Mechanical strain sensing implicated in cell shape recovery in *Escherichia coli*

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The shapes of most bacteria are imparted by the structures of their peptidoglycan cell walls, which are determined by many dynamic processes that can be described on various length scales ranging from short-range glycan insertions to cellular-scale elasticity1–11. Understanding the mechanisms that maintain stable, rod-like morphologies in certain bacteria has proved to be challenging due to an incomplete understanding of the feedback between growth and the elastic and geometric properties of the cell wall2,4,12–14. Here, we probe the effects of mechanical strain on cell shape by modelling the mechanical strains caused by bending and differential growth of the cell wall. We show that the spatial coupling of growth to regions of high mechanical strain can explain the plastic response of cells to bending4 and quantitatively predict the rate at which bent cells straighten. By growing filamentous *Escherichia coli* cells in doughnut-shaped microchambers, we find that the cells recovered their straight, native rod-shapes morphologically when released from captivity at a rate consistent with the theoretical prediction. We then measure the localization of MreB, an actin homologue crucial to cell wall synthesis, inside confinement and during the straightening process, and find that it cannot explain the plastic response to bending or the observed straightening rate. Our results implicate mechanical strain sensing, implemented by components of the elongation machinery yet to be fully characterized, as an important component of robust shape regulation in *E. coli*.

Cell shape, which in many types of bacteria is determined by a mechanically rigid peptidoglycan (PG) cell wall, is crucial for bacterial motility, proliferation, adhesion and survival1–3. Rod-like bacteria maintain their shapes at a fixed diameter with extraordinary precision during growth, and elongate by the action of the peptidoglycan elongation machinery (PGEM), a multi-enzyme complex consisting of penicillin-binding proteins (PBPs) and conserved membrane proteins (MreC, MreD, RodA, RodZ and other shape, elongation, division, sporulation (SEDS) family proteins)4,6,15,16. Recent experimental studies have led to a qualitative description of cell wall growth on a molecular level: the PGEM interacts with the actin homologue MreB to direct the local, circumferential insertion of new glycan strands into the existing PG structure. Although the general roles of PGEM enzymes in cell wall elongation are well studied4–8,17, the feedback mechanism between cell shape—as determined by the geometric and elastic properties of the cell wall—and PGEM-related subcellular components is not understood. It is unclear whether the mechanisms needed to maintain robust cellular morphology detect cellular geometry12,18 or mechanical stresses4,13,14.

Recent progress has been made towards understanding the regulatory mechanisms controlling rod-like cell shape by mechanically perturbing PG, which can be modelled as a partially ordered elastic sheet subject to both plastic and elastic deformations3,4,14,19. *Escherichia coli* cells adapt their growing morphologies to confining environments20,23 or applied hydrodynamic drag forces4,19 by elongating in a manner that results in bending. In several experiments, *E. coli* cells have recovered their straight, native rod-like morphologies upon release from confining environments4,18–21 or disruption of an induced crescentin structure22 after sufficient growth. This striking robustness has led to three prevalent theories of shape regulation: (1) a large processivity—the mean number of subunits incorporated into a glycan strand from initiation to termination of the elongation step—provides a built-in mechanism for straightening23; (2) PGEM-related molecules such as MreB localize, according to cell wall geometry, to regions of negative Gaussian curvature12,18; and (3) new glycan strands are preferentially inserted at regions of high mechanical stress in a manner that straightens the cell3,13,22,23.

By itself, the processivity of PG synthesis cannot explain cell straightening. Although processive glycan insertions into the PG mesh have been shown to yield an exponential decay of curvature23, an exponential increase in length due to growth counteracts the straightening and leads to a self-similar, scale-invariant shape, even in the limit of infinite processivity24,25. The local curvature of a growing, self-similar crescent-shaped cell decays, but in the absence of cell division the cell is always bent and not truly rod-like (Fig. 1a). Similarly, the possible curvature-sensing abilities of PGEM-related subcellular components have been interpreted as a geometry-based feedback mechanism for shape regulation24,18. Such mechanisms would allow the cell to preferentially grow at regions of negative Gaussian curvature and thus result in straightening. However, such a mechanism cannot explain experiments subjecting *E. coli* and *Bacillus subtilis* cells to hydrodynamic drag19. If the local growth of PG were biased towards regions of negative Gaussian curvature, then more growth would occur along the edge facing away from the flow. Upon extinguishing the flow, the cells would bend in the direction opposite the flow because of the stored, anisotropic growth (Fig. 1b). It was observed, on the contrary, that the equilibrated, bent conformations were in the same direction as the flow.

