Topography of Peptidoglycan Synthesis during Elongation and Polar Cap Formation in a Cell Division Mutant of *Escherichia coli* MC4100

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A cell division mutant of *Escherichia coli* K12 *lysA*, the temperature sensitive *ftsZ* strain, was pulse-labelled with [³H]diaminopimelic acid (DAP) during growth in minimal salts medium both at the permissive (28 °C) and restrictive (42 °C) temperature. In contrast to other known cell division mutants, *ftsZ* filaments obtained during growth at 42 °C show no sign of persisting or newly initiated constrictions. The location of the incorporated DAP in dividing cells and in filaments was analysed with an improved autoradiographic method in which preparations of well-spread sacculi are covered with a dry emulsion. From the populations of sacculi complete distributions were obtained, which compared well with those of the intact cells. The grain-density distributions of cells dividing at 28 °C showed that the rate of surface synthesis was strongly increased at the site of constriction at the expense of the activity in the lateral wall, suggesting a redistribution of surface synthesis activity. In individual filaments elongating at 42 °C no indication for the existence of narrow or broad growth zones was found, suggesting a dispersed mode of lateral wall synthesis. These observations are in accordance with theoretical predictions on the rate of surface synthesis during the constriction period in cells which elongate at a constant diameter.

INTRODUCTION

One of the difficulties in studying bacterial growth and division is that the surface biosynthesis necessary for division cannot yet be measured as a specific enzyme activity. Consequently, the division capacity of a population can only be indicated by the rate of increase in cell number, and the state of division of an individual cell can only be described in morphological terms. On the other hand, bacteria have the advantage that their shape is relatively well defined and that the duration of the cell division process, the constriction period, can be easily estimated. These properties make it possible, with assumptions on cell volume growth and cell geometry, for theoretical predictions to be made on the rate of peptidoglycan synthesis during the process of constriction or polar cap formation.

Experimentally, the rate of peptidoglycan synthesis along the cell surface can be measured by autoradiography of cells pulse-labelled with radioactive diaminopimelic acid (DAP; cf. Ryter *et al.*, 1973). In such studies on the topography of peptidoglycan synthesis, an increased incorporation has always been observed in the central area of the cell, whether it was dividing or not (Ryter *et al.*, 1973; Koppes *et al.*, 1978; Verwer & Nanninga, 1980). It has therefore been assumed that this central zone of incorporation represented the site for lateral growth of the cell (Ryter *et al.*, 1973; Schwarz *et al.*, 1975). Verwer & Nanninga (1980), however, interpreted the zone as the present or future site for polar cap formation. Upon reanalysis of the available data, Koch (1982, 1983) concluded that in the Gram-negative rod a diffuse incorporation pattern

Abbreviation: DAP, diaminopimelic acid.

occurs both in the lateral wall and, to a lesser extent, in the old poles. Superimposed on this pattern is an increased incorporation at the sites of constriction (Verwer & Nanninga, 1980; see also Koch, 1983, and Nanninga & Woldringh, 1985). A diffuse pattern during elongation of the cell was also indicated by autoradiography of thin sections of *Escherichia coli* cells starved of DAP (Burman *et al.*, 1983).

The following questions remain, however: (i) whether the increased incorporation at the cell centre is sufficient for the synthesis of polar caps within the time of constriction; (ii) whether it is exclusive for polar caps, i.e. whether the polar caps are entirely made out of new material; and (iii) whether during polar cap formation diffuse incorporation in the lateral wall continues at the same rate. In the present work, we have analysed these questions by measuring the topography of [³H]DAP incorporation in normally dividing and in filamenting cells. The autoradiographic procedure was modified to allow the measurement of a complete size distribution of sacculi, and, to obtain uniform efficiency of grain production in the autoradiograms, an alternative way of applying a dry emulsion was developed.

