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Elasticity and Biochemistry of Growth Relate Replication Rate to Cell Length and Cross-link Density in Rod-Shaped Bacteria

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ABSTRACT In rod-shaped bacteria, cell morphology is correlated with the replication rate. For a given species, cells that replicate faster are longer and have less cross-linked cell walls. Here, we propose a simple mechanochemical model that explains the dependence of cell length and cross-linking on the replication rate. Our model shows good agreement with existing experimental data and provides further evidence that cell wall synthesis is mediated by multienzyme complexes; however, our results suggest that these synthesis complexes only mediate glycan insertion and cross-link severing, whereas recross-linking is performed independently.

INTRODUCTION

To some extent, bacteria are like balloons: Their shape is the consequence of stretching an elastic material (the cell wall) by an internal pressure that is larger than the external pressure. (This pressure difference is the turgor pressure.) However, unlike balloons, bacteria grow. In rod-shaped bacteria, such as *Escherichia coli*, new cell wall material is inserted into the lateral walls, and the bacteria grow in length without changing width (1,2).

The bacterial cell wall is made of a covalently linked, elastic material known as peptidoglycan (PG), which is a network of glycan strands cross-linked by flexible pentapeptide stems (3–5). To insert new material into the PG, existing material must be cleaved (6). Therefore, synthesis of the PG involves a), severing of existing peptide bonds and/or glycan strands; b), insertion of new dissacharide units; and c), recross-linking by formation of new peptide bonds (7). These biochemical processes are driven by enzymes, such as the penicillin-binding proteins. Recent evidence suggests that some aspects of cell wall synthesis are processive and that the enzymes that drive synthesis may be localized in a multienzyme complex (4). However, how biochemistry and biophysics conspire to change the length of the cell without changing the width remains enigmatic, even though this process has received a fair amount of scrutiny in the last 10 years (8–12). For example, computational modeling was used to investigate the effect of insertion and severing on the morphology of rod-shaped bacteria but did not directly consider the biochemical reactions that drive PG synthesis (10). Another group posited that the mechanochemistry of insertion leads to dynamics that attempt to minimize the free energy of the cell wall without describing how this dynamics could arise from biochemical kinetics (11). A recent model also examined processive synthesis of cell wall growth, but did not directly model how cross-link density, elasticity, and replication rate affect cell length (12).

Although efforts to understand bacterial cell width control are ongoing, an equally puzzling phenomenon has received less attention. Bacterial cell morphology is tightly coupled to the rate that cells replicate and divide. It is well known that growth conditions that increase the bacterial replication rate (which is inversely proportional to the doubling time) also lead to populations of cells that have a longer average length (7,13–15). In other words, cells that divide faster are longer. How does faster replication lead to longer cell length?

To address this question, we take an alternative approach to modeling bacterial morphology and construct a simple model for the mechanics and chemistry of PG synthesis. We focus on the fundamental physics and biochemical kinetics to elucidate general principles of rod-shaped growth. Specifically, we address the two experimental observations that cell length increases as the replication rate of the bacteria increases, whereas the fraction of peptides in the PG that are cross-linked decreases (7,13–15).

A MECHANOCHEMICAL MODEL OF BACTERIAL MORPHOLOGY

Bacterial cell length is a consequence of two of things, the total amount of material in the cell wall and how much that material is stretched by the turgor pressure. The amount of PG that is in the cell wall is controlled by the rates that new PG is inserted and old PG is removed. There is then a net insertion rate that is equal to the insertion rate minus the removal rate. How much cell wall material a given cell has is set by the net insertion rate times the doubling time. The PG is elastic and gets stretched by the turgor pressure. The Young's modulus of the cell wall will depend on the extent of the PG cross-linking.

