Mechanical feedback promotes bacterial adaptation to antibiotics

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Abstract

To maximize their fitness, cells must be able to respond effectively to stresses. This demands making tradeoffs between processes that conserve resources to promote survival, and processes that use resources to promote growth and division. Understanding the nature of these tradeoffs and the physics underlying them remains an outstanding challenge. Here we combine single-cell experiments and theoretical modelling to propose a mechanism for antibiotic adaptation through mechanical feedback between cell growth and morphology. Under long-term exposure to sub-lethal doses of ribosome-targeting antibiotics, we find that *Caulobacter crescentus* cells can recover their pre-stimulus growth rates and undergo dramatic cell shape changes. Upon antibiotic removal, cells recover their original forms over multiple generations. These phenomena are explained by a physical theory of bacterial growth, which demonstrates that an increase in cell width and curvature promotes faster growth under protein synthesis inhibition. Shape changes thus make bacteria more adaptive to surviving antibiotics.

How bacteria adapt their growth and biochemical resources to proliferate in a wide variety of environmental conditions is a fundamental question of long standing interest, and of great consequence to human health. Robust bacterial growth implies cellular control mechanisms that couple gene expression with growth rate [1] and cell shape with division control [2]. Maintenance of cell shape and size [3] facilitates adaptation to the local environment [4, 5], which is critical for optimal

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growth [6]. Understanding cellular control mechanisms for antibiotic adaptation is of particular interest due to the growing threat of antibiotic resistance. Recent studies have shown that cell populations that lack a robust mechanism for cell size and division control have heightened antibiotic susceptibility [7], suggesting that cell shape and division are possible therapeutic targets [8].

While previous studies have largely focused on the effect of antibiotics on bacterial gene expression, mutation, and the fraction of surviving cells [1, 9–14], how individual bacterial cells adapt their growth and division dynamics to antibiotic stresses is not well understood. Recent studies have shown that bacterial cell size and shapes are dramatically altered under antibiotic induced perturbations to DNA [15], cell-wall [15, 16] and ribosome biosynthesis [17, 18]. In particular, it has been shown that *Escherichia coli* and *Caulobacter crescentus* cells reduce their surface-to-volume ratios to become spherical with increasing concentration of Chloramphenicol (ribosome-targeting antibiotic) and Fosfomycin (peptidoglycan synthesis inhibitor) [18], indicating a strong coupling between cellular growth rate and surface-to-volume ratio [19]. We have previously found that in the case of *Caulobacter crecentus*, cell shape is a strong predictor of growth and division control [20, 21]. These findings then raise the question of how the feedback between cell shape and growth could facilitate bacterial adaptation to antibiotics.

The results of the present study leads us to propose that a mechanical feedback between cell growth and shape promotes bacterial adaptation to antibiotics. We find that single *Caulobacter crescentus* cells exhibit dramatic shape and size changes over multiple generations in response to the application and removal of antibiotic stresses that inhibit protein translation. To explain this adaptive response, we develop a model for bacterial growth in which a competition between the growth of cell surface area and mechanical stresses determines the rate of cell elongation. The model predicts that under the inhibition of surface area growth, cells undergo an immediate reduction in growth rates. Over longer time scales ($\sim$10 generations) during stressed conditions, cells are able to recover their pre-stimulus growth rates through increases in cell volume and curvature. We confirm these predictions using our statistically large dataset for cell shape and growth rate under time-varying antibiotic perturbations, and show that cell shape changes are reversible upon antibiotic removal. Taken together, our study demonstrates that physical features of cells can provide feedback control for adaptation to growth perturbations.

Antibiotic adaptation occurs via cell shape changes

To investigate cellular response to antibiotic stress, we examined the growth and shape dynamics of single *Caulobacter crescentus* cells in the presence of chloramphenicol using a high-throughput plat-

form that integrates microfluidics, phase-contrast microscopy, and image analysis [20–23] (Fig. 1a). The bacterium *C. crescentus* is an asymmetrically developing organism, whose cell division results in a replication-competent adherent stalk cell, and a motile swarmer cell. We used a genetically modified strain of *C. crescentus* that enables us to control whether cells stick to surfaces. After establishing the initial population of stalked cells, we prevent subsequent generations from sticking so that we can image growth and division under constant conditions without crowding [23]. Chloramphenicol (CHL) is a broad-spectrum antibiotic that targets ribosomes and inhibits protein translation, leading to a monotonic reduction in cell growth rate with increasing drug concentration [1]. The minimum inhibitory concentration (MIC) of CHL is estimated to be 0.7 μg/ml for *C. crescentus* in PYE (Fig. 3e). Upon application of a step stimulus to a low dose of CHL (0.1 μg ml^{-1}), *C. crescentus* stalked cells underwent an immediate reduction in growth rate, κ (Fig. 1c-d). Here, growth rate κ is defined for each individual growth generation as $L(t) = L(0)e^{\kappa t}$, where $L(t)$ is the cell midline length at time t (Fig. 1b). Growth rate κ for each cell recovered to close to its pre-stimulus value, over longer times ($\sim$10 generations) in the presence of 0.1 μg ml^{-1} CHL (Fig. 1c). This long-term adaptive response of cell growth to antibiotic stimulus is clearly evident in the ensemble-averaged dynamics of κ, and the interdivision times, τ, as functions of individual cell generations (Fig. 1d-e). The interdivision times increased proportionally with κ^{-1} before recovering to their pre-stimulus values (Fig. 1e). As a result, $\kappa\tau$ remained invariant throughout the course of the experiment (Fig. 1f). At a higher antibiotic concentration (0.5 μg ml^{-1}), $\kappa\tau$ remained constant within the error bars, while κ and τ did not recover to their pre-stimulus values (Fig. 1d-e, Extended Data Fig 1).

