JOURNAL OF BACTERIOLOGY, June 1997, p. 4075–4079 Vol. 179, No. 12
0021-9193/97/$04.00+0

A Signal Transducer for Aerotaxis in *Escherichia coli*

SERGEI I. BIBIKOV,[1] ROY BIRAN,[1] KENNETH E. RUDD,[2] AND JOHN S. PARKINSON[1]*

Biology Department, University of Utah, Salt Lake City, Utah 84112,[1] and Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, Florida 33101[2]

Received 12 March 1997/Accepted 15 April 1997

The newly discovered *aer* locus of *Escherichia coli* encodes a 506-residue protein with an N terminus that resembles the NifL aerosensor and a C terminus that resembles the flagellar signaling domain of methyl-accepting chemoreceptors. Deletion mutants lacking a functional Aer protein failed to congregate around air bubbles or follow oxygen gradients in soft agar plates. Membranes with overexpressed Aer protein also contained high levels of noncovalently associated flavin adenine dinucleotide (FAD). We propose that Aer is a flavoprotein that mediates positive aerotactic responses in *E. coli*. Aer may use its FAD prosthetic group as a cellular redox sensor to monitor environmental oxygen levels.

Aerotaxis, the movement of a cell or organism toward or away from oxygen, was first described in bacteria more than a century ago by Engelmann (8), Pfeffer (24), and Beijerinck (3), who observed accumulation of cells near air bubbles or other sources of oxygen. Despite considerable study, particularly in *Escherichia coli* (22, 28, 30), the molecular mechanism underlying this behavior has remained elusive. Does the organism detect oxygen directly, or does it instead sense some metabolic consequence of different oxygen environments, such as changes in electron transport activity (19), cellular redox potential (4), or proton motive force (20, 32)? We describe here a gene, dubbed *aer* for aerotaxis, that encodes a likely flavoprotein signal transducer for aerotaxis in *E. coli*. Studies of the Aer protein promise to provide a definitive answer to the long-standing puzzle of how cells detect oxygen gradients during aerotaxis.

Sequence features of the *aer* locus. We initially identified the *aer* gene as an open reading frame (ORF506) discovered in the *E. coli* genome sequencing project (7). Its conceptual translation product, a 506-amino-acid Aer protein, exhibits several hallmarks of an aerosensing function (Fig. 1). Aer residues 10 to 110 are similar to parts of NifL, FixL, and related bacterial proteins that trigger regulatory responses to changes in environmental oxygen levels (5, 11). Residues 168 to 209 are predominantly hydrophobic and could serve to anchor Aer in the cytoplasmic membrane. Aer residues 259 to 506 are about 50% identical to the cytoplasmic signaling domains of methyl-accepting chemotaxis proteins (MCPs), the principal chemoreceptors of *E. coli* (14). These features suggested that Aer might generate chemotactic signals in response to oxygen gradients.

Construction of an *aer* mutant. We constructed a large in-frame deletion lacking codons 5 to 505 of the *aer* coding region by PCR amplification of chromosomal sequences flanking the *aer* locus in strain RP437 (23) by using primer pairs NSB19-NSB20 and NSB25-NSB22 (Fig. 1). The two PCR fragments were ligated at their common *Xba*I site, joining *aer* codon 4 to codon 506, and inserted into the pMAK705 vector, whose replication is temperature-sensitive (13), producing plasmid pSB25. RP437 carrying pSB25 was grown at 44°C to select recombinational insertions and then at 30°C for recombinational excisions. Two reciprocal recombination products were recovered: plasmid pSB27, a pMAK705 derivative carrying the undeleted, wild-type *aer* locus from RP437, and strain UU1117, a Δ*aer-1* derivative of RP437. These constructs were confirmed by plasmid or genomic PCR with NSB26 and NSB27 as primers (Fig. 1). A second *aer*$^+$ plasmid (pSB20) was constructed by PCR amplification of the *aer* locus in RP437, with primers NSB17 and NSB18 (Fig. 1). The *aer* coding region was cloned under control of the p_{tac} promoter in plasmid pCJ30, a derivative of vector pTM30 (21).

