Speculations in Science and Technology, Vol. 6, No. 5 (1983) - p.465-470

MEMBRANE MICROVISCOSITY MIGHT BE INVOLVED IN BACTERIAL MORPHOGENESIS

ARIEH ZARITSKY

Department of Biology, Ben-Gurion University of the Negev, PO Box 653, Beer Sheva 84105, Israel.

Received: 2 September 1983

Abstract

Growth and morphogenesis of rod-shaped bacteria are briefly reviewed, with a special reference to the Gram negative species *Escherichia coli*. Diverse effects of membrane lipid composition or microviscosity (η) on different functions in a bacterial cell are listed, and a new mechanism for homeoviscous adaptation is discussed. A conceivable hypothesis is postulated to explain shape changes of bacillary bacteria, based upon activity changes with η of surface located proteins involved in envelope growth.

1. INTRODUCTION

Heterotrophic bacteria grow and divide in aqueous salts solution. The rate of multiplication depends on temperature and nutrients[1,2]. In *Escherichia coli*, aerobic growth is limited by the rate of respiration[3]. At a constant temperature, doubling time is determined essentially by the quality of the carbon source, but other nutrients enhance growth rate by various mechanisms involved in the genetic flow of information. Repression, attenuation, mRNA processing, metabolic regulation, translational control and feed-back inhibition are examples, all mediated by metabolites in the cytoplasm. On the other hand, a particular medium seems to uniquely define the molecular composition of *E. coli* irrespective of growth temperature between 25° and 37°C (see, for example,[4]). Thus, rate of multiplication as well as cell composition vary with nutritional conditions, but only the former react to cultivation temperature. Cell size and dimensions also vary with nutrition but hardly at all with temperature[1,5,6].

Faster growing bacteria on richer media are larger because a cell grows in mass exponentially[7] while its division is coupled to chromosome replication, which is a linear process[8] initiated at a relatively constant cell size[9,10]. The narrow range of size and composition of wild type *E. coli* cells cultivated unrestrictedly on a particular medium at various temperatures[1,4-6] may be explained by the adjustment of most cellular reactions, including chromosome replication, in proportion to growth rate[4,11].

2. BACTERIAL SHAPE

Rod-shaped bacteria extend by elongation not only during steady-state growth[12,13], but also under unbalanced growth (when DNA but not mass

0155-7785/83/$3.00

synthesis is specifically inhibited)[14], and divide in a perpendicular plane. It is therefore intriguing to find that faster growing *E. coli* cells (due to richer medium, not higher temperature) are also thicker[1,13,15]. In addition to nutrition, there are further means by which cell diameter can be manipulated. These include genetic and pharmacological[16] using certain mutants and drugs, respectively, that affect membrane proteins involved in shape determination.

Most models currently existing to account for the changes observed in cell shape are based upon either cell cycle parameters[12,17,18] or physical forces[15,19-21], but none is able as yet to tie up this end to the biochemical foundations. Those that try[19,20] relate shape determination to cell wall characters. However there is a consensus regarding wall functions in shape maintenance only[22] and a more convincing evidence for cytoplasmic membrane as the envelope constituent responsible for shape determination (at least in *E. coli*)[16].

3. MEMBRANE MICROVISCOSITY: FUNCTIONS AND CONTROL

The structural complexity of a biological membrane can be confined to the hydrocarbon region of its lipids and operationally defined by microviscosity (η), which is expressed in macroscopic units[23]. This physical characteristic of cell membranes seems to play a major role in determining activities of membranal proteins involved in many cellular functions (see[23] for an exhaustive review). In bacteria, the cytoplasmic membrane is a component of the envelope, which mediates many vital functions (for example, see[24]): chromosome replication and segregation[25]; cell division and shape formation[16-22]; energy metabolism, active transport and motility[26,27]; sexual exchange of genetic information[28]; control of homeoviscous adaptation[29,30]; maintaining high levels of protonmotive force[31]. Specifically membrane lipid composition or η has been implicated in the following bacterial processes: initiation of chromosome replication[32], active transport of various solutes into the cell[33], passive permeation and facilitated diffusion of glycerol[34], hyperthermic sensitivity[35], thermosensory response of motile strains[36], homeoviscous adaptation[29,30,37]. It is therefore essential that η be stringently regulated to maintain membrane fluidity at an appropriate range for optimal functionality.

