

Biphasic growth dynamics during *Caulobacter crescentus* division

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1 Cell size is specific to each species and impacts their ability to function. While various
2 phenomenological models for cell size regulation have been proposed, recent work in
3 bacteria have demonstrated an *adder* model, in which a cell increments its size by
4 a constant amount between each division. However, the coupling between cell size,
5 shape and constriction, remain poorly understood. Here, we investigate size control
6 and the cell cycle dependence of bacterial growth, using multigenerational cell growth
7 and shape data for single *Caulobacter crescentus* cells. Our analysis reveals a bipha-
8 sic mode of growth: a *relative timer* phase before constriction where cell growth is
9 correlated to its initial size, followed by a *pure adder* phase during constriction. Cell
10 wall labeling measurements reinforce this biphasic model: a crossover from uniform
11 lateral growth to localized septal growth is observed. We present a mathematical
12 model that quantitatively explains this biphasic *mixer* model for cell size control.

13

14 We recently introduced a technology that enables obtaining unprecedented amounts of precise
15 quantitative information about the shapes of single bacteria as they grow and divide under non-
16 crowding and controllable environmental conditions [1, 2]. Others have developed complementary
17 methods [3–6]. These single-cell studies are generating great interest because they reveal unan-
18 ticipated relationships between cell size and division control [5]. Recent work in bacteria revealed
19 a model of constant size increment between successive generations for a wide range of bacterial
20 species [3–5, 7, 8], as originally proposed in Ref. [9], and recently termed as an *adder* model [5, 10].
21 Competing models for size control include cell division close to a critical size (*sizer*) [11] or at a
22 constant interdivision time (*timer*), equivalent to a critical multiple of the birth size with a constant
23 growth rate [1]. Analysis of single-cell data show that cell size at division is positively correlated
24 with the cell size at birth [1, 4, 5, 12, 13], thus precluding a *sizer* model. In addition, a negative cor-
25 relation between initial cell size and interdivision times, as reported here and in refs [1, 4, 5, 13, 14],
26 is inconsistent with the *timer* model. However, other studies have suggested mixed models of size
27 control, with diverse combinations of *sizer*, *timer* and *adder* models [10, 15–17]. The spatial res-
28 olution and statistically large size of our data now allow us to revisit these issues with greater
29 precision.

30 While cell size serves as an important determinant of growth, the bacterial cell cycle is composed
31 of various coupled processes including DNA replication and cell wall constriction that have to be
32 faithfully coordinated for cells to successfully divide [18]. This raises the question of what other cell
33 cycle variables regulate growth and how the interplay between these variables can be understood

34 quantitatively [19, 20]. Indeed, our recent modeling and analysis of cell shape dynamics revealed
35 how different shape parameters are coupled through growth and division [2, 21]. Here we relate
36 cell size control and cell wall growth to the timing of cell-wall constriction in *C. crescentus* cells.

37 RESULTS

38 We use a combination of microfluidics and phase-contrast microscopy for high-throughput, live-cell
39 measurements of cell shape dynamics of single *C. crescentus* cells [1, 2, 22]. As a population of
40 cells is controllably attached via a stalk and holdfast to the coverslip surface in the microfluidic
41 channel, our measurements allow obtaining accurate and precise data of single cell shape and
42 growth for >10000 generations for >250 cells under steady environmental conditions. From the
43 splined cell contours of the acquired phase-contrast images (Fig. 1a), we determine various cell
44 shape parameters, such as the length of the cell midline axis (l), cell width, and the radius of
45 curvature of the midline. As reported previously, l increases exponentially, $l(t) = l(0)e^{\kappa t}$, with
46 time constant $\langle \kappa \rangle^{-1} = 125 \pm 8$ min and a mean interdivision time $\langle \tau \rangle = 73 \pm 7$ min at 31°C in
47 peptone-yeast extract (PYE) medium, while the average width and the radius of curvature remains
48 approximately constant [2]. Since measurements of the cell area behave the same as the length [1],
49 we use the length as a metric for cell size.

50 **Mixer model of cell size control.** We first analyzed the correlation between cell size at birth,
51 $l(0)$, and at division, $l(\tau)$, which describes the strategy for cell size control. Previously [1], the
52 relationship between cell size at birth and at division was described by fitting the data with only
53 pure timer ($l(\tau) = al(0)$, a is a proportionality constant) and adder ($l(\tau) = l(0) + \delta$) models. Here,
54 consistent with [10], we find that cell size correlation in that same dataset can be best described by
55 a model that combines both adder and timer components: $l(\tau) = al(0) + \delta$, with a slope of $a = 1.25$
56 and an intercept $\delta = 1.39 \mu\text{m}$ (Fig. 1b; Supplementary Note 1; Supplementary Fig. 1). The value
57 of the slope should be contrasted with 1.8 [1], the multiple expected for a size ratio $\simeq 0.55$ between
58 the daughter cells. While the interdivision times, τ , and the growth rates, κ , fluctuate between
59 cells and across generations, positive δ implies that larger cells divide more quickly than smaller
60 cells [4, 5, 7, 13],

$$61 \quad \tau = \kappa^{-1} \ln [a + \delta/l(0)] . \quad (1)$$

62 We find τ to be negatively correlated with $l(0)$ (Eq. (1); Fig. 1c) [4]. As shown in Fig. 1d, the
63 distributions of normalized division cycle times, $\kappa\tau$, are also correlated with the initial lengths