Air bubble assays for aerotaxis. Bacteria were grown at 32°C in tryptone medium containing 100 μg of ampicillin/ml to mid-logarithmic phase (optical density at 595 nm, 0.5), pelleted by centrifugation, and resuspended at the same cell density in motility buffer, with 20 mM sodium lactate as the energy source (23). Cells were placed on a microscope slide, and their distribution in the vicinity of small bubbles trapped under the coverslip was recorded after 15 min at room temperature. Cells of the *aer*$^+$ parental strain congregated around the air bubbles,

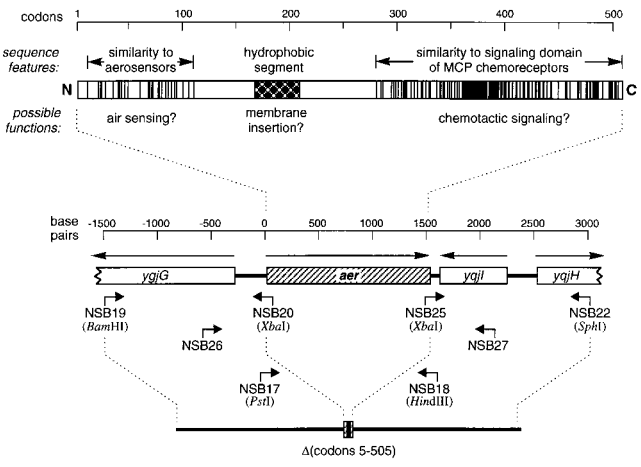

FIG. 1. Physical map of the *E. coli aer* locus and sequence features of the Aer protein. The *aer* gene is located at 69.1 min on the *E. coli* chromosome (GenBank accession number U28379). The flanking genes are open reading frames of unknown function. Arrows above the genes indicate their direction of transcription and translation. Note that *aer* is transcribed independently of neighboring genes. Vertical lines in the Aer protein (listed as AIR_ECOLI in SwissProt, accession number P50466) represent residue identities with the NifL aerosensor and with Tsr, the serine chemoreceptor. Oligonucleotide primers, some with restriction sites at their 5′ ends, are shown below the physical map, and were used to construct the Δ*aer-1* deletion, as described in the text.

* Corresponding author. Mailing address: Biology Department, University of Utah, Salt Lake City, UT 84112. Phone: (801) 581-7639. Fax: (801) 581-4668. E-mail: parkinson@biology.utah.edu.