The constant η in *E. coli* cells cultivated under a wide range of temperatures was originally observed by Sinensky[29], who coined the term, "homeoviscous adaptation". The biochemical foundation to this phenomenon has only recently been elucidated[30]: a temperature change modifies the relative activities of surface enzymes (without de novo protein synthesis[38]) involved in fatty acid metabolism to restore the optimal η (that prevailed before the perturbation) through a new level of acyl chain ordering (proportion of saturated and long fatty acids).

Recent in vivo studies[39] indicate that it is not just temperature that shifts the balance in *E. coli* between synthesis of unsaturated and synthesis of saturated fatty acids (U/S). The ratio U/S was measured in cultures grown at 40°C (U/S = 1.4) and after transfer to 10°C. The large overshoot (U/S = 16, see figure 1 in ref. 39) soon after the downshift and the slow adjustment

during the transition to the new steady-state level (U/S ≅ 3) clearly rule out the possibility[30] that the temperature response of β-keto-acyl-ACP synthase II plays the only role in homeoviscous adaptation. At the present state of our knowledge, there exist numerous conceivable routes by which such adaptation could be exerted, among which are feed-back control loops by various fatty acid metabolites (and see ref. 40). The simplest hypothesis (and equally probable at present) would assume that η itself is involved in this homeostasis by directly affecting activities of the enzymes involved (see Figure 1 below).

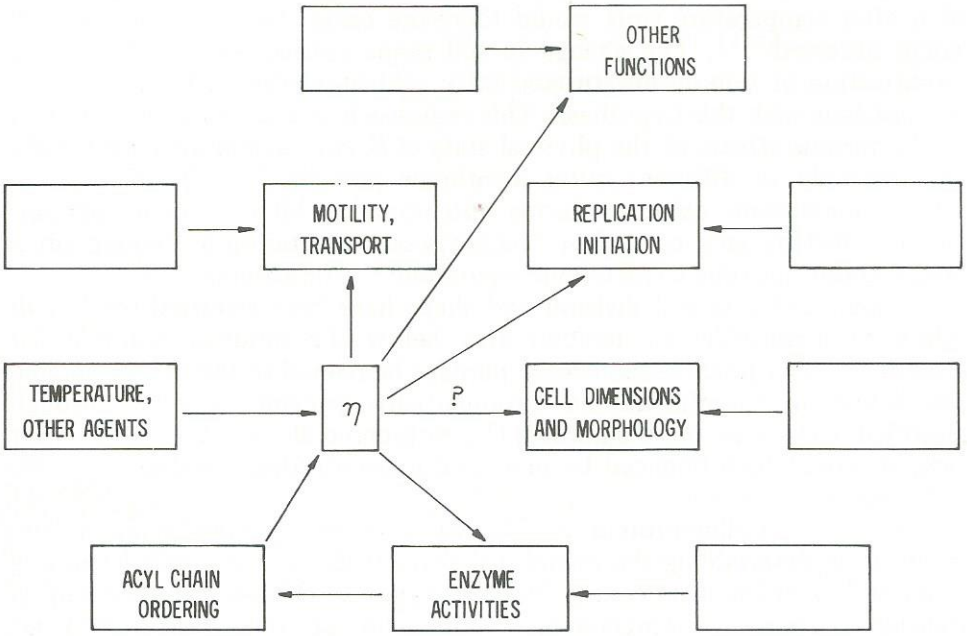

Figure 1. Flow diagram concerning regulation of bacterial membrane microviscosity (η) and some of its functions, including morphogenesis. Empty boxes indicate existence of other factors, including cytoplasmic effectors.

(Activity of Na^+, Mg^{2+}-ATPase in membranes of *Acholesplasma laidlawii B*, for instance, has been modulated by varying their fatty acid composition[41].) This hypothesis is consistent, at least qualitatively, with the change of U/S during the temperature shift mentioned (Fig. 1 in ref. 39); η is maximally stepped-up upon transfer to 10°C, thus maximally affecting the enzymes involved by increasing the ratio U/S. The resultant faster accumulation of unsaturated (compared to saturated) fatty acids is responsible for reduction in η which, in turn, lowers U/S correspondingly. The affected enzymes are thus slowly adjusted to new levels of activities corresponding to the new temperature but with the same η. Hence the differential influence of temperature on the two β-acyl-ACP-synthases[30] is only a minor component in homeoviscous adaptation, and self regulation by η seems to play a major role in the mechanism responsible. The recent observation that

temperature effects on fatty acid composition in *E. coli* are *not* compensated by cholesterol[42] is discordant with this model, but could perhaps be explained by the fact that cholesterol is not a normal metabolite in this organism and its incorporation into cell membrane might cause other, yet unknown effects.