METHODS

Organism and growth medium. *E. coli* MC4100 K12 F⁻ *araD139* Δ (*argF-lac*) U169 *rpsL150 relA1 fibB5301 ptsF25 deoCl lysA* was described by Wientjes *et al.* (1985). An *ftsZ*(ts) derivative of this strain was obtained by P1 transduction of the *ftsZ84*(ts) mutation from JFL100 (Lutkenhaus *et al.*, 1980) to MC4100 *leu*⁺:Tn5 and selecting for *leu*⁺ transductants.

Cells were grown at 28 °C in a waterbath shaker (New Brunswick) in minimal salts medium containing (l⁻¹): K₂HPO₄·3H₂O (3.16 g); KH₂PO₄ (1.47 g); (NH₄)₂SO₄ (1.05 g); MgSO₄·7H₂O (0.1 g); FeSO₄·7H₂O (0.3 mg); Ca(NO₃)₂·4H₂O (7.1 mg); thiamine (1 mg); glucose (5 g); and lysine, threonine and methionine (100 mg of each). Although the strain is not auxotrophic for the last two amino acids, these were added to lower the endogenous synthesis of DAP (see Wientjes *et al.*, 1985). The concentration of phosphate salts was reduced because of the suppressibility of the *ftsZ* mutation at the restrictive temperature by salts.

Growth measurement and temperature shifts. Cells inoculated from a fresh agar slant were cultured at 28 °C to determine the mass doubling time from OD₄₅₀ values measured with a Gilford spectrophotometer. This culture was diluted in fresh medium by a factor of about 50000 to assure continuing exponential growth the next day. At all times, care was taken that cultures never exceeded an OD₄₅₀ of 0.2. The condition of steady-state growth was verified by the constancy with time of average cell mass, expressed as the OD₄₅₀ of a suspension of 10⁹ cells ml⁻¹. Cell number was measured with a Coulter counter (orifice 30 µm). At an OD₄₅₀ value of 0.2, the culture grown at 28 °C was diluted fourfold in pre-aerated and prewarmed medium at 42 °C. About two mass-doublings after this temperature shift the culture was shifted back by twofold dilution in prewarmed medium at 28 °C. The mass-doubling time at 28 °C was about 75 min, and at 42 °C, 45 min. Cells taken for agar filtration were fixed with OsO₄ (final concentration 0.1%) and applied directly to agar filters as described below.

Pulse-labelling and preparation of sacculi. As shown for strain MC4100 *lysA* by Wientjes *et al.* (1985), cells of the present *ftsZ* strain can grow in the absence of DAP, and can therefore be pulse-labelled without any previous washing or starvation steps.

Pulses were started by adding 1.5 ml of the culture to 1.5 ml of fresh prewarmed medium containing 15 µl *meso*-3,4,5-[³H]DAP [47 Ci mmol⁻¹ (1.7 TBq mmol⁻¹); CEA; final concentration 10 µCi ml⁻¹]. A few seconds after the start of the pulse, a control sample of 250 µl was taken (zero-time sample) for measuring the background grain development. From the culture at 28 °C 1 ml samples were taken after a pulse time of 15 min. The culture at 42 °C was sampled after 9 min. For the preparation of clean sacculi, the samples were added to a volume of 20% (w/v) SDS at 80 °C, making the final SDS concentration 4%. Subsequently, the samples were boiled for 1 h (temperature in samples about 95 °C), kept at 80 °C for 18 h, and subsequently at 37 °C for 18 h, until use (see Wientjes *et al.*, 1985).

Agar filtration. The radioactive sacculi in the SDS samples were prepared without any washing steps by applying them directly to agar filters (for preparation see Woldringh *et al.*, 1977) warmed to 37 °C. After filtration the agar filters were cooled to 4 °C. Subsequently, the film carrying the sacculi was cut out and floated off on a series of four to five Petri dishes filled with distilled water at 20 °C. In this way unincorporated [³H]DAP was effectively washed out of the filters. The low temperature causes crystallization of the SDS in the agar layer and slows down its dissolution, which would otherwise interfere with the floating of the film on the water surface. After washing, the films were picked up from below with 200 mesh gold grids, precovered with a thin formvar film. To visualize the almost empty sacculi, the grids were lightly shadowed with platinum-carbon and inspected by electron microscopy before applying the emulsion.

