The mechanical regulation of morphogenesis in plants and fungi

Arezki Boudaoud
Department of Biology
Ecole Normale Supérieure de Lyon

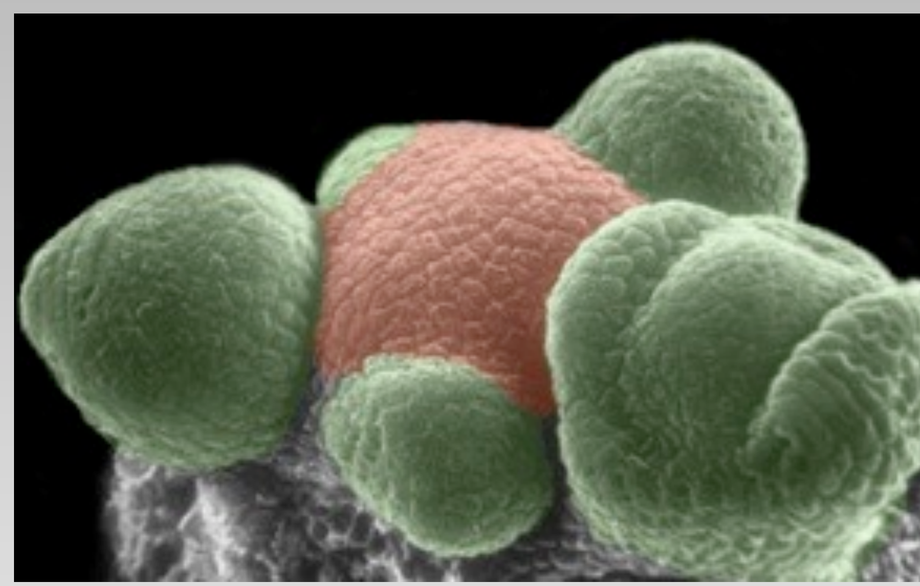
Morphogenesis in plants and fungi

Growth regulation — **Patterning** — **Architecture**

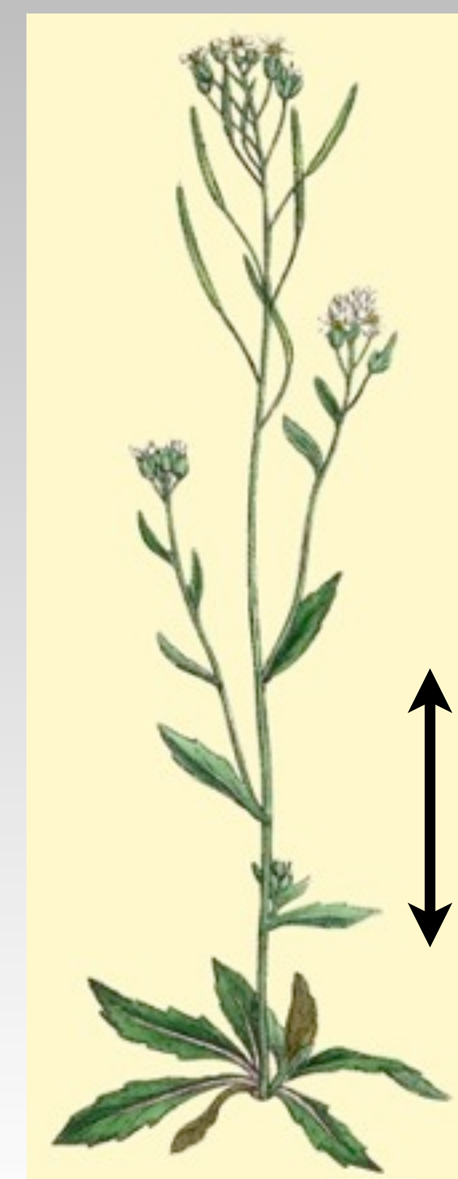
Cell ⟷ *Tissues* ⟷ *Whole organism*

Arabidopsis thaliana

5μm



100μm



5cm

Molecular and genetic regulation

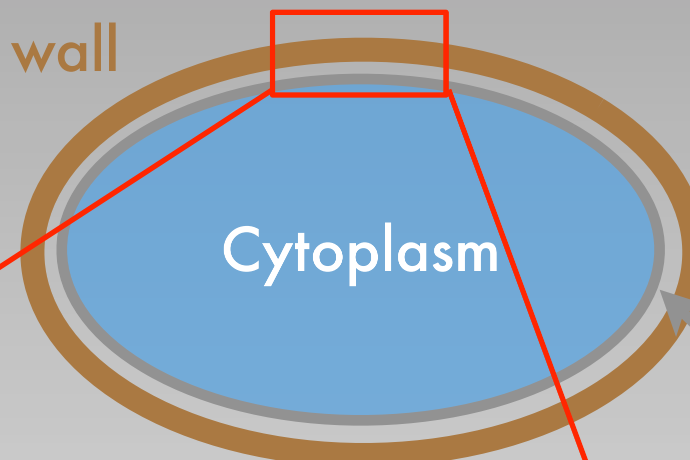
Focus on physical effectors

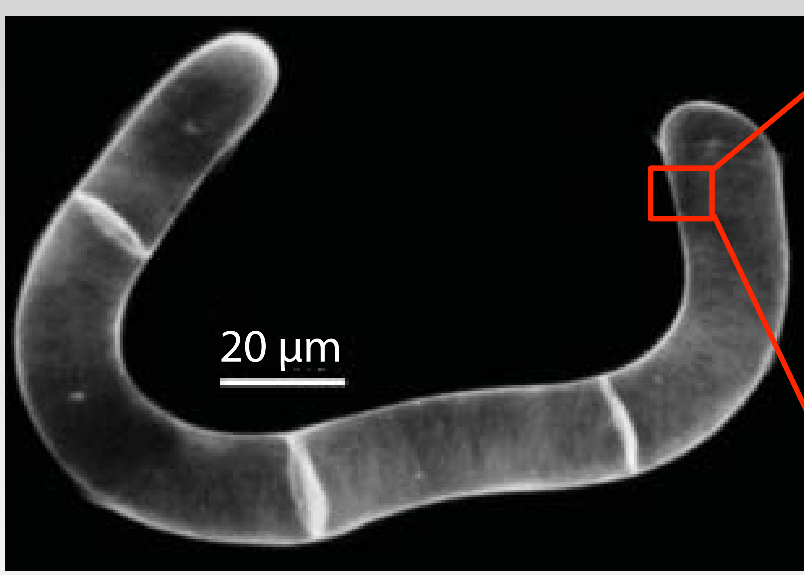
Walled cells

★Walled cells

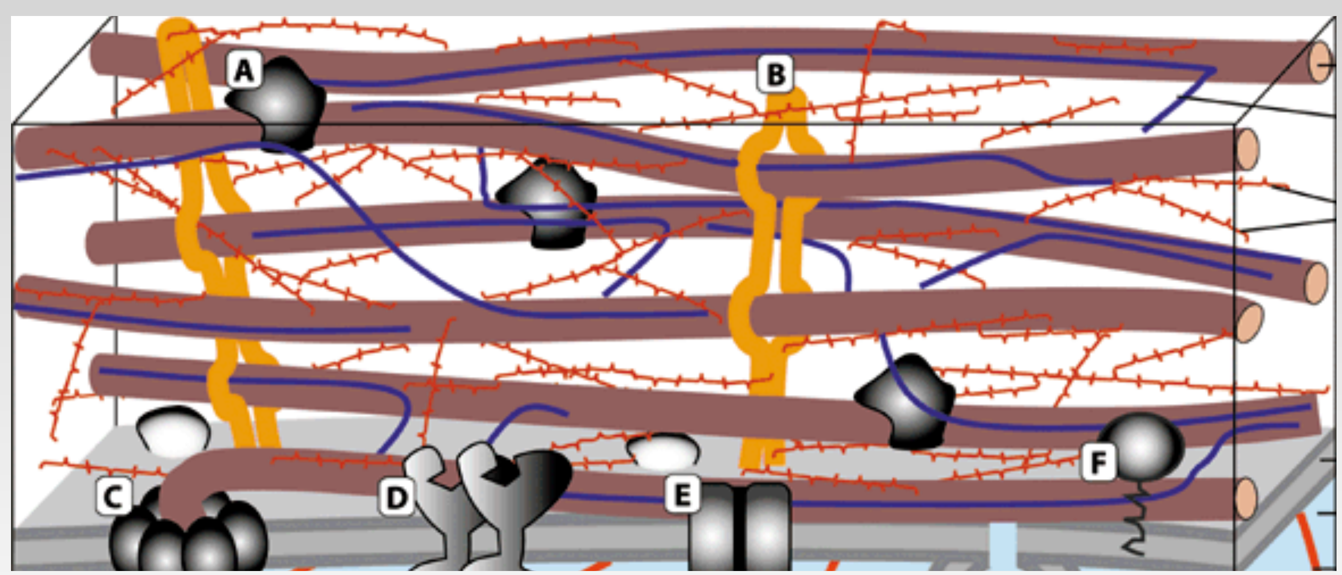
- Green algae and land plants
- Fungi
- Eubacteria
- Archaea
- Red algae
- Brown algae

Stiff casing (no change in shape when depolymerising cytoskeleton)





Tobacco - BY2

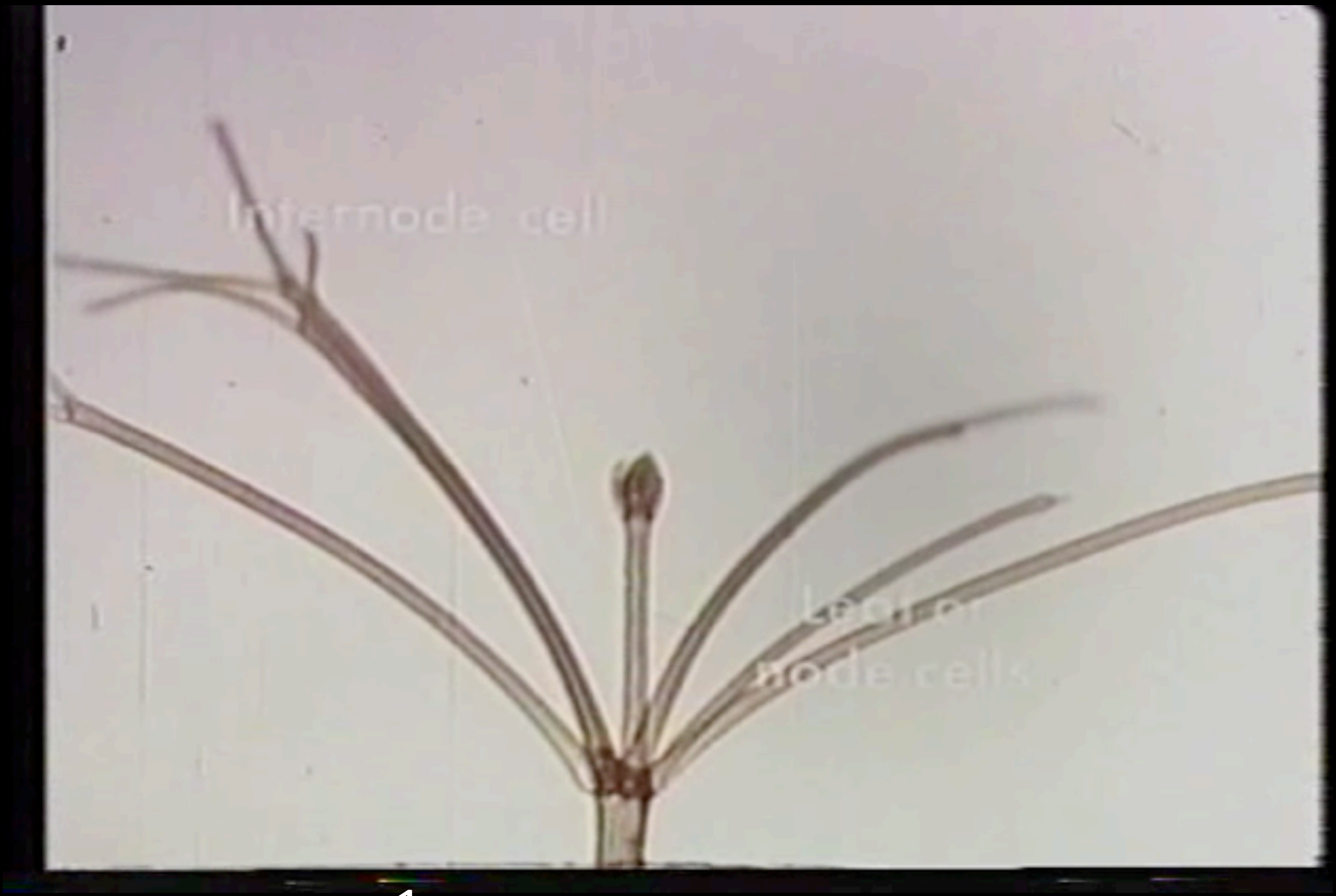


Outline

An introduction to walled cells
Growth mechanics in fission yeast
Growth mechanics in Arabidopsis
Morphogenesis in fission yeast
Growth homogeneity in Arabidopsis
Architecture in Arabidopsis