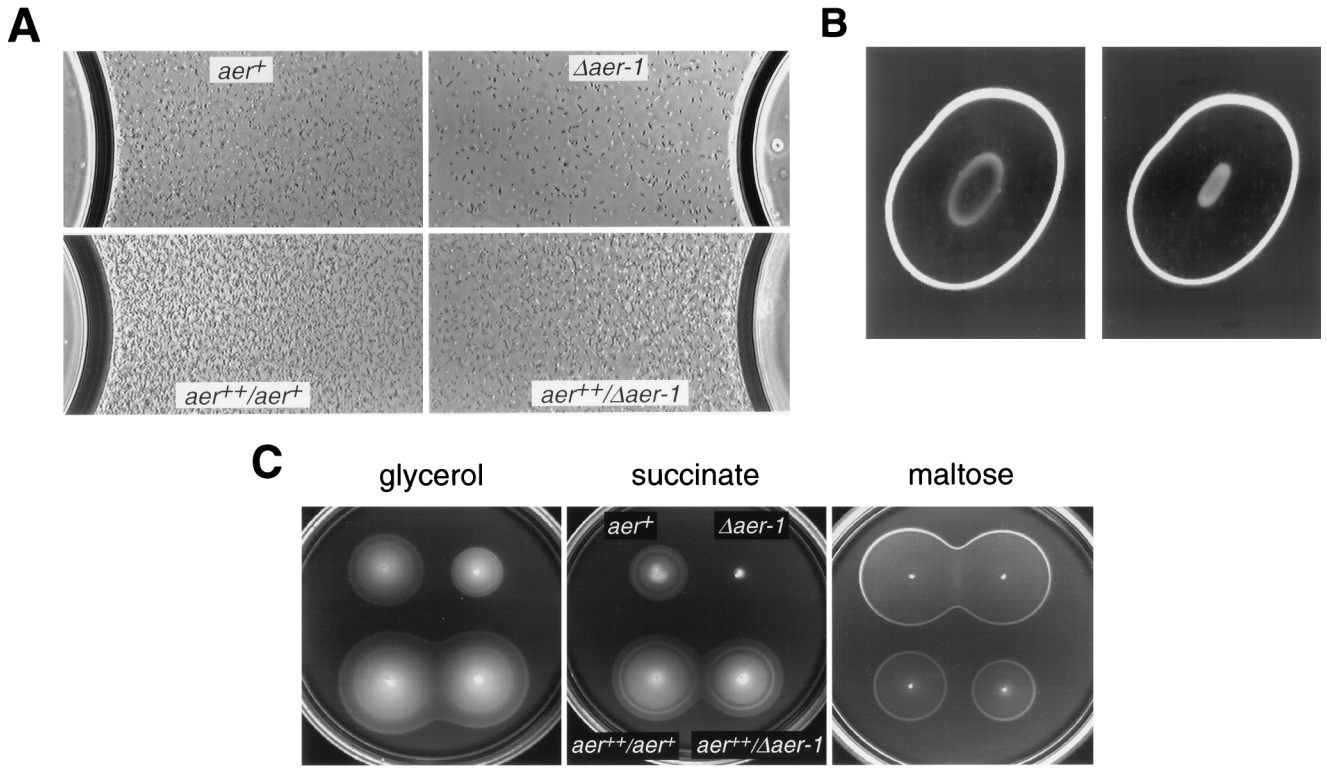

FIG. 2. Aerotaxis assays. (A) Accumulation of *aer*$^+$ cells near air bubbles. The prominent black arcs are the edges of the bubbles. Strains were pCJ30/RP437 (*aer*$^+$), pCJ30/UU1117 (Δ*aer-1*), pSB20/RP437 (*aer*$^{++}$/*aer*$^+$), and pSB20/UU1117 (*aer*$^{++}$/Δ*aer-1*). (B) Air avoidance response of the Δ*aer-1* mutant. Cells of strain pCJ30/UU1117 in motility buffer were observed in sealed chambers at a magnification of ~5×. The air trapped inside the chamber surrounds the cell sample; the white ring is the air/liquid interface. Responses at 60 (left) and 120 min (right) are shown. (C) Colony morphology on soft agar medium. Plates contained minimal swarm agar (23); 1 mg of thiamine/ml and 0.1 mM threonine, leucine, histidine, and methionine to satisfy growth requirements; 50 μg of ampicillin for plasmid maintenance/ml; and either 1 mM glycerol, 30 mM succinate, or 0.1 mM maltose as carbon and energy sources. Cells from freshly transformed colonies were stabbed to the bottom of the agar with a toothpick, and the plates were incubated at 32°C for 12 h. Strains were the same as described for panel A above.

moving closer to the interface as they depleted the oxygen diffusing from the bubble (Fig. 2A). In contrast, cells of the Δ*aer-1* mutant not only failed to gather in the vicinity of air bubbles (Fig. 2A), but actively moved away from them. This repellent response was especially dramatic when cells in a drop of culture fluid were surrounded by air in a sealed chamber consisting of a microscope slide, a Frame-Seal gasket (MJ Research, Inc.), and a coverslip. (Fig. 2B). Within several minutes the Δ*aer-1* cells began a visible retreat from the air/liquid interface, culminating after several hours with the cells huddled in the center of the drop (Fig. 2B). The Δ*aer-1* strain might be responding to changes in proton motive force (16, 17), extracellular or intracellular pH (26, 31), or a more direct repellent effect of oxygen (29). The machinery responsible for this air avoidance response is presumably present in wild-type cells as well but is masked in air bubble assays by the attractant function of the Aer protein. Multicopy plasmids bearing a wild-type *aer* gene complemented the aerotaxis defect of the Δ*aer-1* mutant, confirming that its aberrant behavior reflected the lack of a functional Aer protein (Fig. 2A). In fact, plasmid-containing strains were attracted to air bubbles in higher cell numbers than the *aer*$^+$ parent, indicating that the level of Aer protein in the cell determines the strength of its positive aerotactic response.

