# A Model of Fission-Yeast Cell Shape Driven by Membrane-Bound Growth Factors


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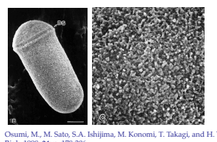
## ABSTRACT

Fission yeast serves as a model for how cellular polarization machinery is used to regulate cell growth. Recent studies identify active Cdc42, found in a cap at the inner membrane of growing tips, as a master growth regulator, likely through control of exocyst tethering and formin-based nucleation of actin cables. To investigate how biochemistry might control shape, we propose a simple model based on the hypotheses that (i) the delivery and internalization rate of wall or membrane components limits cell expansion and (ii) a growth factor, such as Cdc42, signals for delivery of these components. We numerically simulate cell growth according to an axisymmetric, finite-element computational model that couples growth-factor-directed orthogonal expansion of the cell membrane and cell-wall remodeling to reaction and diffusion of the growth factor on that membrane. We explore limiting conditions for polarized growth and consider the additional effects of membrane elasticity and flow. We find a relationship between cap size and diameter, and motivate future experiments on the link between cell signaling and shape. Fission-yeast Cdc42 is regulated by a number of proteins whose absence lead to defects in shape or polarized growth, such as cells of varying diameter, round cells, and branched cells. Among these proteins, Gef1 and Scd1 assist the activation of Cdc42 at the tips and Rga4 restricts the location of its activation. We compare model results to cell morphologies of mutants of Cdc42 regulators and suggest possible mechanistic roles for these regulators.

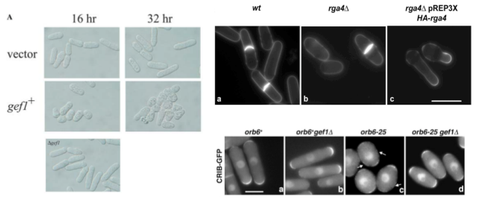

## INTRODUCTION

**Fission-yeast Shape**

- Pill shaped, grows at the tips
- Actin polymerizes near growing tips, symmetrically distributed microtubules throughout growth
- Tea proteins and tip markers accumulate near both cell tips
- Cell wall is an isotropic meshwork of peptogylcans (below, Osumi, *et al.*)



Osumi, M., M. Sato, S.A. Ishijima, M. Konomi, T. Takagi, and H. Yaguchi, Fungal Genet. Biol., 1998. **24**: p. 178-206.

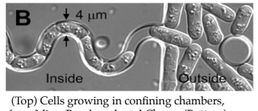



(Left) Overexpression of Cdc42 GEF Gef1 leads to wider cells (Iwaki, *et al.*). (Top Right) Deletion of Cdc42 GAP Rga4 also leads to wider cells, while overexpression gives narrow growth protrusions (Das, *et al.*, 2007). (Bottom Right) Mutations of *orb6*, which codes an NDR kinase and Cdc42 regulator.

References:
Iwaki, N., K. Karatsu, and M. Miyamoto, Biochem. Biophys. Res. Comm., 2003, **213**: p. 414-20.
Das, M., D.J. Wiley, S. Medina, H.A. Vincent, M. Larrea, A. Oriolo, and F. Verde, Mol. Biol. Cell, 2007, **18**: p. 2090-101.
Das, M., D.J. Wiley, X. Chen, K. Shah, and F. Verde, Curr. Biol., 2009, **19**: p. 1314-19.

**Growth depends on turgor pressure, surface factors**

- External force reduces growth rate (see right, top)
- Confined cells buckle, curve (see right)

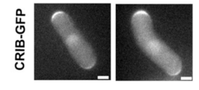




(Top) Cells growing in confining chambers, from Minc, Boudaoud, and Chang. (Bottom) Cells growing in curved confining passages, from Terenna *et al.*

Minc, N., A. Boudaoud, and F. Chang, Curr. Biol., 2009. **19**: p. 1096-101.
Terenna, C.R., *et al.*, Curr. Biol., 2008. **18**: p. 1748-1753.

