JOURNAL OF BACTERIOLOGY, Aug. 1984, p. 440–447
0021-9193/84/080440-08$02.00/0

Vol. 159, No. 2

Topography of the Insertion of LamB Protein into the Outer Membrane of *Escherichia coli* Wild-Type and *lac-lamB* Cells

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Received 10 January 1984/Accepted 13 April 1984

The appearance of newly induced LamB protein at the cell surface of *Escherichia coli* was followed topographically by immuno-electron microscopy. LamB protein was induced in *E. coli* wild-type or *lac-lamB* cells for a short period of time (4 to 6 min), such that the overall level of LamB protein in induced cells was at least twofold higher than that in uninduced cells. Antibodies bound to LamB protein exposed at the cell surface were labeled with a protein A-gold probe, and the probe distribution in briefly induced cells was compared to that in uninduced cells. Analysis of large numbers of cells showed that newly inserted LamB protein appeared homogeneously over the entire cell surface, both in wild-type cells and in *lac-lamB* cells. A peak of insertion which was observed at the division site of the cell was also observed in the absence of induction and in control experiments in which a nonspecific probe was used. It is concluded therefore that insertion of LamB protein into the cell envelope of *E. coli* occurs at multiple sites over the entire cell surface. The average amount of LamB protein which appeared at the cell surface after induction was determined for various cell size classes. It was found that cells of various size classes all synthesized LamB protein after induction, indicating that synthesis of the protein was not restricted to cells in a particular stage of the cell cycle. However, the rate of LamB synthesis was found to vary during the cell cycle: this rate was constant regardless of cell size in nondividing cells, whereas it increased in dividing cells. It is concluded that the accumulation of newly induced LamB protein follows a linear pattern.

During the division cycle, the cell envelope of *Escherichia coli* grows and invaginates as the cell doubles in length and divides into two daughter cells. Since the envelope consists of three layers, i.e., the inner membrane, the peptidoglycan layer, and the outer membrane, this process is a complicated one, requiring a high degree of coordination. Surface extension can be studied at the level of overall cell growth (mass, volume, or surface) or at the level of incorporation of individual envelope components (11). This paper deals with the second approach.

The general problem of where outer membrane components are inserted has been addressed before. Thus, newly synthesized lipopolysaccharide was found to be inserted at sites distributed all over the cell surface (10). Similarly, studies on outer membrane porin suggest that the outer membrane grows predominantly by diffuse intercalation of new components (3, 16). In contrast, however, several phage receptors (generally protein-lipopolysaccharide complexes) have been reported to be inserted either in the vicinity of the septum as it is formed (phage λ receptor [14] and phage T6 receptor [7]) or at the cell poles (phage T6 receptor [4]).

We therefore decided to reinvestigate the insertion pattern of the LamB protein, which is both a phage λ receptor component and an outer membrane component. To specifically follow the insertion of the LamB protein, we used immuno-electron microscopy. To maximize the discrimination between diffuse and zonal insertion, we used conditions resulting in a high sensitivity detection of the newly induced and inserted protein (18). These experiments were carried out with wild-type cells by induction with maltose and cyclic AMP. They were also carried out with a *lac-lamB* fusion strain, which carries the *lamB* gene under *lac* promoter control (9) and provides us with the best LamB induction system available (18).

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The distribution pattern of newly inserted LamB protein in briefly induced cell populations, as revealed by immuno-electron microscopy, was analyzed quantitatively and compared with the pattern found in uninduced cells. All cells in a population were found to synthesize and insert LamB protein after a brief induction. Insertion of LamB protein into the cell envelope occurred at multiple sites without particular preference for the cell septum.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* K-12 pop5217 [genotype: HfrC Δ(*gal-att*$^{\lambda}$*-bio*) *rpoB-500 metA*] is a Rif^{r} *metA* derivative of strain RW 592 (pop5216; 8). It was grown at 37°C in minimal salts medium (19) with 0.5% (wt/vol) glucose as a carbon source and supplemented with thiamine (2 μg/ml) and biotin (20 ng/ml). In addition, Casamino Acids (0.2% [wt/vol]) were added. LamB protein was induced by adding 4 mM cyclic AMP (Sigma Chemical Co., St. Louis, Mo.) and 0.4% (wt/vol) maltose to an exponentially growing culture, at a cell density of 0.2 to 0.25 mg (dry weight) of cell per ml (19).

We also used an operon fusion strain in which the *lamB* gene is expressed under *lac* promoter control. This strain was derived from wild-type strain pop5217 by, first, dele tion of the wild-type *malB* region and, second, introduction of the *lac-lamB* fusion into the bacterial chromosome. The operon fusion strain is called pop5228 [genotype: HfrC Δ(*gal-att*$^{\lambda}$*-bio*) *rpoB-500* Δ*malB107 lacL8UV5p lacZ'*::*mal'K lamB*::*lac'Z*]; details of its construction have been described previously (18). Growth was performed as described for the wild-type strain, except that no supplementary amino acids were added and induction was performed with 1 mM isopropyl-β-D-thiogalactopyranoside (Calbiochem-Behring, La Jolla, Calif.).

Preparation of protein A-colloidal gold. Gold particles with an average diameter of ca. 16 nm were prepared by reducing

chloroauric acid ($HAuCl_4$; Drijfhout & Zn's, Amsterdam, The Netherlands) with sodium citrate by the procedure described by Slot and Geuze (15). After protein A had been complexed to the colloidal gold, the particles were collected by centrifugation (45 min at 15,000 × *g*) and washed once with phosphate-buffered saline. They were suspended in a small volume of phosphate-buffered saline (usually gold particles derived from 30 ml of gold solution were suspended in 1 ml) and stored in 50% (vol/vol) glycerol at −15 to −20°C. Before use, the probe was centrifuged for 5 min in an Eppendorf centrifuge to remove aggregates that might have formed during storage.

