Constriction rate modulation can drive cell size control and homeostasis in *C. crescentus*

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Abstract

Rod-shaped bacteria typically grow first via sporadic and dispersed elongation along their lateral walls, then via a combination of zonal elongation and constriction at the division site to form the poles of daughter cells. Although constriction comprises up to half of the cell cycle, its impact on cell size control and homeostasis has rarely been

considered. To reveal the roles of cell elongation and constriction in bacterial size regulation during cell division, we captured the shape dynamics of *Caulobacter crescentus* with time-lapse structured illumination microscopy and used molecular markers as cell-cycle landmarks. We perturbed constriction rate using a hyperconstriction mutant or fosfomycin inhibition. We report that constriction rate contributes to both size control and homeostasis, by determining elongation during constriction, and by compensating for variation in pre-constriction elongation on a single-cell basis.

Introduction

Cell size regulation is observed nearly universally among prokaryotes [1], allowing them to both control their size at birth and to homeostatically maintain it over multiple generations [2]. Cell size control and homeostasis are critical for survival: once too small, cells lack the volume required to host the essential machinery of life [3] or initiate chromosome segregation [4], whereas cells that are too large may suffer limitations in nutrient uptake [5] and distribution [6] because of their reliance on diffusive transport.

Size regulation is the outcome of multiple sequential processes that take place during the cell division cycle. Progression through the cell cycle is marked by several key processes, including chromosome replication, segregation, and division into two daughter cells. These processes occur once per cell cycle in bacteria such as *Caulobacter crescentus* [7], in contrast to rapidly proliferating organisms such as *Escherichia coli* [8] and *Bacillus subtilis* in which cells often have multi-fork replication and which can, following nutrient up-shifts, initiate replication multiple times in a single cell cycle. In *C. crescentus*, differentiation from a swarmer to a stalked cell and the initiation of chromosome replication and segregation mark the transition from cell cycle phase G1 to S. The completion of replication marks the end of S phase. Once DNA segregation is completed, cells finish cytokinesis to form sibling stalked and swarmer cells during G2/M [9].

From the perspective of the cell wall, individual *C. crescentus* cells elongate exponentially throughout the cell cycle, as is typical for rod-shaped bacteria. Their growth is divided into a stage of pure elongation as peptidoglycan is inserted sporadically along the lateral walls, followed by a stage of mixed elongation and constriction in G2/M phase during which peptidoglycan is inserted at mid-cell to build two new poles [10,11]. Each stage governed by largely independent molecular machines. In *B. subtilis*, when cells are on average longer at the onset of constriction, they are also on average longer at division [12,13]. This suggests a simple model for cell size control, by modifying the cell length at which the divisome, the multi-protein complex which guides division, begins to generate constriction. Another possibility is

62 that the rate of constriction is modulated; this was shown to be the case for MatP, which
63 coordinates chromosome segregation and pole construction in *E. coli* [14].

64 Several checkpoints have been proposed to couple bacterial cell size homeostasis to
65 cell cycle phases. Shortly after entry into S phase, chromosome segregation must initiate
66 before the cytokinetic Z-ring can assemble at mid-cell, coordinated by the gradient-forming
67 FtsZ inhibitor MipZ [15]. In *E. coli*, the initiation of chromosome replication requires a fixed
68 volume per origin of replication, leading to a "sizer" [16]. Chromosome replication in G1/S may
69 take a constant duration, referred to as a "timer" [8]. Putative molecular mechanisms have
70 generally relied on the accumulation of proteins above a threshold, such as an "initiator"
71 triggering replication [17], or excess peptidoglycan (PG) cell wall precursors triggering
72 constriction [18]. A model of the latter case predicts a constant addition of volume per cell
73 cycle, or "adder". Indeed, an adder has been observed for *C. crescentus* under a wide range
74 of growth conditions [19]. Deviations from a pure adder toward a mixed relative timer and adder
75 have also been reported for stalked cells, observed over many generations and a range of
76 different temperatures [20]. Any model incorporating a sizer or adder will allow smaller cells to
77 increase while larger cells decrease in size over generations until both converge to a size set
78 by the constant of addition [21]. Thus, both provide a clear means for a population to achieve
79 size homeostasis.

80 Remarkably, although constriction makes up a significant portion of the cell cycle in
81 many bacteria [22], its impact on cell size control and homeostasis has rarely been considered.
82 Intriguingly, budding yeast may use constriction rate to modulate their size in response to
83 changes in growth conditions [23]. However, a single-cell study of the contribution of the
84 constriction stage in bacteria has been challenging, in part due to the diffraction-limited size of
85 the constriction site, and the need for corroboration by divisome markers to unambiguously
86 identify constriction onset. Furthermore, direct measurement of the instantaneous constriction
87 rate has not been possible.

88 Here, we investigated whether, and how cells adjust their constriction rate to achieve
89 cell size control and homeostasis. We used structured illumination microscopy (SIM) [24] to

resolve the constriction site diameter and measure the size of synchronized *C. crescentus* cells as they progressed through their cell cycle. We show that perturbing the constriction rate changes cell size, independent of elongation rate. Furthermore, we found that within a population the onset of constriction and its rate are coordinated: cells that elongate more than average before constriction undergo a more rapid constriction, leading to less elongation during constriction, and vice-versa. This compensation leads to a higher fidelity adder than permitted by onset control alone, allowing *C. crescentus* to better maintain its size in the face of biological noise.