Swarm plate assays for aerotaxis. Soft agar swarm plates containing glycerol or succinate as carbon source were also used to evaluate the aerotactic ability of the Δ*aer-1* strain. On these plates the respiratory activity of the growing cells creates an oxygen gradient leading outward from the colony (1, 2). Viewed from the side, the gradient profile approximates a truncated cone with its base at the bottom of the plate, where the oxygen is depleted fastest because it is least accessible to atmospheric oxygen diffusing from the surface. Thus, aerotactic colonies expand rapidly in glycerol or succinate soft agar, forming prominent rings of cells at the top and bottom surface of the plate. Strains with at least one functional copy of the *aer* gene produced aerotactic swarms on both glycerol and succinate media (Fig. 2C). Those carrying an *aer*$^+$ plasmid swarmed fastest, consistent with the dose-dependent enhancement of aerotaxis seen in the air bubble assays. In contrast, the Δ*aer-1* mutant formed smaller, cylindrical colonies devoid of aerotactic bands (Fig. 2C). The mutant swarms were especially small on succinate, perhaps owing to the air avoidance effect seen in the bubble assays or to excretion of the attractant aspartate by cells at the center of the colony (6), either of which would slow colony expansion when unopposed by an outward migration to oxygen.

Other behaviors were examined to determine whether the Δ*aer-1* mutant was specifically defective in aerotaxis. The Δ*aer-1* strain formed wild-type chemotactic rings on swarm plates containing conventional attractants, such as maltose (Fig. 2C), aspartate, serine, ribose, or galactose (data not shown). Moreover, the unstimulated swimming and flagellar rotation patterns of the Δ*aer-1* mutant were indistinguishable from those of its *aer*$^+$ parent strain, as were its growth rates in various media (data not shown). When expressed above nor-

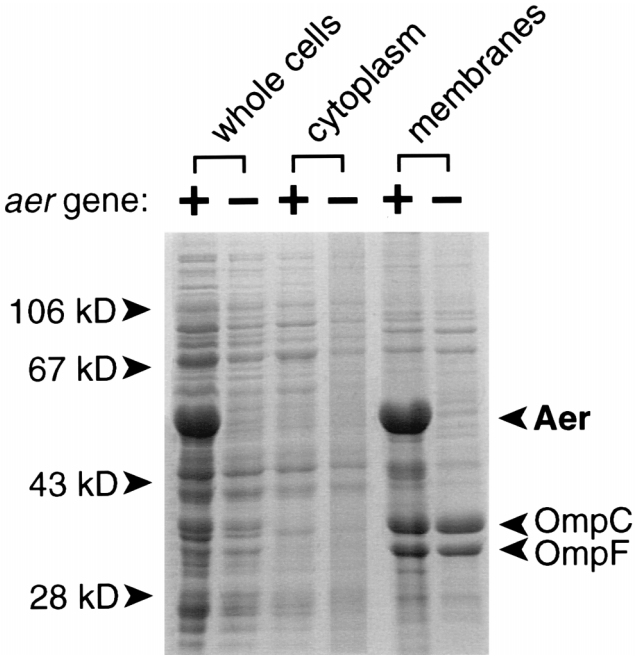

FIG. 3. High-level expression and membrane location of Aer protein. Strain UU1117 carrying either pSB20 (*aer*$^+$) or pCJ30 (*aer*) was grown at 37°C to an optical density at 595 nm of 0.5 to 0.8 in H1 minimal medium (23) supplemented with Casamino Acids. Cells were induced with 2 mM isopropyl-β-D-thiogalactopyranoside for 3 h, collected by centrifugation, and frozen at −70°C. Cells were thawed, washed twice in 50 mM Tris HCl (pH 8.0)–5 mM EDTA–10% glycerol–5 mM phenanthroline–1 mM phenylmethylsulfonyl fluoride, and disrupted in a French press. Membranes were pelleted by ultracentrifugation, washed once in the same buffer with 2 M KCl, and then resuspended in low-salt buffer. Samples of the unfractionated cell lysates, the membrane pellets, and the cytoplasmic supernatants were subjected to electrophoresis on a sodium dodecyl sulfate–10% polyacrylamide gel. Proteins were visualized by Coomassie staining, with the positions of various size markers indicated on the left. OmpC and OmpF are prominent outer membrane proteins.

