Supplemental Experimental Procedures

Strains, Media, and Growth Conditions

After back-dilution, cultures were allowed to grow for the equivalent of at least one doubling time before further treatment. *C. crescentus* (CB15N) and derivatives were grown in PYE (peptone-yeast extract) medium or M2G (M2 minimal salts + glucose) medium as previously described (Harris et al., 2014) at 30°C. *C. crescentus* cells harboring parB::CFP-ParB were strain JWK1322 (Thanbichler and Shapiro, 2006). For perfusion experiments, the HfsA (holdfast)-inducible strain (FC1428, kanamycin resistant, (Iyer-Biswas et al., 2014)) was grown overnight in 5 μg/ml kanamycin, and then back-diluted into medium without drug for the remainder of the experiment. Induction of HfsA was achieved several hours later by adding 0.5 mM vanillic acid. Similarly, for FtsZ-depletion experiments, a *C. crescentus* strain with FtsZ under the inducible xylose promoter (YB1585, kanamycin resistant, (Wang et al., 2001)) was grown overnight in 5 μg/ml kanamycin plus 0.3% xylose to induce expression of FtsZ. In the morning, cells were diluted into medium containing only xylose for several hours before xylose was washed out and 0.2% glucose added to repress the xylose promoter and begin FtsZ depletion. *E. coli* cells were grown in LB at 37°C, and in general strain MG1655 was used for all experiments. One exception was for the overexpression experiment in Figure 5H, which was a BL21(DE3) strain harboring vector pET28 encoding poly-histidine-tagged eGFP (JAT022, kanamycin resistant, from John Dawson). In this case, 30 μg/ml kanamycin was present in both overnight cultures as well as throughout overexpression experiments to maintain the plasmid. Additionally, experiments with GFP-ParB bound to a ParS sequence inserted near the origin of replication (Figure S3D-F) were with strain CC4756 from Stuart Austin (Nielsen et al., 2006). Cells were grown without induction of GFP-ParB and in the presence of 30 μg/ml kanamycin and 100 μg/ml carbenicillin during all growth phases of the experiment. For nutritional upshift (Figure 4), stationary phase MG1655 cells from an overnight culture were used, and cleared supernatant from the same culture was constantly infused before switching to fresh LB. *L. monocytogenes* was grown in BHI (brain-heart infusion) medium at 37°C. Because expression of virulence factors has been shown to be a prerequisite for fosfomycin sensitivity in *L. monocytogenes* (Scortti et al., 2006), a strain with constitutively active virulence factors was used (Leimeister-Wächter et al., 1989). Additionally, the strain contained a deletion in actA to attenuate virulence (DP-L3780, Lauer et al., 2002). For all drug treatments, fresh stocks were made for every experiment. Because chloramphenicol stocks were made in ethanol (as opposed to medium or water for fosfomycin and IPTG respectively), a vehicle control of only ethanol was included in chloramphenicol dose-response experiments.

C. crescentus Perfusion Setup

In an RC-31 flow chamber, two coverslips were held on either side of a 250 μm-thick silicone gasket from which a ~0.5 mm channel had been cut using a scalpel and straight edge. Plastic tubing connected the flow chamber to a source bottle of medium on one side and a syringe pump and waste container on the other. *C. crescentus* cells that had been expressing HfsA (holdfast) for several hours were introduced into the chamber and allowed to adhere for 1-5 minutes. Then, the syringe pump was used to draw fresh medium from the source bottle, across the flow chamber, and eventually into the waste container. The speed of pumping was set based on how much flow was necessary to cause cells to lie flat against the coverslip. For experiments where a switch in medium was required, two lines going to different bottles were primed prior to the start of the experiment, and a valve between the two was flipped when necessary to redirect the flow.

Cell Segmentation

Automatically detected outlines from MicrobeTracker (Sliusarenko et al., 2011) were always verified by visual inspection, and for static images cells that were too close and had interfering phase halos were omitted from analysis, as well as cells that were obviously phase lucent and not viable. Cells that lay end-to-end were counted as having divided if invagination appeared to be complete and there was a visible lightening of the phase signal between the two daughters. For dynamic imaging, individual cell lineages were cropped prior to analysis. Frames where the cell was not in sharp focus were deleted. Daughter cell separation was estimated to have occurred once invagination was complete and there was a lightening of the phase signal between the daughters. In the case of *C. crescentus*, this often occurred before the swarmer cell was swept away, in which case only the remaining stalked cell was included in the analysis. For *E. coli* grown in a microfluidic chamber, automatic detection with MicrobeTracker was not possible due to crowding in the microcolony. We therefore tracked one daughter cell per colony that remained on the colony periphery throughout the movie by adding cell outlines manually and optimizing them using MicrobeTracker.