We therefore hypothesized that a mechanical strain-based, as opposed to geometry-based, pattern of preferential PG elongation could reconcile the aforementioned observations and robustly straighten a cell. The elastic quantity that we examine is the areal...
strain, which measures the local stretching of PG and is defined in terms of the axial and circumferential components of the strain tensor, $u_{xx}$ and $u_{yy}$, respectively, as $A = (1 + u_{xx})(1 + u_{yy}) - 1$. A molecular mechanism that couples growth to PG pore size, for instance, may sense areal strain. As we discuss below, the key assumptions of our model are (1) the elastic properties of the cell wall are unaltered by growth and (2) the number of glycan strand initiations per unit area is modulated by areal strain. With these assumptions, we will show that strain-dependent PG elongation is quantitatively consistent with both the earlier flow-based experiments and experimental measurements of the straightening rate.

To test our hypothesis of strain-dependent PG elongation, we designed an experiment consisting of two phases. In phase 1, filamentous cells are confined and uniformly bent in curved microchambers. In phase 2, the cells are released from captivity and their straightening rates are measured. Before reporting our experimental results, we discuss the theoretical predictions of our model.

As discussed in detail in the Methods, using linear elasticity theory we determined the areal strain experienced by the cell wall at an angle $\theta$ and time $t$ in both phases 1 (‘in’) and 2 (‘out’) as:

$$A^{\text{in}}(\theta, t) \approx A_0 + A_1^{\text{in}}(B_0 - c_0) \sin \theta,$$

$$A^{\text{out}}(\theta, t) \approx A_0 + A_1^{\text{out}}(t) \sin \theta$$

(1)

where $A_0$ is a constant, $B_0$ is the ratio of the cell radius $r$ to the radius of curvature of a bent cell in phase 1, which is assumed small compared to unity and partially relieved by a smaller, constant differential growth parameter $c_0 < B_0$, and both $A_1^{\text{in}} = 1 + \eta - \nu - \eta \nu$ and $A_1^{\text{out}} = \eta(2 + \eta - 2\nu - 4\nu^2 + \nu^2)/2$ are positive for the parameter values relevant to E. coli (Supplementary Table 1). Here $\eta = p r / Y$ is a dimensionless pressure, $p$ is the turgor pressure, $Y$ and $\nu$ are, respectively, the two-dimensional elastic modulus and Poisson ratio of the cell wall, and $\theta = \pi/2$ and $\theta = -\pi/2$ specify, respectively, the outer and inner edges of the cell. The differential growth parameter is defined so that the arclengths of the inner and outer edges differ by $2c_0 L$, where $L$ is the length along the cellular midline, and the assertion that $c_0 < B_0$ is consistent with an elastic snapback wherein removing the bending force results in a sudden decline of curvature from $B_0 / r$ to $c_0 / r$. The variational component $\delta A^{\text{out}} = -A_1^{\text{in}}(t) \sin \theta$ is opposite in sign to

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Figure 1 | Three theories for cellular shape regulation. a. The processivity of glycan insertions provides a robust, built-in mechanism for curvature decay, but even in the infinitely processive limit a cell remains self-similar. b. A geometry-dependent growth mechanism predicts an oppositely bent shape once an applied hydrodynamic drag force is extinguished, which was not observed in previous experiments. c. A mechanical-strain-dependent growth rate can explain both the elastic snapback shown in b and straightening, and the straightening rate can be quantitatively predicted. Left: simulated equilibrium configurations of a bent cylinder (top) and a toroidal shell (bottom) subject to an internal pressure, which respectively describe the cell states under a bending force (phase 1) and in the absence of a bending force (phase 2). The mesh, processed using finite-element software, is coloured by the variational areal strain $\delta A$. Like the differential growth, $\delta A$ flips sign between the two phases. $x$ and $\theta$ denote surface coordinates on the cell. Right: simulated, normalized variational areal strain for a differential growth parameter $c = 0.1$ and varying values of dimensionless pressure $\eta$ plotted against the azimuthal angle $\theta$, along with the linear theory prediction, for a phase 2 cell. Values of $\eta$ are calculated using the radii of deformed states. The Poisson ratio is taken to be $\nu = 0.3$ and the remaining simulation parameters are detailed in the Supplementary Methods.
\[ \delta A^{\text{19}} = A_p^0 (R_k - c_0) \sin \theta \] and is expressed as a function of a time-dependent differential growth parameter \( c(t) \). Importantly, the variational components in both phases 1 and 2 agree in sign with the differential growth profiles (Fig. 1c).

