JOURNAL OF BACTERIOLOGY, Dec. 1988, p. 5507–5511
0021-9193/88/125507-05$02.00/0

Vol. 170, No. 12

Signal Transduction in Chemotaxis to Oxygen in *Escherichia coli* and *Salmonella typhimurium*

JUNICHI SHIOI,† RAJANIKANT C. TRIBHUWAN, SUSAN T. BERG, AND BARRY L. TAYLOR*
Department of Biochemistry, School of Medicine, Loma Linda University, Loma Linda, California 92350

Received 6 June 1988/Accepted 2 September 1988

Pathways previously proposed for sensory transduction in chemotaxis to oxygen (aerotaxis) involved either (i) cytochrome *o*, the electron transport system, and proton motive force or (ii) enzyme $II^{Glucose}$ and the phosphoenolpyruvate:carbohydrate phosphotransferase system for active transport. This investigation distinguished between these possibilities. Aerotaxis was absent in a *cyo cyd* strain of *Escherichia coli* that lacked both cytochrome *o* and cytochrome *d*, which are the terminal oxidases for the branched electron transport system in *E. coli*. Aerotaxis, measured by either a spatial or temporal assay, was normal in *E. coli* strains that had a cyo^+ or cyd^+ gene or both. The membrane potential of all oxidase-positive strains was approximately −170 mV in aerated medium at pH 7.5. Behavioral responses to changes in oxygen concentration correlated with changes in proton motive force. Aerotaxis was normal in *ptsG* and *ptsI* strains that lack enzyme $II^{Glucose}$ and enzyme I, respectively, and are deficient in the phosphotransferase system. A *cya* strain that is deficient in adenylate cyclase also had normal aerotaxis. We concluded that aerotaxis was mediated by the electron transport system and that either the cytochrome *d* or the cytochrome *o* branch of the pathway could mediate aerotaxis.

We previously proposed a sequence for the initial sensory transduction events in the chemotactic response to oxygen (aerotaxis) by *Salmonella typhimurium* and *Escherichia coli* (14–16, 28, 31, 32). According to that model, oxygen increases the flow of reducing equivalents through the respiratory chain and thereby increases the proton motive force across the cytoplasmic membrane. The change in proton motive force is detected by a hypothetical sensing mechanism that transmits a signal to the flagellar motors.

Indirect evidence supports the proposed role of the proton motive force in aerotaxis. The concentration of oxygen which gives a half-maximal behavioral response is similar to the K_m for cytochrome *o*, the terminal oxidase of the respiratory chain in exponentially growing *S. typhimurium* and *E. coli* (14; D. J. Laszlo, Ph.D. dissertation, Loma Linda University, Loma Linda, Calif., 1981). Inhibitors of respiration also inhibit aerotaxis (15, 16). At a constant partial pressure of oxygen, an artificially produced change in proton motive force can cause a behavioral response in *Bacillus subtilis* (18, 20, 21). Brief responses to depolarization or hyperpolarization have also been reported in *S. typhimurium* (16). However, Sherman et al. (25) did not observe a similar response by *E. coli* cells to uncouplers in a temporal assay. They did observe a response to uncouplers in a spatial assay, but the absence of an observed response in a temporal assay raises questions about a primary role of proton motive force in aerotaxis.

An alternative model for sensory transduction in aerotaxis has been proposed by Glagolev and his collaborators (6, 7, 17) in which oxygen alters the redox level of enzyme $II^{Glucose}$ and the latter transmits a signal to the flagellar motors. Enzyme $II^{Glucose}$ is the product of the *ptsG* gene and is the receptor for glucose chemotaxis and transport via the phosphotransferase system (1).