Results

Perturbing constriction rate changes cell length

To test the role of constriction, we perturbed its rate pharmacologically and genetically. Fosfomycin inhibits the peptidoglycan (PG) synthesis enzyme MurA [25], which slows PG synthesis and therefore the constriction rate. Additionally, the divisome includes cell wall remodeling enzymes including the late-arriving FtsW and FtsI. Several point mutants of the trans-glycosylase FtsW [26] and its cognate transpeptidase FtsI [27], referred to as FtsW**I*, resulted in a gain-of-function phenotype in *C. crescentus* [28]. It was hypothesized that these mutations maintain the enzymes in their active state, and thereby would increase the constriction rate [28].

We resolved cell shape dynamics during the cell cycle by performing dual-color imaging of the inner membrane and divisome proteins (FtsZ-GFP, FtsW-GFP) with time-lapse SIM (Figure 1A, Movies S1-S3) on a synchronized population of cells. We used automated image analysis to quantify cell shape parameters during the cell cycle (Figure 1B, Figure S1, Methods). The overall cell length relative to the wild-type strain was shorter for FtsW**I* and longer for fosfomycin-treated cells, consistent with previous studies [18,28] (Figures 2A, S2A, B).

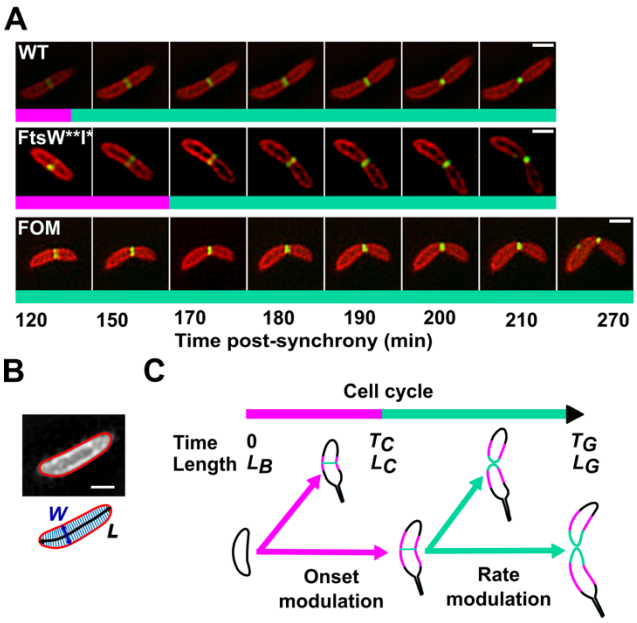


Figure 1: Experimental strategy and constriction-related models for modulation of cell size. (A) Time-lapse SIM images: inner membrane (mCherry-MTS2, red), FtsZ (FtsZ-GFP, green). Shown are example wild-type (WT), FtsW**I* mutant, and fosfomycin-treated cells through constriction, until separation. (B) Analysis of cell shape parameters using sDaDa (see Supplementary methods and Figure S1): the central line (black) is used to measure length (L); the width (W) is extracted from each perpendicular segment; the cell contour defines cell shape (red line). (C) Constriction rate or onset control mechanisms for length. Cells are born at time 0 with length at birth L_B, and elongate exponentially. T_C and L_C are the time and length at constriction onset. T_G and L_G are the time and length at the end of the cell cycle. Magenta parts of the cell contour represent lateral elongation, and cyan parts represent septal elongation. Scale bars: 500 nm. Bicolor bars indicate the stage: pre-constriction (magenta) and post-constriction (cyan).

Could elongation before the onset of constriction (Figure 1C, onset modulation) set the differences in final length between FtsW**I* mutant, fosfomycin-treated and WT cells? The appearance of a measurable constriction in SIM data corresponded well with the arrival of FtsW (Figure S1C), and allowed us to separate elongation before and after constriction onset. Differences in elongation before constriction for all conditions (Figure 2B) were insufficient to account for the observed differences in final length (Figure 2A). Thus, we examined shape changes during constriction (Figure 1C). Individual cells continued to elongate exponentially with the same apparent rate, even as they changed from pure elongation to mid-cell remodeling and constriction (Figures 2 C, E). However, the constriction rate was increased for the FtsW**I* mutant and decreased for fosfomycin-treated cells when compared to WT (Figure 2C), leading to differences in overall cell elongation during constriction (Figure 2D). Thus, we have

demonstrated that constriction rate modulation can be a mechanism for cell size control, independent from onset modulation [12,13].

