

NOTES

Evolutionary Conservation of Methyl-Accepting Chemotaxis Protein Location in *Bacteria* and *Archaea*

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The methyl-accepting chemotaxis proteins (MCPs) are concentrated at the cell poles in an evolutionarily diverse panel of bacteria and an archeon. In elongated cells, the MCPs are located both at the poles and at regions along the length of the cells. Together, these results suggest that MCP location is evolutionarily conserved.

Prokaryotic processes, such as cell division and chemotaxis, often depend on the asymmetric or nonuniform distribution of proteins to subcellular locations (for a recent review on this subject, see reference 31). The methyl-accepting chemotaxis proteins (MCPs) are components of the chemotactic response system in *Bacteria* and *Archaea* (1, 12, 16, 35). The subcellular location of MCPs has been determined for four prokaryotes: *Caulobacter crescentus* (2), *Escherichia coli* (23, 24), *Bacillus subtilis* (17), and *Rhodobacter sphaeroides* (14). In these species, the MCPs are concentrated primarily at the cell poles. The location of the MCPs may be involved in the regulation of *E. coli* chemotaxis (4, 8, 20), as MCP clustering has been implicated in regulating ligand binding and signaling in this bacterium (7, 10, 19, 21, 22, 34). An understanding of MCP localization in a wide range of other bacteria and archaea could illuminate the relationship between MCP location and regulation of chemotaxis.

Previous microscopy studies that have explored the subcellular location of MCPs have utilized anti-MCP antibodies raised against the MCP of the organism being studied (2, 17, 23, 24). Hazelbauer and coworkers have reported that antibodies raised against Trg, an *E. coli* MCP, can be used in Western blottings to identify proteins antigenically related to the *E. coli* MCPs from a number of different organisms (1, 26, 27). These results suggest that anti-Trg antibodies could be used in fluorescence microscopy experiments as convenient reagents to visualize the subcellular location of MCPs in a variety of organisms.

To explore the conservation of MCP location, we selected a representative panel of bacterial species and an archaeon. The species were chosen to represent chemotactically active strains

that, relative to *E. coli*, are either evolutionarily (5, 18) similar (e.g., *Vibrio furnissii* [36]), divergent (e.g., *Spirochaeta aurantia* [11]), or highly divergent (e.g., *Halobacterium salinarium* [29, 33]).

Cells were grown in the following liquid media supplemented with 10 mM D-galactose: *E. coli*, Luria broth (LB) (1% tryptone, 0.5% yeast extract, 0.5% NaCl); *V. furnissii*, high-salt LB (1% tryptone, 0.5% yeast extract, 2% NaCl); *S. aurantia*, GTY (10 mM D-glucose, 4% tryptone, 2% yeast extract, 10 mM phosphate buffer [pH 7.0]); *H. salinarium*, complex medium (27 mM KCl, 166 mM MgSO₄, 10 mM sodium citrate, 4.3 M NaCl, 1% peptone, 2 mM CaCl₂). We used anti-Trg antibodies to determine the location of MCPs by fluorescence microscopy (23, 24).

As has been seen in investigations of *C. crescentus* (2), *E. coli* (10, 23, 24), *B. subtilis* (16), and *R. sphaeroides* (14), fluorescence is observed primarily at the poles of both *V. furnissii* and *S. aurantia* (Fig. 1), which suggests that the MCPs are localized to the cell poles in these bacteria. Similar results were obtained with an anti-Tsr antibody (data not shown). Interestingly, a polarly localized fluorescence pattern also is observed with the archaeal species *H. salinarium* (Fig. 1), despite the divergence between this organism and *E. coli* (5, 18). Thus, in each of the organisms studied, proteins that are antigenically related to the *E. coli* MCPs are localized. The predominant sites of concentration are the cell poles.