Autoradiography. Grids containing sacculi from zero-time samples and of samples taken at the various temperature regimes were stuck to glass slides with double-sided tape. They were then covered with a dry emulsion

prepared in the following way. Ilford L4 emulsion was diluted with 2 vols distilled water at 32 °C. With a semi-automatic dipping apparatus a thin emulsion was applied to glass slides covered with a formvar film. After drying for not longer than 2 h, the naked emulsion layer could be floated off from the formvar surface on distilled water, cooled to about 10 °C (the formvar film sticks to the glass). The dry emulsion stretches out somewhat on the water surface depending on its temperature. By varying the temperature of the water and the initial thickness of the emulsion, thin monolayers could be obtained in a reproducible way. These naked emulsion layers were picked up from below with the glass slide carrying the four grids (see above). To summarize, grids were covered with the following layers from top to bottom: (i) the naked emulsion; (ii) the shadowed sacculi on the agar filter; and (iii) the supporting formvar film on the grid. This new method combines the advantages of the 'stripping film method', i.e. the easy coating of an emulsion monolayer (see Harris & Salpeter, 1983), with those of the 'loop method', i.e. the application of a naked emulsion layer in a dry form. Because of the previous shadowing of the sacculi there is no need to remove an outer collodion layer or the gelatin layer, as done by Ryter *et al.* (1973). The method also meets the criticism by Koch (1983) that, when preparations are directly dipped, the emulsion around the cell is thicker than on top of the cell, causing errors in quantifying the autoradiographic results.

After exposure for 22 to 30 d at -70 °C, the slides were developed for 10 min in Agfa-Gevaert developer (Kopriwa, 1975; Koppes *et al.*, 1978), under slight but continuous agitation for the production of round grains, and directly examined by electron microscopy.

Measurement of cells and sacculi. Agar filters carrying whole cells for determining control distributions, and autoradiograms of sacculi, were photographed at random with a Philips EM300 electron microscope. Dimensions of cells and sacculi and positions of overlying grains were measured from projections of negatives (final magnification about 12000 ×) with a Summagraphics digitizer (Fairfield, Conn., USA), connected to a HP9825A computer.

RESULTS

Rate of mass growth and cell division of E. coli ftsZ during temperature shifts

To make a better distinction between constricting cells and cells involved only in elongation, use was made of the cell division mutant *ftsZ*. In contrast to other cell division mutants like *ftsA* or *pbpB* grown in minimal salts medium, *ftsZ* grows at the restrictive temperature as filaments which lack any persisting or newly initiated constrictions (unpublished). In morphological terms, therefore, surface extension in these filaments merely reflects lateral wall synthesis.

The changes in total cell mass and cell number during a temperature shift up and subsequent shift down are shown in Fig. 1. During the first 15 min after the shift to 42 °C, the average mass of the cells ($\bar{M}$) remained constant as a result of the residual division of, in this experiment, 19% of the population. Thereafter, cell division was completely inhibited resulting in an increase of average cell mass at the same rate as the increase of total cell mass ($\bar{M}$) indicating filamentous growth.

To observe division recovery in the filaments, the population was shifted again to the permissive temperature after about two mass-doublings at 42 °C. This caused an immediate decrease in the rate of mass growth and a recovery of division capacity after about 30 min (Fig. 1). At this time, cells with about twice the length of the new-born cell in the original population were pinched off from the short filaments (see below). Recovery towards the original steady-state cell size seemed to take place in two phases, one rapid lasting about 30 min, followed by a long period of slow recovery.