Introduction

Growth in charales (*Nitella, Chara*)



1mm

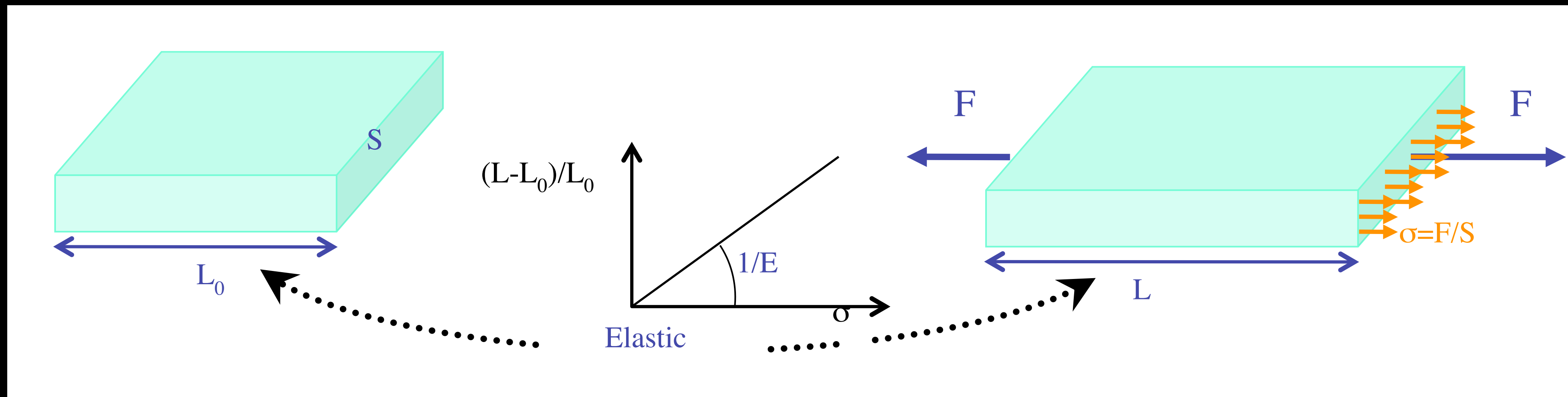
~ day

Nitella axilaris

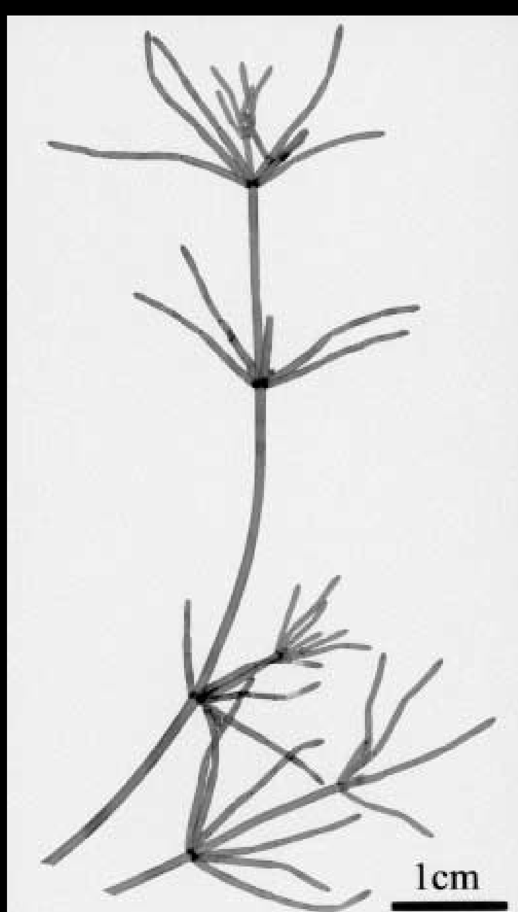
Paul Green 1970s

Introduction

Is the cell wall soft or hard?



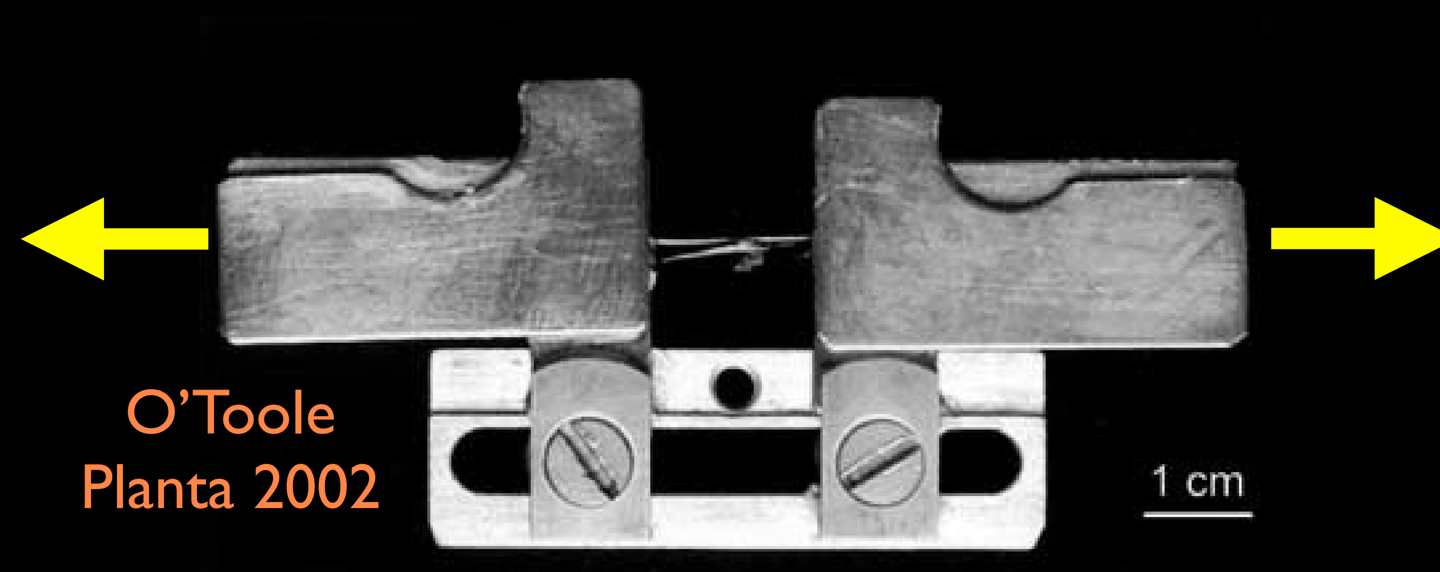
Chara corallina



=> Elastic modulus
(anisotropy?)
E(units of pressure)
Stiff <=> high E
Soft <=> small E

Agar: 0.1-1MPa
PDMS (silicon): ~1MPa
Rubber: 10-100MPa
Plastics: mostly ~1GPa
Metals: ~10GPa

E~100MPa

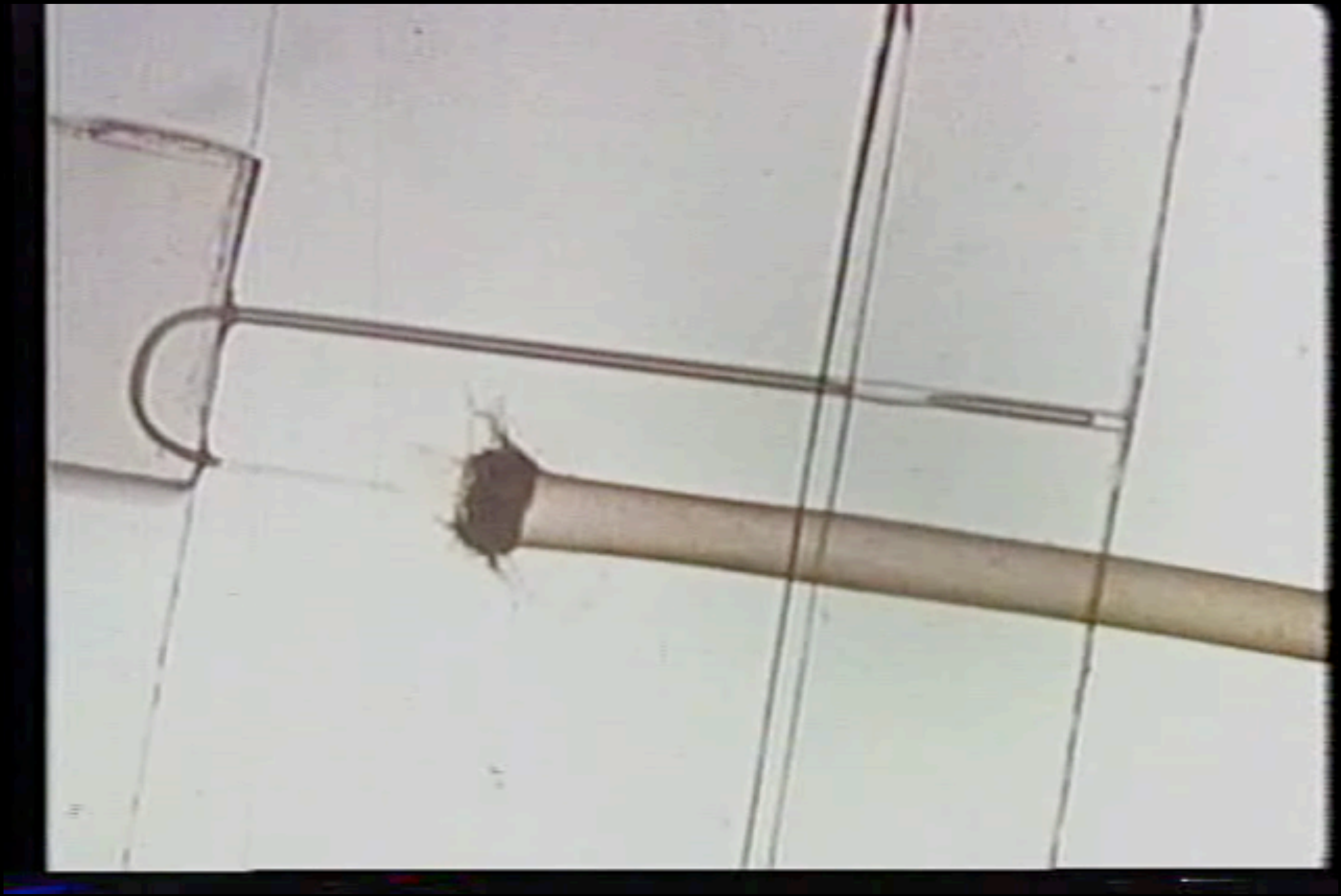


O'Toole
Planta 2002

Introduction

How can they grow within a stiff casing?