We next investigated the location of MCPs in elongated cells. Bacterial cell elongation occurs after treatment with β -lactam antibiotics, such as cephalixin (13, 24, 28, 30), from mutations in the cell division gene *lonS* (32), or upon differentiation to swarmer cells (3, 6, 9, 15, 25). We have previously reported that the MCPs of cephalixin-treated *E. coli* cells are localized not only to the cell poles but also at intervals along the length of the elongated cell (24). Treatment of *E. coli* with cephalixin, therefore, results in deviation from the primarily polar MCP localization observed in the normal-sized cells. To investigate the MCP pattern in elongated cells generated by means other than cephalixin treatment, we conducted fluorescence microscopy experiments in a *lonS* mutant of *Vibrio para-*

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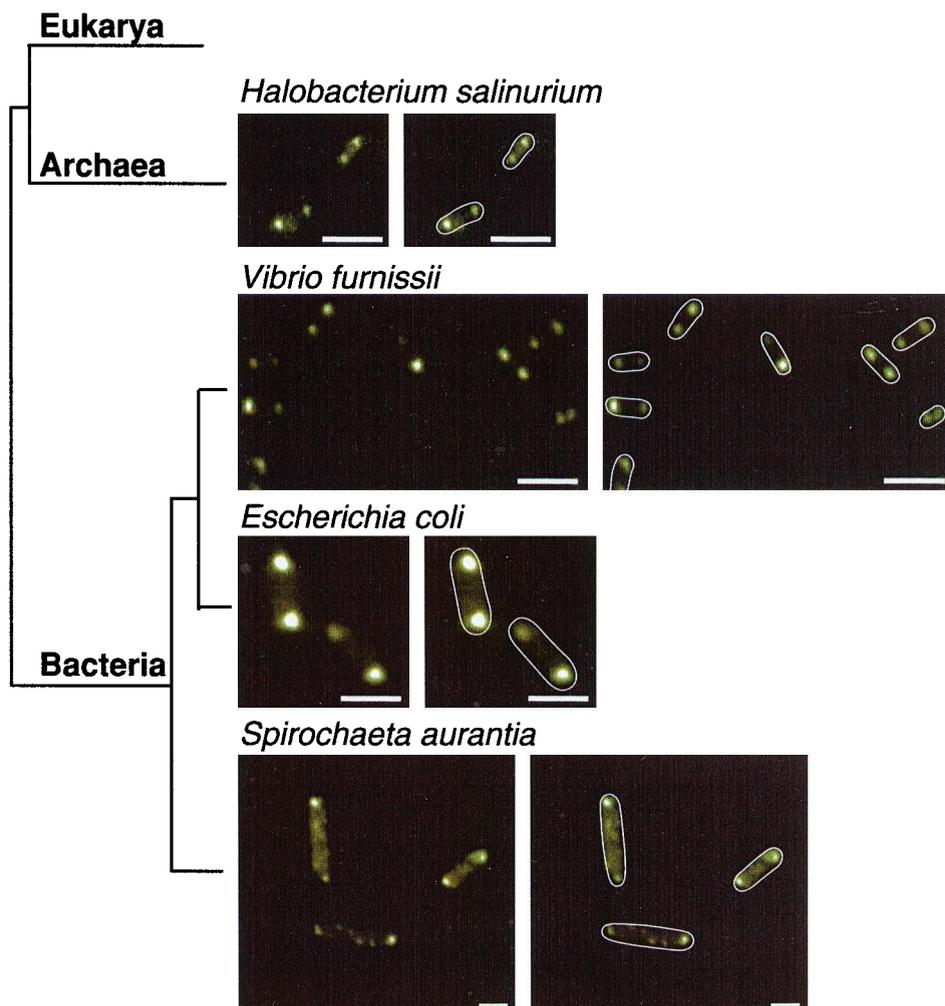


FIG. 1. Schematic representation of relative evolutionary relationships (5, 18) and fluorescence patterns for cells treated with anti-Trg antibody (1:400) followed by a fluorescein-labeled anti-rabbit immunoglobulin G secondary antibody (1:200). Duplicate figures on the right display outlines of the cells. The result with *E. coli* AW405 confirms our previous results (23). Images such as those presented were obtained in >70% of the cells observed in at least three independent experiments. Bars, 2 μ m. Branch lengths are not based on phylogenetic distances.

haemolyticus LM1017 (32) and swarmer cells of *V. parahaemolyticus* BB22, *Proteus mirabilis* BB2000, and *E. coli* ATCC 25922.