Visualization of LamB protein at the cell surface. From an exponentially growing culture, samples were withdrawn 1 min before and a few minutes (see above) after induction of LamB protein. LamB insertion was stopped by direct fixation of the cells with OsO_4, as described in the accompanying paper (18). Since the presence of Casamino Acids during fixation rendered the wild-type cells very dark and thus unsuitable for immuno-electron microscopy, this medium was removed as soon as possible by collecting the cells and resuspending them in fresh fixation medium. LamB protein was probed with anti-LamB trimer antibodies (18), and cell surface-bound antibodies were visualized by using a protein A-gold probe. A saturating amount of the gold probe was added to the cells, in a volume sufficient to decrease the glycerol concentration to 1% or less. Incubation was performed for 15 min at room temperature. Cells were pelleted and carefully washed twice with phosphate-buffered saline. They were prepared for electron microscopy by the so-called agar-filtration technique (17): cells were filtered onto a Parlodion holey film and subsequently deposited on Formvar-carbon-coated grids. They were examined in a Philips EM300 electron microscope at 60 kV.

For quantitation of the protein A-gold distribution, electron micrographs were taken at a magnification of 2,950. Calibration was checked with a cross-grating replica with 2,160 lines per mm (Ladd Research Industries, Burlington, Vt.). Prints were made at a final magnification of 29,500. These were placed against the surface of a data tablet digitizer (Summagraphics, Fairfield, Conn.). Cell diameter, cell length, and the position of grains (i.e., gold particles) on the cell were registered. The data were handled with a Hewlett-Packard HP 9825A calculator (Hewlett-Packard Co., Loveland, Colo.). With respect to nondividing cells, no criterion was applied to distinguish between arbitrary left and right; for dividing cells, the longest cell half was placed to the left.

RESULTS

Choice of cells to be analyzed. To localize the insertion sites of newly induced LamB protein by immuno-electron microscopy, it was important to reduce the level of "old" LamB protein, i.e., LamB protein already present in uninduced cells, to a minimum. In *E. coli lac-lamB* cells, LamB background was found to be extremely low; in wild-type cells, LamB background was generally higher, but it could be reduced to a minimum level (amounting to two- to threefold that in *lac-lamB* cells) by adding Casamino Acids to the growth medium (18).

For the topographical analysis of LamB insertion, induction should be long enough to allow the synthesis and insertion of measurable amounts of LamB protein but short enough to prevent redistribution of newly inserted LamB protein all over the cell surface. We therefore analyzed induced cells which had an average LamB content at least twofold that of uninduced cells; these levels were checked by quantitating the amount of cell surface exposed LamB protein with highly specific anti-LamB trimer antiserum and radioiodinated protein A (18). According to this strategy, we focused our analysis on *E. coli lac-lamB* cells induced for 4 min (average LamB content, 2.1-fold that in uninduced cells) and on *E. coli* wild-type cells induced for 6 min (average LamB content, 2.8-fold that in uninduced cells).

To ensure that the newly inserted protein did not leave its initial insertion site, cells were fixed with OsO_4 immediately after sampling. Fixation with OsO_4 was found to stop LamB insertion effectively and not to prevent subsequent immunological detection of cell surface determinants (18).

Topography of LamB insertion. Figures 1 and 2 show electron micrographs obtained after the labeling of LamB protein at the surface of fixed cells with specific antibodies and 16 nm of protein A-gold particles. By visual inspection of a number of such electron microscopic preparations, the following general observations were made. First, the level of LamB protein detected in *E. coli* wild-type cells was higher than that in *lac-lamB* cells. This difference was observed for both uninduced and induced cell populations (Fig. 1 and 2, panels a and b), and it is consistent with our previous measurements of LamB protein levels, using ^{125}I-protein A (18). Second, the degree of labeling was not uniform for all cells of a population; in particular, for the wild-type cell population (Fig. 1) we, like others (14), observed a high degree of heterogeneity. Third, the overall distribution pattern of LamB protein appeared to be random: gold particles (grains) were found dispersed over the entire cell surface, both in wild-type and in *lac-lamB* cells (Fig. 1 and 2). It is stressed that the observed gold particles are located on either side of the cell, as checked by stereo photography.

The position of the grains on the cells was scored, both for uninduced and for induced cells (Fig. 3). Nondividing and three classes of dividing cells were analyzed separately (Fig. 3d and h, a to c, and e to g, respectively). Our results show that labeling of LamB protein occurs all over the cell surface, both in wild-type cells and in *lac-lamB* cells.

Dividing cells of an induced cell population showed a peak of labeling at the division site; since a similar peak was, however, also observed in uninduced cells (Fig. 3, cf. upper and lower curves), it is clear that this peak cannot be due to preferential insertion of newly induced LamB protein at the division site. Instead, this septal enrichment of label might be either aspecific (i.e., caused by drying artefacts or local sticking of immunoglobulin G's in the division furrow or both) or specific (i.e., representing a high local concentration of LamB protein). To discriminate between these possibilities, we analyzed the distribution pattern of preimmune antibodies over the surface of uninduced cells (in this case, *lac-lamB* cells; Fig. 4). Comparison with the distribution pattern of LamB-specific antibodies clearly showed that the central peak observed in dividing cells is an aspecific one, not related to septal enrichment of LamB protein.

When nondividing cells were subdivided into several size classes, a LamB distribution pattern similar to that in Fig. 3 (d and h) was detected for all size classes (data not shown). In particular, we did not observe a polar enrichment of LamB protein in newborn cells, as reported previously (14).

The LamB distribution patterns shown in Fig. 3 all have a somewhat oval profile, which is related to the shape of the cells. This was shown by determining the distribution pattern in a strip along the length axis of wild-type cells. The flatness of these distribution patterns indicates that the oval profiles of Fig. 3 reflect cell shape.