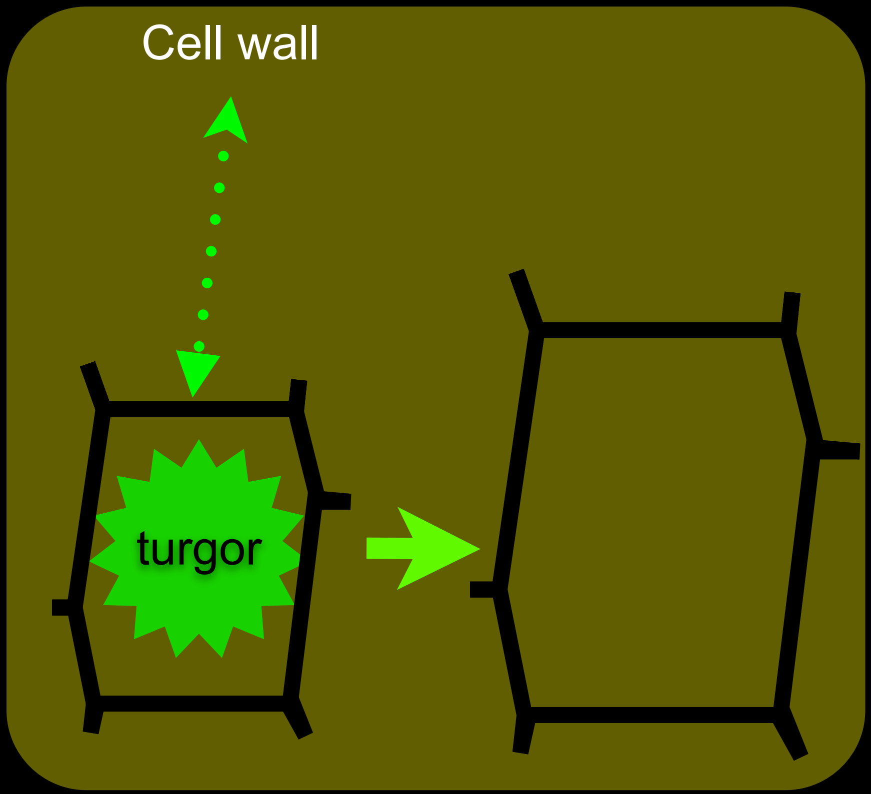
Slower growth in hyperosmotic medium



In walled cells:
turgor pressure 0.5 to 20 atm (0.05 to 2MPa)

Introduction

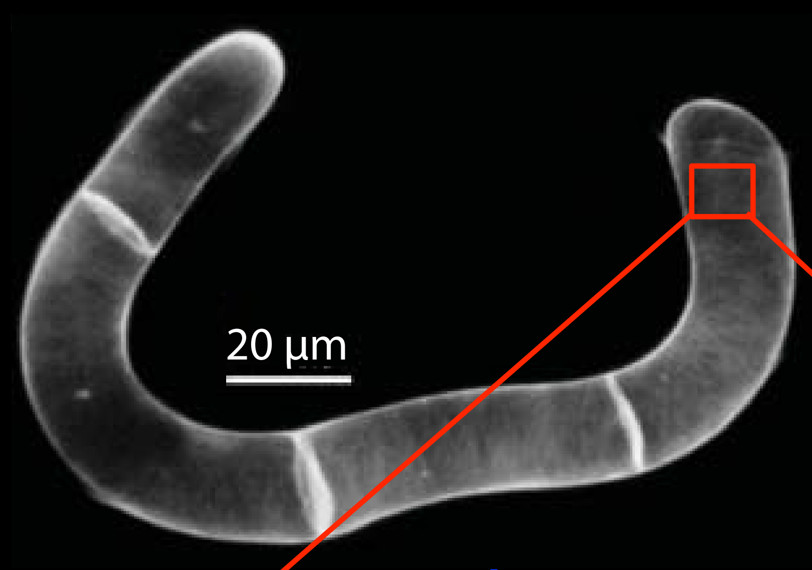
Anisotropic growth?



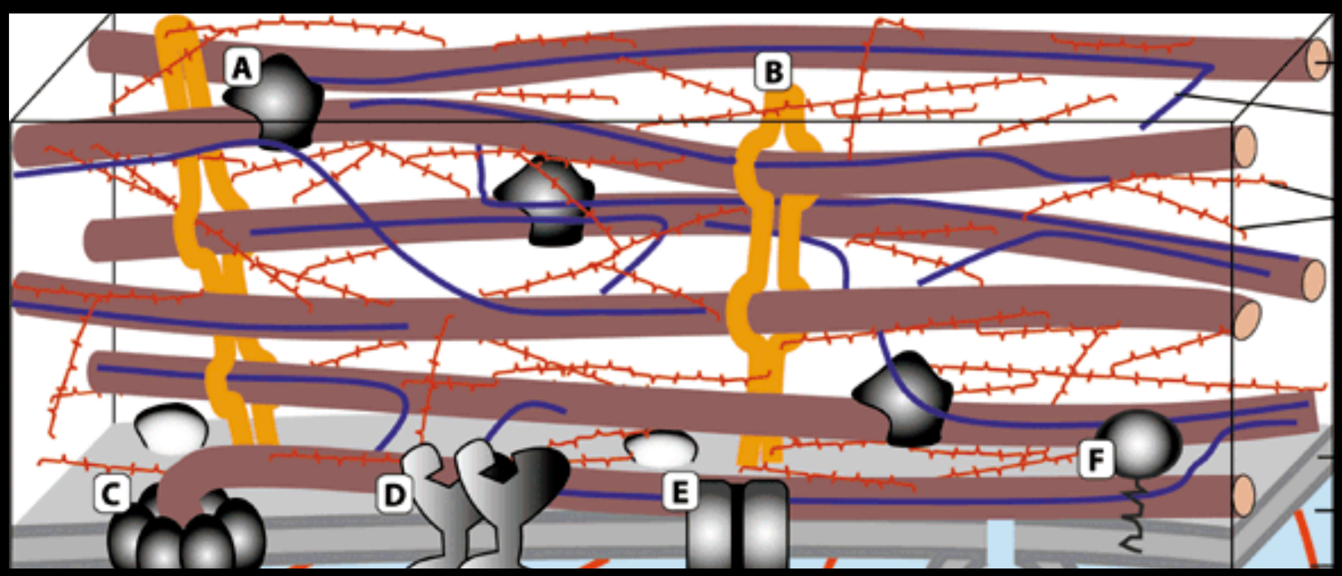
Introduction

Imaging between cross-polarizers

Introduction



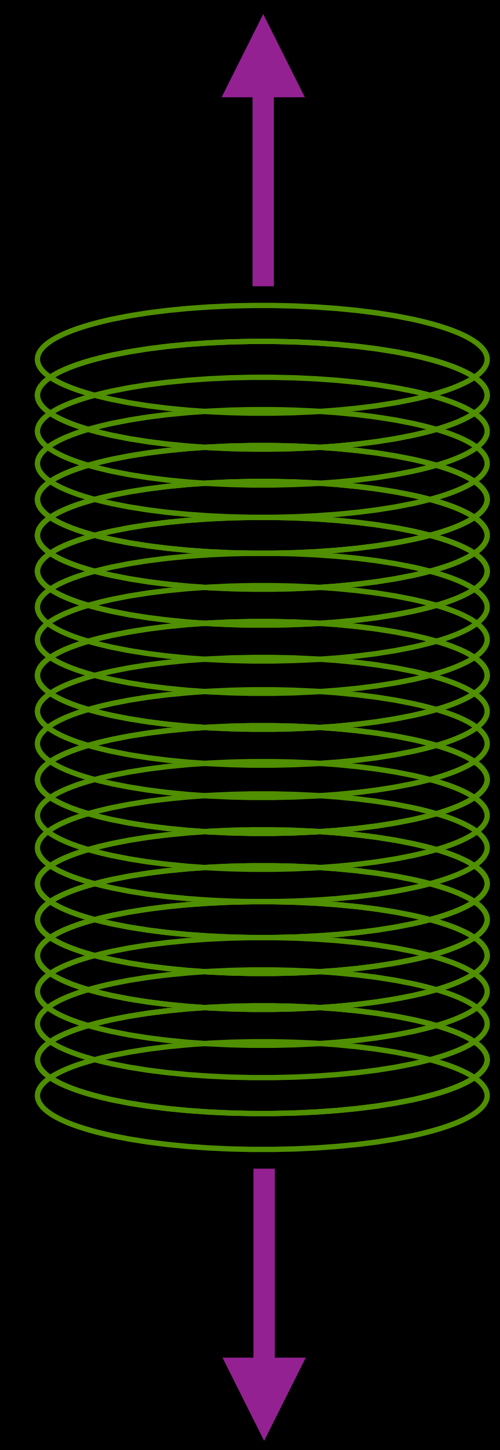
Tobacco - BY2



Cell wall thickness 0.1 to 10µm

Plasma membrane

Cytoplasm

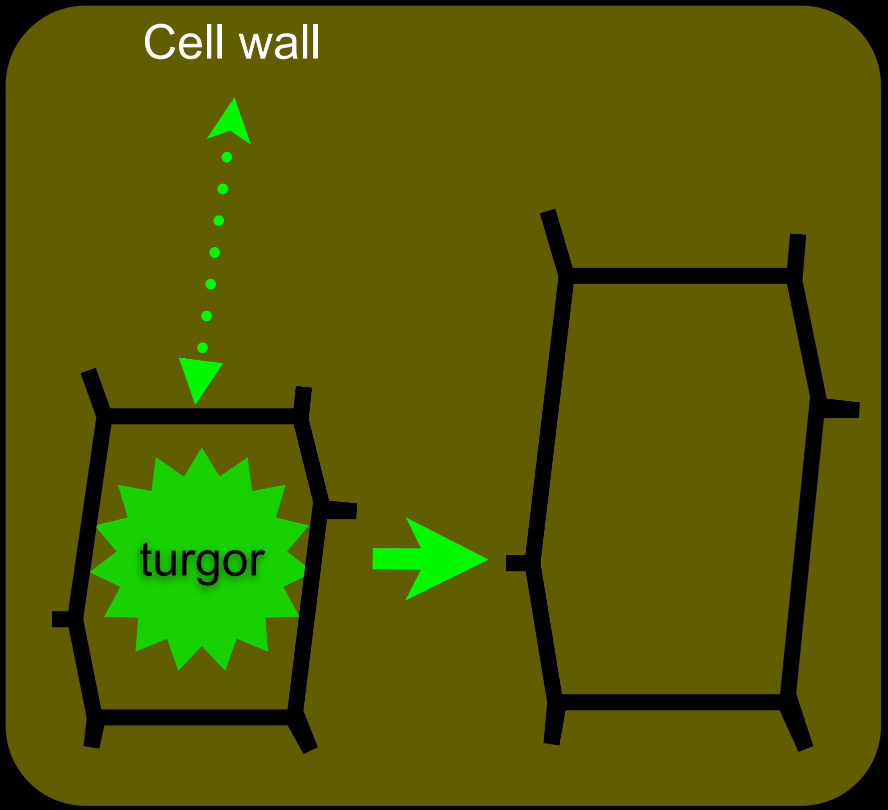


Introduction

The basis of morphogenesis?

Growth of single cell / hypocotyl

- structure: cell wall
- powered by: turgor pressure (osmotic)
- growth rate: soft/stiff wall BUT
- growth orientation: orientation of fibers



How different from animal morphogenesis?

Introduction

Not that much

A directional brake/facilitator:
Cell wall <=> Actomyosin cortex

A power:
Osmotic pressure

But:
adhesion, topology

LETTER

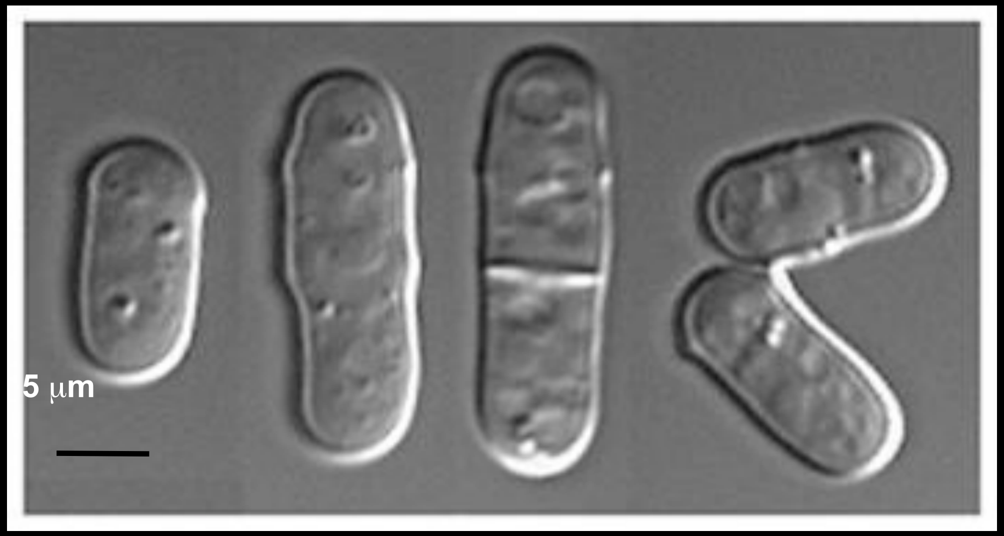
doi:10.1038/nature09642

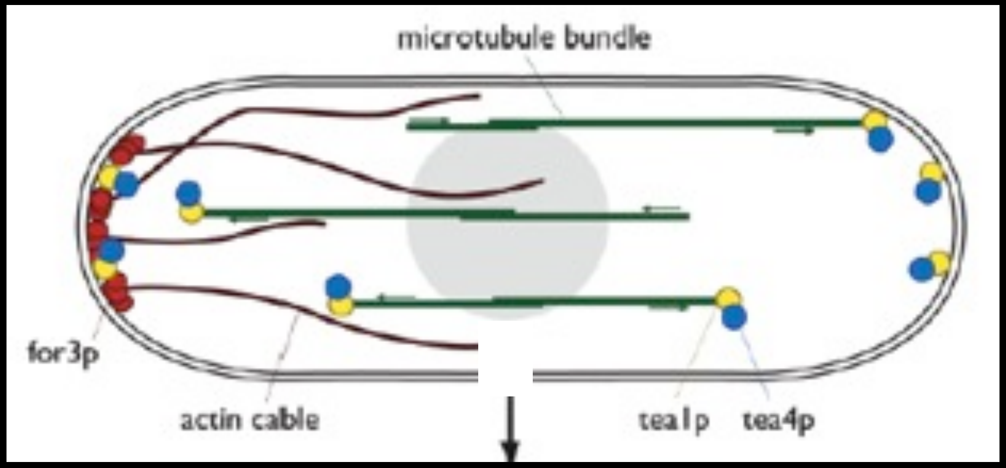
Hydrostatic pressure and the actomyosin cortex drive mitotic cell rounding