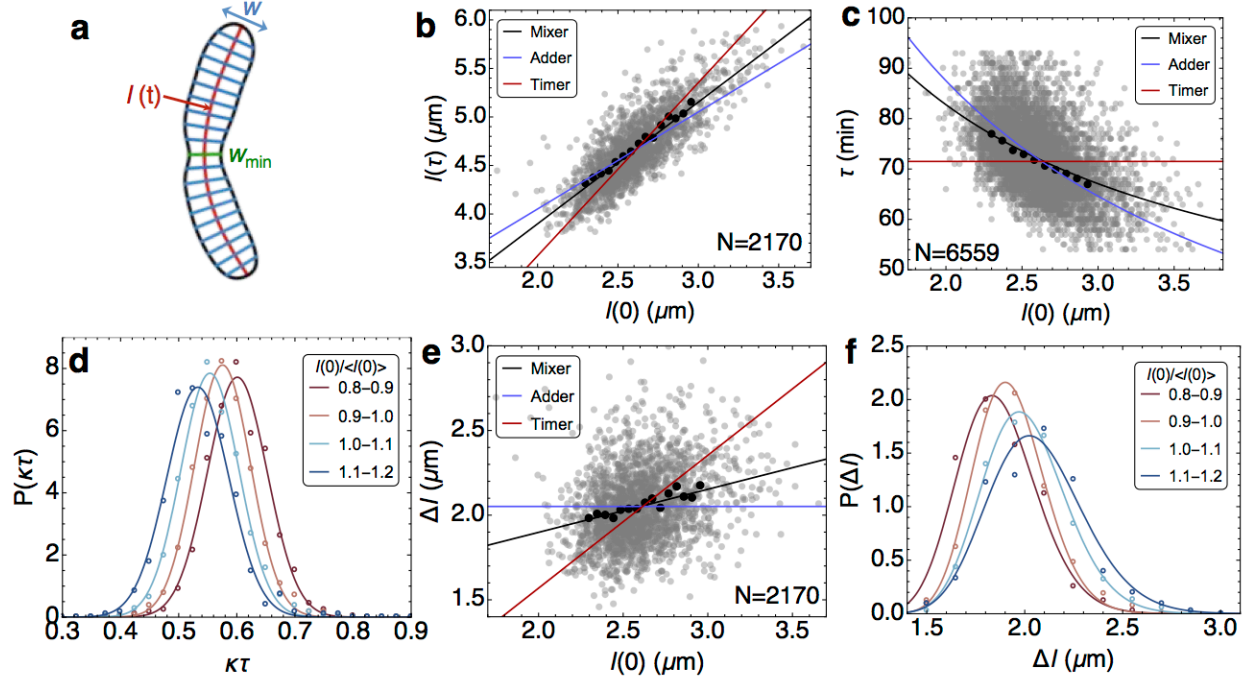


FIG. 1. **Cell size and division control in *C. crescentus*.** **a.** A representative splined contour of a *C. crescentus* cell, illustrating the shape variables. **b.** Cell size at division, $l(\tau)$, vs the cell size at birth, $l(0)$. The black solid line represents a least square linear fit to all single generation data given by the gray scatter cloud i.e. mixer model. Corresponding fits by timer and adder models are given by red and blue lines, respectively. The solid circles represent mean data binned by $l(0)$. **c.** The scatter plot of interdivision times, τ , vs the initial cell size, $l(0)$, exhibits a negative correlation. The black solid line is the prediction based on exponential growth and the mixer model with no adjustable fitting parameters. Predictions from timer and adder models are shown by the red and blue lines, respectively. **d.** The conditional probability density of normalized division cycle time, $\kappa\tau$, given the mean rescaled initial length values, $P(\kappa\tau|l(0)/\langle l(0) \rangle)$, illustrating the negative feedback between τ and $l(0)$. The open circles represent experimental data and the solid curves are Gaussian fits. **e.** Size extension in each generation, Δl is correlated with the initial cell size. The mean trend is described by the linear relationship, $\Delta l = (a - 1)l(0) + \delta$, which is the mixer model. The solid circles represent mean data binned by $l(0)$. **f.** shows the conditional probability density of size extension Δl given the mean rescaled initial cell length, $P(\Delta l|l(0)/\langle l(0) \rangle)$. The open circles represent experimental data and the solid curves are lognormal fits. N indicates number of generations in **b**, **c**, **e**.

64 as shown by the conditional probability $P(\kappa\tau|l(0)/\langle l(0) \rangle)$ for various ranges of $l(0)/\langle l(0) \rangle$. These
65 observations rule out a timer model of size control where the division times would be uncorrelated
66 with the initial lengths [1]. Furthermore, Fig. 1e-f show that the lengths added in each generation,
67 $\Delta l = l(\tau) - l(0)$, are positively correlated with the initial cell lengths, which precludes a pure adder
68 model for cell size control, in contrast to [4]. Our data suggest that *C. crescentus* cells behave

69 with attributes of both timer and adder, i.e. a *mixer*. Furthermore, we find that this mixer model
70 is conserved in the temperature range 14°C-34°C (Supplementary Fig. 2, 3). Interestingly, cells
71 at 37°C behave as a perfect adder, which suggests that adder-like behavior may be elicited by
72 experimental conditions. For *C. crescentus*, 37°C is the extreme upper limit for viable cell growth.

73

74 **Relative timer phase prior to cell wall constriction.** Since a mixer model implements a
75 timer and an adder component serially, we examined our single cell shape data [2] to determine
76 a crossover in growth behavior. We find that the constriction dynamics in individual generations
77 exhibit a biphasic behavior, with an initial period of slow constriction followed by a phase of
78 fast constriction (Fig. 2a). We determine the crossover time, t_c , by fitting piecewise exponential
79 curves to the initial and the later phases of decay in $w_{\min}(t)$ (Methods; Supplementary Fig. 5
80 a-b). We estimate the onset of constriction by t_c , which has a mean value $t_c = 47 \pm 7$ min at 31°C
81 (Supplementary Fig. 5c). The data for w_{\min} across cell lineages collapse to a master crossover
82 curve when time is normalized by interdivision times (Fig. 2b-c), indicating that a single timescale
83 governs constriction initiation. This crossover dynamic is observed in the analogous data obtained
84 at other temperatures of the medium (Supplementary Fig. 6). We find that t_c increases in pro-
85 portion to τ and κ^{-1} as the temperature is decreased (Supplementary Fig. 7). The conditional
86 distributions of the normalized crossover times, t_c/τ , shown in Fig. 2c, collapse to a single curve
87 for various values of $l(0)$, independent of initial cell length. Indeed our data show that t_c/τ is
88 nearly uncorrelated with the initial cell size (Fig. 2d), whereas the cell length at $t = t_c$ increases
89 in proportion to the initial length, $l(t_c) = 1.25 l(0) + 0.43$ (Fig. 2e). By analyzing our shape
90 data at other temperatures we find that t_c/τ remains independent of $l(0)$ (Supplementary Fig.
91 9a), and does not vary with changing temperature of the growth medium (Supplementary Fig. 9b).