Three types of cell sample were pulse-labelled for autoradiographic analysis: (i) the original culture of cells growing at steady-state at 28 °C; (ii) cells growing as short filaments after 70 min at 42 °C; and (iii) short filaments starting constriction during division recovery at 28 °C (Fig. 1, arrowed).

Comparison between size distributions of intact cells and sacculi

From the three different populations mentioned above, intact cells as well as sacculi were prepared, agar-filtered and measured. Complete and comparable length distributions were obtained both from intact cells and sacculi (Fig. 2, Table 1). The latter were either measured directly (Fig. 2*b*) or after preparation and development of the autoradiograms (Fig. 2*c*).

As far as we are aware, no complete distributions of sacculi have been published in similar autoradiographic studies. The parameters of the distributions show that the average length of

Fig. 1. Changes in total cell mass, M ($\circ$), in cell number, N ($\square$), and average cell mass, $\bar{M}$ ($\triangle$), of *E. coli ftsZ* during steady-state growth at 28 °C and after temperature shifts to 42 °C and back to 28 °C. The double arrows indicate the start and end of the radioactive DAP pulses given to cells.

Table 1. Parameters of size distributions (see Fig. 2) obtained from intact cells and sacculi of *E. coli* MC4100 *lysA ftsZ* grown at 28 °C, 42 °C, and 42 °C followed by 28 °C

Growth temperature	Doubling time (min)	Intact cells				Sacculi†						Autoradiograms‡			
		N	$\bar{L}$ (μm)	$2\bar{R}$ (μm)	$p(c)\S$ (%)	N	$\bar{L}$ (μm)	$2\bar{R}$ (μm)	$L^*\parallel$ (μm)	$2R^*\parallel$ (μm)	$p(c)\S$ (%)	N	$\bar{L}$ (μm)	$2\bar{R}$ (μm)	$p(c)\S$ (%)
28 °C	75	450	2.45	0.79	36	790	2.17	0.99	2.90	1.24	36	390	2.08	0.99	34
42 °C	45	514	5.60	0.79	10	478	4.64	0.99	6.05	1.24	14	306	4.63	1.01	10
(42 °C→)28 °C	—	626	5.41	0.83	26	524	4.14	1.30	5.88	1.30	29	372	4.01	0.99	34

† Sacculi not covered with emulsion.

‡ Sacculi covered with emulsion and used for autoradiography.

§ Percentage of constricting cells.

|| L^* and $2R^*$ were calculated from the length and diameter of intact cells assuming complete flattening without shrinkage: $2R^* = \pi R$; $L^* = (L - 2R) + 2R^*$. This calculation leads to a small error of about 5% in the estimation of R^* , because it incorrectly assumes the poles of flattened sacculi to be semicircular.

the sacculi was 12 to 24% smaller than that of the intact cells (Table 1). This decrease could have resulted from a separation of deeply constricted cells during the preparation of the sacculi, resulting in an over-estimation of short, new-born cells. However, as judged from the similar number of constricting cells for both intact cells and sacculi and also from the shrinkage of the filamenting cells, this explanation cannot hold. Alternatively, the sacculi may have shrunk during air-drying. To estimate such shrinkage the diameter of living cells must be known. Cell diameter ($2R$) measured after agar-filtration of intact cells has been found to resemble closely the width of living cells (Woldringh *et al.*, 1977). If we calculate late from this width the diameter of fully flattened sacculi ($2R^*$ in Table 1), the measured diameter of the sacculi appears to be 21 to 28% smaller. This reduction must have been caused by shrinkage during air-drying. The

Fig. 2. Length distributions of intact cells (a) sacculi (b) and sacculi prepared for autoradiography (c) from the three samples (28 °C, 42 °C and 42 °C → 28 °C) indicated by the arrows in Fig. 1. Arrows L_0 and L_d in (a) indicate the length of new-born and dividing cells, respectively, in the steady-state population. The hatched areas represent cells or sacculi showing constriction. See Table 1 for parameters of the distributions.

length of the sacculi (L) is 25 to 30% smaller as compared to the length of flattened sacculi (L^* in Table 1), suggesting a similar extensive shrinkage in the long axis of the cell.

