimurium* in which methylation-deficient strains adapted to low concentrations of serine but not to high concentrations. Since the *cheRB* mutants also adapted to the nonmetabolizable α -aminoisobutyric acid, adaptation was not due to consumption of the attractant. The additivity of responses to serine and to mannose or mannitol suggests that the methylation-dependent and methylation-independent pathways converge to produce a common signal that acts on the switch controlling the direction of flagellar rotation.

Effect of *cheZ* mutation on aerotaxis. The *cheZ* mutant, like the *cheB* mutant, has a constantly tumbling phenotype. However, *E. coli* RP5007 (*cheZ*) had a normal response to oxygen, unlike the *cheB* mutant (Table 2).

DISCUSSION

Adaptation to oxygen and phosphotransferase substrates was previously demonstrated to be methylation independent in cells depleted of *S*-adenosyl-L-methionine (19). The observed adaptation to oxygen and phosphotransferase substrates by a *tsr* Δ (*tar tap cheRB*) mutant (Table 2) confirms that adaptation to these substrates is independent of methylation and that protein methyltransferase and protein methylesterase are not required. On the other hand, it is evident from Table 2 that the loss of the esterase function modifies the responses to phosphotransferase substrates and to oxygen. In some strains, the effect of the *cheB* mutation on the methylation-dependent transduction system is so severe that it masks the response to weaker phosphotrans-

ferase attractants (Table 2). However, a response to phosphotransferase substrate has been detected in these *cheB* mutants when cells were tested under conditions that decreased the extreme clockwise bias of the *cheB* mutants (Tables 3 and 4). The loss of *cheB* function causes a mutant to respond to oxygen as a repellent rather than as an attractant.

There is evidently some feature of the sensory transduction mechanism in *E. coli* and *S. typhimurium* that readily leads to an inverted response when one component of the system is defective. Mutations in *tsr* in *E. coli* can cause inverted responses to benzoate and acetate (18). A *cheB* mutation can invert the response of *S. typhimurium* or *E. coli* to acetate (9, 23) and benzoate as well as oxygen. Inversion of thermotaxis in *E. coli* is observed in *tsr* mutants that have adapted to an attractant, such as aspartate, that is mediated by the *tar* transducer (17). Wild-type *E. coli* strains exhibit inverted thermoresponses after the cells are simultaneously adapted to L-aspartate and L-serine. For thermotaxis, inversion of the response may be the result of overmethylation of the transducing proteins (17). But overmethylation is not essential for inversion of aerotaxis because aerotaxis is also inverted in a *cheRB* mutant which is deficient in methyltransferase activity as well as methylesterase activity (Table 2). The *cheB* product deamidates the nascent transducing proteins (8, 25), and it is possible that the inversion is the result of deficient deamidation of a transducing protein.

One positive finding about the mechanism of inversion of aerotaxis in *cheB* mutants is that it involves an interaction of the Tsr transducer with the aerotactic pathway (Fig. 2). *E. coli cheB* mutants that had inverted aerotaxis became normal for aerotaxis when a *tsr* point mutation was introduced (Table 2). This is surprising because in a *cheB*⁺ cell there is apparently little or no interaction between the Tsr transducer and the aerotactic pathway. *E. coli* AW660 is deficient in *tsr*, *tar*, and *trg* and yet has a normal aerotactic response (19).

The nature of the interaction between the Tsr and CheB proteins of the methylation-dependent transduction system and the aerotaxis system is unexplained at this time. The transducer for aerotaxis responds to changes in proton motive force that result from changes in flux through the electron transport system (35). The Tsr protein might be a weak transducer of changes in proton motive force that becomes a dominant transducer with an inverted signal when the Tsr protein is in the amidated form in *cheB* mutants. It is possible that there is a relationship between the net charge on the Tsr protein and the degree of clockwise bias in the aerotactic response to an increase in oxygen concentration. In strains that are wild type for chemotaxis, the Tsr protein has a basal level of methylation, and there is a short

TABLE 4. Additive responses to methylation-dependent and phosphotransferase attractants in an *E. coli cheB* mutant^a

Attractant (10 μ M each)	Response (s)	
	RP437 (<i>che</i> ⁺)	RP1075 (<i>cheB</i>)
Serine	17.3 $\pm$ 3.5	46.3 $\pm$ 4.1
Mannose	34.3 $\pm$ 5.8	NRD ^b
Mannitol	38.0 $\pm$ 3.0	NRD
Serine and mannose	45.7 $\pm$ 5.7	79.3 $\pm$ 8.1
Serine and mannitol	44.3 $\pm$ 3.8	75.0 $\pm$ 5.0

^a Bacteria were grown and washed as described in Table 3, footnote a.