The MCPs of the normal-sized cells from which the elongated cells were generated were located at the cell poles in each case (Fig. 2A, C, E, and G). The fluorescence patterns of *V. parahaemolyticus* LM4420, an elongated mutant deficient in LonS (32), revealed that the MCPs are localized not only at the poles but also at intervals along the cell (Fig. 2B). Similar results were observed in the wild-type naturally occurring swarmer cells of *V. parahaemolyticus*; the MCPs are concentrated both at the cell poles and at intervals (Fig. 2D). In contrast, the fluorescence intensity at the poles of swarmer cells of *P. mirabilis* (Fig. 2F) and *E. coli* (Fig. 2H) is more pronounced than it is at intervals along the cells. This suggests that the concentration of MCPs at the poles is higher than that along the length of these cells. This pattern of MCP localization may be related to the observation that *E. coli* swarmer cells run essentially all the time (R. Harshey, unpublished data); the probability that the transient phosphorylated CheY (which causes tumbling) can migrate from the site where it is generated (MCPs) to the site where it acts (the flagella) is low.

In contrast, *E. coli* cephalaxin-produced filaments have MCP clusters throughout, and both running and stopping (the equivalent of tumbling in filamentous bacteria) are observed commonly (24).

The regulation of *E. coli* chemotaxis may be modulated by proximity of the MCPs (4, 8, 20). Here we report that the MCPs are polarly localized in a panel of bacteria that are evolutionarily similar (*Vibrio* sp. and *P. mirabilis*) and divergent (*S. aurantia*) from *E. coli*. The MCPs also are localized at the poles in a more distantly related archaeon (*H. salinarium*). Additionally, the MCPs of all of the elongated cells that we studied are located at the poles and at intervals along the cell, although the distribution of MCPs varies (Fig. 2). Moreover, in both normal-sized and elongated cells, the MCPs typically are localized and not distributed randomly. These data suggest that, as with *C. crescentus*, *E. coli*, *B. subtilis*, and *R. sphaeroides* chemotaxis, MCP clustering may be involved, here also, in the regulation of chemotaxis. Investigations of MCP localization in organisms such as *E. coli*, therefore, may lead to a greater understanding of the regulation of chemotactic responses in other bacteria and in archaea.