Given the variational areal strains, we modelled areal strain-dependent growth by assuming that the number of initiated glycan strands also at \( \theta \), which in turn depends on the strain profile of the cell in the past (see also the growth equation in Supplementary Note 2). The growth at angle \( \theta \) at a given time depends on the number of initiated glycan strands also at \( \theta \), which in turn depends on the strain profile of the cell in the past (see also the growth equation in Supplementary Note 2). The predicted straightening rate is consistent with the experimental data shown in Fig. 3c. Here, the symbols \( v \) and \( \kappa \) denote the Poisson ratio and snapback ratio, respectively (to be distinguished from the MreB spot velocity, \( v \), and the strain-independent growth rate, \( k \)).

\[ \gamma \theta(t) = k + \sigma \delta A(\theta, t) \] (2)

where \( k \) is a constant, strain-independent rate, \( \delta A(\theta, t) \) is the variational areal strain as a function of angle \( \theta \) and time \( t \), and the parameter \( \sigma \) quantifies the intensity of growth–strain coupling. The average initiation rate \( k \) can be determined by factors other than strain and need not depend on mechanical stress or turgor pressure. For instance, a growth mechanism may depend only on the abundance of PGEM constituents in maintaining an average initiation rate over the entire cell, but be biased towards regions of high strain in a manner that does not increase the average initiation rate.

To quantitatively characterize the straightening rate arising from cell strain-dependent growth, we coarse-grained the growth dynamics of *E. coli* by first assuming that MreB filaments, which are spatially correlated with new glycan insertions, correspond to PG growth sites. This is consistent with a growth scheme in which MreB filaments orchestrate persistent motion of the PGEM, but differential glycan strand initiation depends on strain-sensitive elongosome components. Although MreB filaments have been observed to move at a helical pitch angle, the small pitch allows us to model them as point molecules moving circumferentially along the inner membrane with a spot velocity of \( v = 5 \text{ mm s}^{-1} \), corresponding to experimentally observed values (Fig. 2d). We further modelled the decay of an MreB filament as a Poisson process with rate \( 1/\kappa \), where \( \kappa \approx 5 \text{ min} \) is the spatial persistence time of membrane-bound MreB (ref. 12), a value that is consistent with our snapshot measurements (Supplementary Discussion). Convolving \( \gamma \) with the width of PG inserted per growth site then yields an integro-differential growth equation describing the pole-to-pole cell length \( L(t) \) at any angle \( \theta \) and time \( t \) (see Supplementary Note 2 for a derivation and solution of this equation). As the pole-to-pole lengths determine the midline curvature \( C(t) \) at any point in time, solving the growth equation results in a theoretical prediction of the straightening rate. In the limit of large processivity, we found an approximate relation between the normalized straightening rate \( \mu = \frac{dC(t)}{dL(t)} \), the normalized growth rate \( \lambda = \frac{dL}{d(L(t))} \) and the snapback ratio \( \kappa = c_0/R_k \) as:

\[ \mu \approx \lambda \left( 1 + \frac{\kappa A_p^0}{(1 - \kappa) A_p^0} \right) \] (3)

Figure 2b,c illustrates the prediction of equation (3), which varies depending on processivity (Fig. 2d), for the parameter values of *E. coli* summarized in Supplementary Table 1 and a snapback ratio obtained from the experiments described below.