In spite of the observed shrinkage, we conclude that the samples of sacculi were representative of the three pulse-labelled populations and could be used for the further analyses described below.

Topographical analysis of DAP incorporation

The autoradiographic data can be analysed to give information both on the kinetics of surface synthesis during the cell cycle (see next section) and on the topography of surface synthesis in elongating or constricting cells. Photographs of sacculi are shown in Fig. 3, and corresponding grain distributions from the three pulse-labelled populations are given in Fig. 4.

The autoradiograms of non-constricting sacculi of the steady-state population growing at 28 °C (Fig. 3a, and Fig. 4a, panels 1 to 3) show a rather diffuse pattern, with the highest grain

Fig. 3. Autoradiograms of sacculi prepared from the three samples indicated in Fig. 1 (see also Fig. 2*c*). (a), (b), Sacculi from the steady-state population at 28 °C; (c) sacculus from a cell filamenting at 42 °C; (d) sacculus from a cell showing constriction after a shift from 42 °C to 28 °C; (e) sacculus from a filamenting cell (42 °C) after prolonged exposure. Magnification bars represent 1 μm .

density in the central area of the cell. In the constricting cells a central peak is evident (Fig. 3*b*, and Fig. 4*a*, panels 4–6).

Because of differences in the thickness of the platinum–carbon shadow, no mutual comparison is possible between Fig. 4*a*, *b* and *c*. The different panels in Fig. 4*a*, *b* or *c*, however, are from the same autoradiogram and can therefore be compared directly. The ordinate grains μm^{-1} , represents the amount of DAP incorporated per pulse time, or the rate of surface synthesis along the normalized length axis of the cell (see also Discussion). When comparing a position between the pole and the cell centre (at 0.25 or 0.75 normalized cell lengths), the rate of surface synthesis can be seen to increase slightly with increasing length classes of non-constricting cells, and to decrease again in the length classes of constricting cells. In other words,

Fig. 4. Silver grain distributions over the sacculi from (a) the steady-state population growing at 28 °C, (b) the culture filamenting at 42 °C and (c) the culture recovering division after a shift from 42 °C to 28 °C. Grains distributed over the sacculi have been plotted as the grain density or grains $(\mu\text{m cell length})^{-1}$ versus normalized cell length (see also Fig. 6). Constricting sacculi have been positioned with the longest cell-half to the left. For each subpanel the total number of cells (N), the total number of counted grains (G) and range of cell lengths (L) considered are indicated. Because (a), (b) and (c) represent different preparations the ordinate values cannot be compared.

there is a redistribution of the activity of surface synthesis when cells are in the process of constriction. The activity in the central area is increased, partly at the expense of activity in the lateral wall (compare Fig. 4a, panels 3 and 4).

The two observations described above, i.e. (i) a diffuse incorporation pattern in non-constricting cells, and (ii) an increased incorporation activity in the constriction site at the expense of lateral wall incorporation, are clearly supported when studying filamenting cells: the three panels in Fig. 4b show relatively long length classes of non-constricting cells growing at 42 °C (see also Fig. 3c). In these short filaments, either observed individually or after normalization and averaging with the computer, no indication was found of a non-random distribution of grains and thus for the presence of growth zones. The same was true for grain distribution over individual sacculi after prolonged exposure of the autoradiograms (Fig. 3e).

The panels in Fig. 4c represent cells which have been shifted back to the permissive temperature and pulse-labelled at the time of increased division activity (arrow 3 in Fig. 1). In contrast to the grain distributions of sacculi growing at 42 °C (Fig. 4b), an increased incorporation at the cell centre of relatively short non-constricting cells is seen in fig. 4c (panel 2). In constricting cells the future polar caps are intensely labelled whereas lateral activity is low (see also Fig. 3d). Also, in the longer filaments, which normally do not divide in the middle but at one-quarter of the cell length (see distributions of constricting sacculi in Fig. 2), an increased incorporation at the constriction site was observed (Fig. 4c, panel 5).