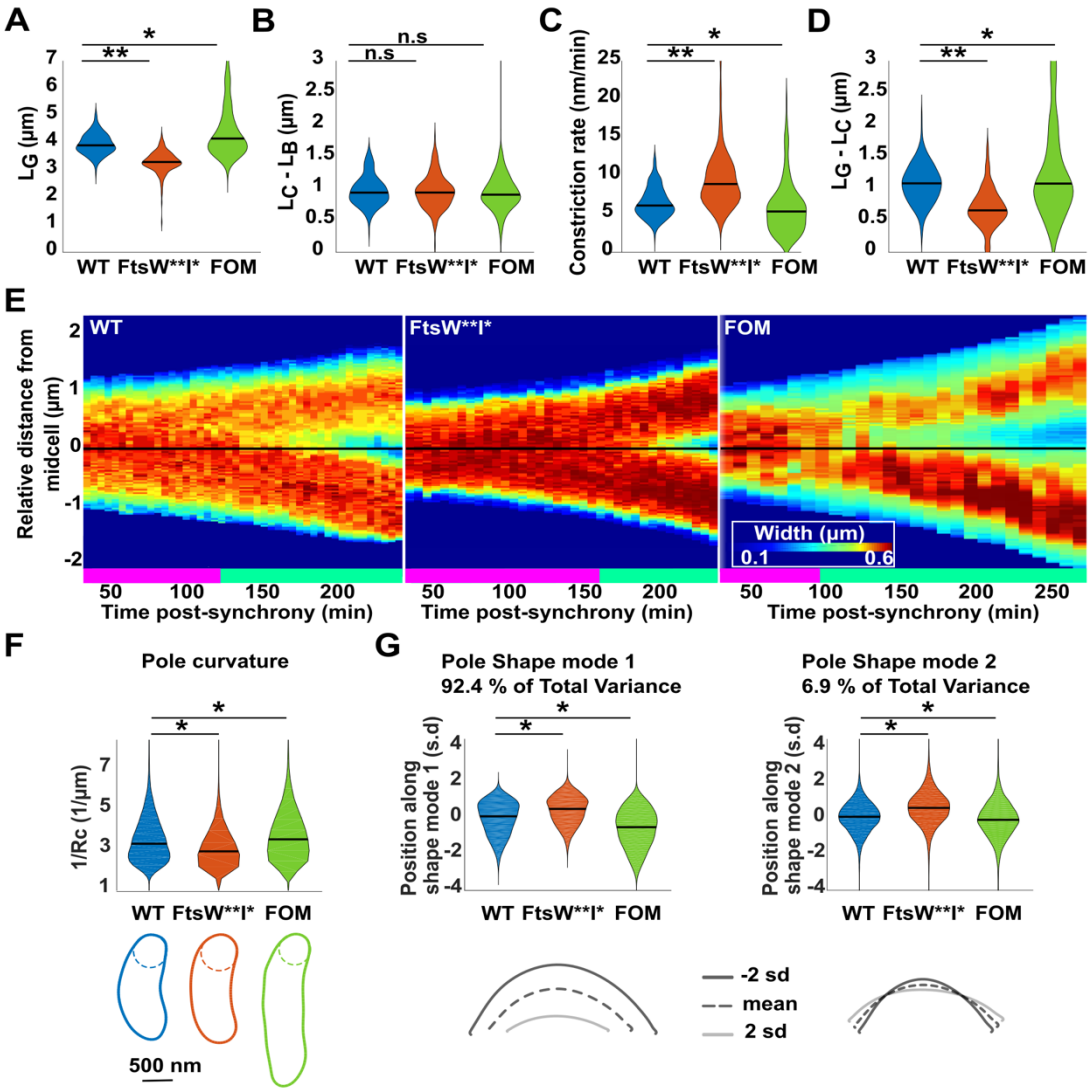


Figure 2. Differences in constriction rate yield different cell sizes and pole shapes. Single-cell distributions of (A) length at division; (B) elongation before constriction; (C) mean constriction rate; (D) elongation during constriction. (A-D), black bars represent the median of the population. Number of cells N: WT: N=208, FtsW**I*: N=212, FOM: N=220. Significance: **: p<0.005, *: p<0.05, n.s.: not significant. (E) Kymographs of representative cells, displaying cell diameter along the cell's length (vertical axis), versus growth time post-synchrony (horizontal axis) , red indicates large diameter, blue small diameter. The middle of the cell is indicated by the black horizontal line. Bicolor bars indicate the stage: pre-constriction (magenta) and post-constriction (cyan). (F) Pole shape analysis. The curvature is the reciprocal of the radius (Rc) of a circle tangent to the curve at a given point, here taken to be the pole. Each cell contour represents a representative single cell from each condition; the distribution of curvatures is plotted above (median value, black bar). (G) The pole region was extracted from each contour (>6000 cells per condition) and analysed using principal component analysis (Celltool, [46]). Shape mode 1 mostly accounts for variation in the length of the pole; shape mode 2 mostly accounts for variation in the bluntness of the pole independent of length. The distributions of each shape mode is plotted, with examples of corresponding shapes. (see also Figure S2)

159 We found that individual cells continued to elongate at the same rate during
160 constriction, although different perturbations modulated their constriction rate. Thus, faster
161 constriction as in the case of FtsW**I* implies cells should have shorter, blunter poles, while
162 slower constriction as in the case of fosfomycin treatment implies they should have longer,
163 sharper poles. Indeed, kymographs show a more extended gradient in cell width at the poles
164 of fosfomycin-treated cells (Figure 2E). In contrast, FtsW**I* cells show a steeper gradient at
165 the poles. This was confirmed quantitatively by measuring the radius of curvature at the poles
166 (Figure 2F). Furthermore, a population-wide analysis of pole shape demonstrated that over
167 95% of the total shape variance is accounted for with two principle shape modes, which
168 primarily capture variation in the length and bluntness of the poles (Figure 2G). FtsW**I*,
169 fosfomycin-treated, and WT cells were all distinct along each of these shape axes. We also
170 observed differences in the width of the Z-ring, which appears laterally extended in the
171 fosfomycin case (Figure S2G). This may result from changes in length at constriction onset,
172 since the region of lowest MipZ concentration will be more extended in longer cells [15].