Evidence presented in support of the enzyme $II^{Glucose}$ redox pathway for aerotaxis included the following. Oxidants such as phenazine methosulfate and benzoquinone enhance aerotaxis, whereas the reductant dithiothreitol attenuates aerotaxis (7). The phosphotransferase substrates glucose and α-methylglucoside inhibit aerotaxis (17). Phosphotransferase mutants that are defective in *ptsG* or *ptsH* (structural gene for the HPr protein) are also deficient in aerotaxis. As originally formulated (6, 7), the alternative sensory transduction pathway from enzyme $II^{Glucose}$ to the flagellar motor involved cyclic GMP and cyclic AMP (cAMP), but this appears to be improbable in the light of later investigations (33, 35).

The present study was designed to further investigate the mechanism of aerotaxis and to distinguish between the proton motive force model and the enzyme $II^{Glucose}$ model.

MATERIALS AND METHODS

Bacterial cells and growth. *S. typhimurium* and *E. coli* strains (Table 1) were grown on Vogel and Bonner minimal medium E (34) supplemented with nutritional requirements and 0.7% glucose or 1% glycerol. Some strains of *E. coli* were grown anaerobically in L broth supplemented with 0.5% potassium nitrate and 0.3% glycerol (8) as indicated. Cultures from the mid-exponential phase of growth were used for the taxis assays. Cells were washed and suspended in chemotaxis buffer consisting of 10 mM phosphate buffer (pH 7.5), 0.1 mM EDTA, and a carbohydrate as indicated.

Aerotaxis assays. Three types of spatial assays were employed. A small drop of bacteria (4 μl, approximately 10^9 cells per ml) was placed on a glass microscope slide and then covered with a cover slip in a manner that trapped one or more air bubbles in the suspension. An aerotactic band of bacteria around a bubble was observed through a phase-contrast or dark-field microscope. In the second method, one drop of bacteria in chemotaxis buffer (approximately 25 μl) was placed between a glass microscope slide and a cover slip, one side of which rested on a piece of cover slip. An aerotactic band along the air-suspension interface was observed and recorded with a video recorder. The third method, employing an air-filled capillary, was described previously (16). A temporal assay was also described previ-

* Corresponding author.
† Present address: Department of Oral Biology, Health Sciences Center, University of Louisville, Louisville, KY 40292.

TABLE 1. Bacterial strains

Strain	Relevant genotype	Reference or source
E. coli		
OW1	*che*$^+$	19
AW581	*ptsG ptsM*	1
RP437	*che*$^+$	22
AW729	RP437 *cya*	5
AN386	*cyo*$^+$ *cyd*$^+$	4
RG98	AN386 *cyd*	4
RG99	AN386 *cyo cyd*	4
RG101	AN386 *cyo*	4
S. typhimurium		
SL3730	*che*$^+$ *galU*	B. A. D. Stocker
LT2	*che*$^+$	N. Zinder via B. N. Ames
LJ45	*che*$^+$ (originally ST1)	D. E. Koshland, Jr. (3), via M. H. Saier, Jr.
LJ130	LJ45 *ptsI18*	M. H. Saier, Jr.
BT57	LJ45 *cya-961* *zid-62*::Tn*10*	33

ously (26). Typically, four assays were repeated on 2 separate days to get an average response time. These assays were performed at 30°C.

Oxygen uptake. Bacterial consumption of oxygen was measured in a closed chamber (Yellow Springs Instrument Co.; model 5301) at 30°C with a Clark-type oxygen electrode (Yellow Springs; model 5331) connected to an oxygen monitor (Yellow Springs; model 53).

Membrane potential. The polyvinyl chloride matrix-type electrode was constructed by the method of Kamo et al. (12). Tetraphenylborate was used as an ion exchanger for tetraphenylphosphonium. Tetraphenylphosphonium ion-selective, oxygen (Clark-type), and reference electrodes were immersed in 5 ml of chemotaxis buffer containing 3 μM tetraphenylphosphonium chloride. Extracellular concentrations of tetraphenylphosphonium and oxygen were measured simultaneously under constant stirring at 30°C. Cells were permeabilized by treatment with EDTA as described previously (27).