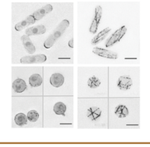
**Polarized growth mediated by Cdc42**

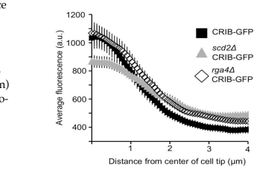
- Cdc42 regulates two modules for polarized growth: actin cables, exocyst (Martin and Bendezú)
- As with Pob1, Cdc42 is required for membrane trafficking and fusion (Estravís, *et al.*)
- Most (7 of 11) wider-than-wild-type deletion mutants lack a gene that controls Cdc42 (Kelly and Nurse; Das, *et al.*)
- Cdc42 relocated during electrical control of growth, but not obviously prior to bending (Minc and Chang, right)
- After wall digestion, can polarize and grow a tip, even without microtubules (Kelly and Nurse)




(Above) From Minc and Chang, CRIB-GFP (active-Cdc42 marker) fluorescence in control cells (left) and cells grown under an electric field (right).

(Below) Fluorescence from CRIB-GFP (left) and Atb1-GFP (right) in wild-type cells (top row) and spheroplasts (bottom) from Kelly and Nurse. Atb1p is a microtubule component.

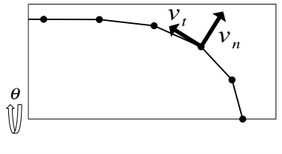





(Above) From Kelly and Nurse, meriodonal profiles of CRIB-GFP fluorescence.

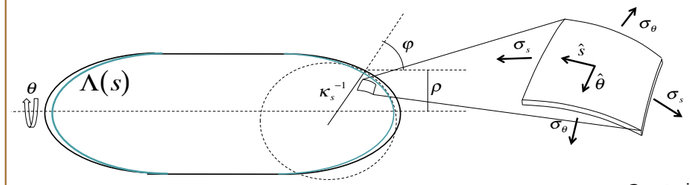
Bendezú, F., and S.G. Martin, Mol. Biol. Cell, 2011, **22**: p. 44-53.
Estravís, M., S.A. Rincón, B. Santos, and P. Pérez, Traffic, 2011, **12**: 1744-58.
Kelly, F. and P. Nurse, Mol. Biol. Cell, 2011, **22**: p. 3801-11.
Minc, N. and F. Chang, Curr. Biol., 2010, **20**: p. 710-716.
Das, M., *et al.*, Science, 2012, **337**: p. 239-43.
Kelly, F. and P. Nurse, PLoS ONE, 2011, **6**: e27977.

## NUMERICAL METHODS




- Axisymmetric cell shape described by contour, broken into discrete segments
- Generalized normal distribution growth-factor signal along meridional contour
- Calculate velocities of vertices, integrate for position
- Rebeading between integration steps
- Whole-cell shapes use steady-state tips, constant volume

## PHYSICAL MODEL FOR CELL GROWTH




$\sigma_i$: stress, direction $i$

$P$: turgor pressure

$\delta$: wall thickness

$\kappa_i$: curvature, direction $i$

**1**. Calculate stresses from shape, force balance:

$$\sigma_s = \frac{P}{2\delta\kappa_\theta}$$

$$\sigma_\theta = \frac{P}{2\delta\kappa_\theta}\left(2 - \frac{\kappa_s}{\kappa_\theta}\right)$$

**2**. From the elastic stress-strain relationship:

$$\varepsilon_s = \frac{1}{E}(\sigma_s - \nu\sigma_\theta)$$

$$\varepsilon_\theta = \frac{1}{E}(\sigma_\theta - \nu\sigma_s)$$

$\varepsilon_i$: strain, direction $i$

$E$: Young's modulus

$\nu$: Poisson's ratio

$\xi_i$: expansion rate, direction $i$

$\Lambda(s)$: cell-wall remodeling rate

$v_t$: velocity, tangential

$v_n$: velocity, normal

**3**. During remodeling, wall expands under turgor pressure in proportion to local strain, corresponding to a mechanism where strained material is replaced by unstrained material. Guarantees constant thickness: $\xi_s = \Lambda(s)\varepsilon_s \quad \xi_\theta = \Lambda(s)\varepsilon_\theta$

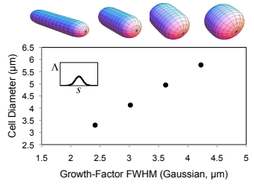
**4**. Differential equation relates velocity to expansion rate:

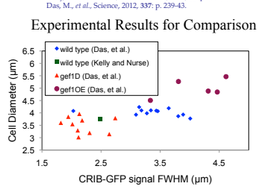
$$\xi_s = v_n\kappa_s + \frac{\partial v_t}{\partial s} \qquad \xi_\theta = v_n\kappa_\theta + \frac{v_t\cos\varphi}{\rho}$$

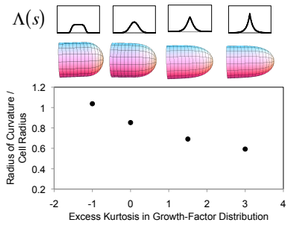
## MODELING RESULTS

**1.** Predicts cell diameter in proportion to the width of growth-factor distribution, consistent with experimentally measured ratio and trend (Kelly and Nurse; Das, *et al.*), and cell-shape dependence on the shape of that distribution.

Kelly, F. and P. Nurse, Mol. Biol. Cell, 2011, **22**: p. 3801-11.
Das, M., *et al.*, Science, 2012, **337**: p. 239-43.

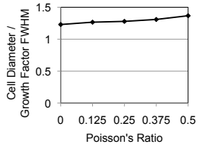


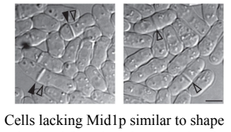




**2.** Predicts ratio of cell diameter to growth-factor full-width half-max of **1.229** to **1.367**, depending on Poisson's ratio, comparing well with experiment (Kelly and Nurse; Das, *et al.*, as above)

Kelly, F. and P. Nurse, Mol. Biol. Cell, 2011, **22**: p. 3801-11.
Das, M., *et al.*, Science, 2012, **337**: p. 239-43.



Cells lacking Mid1p similar to shape calculated from leptokurtotic signal

Lee, I-J. and J.-Q. Wu, J. Cell Sci., 2012, **125**: p. 2973-85.

**3.** Predicts dependence of growth on other parameters, gives estimate for tip remodeling rate.

| Parameter | Value | Source |
|---|---|---|
| $r$, cell radius | 1.6 μm | numerous |
| $P$, turgor pressure | .85 MPa | Minc *et al.*, 2009 |
| δ, cell-wall thickness | 200 nm | Osumi, 1998 |
| $E$, Young's modulus | 101 MPa | Minc *et al.*, 2009 |
| $\Lambda_{max}$, remodeling rate | 1/(60 sec) | estimated here |

$$v_{growth} \propto \frac{Pr^2\Lambda_{max}}{\delta E}$$

$$v_{growth} \approx \frac{L}{\tau}$$

$L$: length at birth

$\tau$: growth duration

**4.** Predicts that points on the cell surface move almost normal to the surface. This agrees with experimental evidence for orthogonal expansion in fungal hyphae (Bartnick-Garcia, *et al.*)

Bartnicki-Garcia, S., C.E. Bracker, G. Gierz, R. López-Franco, and H. Lu, Biophys. J, 2000. **79**: p. 2382-90.

## RELATED MODELS OF TIP EXTENSION

**Models of Pollen-Tube Extension:**

- Dumais *et al.* similar, strain distributed to minimize flow potential, assume delivery must match expansion.
- Fayant *et al.* posit a varying Young's modulus based on cell-wall composition.
- Campas and Mahadevan describe self-similar growth.

**Models of Bacteria Extension:**

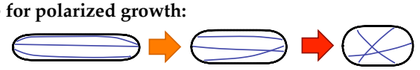
- Huang *et al.* take a molecular approach to describe a similar remodeling mechanism but assume an orientation bias.
- Lan *et al.* consider shape under turgor pressure with a z-ring force.

Dumais, J., S.L Shaw, C.R. Steele, S.R. Long, and P.M. Ray, Int. J Dev. Biol., 2006, **50**: p. 209-22.
Lan, G., C.W. Wolgemuth, and S.X. Sun, Proc. Nat. Acad. Sci. USA, 2007, **104**: 16110-5.
Huang, K.C., R. Mukhopadhyay, B. Wen, Z. Gitai, and N. Wingreen, Proc. Nat. Acad. Sci. USA, 2008, **105**: 19282-7.
Campas, O., and L. Mahadevan, Curr. Biol., 2009, **19**: p. 2102-07.
Fayant, P., O. Girlanda, C.-E. Aubin, I. Villemure, and A. Geitmann, Plant Cell, 2010, **22**: p. 2579-93.