92

93 **Pure adder phase during cell wall constriction.** While the time to the onset of cell wall
94 constriction is uncorrelated with cell size, the added size in the constriction phase, $\delta' = l(\tau) - l(t_c)$,
95 also shows no correlation with $l(t_c)$ (Fig. 2e). This suggests a *pure adder* model of cell size control
96 for $t_c < t < \tau$, such that the distribution of the added size is independent of the initial cell length.
97 This adder behavior is confirmed by the collapse of the conditional distributions of the added size
98 $P(\delta'|l(0))$ to a single curve, approximated by a log-normal distribution (Fig. 2f) [7]. Furthermore,
99 the time to divide after t_c shows negative correlation with $l(t_c)$ (Supplementary Fig. 8). This
100 negative correlation is supported by an *adder* model for $t > t_c$, $\tau = t_c + \kappa^{-1} \ln [1 + \delta'/l(t_c)]$ with
101 $\delta' = 0.97 \mu\text{m}$. We find that the adder phase post constriction is conserved for all temperatures

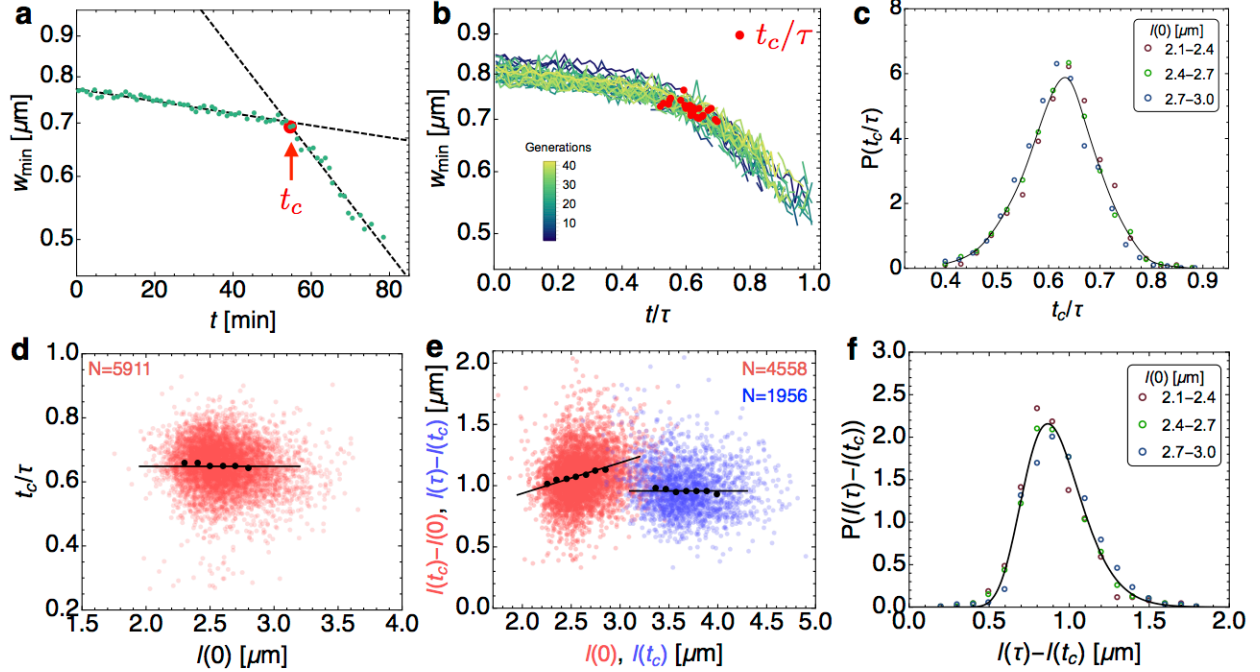


FIG. 2. Crossover from relative timer to adder at the onset of cell wall constriction. **a.** Semi-log plot of the time-dependence of w_{\min} in a representative generation shows two phases of constriction, a slow initial phase followed by a fast constriction phase. We determine t_c by the intersection of the least square exponential fits to the earlier and later portions of the division cycle (Methods). **b.** Dynamics of w_{\min} across generations of a typical cell as functions of the normalized division cycle time. Locations of t_c/τ are marked by red solid circles. **c.** The conditional probability density of the normalized crossover time, t_c/τ , given initial length values, $P(t_c/\tau|l(0))$, shown by colored circles. The density indicates that t_c/τ and $l(0)$ are independent. Solid line is a best fit cubic spline curve. **d.** Red scatter points and mean values (black points) show a lack of correlation between t_c/τ and $l(0)$. Black line represents a relative timer: $t_c = 0.63\tau$. The solid circles represent mean data binned in $l(0)$. **e.** Positive correlation between the added size before constriction, $l(t_c) - l(0)$ and $l(0)$ (red scatter). The black line represents the best fit: $l(t_c) = 1.25l(0) + 0.43$. Added size for $t > t_c$, is uncorrelated with $l(t_c)$ (blue scatter) supporting a pure adder model during the constriction phase: $l(\tau) = l(t_c) + 0.97$. **f.** The conditional probability density of the post-constriction added cell size. Colored circles indicate the ranges of the initial lengths, $l(0)$. The collapse of the distributions indicates the independence of $l(\tau) - l(t_c)$ and $l(0)$. The solid line is a best-fit lognormal distribution. N indicates number of generations in **d** and **e**.