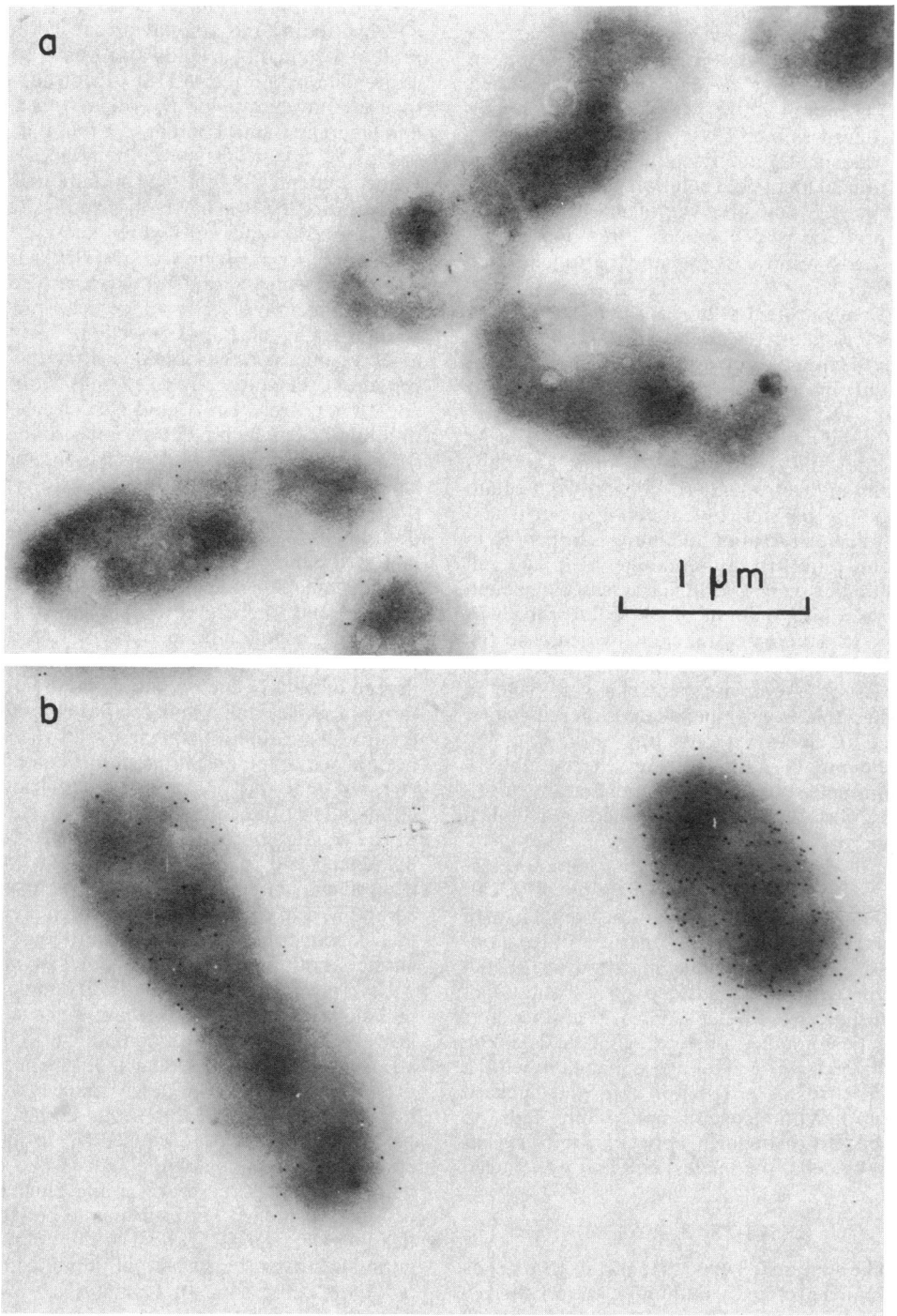

FIG. 1. Visualization of LamB protein at the surface of wild-type cells. Samples of 0.2 mg of OsO_4-fixed cells were labeled with 1.5 μl of anti-LamB trimer antiserum and 10 μl of protein A-gold. *E. coli* pop5217 cells were sampled 1 min before (a) or 6 min after (b) induction of LamB protein.

Heterogeneity of the cell population. Since LamB protein was not homogeneously distributed over various cells of a population, it was important to determine whether different subpopulations of cells showed differences with respect to the LamB insertion pattern. The heterogeneity of a cell population is illustrated in Fig. 5, both for wild-type cells (a and b) and for *lac-lamB* cells (c and d). A comparison of the normalized distributions (number of cells versus the number of gold particles per cell) showed that the heterogeneity was slightly greater in induced wild-type cells than in induced *lac-lamB* cells. Two features probably account for this difference. First, there is a preexisting heterogeneity of the uninduced wild-type cell population which is more extensive than that of the uninduced *lac-lamB* cells (Fig. 5, cf. a and c). Second, wild-type cells synthesize more LamB protein than do *lac-lamB* cells in the induction times used (Fig. 5, b and d): after induction, the average LamB content in wild-type cells induced for 6 min increased 2.8-fold with respect to the