Martin P. Stewart[1,2], Jonne Helenius[1], Yusuke Toyoda[3], Subramanian P. Ramanathan[1], Daniel J. Muller[1] & Anthony A. Hyman[3]

Growth mechanics in fission yeast

A model system for polarised growth



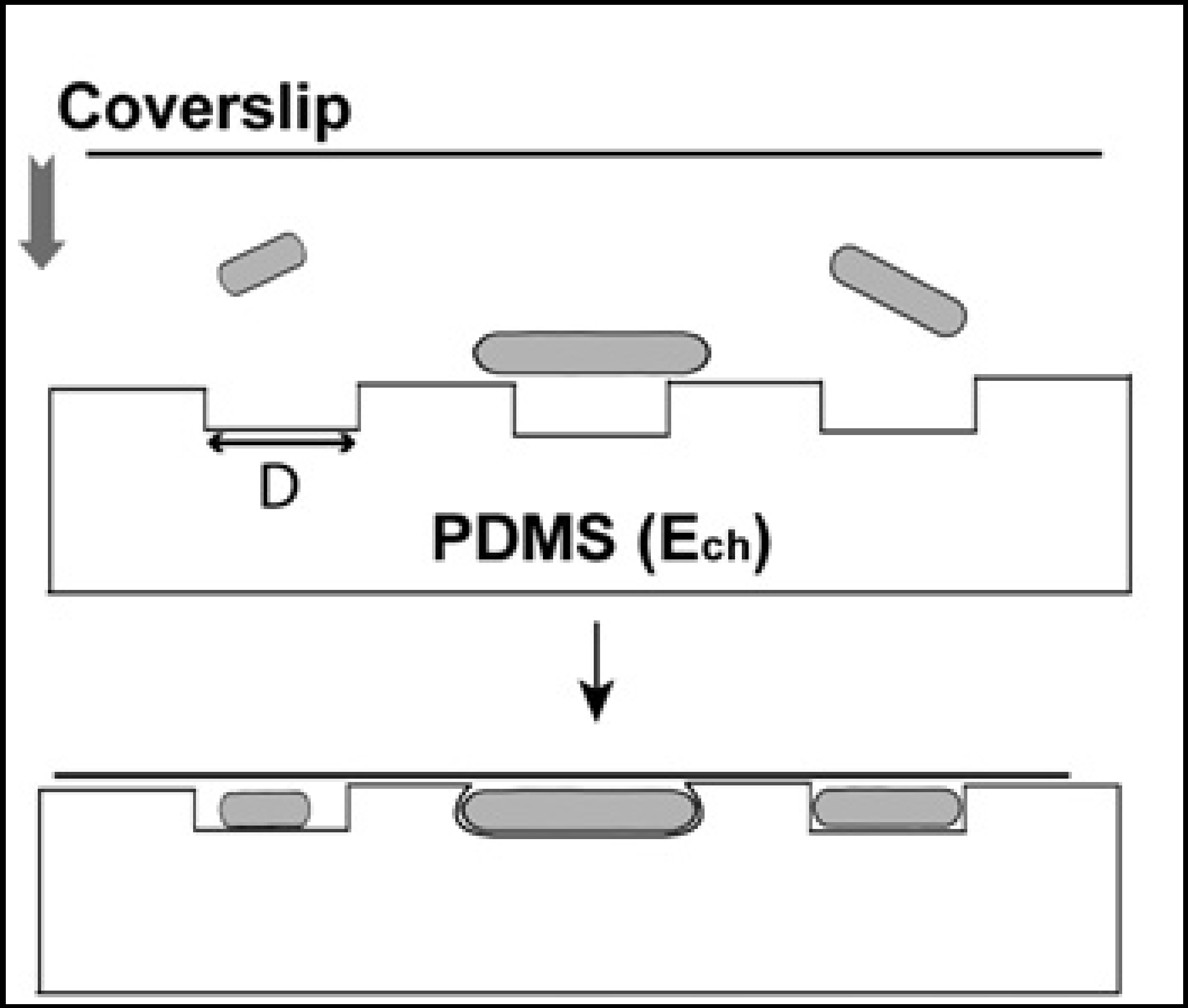


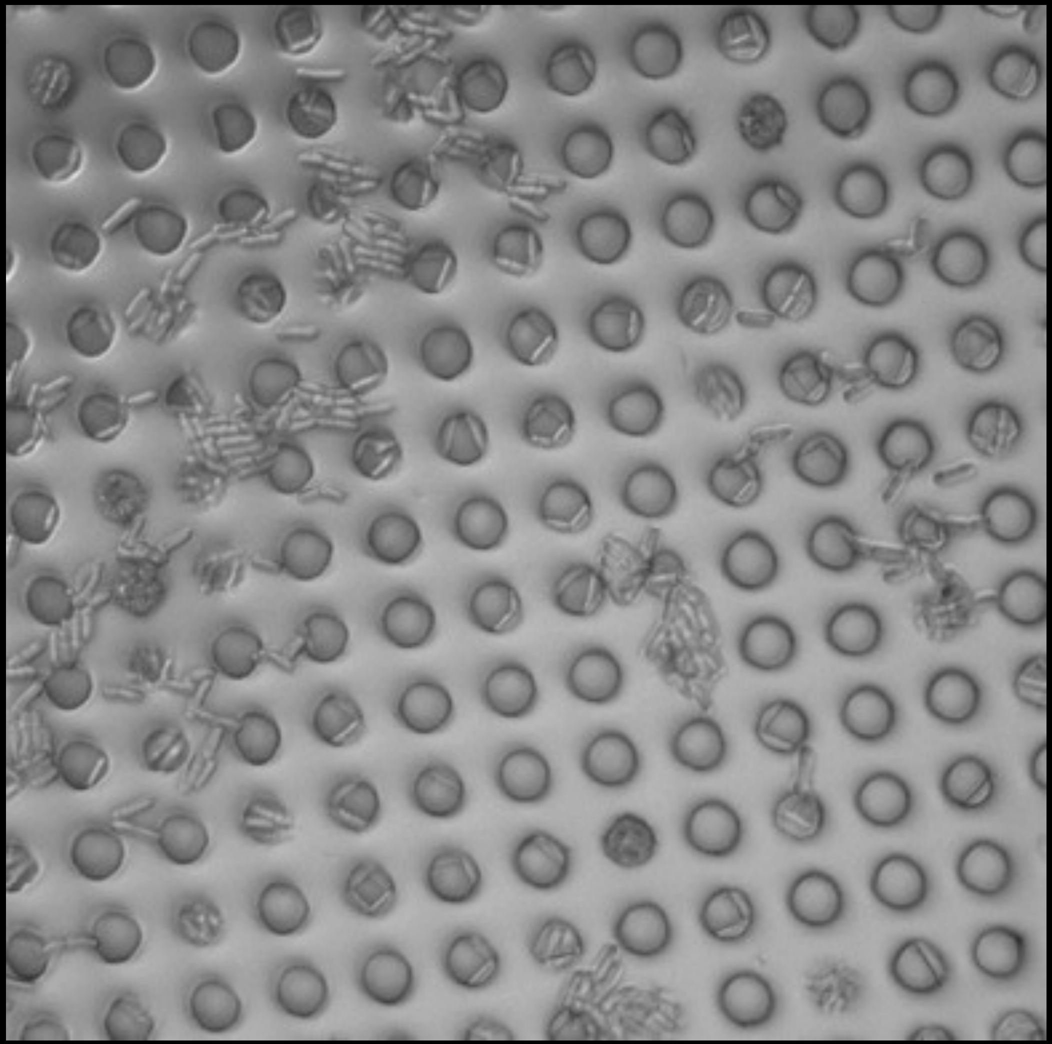
Nicolas MINC
Columbia University
now
Institut Jacques Monod
Paris