RESULTS

The role of cytochrome *o* in aerotaxis has been assigned on the basis of indirect evidence (see above). The subsequent isolation of cytochrome oxidase mutants of *E. coli* by Gennis and co-workers (4, 8) made a direct test of this hypothesis possible. The respiratory chain in *E. coli* branches to two terminal oxidases, cytochrome *o* and cytochrome *d*, the products of the *cyo* and *cyd* genes, respectively (2, 23). Cytochrome *o* is the sole oxidase in exponentially growing cells such as those used in previous studies of aerotaxis (9, 14). Cytochrome *d* is induced in the stationary phase of growth and in low concentrations of oxygen.

Aerotaxis in oxidase-deficient strains. A *cyo cyd* strain (*E. coli* RG99) that was deficient in both oxidases showed a complete loss of aerotactic response in a temporal assay (Table 2). Strains that were deficient in one of the terminal oxidases [*E. coli* RG98 (*cyd*) and RG101 (*cyo*)] responded to changes in oxygen concentration in a manner that was similar to the responses of the parent [*E. coli* AN386 (*cyo*$^+$ *cyd*$^+$)]. That is, an increase in dissolved oxygen concentration from 0 to 225 μM (oxygen concentration in medium equilibrated with air at 30°C) caused transient smooth swimming. A decrease in oxygen concentration from 225 to 0 μM resulted in transient tumbling followed by smooth swimming (data not shown) when the proton motive force fell to a level that did not support tumbling (13, 16). All strains showed a normal chemotactic response to Ni^{2+} (Table 2) and to serine (data not shown).

TABLE 2. Aerotactic response in *E. coli* mutants deficient in cytochrome oxidases[a]

Strain	Relevant genotype	Response time (s)		Oxygen consumption rate (nmol/min per mg of protein)
		N_2→air	0.06 mM Ni^{2+}	
AN386		19 ± 3	25 ± 1	62 ± 10
RG101	*cyo*	14 ± 6	27 ± 1	55 ± 7
RG98	*cyd*	20 ± 2	26 ± 1	55 ± 2
RG99	*cyo cyd*	No response	28 ± 1	2 ± 2

[a] The bacteria were grown anaerobically in L broth supplemented with 0.5% potassium nitrate and 0.3% glycerol. The cells were prepared and the assays were performed as described in Materials and Methods. The responses to oxygen and to Ni^{2+} were smooth swimming and tumbling, respectively. The values are expressed as the means ± standard deviations.

The responses of the oxidase-deficient mutants in a spatial gradient of oxygen were observed after an air-filled capillary was inserted into a drop of suspended cells. The parental strain and the *cyo* and *cyd* strains formed an aerotactic band around the mouth of the capillary, but the *cyo cyd* mutant did not form a band. These data indicate that aerotaxis is observed in strains that have either cytochrome *o* or cytochrome *d* but not in strains without a terminal oxidase.

Respiration and proton motive force in oxidase-deficient strains. The *cyo* and *cyd* strains had respiration rates comparable to those of the parental strain, whereas the *cyo cyd* strain showed little uptake of oxygen (Table 2).

For *E. coli* cells with an external pH of 7.5, the ΔpH component of the proton motive force is approximately zero and it is possible to estimate the magnitude of the proton motive force by measuring the membrane potential, Δψ (24, 29). The membrane potential of the *cyo* and *cyd* strains and of the parental strain underwent large changes when air was replaced by nitrogen or vice versa (Fig. 1). The *cyo cyd* strain showed little change in membrane potential under the same conditions but showed a large increase in potential upon the addition of potassium nitrate, which is an alternative electron acceptor for the electron transport system. The aerobic membrane potential in the oxidase-positive strains was approximately −170 mV, which is similar to the membrane potential measured previously in *S. typhimurium* (28). The small but reproducible change in potential upon aeration of the *cyo cyd* strain was not due to the presence of revertants that had recovered oxidase activity. A sample of the cell population was screened for oxidase activity at the conclusion of each experiment; the frequency of reversion was consistently less than 10^{-4}.