173 **Elongation before and during constriction compensate one another**

174 To better decipher the relative role of the constriction rate in cell size regulation we
175 further analyzed its contribution to cell size homeostasis. Our experiments were designed to
176 precisely measure the relative contributions to total elongation, and not to distinguish between
177 different general models of homeostasis, which would require measurement over thousands
178 of generations. We found that cells elongated with a distinct mean value for each condition
179 (Figure 3), and that the more individual cells elongated before, the less they elongated during
180 constriction across all conditions tested (Figure 3, Figure S3). Indeed, the total elongation was
181 independent of the relative time cells spent in elongation and constriction phases, with the
182 exception of fosfomycin-treated cells (Figure 3), generally consistent with an "adder."
183 Consequently, the variance in the total elongation was lower than the variances in elongation
184 before and during constriction would have independently suggested. This was true for all
185 populations, including under perturbed conditions (Figure S3A). These results demonstrate
186 compensation, or over-compensation in the case of fosfomycin (Figure 3, Figure S3B),

between elongation before and during constriction resulting in a higher fidelity homeostasis for total elongation (Figure S3A-C).

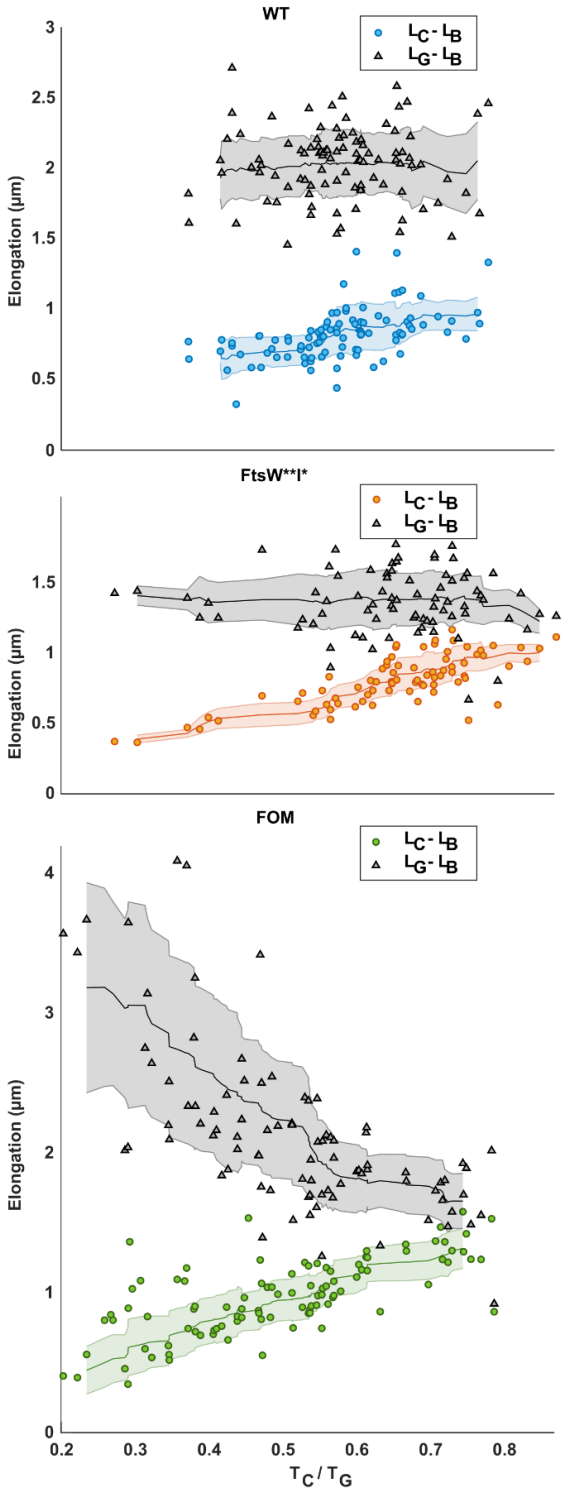


Figure 3. Compensation of elongation before and during constriction contributes to cell size homeostasis.
Total elongation (gray) and elongation before constriction (color) for individual Wild Type, FtsW**I* and fosfomycin (FOM)-treated cells, as a function of normalized onset time (T_C/T_G). Lines represent the 20 cells moving average; the shaded zones represent the moving standard deviation. Extreme outliers, more than 2 standard deviations from the mean, were omitted for the calculation of the moving average (see also Figure S3).

What could be the mechanism for this compensation? Elongation rate and constriction rate together determine elongation during constriction. Compensation could occur if cells which elongate less before onset subsequently elongate more rapidly or constrict more slowly. However, elongation rate during constriction did not negatively correlate with elongation before constriction (Figure S4A). To better understand constriction dynamics, we examined single cell waist widths as a function of relative duration of constriction (Figure 4A). Cells which elongated more before constriction also spent relatively less time constricting, indicating a higher overall constriction rate. The converse was true for cells which elongated less before constriction, confirming that constriction rate was behind the compensatory effect. Single cells constricted with increasing rate until division; thus, we defined two rates, corresponding to early and late constriction (Figure S4B). Interestingly, early instantaneous constriction rate correlated positively with elongation before constriction, but late rate did not (Figure 4B, C). Hence, early constriction rate changes at the single cell level to adjust elongation during and compensate elongation before constriction.