To further quantify cellular-scale response to antibiotic stimulus, we measured the dynamics of bacterial cell shape, quantified by its midline length, L, radius of curvature, R, and the cross-sectional width, w (Fig. 1b) [20]. Cell length at birth, $L(0)$, showed negligible change upon antibiotic application (Fig. 1g, Extended Data Fig 1c), with $8 \pm 4\%$ change at 0.1 μg/ml and $7.1 \pm 6.9\%$ change at 0.5 μg/ml CHL. The correlation between cell length at birth and at division, $L(\tau)$, was well described by the relation [21]: $L(\tau) = 1.1L(0) + 1.75$ μm (Extended Data Fig. 1d). The correlation between $L(\tau)$ and $L(0)$ did not change with CHL concentration, and remained invariant before and after the application of CHL, indicative of an invariant homeostatic value for $L(0)$. However, unlike cell length, cell curvature underwent large irreversible changes (Fig. 1a,i), with $34 \pm 6\%$ change at 0.1 μg/ml and $110 \pm 7\%$ change at 0.5 μg/ml. Cell width changed by $2 \pm 0.5\%$ at 0.1 μg/ml, and by $29 \pm 3\%$ at 0.5 μg/ml of CHL (Fig. 1h). These data indicate a feedback between cell shape and growth rate, such that post-stimulus recovery of κ is accompanied

by a concomitant increase in cell curvature and cross-sectional diameter.

While previous studies have provided evidence for the dependence of bacterial cell shape on growth rate [18, 24–28], the present results provide the first quantitative evidence for the correlation between cell shape and antibiotic adaptation. In the context of our study, we interpret *adaptation* as the tendency for cells to maintain their activity growth rate in response to step changes in external stimulus (antibiotic concentration) [29]. To explain how the combination of cell growth, division, and morphology promotes adaptive stress response, we developed a physical model, where a competition between mechanical energy for cell shape maintenance and chemical energy for cell wall assembly determines the driving forces for cell growth and shape dynamics.

Mechanical feedback promotes adaptive growth

Our model for a growing bacterial cell is based on a Lagrangian formulation of bacterial shape dynamics [20, 30, 31], specified by N shape parameters q_i $(i = 1...N)$, and the velocities dq_i/dt. During cell cycle progression, each shape parameter q_i evolves according to the equation of motion (Supplementary Note 1):

$$\eta_i \frac{1}{q_i}\frac{dq_i}{dt} = -\left(\frac{1}{hA}\right) q_i \frac{\partial E}{\partial q_i}\,, \tag{1}$$

where $q \equiv \{L, R, w\}$, $E(\{q_i\})$ is the free energy of the cell envelope, η_i is an effective viscosity parameter, A is the cell surface area, and h is the thickness of the cell envelope. As shown in Fig. 1b, the geometry of *Caulobacter crescentus* is described by the length of the midline axis, L, the radius of curvature, R, and the radius of cross-section $r = w/2$. The energy function is then given by (Supplementary Note 1),

$$E = -\varepsilon A - PV + \frac{k_L}{2}\int_0^L dL\left(\frac{1}{R-r} - \frac{1}{R_0}\right)^2 + \frac{k_c}{2}\int dA\left(\frac{1}{r} - \frac{1}{r_0}\right)^2\,, \tag{2}$$

where ε is the effective chemical potential for cell surface growth, P is the turgor pressure, and V is the cell volume. During steady-state growth, *C. crescentus* cells elongate in length, while maintaining a constant curvature and width [20]. To constrain cell shape, we include longitudinal and circumferential bending energies in the energy function, with k_L and k_c defining the longitudinal and circumferential bending stiffnesses of the cell envelope. R_0 is the preferred radius of curvature of the cell midline axis and r_0 is the preferred radius of cross-section. Since CHL inhibits translation by inactivating ribosomes, it inhibits the synthesis of all proteins including those making new cell wall materials. It has been previously reported that CHL non-linearly reduces cell growth rate [14]. We modelled this effect using the following form for the chemical potential: $\varepsilon = \varepsilon_0/(1+\phi\Theta(t-t_a))$

(Fig. 2a), where t_a is the time of antibiotic application, ϕ is a monotonically increasing function of antibiotic concentration (Fig. 3e-inset), and Θ is a Heaviside function. This functional form for ε quantitatively captures the dependence of growth rate on CHL concentration (Fig. 3e), with a half-inhibitory concentration $\mathrm{IC}_{50} = 0.42$ μg/ml.

To demonstrate the mechanics of adaptive growth via cell shape changes, we first consider the simplified limit when the radius of the cell cross-section is constant ($r = r_0$). In this limit, exponential elongation of cell length is given by

$$\frac{dL(t)}{dt} = \kappa(R,t)L(t) \ , \tag{3}$$

where $\kappa = -L(\partial E/\partial L)/2\eta_L \pi r h$, the longitudinal growth rate, is a function of cell shape (see Methods). The dynamics of R are described by

$$\frac{dR(t)}{dt} = \frac{R(t)}{h\eta_R}\left[\varepsilon(t) - g(R,t)\right] \ , \tag{4}$$

where, $g = -(\partial E/\partial R + \varepsilon)/Lr$. As a consequence of Eqs. (6) and (8), R evolves to reach a steady-state value determined by the minimum of the energy E (Fig. 2b, grey curve), which depends on the chemical potential ε. Therefore, reducing ε via antibiotics shifts the energy minimum to a new steady-state with increased curvature (Fig. 2b, blue curve). This mechanochemical coupling underlies a built-in adaptive response of the cell. Reducing ε to a value $\varepsilon/(1+\phi)$ results in an initial sharp drop in κ (Fig. 2c-d). Reduction in ε increases R^{-1} to a new steady-state given by the minimum of the shifted energy $E - \phi\varepsilon/(1+\phi)$. As a result, κ recovers close to its pre-stimulus value and the cell resumes fast growth (Fig. 2c-d).