4. POSSIBLE INVOLVEMENT OF η IN SHAPE DETERMINATION

It is postulated that activities of surface enzymes involved in envelope growth too are modulated by temperature, and that this modulation is also directly affected through membrane microviscosity itself. Quick adjustment of η after temperature shift would therefore bring about the constant cell shape observed[1,6]. The change in cell shape consequent to an artificial modification of η in an unsaturated fatty acid auxotroph of *E. coli* K12[43] is consistent with this hypothesis. This response is not surprising also in light of the various effects of the physical state of *E. coli* membrane on synthesis and assembly of different outer membrane proteins[44]. The changes in cellular dimensions obtained during nutritional upshifts[18] could perhaps be explained by lack of a tight homeoviscous adaptation at varying nutritional conditions (due to metabolic regulation[2], for example).

Irregularities in cell division and shape have been reported for *E. coli* following downshifts to temperatures below the minimal required for growth[45]. This phenomenon could perhaps be related to the similar anomalies found in thymine-limited exponentially growing cells[46] through modified activity of the membranal C_{55}-isoprenoid alcohol[47]. In the latter case, it would be influenced by precursor concentrations (and see ref. 48), in the former — by η.

Penicillin-binding-protein 2 (PBP 2) is known to share a major contribution in determining the cylindrical shape of *E. coli* since cells harboring ts mutations in the gene coding for PBP 2 (pbpA) convert to spheres upon transfer to restrictive temperature[49]. It would be interesting to find out whether temperature affects the activity of PBP 2 directly or through the associated instantaneous change in η. (Such an indirect effect, though, would require loss of the ability to restore η by pbpA mutants.)

The stepwise changes observed in synthesis rates of phosphlipids during the cell division cycle of *E. coli* B/r indicate coupling between envelope growth and chromosome replication[50]. It may also be correlated with similar changes and/or qualitative differences in fatty acids incorporated into cytoplasmic membrane, and consequently be reflected by systematic oscillation of η during the cell cycle.

Lipid composition of cytoplasmic membrane, and hence η, could also be involved in helix hand variations of envelope assembly in *Bacillus subtilis*, that have been shown to occur at both ends of the temperature range of growth[51]. In this connection, though, homeoviscous adaptation in membranes of Gram-positive bacteria such as *B. subtilis* should be looked for.

Acknowledgement

This work was partially supported by Ben Gurion University Research Fund. Thanks are extended to Dr Zeev Barak for critically reading the manuscript and to our students for thorough discussions during my course "The Bacterium as a Model System in Biology".