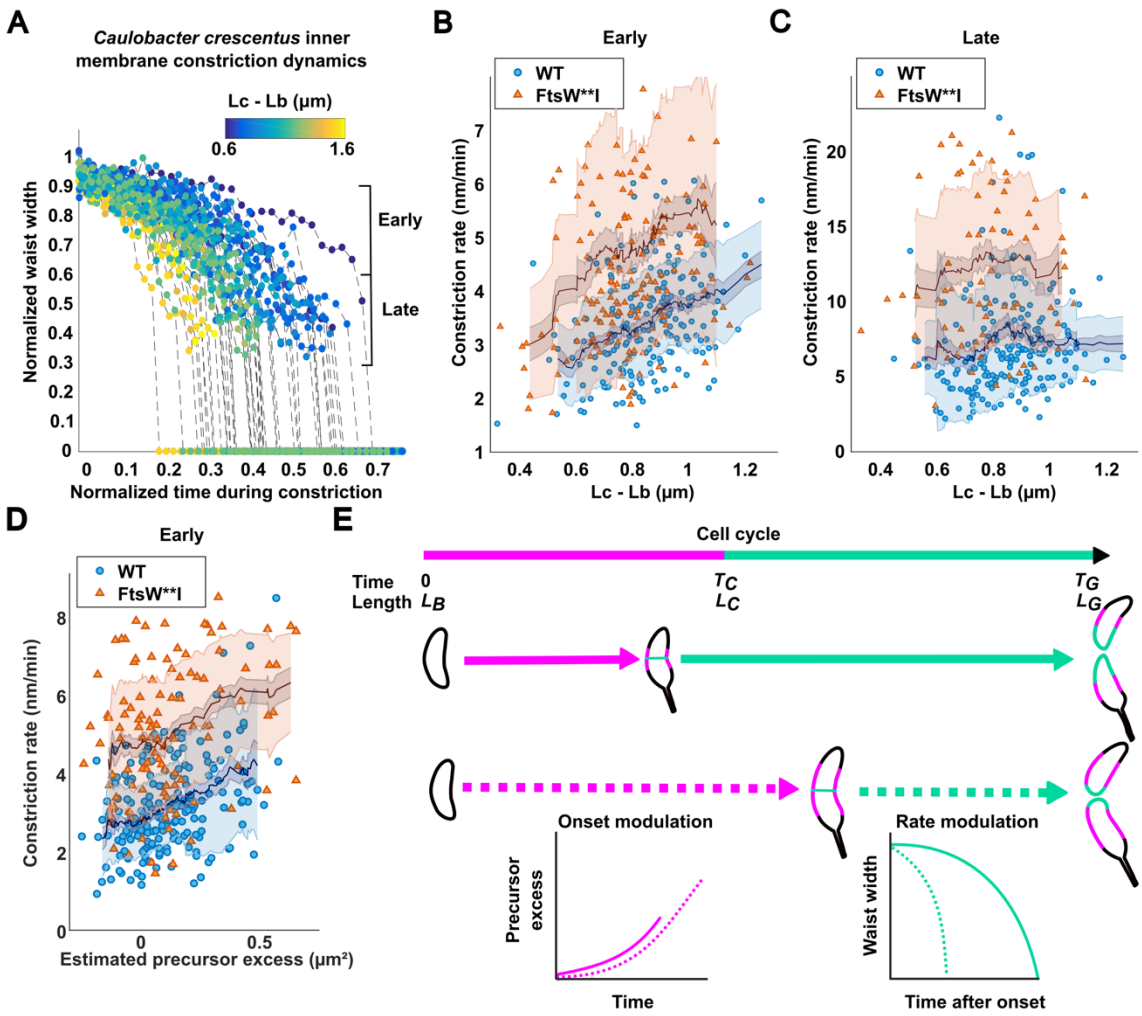
While molecular mechanisms have been proposed for ensuring homeostasis, the identity of the underlying regulatory factors remains controversial. A previous model estimated PG precursor excess amount as a function of cell cycle [18]. Each cell is assumed to be born with negligible excess, and generates an increasing excess of PG precursors. PG precursors are synthesized in the cell volume, at a volume-dependent rate, while being depleted as they become integrated into the cell wall (Supplementary Note 1). Using this model and experimentally measured cell contours to estimate the changes in surface area (ΔA) and volume (ΔV), we calculated the excess precursor area (A_{excess}) at the onset of constriction (T_c) for individual cells at onset of constriction:

$$A_{excess}(T_c) = \langle \frac{\Delta A}{\Delta V} \rangle_{cell\ cycle}\, \Delta V(T_c) - \Delta A(T_c)$$

Here, $\langle\rangle_{cell\ cycle}$ refers to the value averaged over the cell cycle. Since it took on average 30 minutes to set up each experiment, we underestimated the volume and area at birth, leading

223 to an offset toward negative estimated precursor excess (Figure 4D). However, we expect the

224 trends to be insensitive to this shift.



225
226 **Figure 4. Early constriction rates compensate for elongation before onset.**
227 (A) Normalized waist width as a function of normalized time during constriction, colormap represents
228 the elongation before constriction. The measurable constriction was divided into early (0.9-0.6) and
229 late (0.6-0.3) stages. (B) Testing correlation between early constriction rate and elongation before
230 constriction in both WT and mutant strains, WT: r=0.45, p-value<0.01 mutant: r=0.24, p-value<0.01
231 (Pearson correlation coefficient). (C) Testing correlation between late constriction rate and elongation
232 before constriction, WT: r=0.05, p-value>0.48, mutant: r=0.02, p-value>0.8 (Pearson correlation
233 coefficient). (D) Testing correlation between estimated PG precursor excess and early constriction
234 rate, WT: r=0.33, p-value<0.01, mutant: r=0.29, p-value<0.01 (Pearson correlation coefficient).
235 N>=200 for each strain. (E) Schematic of size regulation in *C. crescentus* with mixed modulation of
236 constriction onset and rate. Magenta parts of the cell contour represent lateral elongation, and cyan
237 parts represent septal elongation. Later onset leads to higher PG precursor excess, which drives more
238 rapid initial constriction (dashed trajectories), and vice-versa (see also Figure S4).
239

