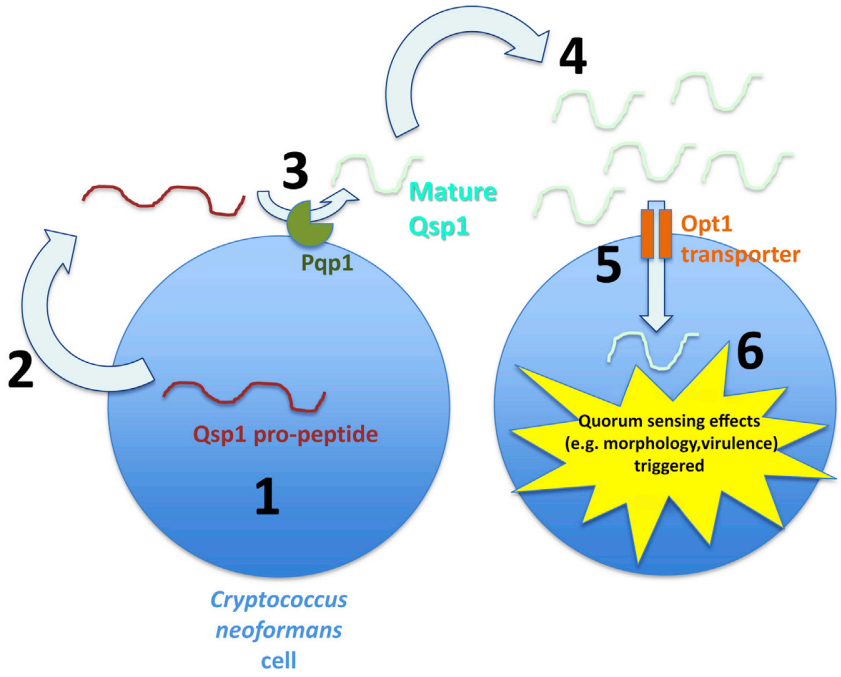


**Figure 1. Qsp1 Quorum-Sensing Pathway in *Cryptococcus neoformans***
The Qsp1 pro-peptide is synthesized (1) and then exported from the fungal cell (2). Once outside, it is cleaved by the cell-associated protease Pqp1 (3) producing the mature Qsp1 peptide (4). This is re-imported into surrounding cryptococcal cells via the oligopeptide transporter Opt1 (5), where it triggers morphological and virulence changes via an as-yet-uncharacterized intracellular receptor (6).

analogous systems may exist in other species and, in particular, in other human fungal pathogens. Because Qsp1 appears to have arisen by convergent evolution, and has no obvious homology outside of this fungal group, identifying similar systems in more distantly related fungi will not be easy. However, it is a fair bet that the biological fascination and clinical opportunities QS offers will mean that Qsp1 is the first, but certainly not the last, species-specific fungal quorum-sensing system to make it into the limelight.

## REFERENCES

Albuquerque, P., and Casadevall, A. (2012). Med. Mycol. *50*, 337–345.

Homer, C.M., Summers, D.K., Goranov, A.I., Clarke, S.C., Wiesner, D.L., Diedrich, J.K., Moresco, J.J., Toffaletti, D., Upadhya, R., Caradonna, I., et al. (2016). Cell Host Microbe, in press. Published online May 19, 2016.

Hornby, J.M., Jensen, E.C., Lisec, A.D., Tasto, J.J., Jahnke, B., Shoemaker, R., Dussault, P., and Nickerson, K.W. (2001). Appl. Environ. Microbiol. *67*, 2982–2992.

Johnston, S.A., and May, R.C. (2013). Cell. Microbiol. *15*, 403–411.

Lee, H., Chang, Y.C., Nardone, G., and Kwon-Chung, K.J. (2007). Mol. Microbiol. *64*, 591–601.

Lee, H., Chang, Y.C., Varma, A., and Kwon-Chung, K.J. (2009). Eukaryot. Cell *8*, 1901–1908.

Ramage, G., Saville, S.P., Wickes, B.L., and López-Ribot, J.L. (2002). Appl. Environ. Microbiol. *68*, 5459–5463.