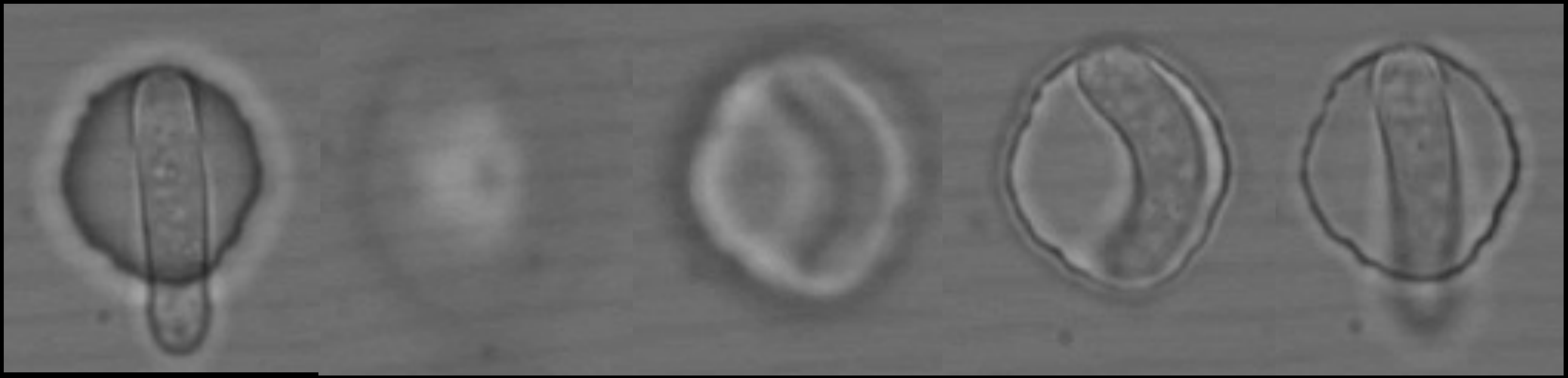
Fred CHANG
Columbia University

Minc et al. Curr. Biol 2009

Growth mechanics in fission yeast

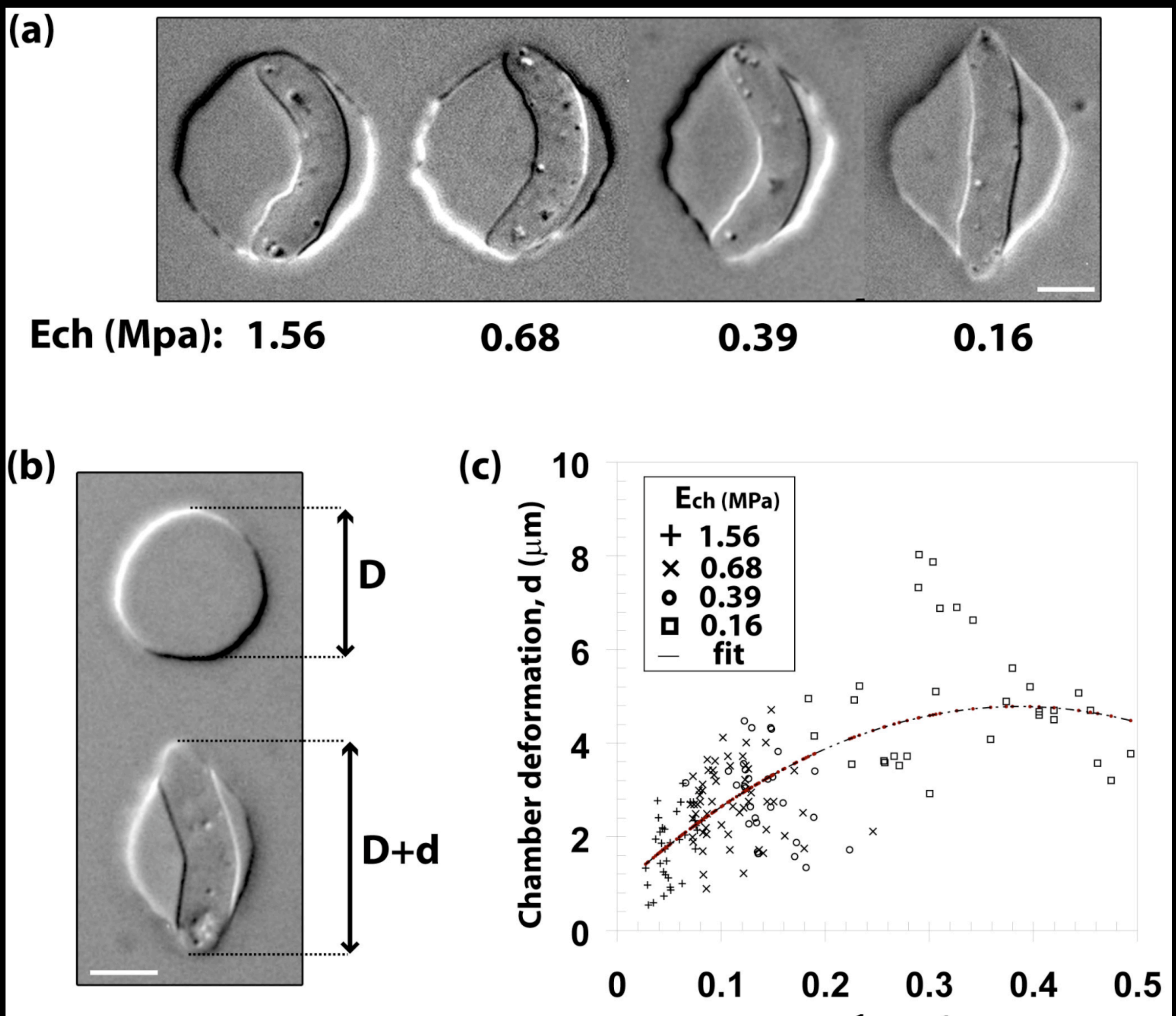






Growth mechanics in fission yeast

- Force deduced from well deformation
- Buckling threshold yields wall stiffnes

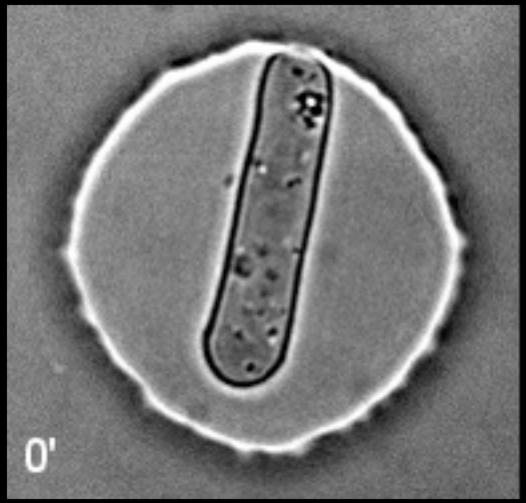


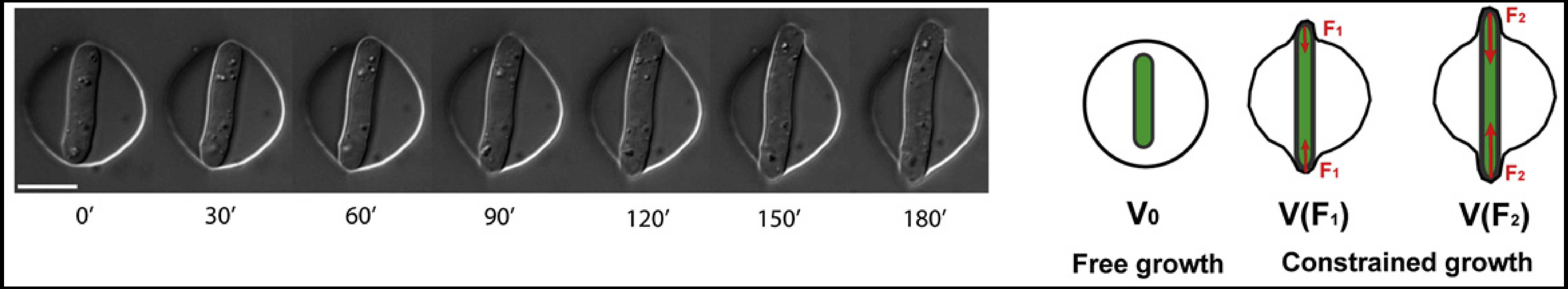
With $E^*=E_{ch}*R^2/d^2$

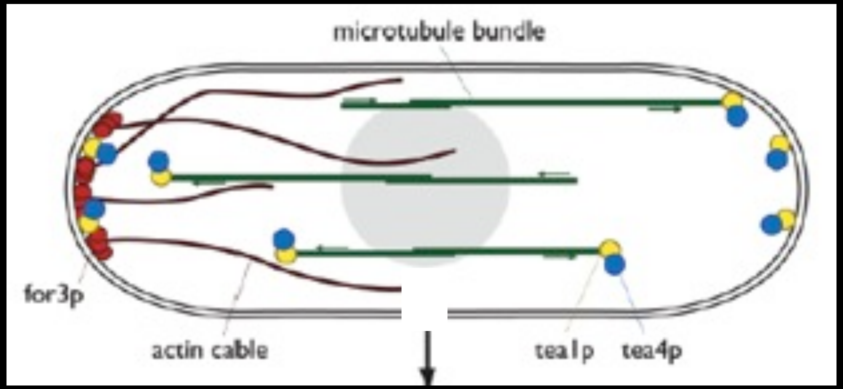
$E_{fission\ yeast} = 100 \pm 30$ MPa

Confirmed by 'swelling-shrinking' experiments

Growth mechanics in fission yeast

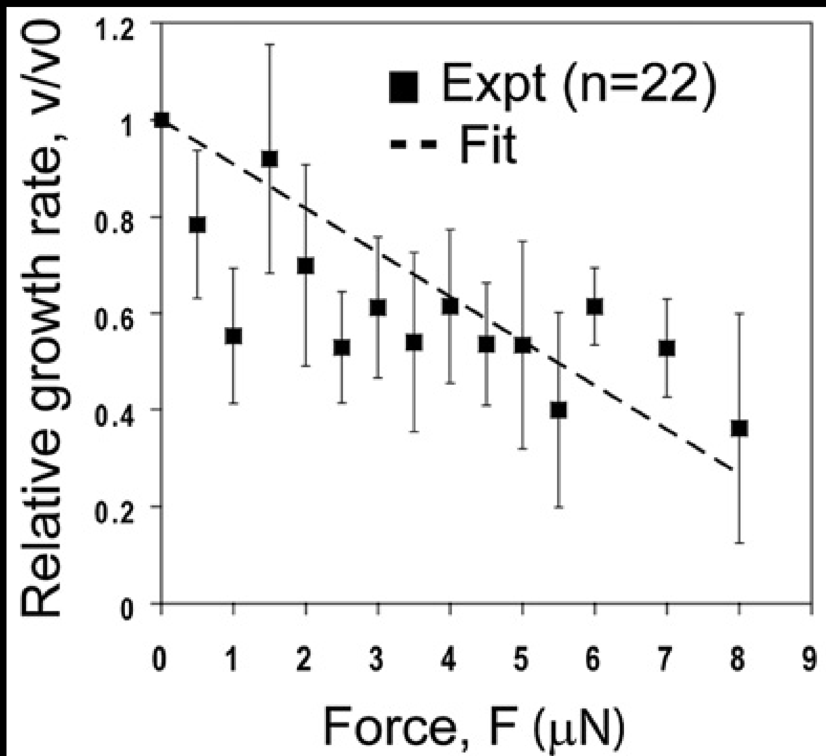


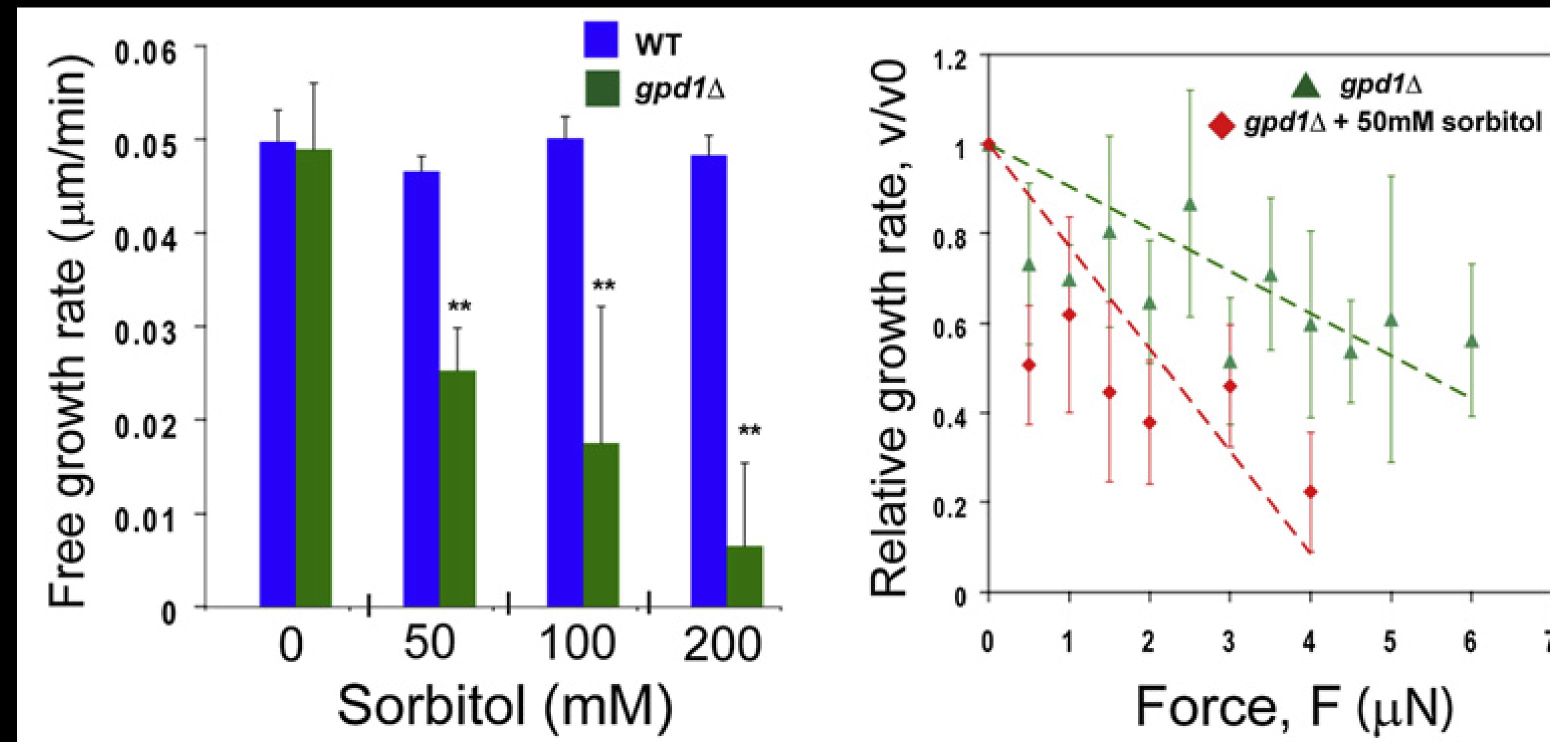