In our model, the feedback between growth rate and curvature can be intuitively understood from the following mechanical argument (Fig. 2d). A reduction in chemical potential reduces the rate of addition of new cell surface material, leading to an initial fast drop in growth rate. The reduced rate of surface area addition also reduces the effective growth pressure working against the compressive bending forces acting on the cell surface (Eq. (2)). As a result, reduced chemical potential leads to further cell wall bending, until a new mechanical equilibrium is reached with lower tension and a higher curvature. This restores the growth rate to its pre-stimulus value (Fig. 2d). The growth-curvature feedback is supported by data showing that the intergenerational change in growth rate is positively correlated with curvature (Fig. 2e). When the feedback loop between growth rate and curvature (Fig. 2d, right) is broken in the model, growth rate does not show any recovery after CHL application (Fig. 2f, dashed line). This feedback can be further tested in experiments by studying the CHL-induced growth response of rod-shaped crescentin mutants.

By simulating the full dynamic model with variable cell cross-section and curvature, we are able to capture the experimental observations, including the adaptive dynamics of κ (Fig. 2f), and the increase in cell curvature (Fig. 2g), and cell width (Fig. 2h) in response to a step decrease in chemical potential. Achieving the observed increase in cell width necessitates softening the cell bending stiffness in response to CHL, i.e., $k_c \to k_c/(1+\phi)$ (Extended Data Fig. 2). This softening can arise from global inhibition of translation affecting the synthesis of MreB, the Rod system, and penicillin-binding proteins that control cell width [32, 33]. CHL could also modulate turgor pressure via a regulatory response [34, 35]. Our modeling suggests that the experimental data are consistent with a moderate increase in turgor pressure that would increase internal stress and cell width (Extended Data Fig. 3). It is important to note that cell width modulation alone cannot achieve growth rate adaptation under stress (Extended Data Fig. 4).

While mechanical feedback is sufficient to promote growth rate recovery and cell shape changes under antibiotic stress, it does not capture the trend that cell curvature and width undergo a transient increase immediately after drug treatment, followed by a relaxation phase (Fig. 1h-i). This behavior may be a consequence of active feedback mechanisms (e.g., ribosome synthesis, efflux pumps) that act to increase cell growth rate under stress. We therefore consider a model where ε recovers to a value $\alpha\varepsilon_0 + (1-\alpha)\varepsilon_1$, where ε_0 is the pre-stimulus chemical potential and $\varepsilon_1 = \varepsilon_0/(1+\phi)$ (Fig. 2i). For $\alpha = 0$, there is no active feedback, whereas for $\alpha = 1$, ε fully recovers to its initial value (Fig. 2j-k). The latter results in complete recovery in cell shape and growth rate, irrespective of the amplitude of applied stress. However, smaller non-zero values of the active feedback parameter α results in partial recovery in cell shape and growth rate, with non-monotonic changes in cell curvature and width, as observed experimentally. Thus, active feedback mechanisms likely contribute to the adaptive growth response, consistent with the predictions of our biochemical model combining drug transport and binding with cell shape and translational feedback (Supplementary Note 2, Extended Data Fig. 5).

Comparing single-cell simulations to experimental data

While our theory can account for the mechanics of growth homeostasis and cell shape, it does not treat cell division and size control. To compare our model quantitatively with experimental data, we turn to single-cell simulations that allow us to extend the mechanical model to count a division event when cells grow to a size $L(\tau) = aL(0) + \delta$, with $a = 1.1$ and $\delta = 1.75$ [21]. In this model (see Methods for details), the i^{th} cell shape parameter in generation j, q_{ij}, evolves according to the

equation of motion

$$\eta_i \frac{1}{q_{ij}} \frac{dq_{ij}}{dt} = -\left(\frac{q_{ij}}{hA_j}\right) \frac{\partial E}{\partial q_{ij}} , \tag{5}$$

for a step stimulus in chemical potential, $\varepsilon \to \varepsilon/(1+\phi)$, applied at $t = t_a$. We introduce a phenomenological model for the control of division times. Namely, we note that experimentally the relation between cell length at birth, $L_j(0)$, and cell length at division, $L_j(\tau_j)$, is invariant to CHL application (Extended Data Fig. 1d). As a result, we take the interdivision times to be $\tau_j = \kappa_j^{-1} \ln\left(a + \delta/L_j(0)\right)$, where $\kappa_j = L_j^{-1} dL_j/dt$.

So defined, our single-cell model quantitatively captures the experimental data for antibiotic dose of 0.1 μg/ml ($\phi = 0.8$; Fig. 3a), 0.2 μg/ml ($\phi = 1.8$), and 0.5 μg/ml ($\phi = 3.0$). The simulated cell growth rate drops sharply at $t = t_a$, followed by slow recovery for $t > t_a$ (Fig. 3a). As ϕ increases, the accuracy of adaptation decreases monotonically (Fig. 3a, Extended Data Fig. 6). Consistent with experimental data, the interdivision time, τ, increases upon application of the growth inhibitory stress, but recovers over tens of generations. However, the adaptation is not perfect. Rather, the percentage change in τ or κ, defined as the percent difference between the post-stimulus and pre-stimulus steady state values, increases with ϕ (Fig. 3a-b, Extended Data Fig. 6a-b). In contrast to τ and κ, cell length recovers (Extended Data Fig. 6a-b) due to the invariance of the parameters of the size control model (Extended Data Fig. 1d). Growth rate adaptation is much weaker in the model with constant curvature, since there is no feedback between cell elongation rate and curvature (Extended Data Fig. 4).