^b NRD, No response detected.

counterclockwise aerotactic response (Table 2). In a *cheR* strain, there is an absence of methylation and a net negative charge on the Tsr protein relative to wild-type cells. The *cheR* cells have a prolonged counterclockwise aerotactic response. On the other hand, as a result of complete methylation of glutamyl residues, there is a net positive charge on the Tsr protein of *cheB* strains relative to wild-type strains. The *cheB* cells have a prolonged clockwise aerotactic response (Table 2). As an alternative to this second transducer model, a direct interaction between the Tsr transducer and the aerotaxis transducer might influence the aerotactic response.

Plasmodia of *Physarum polycephalum* exhibit changes in chemotaxis from positive to negative when the concentration of calcium is increased (24). It is possible in *E. coli* that the increased amidation of the Tsr transducer in *cheB* mutants could result in a change in the intracellular environment that in turn would modify the aerotactic transducer. This could be readily achieved if the Tsr protein was an ion channel, but at present, there is no evidence that the chemotactic transducers are ion channels.

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LITERATURE CITED

- Adler, J., and W. Epstein. 1974. Phosphotransferase-system enzymes as chemoreceptors for certain sugars in *Escherichia coli* chemotaxis. *Proc. Natl. Acad. Sci. USA* 71:2895-2899.
- Aswad, D., and D. E. Koshland, Jr. 1975. Isolation, characterization and complementation of *Salmonella typhimurium* chemotaxis mutants. *J. Mol. Biol.* 97:225-235.
- Davis, R. W., D. Botstein, and J. R. Roth. 1980. Advanced bacterial genetics, p. 201. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- DeFranco, A. L., J. S. Parkinson, and D. E. Koshland, Jr. 1979. Functional homology of chemotaxis genes in *Escherichia coli* and *Salmonella typhimurium*. *J. Bacteriol.* 139:107-114.
- Goy, M. F., M. S. Springer, and J. Adler. 1977. Sensory transduction in *Escherichia coli*: role of a protein methylation reaction in sensory adaptation. *Proc. Natl. Acad. Sci. USA* 74:4964-4968.
- Hazelbauer, G. L., P. Engstrom, and S. Harayama. 1981. Methyl-accepting chemotaxis protein III and transducer gene *trg*. *J. Bacteriol.* 145:43-49.
- Imae, Y., T. Mizuno, and K. Maeda. 1984. Chemosensory and thermosensory excitation in adaptation-deficient mutants of *Escherichia coli*. *J. Bacteriol.* 159:368-374.
- Kehry, M. R., M. W. Bond, M. W. Hunkapiller, and F. W. Dahlquist. 1983. Enzymatic deamidation of methyl-accepting chemotaxis proteins in *Escherichia coli* catalyzed by the *cheB* gene product. *Proc. Natl. Acad. Sci. USA* 80:3599-3603.
- Kihara, M., and R. M. Macnab. 1981. Cytoplasmic pH mediates pH taxis and weak-acid repellent taxis of bacteria. *J. Bacteriol.* 145:1209-1221.
- 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.
- Koshland, D. E., Jr. 1983. The bacterium as a model neuron. *Trends Neurosci.* 6:133-137.
- 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.
- Laszlo, D. J., B. L. Fandrich, A. Sivaram, B. Chance, and B. L. Taylor. 1984. Cytochrome *o* as a terminal oxidase and receptor for aerotaxis in *Salmonella typhimurium*. *J. Bacteriol.* 159:663-667.
- Laszlo, D. J., and B. L. Taylor. 1981. Aerotaxis in *Salmonella typhimurium*: role of electron transport. *J. Bacteriol.* 145:990-1001.
- Macnab, R., and D. E. Koshland, Jr. 1974. Bacterial motility and chemotaxis: light-induced tumbling response and visualization of individual flagella. *J. Mol. Biol.* 84:399-406.
