JOURNAL OF BACTERIOLOGY, Aug. 1978, p. 307-310
0021-9193/78/0135-0307$02.00/0

Vol. 135, No. 2
Printed in U.S.A.

Cell Surface Growth in *Escherichia coli*: Distribution of Matrix Protein

K. J. BEGG

Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland

Received for publication 27 February 1978

Autoradiography of cell envelope "ghosts" from *Escherichia coli* was used to demonstrate that newly synthesized molecules of "matrix" protein are inserted at random locations over the entire surface of the outer membrane and that, once inserted, these molecules are not thereafter conserved in any fixed spatial location.

The cell envelope of *Escherichia coli* is a complex structure which is basically composed of three distinct layers: (i) the cytoplasmic membrane consisting of proteins and phospholipids, (ii) the peptidoglycan layer, and (iii) an outer membrane containing proteins, lipopolysaccharide, and phospholipids (15). Many growth studies have been carried out on the cell envelope; nevertheless, contradictory conclusions have been drawn from these studies. The evidence in this field can be divided into two broadly based contradictory groups: (i) the evidence that supports the view that cell wall growth is an intercalary process which allows components to diffuse into the wall at random (3, 8, 12, 18), and (ii) the evidence which forms the tenet that envelope growth involves localized insertion of new material into a few "growth zones" per cell (4, 5, 7, 11).

Rosenbusch (16) has described a technique which produces rod-shaped cell "ghosts" containing the peptidoglycan layer of the cell envelope surrounded by one major outer membrane protein (matrix protein). This indicates that such ghosts could be used to determine the conservation or dispersion of matrix protein during growth of the cell. Lugtenberg et al. (13) have shown that this matrix protein corresponds with their protein b when isolated from B strains of *E. coli* but consists of two proteins, b and c, when isolated from K-12 strains. B strains of *E. coli* do not contain protein c, and the original report of matrix protein was from a B-derived strain of *E. coli*.

This report describes results obtained when cells of *E. coli* K-12 and B/r were pulse-labeled with [^{3}H]histidine and subsequently allowed to continue growing in the presence of low concentrations of penicillin to inhibit division (17). An experiment involving pulse-labeling of preformed filaments to determine any localization at the time of incorporation is also described.

MATERIALS AND METHODS

Strains and growth conditions. The strains used were *E. coli* KM7 K-12 Thr$^-$ Leu$^-$ (14) and *E. coli* B/r ATCC 12407 (9).

Cells were grown in minimal medium (M9 salts [1]) plus 0.2% glucose plus required amino acids at 20 μg/ml in a shaking water bath at 37°C. Doubling times were 60 min for KM7 and 41 min for B/r.

Penicillin was used at concentrations of 60 U/ml for KM7 and 30 U/ml for B/r.

Labeling with [^{3}H]histidine. Radioactive histidine was chosen as the label because it is not inserted into the lipoprotein described by Braun and Rehn (6) which is covalently bound to the mucopeptide.

Growing cells were concentrated on a HAWP filter membrane (Millipore Corp.) (pore size, 0.45 μm) and resuspended in a small volume of growth medium containing [^{3}H]histidine (47 Ci/mmol) at 20 μCi/ml (0.066 μg/ml) for the required pulse time. The pulse was stopped by washing the cells on a Millipore membrane filter with phosphate-buffered saline containing nonradioactive histidine at 100 μg/ml.

Ghost preparation. Washed cells were suspended in extraction buffer containing 2% sodium dodecyl sulfate in 10 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.3, containing 10% glycerol and 0.7 M 2-mercaptoethanol and heated to 60°C for 30 min. The resulting ghosts were washed three times with distilled water. Ghosts prepared by this technique were analyzed on polyacrylamide gels, and it was found, as other workers have found (13, 16), that matrix protein was the only protein present.

Sacculi controls were prepared by heating a portion of each cell sample to 100°C for 30 min.

Autoradiography. Ghosts were spread on clean glass microscope slides, dried, and overlaid with a thin layer of Ilford L-4 nuclear emulsion. Slides were kept in light-proof boxes and subsequently developed using Kodak D19b developer and Kodak F24 fixer. Autoradiograms were stained for 30 min with 1% methyl violet.

Collection of data. Autoradiograms were examined in a Zeiss Photo-Microscope, and photographs were taken by using Pan-F film (Ilford). Prints were made, and the positions of the grains along each filament were measured. Grain positions were ex-

pressed as fractional distances from the nearer of the two cell poles.