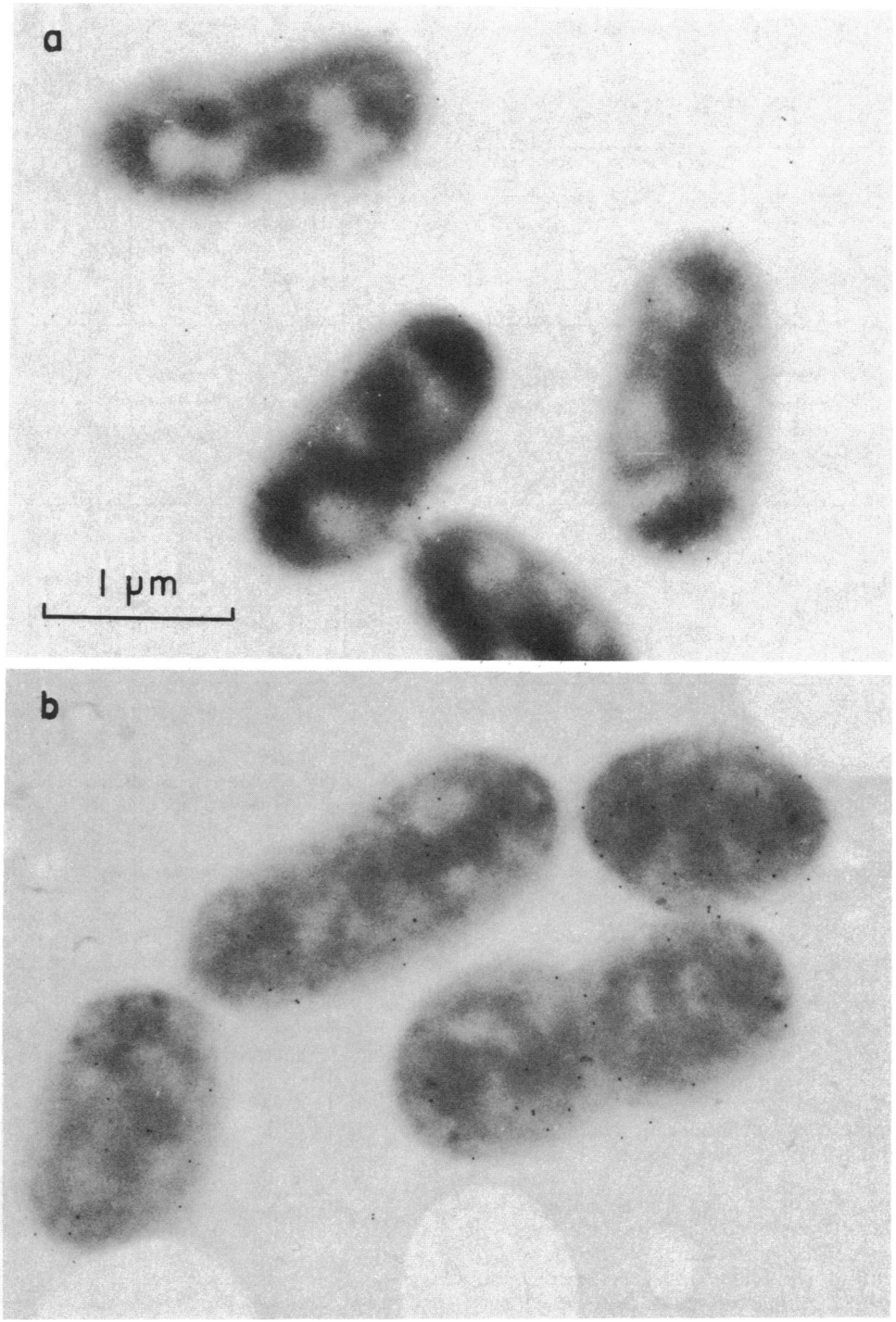

FIG. 2. Visualization of LamB protein at the surface of *lac-lamB* cells. E. coli pop5228 cells were sampled 1 min before (a) or 4 min after (b) induction of LamB protein, and cells were prepared for immunoelectron microscopy as described in the legend to Fig. 1.

LamB background level, whereas the LamB content in *lac-lamB* cells induced for 4 min was only 2.1-fold higher than that in uninduced cells.

Subpopulations of induced wild-type cells having different amounts of LamB protein were selected, and the topography of LamB insertion of these subpopulations was determined. It was found that cells of all subpopulations tested showed a diffuse insertion pattern, analogous to the overall patterns in Fig. 3.

In conclusion, our results with respect to the overall pattern of LamB insertion show that after induction a random pattern of LamB insertion is superimposed on the preexisting one; this result applies to all cell size classes analyzed. Possible interpretations will be discussed below.