Previous studies examined the plastic deformation of *E. coli* cells under flow, but prior experiments were limited to several cells.
and the areal strains were non-uniform in the axial directions of the cells. To test our theory of strain-dependent growth, we therefore conducted experiments following the two phases described above. In phase 1, filamentous *E. coli* cells grew in toroidal microchambers with constant diameters of \( d = 8 \mu m \) (Fig. 3a). The cells were confined to the microchambers during growth and extracted into a larger, square-shaped microchamber once they filled over 90% of the microchamber circumference. Elastic snapbacks of the cells were observed after extraction, in agreement with flow-based experiments\(^4\) (Supplementary Discussion). In phase 2, we imaged the shape recovery process of unconstrained cells in 2 min intervals for over 40 min (Supplementary Videos 1–10).

On imaging the recovery process, we quantitatively analysed the straightening dynamics of unconstrained phase 2 cells (the methodology and results are detailed in the Supplementary Methods and Supplementary Fig. 7). We extracted the normalized growth rate \( \lambda \)
from the arclengths of the resulting fits for 60 cells and found a population-averaged value of $\langle \lambda \rangle = 0.021 \text{ min}^{-1}$ (Fig. 3b,c and Supplementary Fig. 8). This value of $\langle \lambda \rangle$ reflects a doubling time of $t_d = 33 \text{ min}$, in agreement with bulk culture growth measurements30,31. Similarly, we extracted the normalized straightening rate $\mu$ from the curves of the fits and found a population-averaged value of $\langle \mu \rangle = 0.038 \text{ min}^{-1}$, 1.8 times larger than the growth rate. Extrapolating the population-averaged curvature to the time of release, two minutes before the first frame, yields a mean elastic snapback ratio of $\kappa = 78\%$, with an extrapolated standard deviation of 9% (Fig. 3d). The observed straightening rate and snapback ratio are consistent with equation (3) (Fig. 2b,c) and numerical simulations of the growth process (Supplementary Figs 5 and 6 and Supplementary Video 11) for the material values of E. coli summarized in Supplementary Table 1.

We next wondered whether MreB—which is believed to localize to regions of negative Gaussian curvature in cells with submicrometre-scale indentations12—could also sense strain or otherwise account for straightening by localizing to the inner edge in unconfined phase 2 cells. To test the possibility that MreB localization could explain cell straightening, we repeated the foregoing experiments with a fully functional and complementing MreB-msGFP fusion expressed from the native mreB locus32. We measured MreB fluorescence intensities at the inner and outer edges of both phase 1 and 2 cells using an approach similar to previous work12 (see Supplementary Methods and Supplementary Figs 9 and 10 for details). In qualitative agreement with previous work12, we found increased MreB-msGFP intensities at the inner edges of confined phase 1 cells (Fig. 4a), with an enrichment positively correlated with the centreline curvature (Fig. 4b), indicating that MreB localization alone cannot account for growth inside confinement in phase 1. We also found an increased MreB-msGFP intensity at the inner edges of recovering phase 2 cells. However, the MreB enrichment is not sufficient to explain a straightening ratio of 1.8 based on a model in which cell elongation is proportional to MreB density (Fig. 4a). Together, these results indicate that MreB localization cannot explain differential growth in both phases 1 and 2. Furthermore, although we do not rule out an active mechanism for curvature-sensing in submicrometre-scale indentations, the observed MreB localization between the inner and outer edges of bent cells does not require an active sensing mechanism but can be explained by constant initiation and persistent circumferential motion alone (Fig. 4a).

The consistency of our strain-based model with experimental results suggests that mechanical strains arising from differential growth can act as a sensory cue for robust shape regulation. Subsequent simulations and stress analyses indicate that other sources of variation in elastic quantities, particularly non-uniform crosslinking of glycan strands or cleavage of peptide bonds, cannot explain the observed straightening rate (Supplementary Note 1 and Supplementary Figs 3 and 4). However, while our results constrain models that can explain straightening, further work will be needed to experimentally demonstrate that mechanical strain mediates PG elongation and uncover the molecular mechanisms responsible for mechanosensitive strain sensing. One intriguing possibility for such a mechanism is the lipoprotein–PBP interaction, which may be sensitive to PG pore size33.35. We anticipate future experiments to determine possible effects of the lipoprotein–PBP interaction and other perturbations, such as osmotic shock (Supplementary Discussion and Supplementary Fig. 11), on straightening.