Effect of an upcoupler on respiration rate and tactic behavior. Preliminary studies sought to find conditions that would partially depolarize a membrane-permeable strain, *S. typhimurium* SL3730, but would permit the proton motive force to remain above the level required for maximal motility (13). The uncouplers CCCP (carbonyl cyanide *m*-chlorophenylhydrazone), FCCP [carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone], and 1799 [2,6-dihydroxy-1,1,1,7,7,7-hexafluoro-2,6-bis(trifluoromethyl)heptan-4-one] were investigated at various concentrations. Each abruptly increased bacterial respiration, indicating uncoupling of respiration, but motility was severely affected. The uncoupler 2,4-dinitrophenol increased respiration by 25% at a concentration of

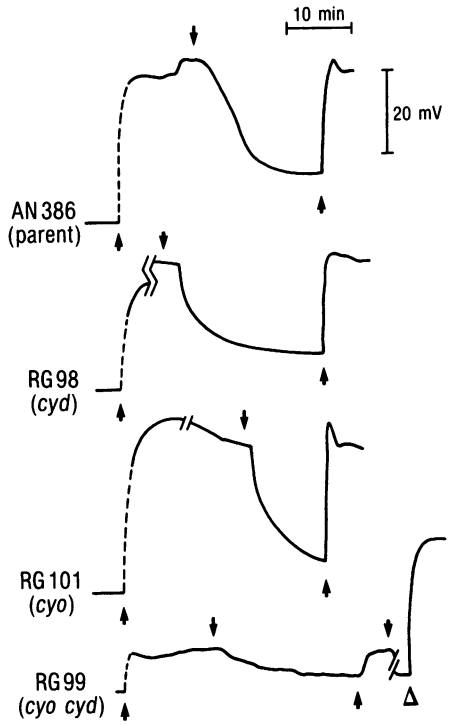

FIG. 1. Comparison of aerobic and anaerobic membrane potential in *E. coli* mutants deficient in the terminal oxidases of the electron transport system. The bacteria were grown anaerobically in L broth supplemented with glycerol and nitrate. After harvesting, the bacteria were permeabilized by treatment with EDTA, and 50 μl of a dense suspension was added to a closed chamber that contained 3 μM tetraphenylphosphonium and 0.1% glycerol in 5 ml of chemotaxis buffer. The chamber was initially sparged with air. The arrows indicate the times at which the cells were introduced, the gas was changed from air to nitrogen, and air was reintroduced. Potassium nitrate (2 mM) was added to strain RG99 at the time indicated (Δ). Membrane potential was measured with a tetraphenylphosphonium-selective electrode as described in Materials and Methods. The final cell concentrations (milligrams of dry weight per milliliter) in the chamber were as follows: AN386, 2.05; RG98, 2.05; RG101, 1.94; and RG99, 2.54.

300 μM and did not impair motility at that concentration. Dinitrophenol was selected for further investigation of the effect of uncouplers on the behavior of *S. typhimurium*.

In a temporal assay of behavior, the addition of 300 μM dinitrophenol caused a strong tumbling response (Fig. 2), which was similar to the behavioral response to a decrease in oxygen concentration from 225 to 0 μM. When the dinitrophenol was decreased by a 10-fold dilution, the bacteria showed a transient smooth swimming response.

Aerotaxis in phosphotransferase-deficient mutants. Evidence supporting the enzyme $II^{Glucose}$ model for aerotaxis was reexamined. Mutants that were defective in enzyme $II^{Glucose}$ [*E. coli* AW581 (*ptsG*)] or in enzyme I [*S. typhimurium* LJ130 (*ptsI*)] gave normal aerotactic responses in a temporal assay (Table 3). These strains are defective in chemotaxis to glucose or to all substrates for the phosphotransferase system, respectively (1). Glucose (35 mM) did not inhibit aerotaxis of *S. typhimurium* LT2 in spatial (capillary and air bubble) assays for aerotaxis (Fig. 3).