RESULTS

Figures 1 and 2 show the grain distribution obtained when KM7 cells were pulsed with [^{3}H]histidine for 10 min, chased with unlabeled histidine, and allowed to continue growing for two mass doublings in the presence of penicillin (60 U/ml). Autoradiograms of ghosts were prepared and grain positions were measured as described above. The grains appear to be randomly distributed over the entire surface of the ghosts.

Figure 3 shows the grain distribution obtained when B/r cells were pulsed with [^{3}H]histidine for 5 min and treated as above. Again the grains appear to be distributed at random over the surface of the ghosts.

Figure 4 shows the grain distribution given when filaments of KM7 cells, formed by treating growing cells with penicillin (60 U/ml) for two mass doublings, were pulsed with [^{3}H]histidine for 1 min immediately before ghost preparation. The grains are randomly located over the surface of the ghosts.

Figure 5 shows a control preparation of sacculi prepared from KM7 cells by heating Rosenbusch ghosts to 100°C for 30 min to remove matrix protein. The peptidoglycan sacculi can clearly be seen to be unlabeled.

Figure 6 shows a control preparation obtained by mixing equal proportions (mass) of penicillin-induced filaments labeled with [^{3}H]histidine and unlabeled normal-sized cells of *E. coli* KM7 before ghost preparation. The label is clearly confined to the filaments, indicating that matrix protein does not dissociate and reassociate during ghost preparation.

DISCUSSION

Matrix protein (b and c) represents approximately 35% of the total outer membrane protein

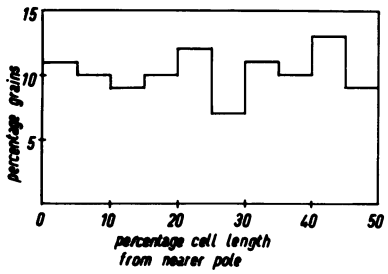

FIG. 1. *Distribution of grains (304 grains measured) on the surface of cells ghosts of E. coli KM7. Cells were pulse-labeled with [^{3}H]histidine for 10 min and subsequently grown for two mass doublings in the presence of 60 U of penicillin per ml. The distance of each grain from the nearer pole was measured and expressed as a fraction of the total cell length.*

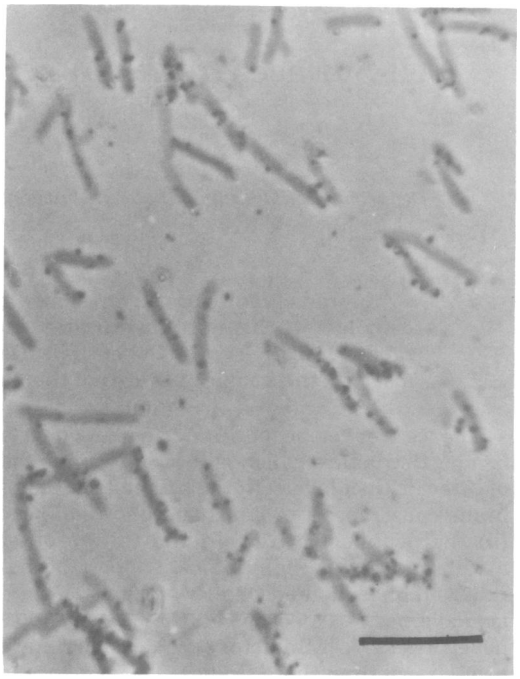

FIG. 2. *Photomicrograph of ghosts described in Fig. 1. Bar is 10 μm.*

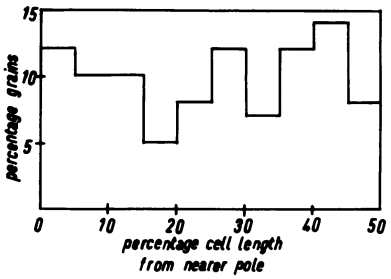

FIG. 3. *Distribution of grains (102 grains measured) on the surface of cell ghosts of E. coli B/r. Cells were pulse-labeled with [^{3}H]histidine for 5 min and subsequently grown for two mass doublings in the presence of 30 U of penicillin per ml. Data expressed as in Fig. 1.*

of the cell envelope in *E. coli* (13) and appears to be arranged in a lattice structure of hexagonal symmetry (16). It has been shown that matrix protein is closely associated with lipopolysaccharide, and it has been suggested that matrix protein, together with lipopolysaccharide, forms hydrophilic pores in the outer membrane (13).