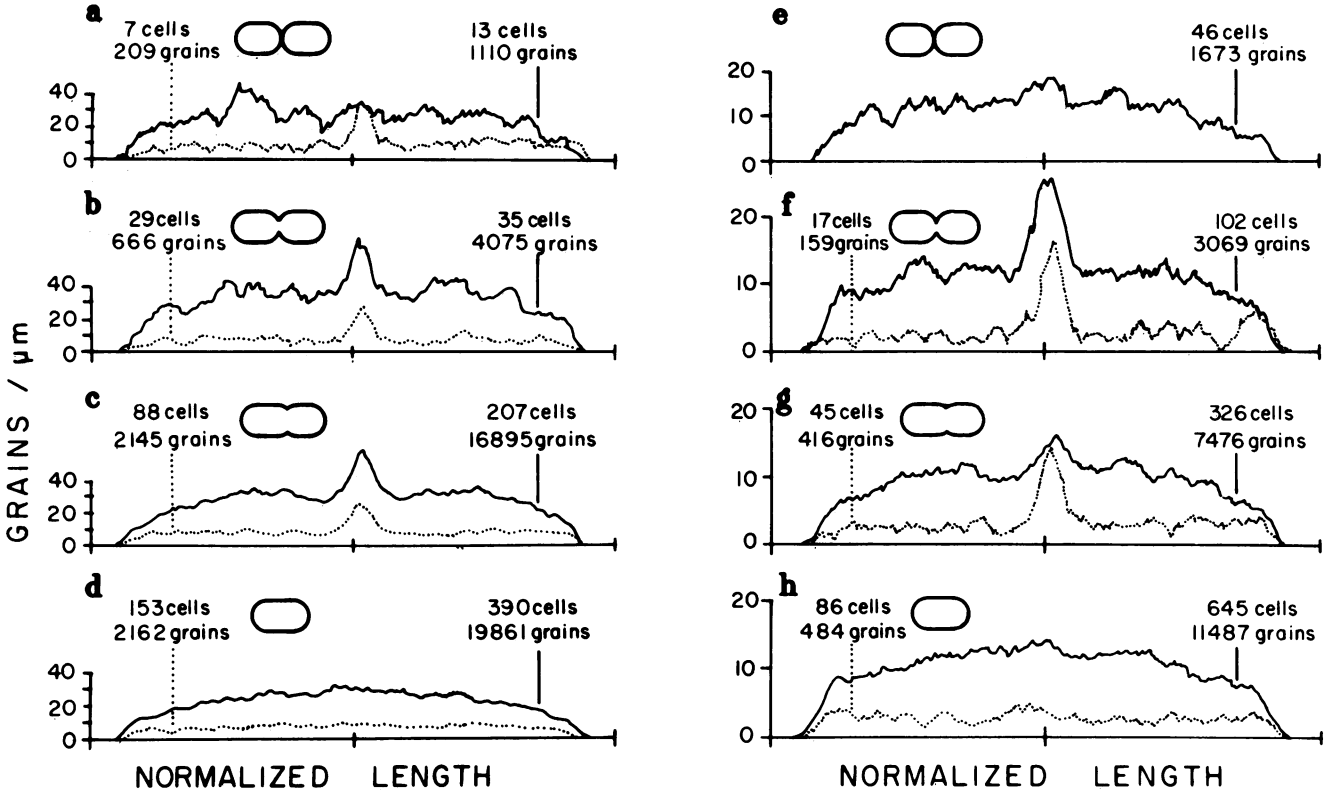

FIG. 3. Topography of LamB insertion in wild-type (a to d) and *lac-lamB* (e to h) cells. The grain distribution of uninduced cells (lower curves) and of cells induced for 6 min (wild type; a to d, upper curves) or for 4 min (*lac-lamB*; e to h, upper curves) was analyzed. Cells were classified into four groups: nondividing cells (d and h), cells with a just-visible constriction (c and g), cells which have progressed halfway towards division (b and f), and cells which have nearly separated (a and e). The grain density (number of grains per µm of cell length) is plotted as the running mean over 4% of the total cell length. Dividing cells were placed with their longest cell halves to the left; as a consequence, the site of division is shifted slightly to the right with respect to the center of the profile (indicated on the horizontal axis). For almost-divided *lac-lamB* cells (panel e), insufficient data of uninduced cells were available to make a comparable plot.

Rate of LamB synthesis. After induction, the number of grains per cell increased to higher values (Fig. 5). This increase was largest for dividing cells, as became clear when the average number of grains per cell was plotted against cell length. Since the number of grains per cell represents the number of LamB molecules or trimers synthesized and inserted during a short induction period, the average number of grains per cell indicates the rate of LamB synthesis in a cell.

By using this approach, the rate of LamB synthesis was found to be constant and independent of cell length in induced nondividing cells; as a result, the surface density of newly induced LamB protein (defined here as the number of grains per µm of cell length) decreased as cells grew longer (Fig. 6, ▲ and ■). By contrast, in dividing cells, the rate of newly induced LamB synthesis increased sufficiently with length so that the surface density of the protein also increased (Fig. 6, △ and □). It was determined that the central peak found in dividing cells (Fig. 3) hardly contributed to this phenomenon: when the central area was taken out in induced *lac-lamB* cells, a grain density pattern similar to that in Fig. 6 was still observed. This indicates that the grain density in dividing cells also increased with cell length outside the central area.

Thus, the rate of newly induced LamB synthesis appears to follow a linear pattern (5): it is constant during the major part of the cell cycle and increases around the initiation of cell division. The increase in LamB production seems to precede initiation of cell division in wild-type cells, whereas it closely follows this event in *lac-lamB* cells.

It is stressed that the above effect is only observed for the rate of newly induced LamB synthesis; considering the

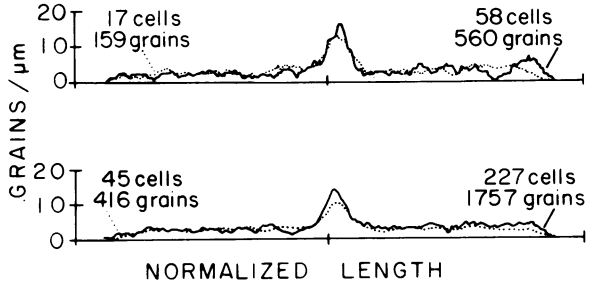

FIG. 4. Characterization of the central peak detected in dividing cells. Uninduced *lac-lamB* cells were labeled with either preimmune serum (i.e., serum collected from the rabbit used for LamB immunization, before immunization; solid lines) or with LamB-specific antiserum (dotted lines) and, subsequently, with protein A-gold. The upper panel represents the grain distribution in cells progressed halfway towards division (comparable to Fig. 3b), and the lower panel shows the distribution profile of cells which had just started constriction (comparable to Fig. 3c). Details of the plots are as in Fig. 3.