In summary, we have used a combination of theory and experiment to quantitatively reveal and explain shape recovery in cells that have been released after growing in a confined environment. Our findings underscore how perturbing cells using physical, in contrast to biological, approaches can uncover how cells function in their native conformations. Because cell wall strains are determined by the entire deformation history of the cell, strain-sensitive growth can enable the robust recovery of native rod shape3,4,13,14, in addition to allowing cells to adapt to growth in various geometries by relieving cell wall stresses and regulating cell wall thickness by localizing growth to thinner regions of PG, where the areal strains are larger. By showing that coupling growth to mechanical strain can quantitatively explain shape recovery, our analysis contributes to our understanding of the possible biophysical mechanisms that underlie the remarkable diversity and robustness of cellular morphology.

**Methods**

**Areal strain profiles of a cell in phases 1 and 2.** To quantify the areal strain incurred by a bent, filamentous cell due to non-uniform growth, we modelled the bacterial cell...
as a homogeneous, isotropic, linear-elastic shell under pressure. For the spatial couple of Service for determinations of high areal strain to be consistent with the elastic snapback observed in previous flow-based experiments (Fig. 1b), it is necessary that a bending force makes the areal strain larger on the outer edge of the cell. It is also necessary that the residual stresses caused by turgor pressure and anisotropic growth during bending make the areal strain smaller on the outer edge once the bending force is removed. A mechanical-strain-dependent growth rate, in and outside the initiation rate of new glycan strands quantitatively depends on the areal strain, would then explain the ability of the cell to both plastically adapt to a bending force and straighten in the absence of external forces.

When a cell is bent uniformly by an external force (phase 1), the areal strain, which is proportional to the bending rigidity, varies in the azimuthal coordinate $\theta$ and constant in the axial direction, is readily determined by elasticity theory and is larger on the outer edge due to the axial stresses incurred by bending (Supplementary Note 1 and Supplementary Fig. 1). Determining the areal strain once the bending force is removed (phase 2) requires consideration of growth as the cell is bent. It is convenient to model growth by changing the intrinsic geometry of the cell, which is the shape a cell would assume in the absence of external forces such as pressure. Any growth in the axial direction that couples to a sinusoidally varying areal strain profile is also sinusoidally varying, so the intrinsic pole-to-pole PG length becomes larger at the outer edge and smaller at the inner edge in phase 1 (Fig. 1c). As a result of this differential growth, the intrinsic geometry of the cell evolves from that of a cylinder to that of a torus, a geometry for which the pole-to-pole lengths are sinusoidally varying. The toroidal geometry is described by the cell radius $r$ and a differential growth parameter $c$, which quantifies the cellular growth asymmetry. Assuming that growth does not change the elastic properties of a cell, the areal strain will be determined. Although the shape of a cell would exactly realize the intrinsic geometry resulting from differential growth, the presence of a turgor pressure can result in a different geometry and stress state. We therefore undertook finite-element stress analyses of a closed, circular toroidal shell subject to internal pressure (see Supplementary Methods for a detailed discussion of the simulation methodology and results). We found that, for infinitesimal and moderate strains, the stress profiles were well approximated by the linear theory result in which the deformed geometry remains that of a circular torus (Fig. 1c and Supplementary Fig. 2), with a circumferential stress component that is larger on the inner edge. Interestingly, the sources of the variational terms $\delta a$ and $\delta a'$ are the axial and circumferential components of the stress tensor, respectively, and not the differential stress components alone flips signs between phases 1 and 2 (Supplementary Note 1).

Equilibrium simulations of an elastic shell. Stress analyses of closed cylindrical and toroidal shells subject to internal pressure were computationally undertaken with finite-element simulations using Abaqus FEA (Dassault Systems). Explicit details of these simulations are discussed in the Supplementary Methods. Abaqus input files were created with MATLAB, with shells being discretized uniformly into approximately 10,000 54R elements, assigned material properties faithful to that of the E. coli cell wall, and equilibrated with respect to a range of internal pressures and material parameters. As previously described, we then extracted from the deformed state and used to compute the areal strain.