Measurement of Cell Shape, Fluorescence Intensity, and Chromosome Number

MicrobeTracker was also used to divide cells into 1-pixel-thick segments (Sliusarenko et al., 2011). The width of each segment was estimated by dividing segment area by the step length along the cell backbone associated with that segment. To find the predominant width for each cell, we binned all segment widths for a particular cell and approximated their distribution using a kernel density estimator function in MATLAB (Botev, 2007), taking the value of width associated with the maximum density as the predominant width for that cell. The maximum width of a cell was simply the width of the widest segment. Cell length and segment volume were calculated directly using MicrobeTracker, and SA was calculated by assuming that segments were cylindrically symmetric. We have previously demonstrated that this method of estimating SA/V is consistent with 3D deconvolution imaging of cell surfaces (Harris et al., 2014). Additionally, fluorescence intensities in Figure 5H were determined using MicrobeTracker. Fluorescence intensity was determined by dividing the integrated fluorescence signal within each cell by the area of that cell. Background subtraction was achieved by creating an additional cell mesh in each frame in a region without cells, and subtracting the intensity in this region from that measured for the cells. Finally, images of fluorescent ParB bound near the origin of replication in *C. crescentus* and *E. coli* were initially processed using ImageJ. First the background was subtracted using a "rolling ball" algorithm with a radius of 8 or 9 pixels for *C. crescentus* and *E. coli* respectively. Then images were filtered using a Gaussian blur with a 1 pixel radius. Spots were detected using the SpotFinderZ function in MicrobeTracker, and detection parameters were optimized using the graphical user interface and held constant for each species.

Analysis of Long-Term Growth Dynamics and Simulation of SA/V for Changing α and β

For perfusion experiments (Figures 3, 4, 5C-G), prior to averaging, cell shape and growth parameters were smoothed using a sliding time window. Briefly, the average value of all measurements within a particular window, disregarding frames where the cell was out of focus, was assigned to the time point at the center of the window. For *C. crescentus*, the window size was 21 frames (spanning 100 minutes), and for *E. coli* it was 5 frames (spanning 8 minutes). To fit a decaying exponential to the SA/V over time (Figures 3I, 4D, 5E), the decay constant and associated error were found through bootstrapping, fitting a decaying exponential to N smoothed, single-cell traces sampled with replacement from the total available N single-cell traces for a given condition using the built-in nonlinear regression function in MATLAB. This process was repeated 2000 times, and the mean of all measured decay constants was reported as the final decay constant, while the standard deviation of this distribution was reported as the estimated uncertainty. To simulate SA/V according to the "relative rates" model when α and β are changing (Figure S1), idealized traces of α and β were approximated from Figures 3L and 4F. The initial volume, V_0, was set to 1.5 μm^3, though this number does not affect the simulation. The initial value of SA was then calculated by assuming that SA/V started at the "steady state" SA/V given $\beta_{initial}$ and $\alpha_{initial}$: $(SA/V)_{initial} = \beta_{initial}/\alpha_{initial}$. Then the simulation was progressed according to the model in Figure 1A with time increments of 0.001 hour, and volume and SA were increased at rates equal to the current volume times the local values of α and β respectively. The resulting SA and volume values were recorded, and the predicted behavior of SA/V over time reported.

Cell Cycle-Dependent Averaging

Cell cycle-dependent properties (Figure 6A) were determined from perfusion data, selecting lineages with the best continuous focus. Cell division events were automatically detected by using large decreases in cell volume as an indicator of cell division. Occasional gaps in cell volume and SA where cells had been out of focus were filled in from the end of the gap backward, extrapolating using the average values of α and β for that cell cycle and the equations for SA and volume in Figure 1A. Cell cycles that contained gaps longer than 3 frames were omitted from analysis. Before averaging, the first 3 measurements for α and β were removed from each cell cycle because segmentation around this time was less accurate, often producing anomalous rate values. In deciding how best to align individual cell cycles within a given condition, we monitored the progress of invagination (Figure S7A) and found that, although there was considerable variation in the amount of time that elapsed before constriction initiation, there was much less variation in the amount of time it took for constriction to finish (Figure S7B-C). We therefore aligned all cell cycles within a given condition to the end of the cell cycle before averaging to ensure that constriction initiation events were roughly lined up. After this, the mean values for SA/V, maximum width, α, and β at each time point were calculated. Because some cell cycles were longer than others, early time points had fewer values to average, and points where less than 1/3 of the total number of cell cycles had values were not included in the final analysis. Furthermore, comparing across different conditions and growth rates, the average amount of time that elapsed before constriction initiation varied much more widely than the average amount of time spent completing constriction once it had started (Figure S7D-E). This suggested that variations in cell cycle length could be due to delays in constriction initiation, while the process of constriction, once initiated, generally completed in a

set amount of time. For these reasons, when comparing across conditions, we chose to center each condition about constriction initiation to emphasize this natural turning point in the cell cycle. Finally, for FtsZ-depletion experiments in Figure S4, each population of cells was binned into 12 bins according to length, and the average SA/V for each bin was reported. Bins containing fewer than 4 cells were omitted.