The *cya* mutant, which is deficient in adenylate cyclase, has been reported to be defective in aerotaxis (7). *S. typhimurium* BT57 (*cya*) and *E. coli* AW729 (*cya*) were aerotactic in a temporal assay (Table 3) and in a spatial bubble assay

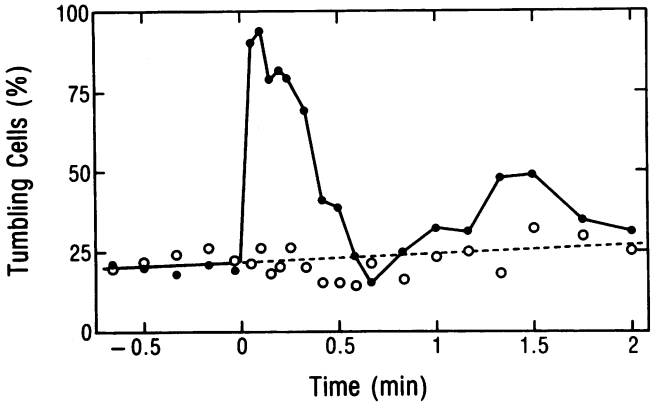

FIG. 2. Effect of an uncoupler on the tumbling frequency of *S. typhimurium* SL3730. A sample (9 μl) of *S. typhimuirum* SL3730 suspended in chemotaxis buffer containing 0.7% glucose (5×10^8 cells per ml) was rapidly mixed with 1 μl of 3 mM, 2,4-dinitrophenol in 1% methanol on a glass microscope slide. The behavioral response was observed through the microscope and videotaped. The percentage of cells that tumbled in a 1-s interval was determined as described previously (28). Symbols: ●, dinitrophenol added; ○, 1% methanol in chemotaxis buffer added.

(data not shown). Chemotaxis to phosphotransferase sugars in the *cya* strain BT57 is dependent on growth in the presence of cAMP (33); aerotaxis in strain BT57 was independent of cAMP (Table 3).

DISCUSSION

Strains that were defective in enzyme $II^{Glucose}$ or in enzyme I of the phosphotransferase transport system were normal in responses to oxygen measured by either a temporal assay (Table 3) or a spatial assay (data not shown). The cell suspensions in which the behavioral responses were measured were streaked on MacConkey agar and confirmed to have the predicted fermentation phenotype. The cultures were essentially free of revertants. These results disprove the hypothesis that enzyme $II^{Glucose}$ is the receptor for oxygen and that aerotaxis is mediated through the phosphotransferase pathway.

Glucose (35 mM), at concentrations that would saturate the phosphotransferase transport system, did not inhibit aerotaxis in a temporal (16) or spatial (Fig. 3) assay. Chemotaxis to phosphotransferase substrates requires cAMP for

TABLE 3. Aerotactic response in *pts* mutants and *cya* mutants of *E. coli* and *S. typhimurium*[a]

Strain	Response time (s) ($N_2 \rightarrow$air)
E. coli	
OW1	17 ± 7
AW581 (*ptsG*)	23 ± 5
AW729 (*cya*)	20 ± 3
S. typhimurium	
LJ45	15 ± 2
LJ130 (*ptsI*)	14 ± 2
BT57 (*cya*) (with 5 mM cAMP)	12 ± 3
BT57 (*cya*) (without cAMP)	11 ± 2

[a] The bacteria were grown in minimal medium with glycerol as the carbon source. The temporal assay for aerotaxis was described in Materials and Methods. The values are expressed as the means ± standard deviations.