To construct a mathematical model that incorporates these features, we begin by considering a single growing

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Gram-negative cell with a single layer of PG. Although conclusive evidence on the orientation of the glycan strands is lacking, the predominant view favors alignment of the glycan strands about the circumference of the cell with the peptide chains oriented parallel with the long axis of the cell (16,17)(Fig. 1). As mentioned previously, the cell length is a function of the amount of material and how much the material has been stretched. Therefore, we consider the glycan strands to be hoops about the cell circumference with each hoop defining a cross section (Fig. 1 *B*). We further consider that the cell maintains a fixed number of disaccharides, $4N$, per cross section. The total amount of material is then proportional to the total number of cross sections, which we denote as X. Each dissacharide in a glycan strand has one peptide stem; however, the peptide stems rotate ~90° per dissaccharide (18). Therefore, we assume that only one in four peptides are available to cross-link any two cross sections, which implies that there are only N possible cross-linking sites between each cross section. If the average fraction of bound cross-links per cross section is ψ and each bound peptide chain acts like a linear elastic spring with spring constant k and rest length a, the turgor pressure P will induce a strain between cross-sections ε given by

$$\pi R^2 P - akN\psi\varepsilon = 0, \tag{1}$$

where R is the radius of the bacterium. Here, we have assumed that the elastic stress in the cell wall equilibrates fast compared to the biochemical reactions, which is a standard assumption (10,11). The total length of the growing cell is then equal to the number of cross sections times the distance between cross sections, $L = aX(1 + \varepsilon)$.

To complete our model, we need to describe the biochemical reactions that occur during cell wall synthesis (Fig. 1). Because our simple description of the cell wall ignores glycan strand length, we only consider the kinetics of peptide severing and cross-linking, along with insertion of new material. We define the rate constants of severing and cross-linking to be k_{off} and k_{on}, respectively. New glycan monomers are inserted between existing cross sections at a net rate R_{I} (which includes insertion and turnover). Insertion can occur in one of two ways, the new dissacharide can be inserted either with or without cross-linking it to a neighboring strand. We, therefore, define f to be the fraction of new monomers that are inserted and cross-linked simultaneously. The fraction of peptide bonds then obeys the kinetic equation,

$$\frac{\partial\psi}{\partial t} = -k_{\text{off}}\psi + k_{\text{on}}(1-\psi) + \frac{fR_{\text{I}}}{N}. \tag{2}$$

The physics and kinetics just described hold for the cross-linking and strain between any two cross sections. Insertion