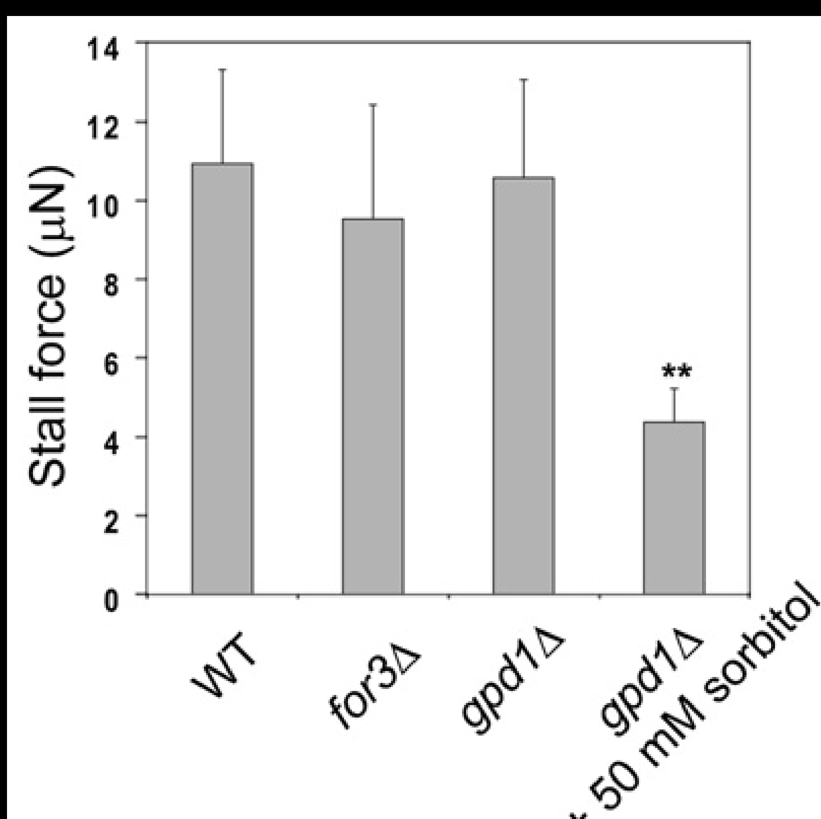


Force generation by MTs?
Max ~ 50nN

Growth mechanics in fission yeast





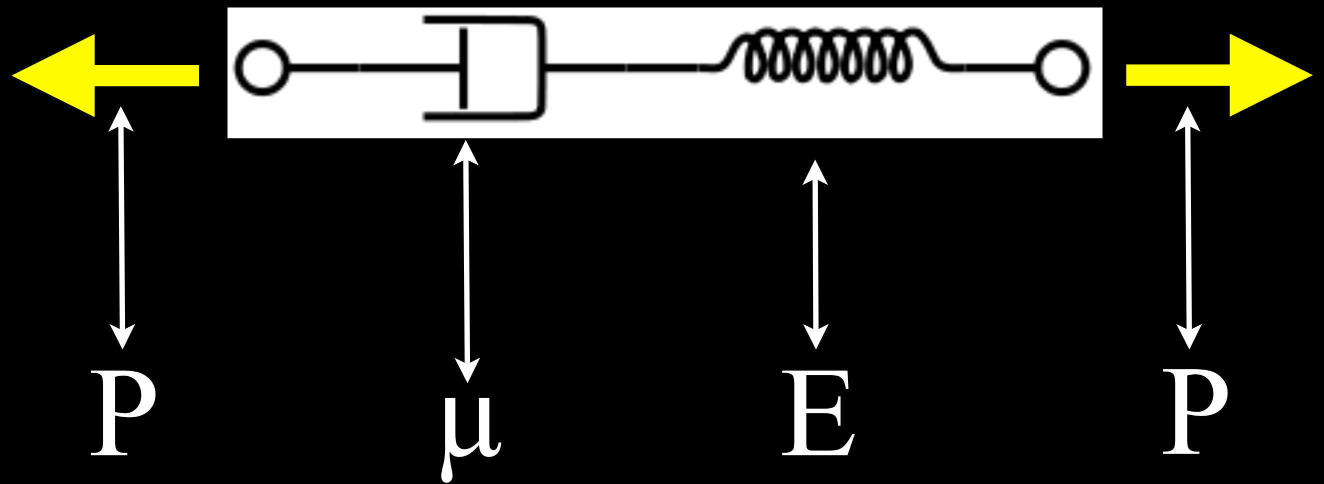


Stall force F=11µN
Cross section S=3.14x2^2=12.6µm^2
Corresponding pressure P=F/S
WT: P=0.9MPa (=9bars)

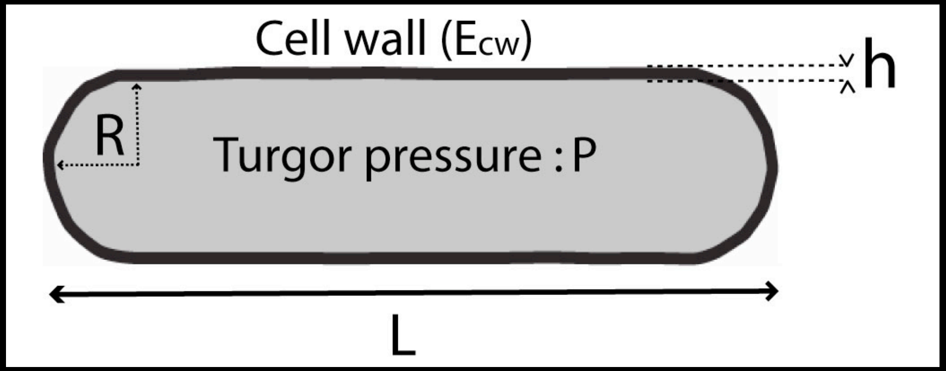
Growth mechanics in fission yeast

Turgor-powered growth

Simplest model



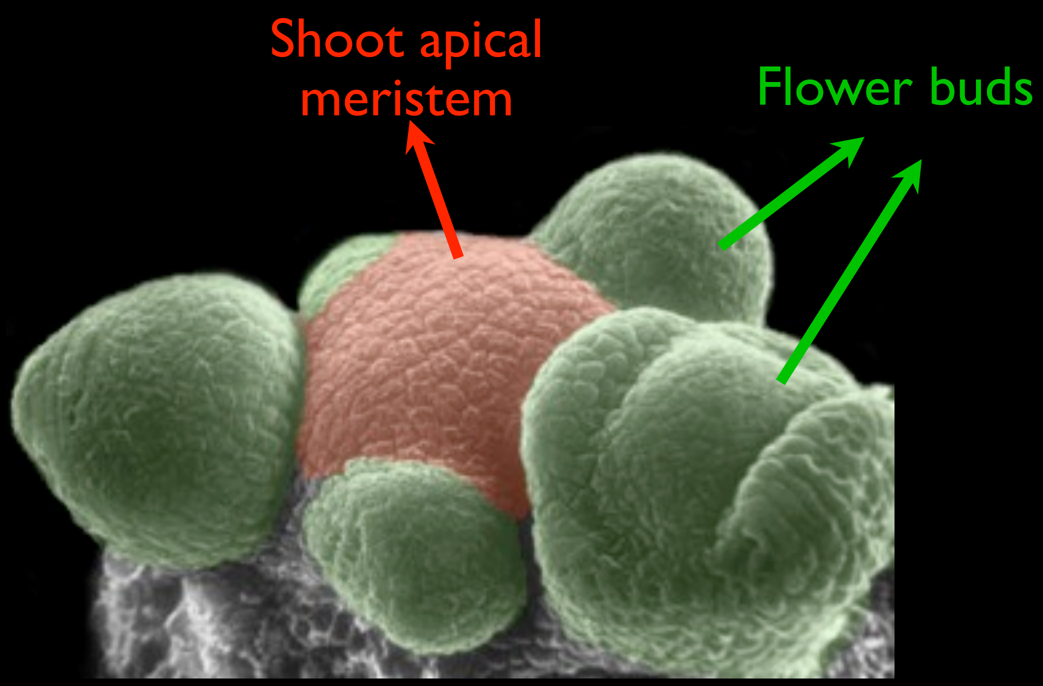
Geometry, wall thickness
=> turgor, wall properties



Multicellular context?

Growth mechanics in Arabidopsis

The shoot apex

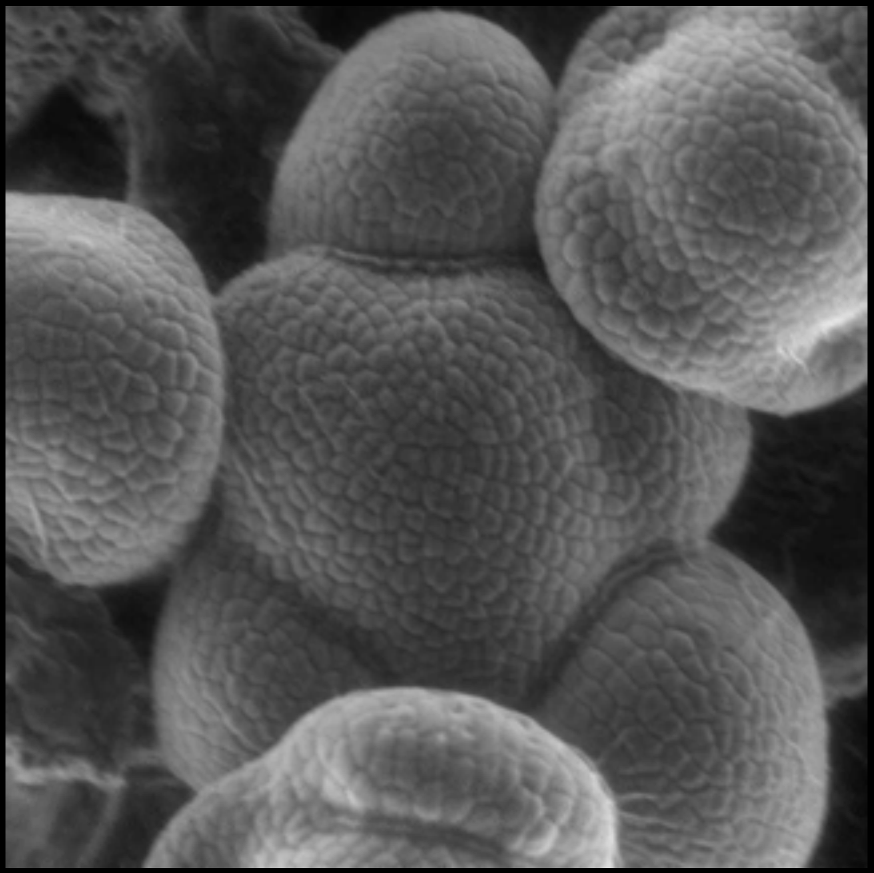


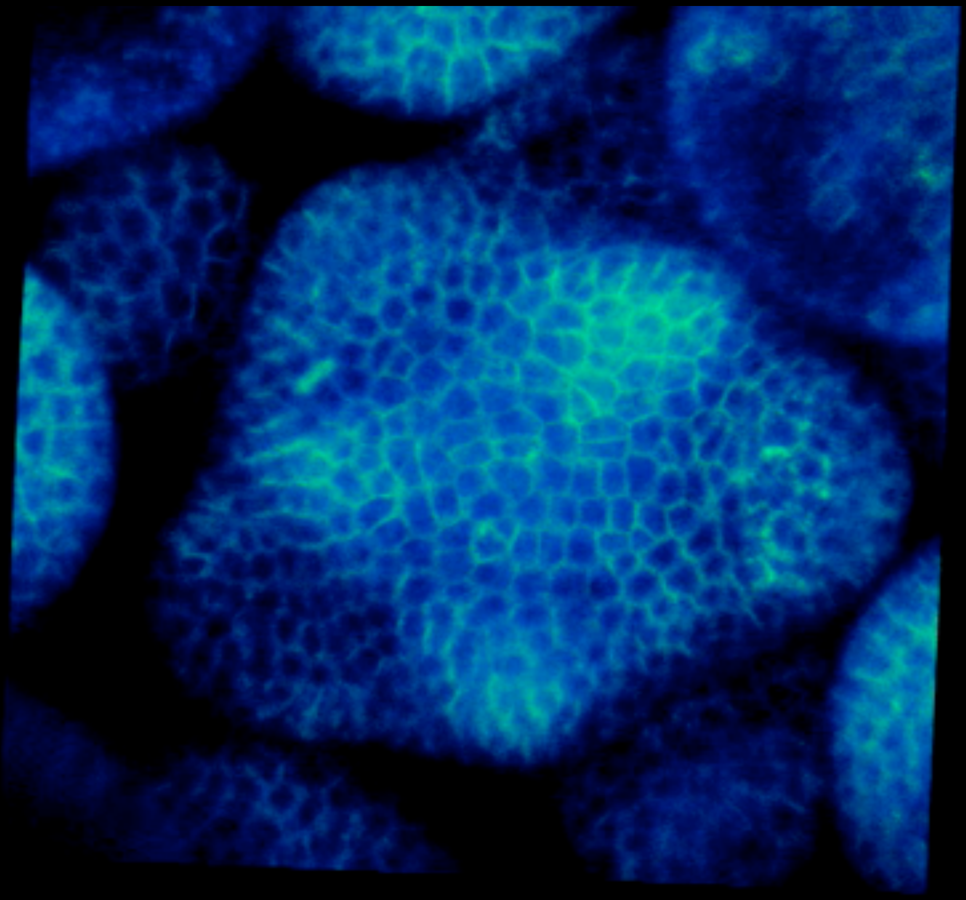
An ideal system:

- well-characterised molecularly/genetically
- determines aerial architecture
- accessible in the reproductive state

Growth mechanics in Arabidopsis

Continuous development

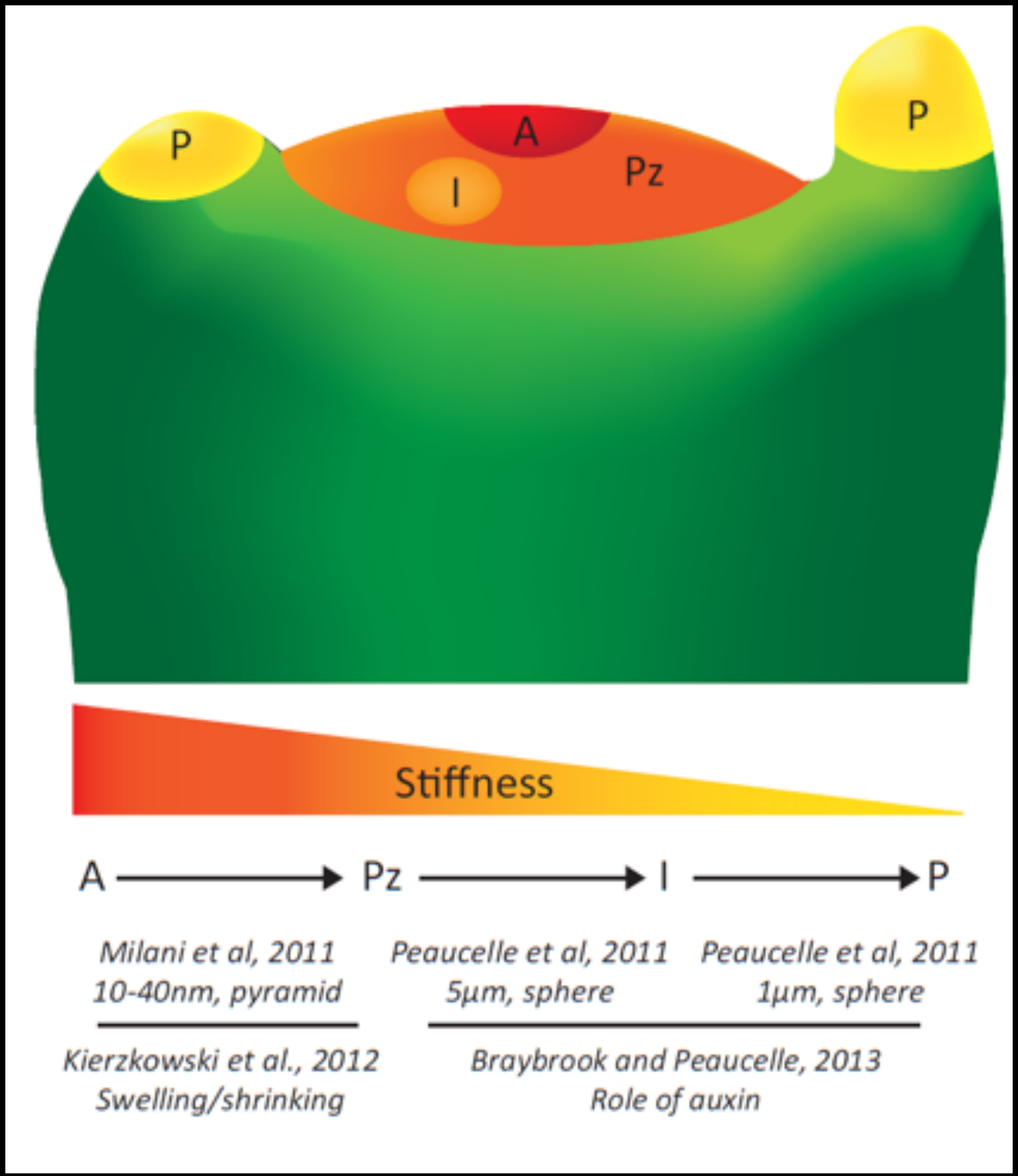




Heisler, 2005

Growth mechanics in Arabidopsis

Appropriate approaches: indentation (eg AFM); swelling-shrinking



Growth mechanics in Arabidopsis

Does this stiffness pattern correspond to cell identity?