102 with a mean added size $\approx 1 \mu\text{m}$ (Supplementary Fig. 9 c-d).

103 **Crossover in cell wall growth dynamics.** We conducted fluorescence labeling experiments to
 104 determine if we can visualize a crossover from timer to adder growth phase by selective labeling. We
 105 sought to examine the division cycle dependence of peptidoglycan synthesis, by using a fluorescent

106 construct of lectin wheat germ agglutinin (fWGA) that has been shown to label the cell wall of
 107 Gram-negative bacteria [23]. Consistently, our experiments measuring the WGA fluorescence of
 108 stalked *Caulobacter* cells showed peripheral WGA localization in confocal slices (Supplementary
 109 Fig. 10). Using our microfluidics platform [1], *C. crescentus* cells were initially incubated in media
 110 with PYE and fWGA for 15 minutes without flow, allowing the cells to be covered with fWGA.
 111 PYE media was then flowed into the microfluidics channel and image stacks of stalked cells were
 112 acquired every 10 minutes within the fields of view. The deconvolved images in Fig. 3a show that
 113 the fWGA intensity is spatially uniform prior to constriction (i.e., for samples at $t < 50$ min),
 114 but exhibits a pronounced minimum at the septum as the cell-wall is invaginated ($t > 50$ min).
 115 Moreover, the 70 and 80 min images even hint at the secondary invaginations in a predivisional
 116 cell, consistent with our previous report [2]. For each of these images, Fig. 3b shows the intensity
 117 along the centerline axis, averaged over the cell cross-section at each position and then normalized
 118 by the maximum value for each time. Because we account for variation of the cell cross-section,
 119 the appearance of the minimum in the intensity at the septum is not an artefact of its diminishing
 120 width. The spatial distribution of fWGA intensity suggests that growth is spatially uniform for
 121 $t < 50$ min and new cell-wall material is primarily synthesized at the invagination for $t > 50$ min.
 122 This septal mode of growth has been reported earlier with D-amino acid cell wall labeling [24, 25].

123 To quantify the spatial uniformity of cell-wall deposition for each cell in the ensemble we intro-
 124 duce an *intensity uniformity index*, D , given by the ratio of the intensity at the site of the septum
 125 (I_{\min}) to the mean of the maximum intensities ($I_{\max,1,2}$) on the stalked and swarmer sides of that
 126 site (Fig. 3c-inset). D is close to unity for $t < 50$ min (since $I_{\min} \simeq I_{\max,1,2}$), indicating spatially
 127 uniform growth (Fig. 3c). For $t > 50$ min, D drops sharply to lower values, suggesting that cell
 128 wall growth is localized to the septum. Fig. 3d shows the ensemble averaged intensity uniformity
 129 index, $\langle D \rangle$, exhibiting a smooth crossover to septal growth for $t > 0.6\tau$.

130 **Septal growth model describes biphasic constriction.** To examine whether septal cell wall
 131 synthesis (Fig. 3) can reproduce the observed crossover dynamics of constriction (Fig. 2), we
 132 consider a quantitative model for cell wall constriction driven by septal growth [21, 26]. We
 133 assume that the shape of the constriction zone is given by two intersecting hemispherical segments
 134 with diameter w , and constriction proceeds by completing the missing parts of the hemispheres
 135 while maintaining the curvature of the preformed spherical segments. The total surface area of the
 136 septum is given by $S(t) = \pi w l_s(t)$, where $l_s(t)$ is the total length of the hemispherical segments