Rutherford, S.T., and Bassler, B.L. (2012). Cold Spring Harb. Perspect. Med. *2*, http://dx.doi.org/10.1101/cshperspect.a012427.

# Size Regulation: Big Insights from Little Cells

**Hui Chen[1] and Matthew C. Good[1,2,*]**
[1]Department of Cell and Developmental Biology
[2]Department of Bioengineering
University of Pennsylvania, 421 Curie Boulevard, 1151 BRB II/III, Philadelphia, PA 19104, USA
*Correspondence: mattgood@upenn.edu
http://dx.doi.org/10.1016/j.devcel.2016.05.020

**Reporting in *Cell*, Harris and Theriot (2016) use modeling and quantitative imaging to analyze bacterial cell growth and division. By manipulating surface and volume growth rates, the authors provide insight into bacterial cell size regulation and propose that a threshold level of unincorporated cell wall material specifies when cells divide.**

How cells sense and regulate their size is a fundamental question in eukaryotic and prokaryotic cell biology. Cells precisely control their size during the cell cycle, coordinating growth with cell division to limit overall population variance (Ginzberg et al., 2015; Jorgensen et al., 2002; Taheri-Araghi et al., 2015). Additionally, cells adapt their dimensions to variations in the extracellular environment, integrating information about nutrient availability in the decision to grow or divide (Weart et al.,

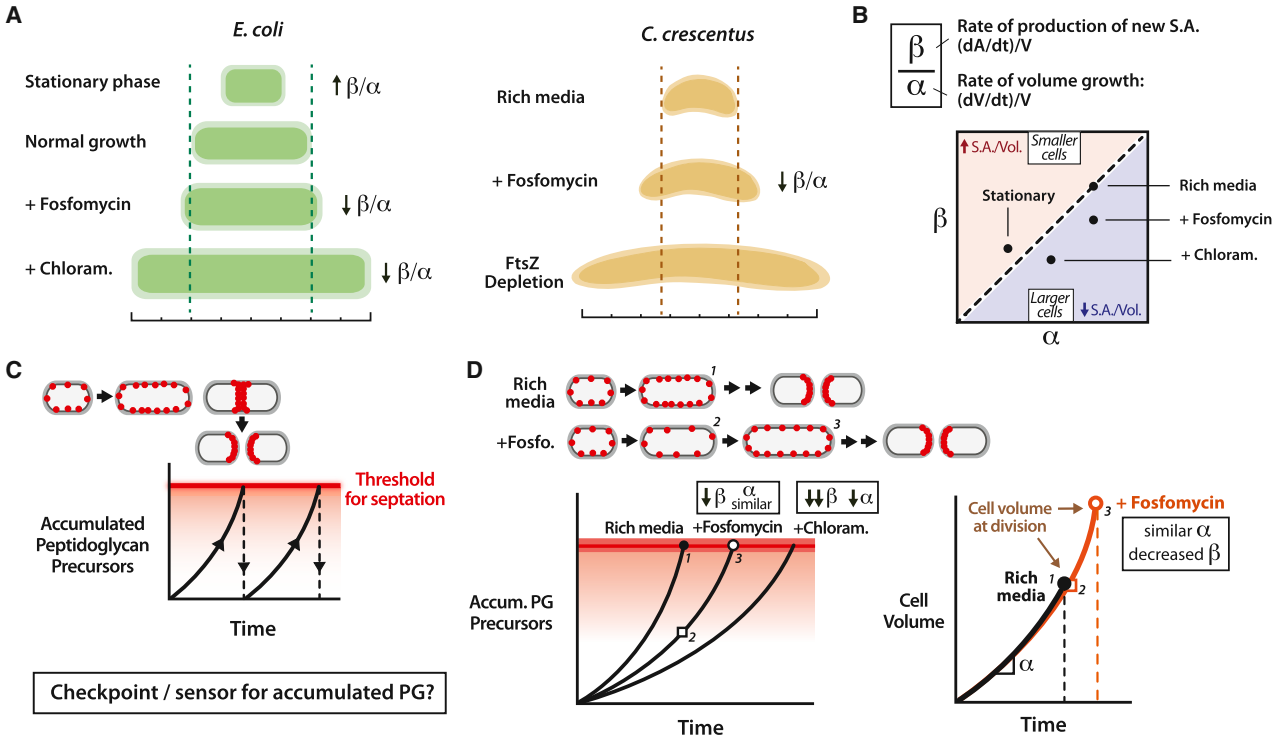


**Figure 1. Regulation of Cell Size in Bacteria**

(A) Cell size regulation: experimental perturbations that alter the average size of *E. coli* and *C. crescentus* cells. Fosfomycin inhibits peptidoglycan (PG) synthesis. Chloramphenicol inhibits translation. Increased cell length in *C. crescentus* depleted of FtsZ results from inability to divide. Ruler denotes 8 μm.