The experiments described in this communication suggest that matrix protein is not spatially conserved in the cell envelope and tends to move freely and at random over the surface. In addition, it would appear from the experiments with preformed filaments that it is not even localized

for short periods at the time of incorporation. Although matrix protein does not appear to dissociate and reassociate during ghost preparation (Fig. 6) and has been shown to maintain a regular pattern when isolated in conjunction with mucopeptide (16), the possibility still exists that the random distribution of label observed in this communication is a result of procedural artifacts which cause relocation of matrix protein within individual cells, and unfortunately the technology to prove or disprove this does not as yet exist.

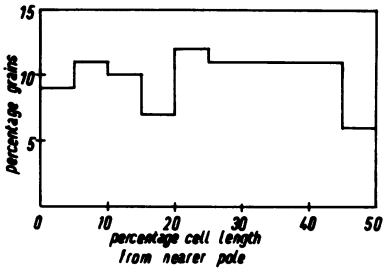

FIG. 4. *Distribution of grains (364 grains measured) on the surface of cell ghosts of E. coli KM7. Cells were grown for two mass doublings in the presence of 60 U of penicillin per ml before being pulse-labeled with [^{3}H]histidine for 1 min immediately before ghost preparation.*

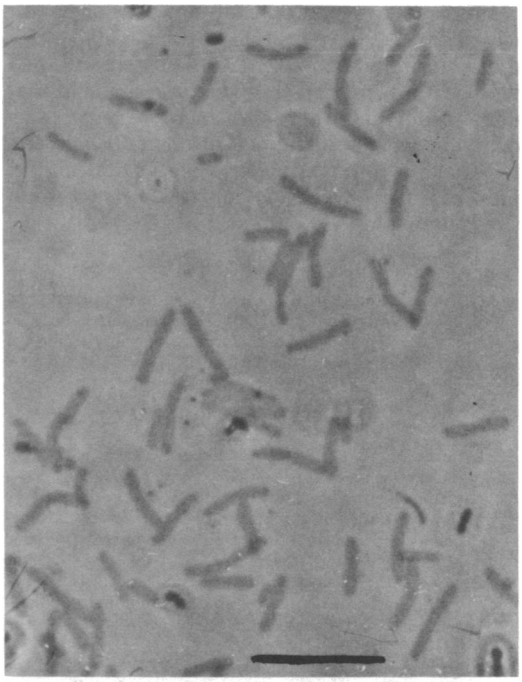

FIG. 5. *Photomicrograph of sacculi prepared from [^{3}H]histidine-labeled ghosts of E. coli KM7 cells by heating to 100°C in sodium dodecyl sulfate to remove matrix protein. Bar is 10 μm.*

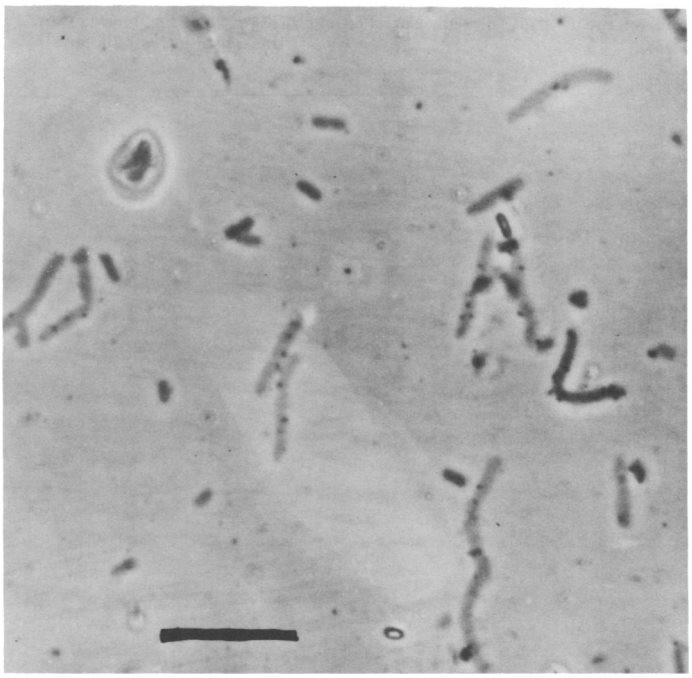

FIG. 6. *Photomicrograph of ghosts prepared from a mixture of [^{3}H]histidine-labeled filaments and unlabeled normal-sized cells of E. coli KM7 mixed in equal proportions (mass) before ghost preparation and autoradiography.*

It has been shown that mutant cells of *E. coli* which lack matrix protein can exist and grow quite normally without it (2, 10). This, combined with the information that the proportion of matrix protein and other outer membrane proteins can vary with cultural conditions (13), and the results shown in this paper, suggest that matrix protein is not part of any conserved structure in the cell envelope.