Numerical solutions of the growth equation. Numerical solutions of the growth equation were found in MATLAB by discretizing the integral as a Riemann sum and solving the finite-element stress analyses of a closed, circular toroidal shell subject to internal pressure (see Supplementary Methods for a detailed discussion of the simulation methodology and results). We found that, for infinitesimal and moderate strains, the stress profiles were well approximated by the linear theory result in which the deformed geometry remains that of a circular torus (Fig. 1c and Supplementary Fig. 2), with a circumferential stress component that is larger on the inner edge. Interestingly, the sources of the variational terms $\delta a$ and $\delta a'$ are the axial and circumferential components of the stress tensor, respectively, and not the differential stress components alone flips signs between phases 1 and 2 (Supplementary Note 1).

Microfabrication. We designed patterns of microchambers in doughnut-shaped designs in CleWin (Delta Mask) and square-shaped designs in Adobe Illustrator (Adobe Systems). The design of the doughnut-shaped microchambers was inspired by previous work. We created designs of doughnuts with outer diameters of 8 $\mu$m (channel width 2 $\mu$m), corresponding to circumferences of $\sim 25$ $\mu$m (Fig. 2a). The number of doughnut-shaped microchambers was $\sim 40,000$ per array. The fabrication process has been reviewed in detail in ref. 38 and previously described for microchannels and microchambers. Pristine silicon dioxide wafers were cleaned in isopropanol and $\text{D}_{2}\text{O}$ repeatedly. For doughnut-shaped microchambers, we used positive photoresist Shipley 1813 (MicroChem). Before spincoating, a vapour of hexamethyldisilazane (HMDS) was deposited on the clean silicon dioxide wafer substrate to prime it for adhesion of the photoresist. The spincoating resulted in $\sim 1.3$-$\mu$m-thick layers of photoresist. For square-shaped microchambers, we used negative photoresist SU-8 3010 (MicroChem) directly spincoated onto clean silicon dioxide wafers to produce $\sim 20$-micron-thick polymer layers. Layer thicknesses were confirmed using a surface profilometer (Tencor AlphaStep 200). Doughnut-shaped microchambers were directly written onto the photoresists by laser lithography ($\mu$PG 101, Heidelberg Instruments). Photomasks (C达到了工业级的精度) were used for the transmission of the phototopes onto the photoresists using UV lithography. The patterns were developed with MF-321 and SU-8 developer (MicroChem), respectively. The resulting phototopes were used as master molds for the embossing of PDMS (Sylgard 184, Dow Corning) using a ratio of 101:1 (base to curing agent) and cured the polymer overnight at 60 $^\circ$C. The resulting PDMS layer contained patterns of microchambers in bas-relief and was used as a stamp to emboss a layer of agarose with $r$-wt% agarose to $c$-wt% agarose. We poured a hot solution (65 $^\circ$C) of 4% lysogeny broth (LB)-agarose (EM-2120, Omnipur, EM Biosciences) containing isopropyl-$\beta$-D-thiogalactoside (IPTG, Sigma Aldrich) and antibiotics (if required) on PDMS stamps oriented with the features facing up and cooled them to room temperature to gel the agarose. We cut out the layer of LB-agarose covered with microchambers using a scalpel and prepared the microchambers for growth experiments.

Bacterial strains and growth. E. coli MG1655 was used with plasmid encoding SulA (a cell division inhibitor) under an inducible lac promoter to induce filamentation with 1 mM IPTG. Bacteria were grown in liquid LB medium (10 g l$^{-1}$ tryptone, 5 g l$^{-1}$ yeast extract, 10 g l$^{-1}$ NaCl) and, if required, supplemented with appropriate antibiotics. LB medium containing 1.5% Difco agar (wt/vol) was used to grow individual colonies. Tryptone, yeast extract, peptone, Petri dishes and bacteriological agar were purchased from Becton Dickinson and sodium chloride from Fisher Scientific. Bacteria were grown from a single colony in LB at 30 $^\circ$C overnight and the medium was supplemented with 100 $\mu$m l$^{-1}$ ampicillin (Sigma Alrich) for plasmid selection. We used a 1:10 dilution to inoculate fresh liquid LB medium. The culture was grown at 30 $^\circ$C with shaking at 200 r.p.m. to an absorbance of $\sim 0.6$ (610 nm). At this point, we added IPTG to a final concentration of 1 mM and incubated the solution for another 5 min to initiate filamentation (expression of SulA) under shaking at 30 $^\circ$C. Subsequently, we added 3–5 $\mu$L of the bacterial culture to the top of the doughnut-shaped microchambers (embossed in LB agarose with 1 mM IPTG, no antibiotics), incubated the agarose slab for 30 min, and sealed the microchambers with a #1.5 cover slip (12S-548-3g, Fisher Scientific). The microchambers were incubated in a static incubator at 30 $^\circ$C for at least 30 min. Continuous observation of these cells was performed for 40 min thereafter. Once the majority of cells were sufficiently filamented, we cut out the doughnut-shaped microchambers from the LB-agarose slab with a scalpel. We placed a 10 $\mu$L drop of LB-IPTG solution on a clean glass coverslip, used tweezers to hold the LB-agarose slab upside down on top of the drop and within the drop we released the cells from the microchambers. We repeated this step at least 20 times. The remaining drop was pipetted carefully, added on top of the square-shaped microchambers and sealed with a coverslip. We then immediately started imaging of the recovery process. Note that we also used E. coli MG1655 with the addition of 20 $\mu$m l$^{-1}$ cephalaxin as we have done successfully before for spheroplast formation.

