The Rate and Topography of Cell Wall Synthesis during the Division Cycle of *Escherichia coli* Using *N*-Acetylglucosamine as a Peptidoglycan Label

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The rates of synthesis of peptidoglycan and protein during the division cycle of *Escherichia coli* were measured by the membrane elution technique using cells differentially labelled with *N*-acetylglucosamine and leucine. During the first part of the division cycle the ratio of the rates of protein and peptidoglycan synthesis was constant. The rate of peptidoglycan synthesis, relative to the rate of protein synthesis, increased during the latter part of the division cycle. These results support a simple, bipartite model of cell surface increase in rod-shaped cells. Prior to the start of constriction the cell surface increases only by lateral wall extension. After cell constriction starts, the cell surface increases by both lateral wall and pole growth. The increase in surface area is partitioned between the lateral wall and the pole so that the volume of the cell increases exponentially. No variation in cell density occurs, because the increase in surface allows a continuous exponential increase in cell volume that accommodates the exponential increase in cell mass. The results are consistent with the constant density of the growing cell and the surface stress model for the regulation of cell surface synthesis. In addition, the elution pattern suggests that the membrane elution method does work by having the cells effectively bound to the membrane by their poles.

INTRODUCTION

Woldringh *et al.* (1987) studied the sites of peptidoglycan synthesis in *Escherichia coli* and made the remarkable observation that in the later stages of the division cycle, as defined by cells with constrictions, there is a decrease in the rate of peptidoglycan synthesis in the lateral cell wall. As the total rate of peptidoglycan synthesis continuously increases during the division cycle, they interpreted their results as indicating that the decrease occurs because pole synthesis occurs 'at the expense of the activity in the lateral wall.' Investigations of the rate of peptidoglycan synthesis in *Salmonella typhimurium* (Cooper, 1988) have led to a slightly different model and explanation but one that supports and explains the findings of Woldringh *et al.* (1987). In this paper we wish to extend the results to *E. coli*, and show that cell wall synthesis can be studied using *N*-acetylglucosamine (GlcNAc) as a peptidoglycan label. (For a complete review of previous work on this problem see Woldringh *et al.*, 1987; Cooper, 1988.)

S. typhimurium is superior to *E. coli* in its ability to incorporate exogenous diaminopimelic acid (DAP) into its peptidoglycan (Cooper & Metzger, 1986). When the rate of incorporation of DAP during the division cycle of *S. typhimurium* was compared to the rate of incorporation of a marker of cell protein synthesis, a simple bipartite model of cell wall synthesis was developed (Cooper, 1988). During the first part of the division cycle prior to invagination, cell growth occurs only by lateral wall extension and there is a constant ratio of DAP to leucine incorporation. When invagination starts the ratio of the rates increases because of the different

Abbreviations: DAP, diaminopimelic acid, GlcNAc, *N*-acetylglucosamine.

geometry of the pole compared to the cylinder. The ratio of the rates can be described by the equation

$$\frac{dA/dt}{dM/dt} = 1 + \frac{12h_{\alpha}^2}{2^{\alpha} \ln 2 (1 - T_c)(8r^2 + 6L_{0cy})}$$

where dA/dt is the rate of area increase and dM/dt is the rate of mass synthesis; α is the age of the cell during the division cycle; h_{α} is the height of the newly forming poles; T_c is the age the start of invagination and L_0 and r are the initial length of the side wall and the radius of the cell. Before invagination starts the heights of the new poles are zero and the ratio of the rates is constant. After the poles begin to form, the value of the ratio increases. This equation was derived assuming that the poles grew with the area of the poles increasing at a constant rate.

Because *E. coli* is difficult to label with DAP (Cooper & Metzger, 1986) we turned to GlcNAc as a specific label for peptidoglycan. The pattern of wall synthesis during the division cycle of both *E. coli* and *S. typhimurium* has now been determined using GlcNAc as a specific label for cell wall synthesis. The results are consistent with the model of peptidoglycan synthesis proposed earlier (Cooper, 1988).