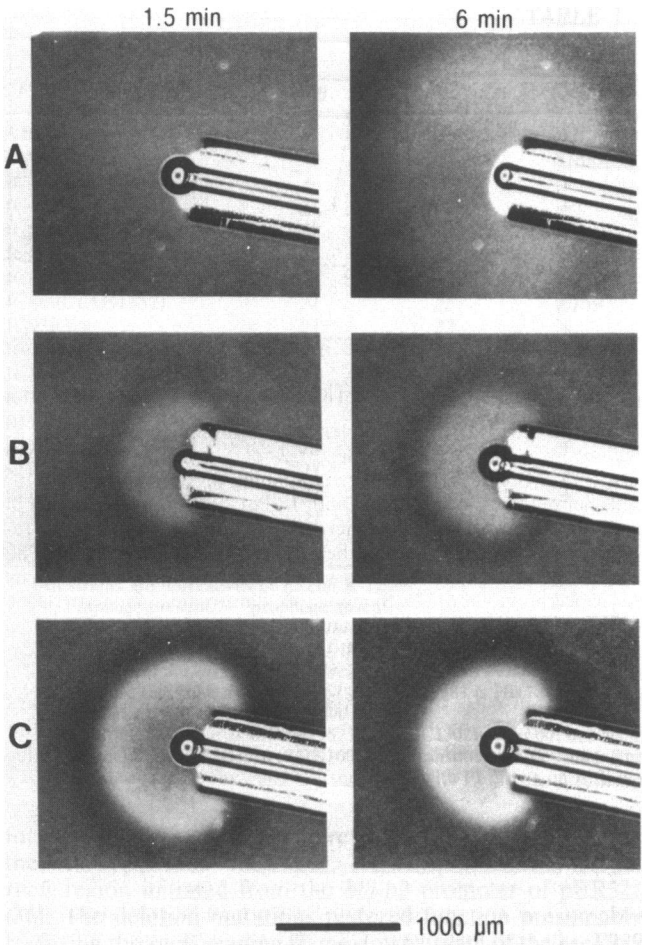

FIG. 3. Effect of glucose on aerotaxis of *S. typhimurium* LT2. Bacteria were grown in a minimal medium containing glycerol and then washed and suspended (2.1×10^9 cells per ml) in chemotaxis buffer containing glycerol, glucose, or no carbon source. The capillary assay was performed as described in Materials and Methods. Photographs were then taken through a phase-contrast microscope at the indicated times. (A) Cells suspended in chemotaxis buffer without a carbon source. (B) Cells suspended in chemotaxis buffer with 35 mM glycerol. (C) Cells suspended in chemotaxis buffer with 35 mM glucose.

induction of a chemotaxis protein in addition to the proteins of the flagellar motor and the proteins in the pathway for methylation-dependent chemotaxis (33, 35). With strains that are constitutive for the flagellar apparatus, the present study found no requirement for cAMP in aerotaxis. This is a further indication that there are different pathways for phosphotransferase chemotaxis and aerotaxis.

The results obtained with the *cyo* and *cyd* strains confirm a requirement for the electron transport system in aerotaxis. A new finding is that cytochrome *d* in addition to cytochrome *o* can function as a "receptor" for aerotaxis in both temporal and spatial assays. Cytochrome *o* and cytochrome *d* are not receptors in the same class as receptors such as the serine or ribose receptor. From previous studies, it is evident that oxygen binding to the terminal oxidase is required but not sufficient for signal transduction in aerotaxis (15, 16). Metabolism of oxygen is an integral part of the sensing mechanism.

Glagolev and Sherman have proposed that a change in the redox potential of a signaling component rather than a change in proton motive force mediates aerotaxis (6, 7). Neither the present study nor previous investigators can conclusively distinguish between those possibilities. Previous studies were equivocal on the question of whether an uncoupler of oxidative phosphorylation could cause a repellent (tumbling) response in a temporal assay of enteric bacteria (16, 21, 25). Failure to observe a response to a temporal gradient of an uncoupler would cast serious doubts on the proposed role of the proton motive force in aerotaxis. The observed response to a temporal gradient of 2,4-dinitrophenol in Fig. 2 was entirely consistent with the proton motive force hypothesis. So too, were the observed changes in proton motive force in the *cyo*, *cyd*, and *cyo cyd* strains (Fig. 1).