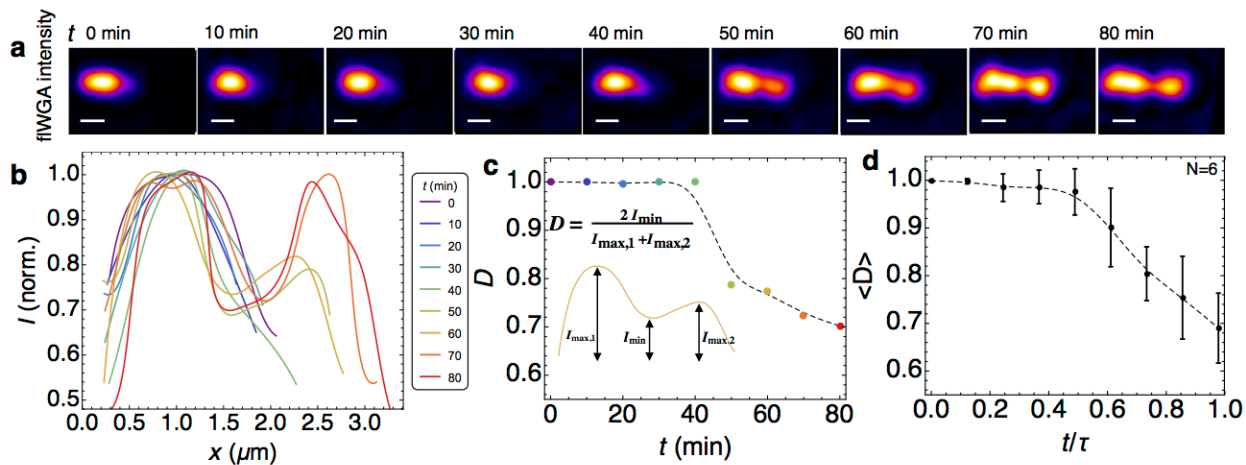


FIG. 3. **Crossover in cell wall growth dynamics at the onset of constriction.** **a.** Confocal fluorescent images of a representative *C. crescentus* cell in a microfluidic flow cell labeled with fluorescent WGA taken after 0, 10, 20, 30, 40, 50, 60, 70 and 80 min of growth in PYE medium. The fluorescence intensity is averaged over cell thickness (see Supplementary Fig. 10 for mid-plane fluorescence). The images are deconvoluted using the Huygens software package (Methods). The scale bars represent $1 \mu\text{m}$. The depletion of fluorescence reveals the underlying spatial pattern of growth, i.e. growth occurs where the fluorescence is minimized. **b.** Spatial distribution of fWGA intensity along the centerline axis, averaged over the cell cross-section at each position. We then normalized by the maximum value for each time to account for variations in fWGA labeling. The cross-section averaging accounts for the change in surface area reduction in the septal region. The time points are indicated with colors progressing from purple to red. **c.** Inset: A typical intensity profile is characterized by one minimum at the septum (I_{\min}) and two maxima near either pole ($I_{\max,1}$, $I_{\max,2}$). We define the index of uniformity as $D = 2I_{\min}/(I_{\max,1} + I_{\max,2})$ (Methods). $D(t)$ is shown for a representative cell in (A), revealing a crossover from uniform growth ($\langle D \rangle \simeq 1$) to localized septal growth at $t \sim 50$ min. **d.** Ensemble averaged dynamics of the growth uniformity index, $\langle D \rangle$, as a function of time normalized by the division time. Error bars indicate ± 1 standard deviation.

137 (Fig. 4a). Exponential growth of septal surface area implies [2]:

138
$$\frac{dl_s}{dt} = \kappa l_s + v_0, \quad (2)$$

where v_0 is the speed of septum synthesis at $t = 0$, which we determine by fitting our model to the data for $w_{\min}(t)$. Eq. (2) can be solved using the initial condition, $l_s(0) = 0$, to derive the time-dependence of $w_{\min}(t)$,

$$w_{\min}(t) = w_{\min}(0) \sqrt{1 - (l_0/w_{\min}(0))^2 (e^{\kappa t} - 1)^2},$$

139 where $l_0 = v_0 \kappa^{-1}$. As a result, the dynamics of constriction are controlled by the dimensionless
 140 parameter, aspect ratio $l_0/w_{\min}(0)$, exhibiting a crossover point (Fig. 4b). As shown in Fig. 4b,

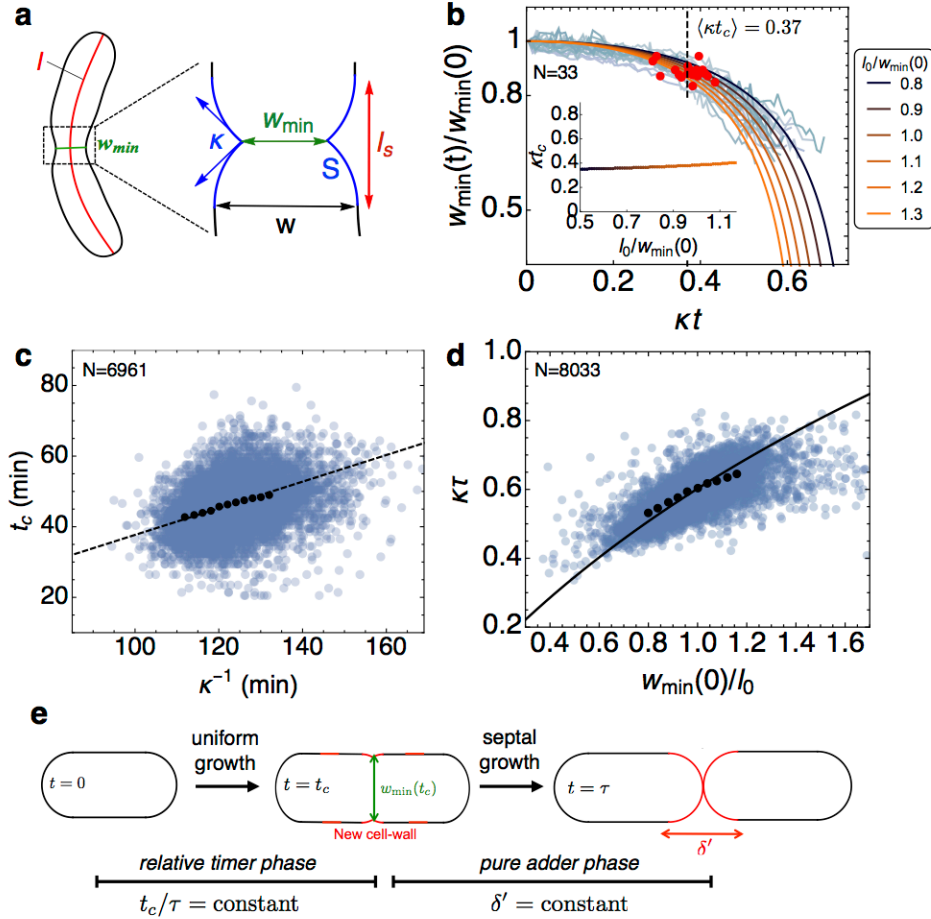


FIG. 4. Septal growth model predicts the onset of cell wall constriction and interdivision times.