As in the experimental data, average cell curvature does not return to its prestimulus value for all values of ϕ (Fig. 3c). Both cell curvature (Fig. 3c), and cell width (Fig. 3d) increases upon antibiotic stress, with their steady-state values increasing monotonically with ϕ. By calibrating our model parameters with experimental data for cell growth and shape for three different antibiotic concentrations, we determined the dependence of ϕ on CHL concentration (Fig. 3e-inset). This allowed us to compute the bacterial growth inhibition curve by varying ϕ (Fig. 3e), showing the predicted dependence of final growth rate, κ_∞, on CHL concentration. From the growth inhibition curve, we predict that the MIC of CHL acting on *C. crescentus* cells growing in PYE is 0.7 μg/ml. With no additional parameter adjustments, our model quantitatively captures the experimentally reported trend [18] for the percentage change in *C. crescentus* cell curvature and width, at different CHL concentrations (Fig. 3f). Put together, these results indicate that changes in cell shape coordinate the adaptive dynamics of cell growth rate under antibiotic induced stress.

Adaptation to time-varying antibiotic stresses

We also considered responses to finite-duration pulses of antibiotic, to examine if the shape changes were reversible as would be predicted by a model of mechanical feedback. We subjected *C. crescentus* cells growing in PYE medium to three consecutive pulses of chloramphenicol (0.1 and 0.5 μg ml^{-1} concentrations) (Fig. 4a). In response to pulsatory antibiotic stress, both the growth rates (Fig. 4a) and the interdivision times (Fig. 4b) underwent pulsatory changes, fully recovering to their pre-stimulus values when the antibiotic was removed for both concentrations studied. Concomitantly with the changes in growth rates and interdivision times, the cells underwent pulsatory shape changes (Fig. 4c). Our simulations quantitatively capture these behaviors (Fig. 4d-f, Extended Data Fig. 7). Interestingly, both simulated and experimental data show a memory effect (Fig 4a,d), such that κ decreases progressively less during each successive antibiotic pulse. This is accompanied by a decrease in peak cell curvature and width (Fig 4c,f), indicating the coupling between antibiotic tolerance and cell shape. The memory effect arises in the model due to disparate timescales for recovery in chemical potential and relaxation of cell shape parameters upon antibiotic stress release.

Discussion

Cells harness feedback control to survive and thrive in varying environments [36]. Biochemical networks have been shown to provide this feedback and thus enable adaptation to perturbations [29, 37, 38]. The present work demonstrates that physical features of cells can also provide feedback control. In particular, we propose that a competition between the mechanical energy associated with cell shape and the chemical energy associated with addition of cell surface material enables adaptation of growth rate and interdivision time of bacterial cells (Fig. 2). The ability of cells to maintain a homeostatic growth rate under perturbations arises in our model from a negative feedback between cell growth rate and mechanical stress at the cell surface (Supplementary Fig. 1), consistent with recent phenomenological models of feedback between outer membrane tension and cell elongation rate [39]. By comparing our theoretical predictions against single-cell experiments on *C. crescentus* under long-term exposure to ribosome-targeting antibiotics, we establish that bacteria can recover their pre-stimulus growth rates by increasing their cell curvatures and widths (Fig. 3). Furthermore, the cells anticipate successive antibiotic pulses (Fig. 4) and retain memory of growth inhibition.

Our theory for cell growth and shape control has broad applicability beyond predicting cellular response to chloramphenicol. To demonstrate this, we used our model to predict the dynamics

of cell growth and morphology under translation inhibition (Extended Data Fig. 5), osmotic shocks (Supplementary Fig. 1), nutrient shifts (Supplementary Fig. 2), inhibition of peptidoglycan synthesis (Supplementary Fig. 3). Under nutrient shifts we find that cell volume increases and surface-to-volume ratio decreases with increasing nutrient-specific growth rate of the medium [18, 40–42] (Supplementary Fig. 2). These data are in agreement with the phenomenological *nutrient growth law* [43] that cell volume increases with nutrient-specific growth rate (Supplementary Fig. 2e). Further experiments manipulating cellular mechanics are needed to test theoretical predictions about the role of cell mechanical properties in growth rate adaptation.

Our physical model naturally leads to the phenomenological surface/volume model proposed recently by Harris and Theriot [18]. We derive that cell surface area is produced at a rate proportional to cell volume (Supplementary Note 1), where the volume-specific surface synthesis rate depends on cell shape, surface mechanical stress, growth rate, as well as the chemical potential for area synthesis. When peptidoglycan synthesis is inhibited (e.g., by Fosfomycin) we expect a reduction in both chemical potential and surface stress, leading to the maintenance of a homeostatic growth rate (Supplementary Fig. 3). In addition, a softer cell wall promotes a larger area synthesis rate, leading to an increase in cell diameter.

Increases in average cell diameter and cell length in response to CHL have been reported in *C. crescentus* [18] and *E. coli* [17, 18]. Other studies have reported invariance of average cell volume [44] and aspect ratio [28] with increasing CHL concentration. Here, we report increases in cell diameter and curvature in the presence of CHL, associated with long-term changes in cell growth rate and their ability to adapt. This raises the question of why specific shape changes may be beneficial for antibiotic tolerance. First, an increase in cell volume via changes in cell width should lead to dilution of intracellular antibiotic molecules at a rate proportional to the cell growth rate. Second, lowering surface-to-volume ratio should reduce antibiotic influx through the cell surface leading to a further dilution of intracellular antibiotic concentration, for a given cell size. This hypothesis is consistent with predictions of our biochemical model that combines drug transport and binding with cell shape and ribosome regulation (Supplementary Note 2). This model predicts that at sub-MIC concentrations of CHL, reduction in cell surface-to-volume ratio could lead to a significant dilution of intracellular CHL concentration (Extended Data Fig. 5). This result suggests a new mechanistic mode of adaptation that bacteria may harness to counter antibiotics, opening doors to future molecular studies into the role of cell shape on antibiotic response.