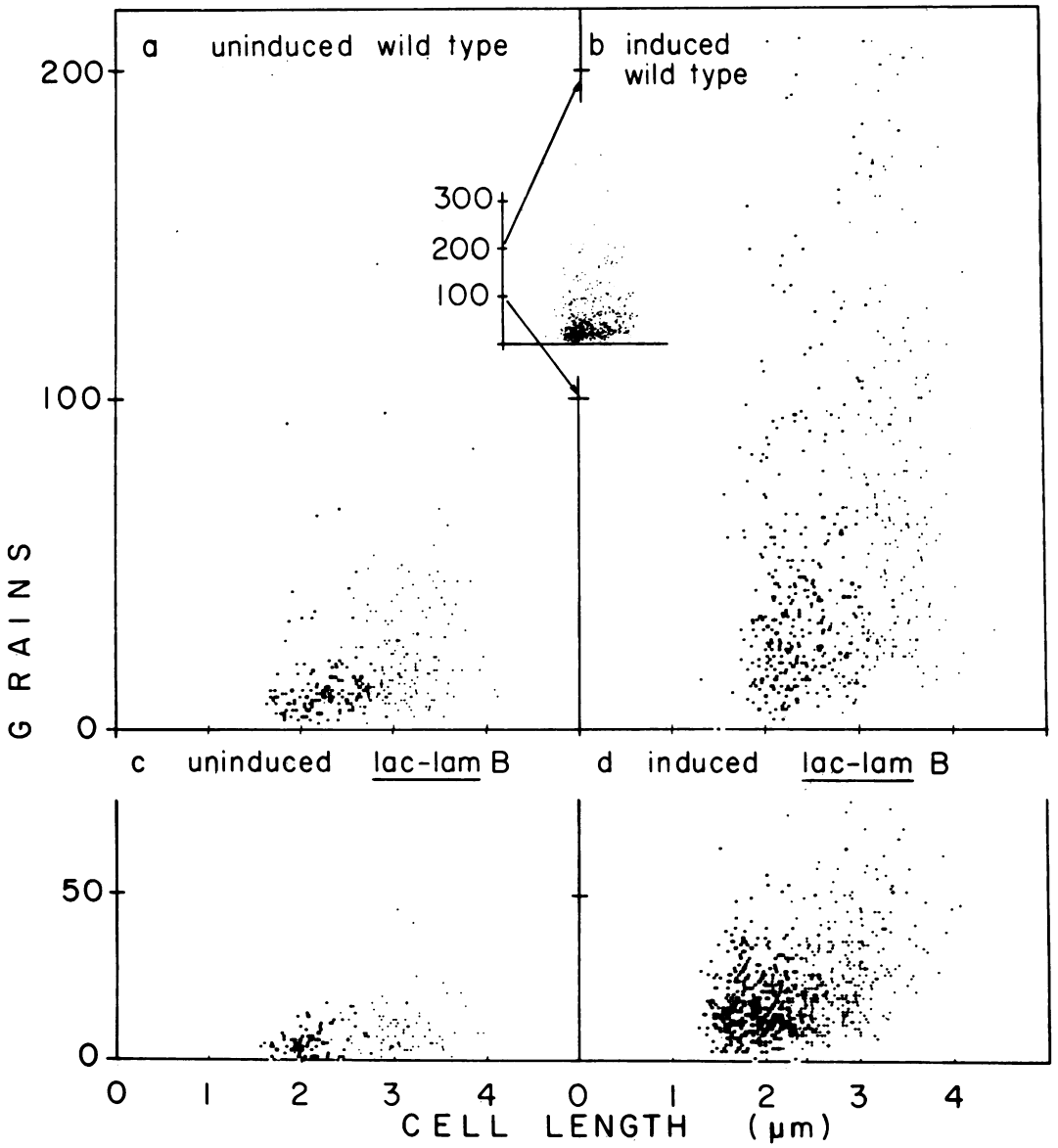

FIG. 5. Heterogeneity of a cell population with respect to the LamB contents of individual cells. Uninduced and induced cells of a wild-type (a and b) or of a *lac-lamB* (c and d) cell population were probed for the amount of cell surface exposed LamB protein, using specific antibodies and protein A-gold particles. Induction was performed for 6 min (wild-type cells [b]) or for 4 min (*lac-lamB* cells [d]); uninduced cells were sampled from the same culture 1 min before induction was started. The number of grains per bacterium is plotted against cell length. Due to extreme scattering, panel b does not comprise the cells with the largest LamB contents; the complete distribution of induced wild-type cells is shown in the inset of this panel. Nondividing (*) and dividing (+) bacteria are indicated separately.

continuous accumulation of LamB protein in uninduced wild-type cells (Fig. 6, ● and ○) or the continuous accumulation of OmpF protein in *lac-lamB* cells (Fig. 7), no significant variations in outer membrane protein density could be observed, either by us or by others (1, 5).

DISCUSSION

Topography of LamB insertion. LamB protein was found to be distributed homogeneously over the entire cell surface as soon as it could be detected; this was found for both LamB protein produced under wild-type control and for LamB protein produced under *lac* promoter control.

Our results are therefore at variance with those of Ryter et al. (14), who reported that integration of phage λ receptor (i.e., LamB protein probably complexed with lipopolysaccharide; 20) occurs preferentially in the vicinity of the constriction site. Although we have also detected a central peak of label in dividing cells, this peak was found not to be related to LamB protein.

There are several methodological differences in the experiments of Ryter et al. (14) and those described here, some of which might help explain these seemingly incompatible results. One of these is that Ryter et al. induced for 9 min, compared with the 4 to 6 min used in this study. However, since a zonal insertion peak of LamB protein would be expected to be sharper at 4 to 6 min than at 9 min and the opposite was in fact observed, the different induction times