- Miller, J. H. 1972. Experiments in molecular genetics, p. 201-205. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Mizuno, T., and Y. Imae. 1984. Conditional inversion of the thermoresponse in *Escherichia coli*. *J. Bacteriol.* 159:360-367.
- 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.
- Niwano, M., and B. L. Taylor. 1982. Novel sensory adaptation mechanism in bacterial chemotaxis to oxygen and phosphotransferase substrates. *Proc. Natl. Acad. Sci. USA* 79:11-15.
- Parkinson, J. S. 1978. Complementation analysis and deletion mapping of *Escherichia coli* mutants defective in chemotaxis. *J. Bacteriol.* 135:45-53.
- Parkinson, J. S., and G. L. Hazelbauer. 1983. Bacterial chemotaxis: molecular genetics of sensory transduction and chemotactic gene expression, p. 293-318. In J. Beckwith, J. Davies, and J. A. Gallant (ed.), *Gene function in prokaryotes*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Parkinson, J. S., and S. E. Houts. 1982. Isolation and behavior of *Escherichia coli* deletion mutants lacking chemotaxis functions. *J. Bacteriol.* 151:106-113.
- Repaske, D. R., and J. Adler. 1981. Change in intracellular pH of *Escherichia coli* mediates the chemotactic response to certain attractants and repellents. *J. Bacteriol.* 145:1196-1208.
- Sato, H., Y. Mito, T. Ueda, K. Kurihar, and Y. Kobatake. 1980. Conversion of positive to negative chemotaxis by Ca^{2+} ionophore treatment in plasmodium of *Physarum polycephalum*. *Biochim. Biophys. Acta* 633:436-443.
- Sherris, D., and J. S. Parkinson. 1981. Posttranslational processing of methyl-accepting chemotaxis proteins in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 78:6051-6055.
- Shioi, J., and B. L. Taylor. 1984. Oxygen taxis and proton motive force in *Salmonella typhimurium*. *J. Biol. Chem.* 259:10983-10988.
- Silverman, M., P. Matsumura, M. Hilmen, and M. Simon. 1977. Characterization of lambda *Escherichia coli* hybrids carrying chemotaxis genes. *J. Bacteriol.* 130:877-887.
- 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.
- 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.
- Springer, M. S., M. F. Goy, and J. Adler. 1979. Protein methylation in behavioural control mechanisms and in signal transduction. *Nature (London)* 280:279-284.
- 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.
- Spudich, J. L., and D. E. Koshland, Jr. 1975. Quantitation of the sensory response in bacterial chemotaxis. *Proc. Natl. Acad. Sci. USA* 72:710-713.
- Stock, J., G. Kersulis, and D. E. Koshland, Jr. 1985. Neither methylating nor demethylating enzymes are required for bacterial chemotaxis. *Cell* 42:683-690.
- Stock, J. B., and D. E. Koshland, Jr. 1978. A protein

- methylesterase involved in bacterial sensing. *Proc. Natl. Acad. Sci. USA* **75**:3659–3663.
34. Stock, J. B., A. M. Maderis, and D. E. Koshland, Jr. 1981. Bacterial chemotaxis in the absence of receptor carboxyl-methylation. *Cell* **27**:37–44.
35. Taylor, B. L. 1983. Role of proton motive force in sensory transduction in bacteria. *Annu. Rev. Microbiol.* **37**:551–573.
36. Taylor, B. L. 1983. How do bacteria find the optimal concentration of oxygen? *Trends Biochem. Sci.* **8**:438–441.
37. Taylor, B. L., and S. M. Panasencko. 1984. Biochemistry of chemosensory behavior in prokaryotes and unicellular eukaryotes, p. 71–112. *In* G. Colombetti and F. Lenci (ed.), *Membranes and sensory transduction*. Plenum Publishing Corp., New York.
38. Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. *J. Biol. Chem.* **218**:97–106.
39. Yonekawa, H., H. Hayashi, and J. S. Parkinson. 1983. Requirement of the *cheB* function for sensory adaptation in *Escherichia coli*. *J. Bacteriol.* **156**:1228–1235.