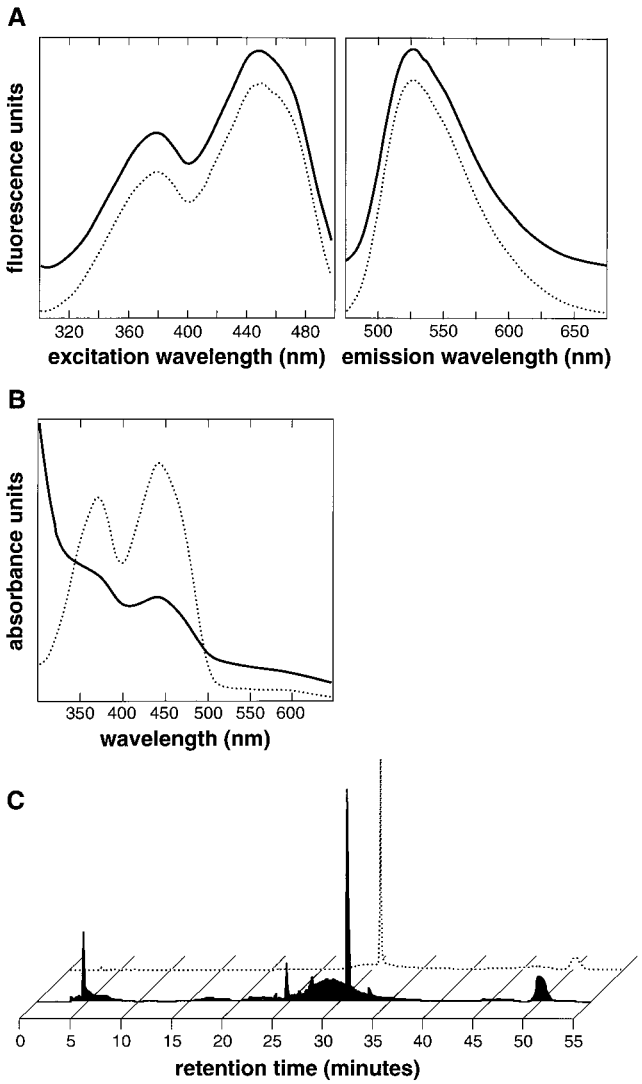

FIG. 4. Identification of FAD in Aer extracts. Membrane preparations from strains overexpressing Aer protein were prepared as described in the legend for Fig. 3, resuspended in water, and treated five times with $CHCl_3$ to remove proteins and lipids. Samples of the aqueous extract were concentrated on a Speedvac centrifuge and analyzed by various methods. (A) Fluorescence excitation and emission spectra of the Aer extract (solid line) and an aqueous solution of flavin adenine mononucleotide (FMN) (dotted line). (B) Absorbance spectra of Aer-containing membranes (solid line) and FMN (dotted line). (C) HPLC profiles of the Aer extract (solid profile) and FAD (dotted line). The HPLC analysis was done on a Waters 600 system and Microsorb C_{18} reversed-phase column. The elution procedure was adapted from one described previously (35). The column eluate was monitored by absorbance at 260 nm.

mal levels in wild-type cells, the Aer protein inhibited chemotaxis generally (Fig. 2C), presumably by competing with other chemoreceptors for common signaling components, particularly CheA and CheW, which are needed for aerotaxis (27) as well as for MCP-mediated behaviors. In cells lacking other chemoreceptors, overexpression of Aer protein produced episodes of clockwise flagellar rotation, consistent with activation of the CheA-CheW signaling pathway (data not shown). We conclude that the Aer protein is essential for aerotactic responses in bubble and swarm assays and that it uses the CheA-CheW signaling pathway to communicate with the flagellar motors. Aer seems to play no important role in other chemotactic responses or in general cellular metabolism.