References

1. Schaechter, M., Maaloe, O. and Kjeldgaard, N.O., *J. Gen. Microbiol.*, **19**, 592 (1958).
2. Maaloe, O., in *Biological Regulation and Development*, R.F. Goldberger, Ed., Vol. 1, 487, Plenum Publ. Corp. New York (1979).
3. Anderson, K.B. and v. Meyenburg, K., *J. Bacteriol.*, **144**, 114 (1980).
4. Zaritsky, A., *J. Bacteriol.*, **151**, 485 (1982).
5. Shehata, T.E. and Marr, A.G., *J. Bacteriol.*, **124**, 857 (1975).
6. Trueba, F.J., v. Spronsen, E.A., Traas, J. and Woldringh, C.L., *Arch. Microbiol.*, **131**, 235 (1982).
7. Ecker, R.E. and Kokaisel, G., *J. Bacteriol.*, **98**, 1219 (1969).
8. Helmstetter, C.E., *J. Molec. Biol.*, **24**, 417 (1967).
9. Pritchard, R.H., Barth, P.T. and Collins, J., *Symp. Soc. Gen. Microbiol.*, **19**, 263 (1969).
10. Zaritsky, A. and Zabrovitz, S., *Molec. Gen. Genet.*, **181**, 564 (1981).
11. Pierucci, O., *J. Bacteriol.*, **109**, 848 (1972).
12. Donachie, W.D. and Begg, K.J., *Nature*, **227**, 1220 (1970).
13. Trueba, F.J. and Woldringh, C.L., *J. Bacteriol.*, **142**, 869 (1980).
14. Sargent, M.G., *J. Bacteriol.*, **123**, 7 (1975).
15. Rosenberger, R.F., Grover, N.B., Zaritsky, A and Woldringh, C.L., *Nature*, **271**, 244 (1978).
16. Spratt, B.G., *Sci. Prog., Oxf.*, **65**, 101 (1978).
17. Pierucci, O., *J. Bacteriol.*, **135**, 559 (1978).
18. Zaritsky, A., Woldringh, C.L., Grover, N.B., Naaman, J. and Rosenberger, R.F., *Comments Molec. Cell. Biophys.*, 1, 261 (1982).
19. Mendelson, N.H, *Microbiol. Rev.*, **46**, 341 (1982).
20. Koch, A.L., *Adv. Microbial Physiol.*, **24**, 301 (1983).
21. Harold, R.L. and Harold, F.M., *J. Bacteriol.*, **144**, 1159 (1980).
22. Henning, U., *Ann. Rev. Microbiol.*, **29**, 45 (1975).
23. Shinitsky, M., *Physiol. Rev.*, in press.
24. Cronan, J.E. Jr. and Gelman, E.P., *Bacteriol. Rev.*, **39**, 232 (1975).
25. Leibowitz, P.J. and Schaechter, M., *Interntl. Rev. Cytol.*, **41**, 1 (1975).
26. Harold, F.M., *Ann. Rev. Microbiol.*, **31**, 181 (1977).
27. Macnab, R.M., *Crit. Rev. Biochem.*, **5**, 291 (1978).
28. Chaustova, L.P., Grinius, L.L., Griniuviene, B.B., Jasaitis, A.A., Kadziauskas, J.P. and Kiausinyte, R.J., *Eur. J. Biochem.*, **103**, 249 (1980).
29. Sinensky, M., *Proc. Nat. Acad. Sci. USA*, **71**, 522 (1974).
30. de Mendoza, D. and Cronan, J.E. Jr., *Trends Biochem. Sci.*, **8**, 49 (1983).
31. Khan, S. and Macnab, R.M., *J. Molec. Biol.*, **138**, 599 (1980).
32. Fralick, J.A. and Lark, K.G., *J. Molec. Biol.*, **80**, 459 (1973).
33. Fox, C.F., in *Biochemistry of Cell Walls and Membranes*, C.F. Fox, ed., Vol. 2, Buttersworth, London, p.279 (1975).

34. Eze, M.O. and McElhaney, R.N., *J. Gen. Microbiol.*, 124, 299 (1981).
35. Dennis, W.H. and Yatvin, M.B., *Interntl. J. Radiat. Biol.*, 111, 183 (1981).
36. Maeda, K. and Imae, Y., *Proc. Natl. Acad. Sci. USA*, 76, 91 (1979).
37. Tsien, H., Panos, C., Shockman, G.D. and Higgins, M.L., *J. Gen. Microbiol.*, 121, 105 (1980).
38. Garwin, J.L. and Cronan, J.E. Jr., *J. Bacteriol.*, 141, 1457 (1980).
39. Okuyama, H., Saitoh, M. and Hiramatsu, R., *J. Biol. Chem.*, 257, 4812 (1982).
40. Nunn, W.D., Giffin, K., Clark, D. and Cronan, J.E. Jr., *J. Bacteriol.*, 154, 554 (1983).
41. Silvius, J.R. and McElhaney, R.N., *Proc. Natl. Acad. Sci. USA*, 77, 1255 (1980).
42. Eaton, L.C., Erdos, G.W., Vreeland, N.L. and Ingram, L.O., *J. Bacteriol.*, 146, 1151 (1981).
43. Kuriki, Y., *J. Bacteriol.*, 147, 1121 (1981).
44. DiRienzo, J.M. and Inouye, M., *Cell*, 17, 155 (1979).
45. Shaw, M.K., *J. Bacteriol.*, 95, 221 (1968).
46. Zaritsky, A., *FEMS Microbiol. Lett.*, 2, 65 (1977).
47. Ghuysen, J.-M. and Shockman, G.D., in *Bacterial Membranes and Walls*, L. Leive (ed.), Marcel Dekker, New York, p. 37 (1973).
48. Zaritsky, A. and Woldringh, C.L., *J. Bacteriol.*, 135, 581 (1978).
49. Spratt, B.G., Boyd, A. and Stoker, N., *J. Bacteriol.*, 143, 569 (1980).
50. Pierucci, O., *J. Bacteriol.*, 138, 453 (1979).
51. Mendelson, N.H. and Karamata, D., *J. Bacteriol.*, 151, 450 (1982).

Note Added in Proof

The recent finding (A. Zaritsky, A.H. Parola, H. Masalha and M. Abdah, to be published elsewhere), that η (estimated by fluorescence polarization) is inversely correlated to cell diameter in *E. coli* B/r ($r = 0.95$), is consistent with the hypothesis postulated here.