In our view, the results obtained with dinitrophenol favor the proton motive force hypothesis over a redox signaling mechanism. In well-aerated unstarved bacteria, there appears to be some inhibition of electron transport by a large proton motive force. When dinitrophenol partially depleted the proton motive force, there was an increase in respiration. The change in redox potential that accompanied the observed 25% increase in respiration was probably quite small, with cytochrome *o* being slightly reduced and other components being slightly oxidized. The change in proton motive force is more likely to have the characteristics required for signaling. However, historically it has proved difficult in studies of oxidative phosphorylation to eliminate localized phenomena that are in equilibrium with the bulk phase as the driving force for ATP synthesis. There is a similar dilemma in the mechanism for aerotaxis.

Recent investigations of the basis for an ATP requirement (27, 30) in chemotaxis revealed a protein transphosphorylation mechanism that may be the signal transduction pathway (11). The *cheA* gene product is autophosphorylated, and the phosphoryl residue can be transferred to the *cheY* gene product (10, 36). Investigations in this laboratory have shown that the pathways for phosphotransferase (mannose) chemotaxis and for aerotaxis converage at the CheA-CheW step in the methylation-dependent pathway (E. H. Rowsell, J. M. Smith, A. J. Wolfe, M. P. Conley, B. L. Taylor, and H. C. Berg, FASEB J. **2**:A575, 1988).

ACKNOWLEDGMENTS

Strains were kindly donated by Robert B. Gennis at the University of Illinois.

This investigation was supported by Public Health Service grant GM29481 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **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.
2. **Anraku, Y., and R. B. Gennis.** 1987. The aerobic respiratory chain of *Escherichia coli*. Trends Biochem. Sci. **12**:262–266.
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. **Au, D. C.-T., R. M. Lorence, and R. B. Gennis.** 1985. Isolation and characterization of an *Escherichia coli* mutant lacking the cytochrome *o* terminal oxidase. J. Bacteriol. **161**:123–127.
5. **Black, R. A., A. C. Hobson, and J. Adler.** 1983. Adenylate cyclase is required for chemotaxis to phosphotransferase system sugars by *Escherichia coli*. J. Bacteriol. **153**:1187–1195.
6. **Glagolev, A. N.** 1984. Bacterial $\Delta\mu H^+$-sensing. Trends Biochem. Sci. **9**:397–400.
7. **Glagolev, A. N., and M. Y. Sherman.** 1983. The glucose phosphotransferase system is involved in *Escherichia coli* oxygen taxis. FEMS Microbiol. Lett. **17**:147–150.