Aer is probably a flavoprotein. The NifL and FixL proteins, partial homologs of Aer, use prosthetic groups for aerosensing. FixL has a noncovalently associated heme moiety for direct detection of oxygen (12), whereas NifL binds flavin adenine dinucleotide (FAD), perhaps to sense oxygen-related changes in redox potential (15). Plasmid pSB20, which expresses the *aer* gene under control of an inducible promoter, was used to look for evidence of a prosthetic group in the Aer protein. Upon induction in a Δ*aer-1* host, pSB20 produced large amounts of an approximately 55-kDa protein (Fig. 3), which cross-reacted with polyclonal antiserum raised against the highly conserved portion of the Tsr signaling domain (data not shown). The Aer protein in these cells was associated with the membrane fraction (Fig. 3), which, unlike control membranes, had a distinct greenish yellow color (data not shown), implying that Aer might contain a chromophore. Conceivably, high-level expression of the Aer protein sequesters the chromophore, causing the cells to compensate by increasing its production.

Aqueous solutions of the colored substance(s) in Aer-containing membranes could be produced by removal of proteins and lipids with a chloroform extraction, implying a noncovalent association between Aer and the chromophore. Spectral analyses of the aqueous material, hereafter referred to as Aer extract, indicated that the Aer-related chromophore was fluorescent, with excitation and emission maxima identical to those of flavin compounds (Fig. 4A). Aer-containing membranes ex-

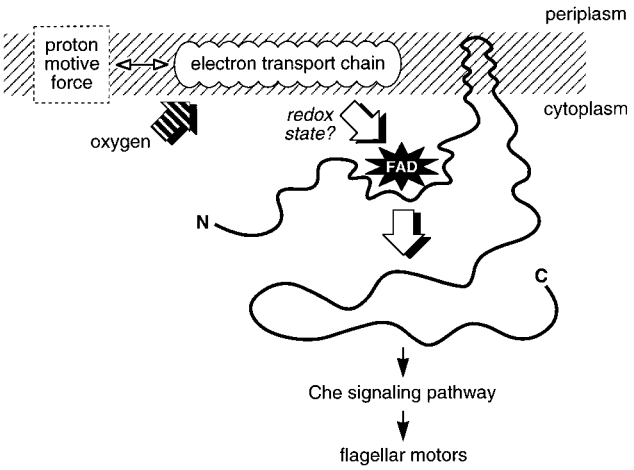

FIG. 5. Working model of aerotactic sensing and signal transduction by the Aer protein. See text for details.

hibited two prominent absorbance signals at wavelengths characteristic of flavin compounds (Fig. 4B), indicating that this putative Aer chromophore was a major constituent of the membrane preparation. On thin-layer chromatographic plates, the fluorescent compound in Aer extract comigrated with FAD (data not shown). In a high-performance liquid chromatography (HPLC) system developed for identifying flavin compounds (35), the major peak in the water-soluble Aer extract had the same retention time as an FAD standard (Fig. 4C). Finally, electrospray mass spectrometry revealed the presence of a compound in the Aer extract with the same molecular weight as FAD (data not shown). These findings indicate that Aer may use an FAD prosthetic group to sense aerotactic stimuli.

Aer may function as a redox sensor. A working model of the Aer protein is summarized in Fig. 5. Aer most likely associates with the cytoplasmic membrane through its central hydrophobic segment, which is just long enough to traverse the membrane twice. Both its N and C termini are probably located in the cytoplasm. The C-terminal domain is highly similar to the signaling domains of the MCP family of chemoreceptors, which associate with the cytoplasmic CheW and CheA proteins to generate signals that control the flagellar motors (14). Aer appears to have a similar signaling capability. The N-terminal portion of Aer may bind FAD and function as an input domain. The FAD moiety could serve to sense aerotactic stimuli and perhaps blue light as well (33).