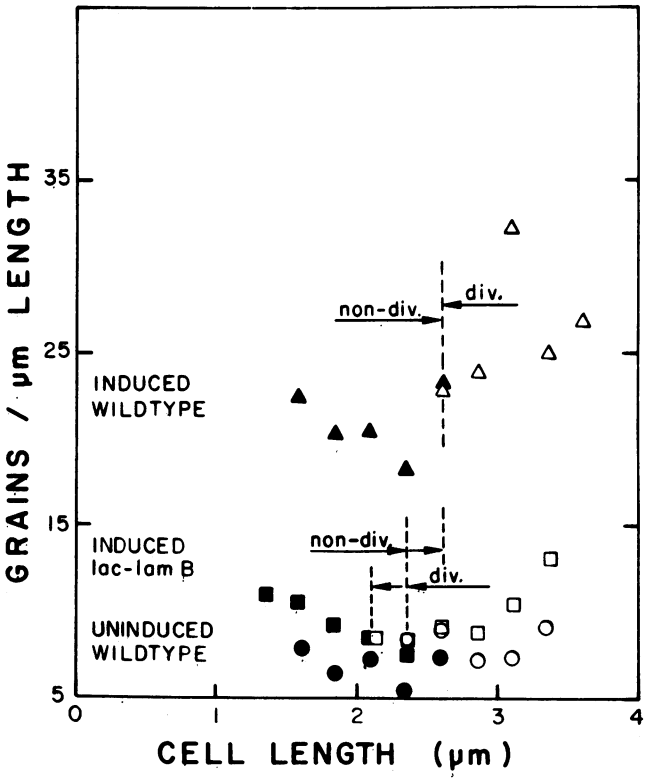

FIG. 6. Average LamB density as a function of cell length. The average number of grains per µm of cell length (indicating LamB density) was determined for various size classes of dividing (open symbols) and nondividing (closed symbols) cells of an uninduced wild-type population (circles), a wild-type population induced for 6 min (triangles), and an *lac-lamB* population induced for 4 min (squares).

do not explain the different results obtained. Another possibility, i.e., redistribution of LamB protein during sample handling in our experiments, is also unlikely since samples were stopped by cooling and direct fixation of the cells. Moreover, if there were redistribution of LamB protein, it would be expected to occur in the experiments of Ryter et al. (14) as well, given the labeling and fixation procedures used.

The most likely explanation for the differences observed is that the LamB protein is inserted at multiple sites over the entire cell envelope and is detected as such with anti-LamB trimer antibodies in our experiments, but that phage λ does not detect the resulting phage λ receptors with equal efficiency at all sites on the cell envelope. According to the results of Ryter et al. (14), LamB molecules inserted at the septum evidently form more efficient phage receptors than do LamB molecules inserted elsewhere at the cell surface. This could be due to varying receptor accessibilities (with fewer obstructions to phage binding near the septum) or to varying rates of assembly of LamB protein and lipopolysaccharide to functional receptors (with faster receptor assembly at the cell septum). The effects of varying rates of receptor assembly should decrease as induction times increase, because newly inserted LamB protein contributes progressively less to total receptor activity. In contrast, variations in receptor accessibility should in principle be seen at all induction times, since reduced accessibility must be due to other cell surface components rather than to the phage receptor itself.

More detailed comparisons of phage λ binding to complete receptor and of anti-LamB antibody binding to LamB protein are needed to clarify these points. For now, however, we believe that the results of Ryter et al. (14) are best explained by better accessibility of the phage λ receptor near the cell septum and relatively poor accessibility elsewhere, since their electron micrographs suggest that septal enrichment of phage λ is seen at later as well as early induction times.

Insertion of LamB protein as a function of cell cycle. It has been reported that the synthesis of a number of envelope proteins, including the outer membrane LamB protein, is cell cycle dependent (12). In fact, Ryter et al. (14) have interpreted their results to indicate that integration of the phage λ receptor, and probably its synthesis as well, is coupled to cell division. However, our results clearly show that LamB protein is synthesized and inserted during the entire cell cycle, as indicated by the probe distribution patterns of different cell size classes.

Despite the fact that all cells synthesized LamB protein immediately after induction, the rate of LamB synthesis was highest in dividing cells, suggesting that variations in the rate of synthesis, rather than exclusive synthesis at one particular cell age, might be responsible for the cell cycle-dependent patterns of *lamB* mRNA synthesis observed previously (13).

To check this point, we classified cells by size (roughly corresponding to age) and the relative rates of LamB synthesis. This was possible because we have found in other experiments that the appearance of LamB molecules at the cell surface follows the appearance of total LamB protein in cell lysates within 1 min. Moreover, the linear appearance of LamB at the cell surface reflects the linear synthesis of total cellular LamB (manuscript submitted). Thus, the number of LamB molecules detected at the cell surface of individual cells provides information on the number of LamB molecules synthesized by each cell during the first 4 to 6 min after induction.

Analysis of the average number of LamB molecules detected versus cell size showed that the rate of LamB protein synthesis per cell is essentially constant in nondivid-

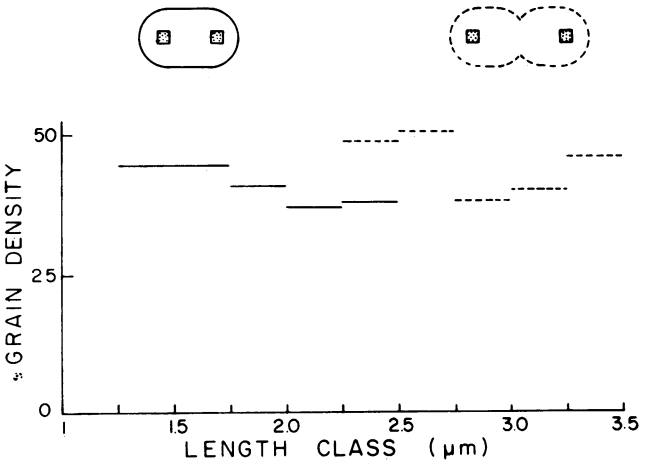

FIG. 7. Average OmpF density as a function of cell length. Uninduced pop5228 cells were fixed with OsO_4 and labeled with saturating amounts of anti-OmpF trimer antiserum and protein A-gold. The number of grains indicated on the vertical axis represents the number of grains present in two square areas of the cell surface, which occupy a total area of 0.08 μm^2. Five classes of nondividing cells (——) and of dividing cells (– – –) were analyzed separately.

ing cells and increases as cells begin to divide, thus supporting a linear growth model (5) for newly induced LamB protein. The increased rate of LamB synthesis which occurs during the invagination process may reflect a gene dosage effect, or it may reflect other effects associated directly or indirectly with invagination or septation. Efforts to distinguish between these possibilities are under way.