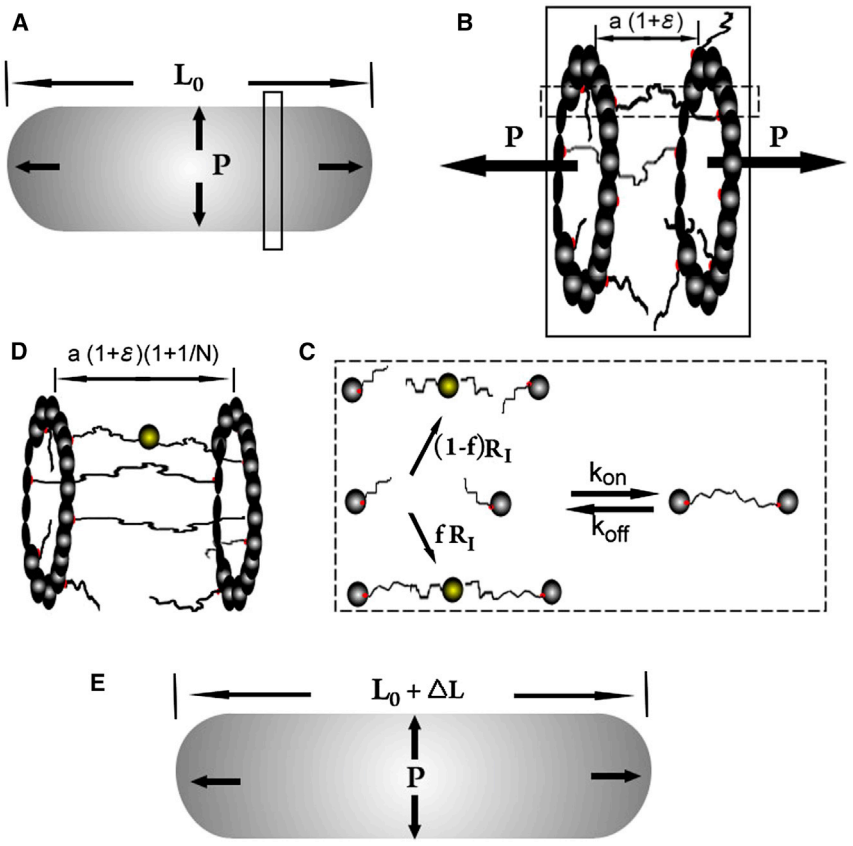

FIGURE 1 Schematic of bacterial growth. (*A*) A bacterium of initial length, L_0, is inflated by the turgor pressure, P. (*B*) The glycan strands are assumed to form circumferential hoops about the cell that are connected by peptide chains (*thin lines*) that are strained an amount ε by the turgor pressure. (*C*) Cell wall synthesis includes severing and re-cross-linking of peptides and insertion of new disaccharide units, which build new cross sections between existing ones (*D*). (*E*) Insertion of new material, therefore, leads to lengthening of the bacterium.

of new dissacharide units between existing cross sections also leads to an increase in the number of cross sections. Because there are $4N$ dissacharides per cross section, the time rate of change of the cross sections is $\partial X/\partial t = R_I X/4N$.

To finish our description of a single growing cell, we need to define the local insertion rate, R_I. It is important to note that this insertion rate is the rate that new material is inserted between a given cross section. The cell-level insertion rate is then XR_I. Bacterial cell wall synthesis involves the production of precursor molecules and enzymes in the cytoplasm. The precursor molecules are then transported across the inner membrane in a lipid-bound form and are then incorporated into the existing PG structure by enzymes, such as the penicillin-binding proteins (4). Transport of the precursor molecules across the membrane is believed to occur at a sufficient rate to match the precursor production rate (4). We, therefore, expect that the transport rate should be proportional to the concentration of precursor molecules times the surface area of the cell, and the local insertion rate (which is the insertion rate per length) should be proportional to the flux of precursors out of the cytoplasm, which depends on the density of the precursor molecules, the cell circumference, and the number of available insertion sites. Recent experiments and modeling have shown that ribosome production, protein production, and the replication rate of bacteria are tightly coupled (19). Indeed, these results suggest that the total number of molecules of a given protein in the cell should be proportional to $e^{\lambda t}$, where λ is the replication rate (19). Note that our model makes no assumption on what sets the replication rate. This rate is likely determined by the nutrient capacity of the organism in the environment (19). The cell volume is proportional to XN^2, and the cell circumference is proportional to N. Therefore, the flux of precursor molecules out of the cytoplasm is $\lambda p_0 e^{\lambda t}/XN$, where p_0 is a constant that is proportional to the initial concentration of the precursors. We assume that new strands can only be inserted between cross sections at locations that are not currently cross-linked. Therefore, the insertion rate should be $R_I = K_0 \lambda p_0 e^{\lambda t}(1-\psi)/X$, where K_0 is a rate constant. The elongation of the cell is then dictated by the coupled set of equations for the number of cross sections and the fraction of bound cross-links:

$$\begin{aligned} \frac{\partial X}{\partial t} &= \frac{K_0 \lambda p_0 e^{\lambda t}(1-\psi)}{4N} \\ \frac{\partial \psi}{\partial t} &= -k_{\text{off}}\psi + k_{\text{on}}(1-\psi) + \frac{fK_0 \lambda p_0 e^{\lambda t}(1-\psi)}{NX}. \end{aligned} \tag{3}$$

The model described so far considered a single growing cell that does not divide. For cells that are growing in length while replicating and dividing, the exponential rate of division can balance elongation and lead to a well-defined average length of the cells in the population. This average length can be determined by the total length defined previously divided by the total number of cells, $\overline{L} = L/M = aX(1+\varepsilon)/M$, where $M = M_0 e^{\lambda t}$ is the total number of bacteria.