a. A representative splined contour of a *C. crescentus* cell, illustrating the shape parameters, l (red) and w_{\min} (green). The region inside the dashed rectangle represents the constriction zone, where S is the surface area of the septal cell-wall (blue) synthesized after constriction. **b.** Dynamics of the width of the pinch-off plane (normalized by $w_{\min}(0)$), with time normalized by κ^{-1} . Predictions of the septal growth model are shown by solid curves at various values of the dimensionless parameter, $l_0/w_{\min}(0)$. Experimental data for different generations of a representative cell are shown in light blue with the locations of the crossover marked by solid red circles. Inset: No dependence of κt_c on $l_0/w_{\min}(0)$, as predicted by the theoretical model (Supplementary Note 2). **c.** Positive correlation between t_c and κ^{-1} (blue scatter). The solid black circles represent mean data binned in κ^{-1} and the dashed line represents the best fit with $t_c = 0.37\kappa^{-1}$. **d.** Normalized interdivision times ($\kappa\tau$) vs the normalized initial width of the pinch-off plane (binned mean data as solid black circles; model prediction as solid curve; see Supplementary Note 4; Supplementary Fig. 13a). **e.** Schematic for spatiotemporal coordination of cell wall growth in *C. crescentus* cells. Growth is spatially uniform for $t < t_c$ when cell wall deposition occurs along the entire cell length. For $t > t_c$, cell wall growth is dominated by septal cell wall synthesis that leads to constant size extension determined by the surface area of the new poles. N indicates number of generations in **b**, **c** and **d**.

141 the model prediction for the crossover time is in excellent agreement with our experimental data,
 142 and the time to the onset of constriction is insensitive to variations in $l_0/w_{\min}(0)$ (Supplementary
 143 Note 2; Fig. 4b-inset). This implies that t_c is controlled by κ , which is consistent with the positive
 144 correlation between t_c and κ^{-1} (Fig. 4c). The septal growth model describes a smooth transi-
 145 tion from predominantly lateral growth to predominantly septal growth, consistent with the data.
 146 We explicitly show how the dynamics depend on the rate of transition in Supplementary Note 3
 147 (Supplementary Fig. 12).

148 Another prediction of the septal growth model is that the interdivision times increase with
 149 $w_{\min}(0)/l_0$, because wider cells require more material to close the septum. Based on our model,
 150 we predict a simple relation between τ and $w_{\min}(0)$ (Supplementary Note 4),

$$151 \quad \tau = \kappa^{-1} \ln \left(1 + \frac{w_{\min}(0)}{l_0} \right). \quad (3)$$

152 Thus, the interdivision time is predicted to be longer for larger $w_{\min}(0)$. We find a positive
 153 correlation in our data between $\kappa\tau$ and $w_{\min}(0)/l_0$, and the mean trend in our data is in good
 154 quantitative agreement with our model prediction (Fig. 4d). Since constricting cells primarily
 155 grow from the septum (Fig. 3), the added size in the constriction phase, δ' , is expected to be
 156 proportional to the width of the septal plane. We find that δ' is positively correlated with $w_{\min}(t_c)$
 157 (Supplementary Fig. 13b). Furthermore, Eqs. (1) and (3) together imply a negative correlation
 158 between initial septal width, $w_{\min}(0)$, and the cell size at division, $l(\tau)$, in quantitative agreement
 159 with our data (Supplementary Note 5; Supplementary Fig. 13c).

160 DISCUSSION

161 The adder phase of cell-wall growth ($t > t_c$) coupled with relative timer prior to constriction
 162 ($t < t_c$) comprise a biphasic growth model for *C. crescentus* (Fig. 4e). Newborn cells exhibit
 163 uniform patterning of cell wall synthesis prior to constriction ($t < t_c$), and initiate constriction
 164 at a fixed phase in the division cycle. During the constriction phase ($t > t_c$), cells primarily add
 165 new cell wall material at the septum. This phase of growth is characterized by a constant cell size
 166 extension, proportional to the cell width. The length added after $t > t_c$ originates primarily from
 167 the surface area of the daughter cell poles. Taken together, the crossover from uniform cell wall
 168 growth to localized septal growth provides a physical basis for the *mixer model* of cell size control.

169 Like the adder model, the mixer model ensures cell size homeostasis: with each division, the cell
 170 length regresses to the ensemble average [10]. It is interesting to consider why *E. coli* exhibits a

171 pure adder behavior while *C. crescentus* exhibits a mixer behavior. One notable difference between
172 the species is that *E. coli* can initiate multiple rounds of DNA replication per cell cycle while *C.*
173 *crescentus* has a strict one-to-one correspondence between these processes. While the molecular
174 basis for the biphasic cell size control is unknown, the relative timer phase may be related to
175 the duration of chromosome replication, which is independent of cell size. Alternatively, it has
176 been suggested that nutrient uptake imposes condition-dependent constraints on surface-area-to-
177 volume ratios and in turn the growth mechanism [17]. Our study provides additional insights for
178 investigating the molecular candidates regulating cell size and division control in bacteria.

179 **METHODS**

180 **Acquisition of Experimental Data and Cell Shape Analysis.** Experimental data were
181 acquired as described in [1]. In the main article we use the exact same dataset as in refs. [1, 2],
182 consisting of 260 cells, corresponding to 9672 generations (division events) at 31°C. Corresponding
183 data and analysis of cell shape for other temperature are provided in the Supplementary Figures.
184 The acquired phase-contrast images were analyzed using a custom routine in Python [1, 2]. See
185 Supplementary Methods for further details.

186 **WGA Fluorescence Microfluidics Assay.** To investigate the dynamics of cell wall growth over
187 time in *Caulobacter crescentus*, we monitored the localization of fluorescent wheat germ agglutinin
188 (fWGA) on the cell wall of the bacteria using the microfluidics platform we previously developed [1].
189 A 5 mL liquid culture of *C. crescentus* in PYE was prepared overnight and diluted the following
190 morning to an optical density at 660 nm of 0.1. Vanillate was added to this diluted culture at a
191 final concentration of 0.5 mM to induce the production of the holdfast hfsA for 3 hrs. 1 mL of
192 this culture was flowed into a cleaned microfluidics chip and allowed to incubate for 1 hr. A 20
193 mL syringe of PYE and a 3 mL syringe with 2 mL of PYE and 1 mL of fWGA were attached to
194 two separate input ports into the microfluidics channel. Flow into the channel was resumed with
195 media from the 3 mL syringe at a rate of 3.5 L/min for 15 minutes. Flow was then halted for 15
196 minutes to allow the cells to be covered with the fWGA. Media from the 20 mL syringe was flowed
197 into the channel at a rate of 3.5 L/min. Image stacks with a 100 nm spacing were acquired every
198 10 minutes (using MicroManager) at a position along the microfluidics channel with sufficient cell
199 coverage.

