Chiral self-organization of the bacterial cell wall

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Thanks

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Steven Wang
What rules govern how cells choose their shape?
What rules govern how cells grow?
The Selective Value of Bacterial Shape

Kevin D. Young

INTRODUCTION

It’s not in the open we feel comforted but in the shadows. . . . We can’t feel at home with the infinite sky above and around us. Space must be cut off, shaped, defined, for us to inhabit. From cradle to coffin, it’s enclosure that defines us.
—Robert Morgan (221)

To be brutally honest, few people care that bacteria have different shapes. Which is a shame, because the bacteria seem to care very much. A simple way to verify this is to take a leisurely stroll through Bergey’s Manual of Determinative Bacteriology (133) or The Prokaryotes (65, 313), pausing to admire the surprising and bewildering riot of shapes, sizes, and aggregates, some of which are illustrated in Fig. 1. There are cells
(Champion PNAS 2006)
Motile

Nonmotile
• Importance of shape to cellular processes
  – Predation, immunity, differentiation, …
• Introduction to the bacterial cell wall
• Modeling the *E. coli* cell wall
  – Robustness of cell shape to damage
  – Cell shape determination
  – Cell shape maintenance
• How do cells grow?
Cell wall determines cell shape

- Outer membrane
- Peptidoglycan cell wall
- Periplasm
- Cytoplasmic membrane

Cytoplasm

\[ \text{Turgor pressure} (>1 \text{ atm}) \]
Cell wall determines cell shape

Outer membrane

Peptidoglycan cell wall

Periplasm

Cytoplasmic membrane

Cytoplasm

↑  ↑  ↑  ↑

Turgor pressure (>1 atm)
Cell wall determines cell shape

The cell wall is necessary and sufficient for cell shape determination
Wild type

Point mutations

Stationary phase, MreB knockout

Shih *MolMicro* 2005

Stationary phase, knockout

Varma *JBact* 2007

Astrographics

Exponential phase
Wild type

Stationary phase, MreB knockout

Genetic knockout

Point mutations

Chemical perturbation

Exponential phase

Astrographics

Shih *MolMicro* 2005

Varma *JBact* 2007

Varma *JBact* 2008

Varma *JBact* 2007
Takeuchi
NanoLett
2006

(a) microconfinement in agarose chamber

E. coli

20 μm

0 min 10 20 30 40 50 60

70 80 90 100 108 110 118

donut pattern
Cells grow into (and maintain) shapes defined by the chamber
Cell wall is the stress-bearing structure

Cell wall is a peptide-sugar network (peptidoglycan)
• *E. coli* cell wall is 75-80% single layered (2-3nm thickness).
• Glycans polymerized as strands of 50-60 subunits.
• Approximately 50% of peptides are crosslinked.
• Isolated cell walls (sacculi) are 2-3 times more deformable along the long axis than in the perpendicular direction.
• Spring network of peptides and glycans with hoops of glycans oriented along the circumference
• Osmotic pressure acts on the surface \( \mathbf{F} = \Pi \, d\mathbf{V}/d\mathbf{r} \)

(Holtje, Koch, Beveridge, Pink, …)
Macroscopic observables lead to microscopic parameters

- Lengths ($d_p, d_g \sim 1\text{nm}, 2\text{nm}$)
- Young’s modulus $\rightarrow$ stiffness
  
  ($k_p \sim 10\text{pN/nm}, k_g \gg k_p$)
- Persistence length $\rightarrow$ bending
  
  ($\zeta \sim 1-10\text{nm}, \kappa \sim 10\text{pNnm}$)
- Glycan strand lengths $\rightarrow$ organization
Bacterial cell wall
In response to vancomycin, cells “crack” around the bulge

*(imp4213 strain: Sampson et al, Genetics 1989, Tom Silhavy)*
Normal modes of cell shape

• Single peptide defect couples strongly to the bend mode

• Single glycan defect couples to both twist and bend modes
Common cell shapes via spatial patterning of defects

*Caulobacter crescentus*
Diane Caporale

**Spirochaetes**
Peptidoglycan Crosslinking Relaxation Promotes *Helicobacter pylori*’s Helical Shape and Stomach Colonization

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Hypothesis:
Spatial patterning of new insertion affects shapes of growing bacteria
Modes of growth

Elongation (PBP2)

Division (PBP3)
Modes of growth

Elongation (PBP2)

Division (PBP3)
Insertion of new material

Vary spatial, biochemical, and mechanical characteristics
Random insertion
Random strand insertion

6X growth
Decouple wall density and insertion
Decouple wall density and insertion
Decouple wall density and insertion
Uniform density insertion preserves cell shape
What biological factor could be responsible?

- Patterns cell-wall growth
- Insensitive to fluctuations in cell shape
- Deletion affects cell shape
- Conserved across bacteria
Cell growth without MreB

- Bacterial actin homolog
- Found in rod-shaped bacteria
- ATP binding, inhibited by A22
Cell growth without MreB

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Cell growth without MreB

• Bacterial actin homolog
• Found in rod-shaped bacteria
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Role of MreB in rod-shaped growth

Stiffness is comparable to cell wall

MreB controls PBP2 localization

MreB is left-handed!
Helically patterned insertion
Helical-insertion growth preserves shape

6X growth
What is the effect of helical insertion?
New strand reorientation

Old strands

New strands

Helical pattern
Cell wall rotation
Left-handed rotation during growth

(Steven Wang and Josh Shaevitz, Princeton)
Rotation is always left-handed!
Bead twisting
Helix angle vs. twist angle
Rotation is MreB-dependent

0 hours  5 hours  7 hours

Circumferential distance (μm) vs Time (min)
Rotation is MreB-dependent

0 hours 5 hours 7 hours
Osmotic shock

low turgor pressure

high turgor pressure
Osmotic shock
Osmotic shock
Twist in other species

*B. Subtilis* (right-handed cytoskeleton)

18±6 degrees/µm right-handed
Can we design the shape of bacteria?
Conclusions

• Cell shape maintenance relies on a combination of spatial patterning, biochemical regulation, and mechanical force
• Modeling can be used to interpret molecular mechanisms underlying cell growth
• Predictions about mechanisms for altering cellular dimensions