240 To explain our data, we speculate on a parsimonious model in which PG precursor

241 excess also sets the rate of PG remodeling at the constriction site, and therefore the rate of

242 constriction: the higher the excess the shorter the constriction duration. Indeed, we observed

243 a negative correlation between rate of constriction and estimated excess PG precursor for WT
244 and FtsW**I* cells (Figure 4D, Figure S4). Furthermore, as the new cell poles are built, the
245 excess PG precursor should diminish, leading to a decreased creation of area per time. This
246 is indeed what we observe (supplementary note 2), consistent with models of constriction rate
247 in *E. coli* [14]. Fosfomycin inhibits PG synthesis, so we can no longer use the same
248 mathematical expression to estimate precursor excess, since the activity of fosfomycin would
249 introduce an extra depletion term. Interestingly, within our model this should lead to a slower
250 constriction, consistent with our observations (Figure 2C). Although we have posed the
251 regulatory factor to be PG precursors, this remains controversial because there is only indirect
252 evidence for their role. Any "X-factor" regulatory molecule for constriction rate following the
253 functional relationship described for surface area and volume would fit within the model we
254 suggest.

255 **Discussion**

256 *Salmonella, E. coli* and *B. subtilis* coordinate their cell size with nutrient availability,
257 perhaps to allow sufficient room for multi-fork replication [4,29,30]. *C. crescentus* shows no
258 such adaptation [19,31] , and how its size is modulated has remained a mystery. Our findings
259 show a clear contribution to cell size control from growth during the final constriction stage of
260 cell cycle. Modulation of constriction dynamics changes the overall length of cells, in a manner
261 which has implications for cell shape. In the hypothetical case of extremely rapid constriction,
262 the cell length would be set almost entirely by the growth during the pure elongation stage,
263 leading to short cells with blunt poles. By modulating constriction onset and rate together
264 (Figure 4E), cells may arrive at a variety of pole shapes, an unexpected control mechanism for
265 bacterial cell shape.

266 Intriguingly, the cell wall itself can have differential properties at the division site. In *B.*
267 *subtilis*, the division septum contains an enrichment of pentapeptides compared to the rest of
268 the cell envelope [32], perhaps due to a change in the crosslinking or PG composition. In *E.*
269 *coli*, glycan strands lacking stem peptides are enriched at the septum, allowing proteins
270 containing the PG binding (SPOR) domain to be recruited [33]. In *C. crescentus,* the hydrolase

271 DipM is recruited to the division site by its PG binding LysM domains, suggesting a distinct PG
272 chemistry [32–34] . Consistently, we observed a reduction in staining by wheat germ agglutinin
273 (WGA) at midcell for later stages of the cell (Figure S4I) [37]. Since cells were fixed prior to
274 staining, this implies a difference in the chemistry of the division site, but confounds the
275 interpretation of WGA staining in living cells [20]. We expect that in future studies it will be
276 important to use fluorescent cell-cycle markers in conjunction with fluorescent D-amino acids
277 [11], which together can identify cell-cycle timing and modes of growth. It would be interesting
278 to investigate whether the rate of constriction also affects cell wall chemistry at the division
279 site.

280 Different factors have been demonstrated to be important for determining constriction
281 dynamics. Before cells can build a septal wall, chromosomes must be partitioned; accordingly,
282 machinery which coordinates the two such as MatP in *E. coli* can also modulate constriction
283 rate [14]. Similarly, dynamically treadmilling FtsZ filaments act as a scaffold to direct cell wall
284 remodelers to the division site, and modulate their rate [38,39]. Nevertheless, the activity of
285 PG remodeling enzymes involved in constriction might depend on PG precursor amounts, and
286 thus may act as a part of a responsive machine. Since, for individual cells, elongation proceeds
287 exponentially with a single rate constant even as PG precursor excess is predicted to increase
288 over cell cycle, the elongation machinery is presumably relatively insensitive to changes in PG
289 precursor amounts. By coupling the elongation machinery to the PG precursor-sensitive
290 constriction machinery, the cell may have arrived at a simple means of compensating for
291 fluctuations in elongation during different phases of the cell cycle. This compensation still has
292 its limitations as we observed in the case of FtsW**I* (Figure S3B); in the case of large
293 elongation before onset, cells must still elongate by a minimum amount during Z-ring
294 maturation (Figure S2H) and constriction. In the future, it will be interesting to identify the
295 molecular partners responsible for constriction rate modulation and PG sensing, and to
296 experimentally investigate the mechanism behind compensation of elongation.

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Author contributions

A.L., A.V., S.M., E.G., M.T.L., S.H. conceived, designed and/or performed experiments, A.L., A.V., A.A., S.M., F. S., S.H., Z.P. created analysis tools and analyzed the data, and all authors contributed to the writing of the manuscript.

Declaration of interests

The authors declare no competing interests.

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428

429 **STAR Methods**

430 **CONTACT FOR REAGENT AND RESOURCE SHARING**

431 Further information and requests for resources and reagents should be directed to and will be
432 fulfilled by the Lead Contact, Suliana Manley (suliana.manley@epfl.ch).