Insertion of LamB protein at insertion regions. Several laboratories have tried to define insertion regions. Insertion of various outer membrane components has been described to take place at the cell septum (14), at some 200 sites of adhesion between the cytoplasmic and the outer membranes (2), at multiple discrete patches probably related to adhesion sites (16), and above mobile polysomes (6).

Our results show that at least for the LamB protein insertion occurs at many points over the entire cell surface of all cells in a population. Whether insertion occurs at specific structural sites (fixed or mobile) as reported for outer membrane porin (16) or whether insertion occurs at essentially any site on the cell envelope remains to be determined. Our results do not exclude the possibility that insertion regions are located in multiple specific and reproducibly placed regions in every single cell; they do, however, exclude zonal insertion of LamB protein.

ACKNOWLEDGMENTS

All bacterial strains used in this study were generous gifts of M. Hofnung (Institut Pasteur, Paris). We acknowledge the interest and help of C. L. Woldringh during the initial phase of this study and J. F. L. van Breemen for his assistance with electron microscopy. We also thank J. D. Leutscher for drawing and J. Woons for photography.

LITERATURE CITED

1. **Aldea, M., E. Herrero, M. I. Esteve, and R. Guerrero.** 1980. Surface density of major outer membrane proteins in *Salmonella typhimurium* in different growth conditions. J. Gen. Microbiol. **120**:355–367.
2. **Bayer, M. E.** 1979. The fusion sites between the outer and cytoplasmic membrane of bacteria; their role in membrane assembly and virus infection, p. 167–202. *In* M. Inouye (ed.), Bacterial outer membranes: biogenesis and functions. John Wiley & Sons, Inc., New York.
3. **Begg, K. J.** 1978. Cell surface growth in *Escherichia coli*: distribution of matrix protein. J. Bacteriol. **135**:307–310.
4. **Begg, K. J., and W. D. Donachie.** 1977. Growth of the *Escherichia coli* cell surface. J. Bacteriol. **129**:1524–1536.
5. **Boyd, A., and I. B. Holland.** 1979. Regulation of the synthesis of surface protein in the cell cycle of *E. coli* B/r. Cell **18**:287–296.
6. **De Leij, L., J. Kingma, and B. Witholt.** 1979. Nature of the regions involved in the insertion of newly synthesized protein into the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta. **553**:224–234.
7. **Leal, J., and H. Markovich.** 1974. Expression of T6 sensitivity in *Escherichia coli* K12 F^- bacteria after mating with Hfr cells. Mol. Gen. Genet. **130**:345–359.
8. **Marchal, C., J. Greenblatt, and M. Hofnung.** 1978. *malB* region in *Escherichia coli* K12: specialized transducing phages and first restriction map. J. Bacteriol. **136**:1109–1119.
9. **Marchal, C., D. Perrin, J. Hedgepeth, and M. Hofnung.** 1980. Synthesis and maturation of λ receptor in *Escherichia coli* K-12: *in vivo* and *in vitro* expression of gene *lamB* under *lac* promoter control. Proc. Natl. Acad. Sci. U.S.A. **77**:1491–1495.
10. **Mühlradt, P. F., J. Menzel, J. R. Golecki, and V. Speth.** 1973. Outer membrane of *Salmonella*: site of export of newly synthesized lipopolysaccharide in the bacterial surface. Eur. J. Biochem. **35**:471–481.
11. **Nanninga, N., C. L. Woldringh, and L. J. H. Koppes.** 1982. Growth and division of *Escherichia coli*, p. 225–270. *In* Nicolini (ed.), Cell growth. Plenum Publishing Corp., New York.
12. **Ohki, M.** 1979. The cell cycle-dependent synthesis of envelope proteins in *Escherichia coli*, p. 293–315. *In* M. Inouye (ed.), Bacterial outer membranes: biogenesis and functions. John Wiley & Sons, Inc., New York.
13. **Ohki, M., H. Ogawa, and S. Nishimura.** 1982. Synthesis of mRNA of *malB* operons at specific stages in the cell cycle of *Escherichia coli*. Ann. Microbiol. (Paris) **133A**:71–75.
14. **Ryter, A., H. Shuman, and M. Schwartz.** 1975. Integration of the receptor for bacteriophage lambda in the outer membrane of *Escherichia coli*: coupling with cell division. J. Bacteriol. **122**:295–301.
15. **Slot, J. W., and H. J. Geuze.** 1981. Sizing of protein A-colloidal gold probes for immunoelectron microscopy. J. Cell Biol. **90**:533–536.
16. **Smit, J., and H. Nikaido.** 1978. Outer membrane of gram-negative bacteria. XVIII. Electron microscopic studies on porin insertion sites and growth of cell surface of *Salmonella typhimurium*. J. Bacteriol. **135**:687–702.
17. **Trueba, F. J., and C. L. Woldringh.** 1980. Changes in cell diameter during the division cycle of *Escherichia coli*. J. Bacteriol. **142**:869–878.
18. **Vos-Scheperkeuter, G. H., M. Hofnung, and B. Witholt.** 1984. High-sensitivity detection of newly induced LamB protein on the *Escherichia coli* cell surface. J. Bacteriol. **159**:435–439.
19. **Witholt, B., M. Boekhout, M. Brock, J. Kingma, H. van Heerikhuizen, and L. de Leij.** 1976. An efficient and reproducible procedure for the formation of spheroplasts from variously grown *Escherichia coli*. Anal. Biochem. **74**:160–170.
20. **Yamada, H., T. Nogami, and S. Mizushima.** 1981. Arrangement of bacteriophage lambda receptor protein (LamB) in the cell surface of *Escherichia coli*: a reconstitution study. J. Bacteriol. **147**:660–669.