The membrane elution technique is a method for measuring the rate of synthesis of a particular macromolecule during the division cycle. Exponentially growing, unperturbed cells are labelled for a short time with a label specific for the molecule of interest. The cells are filtered onto a cellulose nitrate membrane, the membrane is inverted and fresh medium is pumped through the membrane. A fraction of the labelled cells bind to the membrane and grow in the presence of the medium pumped over the cells. Only new-born cells are released from the membrane. The first cells released by division arise from the oldest cells in the labelled culture, that is, those cells that were just about to divide at the time the cells were labelled. With further incubation the new-born cells released from the membrane are the products of cells that are increasingly younger at the time the cells were labelled. Cells are therefore released from the membrane with radioactive label that reflects the labelling of the cells, during balanced and unperturbed growth, and they are released sequentially as a function of their position between divisions. By considering cells in reverse order of elution, and over one generation of elution, the rate of incorporation of a radioactive label as a function of the division cycle can be determined.

METHODS

Bacterial strains. *Escherichia coli* B/r (*lys*) is a lysine-requiring derivative of the classic strain that was used to determine the rate of DNA synthesis during the division cycle (Helmstetter, 1967; Helmstetter & Cooper, 1968; Cooper & Helmstetter 1968). The lysine requirement was introduced by transduction and penicillin selection. The *Salmonella typhimurium* strain 2616 (*lys*) was described by Cooper (1988).

Membrane elution method. Medium C (Helmstetter, 1967) supplemented with 0.2% of the noted carbon source (glucose, glycerol) and 40 μg lysine ml^{-1} was used throughout. The bacteria was grown at 37 °C with rotary shaking for at least 15 h prior to the start of the elution experiment. Exponentially growing cells were labelled with [^3H]GlcNAc and [^{14}C]leucine for approximately 5% of the generation time. The cells were filtered on a nitrocellulose membrane (Millipore, GSWP). The filter was washed with pre-warmed medium and then inverted. Fresh warm medium was then pumped through the membrane. After an initial release of unbound and loosely bound cells, the eluate contained only new-born cells released from the membrane by cell division. A fraction of each sample was taken for a cell count using a Coulter counter. A larger fraction (4–5 ml) was taken for the determination of the amounts of GlcNAc and leucine incorporated. As the initial washing on the membrane removed unincorporated radioactivity, the eluted material was counted without filtration. Control experiments indicated that there was no significant 'pool' of unincorporated material in the cells eluted from the membrane.

Radioactivity sources and measurements. [^{14}C]Leucine [0.3078 Ci mmol^{-1} (11.39 GBq mmol^{-1})] was purchased from New England Nuclear. [^3H]GlcNAc [33.1 Ci mmol^{-1} (1.225 TBq mmol^{-1})] was from Research Products International. Scintillation fluids which are compatible with aqueous samples (Safety Solve, Research Products International) allowed the counting of up to 5 ml of eluate without filtration of the cells. A Beckman 3801 scintillation counter was used to count the samples; this instrument corrected for quench in different samples. The counts were also automatically corrected for spillover of the tritium and carbon labels into the alternate windows.

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Rate of incorporation of GlcNAc and leucine during the division cycle of *E. coli* B/r (*lys*) growing in medium C (100 ml) with glucose and lysine was labelled at a cell concentration of 1.3×10^8 cells ml^{-1} for 3 min with $2.0 \mu\text{Ci}$ (74 kBq) [^{14}C]leucine and $100 \mu\text{Ci}$ (3.7 MBq) [^3H]GlcNAc. The cells were then filtered and bound to a membrane and analysed as described in Methods.

Fig. 2. Rate of incorporation of GlcNAc and leucine during the division cycle of *E. coli* B/r growing in minimal medium with glycerol and lysine. The procedure was the same as described in Fig. 1 (50 ml of cells at 1.5×10^8 cells ml^{-1} labelled for 3 min).