433 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

434 **Bacterial strains and growth conditions**

435 **Strains and plasmids**

436 The strains, plasmids, oligonucleotides, restriction sites and modes of constructions used for this study
437 are summarized in key resources table. The WT and mutant strains were electroporated with the P_{van}
438 mCherry-MTS2 plasmid to yield the P_{van} mCherry-MTS2 strain. These strains were then electroporated
439 with the P_{xyl} FtsZ-GFP, P_{xyl} FtsW-GFP or P_{xyl} FtsW**-GFP plasmid to yield the respective dual color
440 strains.

441 **Growth conditions**

442 Liquid cultures were grown overnight at 28°C with 15 mL of M2G minimal media under mechanical
443 agitation (180 rpm). Each specific inducer for every different condition is described below. Fosfomycin
444 perturbation was achieved with a subminimal inhibitory concentration of 12.5 µg/ml added one hour prior
445 synchronization. To induce the expression of mCherry-MTS2 from the P_{van} promoter, 0.5 mM of vanillate
446 was added to the culture before overnight growth. For the expression of FtsW-eGFP, xylose was added
447 to reach a final concentration of 0.3 % (mass per volume) 2 hours before synchrony as optimized
448 previously [40]. P_{xyl} FtsZ-eGFP was induced overnight at 0.003 % xylose in M2G as optimized in a
449 previous study [41].

450 **METHOD DETAILS**

451 **Sample preparation**

452 Cells were synchronized at 4°C by Percoll density gradient [42] when they reached mid-exponential
453 phase (OD_{660}=0.3-0.5). A silicone gasket (Grace Biolabs, 103280) was placed on a rectangular cover
454 slide, and filled with 1% M2G agarose (Ultra PureTM Agarose, Sigma) containing fosfomycin, xylose
455 and vanillate at the appropriate concentrations when needed. Vanillate was present at 0.5 mM in all
456 experiments, xylose was present at 0.003% for induction of FtsZ-eGFP, but absent for the FtsW-eGFP
457 experiments. Fosfomycin was added to a final concentration of 10 µg/ml for drug perturbation
458 experiments. A cover slide was placed on top of the silicone gasket before solidification of the agarose

459 to achieve a flat agarose pad. After 5 min, the top cover slide was removed, and a 1 µL drop of a
460 synchronized cells suspension was placed on the pad. A small piece of agarose (~1 mm) was cut out
461 on two opposing sides to ensure aerobic conditions during imaging. After absorption of the droplet, the
462 pad was sealed with a plasma-cleaned #1.5 round coverslip with a diameter of 25 mm.

463 **Image acquisition**

464 **Microscope set up**

465 SIM microscopy was performed on the 3D NSIM Nikon microscope, with a CFI Apochromat TIRF
466 objective (100 x, NA 1.49, Nikon). The microscope was equipped with 400 mW, 561 nm and 480mW,
467 488 nm lasers (Coherent Sapphire) and a back-illuminated EMCCD camera (iXon 3, Andor Technology)
468 with a 512x512 pixel CCD sensor.

469 **Acquisition settings**

470 Dual color imaging of the cells was performed at 28°C using the 488 nm and 561 nm lasers for the
471 divisome protein-eGFP channel and the mCherry-MTS2 channel respectively. The camera was
472 operated with a readout speed of 1 MHz and a dynamic range of 16 bit to have the maximum pixel
473 readout speed at the highest dynamic range. The preamplifier gain and the electron multiplication gain
474 were set to 1 and 200 respectively to maximize the signal to noise at the chosen dynamic range. All raw
475 SIM images were acquired with a camera acquisition time of 200 ms and 100 ms (5 fps and 10 fps) for
476 the 561 and 488 channels. The laser power for both channels was 4 W/cm2. These settings yielded a
477 good balance between image quality and photo-bleaching.

478 All the raw images were acquired in 3D SIM image mode to ensure the highest signal to noise
479 ratio and lateral resolution. Fifteen images were captured of each 30.7×30.7 µm field of view, five phase-
480 shifted images per angle at each of three interference pattern angles. A full raw dual color image stack
481 was acquired in 17s.

482 Live-cell fluorescence microscopy over the cell cycle was achieved by performing time-lapse imaging.
483 3D SIM snapshots were captured at 5 min or 10 min (for fosfomycin-treated cells) time intervals to follow
484 dynamics while minimizing photo-bleaching of the sample during the image acquisition. Multiple fields
485 of view were imaged sequentially at each time point, allowing following up to 200 cells per experiment.
486 Super-resolved SIM images were reconstructed by the Nikon NIS-Elements software.

487 **QUANTIFICATION AND STATISTICAL ANALYSIS**

488 **Analysis of cell shape dynamics**

489 The super-resolved SIM images were processed via a custom-made software package called sDADA
490 (Shape Dynamics Automated Data Analysis). sDADA generates scatter plots, histograms and violin
491 plots in order to study key parameters controlling the cell size and homeostasis, such as: elongation
492 rate, constriction duration, length at birth, onset, onset time. sDADA extracts these parameters from the
493 analysis of the cell shape dynamics thanks to semi-interactive modules for image segmentation, edge
494 detection, cell filtering, cell tracking and statistical analysis (Supplementary Methods). The MATLAB-
495 based software package is available together with its documentation upon request.

496 **Parameter definition**

497 We assumed as time zero (T_0) the time at which the suspension of synchronized bacteria is added to
498 the agarose pad. This occurred approximately 20-40 minutes before starting time-lapse SIM acquisition.
499 T_C, T_Z, and T_W refer to the constriction onset time measured with different approaches. T_Z is the time of
500 the FtsZ assembly, which we assumed to occur when the fluorescence intensity of FtsZ-eGFP at mid-
501 cell was three times higher than elsewhere. T_W is the FtsW arrival time measured as the moment at
502 which the FtsW signal appeared stable at midcell (Figure S1C).