Estimation of Pre-Constriction SA Material Accumulation

To estimate the amount of pre-constriction SA material accumulation (Figure 7B), we first developed a method to approximate when constriction initiation occurred on an individual cell basis. Invagination progress for single cells over time was monitored using the following metric: 7 segments were removed from each pole and the difference between the maximum and minimum remaining widths was calculated (see Figure S7A). As cells began to constrict in the middle, this metric increased steadily, and constriction initiation was estimated to have occurred 3 frames (15 minutes) before the metric went over an empirically determined threshold of 0.18 μm, such that the estimate was robust to noise but still approximated when the cell had initially begun invaginating. The pre- and post-constriction volume integrals were then calculated by multiplying the volume at each time point either before or after constriction initiation by the amount if time that elapsed between time points. The average α for a cell cycle was the average of the α values for all the time points in that cell cycle excluding the initial 3, while the average β was the average of the β values for the final 6 frames, or 30 minutes, of the cell cycle. To calculate the pre-constriction ΔSA, we first extrapolated SA backwards as we had when there were gaps in the data, replacing the SA values for the first ~1/6 of the cell cycle, and then subtracted the estimated initial value of SA from the SA present at constriction initiation. Correlations between variables were determined by calculating the Pearson's linear correlation coefficient, and p-values were found by creating a t-statistic with two degrees of freedom.

Simulation of SA Material Accumulation and Division Timing

We simulated the accumulation of excess SA material during elongation for cells of different initial sizes by varying the initial length of cells while holding the width constant (0.81 μm unless otherwise stated, typical for *C. crescentus*) and allowing cells to elongate. The rate of elongation was determined by the exponential volume growth rate, α (0.6 h^{-1}, typical for *C. crescentus*), and with each time step (0.0001 h), the amount of additional length required to accommodate the accrued volume as part of the cylindrical cell body was added. Additionally, SA material was created at a rate linearly proportional to volume according to our model (Figure 1A), with scaling factor β (3.3 μm^{-1} h^{-1}, also typical of *C. crescentus*). Some of this material was used to build sufficient sidewall to encapsulate the newly added volume, while the rest was stored as excess material. This process was repeated until the concentration or amount of accumulated SA material had reached a specific threshold, and the total delta volume added up to that point was recorded. The specific threshold was determined by calculating how much excess SA material a cell starting at the typical initial length for *C. crescentus* (2.4 μm) would have accumulated by the time it reached a critical multiple of its initial volume – 1.8 in the case of *C. crescentus* (Iyer-Biswas et al., 2014), but the simulation would work similarly for species like *E. coli* where this multiple would be closer to 2. This "ideal" concentration or amount was then used as the threshold for successive simulations where a variety of starting lengths were used. In the case where surface material was destroyed at a rate dependent on surface material concentration (Figure S6D), an integrated first order rate law was used to approximate the new concentration at each step. In the case of cell thinning (Figure S6E), the rate at which the radius thinned was linearly proportional to the amount of accumulated surface material. For each time step, after the rate of thinning was calculated, the resultant change in radius over the time step was determined. Cells were assumed to become thinner without changing their volume, and both length and SA increased as a result. The amount of SA required to achieve this thinning was then removed from the cell's accumulated excess SA material, the width and length updated, and the simulation continued. In the case where cell width was altered and the effect on delta length monitored (Figure S6F), the trigger concentration was held constant at an ideal amount based on a cell of width 0.9 μm.

Analytical Modeling of Surface Material Accumulation

First, demonstrate that division upon reaching a threshold amount of a generic factor X that accumulates at a rate proportional to volume gives rise to an "adder" growth mechanism. Assume that volume grows exponentially:

$$V(t) = V_o e^{\alpha t} \tag{1}$$

where $V(t)$ is the volume at time t, V_o is the initial volume, and α is the exponential volume growth rate. Also, assume that a factor X increases at a rate proportional to volume:

$$\frac{dX}{dt} = \gamma V(t) \tag{2}$$

where γ is the scaling factor between the rate of X production and volume. Substituting (1) into (2) and integrating gives:

$$X(t) = \frac{\gamma}{\alpha} V_o e^{\alpha t} + c \tag{3}$$

with constant of integration c. If X is zero at the initial time point:

$$X(t) = V_o \frac{\gamma}{\alpha} (e^{\alpha t} - 1) \tag{4}$$

To define the ideal amount of accumulation, find how much $X(t)$ has been produced after a cell starting out at the ideal volume has grown for an ideal amount of time:

$$X(t)_{ideal} = V_{o\ ideal} \frac{\gamma}{\alpha} (e^{\alpha t_{c\ ideal}} - 1) \tag{5}$$