Data availability

Source data are available for this paper. All other data that support the plots within this paper and other findings of this study are available from the corresponding author upon reasonable request.

Code availability

Custom computer codes that were used in this paper are available from the corresponding authors upon reasonable request.

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Author Contributions

SB, NFS and ARD designed the study. ARD and NFS designed the experiments. SB developed the theory. KL performed experiments; SB, NO and RS performed model simulations. SB, KL, NO and RS analyzed data. SB, NFS and ARD wrote the manuscript.

Competing Interests

The authors declare no competing interests.

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Figure Legends

FIG. 1. **Adaptive growth of *C. crescentus* under antibiotic stress**. (a) Left: A representative phase contrast image of one field of view of *C. crescentus* cells. Right: Magnified images of the yellow highlighted cell, showing a single generation of growth in rich medium (PYE; peptone-yeast extract) and later in the presence of chloramphenicol (CHL). Scale bar represents 1 μm. Time is indicated in hours:minutes since the start of the experiment. (b) Definition of cell shape parameters. (c) Cell length as a function of time for many generations of a single cell (points). A step dose of 0.1 μg ml^{-1} CHL is applied at $t = 450$ min. (d) Recovery curve of cell growth rate, κ, as a function of generations for CHL concentrations: 0.1 μg ml^{-1} (blue, Number of cells $n = 40$, Total number of generations $g = 941$), 0.2 μg ml^{-1} (green, Number of cells $n = 20$, Total number of generations $g = 280$, and 0.5 μg ml^{-1} (red, $n = 135$, $g = 986$). 0.2 μg/ml data is taken from Ref [18]. Generation '0' denotes the first generation after CHL application. (e) Interdivision time, τ, as a function of generation, showing concomitant increase in response to antibiotic, followed by slow recovery. (f) $\kappa\tau$ remains constant irrespective of CHL concentration. (g) Cell length at birth ($L(0)$) (h) spatially averaged and cell cycle averaged cell width (w), and (i) cell-cycle averaged cell curvature ($\langle R^{-1}\rangle$) as a function of generation. Error bars indicate ± 1 standard deviation in cell-to-cell variations.

FIG. 2. **Mechanics of antibiotic adaptation.** (a) Top: A step perturbation to the chemical potential, ε. Bottom: Step increase in ϕ, showing the protocol for antibiotic application. (b) Cell surface energy density, $U = E/\theta$, as a function of the midline curvature, R^{-1}, for $\phi = 0$ and $\phi = 0.42$ with w fixed. (c) Longitudinal growth rate, κ, as a function of R^{-1}. The dashed arrows indicate the pathway to adaptation by relaxation of cell shape to a new energy minimum after antibiotic stimulus. (d) Schematic illustrating the mechanics of antibiotic adaptation by growth-curvature feedback. Arrow thicknesses scale with growth rate. (e) Correlation between change in growth rate, $\Delta\kappa$ (between successive generations), and curvature. (f) Adaptive dynamics of the longitudinal growth rate, κ, for two non-zero values of ϕ, according to Eq. (3). Dashed line indicates growth rate dynamics when the growth-curvature feedback loop is disabled in the model. (g) Dynamics of curvature, $R(t)^{-1}$, relaxing to a higher value post-stimulus, as determined by Eq. (8). (h) Dynamics of the cell width, $w(t) = 2r(t)$, increasing to larger values post-stimulus. (i) Model for active feedback in chemical potential, restoring its post-stimulus value to $\alpha\varepsilon_0 + (1-\alpha)\varepsilon_1$, where $0 \leq \alpha \leq 1$. (j-k) Adaptive dynamics of growth rate, cell curvature and width for different values of the active feedback parameter α, and for $\phi = 1.0$. Other parameter values are the same as (a-h). See Supplementary Table 1 for a list of model parameters.

FIG. 3. **Single-cell simulations reproduce experimentally measured growth and cell shape dynamics in response to antibiotic application**. (a) Population-averaged cell growth rate κ vs. generation in single-cell simulations for: $\phi = 0.8$ (blue, dashed), $\phi = 1.8$ (green, dashed), and $\phi = 3.0$ (red, dashed). Corresponding experimental data are shown in solid circles, with [CHL]=0.1 μg/ml (blue), [CHL]=0.2 μg/ml (green) [18] and [CHL]=0.5 μg/ml (red). Error bars are Standard Error of Mean (SEM). (b-d) Intergenerational dynamics of population-averaged interdivision time τ (b), mean cell curvature (c), and mean cell width (d). Population-averaged model data for different values of ϕ are shown by dashed lines, whereas the experimental data are shown by solid circles. Error bars are SEM. (e) Population-averaged cell growth rate (after long-term antibiotic exposure) vs CHL concentration. Open circle: model data, Solid circles: experimental data. Error bars, SEM. Inset: Dependence of ϕ of CHL concentration after calibrating model to experimental data. Predicted MIC of CHL is ≈ 0.7 μg/ml. (f) Percentage change in cell shape parameters R^{-1} (red) and w (green), as a function of antibiotic concentration post long-term exposure. Experimental data (solid circles) are compiled from our experiments and those by Harris and Theriot [18]. Model data (open circles) are obtained by varying ϕ. Number of cells simulated = 40. See Supplementary Table 2 for a list of simulation parameters.