Theoretical rate of surface synthesis during polar cap formation

In contrast to *E. coli* B/r cells grown in minimal medium (Trueba & Woldringh, 1980) the present *E. coli* K12 cells have been found to elongate at constant diameter (result not shown). This means, for geometrical reasons, that the relative rate of surface synthesis will lag behind the rate of volume synthesis during elongation. It has to increase again for polar cap synthesis in order to recover the deficit in surface (for a graphical representation see Woldringh *et al.*, 1985).

By definition, polar cap synthesis occurs during the constriction period. The duration of this period can be calculated from the percentage of cells engaged in the constriction process during steady-state growth (i.e. 36%; see Table 1) by making use of the age distribution equation (Powell, 1956). For the present cells the period is 33 min. With the assumptions formulated below we can now calculate both the amount of surface that has to be synthesized in this period, and the rate increase of surface synthesis necessary for polar cap formation.

If we assume an exponential volume increase for the average individual cell, cell volume, $V(a)$, at age a , is given by

$$V(a) = V_0 2^{a/t_d} \quad (1)$$

in which V_0 is the volume of a new-born cell and t_d the generation time.

Given that the geometry of a cell is a cylinder with hemispherical polar caps and elongation at constant diameter, we can derive from equation (1) the following expression for the increase in surface, $A(a)$, of a cell which does not form polar caps

$$A(a) = (A_0 - 4/3\pi R^2)2^{a/t_d} + 4/3\pi R^2 \quad (2)$$

In the case of the steady-state population growing at 28 °C (see Table 1, intact cells), cell diameter $R = 0.8 \mu\text{m}$, the surface of a new-born cell $A_0 = 2\pi RL_0 = 4.62 \mu\text{m}^2$, and that of a dividing cell $A_d = 9.24 \mu\text{m}^2$. From equation (2) the total cell surface synthesized during the constriction period can be calculated to be $2.7 \mu\text{m}^2$. If the polar caps, which represent a surface of $2 \mu\text{m}^2$, are entirely made out of newly synthesized material the following prediction on the localization of surface synthesis can be made: 74% of the total surface synthesized during constriction has to be invested in the formation of polar caps, whereas 26% remains available for further elongation. A similar prediction is obtained when assuming linear volume growth. The present autoradiographic results clearly support the above prediction that for polar cap synthesis most of the synthetic activity of the cell is concentrated at the constriction site.

Another prediction that can be made concerns the rate of surface synthesis during elongation and constriction. From equation (2) it can be calculated that the cell surface of a non-

Fig. 5. Comparison of theoretical (—) and experimental (---) rates of surface synthesis plotted as a function of cell length. The theoretical lines, calculated for the present conditions with equations given in the text, predict a slightly larger increase in the rate of surface synthesis with cell length than shown by the regression lines obtained from the observed grain number per individual sacculus (not shown). The regression coefficient and linear correlation coefficient for sacculi that were merely elongating were 2.89 and 0.19, and for constricting sacculi, 3.59 and 0.62, respectively. The theoretical and experimental lines were normalized at the cell length corresponding to the start of constriction (○). The two cell figures represent cells that have doubled the volume, V_0 , of a new-born cell. The hatched areas reflect the surface synthesized during the constriction period. The black area indicates the extra surface necessary to make polar caps.