Fig. 3. Rate of incorporation of GlcNAc and leucine during the division cycle of *E. coli* B/r growing in minimal medium with succinate and lysine. The procedure was as described in Fig. 1 (50 ml of cells at 3.0×10^8 cells ml^{-1} labelled for 4 min).

Fig. 4. Predicted pattern of ratios of cell wall to protein synthesis rates during an elution experiment. The values were calculated for a cell with a side wall length of 1, a radius of 0.5, and with invagination starting at age 0.5.

RESULTS

Rate of peptidoglycan and protein synthesis during the division cycle of E. coli

E. coli B/r (*lys*) growing in different media was labelled with [^{14}C]leucine and [^3H]GlcNAc and analysed by the membrane elution technique. Representative results are shown in Figs 1–3. It can be seen that:

- (a) the ratio of GlcNAc to leucine in the first cells eluted is elevated and rapidly drops to a lower, constant level approximately one-half a generation after elution begins;
- (b) one can observe a slight elevation in the ratio at the start of the second generation of elution;
- (c) the ratio remains relatively constant for the remainder of the elution period.

DISCUSSION

The results can be interpreted as follows.

(a) There is a constant ratio of cell wall synthesis to mass synthesis during the first part of the division cycle and this is elevated during the last half of the division cycle. This is consistent with lateral wall growth during the first part of the division cycle (before invagination starts) and pole formation along with lateral wall synthesis in the last part of the division cycle (Cooper, 1988).

(b) The 'bump' in the ratio at the start of the second generation is interpreted as the elevated incorporation due to the release, from the membrane, of the pole material that was labelled and that remained on the membrane after the first cell division. As the newly forming poles at the time label is added are 'interior' compared to the older 'outer' poles, there is a preferential release of these poles during the first and second generations (Cooper, 1988).

(c) The remaining elution, during the succeeding generations, is due to the subdivision of the peptidoglycan in the lateral wall and the subdivision of the protein in the cytoplasm. The constant ratio with extended elution indicates that there is no measurable turnover of peptidoglycan (Cooper, 1988).

In Fig. 4 the predicted ratio according to this model is plotted. The results are normalized so that the ratio of the differential rates of peptidoglycan to protein synthesis is unity. The second generation gives a higher predicted peak because the amount of leucine label in the second generation is precisely half that in the first generation, while the peptidoglycan label due to pole synthesis is the same in the first and second generation (Cooper, 1988). The finding of this elution pattern is the first indication that cells bind to the membrane as though they were bound by their pole. This binding pattern allows the incipient poles to be completely eluted during the first and second generations of growth on the membrane and thus gives the elution pattern observed.

The results are consistent with a model that predicts a constant ratio of the rate of cell surface increase to the rate of volume or mass increase prior to the start of constriction, and an increase

in the ratio after the start of constriction. The experiments are not able to distinguish the precise mode of new pole formation, but the results fit a model of a constant rate of pole area increase. Throughout the cell cycle there is no change in density since the cell volume and mass both increase exponentially.

The constant ratio at extended elution times also indicates that there is very little turnover and release of the peptidoglycan in this strain of *E. coli*. This was also observed to be true for *S. typhimurium* (Cooper, 1988). We do note that a different result has been reported by Greenway & Perkins (1985). They presented evidence that the peptidoglycan of *E. coli* labelled with glucosamine was unstable, while peptidoglycan labelled with DAP was stable. Aside from the problem of explaining how one could excise the glycan chain without disturbing the peptide chain, we would merely note that the experimental data presented by Greenway & Perkins (1985) to support turnover is heavily determined by a single original point and that there is enough variability in the data (as evidenced by observed 50% increases in label after label is removed) to suggest that their results may not be in contradiction to the conclusions reached here. Further, we have previously noted that strain W7, a DAP-requiring strain, which they used in their studies, may have an abnormal pattern of turnover (Cooper, 1988).

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