## COUPLING GROWTH FACTOR AND SHAPE

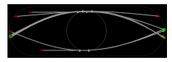
**More than positive feedback loop for polarized growth:**

Alone, feedback between shape and microtubule distribution is probably unstable, as small changes in shape lead to less-focused growth factors.



(Above) Cells that become a bit wider focus microtubules less efficiently. The resulting spread of growth factors further widens the cell.

Detailed model of Foethke and others to simulate microtubules in cells.

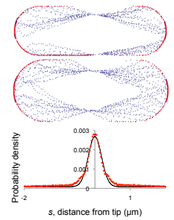


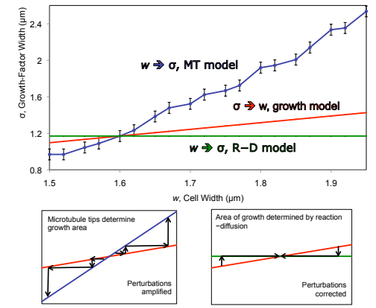
downloaded from www.cytosim.org

Foethke, D., T. Makushok, D. Brunner, and F. Nédélec, Mol. Sys. Biol., 2009, **5**: 241.

**Microtubule-based delivery model**

- Many simulations, microtubule tip positions recorded
- Width of cell varied
- Frequency of cortex-touching microtubules versus distance from tip fitted to Gaussian
- Growth-factor width assumed proportional to MT profile width



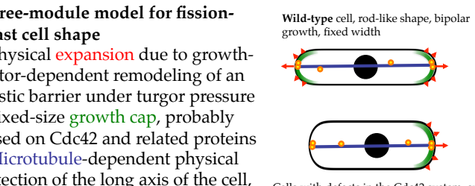




Mapping above shows interplay between growth model and either microtubule model or a fixed width as from a reaction–diffusion system. The microtubule profile width has been scaled up to match the growth model at wild-type width.

## THEORETICAL FRAMEWORK FOR UNDERSTANDING KNOWN SHAPE MUTANTS

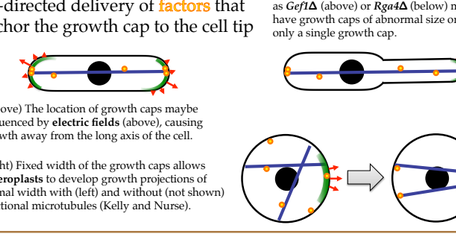
**Three-module model for fission-yeast cell shape**

- Physical expansion due to growth-factor-dependent remodeling of an elastic barrier under turgor pressure
- Fixed-size growth cap, probably based on Cdc42 and related proteins
- Microtubule-dependent physical detection of the long axis of the cell, tip-directed delivery of factors that anchor the growth cap to the cell tip




Cells missing components delivered to the tips along microtubules, such as *Tea1Δ* (above) may have problems anchoring growth caps to normal cell tips, leading to ectopic growth and possible T-shape morphology (Mata and Nurse).

Cells with defects in the Cdc42 system, such as *Gef1Δ* (above) or *Rga4Δ* (below) may have growth caps of abnormal size or have only a single growth cap.




(Above) The location of growth caps maybe influenced by **electric fields** (above), causing growth away from the long axis of the cell.

(Right) Fixed width of the growth caps allows **spheroplasts** to develop growth projections of normal width with (left) and without (not shown) functional microtubules (Kelly and Nurse).

Computational model. Microtubule (arrow) detects long axis of cell (outline), provides landmarks for diffusing growth zone (circle), and cell expands.

See Introduction panel for most references.
Kelly, F. and P. Nurse, PLoS ONE, 2011, **6**: e27977
Mata, J. and P. Nurse, Cell, 1997, **89**: p. 939-49

## SUMMARY AND CONCLUSIONS

**In this Work:**

- Physical model for fission-yeast cell growth due to surface remodeling under turgor pressure
- Explored how shape and diameter depend on parameters and growth-factor distribution
- Investigated stability of microtubule-dependent growth-factor profile width
- Proposed framework for understanding many of the known shape mutants or defects

**Future Work:**

- Finish computational implementation and investigation of three-module framework
- Propose experiments to test hypotheses that (i) Cdc42 is a master control for growth and that (ii) microtubule-delivered factors anchor the growth cap to the tip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