200 **Deconvolution of Images.** Captured images of an object (I) are a convolution of the actual

201 object (f) and the point spread function (PSF) of the microscope (h):

$$202 \quad I = \int_{-\infty}^{\infty} f(\vec{x})h(\vec{x} - \vec{x}')d^3\vec{x}' . \quad (4)$$

203 Given a measurement of the PSF and the acquired images of the object, our deconvolution
204 approach employs a classic maximum-likelihood estimation algorithm that calculates the most
205 likely object to produce the acquired images [27]. This calculation is performed relatively quickly in
206 the Fourier domain, where the integral (4) is transformed into simple multiplication. Deconvolution
207 microscopy is widely used to remove the blurring imposed by the PSF of the microscope.

208 An image stack from each time point was deconvolved individually using commercial software
209 (Huygens Deconvolution; Scientific Volume Imaging). Before performing the deconvolution, the
210 3D PSF of our oil immersion objective (Nikon), with a magnification of 100X and $NA = 1.49$,
211 was measured by imaging static 100-nm-diameter polystyrene beads coated with green fluorescent
212 protein (Thermofisher). The PSF was sampled at 72 nm by 72 nm in the x-y plane and 50 nm in
213 the z direction, thus satisfying the Nyquist criteria for our particular objective. Next, 17 image
214 stacks corresponding to 17 time points were loaded into Huygens. Parameters such as background
215 intensity, spatial sampling, objective NA, immersion index of refraction, and the signal-to-noise
216 ratio (SNR) of objects were entered manually.

217 The background intensity was determined by calculating the mean intensity in an area of the
218 image where there is no signal. The bacterial image stacks were sampled at 72 nm by 72 nm in
219 the x-y plane and 100 nm in the z-direction. Because of photo-bleaching, the SNR of the bacteria
220 will change as a function of time. At each time point, the SNR was calculated using the following
221 equation: $SNR = \sqrt{N} = \sqrt{i_{\max}/i_{\text{single}}}$, where i_{\max} is the maximum grayscale value of a pixel in
222 the bacteria and i_{single} is the grayscale value due to a single photon incident on our detector. The
223 value of i_{mean} is obtained at each time point from the measured images. The value of i_{single} is a
224 calculated quantity using the parameters of our camera (Andor iXon EMCCD) such as quantum
225 efficiency, A/D conversion, and system gain. A maximum number of 40 iterations was allowed for
226 the deconvolution, but Huygens reached a global minimum at ~ 30 iterations for each time point.

227 **Intensity uniformity index.** A typical intensity profile at early times ($t < 50$ min) is spatially
228 uniform around the cell center and then decays towards the poles. At later times, $t > 50$ min, the
229 intensity profile is characterized by one minimum at the septum, given by I_{\min} , and two maxima
230 at the stalked and the swarmer components, given by $I_{\max,1}$ and $I_{\max,2}$ respectively (Fig. 3c -
231 inset). At each time point, we define the growth uniformity index for each intensity profile as,
232 $D = 2I_{\min}/(I_{\max,1} + I_{\max,2})$. I_{\min} is defined as the minimum in the intensity profile for $r - 2\sigma <$

233 $x/l < r + 2\sigma$, where x is the coordinate along the centerline, r is the mean ratio of the daughter cell
 234 lengths, and σ is the standard deviation in daughter cell length ratio. Let x_{\min} denote the location
 235 of I_{\min} along the centerline coordinate. Then $I_{\max,1}$ is defined as the maximum in the intensity
 236 for $x < x_{\min}$ and $I_{\max,2}$ is the maximum in the intensity profile for $x > x_{\min}$. Thus, for $t \leq 50$,
 237 $I_{\min} \simeq I_{\max,1} \simeq I_{\max,2}$ and $D \simeq 1$. Whereas for $t \geq 50$ min, I_{\min} represents the fWGA intensity
 238 value at the septum and is lower than both $I_{\max,1}$ and $I_{\max,2}$.

239 **Crossover analysis from experimental data.** To determine the crossover time, t_c , from the
 240 data on w_{\min} , we fit the following piecewise linear function to $\ln(w_{\min})$:

$$241 \quad \ln(w_{\min}) = \begin{cases} at + b & \text{if } t < t'_c \\ b + (a - c)t'_c + ct'_c & \text{if } t \geq t'_c, \end{cases} \quad (5)$$

242 with four undetermined parameters a , b , c and t'_c obtained using a built-in curve fitting function
 243 in Mathematica. A representative fit is given in Supplementary Fig. 5a, where t'_c is the point of
 244 intersection of the two lines. We then compute the metric $Dw(t) = w_{\min}^i(t) - w_{\min}(t)$, where $w_{\min}^i =$
 245 e^{at+b} (Supplementary Fig. 5b). Constriction is estimated to initiate when the metric Dw exceeds
 246 a threshold of $0.05 \mu\text{m}$, which is equal to a single image pixel. The crossover time, t_c , is taken to
 247 be 3 frames prior to the frame when Dw crosses the threshold value (Supplementary Fig. 5b), such
 248 that the determination of t_c is robust to noise. We find the location for t_c is significantly spread out
 249 across generations when $w_{\min}(t)$ is plotted against absolute time (Supplementary Fig. 5c). When
 250 the constriction curves are aligned from the end of the cycle, as in Ref. [17], the individual curves
 251 collapsed, although the spread for t_c is significant (Supplementary Fig. 5d). By contrast, when
 252 the constriction curves are plotted against relative time, as shown in Fig. 2b, the locations of the
 253 crossover, t_c/τ , are much better aligned across generations.