(B) Relative rates concept. α: rate of volume addition, β: rate of production of new surface area (SA). Ratio of β/α dictates cell size at time of division. Plot of β versus α: if β/α ratio decreases below the dotted line, cells enlarge; if ratio increases above diagonal, cell size reduces. Trends based on *E. coli* data.

(C) Growing cells divide once they have accumulated a threshold amount of peptidoglycan precursors (red circles) necessary for septum formation. Checkpoint or sensor for accumulated PG is unknown.

(D) Plots depicting accumulated PG and cell volume as a function of time. Fosfomycin reduces β, while α remains constant. Compared to cells growing in rich media, fosfomycin-treated cells must grow for longer, and to a larger size, to accumulate the threshold level of PG required for septation.

2007). But how is size regulation achieved? "Timer" hypotheses propose that newly born cells grow for a defined period of time before dividing. However, these models are inconsistent with observations of cell size-dependent growth rates; large cells grow faster. Instead, cells must have evolved mechanisms for sensing their absolute size or the amount of growth since birth. In mammalian cells, although cell sizes have been carefully measured in culture (Kafri et al., 2013), the molecular identity of the cell size sensor has remained elusive. However, recently, a volume sensor for spherical budding yeast cells was discovered (Schmoller et al., 2015). Newborn cells inherit a defined amount of Whi5, and express and accumulate Cln3 while growing during G1. Surpassing a threshold Cln3/Whi5 ratio triggers the decision to divide by entering G1/S START. Furthermore, in fission yeast, the Pom1 kinase has been shown to sense pole-to-pole length (Moseley et al., 2009). In rod-shaped bacteria such as *E. coli* and *C. crescentus*, cell length is primarily determined by how much elongation occurs before cells divide. Therefore, it is tempting to speculate that these cells measure their one-dimensional (1D) length using protein gradients analogous to Pom1. However, recent data supporting an "adder" mechanism for bacterial cell size regulation argue against the idea of a critical length prompting division. "Adder" models suggest that bacteria add a constant amount of cell volume during each cell cycle, independent of their size at birth (Campos et al., 2014). How this is achieved—how bacterial cells measure volume added—is an open question.

In a study published in *Cell*, Harris and Theriot (2016) image bacterial growth and division under conditions that perturb cell size and develop a mathematical model to explain how the relative rates of surface area and volume growth predict cell size at division. Although changing surface area growth rate (β) can affect volume growth rate (α), and vice versa, the authors identify conditions that differentially alter each of these variables. They identified four experimental conditions in which the β/α ratio was perturbed and cell size altered (Figure 1A). Using fosfomycin, a potent inhibitor of the first step of peptidoglycan (PG) synthesis, the authors manipulated the production of cell wall material (Harris and Theriot, 2016). This caused β to decrease without altering volume growth rate, α, causing a reduction in the β/α ratio (Figure 1B) and leading to a dose-dependent increase in cell length and cell width. This result was confirmed in *C. crescentus*, *E. coli*, and *L. monocytogenes*. Additionally, the authors were able to reduce the β/α ratio and increase cell length and width by treating *C. crescentus* and *E. coli* cells

with chloramphenicol, a ribosome inhibitor. Although this treatment lowered volume growth rate, $\alpha$, it more drastically attenuated $\beta$, causing a cell size increase and subsequent drop in surface area-to-volume ratio. They also observed a similar decrease in surface area-to-volume ratio in *E. coli* cells whose synthesis activities were diverted by GFP overexpression. Conversely, growing cells overnight until they reach stationary phase increases $\beta/\alpha$ ratio and decreases cell size (Figure 1A). Importantly, in all cases, the authors' relative rates model successfully predicted cell size trends (Figure 1B) and time required to reach a new steady state.