8. **Green, G. N., and R. B. Gennis.** 1983. Isolation and chracterization of an *Escherichia coli* mutant lacking cytochrome *d* terminal oxidase. J. Bacteriol. **154**:1269–1275.
9. **Haddock, B. A., J. A. Downie, and P. B. Garland.** 1976. Kinetic characterization of the membrane-bound cytochromes of *Escherichia coli* grown under a variety of conditions by using a stopped-flow dual-wavelength spectrophotometer. Biochem. J. **154**:285–294.
10. **Hess, J. F., K. Oosawa, N. Kaplan, and M. I. Simon.** 1988. Phosphorylation of three proteins in the signaling pathway of bacterial chemotaxis. Cell **53**:79–87.
11. **Hess, J. F., K. Oosawa, P. Matsumura, and M. I. Simon.** 1987. Protein phosphorylation is involved in bacterial chemotaxis. Proc. Natl. Acad. Sci. USA **84**:7609–7613.
12. **Kamo, N., M. Muratsugu, R. Hongoh, and Y. Kobatake.** 1979. Membrane potential of mitochondria measured with an electrode sensitive to tetraphenyl phosphonium and relationship between proton electrochemical potential and phosphorylation potential in steady state. J. Membr. Biol. **49**:105–121.
13. **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.
14. **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.
15. **Laszlo, D. J., M. Niwano, W. W. Goral, and B. L. Taylor.** 1984. *Bacillus cereus* electron transport and proton motive force during aerotaxis. J. Bacteriol. **159**:820–824.
16. **Laszlo, D. J., and B. L. Taylor.** 1981. Aerotaxis in *Salmonella typhimurium*: role of electron transport. J. Bacteriol. **145**:990–1001.
17. **Milgrom, E. M., M. Y. Sherman, and A. N. Glagolev.** 1985. Functional interaction between information processing pathways in *Escherichia coli* and the nature of Δ-H^+ (redox)-sensing. FEMS Microbiol. Lett. **26**:211–216.
18. **Miller, J. B., and D. E. Koshland, Jr.** 1977. Sensory electrophysiology of bacteria: relationship of the membrane potential to motility and chemotaxis in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **74**:4752–4756.
19. **Ordal, G. W., and J. Adler.** 1974. Isolation and complementation of mutants in galactose taxis and transport. J. Bacteriol. **117**:509–516.
20. **Ordal, G. W., and D. J. Goldman.** 1975. Chemotaxis away from uncouplers of oxidative phosphorylation in *Bacillus subtilis*. Science **189**:802–805.
21. **Ordal, G. W., and D. J. Goldman.** 1976. Chemotactic repellents of *Bacillus subtilis*. J. Mol. Biol. **100**:103–108.
22. **Parkinson, J. S.** 1978. Complementation analysis and deletion mapping of *Escherichia coli* mutants defective in chemotaxis. J. Bacteriol. **135**:45–53.
23. **Poole, R. K., and W. J. Ingledew.** 1987. Pathways of electrons to oxygen, p. 170–200. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
24. **Ramos, S., S. Schuldiner, and H. R. Kaback.** 1976. The electrochemical gradient of protons and its relationship to active transport in *Escherichia coli* membrane vesicles. Proc. Natl. Acad. Sci. USA **73**:1892–1896.
25. **Sherman, M. Y., E. O. Timkina, and A. N. Glagolev.** 1981. Sensing of the proton motive force in *Escherichia coli* chemotaxis. FEMS Microbiol. Lett. **12**:121–124.
26. **Shioi, J., C. V. Dang, and B. L. Taylor.** 1987. Oxygen as attractant and repellent in bacterial chemotaxis. J. Bacteriol. **169**:3118–3123.
27. **Shioi, J., R. J. Galloway, M. Niwano, R. E. Chinnock, and B. L. Taylor.** 1982. Requirement of ATP in bacterial chemotaxis. J. Biol. Chem. **257**:7969–7975.
28. **Shioi, J., and B. L. Taylor.** 1984. Oxygen taxis and proton motive force in *Salmonella typhimurium*. J. Biol. Chem. **259**: 10983–10988.
29. **Slonczewski, J. L., B. P. Rosen, J. R. Alger, and R. M. Macnab.** 1981. pH homeostasis in *Escherichia coli*: measurement by ^{31}P nuclear magnetic resonance of methylphosphonate and phosphate. Proc. Natl. Acad. Sci. USA **78**:6271–6275.
30. **Smith, J. M., E. H. Rowsell, J. Shioi, and B. L. Taylor.** 1988. Identification of a site of ATP requirement for signal processing in bacterial chemotaxis. J. Bacteriol. **170**:2698–2704.
31. **Taylor, B. L.** 1983. Role of proton motive force in sensory transduction in bacteria. Annu. Rev. Microbiol. **37**:551–573.
32. **Taylor, B. L.** 1983. How do bacteria find the optimal concentration of oxygen? Trends Biochem. Sci. **8**:438–441.
33. **Tribhuwan, R. C., M. S. Johnson, and B. L. Taylor.** 1986. Evidence against direct involvement of cyclic GMP or cyclic AMP in bacterial chemotactic signaling. J. Bacteriol. **168**:624–630.
34. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
35. **Vogler, A. P., and J. W. Lengeler.** 1987. Indirect role of adenylate cyclase and cyclic AMP in chemotaxis to phosphotransferase system carbohydrates in *Escherichia coli* K-12. J. Bacteriol. **169**:593–599.
36. **Wylie, D., A. Stock, C.-Y. Wong, and J. Stock.** 1988. Sensory transduction in bacterial chemotaxis involves phosphotransfer between Che proteins. Biochem. Biophys. Res. Commun. **151**: 891–896.