constricting cell at the end of the cycle ($a = t_d$) is only $8.57 \mu\text{m}^2$, giving a deficit in surface of $0.67 \mu\text{m}^2$ (i.e. the term $4/3\pi R^2$ in equation 2). In order to quantify the necessary increase in the differential rate of surface synthesis during constriction, we assumed that an extra exponential surface synthesis takes place between the onset of constriction (t_1) and the end of constriction (t_d). Such surface growth is described by $A'(a)$, defined as follows:

$$A'(a) = A(a) \quad \text{for } 0 < a < t_1$$

$$\text{and } A'(a) = A(a) \cdot \exp[(a - t_1) \cdot Q] \quad \text{for } t_1 < a < t_d \quad (3)$$

with $Q = \frac{1}{t_d - t_1} \cdot \ln \left[\frac{A(t_d) + P}{A(t_d)} \right]$ in which P is the difference between the actual surface of a constricted cell and $A(t_d)$: $P = 4/3\pi R^2$. After differentiation with respect to a of $A(a)$ and $A'(a)$, we find that the differential surface growth rate between $A(a)$ and $A'(a)$ is given by

$$\frac{\delta A'(a)}{\delta a} \bigg/ \frac{\delta A(a)}{\delta a} = 1 \quad \text{for } 0 < a < t_1$$

$$\frac{\delta A'(a)}{\delta a} \bigg/ \frac{\delta A(a)}{\delta a} = \left[1 + \frac{t_d \cdot Q(1 + T)}{\ln 2} \right] \cdot \exp[(a - t_1)Q] \quad \text{for } t_1 < a < t_d \quad (4)$$

where $T = 4/3\pi R^2 / (A_0 - 4/3\pi R^2) \cdot 2^{a/t_d}$.

From equation (4) we can calculate that the necessary differential rate of cell surface growth during constriction increases slowly from 1.28 at $a = t_1$ to 1.37 at $a = t_d$ for the present conditions. In Fig. 5 the absolute rate of surface synthesis during elongation and constriction, as calculated from the above equations, is plotted as a function of cell length (solid lines).

Kinetics of surface synthesis during the cell cycle

Information on the kinetics of surface synthesis can be obtained by plotting the grain number per sacculus, which reflects the amount of DAP incorporated per pulse time, and thus the rate of peptidoglycan synthesis, as a function of cell length. In such a plot (not shown) an extensive dispersion of the grain number over individual sacculi becomes apparent. In previous studies (Ryter *et al.*, 1973; Koppes *et al.*, 1978) the grain number per cell was averaged over broad length classes and no distinction was made between non-constricting and constricting

cells. These plots clearly showed an increase in the rate of peptidoglycan synthesis during the cell cycle. A similar increase for both non-constricting and constricting cells is suggested by the regression lines in Fig. 5 (dashed lines) obtained by plotting the observed grain number per individual sacculus. Such an increase was also observed in plots of data obtained from filaments at 42 °C (not shown) and is predicted by our theoretical model.

Further comparison of the theoretical rates of surface synthesis in non-constricting and constricting cells (solid lines in Fig. 5) with the experimental rates (dashed lines in Fig. 5) shows a similar jump in the rate of surface synthesis at the start of constriction. There is also a discrepancy: the experimental rates increase less with cell length than predicted. This could indicate that the assumptions made on volume growth and cell geometry during elongation are incorrect, or that in young cells the incorporated DAP does not directly reflect an extension of the surface (see Discussion).

DISCUSSION

With respect to the much debated mode of lateral wall synthesis, our results confirm earlier interpretations: elongation of the cylindrical *E. coli* cell takes place by diffuse incorporation of peptidoglycan precursors (Verwer & Nanninga, 1980; Burman *et al.*, 1983; Koch & Burdett, 1984; Nanninga & Woldringh, 1985). Our observations also seem to agree with theoretical predictions on the rate of surface synthesis during polar cap formation (Woldringh *et al.*, 1985): in cells growing at constant diameter, the rate has to increase during constriction for geometrical reasons, whereas the synthetic activity has to be largely restricted to the constriction site.