ACKNOWLEDGMENTS

My thanks are due to W. D. Donachie, J. F. Lutkenhaus, and H. Wolf-Watz for much helpful discussion. I am also grateful to L. Richardson for performing polyacrylamide gel analysis, and to the Institute of Medical Laboratory Sciences for providing financial assistance to this work.

LITERATURE CITED

1. **Adams, M. H.** 1959. Bacteriophages, 1st ed., p. 466. Interscience, New York.
2. **Alphen, W., B. Lugtenberg, and W. Berendsen.** 1976. Heptose deficient mutants of *Escherichia coli* K12 deficient in up to three major outer membrane proteins. Mol. Gen. Genet. **147**:263–269.
3. **Beachey, E. H., and R. M. Cole.** 1966. Cell wall replication in *Escherichia coli* studied by immunofluorescent and immunoelectron microscopy. J. Bacteriol. **92**:1245–1251.
4. **Begg, K. J., and W. D. Donachie.** 1977. Growth of the *Escherichia coli* cell surface. J. Bacteriol. **129**:1524–1536.
5. **Bisset, K. A., and P. E. Pease.** 1957. The distribution of flagella in dividing bacteria. J. Gen. Microbiol. **16**:382–384.
6. **Braun, V., and K. Rehn.** 1969. Chemical characterization, spatial distribution and function of a lipoprotein (murein lipoprotein) of the *E. coli* cell wall. Eur. J. Biochem. **10**:426–438.
7. **Donachie, W. D., and K. J. Begg.** 1970. Growth of the bacterial cell. Nature (London) **227**:1220–1224.
8. **Green, E. W., and M. Schaechter.** 1972. The mode of segregation of the bacterial cell membrane. Proc. Natl. Acad. Sci. U.S.A. **69**:2312–2316.
9. **Helmstetter, C. E.** 1967. Rate of DNA synthesis during the division cycle of *E. coli* B/r. J. Mol. Biol. **24**:417–428.
10. **Henning, U., and I. Haller.** 1975. Mutants of *Escherichia coli* K12 lacking all "major" proteins of the outer cell envelope membrane. FEBS Lett. **55**:161–164.
11. **Leal, J., and H. Marcovich.** 1971. Segregation of phage receptors T6 during cell division in *Escherichia coli* K12. Ann. Inst. Pasteur Paris **120**:467–474.
12. **Lin, E. C. C., Y. Hirota, and F. Jacob.** 1971. On the process of cellular division in *Escherichia coli*. J. Bacteriol. **108**:375–385.
13. **Lugtenberg, B., R. Peters, H. Bernheimer, and W. Berendsen.** 1976. Influence of cultural conditions and mutations on the composition of the outer membrane proteins of *Escherichia coli*. Mol. Gen. Genet. **147**:251–262.
14. **Matzura, H., S. Molin, and O. Maaløe.** 1971. Sequential biosynthesis of the β and β' subunits of the DNA dependent RNA polymerase from *Escherichia coli*. J. Mol. Biol. **59**:17–25.
15. **Osborn, M. J., J. E. Gander, E. Parisi, and J. Carson.** 1972. Mechanism of assembly of the outer membrane of *Salmonella typhimurium*. J. Biol. Chem. **247**:3962–3972.
16. **Rosenbusch, J. P.** 1974. Characterization of the major envelope protein from *Escherichia coli*. Regular arrangement on the peptidoglycan and unusual dodecyl sulphate binding. J. Biol. Chem. **249**:8019–8029.
17. **Starka, J.** 1971. Formation et stabilite osmotique des formes filamenteuses d'*Escherichia coli* induites par la penicilline. Ann. Inst. Pasteur Paris **121**:149–159.
18. **Tsukagoshi, N., P. Fielding, and C. F. Fox.** 1971. Membrane assembly in *Escherichia coli*. Segregation of preformed and newly formed membrane into daughter cells. Biochem. Biophys. Res. Commun. **44**:497–502.