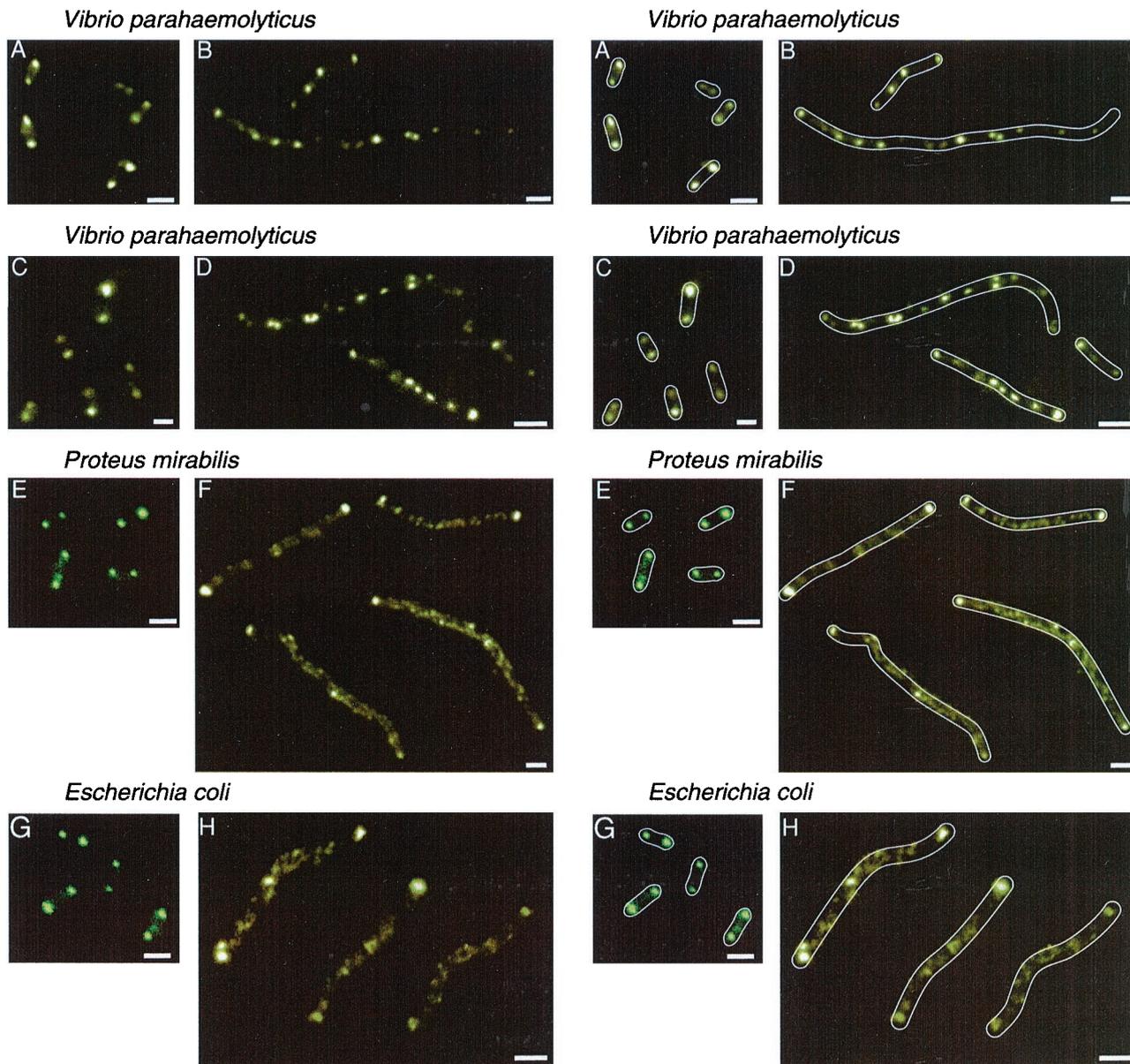


FIG. 2. Comparison of the MCP fluorescence patterns in normal-sized and elongated cells. (A) Normal-sized *V. parahaemolyticus* LM1017. (B) *V. parahaemolyticus* *lonS* mutant LM4420 derived from LM1017. (C) Normal-sized *V. parahaemolyticus* BB22. (D) Elongated swarmer cells of *V. parahaemolyticus* BB22 taken from 2% agar high-salt LB plates at 30°C. (E) Normal-sized *P. mirabilis*. (F) *P. mirabilis* swarmer cells from 1.5% agar LB plates at 37°C. (G) Normal-sized *E. coli* ATCC 25922. (H) *E. coli* ATCC 25922 swarmer cells from 0.55% agar LB plates supplemented with 0.5% glucose at 30°C. Duplicate figures on the right display drawn outlines of the cells. Images such as those presented were obtained in >90% of the cells observed in three independent experiments. Bars, 2 μ m.