The Aer protein could conceivably detect oxygen directly, for example, as a flavin hydroperoxide complex, which is thought to form in some flavoprotein oxygenases and bacterial luciferases (9, 34). However, it seems more likely that Aer uses bound FAD to measure redox potential in the cell, with the terminal oxidases of the electron transport chain serving as the primary oxygen sensors (18). Aer might, for example, monitor electron transport activity by exchanging electrons with one of the membrane carriers of the respiratory chain. Oxidation or reduction of the FAD moiety might in turn trigger a conformational change that alters the activity of the Aer signaling domain to control flagellar movement. Fu et al. (10) have described a heme-containing MCP (DcrA) in anaerobic *Desulfovibrio* species that may perform a similar role. Its methylation state changes in response to oxidizing or reducing environments, but it is not yet known if DcrA mediates an aerotactic response.

This report and work by Rebbapragada et al. (25) support the view that the Aer protein is primarily an energy sensor designed to monitor the cell's respiratory activity. Thus, Aer should prove an excellent prototype for exploring not only aerotactic behavior but also the more general question of how cells evaluate their internal physiological state.

We thank John Spikes for performing the fluorescence and absorbance measurements on Aer extracts, Emma Lou Bardall for performing flagellar rotation assays, Peter Ames for antisera and technical advice, Marian Price-Carter for help with HPLC, the David Goldenberg lab for use of their video capture equipment, David Blair for valuable advice and the loan of video equipment, Larry Okun for helpful comments on the manuscript, and Barry Taylor and Igor Zhulin for communicating results prior to publication.

The Protein-DNA Core Facility and the Mass Spectrometry Facility at the University of Utah receive support from National Cancer Institute grant CA42014 to the Huntsman Cancer Institute. This research was supported by grant GM19559 from the National Institute of General Medical Sciences, National Institutes of Health.

REFERENCES

1. **Adler, J.** 1966. Chemotaxis in bacteria. Science **153:**708–716.
2. **Adler, J.** 1966. The effect of amino acids and oxygen on chemotaxis in *Escherichia coli*. J. Bacteriol. **92:**121–129.
3. **Beijerinck, M. W.** 1893. Über Atmungsfiguren beweglicher Bakterien. Z. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. **14:**827–845.
4. **Bespalov, V. A., I. B. Zhulin, and B. L. Taylor.** 1996. Behavioral responses of *Escherichia coli* to changes in redox potential. Proc. Natl. Acad. Sci. USA **93:**10084–10089.
5. **Blanco, G., M. Drummond, P. Woodley, and C. Kennedy.** 1993. Sequence and molecular analysis of the *nifL* gene of *Azotobacter vinelandii*. Mol. Microbiol. **9:**869–879.
6. **Budrene, E. O., and H. C. Berg.** 1995. Dynamics of formation of symmetrical patterns by chemotactic bacteria. Nature **376:**49–53.
7. **Daniels, D. L., G. Plunkett, V. Burland, and F. R. Blattner.** 1992. Analysis of the *Escherichia coli* genome: DNA sequence of the region from 84.5 to 86.5 minutes. Science **257:**771–778.
8. **Engelmann, T. W.** 1881. Neue Methode zur Untersuchung der Sauerstoffausscheidung pflanzlicher und thierischer Organismen. Pfluegers Arch Gesamte Physiol. Menschen Tiere **25:**285–292.
9. **Ermler, U., R. A. Siddiqui, R. Cramm, D. Schröder, and B. Friedrich.** 1995. Crystallization and preliminary X-ray diffraction studies of a bacterial flavohemoglobin protein. Proteins Struct. Funct. Genet. **21:**351–353.
10. **Fu, R., J. D. Wall, and G. Voordouw.** 1994. DcrA, a *c*-type heme-containing methyl-accepting protein from *Desulfovibrio vulgaris* Hildenborough, senses the oxygen concentration or redox potential of the environment. J. Bacteriol. **176:**344–350.
11. **Gilles, G. M., G. S. Ditta, and D. R. Helinski.** 1991. A haemoprotein with kinase activity encoded by the oxygen sensor of *Rhizobium meliloti*. Nature **350:**170–172.
12. **Gilles, G. M., G. Gonzalez, M. F. Perutz, L. Kiger, M. C. Marden, and C. Poyart.** 1994. Heme-based sensors, exemplified by the kinase FixL, are a new class of heme protein with distinctive ligand binding and autoxidation. Biochemistry **33:**8067–8073.
13. **Hamilton, C. M., M. Aldea, B. K. Washburn, P. Babitzke, and S. R. Kushner.** 1989. New method for generating deletions and gene replacements in *Escherichia coli*. J. Bacteriol. **171:**4617–4622.
14. **Hazelbauer, G. L.** 1992. Bacterial chemoreceptors. Curr. Opin. Struct. Biol. **2:**505–510.
15. **Hill, S., S. Austin, T. Eydmann, T. Jones, and R. Dixon.** 1996. *Azotobacter vinelandii* NifL is a flavoprotein that modulates transcriptional activation of nitrogen-fixation genes via a redox-sensitive switch. Proc. Natl. Acad. Sci. USA **93:**2143–2148.
16. **Khan, S., and R. M. Macnab.** 1980. Proton chemical potential, proton electrical potential, and bacterial motility. J. Mol. Biol. **138:**599–614.
17. **Khan, S., and R. M. Macnab.** 1980. The steady state counterclockwise/clockwise bias of bacterial flagellar motors is regulated by proton motive force. J. Mol. Biol. **138:**563–597.
18. **Laszlo, D. J., B. L. Fandrich, A. Siraram, 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.
19. **Laszlo, D. J., and B. L. Taylor.** 1981. Aerotaxis in *Salmonella typhimurium*: role of electron transport. J. Bacteriol. **145:**990–1001.
20. **Miller, J. B., and D. E. Koshland, Jr.** 1980. Proton motive force and bacterial sensing. J. Bacteriol. **141:**26–32.