FIG. 4. **Adaptation to pulsatory antibiotic stress**. (a-b) Experimental data for cell growth rate (a) and interdvision times (b), vs generation number under exposure to three discrete pulses of 0.1 and 0.5 μg ml^{-1} concentrations of chloramphenicol. For 0.1 μg ml^{-1} of CHL, n=22 and $n_g = 1085$. For 0.5 μg ml^{-1} of CHL, n=19 and $n_g = 532$. Error bars indicate ± 1 standard deviation in cell to cell variations. (c) Intergenerational dynamics of mean cell curvature for chloramphenicol concentration 0.1 μg ml^{-1}. Experimental data: blue solid circles, model prediction: orange. (d-e) Simulated data for longitudinal growth rate κ (d), and interdivision time (e) vs generations in growth simulations for different values of ϕ (fractional reduction in chemical potential): $\phi = 0.8$ (blue) and $\phi = 3.0$ (red). See Methods for the determination of model parameters. Model simulations predict full recovery in growth rate after release of antibiotic stress. (f) Cell width vs. generations. Experimental data: blue solid circles, model prediction: orange. Panels (c) and (c) show reversible shape changes upon application and removal of antibiotic stress. Number of cells simulated = 40. See Supplementary Table 2 for a list of model parameters.

Extended Data Figure 1. **Cell shape, size control and growth dynamics during antibiotic adaptation, shown in real time**. (a) Cell elongation rate, κ, as a function of absolute time for CHL concentrations: 0.1 μg ml^{-1} (blue, Number of cells n=40, Total number of generations $g = 941$) and 0.5 μg ml^{-1} (red, $n = 135$, $g = 986$). Error bars indicate ± 1 standard error of mean. (b) Interdivision time, τ, as a function of absolute time. (c) Cell length at birth, $L(0)$, as a function of absolute time. (d) Correlation between cell length at division, $L(\tau)$, and cell length at birth, $L(0)$, is best described by a mixer model: $L(\tau) = 1.1L(0) + 0.75$ μm. (e) Spatiotemporally averaged cell diameter (width), w, as a function of absolute time. (f) Cell-cycle averaged cell curvature, R^{-1}, as a function of absolute time.

Extended Data Figure 2. **Dynamics of cell shape and growth rate in response to mechano-chemical perturbations**. Model predictions for the response of (a) growth rate κ, (b) curvature R^{-1}, and (c) width w, to perturbations in parameters: $\{\varepsilon, k_c\}$ (blue), $\{\varepsilon\}$ (green), $\{\varepsilon, k_L\}$ (purple), $\{\varepsilon, k_c, k_L\}$ (red), and $\{\varepsilon, k_c, P\}$ (black). Perturbation to a particular parameter μ is of the form $\mu \to \mu/(1+\phi)$ for $t > t_a$, where $\mu \in \{\varepsilon, k_c, k_L, P\}$. Comparing to experimental results (Figure 1), translation inhibitory antibiotics likely affect parameters ε and k_c. Perturbation to turgor pressure P is qualitatively similar to perturbing ε.

Extended Data Figure 3. **Effect of turgor pressure on cellular response to chloramphenicol**. Intergenerational dynamics of (a) growth rate κ, (b) average cell width w, (c) average curvature R^{-1} and (d) length at birth $L(0)$ in response to a step pulse of 0.1 μg/ml CHL applied at $t = 450$ min for three different cases – turgor pressure remains unchanged (blue solid circles), turgor pressure is reduced by 25% by CHL (red solid circles), and turgor pressure is increased by 25% by CHL (green data points). Turgor pressure reduction leads to a decrease in cell diameter, inconsistent with experimental data. Moderate increase in turgor pressure is consistent with experimental data.

Extended Data Figure 4. **Cell width modulation alone is not sufficient to achieve growth rate adaptation**. Intergenerational dynamics of (a) growth rate κ, (b) average cell width w, and (c) average curvature R^{-1} in response to a step pulse of 0.1 μg/ml CHL applied at $t = 450$ min for two different cases – Cell curvature is variable and adapts to CHL-induced growth inhibition (blue data points), and curvature is constant and not affected by CHL (red data points). In the absence of curvature modulation, adaptive response is much weaker.

Extended Data Figure 5. **Coupling the physical model for bacterial growth with a biochemical model for chloramphenicol-ribosome interactions.** (a) Schematic of the biochemical pathway of ribosome-CHL interaction. CHL with extracellular concentration $a_{\rm ex}$ enters the cell with net flux proportional to $(P_{\rm in}a_{\rm ex} - P_{\rm out}a_{\rm in})A/V$ where $P_{\rm in}$ and $P_{\rm out}$ are the inward and outward permeabilities of the cell envelope. CHL binds to ribosomes at a rate $k_{\rm on}$ and unbinds with a rate $k_{\rm off}$. Growth rate is linearly proportional to the fraction of unbound ribosomes. Ribosomes upregulate their synthesis when a fraction of them are bound to CHL. Model A: No mechanical feedback between cell shape and growth rate. Model B: Cell elongation promotes an increase in surface stress σ which in turn inhibits growth rate. (b-f) Intergenerational dynamics of (b) growth rate κ, (c) intracellular CHL concentration $a_{\rm in}$, (d) concentration of active ribosomes, (e) average cell width w, and (f) average curvature R^{-1} in response to a step pulse of 0.1 μg/ml CHL applied at $t = 450$ min for Model A (blue) and Model B (red). (g) Cell shape evolution simulated using Model B (time progression: left-to-right and top-to-bottom), shows antibiotic dilution. Color coding indicates the intracellular concentration of CHL.

Extended Data Figure 6. **Speed-accuracy tradeoff in antibiotic adaptation**. (a) Adaptation error (post-stimulus recovery error %) for κ, R, w and L as a function of antibiotic stress, ϕ. (b) Rate of adaptation (in units of generation^{-1}) as a function of ϕ. (c) Trade-off between adaptation speed (defined as the rate of recovery) and adaptation accuracy (defined as 100-Error%).