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REFERENCES

1. Alam, M., and G. L. Hazelbauer. 1991. Structural features of methyl-accepting taxis proteins conserved between archaeobacteria and eubacteria revealed by antigenic cross-reaction. *J. Bacteriol.* **173**:5837–5842.
2. Alley, M. R. K., J. R. Maddock, and L. Shapiro. 1992. Polar localization of a bacterial chemoreceptor. *Genes Dev.* **6**:825–836.
3. Belas, R., D. Erskine, and D. Flaherty. 1991. *Proteus mirabilis* mutants defective in swarmer cell differentiation and multicellular behavior. *J. Bacteriol.* **173**:6279–6288.
4. Bray, D., M. D. Levin, and C. J. Morton-Firth. 1998. Receptor clustering as a cellular mechanism to control sensitivity. *Nature* **393**:85–88.
5. Brown, J. R., and W. F. Doolittle. 1997. *Archaea* and the prokaryotic-to-eukaryotic transition. *Microbiol. Mol. Biol. Rev.* **61**:456–502.
6. Burkart, M., A. Toguchi, and R. M. Harshey. 1998. The chemotaxis system, but not chemotaxis, is essential for swarming motility in *Escherichia coli*.

- Proc. Natl. Acad. Sci. USA **95**:2568–2573.
7. Cochran, A. G., and P. S. Kim. 1996. Imitation of *Escherichia coli* aspartate receptor signaling in engineered dimers of the cytoplasmic domain. *Science* **271**:1113–1116.
 8. Duke, T. A. J., and D. Bray. 1999. Heightened sensitivity of a lattice of membrane receptors. *Proc. Natl. Acad. Sci. USA* **96**:10104–10108.
 9. Fraser, G. M., and C. Hughes. 1999. Swarming motility. *Curr. Opin. Microbiol.* **2**:630–635.
 10. Gestwicki, J. E., L. E. Strong, and L. L. Kiessling. 2000. Tuning chemotactic responses with synthetic multivalent ligands. *Chem. Biol.* **7**:583–591.
 11. Goulbourne, E. A., Jr., and E. P. Greenberg. 1981. Chemotaxis of *Spirochaeta aurantia*: involvement of membrane potential in chemosensory signal transduction. *J. Bacteriol.* **148**:837–844.
 12. Grebe, T. W., and J. Stock. 1998. Bacterial chemotaxis: the five sensors of a bacterium. *Curr. Biol.* **8**:R154–R157.
 13. Greenwood, D., and F. O'Grady. 1973. Comparison of the response of *Escherichia coli* and *Proteus mirabilis* to seven β -lactam antibiotics. *J. Infect. Dis.* **128**:211–222.
 14. Harrison, D. M., J. Skidmore, J. P. Armitage, and J. R. Maddock. 1999. Localization and environmental regulation of MCP-like proteins in *Rhodobacter sphaeroides*. *Mol. Microbiol.* **31**:885–892.
 15. Harshey, R. M. 1994. Bees aren't the only ones: swarming in Gram-negative bacteria. *Mol. Microbiol.* **13**:389–394.
 16. Kathariou, S., and E. P. Greenberg. 1983. Chemoattractants elicit methylation of specific polypeptides in *Spirochaeta aurantia*. *J. Bacteriol.* **156**:95–100.
 17. Kirby, J. R., T. B. Niewold, S. Maloy, and G. W. Ordal. 2000. CheB is required for behavioral responses to negative stimuli during chemotaxis in *Bacillus subtilis*. *Mol. Microbiol.* **35**:44–57.
 18. Koonin, E. V., and M. Y. Galperin. 1997. Prokaryotic genomes: the emerging paradigm of genome-based microbiology. *Curr. Opin. Genet. Dev.* **7**:757–763.
 19. Le Moual, H., T. Quang, and D. E. Koshland, Jr. 1997. Methylation of the *Escherichia coli* chemotaxis receptors: intra- and interdimer mechanisms. *Biochemistry* **36**:13441–13448.