21. **Morrison, T. B., and J. S. Parkinson.** 1994. Liberation of an interaction domain from the phosphotransfer region of CheA, a signaling kinase of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **91:**5485–5489.
22. **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.
23. **Parkinson, J. S., and S. E. Houts.** 1982. Isolation and behavior of *Escherichia coli* deletion mutants lacking chemotaxis functions. J. Bacteriol. **151:**106–113.
24. **Pfeffer, W.** 1888. Über chemotaktische Bewegungen von Bakterien, Flagellaten und Volvocineen. Untersuch. Bot. Inst. Tübingen. **2:**582–663.
25. **Rebbapragada, A., M. S. Johnson, I. B. Zhulin, G. P. Harding, A. J. Zuccarelli, H. M. Fletcher, and B. L. Taylor.** Personal communication.
26. **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.
27. **Rowsell, E. H., J. M. Smith, A. Wolfe, and B. L. Taylor.** 1995. CheA, CheW, and CheY are required for chemotaxis to oxygen and sugars of the phosphotransferase system in *Escherichia coli*. J. Bacteriol. **177:**6011–6014.
28. **Shioi, J., and B. L. Taylor.** 1984. Oxygen taxis and proton motive force in *Salmonella typhimurium*. J. Biol. Chem. **259:**10983–10988.
29. **Shioi, J. C., V. Dang, and B. L. Taylor.** 1987. Oxygen as attractant and repellent in bacterial chemotaxis. J. Bacteriol. **169:**3118–3123.
30. **Shioi, J. C., R. C. Tribhuwan, S. T. Berg, and B. L. Taylor.** 1988. Signal transduction in chemotaxis to oxygen in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **170:**5507–5511.
31. **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.
32. **Taylor, B. L.** 1983. Role of proton-motive force in sensory transduction in bacteria. Annu. Rev. Microbiol. **37:**551–573.
33. **Taylor, B. L., J. B. Miller, H. M. Warrick, and D. E. Koshland, Jr.** 1979. Electron acceptor taxis and blue light effect on bacterial chemotaxis. J. Bacteriol. **140:**567–573.
34. **Vervoort, J., F. Müller, J. Lee, W. A. M. V. de Berg, and C. T. W. Moonen.** 1986. Identifications of the true carbon-13 nuclear magnetic resonance spectrum of the stable intermediate II in bacterial luciferase. Biochemistry **25:** 8062–8067.
35. **Willie, A., and M. S. Jorns.** 1995. Discovery of a third coenzyme in sarcosine oxidase. Biochemistry **34:**16703–16707.