Extended Data Figure 7. Quantitative comparisons between single-cell simulations and experimental data for pulsatory chloramphenicol dose. (a-b) Cell growth rate κ (a) and interdivision time τ (b) upon application of a step dose of 0.1 μg ml^{-1} chloramphenicol. Blue: experimental data, Orange: Simulation data with $\phi = 0.8$. (c-d) Cell growth rate (c) and interdivision time (d) for a pulsatile antibiotic dose of 0.5 μg ml^{-1}. Blue: experimental data, Orange: Simulation data with $\phi = 3.0$. Error bars indicate ± 1 standard deviation.

Methods

Acquisition of Experimental Data. As described in [20, 23], the inducibly-sticky *Caulobacter crescentus* strain FC1428 was introduced into a microfluidic device and cells were incubated in the presence of the vanillate inducer for one hour. The microfluidic device was placed inside a home-made acrylic microscope enclosure ($39'' \times 28'' \times 27''$) equilibrated to 31°C (temperature controller: CSC32J, Omega and heater fan: HGL419, Omega). At the start of the experiment, complex medium (peptone-yeast extract; PYE) was flowed through the channel at a constant rate of 7 μL/min using a synringe pump (PHD2000, Harvard Apparatus), which flushed out non-adherent cells. We initially imaged cells in medium without chloramphenicol to measure the drug-free growth rate and cell shape. We then switched to medium with chloramphenicol (concentration in the range 0.1-0.5 μg/ml) at 450 min. Phase-contrast images were acquired using a microscope (Nikon Ti Eclipse with perfect focus system) and robotic XY stage (Prior Scientific ProScan III) under computerized control (LabView 8.6, National Instrument). Images were acquired at a magnification of 250X (EMCCD, Andor iXon+ DU888 1k $\times$ 1k pixels; objective, Nikon Plan Fluor 100X oil objective plus 2.5X expander; lamp, Nikon C-HFGI) and a frame rate of 1 frame/min.

Cell Shape Analysis. Phase contrast images of single *C. crescentus* cells were processed with a pixel-based edge detection algorithm that applied a local smoothing filter, followed by a bottom-hat operation [20]. We identified the boundary of each cell by thresholding the filtered image. Individual cell contours were constructed by interpolating a smoothing B-spline through the boundary pixels. Each identified cell was then tracked over time to construct the full time trajectory for its growth and division cycle over consecutive generations. We applied a minimal amount of filtering for each growth curve to remove spurious points, such as those arising from cells touching or twisting out-of-plane. We manually checked the timing of each cell division to ensure that the precision in determining the inter-division times results from the frame rate and not limitations of the automated image analysis. For the phase contrast images of *C. crescentus* cells obtained from Harris and Theriot [18], we also used the Fiji plug-in MicrobeJ [45] to extract the cell midline

lengths at birth and division, cell width profile and the midline curvatures at the mid focal plane. Data were aligned in time based on the time point when chloramphenicol was added, and mean ± SD (or SEM) were calculated for different cells (Fig. 1).

Mathematical model for cell growth and shape dynamics. As described in the main text, the geometry of a growing *C. crescentus* cell is parameterized by the length of the cell's midline axis L, radius of curvature R, and radius of cross-section r. During each growth cycle, the dynamics of length L is given by

$$\frac{dL}{dt} = \kappa(R, r)L \ , \tag{6}$$

where the longitudinal growth rate κ is given by

$$\kappa(R, r) = \left[\frac{Pr}{2} + \varepsilon - \frac{k_L}{4\pi}\left(\frac{1}{r} - \frac{1}{R}\right)\left(\frac{1}{R-r} - \frac{1}{R_0}\right)^2 - \frac{k_c}{2}\left(\frac{1}{r} - \frac{1}{r_0}\right)^2\right] / h\eta_l \ , \tag{7}$$

Radius of curvature evolves in time as,

$$\frac{dR}{dt} = \frac{R}{h\eta_R}\left(\frac{Pr}{2} + \varepsilon - g_R(R, r)\right) \ , \tag{8}$$

where,

$$g_R(R, r) = \frac{k_L}{4\pi r}\left(-\frac{1}{(R-r)^2} + \frac{1}{R_0^2}\right) + \frac{k_c}{4}\left(\frac{1}{r} - \frac{1}{r_0}\right)^2 \ .$$

Dynamics of the cell's radius of cross-section is given by,

$$\frac{dr}{dt} = \frac{r}{h\eta_r}\left(\frac{Pr}{2} + \varepsilon - g_r(R, r)\right) \ , \tag{9}$$

with,

$$g_r(R, r) = -\frac{k_L}{4\pi R}\left(\frac{1}{R-r} - \frac{1}{R_0}\right)^2 + \frac{k_L}{4\pi R(R-r)}\left(\frac{1}{R-r} - \frac{1}{R_0}\right) - \frac{k_c}{2r}\left(\frac{1}{r} - \frac{1}{r_0}\right) + \frac{k_c}{4}\left(\frac{1}{r} - \frac{1}{r_0}\right)^2 \ .$$