RESULTS AND DISCUSSION

We begin by examining the behavior of the model for a single growing cell that does divide. It is straightforward to show that for timescales much longer than the replication rate, the elongation model (Eq. 3) predicts that the number of cross sections and the cross-link fraction should be

$$\begin{aligned} X &\approx \frac{K_0 p_0 e^{\lambda t}(1-\psi)}{4N} \\ \psi &= \frac{k_{\text{on}} + 4f\lambda}{k_{\text{on}} + k_{\text{off}}}. \end{aligned} \tag{4}$$

Therefore, the length of the cell grows exponentially in time as

$$L = a(1+\varepsilon)X = \frac{aK_0 p_0 e^{\lambda t}}{4N}(1-\psi)\left(1 + \frac{\pi R^2 P}{akN\psi}\right). \tag{5}$$

This result is consistent with the finding that when septation is blocked (such as by inhibiting FtsZ, the protein that initiates the cytokinetic ring) the length of rod-shaped bacteria increases exponentially in time (20).

For a population of bacteria, the initial amount of precursor molecules, p_0, is proportional to the initial number of cells, M_0. Therefore, our model predicts that the average length of a population of cells is

$$\overline{L} = \frac{K}{N}(1-\psi)\left(1 + \frac{\pi R^2 P}{akN\psi}\right), \tag{6}$$

where $K = aK_0 p_0/4M_0$. Note that the ratio p_0/M_0 reflects the average number of precursor molecules per cell. Therefore, the factor K is a constant that depends on the average behavior of the population and is not dependent on the initial conditions.

Experimental data suggest that the average cell length of *E. coli* and *Bacillus subtilis* increases with the division rate (13–15), whereas the degree of cross-linking decreases (7). (The width also increases, but modeling that effect is outside the scope of our simple model.) The force balance equation (Eq. 1) gives that the strain in the cell wall is only a function of the turgor pressure and the fraction of cross-links. Therefore, at constant turgor pressure, the average length of the cell only depends on the degree of cross-linking, which can be determined by Eq. 4. If severing and/or cross-linking of the peptides during cell wall synthesis are mediated by a multienzyme synthesis complex, we would then expect that the rates k_{off} and k_{on}, respectively, would be proportional to the insertion rate and would, therefore, depend, directly or indirectly, on the growth rate. We, therefore, consider four scenarios: A), severing and cross-linking are independent

of insertion (k_{off} and k_{on} are constant); B), severing; or C), cross-linking is linked to insertion (k_{off} or k_{on}, respectively, are linearly functions of λ); and D), both rates are proportional to the division rate. Note that the rate constants may also depend on the stress or strain, but here, for simplicity, we ignore these effects.

From Eqs. 4 and 6, one way to get an increase in length with the division rate is if k_{off} alone is dependent on λ (i.e., Scenario B) (Fig. 2 *A*). This naturally leads to the result that the degree of cross-linking will decrease with the division rate (Fig. 2 *B*). This mechanism suggests that severing and insertion are linked together during synthesis; however, cross-linking is independent. Basically, as the cell grows faster, severing occurs more rapidly, whereas the rate of re-cross-linking remains fixed. Consequently, faster replicating cells have a lower fraction of bound peptides, and there is a larger strain in the existing peptide cross-links. Another mechanism that would also work is if $k_{on} = A - B\lambda$, where A and B are positive constants. This mechanism assumes that peptide cross-linking decreases as the replication rate increases, which also suggests that peptide cross-linking does not occur simultaneously with insertion. We favor the first mechanism, as it seems more likely that a bacterium would sever old cross-links to insert new material, rather than inserting new material at locations that have not yet been cross-linked.

To validate this model, we set $k_{off} = k_a\lambda + c$, where k_a and c are constants, and compare the results of Eq. 6 to the experimental measurements of *E. coli* length versus division rate given in (13,14) (Fig. 2 *A*). Our model depends on four parameters, a dimensionless pressure, $\pi R^2 P/akN$, and the ratios k_{on}/k_a, c/k_a, and $4f/k_a$. We find good agreement between our model and the experimental results when $\pi R^2 P/akN \sim 0.3$, $k_{on}/k_a \sim 3.5$, $c/k_a \sim 0.5$, and $4f/k_a \sim 0.4$. These parameters suggest that ψ is on order between 0.7–0.8. Because our model only considers the peptides that are parallel to the long axis of the cell (i.e., half the total peptide chains), we predict that the degree of cross-linking is around 35–40%, which is consistent with experimental measurements in *E. coli* (7). In addition, our model predicts that the strain in the cell wall should be ~50%, which is consistent with the decrease in length that is observed upon osmotic shock (21,22).