Harris and Theriot also identify a potential mechanism, a threshold amount of accumulated peptidoglycan precursor, to explain how cells know when to divide (Figure 1C). By analyzing bacterial growth data, the authors defined the total amount of surface area material synthesized prior to constriction. They found that, independent of pre-constriction cell volume, volume growth rate, and surface area growth rate, *C. crescentus* cells accumulated almost identical amounts of surface area material (0.4–0.5 $\mu m^2$) prior to constriction. Intriguingly, the accumulated amount is nearly identical to the amount of surface area required to generate two new polar end caps. To explore the implications of this finding, consider cells treated with fosfomycin. Although volume growth rate, $\alpha$, is unchanged, $\beta$ is decreased, and therefore cells must grow for a longer period of time to accumulate the requisite level of peptidoglycan precursors needed for septation (Figure 1D). The result of this extended period of growth is increased cell length and width. Alternatively, for cells whose $\beta/\alpha$ ratio is increased, peptidoglycan will accumulate more quickly and cells will reach the critical threshold at a smaller volume (i.e., stationary phase in Figures 1A and 1B).

The findings from this study represent an important advance in our understanding of cell size regulation and potentially explain "adder" models of bacterial cell growth. Additionally, the work from Harris and Theriot raises some interesting questions. For example, what is the identity of the molecular sensor for accumulated peptidoglycan, and how does this sensor signal to the FtsZ ring, the machinery that constricts a cell into two daughters, at the checkpoint for constriction initiation? FtsN and other proteins recruited at the late stages of FtsZ ring assembly (Chung et al., 2009) are potential candidates for either directly sensing PG levels or transducing the signal to trigger invagination. More broadly, how do cells integrate information from nutrient sensors with the signals from accumulated peptidoglycan in the decision to divide (Weart et al., 2007)? A key question not addressed in the current study is whether the PG threshold varies with cellular or environmental conditions. Because the deduced amount of accumulated surface area material prior to constriction is similar to the size of the end caps, the PG threshold for triggering cell division may depend on cell width. For example, if cell volume is dramatically increased but the $\beta/\alpha$ ratio is only modestly reduced, an increase in the PG threshold would account for a larger cell size. Development of a sensor for unincorporated peptidoglycan precursor would also greatly aid in determining both the threshold and whether any material is inherited by daughter cells. Future studies should tackle some of these questions. In the meantime, careful studies, such as this one from Harris and Theriot (2016), are taking us closer to a breakthrough in understanding cell size regulation.

## REFERENCES

Campos, M., Surovtsev, I.V., Kato, S., Paintdakhi, A., Beltran, B., Ebmeier, S.E., and Jacobs-Wagner, C. (2014). Cell *159*, 1433–1446.

Chung, H.S., Yao, Z., Goehring, N.W., Kishony, R., Beckwith, J., and Kahne, D. (2009). Proc. Natl. Acad. Sci. USA *106*, 21872–21877.

Ginzberg, M.B., Kafri, R., and Kirschner, M. (2015). Science *348*, 1245075.

Harris, L.K., and Theriot, J.A. (2016). Cell *165*, 1479–1492.

Jorgensen, P., Nishikawa, J.L., Breitkreutz, B.J., and Tyers, M. (2002). Science *297*, 395–400.

Kafri, R., Levy, J., Ginzberg, M.B., Oh, S., Lahav, G., and Kirschner, M.W. (2013). Nature *494*, 480–483.

Moseley, J.B., Mayeux, A., Paoletti, A., and Nurse, P. (2009). Nature *459*, 857–860.

Schmoller, K.M., Turner, J.J., Kõivomägi, M., and Skotheim, J.M. (2015). Nature *526*, 268–272.

Taheri-Araghi, S., Bradde, S., Sauls, J.T., Hill, N.S., Levin, P.A., Paulsson, J., Vergassola, M., and Jun, S. (2015). Curr. Biol. *25*, 385–391.

Weart, R.B., Lee, A.H., Chien, A.C., Haeusser, D.P., Hill, N.S., and Levin, P.A. (2007). Cell *130*, 335–347.