Model parameters. For the simulations shown in Fig. 2, we assume that the simulated cell is in a steady-state exponential growth phase before the antibiotic stress is applied at $t = t_a$. The initial conditions are $\varepsilon(t < t_a) = \varepsilon_0$, $\kappa(t < t_a) = \kappa_0$, $r(t < t_a) = r_i$, $R(t < t_a) = R_i$, where we take $\kappa_0 = 0.01$ min^{-1}, $R_i = 5$ μm, and $r_i = 0.36$ μm, calibrated from the average growth rate and shape parameters for *C. crescentus* growing in PYE at 31°C. Therefore, for $t < t_a$, the bacterium is subjected to the following constraints:

$$\kappa(r_i, R_i) = \kappa_0 \ , \tag{10}$$

$$\partial E/\partial r|_{r=r_i, R=R_i} = 0 \ , \tag{11}$$

$$\partial E/\partial R|_{r=r_i, R=R_i} = 0 \ . \tag{12}$$

For $t \geq t_a$ we solve Eqs. (8)-(9) subject to the above initial conditions and $\varepsilon(t \geq t_a) = \varepsilon_0/(1+\phi)$. For simplicity, we assume $\eta_R = \eta_r = \eta$, $P = 0.3$ MPa [46], and set $k_L = 1$. With time expressed in min, and length in μm, Force is expressed in units of k_c, such that [Force]=$[k_L]/\mu\text{m}^2$, where square brackets represent the dimensions. The undetermined parameters $\{k_c/k_L, \varepsilon_0/k_L, h\eta_l/k_L\}$ are obtained by solving Eq. (10)-(12) at $t = t_a$. The remaining parameters ϕ, R_0, and r_0 are obtained by multi-parameter fitting of the solutions to equations (6)-(9), to the ensemble averaged time course data for κ, r and R in experiments with CHL concentration 0.1 μg/ml. The resultant set of parameters are listed in Supplementary Table 1.

Single-cell simulations. In Figures 3 and 4, we simulate the stochastic growth and shape dynamics of a total of $n = 40$ cells, for $g = 100$ generations each. We initialize the shape of cell j ($1 \leq j \leq n$) by prescribing its length, L_j, radius of curvature R_j and radius of cross-section r_j, at the start of the first generation ($t = 0$). We draw $L_j(t = 0)$, $R_j(t = 0)$ and $r_j(t = 0)$ from a Gaussian distribution with mean values 2.6 μm, 3.9 μm and 0.365 μm, respectively. The standard deviations of the distributions, σ_l, σ_R and σ_r, are determined from our experimental data for cell shape distribution prior to antibiotic application. These values also set the noise amplitude in the shape equations (Supplementary Note 1). We set the longitudinal growth rate in the first generation equal to mean growth rate: $\kappa_0 = 0.01$ min^{-1}. By simultaneously solving the equations (for $t < t_a$) $\kappa_j = \kappa_0$ (Eq. (7)), $\partial E/\partial R = 0$, and $\partial E/\partial r = 0$ we fix the model parameters, ε/k_L, k_c/k_L and $k_L/h\eta_l$. The undetermined parameters, c_0 (spontaneous curvature), $(h\eta_R)^{-1}$ (rate of curvature relaxation), and $(h\eta_r)^{-1}$ (rate of width relaxation) are obtained by fitting the model predictions for shape dynamics to the experimental data for step stimulus of 0.1 μg ml^{-1} of chloramphenicol (see Extended Data Fig. 7). The parameters are listed in Supplementary Table 2.

In generation k, cell length, L_{jk}, radius of curvature R_{jk}, and radius of cross-section r_{jk} evolve according to Eqs. (6), (8), and (9), respectively. We solve Eqs. (6), (8) and (9) for $t \leq \tau_{jk}$, where τ_{jk} is the division time in the k^{th} generation for the j^{th} cell. Division time in the k^{th} generation is given by,

$$\tau_{jk} = \kappa_{jk}^{-1}\left(a + \frac{\delta}{L_{jk}(0)}\right), \tag{13}$$

such that $L_{jk}(\tau_{jk}) = aL_{jk}(0)+\delta$, assuming a mixer model [21], where parameters a and δ are determined from experimental data (Extended Data Fig. 1d). Cell j divides at a ratio D_{jk} in generation k. Therefore, $L_{j(k+1)}(t = 0) = D_{jk}L_{jk}(t = \tau_{jk})$. We draw D_{jk} from a normal distribution with mean and standard deviation given by, $\langle D_{jk}\rangle = 0.54$ and $\sqrt{\langle (D_{jk})^2\rangle} = 0.04$ [20]. Furthermore,

cell shape parameters in consecutive generations are related as $R_{j(k+1)}(t=0) = R_{jk}(t=\tau_{jk})$, and $r_{j(k+1)}(t=0) = r_{jk}(t=\tau_{jk})$.

We subject the cells to antibiotic stress for $t \geq t_a$. We assume that under antibiotic treatment the chemical potential for growth, ε, reduces in proportion to the concentration of the antibiotic. As a result, the cell responds initially by reducing its growth rate, κ, which is proportional to ε. Therefore, $\varepsilon(t) = \varepsilon_0 / [1 + \Theta(t - t_a)\phi]$, where $0 \leq \phi \leq 1$. We also assume, $k_c(t) = k_c(0) / [1 + \Theta(t - t_a)\phi]$, to capture the effect of increasing width. Under the pulsatile stress protocol, we subject the cells to antibiotic stress for time periods satisfying the condition $t_a + (2x-2)t_d \leq t \leq t_a + (2x-1)t_d$, where t_a is the application time of the first pulse, t_d is the pulse duration and x is the pulse number (starting at $x = 1$ for the first pulse). Chemical potential ε is fractionally reduced by ϕ as in the step pulse model. Upon release of an antibiotic pulse, the chemical potential increases to its original value ε_0 at a rate equal to the cell growth rate κ: $\varepsilon(t) = \varepsilon_2 + (\varepsilon_0 - \varepsilon_2)(1 - e^{-\kappa t})$, where ε_2 is the chemical potential at the time of antibiotic removal. This models the reversibility of chloramphenicol induced effect upon stress removal.