From Eq. 1, the effective Young's modulus for the cell wall is $E = akN\psi/2\pi Rl$, where l is the thickness of the PG, which is ~4.5 nm for *E. coli* (23,24). Measurements of the Young's modulus in *E. coli* suggest that $E \sim 25$ MPa (23,24). Therefore, our model predicts that the turgor pressure is $P \sim 3lE/5R\psi \sim 0.2$ MPa, which falls in the range of a number of experimentally based estimates (21,24).

As described, our model assumes that the number of disaccharide subunits about the circumference of the cell is fixed. This assumption may not be entirely valid as cell width also increases with the replication rate. However, cell width is less affected by cell growth than cell length. Using a linear function to fit the previous data on cell length and width as a function of replication rate that was reported in (14) suggests that $L = L_0(1 + 0.42\lambda)$ and $W = W_0(1 + 0.19\lambda)$. Therefore, a 42% change in length only corresponds with a 19% change in cell width (the width changes by ~50% over the range of replication rates in the experimental data, whereas the length changes by a factor of 2). In addition, it is not clear how much of this change in width is due to an increase in the number of disaccharides about the circumference and how much is due to additional strain in the cell wall due to a reduction in the PG elasticity. It is then likely that the number of disaccharides about the cell circumference (i.e., N) does not change significantly with the replication rate.

Here, we have developed a simple model for bacterial cell wall synthesis that couples the biochemistry of synthesis with the biomechanics of the cell wall. The model reproduces the observation that the average cell length in a population increases roughly linearly with the replication rate of the bacteria. This result is a consequence of the increase in the number of unbound peptides. Decreasing the number of bound peptides does two things. First, it reduces the Young's modulus for the cell, and, therefore, the turgor pressure further strains the cell wall. Second, it increases the number of sites where new strands can be inserted, which increases the rate that new material is incorporated into the cell wall. For the model to predict an increase in unbound peptides

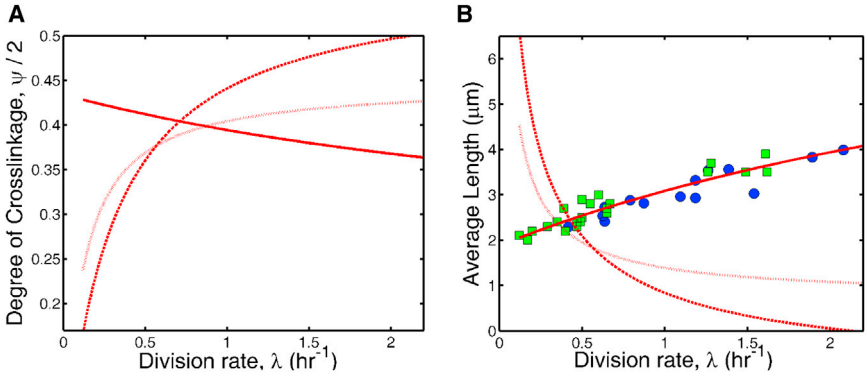

FIGURE 2 Mean cell length only increases with division rate for Scenario B (*solid red line*), which is consistent with the experiments on *E. coli* (data taken from (13) (*blue circles*) and (14) (*green squares*)). Scenarios C (*dashed line*) and D (*dotted line*) led to decreases in length with division rate. (*B*) The degree of cross-linking decreases for Scenario B (*solid line*), but increases for the other scenarios. The parameter values are as given in the text.

requires that severing and insertion are coupled and independent of re-cross-linking. This result is not consistent with the 3-for-1 model of synthesis that suggests that all three processes occur simultaneously (4). It should be possible to test this model by osmotically shocking cells that are growing at different rates to determine how strain in the cell wall depends on the rate of division.

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