Assuming that non-ideal cells starting at V_o different from the ideal case will still grow until reaching the same $X(t)_{ideal}$, how long will it take for a generic non-ideal cell to reach this threshold? Use t_c to denote the time until a generic cell with initial volume V_o reaches the threshold $X(t)_{ideal}$:

$$V_o \frac{\gamma}{\alpha} (e^{\alpha t_c} - 1) = V_{o\ ideal} \frac{\gamma}{\alpha} (e^{\alpha t_{c\ ideal}} - 1) \tag{6}$$

Simplifying (6) gives:

$$t_c = \frac{\ln(\frac{V_{o\ ideal}}{V_o} (e^{\alpha t_{c\ ideal}} - 1) + 1)}{\alpha} \tag{7}$$

With this general expression for t_c, determine how much ΔV has been added for the generic cell once it has reached the threshold:

$$\Delta V = V_o e^{\alpha t_c} - V_o \tag{8}$$

Substituting (7) into (8) and simplifying gives:

$$\Delta V = V_{o\ ideal} (e^{\alpha t_{c\ ideal}} - 1) \tag{9}$$

ΔV therefore only depends on parameters that will remain constant regardless of the initial size of the generic cell, meaning that ΔV is independent of initial volume, consistent with an "adder" mechanism of cell growth.

Now, perform a similar analysis specifically for accumulation of excess SA material. As with our simulations, we will assume that cells elongate without changing their radius, that volume grows exponentially with growth rate α:

$$V(t) = V_o e^{\alpha t} \tag{10}$$

and that SA material (A) accumulates at a rate proportional to volume scaled by β:

$$\frac{dA}{dt} = \beta V(t) \tag{11}$$

Substituting (10) into (11) and integrating gives:

$$A(t) = \frac{\beta}{\alpha} V_o e^{\alpha t} + c \tag{12}$$

Assuming that A is zero at the initial time point:

$$A(t) = V_o \frac{\beta}{\alpha} (e^{\alpha t} - 1) \tag{13}$$

Additionally, the amount of SA used up in new sidewall synthesis to encapsulate the volume that has been added at any time must be deducted from this accumulated amount. To do this, we first find an expression for how much SA material (ΔA) must be subtracted given a specific amount of volume addition (ΔV). For an elongating cylinder with radius r and length l:

$$\Delta V = \Delta l\, \pi\, r^2 \tag{14}$$

and:

$$\Delta A = 2\, \pi\, r\, \Delta l \tag{15}$$

Solving (14) for Δl and substituting into (15) gives:

$$\Delta A = \frac{2}{r} \Delta V \tag{16}$$

This means that:

$$\Delta A = \frac{2}{r} (V(t) - V_o) \tag{17}$$

Substituting (10) into (17) gives:

$$\Delta A = \frac{2\, V_o}{r} (e^{\alpha t} - 1) \tag{18}$$

We can now subtract this expression for the SA material used up in sidewall building from the expression for accumulated material (13):

$$A(t) = V_o \frac{\beta}{\alpha}(e^{\alpha t} - 1) - \frac{2\,V_o}{r}(e^{\alpha t} - 1) \tag{19}$$

which simplifies to:

$$A(t) = V_o(e^{\alpha t} - 1)(\frac{\beta}{\alpha} - \frac{2}{r}) \tag{20}$$

As before with the generic factor X, find the time, t_c when a general case reaches the ideal amount $A(t)_{ideal}$. First, determine $A(t)_{ideal}$:

$$A(t)_{ideal} = V_{o\ ideal}(e^{\alpha t_{c\ ideal}} - 1)(\frac{\beta}{\alpha} - \frac{2}{r}) \tag{21}$$

Now, set the general case of a cell starting at V_o and dividing at t_c equal to this ideal amount:

$$V_o(e^{\alpha t_c} - 1)\left(\frac{\beta}{\alpha} - \frac{2}{r}\right) = V_{o\ ideal}(e^{\alpha t_{c\ ideal}} - 1)(\frac{\beta}{\alpha} - \frac{2}{r}) \tag{22}$$

Solving for t_c gives:

$$t_c = \frac{\ln(\frac{V_{o\ ideal}}{V_o}(e^{\alpha t_{c\ ideal}} - 1) + 1)}{\alpha} \tag{23}$$

This is the same t_c as before (7), and substituting it into (8) also produces (9). This means that again the amount of volume added is predicted to be independent of the initial cell volume, consistent with an "adder" mechanism of cell growth.

Supplemental References

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