254 **Data availability.** The data that support the findings of this study are available from the corre-
 255 sponding authors upon request.

256 REFERENCES

- 257 [1] Iyer-Biswas, S. *et al.* Scaling laws governing stochastic growth and division of single bacterial cells.
 258 *Proceedings of the National Academy of Sciences* **111**, 15912–15917 (2014).
 259 [2] Wright, C. S. *et al.* Intergenerational continuity of cell shape dynamics in *caulobacter crescentus*.
 260 *Scientific Reports* **5**, 9155 (2015).
 261 [3] Wang, P. *et al.* Robust growth of *Escherichia coli*. *Current Biology* **20**, 1099–1103 (2010).

- 262 [4] Campos, M. *et al.* A constant size extension drives bacterial cell size homeostasis. *Cell* **159**, 1433–1446
263 (2014).
- 264 [5] Taheri-Araghi, S. *et al.* Cell-size control and homeostasis in bacteria. *Current Biology* **25**, 385–391
265 (2015).
- 266 [6] Sauls, J. T., Li, D. & Jun, S. Adder and a coarse-grained approach to cell size homeostasis in bacteria.
267 *Current opinion in cell biology* **38**, 38–44 (2016).
- 268 [7] Amir, A. Cell size regulation in bacteria. *Physical Review Letters* **112**, 208102 (2014).
- 269 [8] Deforet, M., van Ditmarsch, D. & Xavier, J. B. Cell-size homeostasis and the incremental rule in a
270 bacterial pathogen. *Biophysical Journal* **109**, 521–528 (2015).
- 271 [9] Voorn, W., Koppes, L. & Grover, N. Mathematics of cell division in Escherichia coli. *Curr. Top. Mol.*
272 *Genet* **1**, 187–194 (1993).
- 273 [10] Jun, S. & Taheri-Araghi, S. Cell-size maintenance: universal strategy revealed. *Trends in Microbiology*
274 **23**, 4–6 (2015).
- 275 [11] Schaechter, M., Williamson, J. P., Hood Jr, J. & Koch, A. L. Growth, cell and nuclear divisions in
276 some bacteria. *J Gen Microbiol* **29**, 421–434 (1962).
- 277 [12] Koppes, L., Meyer, M., Oonk, H., De Jong, M. & Nanninga, N. Correlation between size and age at
278 different events in the cell division cycle of Escherichia coli. *Journal of Bacteriology* **143**, 1241–1252
279 (1980).
- 280 [13] Osella, M., Nugent, E. & Lagomarsino, M. C. Concerted control of Escherichia coli cell division.
281 *Proceedings of the National Academy of Sciences* **111**, 3431–3435 (2014).
- 282 [14] Tanouchi, Y. *et al.* A noisy linear map underlies oscillations in cell size and gene expression in bacteria.
283 *Nature* **523**, 357–360 (2015).
- 284 [15] Donachie, W., Begg, K. & Vicente, M. Cell length, cell growth and cell division. *Nature* **264**, 328–333
285 (1976).
- 286 [16] Kubitschek, H. Bilinear cell growth of Escherichia coli. *Journal of Bacteriology* **148**, 730–733 (1981).
- 287 [17] Harris, L. K. & Theriot, J. A. Relative rates of surface and volume synthesis set bacterial cell size. *Cell*
288 **165**, 1479–1492 (2016).
- 289 [18] Wang, J. D. & Levin, P. A. Metabolism, cell growth and the bacterial cell cycle. *Nature Reviews*
290 *Microbiology* **7**, 822–827 (2009).
- 291 [19] Mitchison, J. M. *The biology of the cell cycle* (CUP Archive, 1971).
- 292 [20] Marshall, W. F. *et al.* What determines cell size? *BMC Biology* **10**, 101 (2012).
- 293 [21] Banerjee, S., Scherer, N. F. & Dinner, A. R. Shape dynamics of growing cell walls. *Soft Matter* **12**,
294 3442–3450 (2016).
- 295 [22] Lin, Y., Li, Y., Crosson, S., Dinner, A. R. & Scherer, N. F. Phase resetting reveals network dynamics
296 underlying a bacterial cell cycle. *PLoS Comput Biol* **8**, e1002778 (2012).
- 297 [23] Ursell, T. S. *et al.* Rod-like bacterial shape is maintained by feedback between cell curvature and
298 cytoskeletal localization. *Proceedings of the National Academy of Sciences* **111**, E1025–E1034 (2014).

- 299 [24] Aaron, M. *et al.* The tubulin homologue ftsz contributes to cell elongation by guiding cell wall precursor
300 synthesis in caulobacter crescentus. *Molecular Microbiology* **64**, 938–952 (2007).
- 301 [25] Kuru, E. *et al.* In situ probing of newly synthesized peptidoglycan in live bacteria with fluorescent
302 d-amino acids. *Angewandte Chemie International Edition* **51**, 12519–12523 (2012).
- 303 [26] Reshes, G., Vanounou, S., Fishov, I. & Feingold, M. Cell shape dynamics in Escherichia coli. *Biophysical*
304 *Journal* **94**, 251–264 (2008).
- 305 [27] Sibarita, J.-B. Deconvolution microscopy. In *Microscopy Techniques*, 201–243 (Springer, 2005).

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315 **AUTHOR CONTRIBUTIONS**

316 S.B., K.L., A.R.D. and N.F.S. designed research; S.B., K.L., A.S., M.D. and T.K. performed
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318 **COMPETING FINANCIAL INTERESTS**

319 The authors declare no competing financial interests.