503 T_C is defined as the time at which the constriction invagination depth is equal to a predetermined
504 normalized waist width threshold. To find the optimal threshold, we tested different thresholds in the
505 reasonable range from 80% up to 99% of the maximum diameter, with step sizes of 1 %. Since FtsW
506 arrival time is an alternative readout of the constriction start the Tc values computed from the waist
507 diameter versus time should strongly and robustly correlate with the Tw values. For each threshold, we
508 computed the Pearson's correlation coefficient for the scatter plot of the Tc values versus the Tw values
509 of all the cells. We found that a threshold of 92% had the best correlation coefficient and minimal least
510 square error for Tc versus Tw.

511 Generation time (T_G) and final length (L_G) are the time and the length at which the cell divide.
512 Constriction duration τ is the difference between T_G and T_C, or when specified, T_W. The length at birth,
513 L_B and the elongation rate k are extracted from the exponential fitting of the elongation: $L(t) = L_B \cdot$
514 e^{kt}.The length at the constriction onset, L_C, L_Z or L_W, were measured as the lengths at T_C, T_Z and T_W
515 respectively. The total elongation is the difference between L_G and L_B. Elongation before constriction
516 and during constriction are defined respectively as the difference between L_G and L_C, and between L_C
517 and L_B. When specified, L_W can be used instead of L_C.

518 **Statistical tests**

519 For each parameter defined in the section above, the statistical significance of observed differences
520 between strains or conditions was tested. We compared the means of the repeats using Mood's median
521 test (Supplementary Methods). The correlation between variables was analyzed using Pearson's
522 correlation coefficient in the presence of a linear trend or the Spearman's correlation coefficient for non-
523 linear relationship. The experiments were performed in two independent replicates per condition with a
524 minimum of 100 cells analyzed per replicate, and 200 minimum per condition.

525 **DATA AND SOFTWARE AVAILABILITY**

526 All data and software used to support the results of this manuscript are available from the Lead Contact
527 upon reasonable request. Original data is available on Zenodo, DOI: 10.5281/zenodo.1172042 and
528 10.5281/zenodo.1172072

529 **KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
NA1000, synchronizable derivative of wild-type CB15	[43]	CB15N
CB15N *ftsW(F145L,A246T); ftsI(I45V)*	[28]	ML2159
WT *Pxyl::ftsZ-GFP Pvan::MTS2-CHY (GmR/KmR)*	This paper	CB15NVL1
ML2159 *Pxyl::ftsZ-GFP Pvan::MTS2-CHY (GmR/KmR)*	This paper	CB15NVL2
WT *Pxyl::ftsW-GFP Pvan::MTS2-CHY (GmR/KmR)*	This paper	CB15NVL3
ML2159 *Pxyl::ftsW**-GFP Pvan::MTS2-CHY (GmR/KmR)*	This paper	CB15NVL4
Oligonucleotides		
5'-AACTTGGTACCTCTAGAGGAAGATCTTTCATCGAGGAG-3'	This paper	MTS2_KpnI-F
5'-AACTTGAATTC AAGCTTCTAGGATCCACCGCCG-3'	This paper	MTS2_EcoRI-R
5'-CTCGAGCTCCGATGGCCTCCAACGCG-3'	This paper	oEG035
5'-GTTCGAATTCTCTCAGGCGTCCGCGCGACC-3'	This paper	oEG036
5'-AACTTGGTACCTCTAGAGGAAGATCTTTCATCGAGGAG-3'	This paper	MTS2_KpnI-F
Plasmids		
Pxyl::VENN-2 FtsW (KmR)	[40]	pEG105
*Pxyl::VENN-2 FtsW** (KmR)*	This paper	pEG1224
Pxyl:: GFPN-2*(KmR)*	[44]	pXGFPN-2
Pxyl:: GFPC-2*(KmR)*	[44]	pXGFPC-2
Pxyl::VENN-2(KmR)	[44]	PXVENN-2
E. coli MTS2 fused to GFP pBAD33 derived plasmid *Para-GFP::EcMTS::EcMTS (CmR)*	[45]	pSLR92
Pvan:: (KmR)	[44]	pVCHYC-4
Pxyl::ftsZ-dendra2 (KmR) used to create pVL1	[41]	pX-Ftsz-Dendra2
Pxyl::ftsZ-GFP (KmR)	This study	pVL1
Pvan::MTS2-CHY(GmR)	This study	pVL2
*Pxyl::GFPN-2 FtsW** (KmR)*	This study	pEG1308
Pxyl::VENN-2 FtsW (KmR)	[40]	pEG105

*Pxyl::VENN-2 FtsW** (KmR)*	This study	pEG1224
used to create pEG1305,pEG1308 (KmR)	This study	pXGFPN-2
Software and Algorithms		
sDaDa	This paper	See Supplementary Note
Celltool	[46]	http://zplab.wustl.edu/celltool/
ImageJ	[47]	https://imagej.nih.gov/ij/
MicrobeTracker	[48]	http://microbetracker.org/
MATLAB	The MathWorks, Natick, MA	https://ch.mathworks.com/products/matlab.html
Original data	This study	10.5281/zenodo.1172042 and 10.5281/zenodo.1172072