However, genetic manipulation generated fewer efficient filamentation and process we observed frequent cell lysis under antibiotic pressure even at low cephalaxin concentrations.

The MreB-msfGP strain used for MreB localization experiments carries a functional MreB-msfGP translational sandwich fusion in the native mreB locus (MG1655) under regulation of the mreB promoter. The same MSF-mgf strain was grown overnight from frozen stock at $37^\circ$C in LB plus ampicillin (100 $\mu$m l$^{-1}$) in a shaking incubator. The overnight culture was washed and diluted 1:500 in LB minimal medium (0.2% glucose, 0.5% casamino acids) and incubated at $37^\circ$C. The same MSF-mgf strain was grown in minimal medium. The culture was grown at 30 $^\circ$C with shaking at 200 r.p.m. to an optical density at 600 nm ($OD_{600}$) of 0.1 in the shaker. Then, 1 mM IPTG was added to induce SulA expression and cells were grown for approximately half a doubling time (~20 min at $37^\circ$C or ~40 min at $30^\circ$C) in the shaker before being collected for microchamber confinement and microscopy. MreB-msfGP intensity measurements were performed in microchambers grown in minimal medium to reduce autofluorescence and at $37^\circ$C for increased growth rates. We confirmed that cells grown at $30^\circ$C behave quantitatively similar in terms of their average curvature and MreB enrichment, during confinement and after release from the microchambers (Supplementary Table 2).

Microscopy. We used a Zeiss Axiosvert 200 inverted microscope with an enclosing custom-made incubation chamber (at 30 and $37^\circ$C) equipped with an AxioCam 503 mono charge-coupled device (CCD, Zeiss) and a 40$\times$ objective (EC Plan-neofluar, NA 0.75). Imaging was confined to square microchambers of length 40 $\mu$m. The time-lapse images were acquired on an AxiosImager.Z1 (Carl Zeiss, Jena, Germany) with a 10-min exposure time using AxioVision (v.4.8, Zeiss). ImageJ (NIH) was used for cropping raw time-lapse images and to export the files as image sequences.

MreB-msfGP cells were confined in toroidal microchambers of 8 $\mu$m outer diameter and 2 $\mu$m inner diameter, similar to the experiments described above (but with a smaller inner diameter). However, the agarose microchambers were made from M63 minimal medium containing IPTG. To study MreB localization we used...
We found that pre-existing software, such as MicrobeTracker, did not provide curvature measurements and that curvature measurements were performed using a custom-made, MATLAB–34, 30 and 35 MreB shape analysis.

MATLAB = C

Experiments with 34, 18, 48 and 19 MreB–7. Lee, T. K.

agarose pads in the same manner as immediately after extraction. MreB–6. Domínguez-Escobar, J. et al.

found similar results for the global findings of this study are available from corresponding authors upon request.

Data availability. The data that support the findings of this study are available from the corresponding authors upon request.

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Author contributions
F.W. and A.A. developed the model of straightening. F.W. and J.P. performed simulations. L.D.R., G.O., D.B.W., S.v.T. and A.A. designed the experiments. L.D.R. and G.O. performed the experiments. F.W., L.D.R. and G.O. analysed the data. F.W. and G.O. wrote cell-tracking software. F.W., L.D.R., G.O., S.v.T. and A.A. wrote the paper.

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