 20. Levit, M. N., Y. Liu, and J. B. Stock. 1998. Stimulus response coupling in bacterial chemotaxis: receptor dimers in signaling arrays. *Mol. Microbiol.* **30**:459–466.
 21. Li, G., and R. M. Weis. 2000. Covalent modification regulates ligand binding to receptor complexes in the chemosensory system of *Escherichia coli*. *Cell* **100**:357–365.
 22. Liu, Y., M. Levit, R. Lurz, M. G. Surette, and J. B. Stock. 1997. Receptor-mediated protein kinase activation and the mechanism of transmembrane signaling in bacterial chemotaxis. *EMBO J.* **16**:7231–7240.
 23. Maddock, J. R., and L. Shapiro. 1993. Polar location of the chemoreceptor complex in the *Escherichia coli* cell. *Science* **259**:1717–1723.
 24. Maki, N., J. E. Gestwicki, E. M. Lake, L. L. Kiessling, and J. Adler. 2000. Motility and chemotaxis in filamentous cells of *Escherichia coli*. *J. Bacteriol.* **182**:4337–4342.
 25. McCarter, L., and M. Silverman. 1990. Surface-induced swarmer cell differentiation of *Vibrio parahaemolyticus*. *Mol. Microbiol.* **4**:1057–1062.
 26. Morgan, D. G., J. W. Baumgartner, and G. L. Hazelbauer. 1993. Proteins antigenically related to methyl-accepting chemotaxis proteins of *Escherichia coli* detected in a wide range of bacterial species. *J. Bacteriol.* **175**:133–140.
 27. Nowlin, D. M., D. O. Nettleton, G. W. Ordal, and G. L. Hazelbauer. 1985. Chemotactic transducer proteins of *Escherichia coli* exhibit homology with methyl-accepting proteins from distantly related bacteria. *J. Bacteriol.* **163**:262–266.
 28. Rolinson, G. N. 1980. Effect of β -lactam antibiotics on bacterial cell growth rate. *J. Gen. Microbiol.* **120**:317–323.
 29. Rudolph, J., and D. Oesterhelt. 1996. Deletion analysis of the *che* operon in the archaeon *Halobacterium salinarum*. *J. Mol. Biol.* **258**:548–554.
 30. Segall, J. E., A. Ishihara, and H. C. Berg. 1985. Chemotactic signaling in filamentous cells of *Escherichia coli*. *J. Bacteriol.* **161**:51–59.
 31. Shapiro, L., and R. Losick. 2000. Dynamic spatial regulation in the bacterial cell. *Cell* **100**:89–98.
 32. Stewart, B. J., J. L. Enos-Berlage, and L. L. McCarter. 1997. The *lonS* gene regulates swarmer cell differentiation of *Vibrio parahaemolyticus*. *J. Bacteriol.* **179**:107–114.
 33. Sundberg, S. A., M. Alam, M. Lebert, J. L. Spudich, D. Oesterhelt, and G. L. Hazelbauer. 1990. Characterization of *Halobacterium halobium* mutants defective in taxis. *J. Bacteriol.* **172**:2328–2335.
 34. Tatsuno, I., M. Homma, K. Oosawa, and I. Kawagishi. 1996. Signaling by the *Escherichia coli* aspartate chemoreceptor Tar with a single cytoplasmic domain per dimer. *Science* **274**:423–425.
 35. Yao, V. J., and J. L. Spudich. 1992. Primary structure of an archaeobacterial transducer, a methyl-accepting protein associated with sensory rhodopsin I. *Proc. Natl. Acad. Sci. USA* **89**:11915–11919.
 36. Yu, C., B. L. Bassler, and S. Roseman. 1993. Chemotaxis of the marine bacterium *Vibrio furrens* to sugars: a potential mechanism for initiating the chitin catabolic cascade. *J. Biol. Chem.* **268**:9405–9409.