This mode of growth and division does not need to hold under all circumstances. In those strains or under those conditions where cell diameter increases during constriction (Trueba & Woldringh, 1980), diffuse peptidoglycan synthesis may have to continue over the entire cell surface during polar cap formation too (see Woldringh *et al.*, 1985). Experiments are in progress to test this possibility of alternative polar cap formation in a different *E. coli* strain. The present results with the *ftsZ* strain contrast with our preliminary data obtained from the parent strain MC4100 *lysA*, in which we found no increase of grain number with sacculus length (Woldringh *et al.*, 1985). We must conclude, however, that reliable interpretations of such quantitative analyses have to await a reduction in the high degree of scatter inherent in these experiments (results not shown).

It should be noted that any comparison between theoretical predictions and our observations presupposes that the sacculus contains peptidoglycan of a uniform thickness all over the cell and that the incorporation of DAP directly reflects extension of the stress-bearing cell surface. Although from chemical determinations (Braun *et al.*, 1973; Labischinski *et al.*, 1979) it has been deduced that the Gram-negative peptidoglycan layer is very thin (monolayer), other organizations cannot be excluded (Hobot *et al.*, 1984).

An important difference between this study and all previous experiments is that we could add the radioactive DAP to cells growing in steady-state, without having to wash or starve the cells (see Wientjes *et al.*, 1985), and that we analysed complete length distributions of sacculi. In spite of these improvements the variation in the number of grains lying over cells of the same length class (results not shown) was too high to allow a reliable analysis of the kinetics of surface synthesis during the cell cycle.

When comparing our topographical results with those in previous studies, differences in the presentation of the data should be taken into account. In Fig. 6 it is shown how plotting the number of grains (as a percentage of total grains) versus cell length (cf. Ryter *et al.*, 1973; Schwarz *et al.*, 1975) leads to an overestimation of central grains relative to polar grains (Fig. 6e). This does not occur when plotting grain density per length unit (μm) versus normalized cell length (compare Fig. 6c with 6e). We chose this presentation because it shows directly the rate of surface synthesis at any position along the long axis of the cell. In addition, we did not make the figure symmetrical as in Fig. 6(c) and 6(e), thus preserving the ability to control the significance of possible peaks occurring in the cell halves (see Fig. 6 legend). Nevertheless, normalizing and

Fig. 6. Comparison of two different ways to present the distribution of silver grains. (a), (d) Cells of 3 and 4 μm long randomly covered with grains. The two cells would belong to size class 'c' as distinguished by Schwarz *et al.* (1975). (b) Distribution of grains as plotted in the present work (Fig. 4) as grain density versus normalized cell length. (c) The same plot as in (b) but redrawn on a scale comparable to the one in (e). (e) Distribution of grains as plotted by Schwarz *et al.* (1975) as percentage of total grains versus cell length (distance from cell centre). Note the different appearance of the same grain distribution in (c) and (e). The 'peak' in (c) on one-quarter of the cell length is not significant because it is not found in the two cell halves in (b).

averaging cells of different lengths in length classes may obscure the grain pattern in any individual cell. We therefore examined autoradiograms of individual cells after prolonged exposure (Fig. 3e) but found no indication of the presence of growth zones.

The questions posed in the Introduction can now be answered in a qualitative way. Because of the localized synthesis at the constriction site it seems probable that polar caps are to a large extent made out of new material. In addition, diffuse incorporation in the lateral wall continues at a lower rate. The simultaneous synthesis of lateral wall and polar caps has also been observed by Burman *et al.* (1983) in *E. coli*, and contradicts the existence of alternating processes suggested on the basis of studies on a mutant of *Klebsiella pneumoniae* (Satta *et al.*, 1979). The question whether the increased incorporation observed at the constriction site is sufficient for the synthesis of polar caps within the time of constriction still remains. More accurate data than presently obtained (Fig. 5) will be necessary to calculate reliable rates of surface synthesis during elongation and during constriction. The present autoradiographic data could easily be compared with results on DAP incorporation in synchronized cells. Experiments are in progress to apply both techniques to the same strain.

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