Pascale MILANI

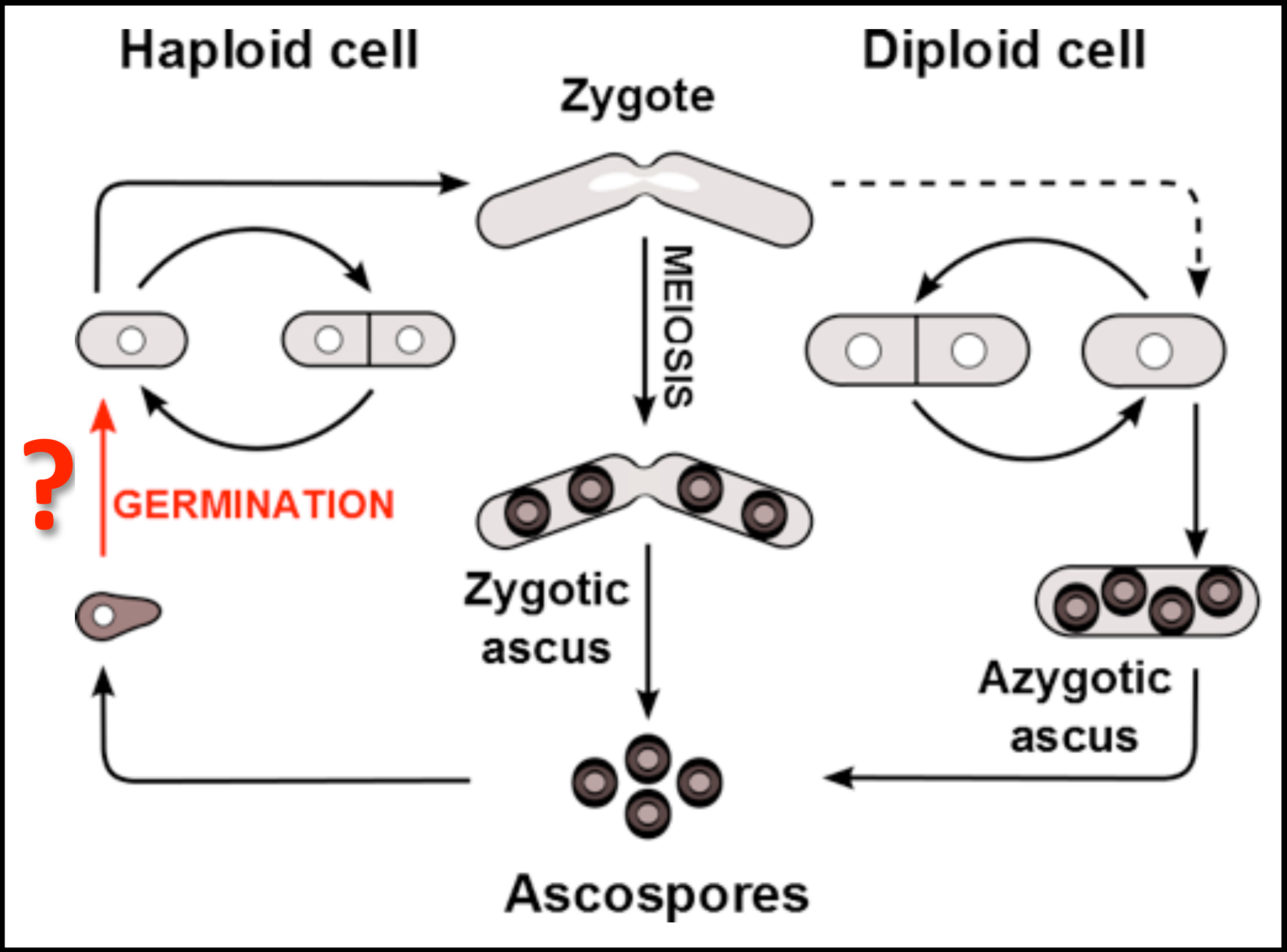
Vincent MIRABET

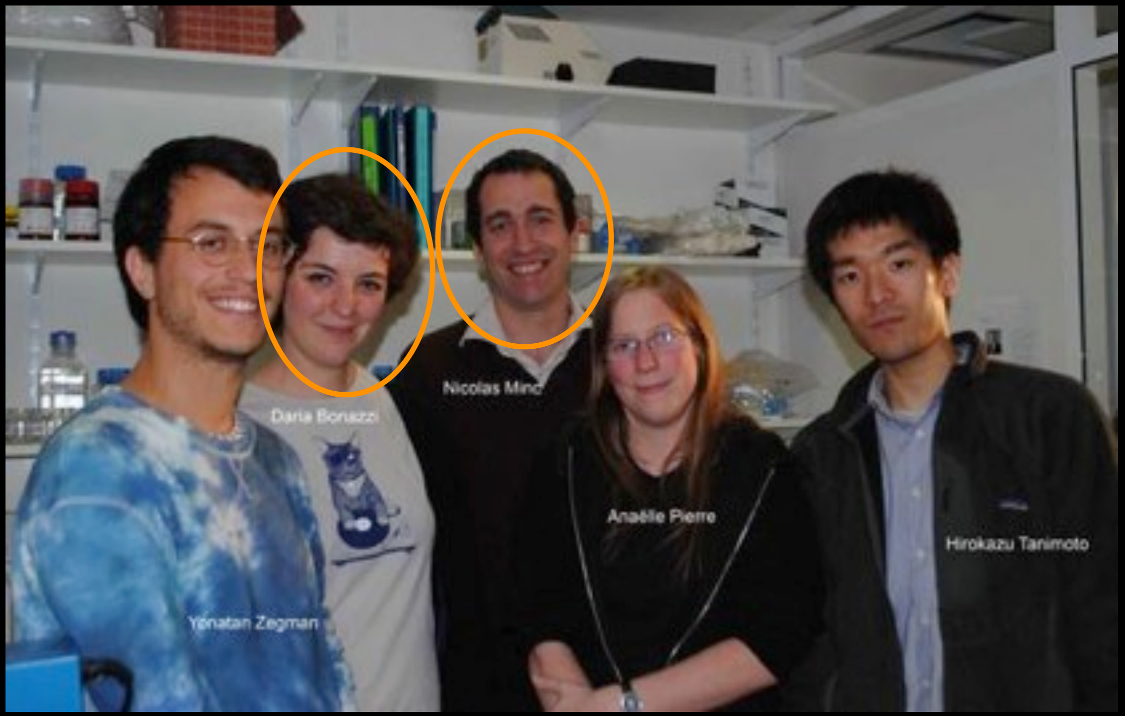
Pradeep DAS

and Coralie CELLIER and Olivier HAMANT

P. Milani et al., unpublished

Morphogenesis in fission yeast

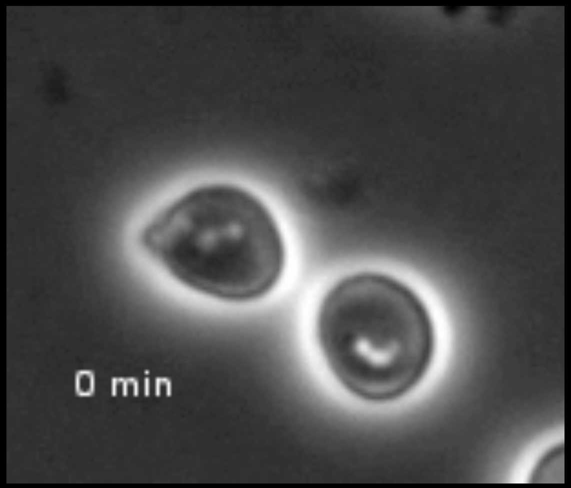




Daria BONAZZI
Nicolas MINC's group
Institut Jacques Monod, Paris ;
Matthieu PIEL

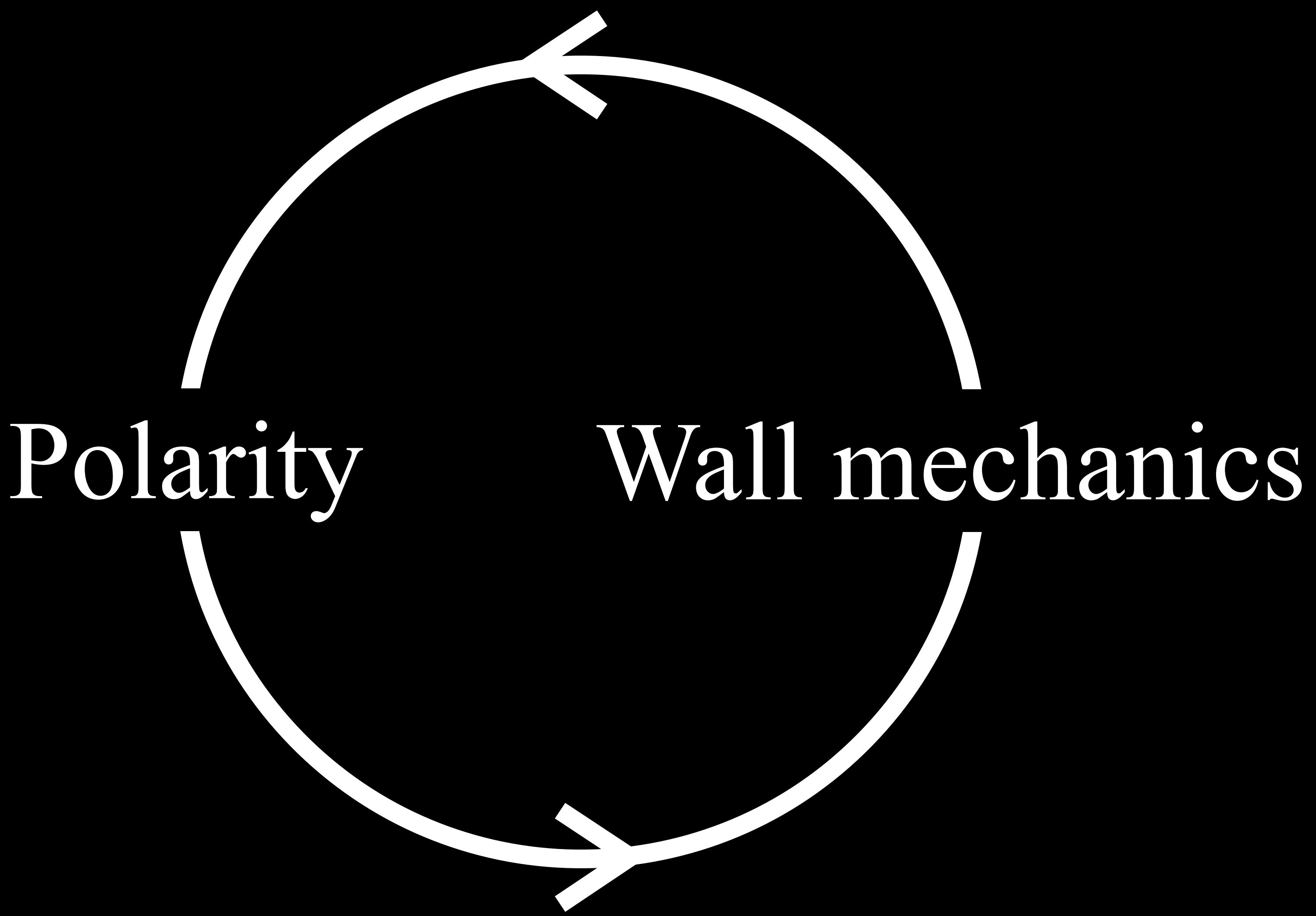
How do spores become vegetative cells?

D. Bonazzi, JD Julien et al., unpublished



Jean-Daniel JULIEN

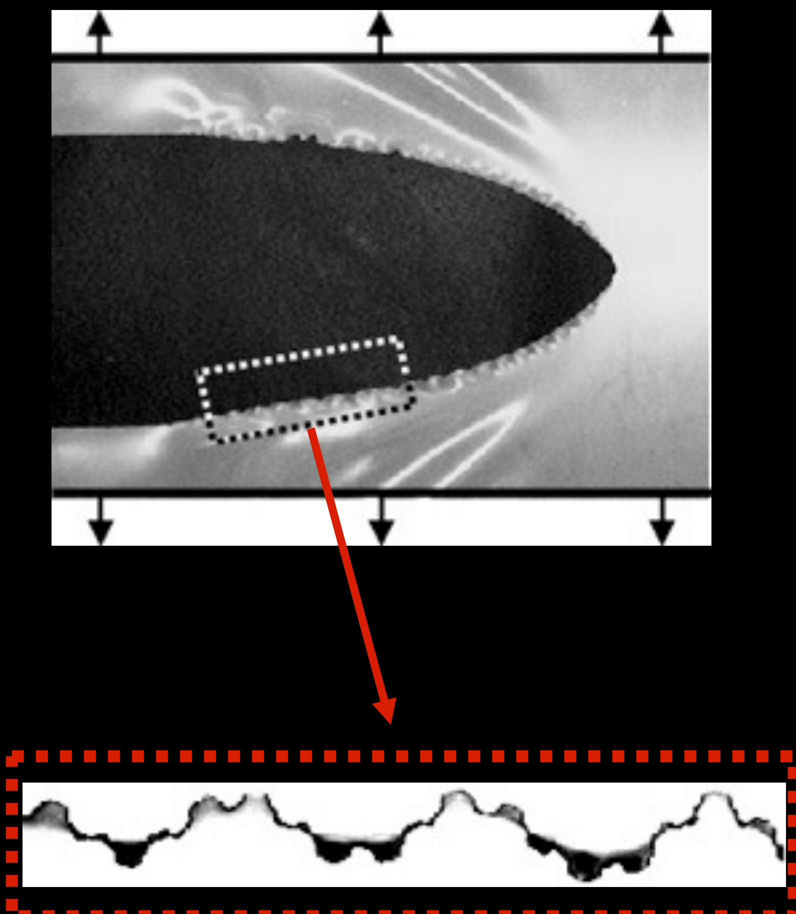
Morphogenesis in fission yeast



Flatness of leaves and petals

Why are leave flat?

Flatness of leaves and petals



Torn plastic sheets
and beet leaves
Sharon *Nature* 2002

Antirrhinum *cin*
Nath *Science* 2003

African tulip tree
UCSB campus

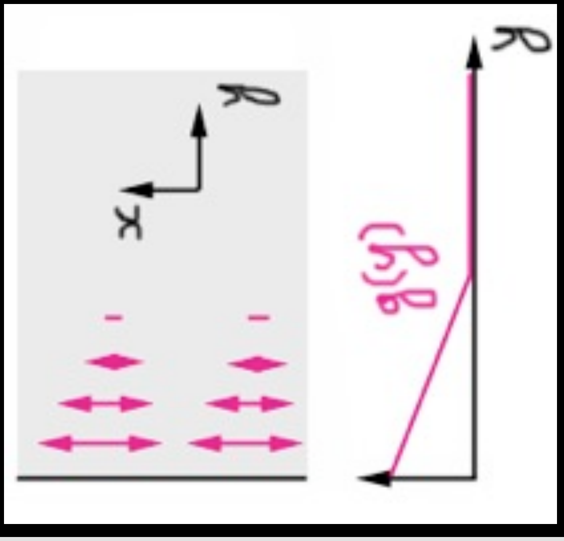
Arabidopsis *jaw-D*
Palatnik *Nature* 2003

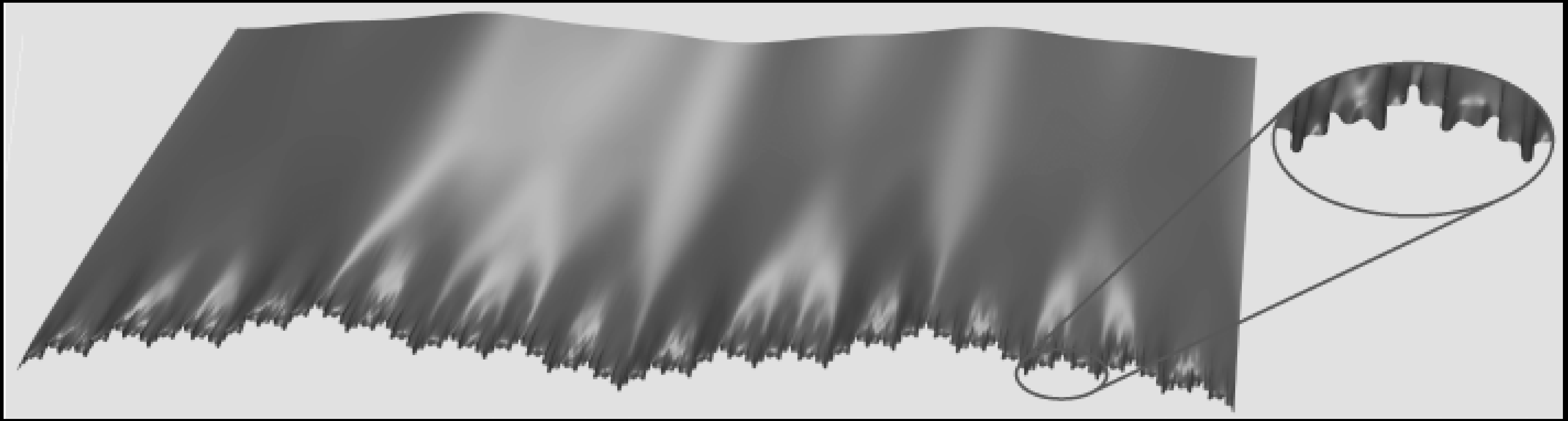
Arabidopsis *Δppd*
White PNAS 2006

Flatness of leaves and petals

- A thin elastic body
  - enhanced growth at the edge
  - mechanical equilibrium

with Basile AUDOLY, PRL 2003

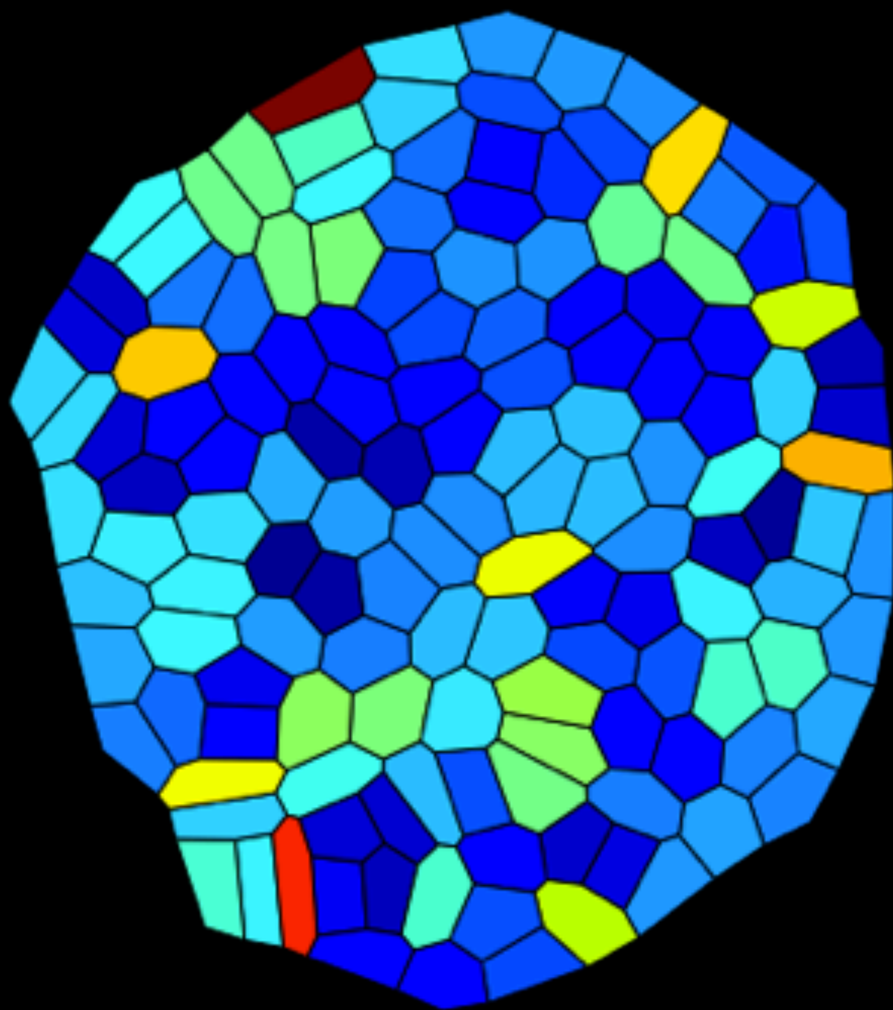




By default: leaves are not flat

Growth homogeneity in Arabidopsis

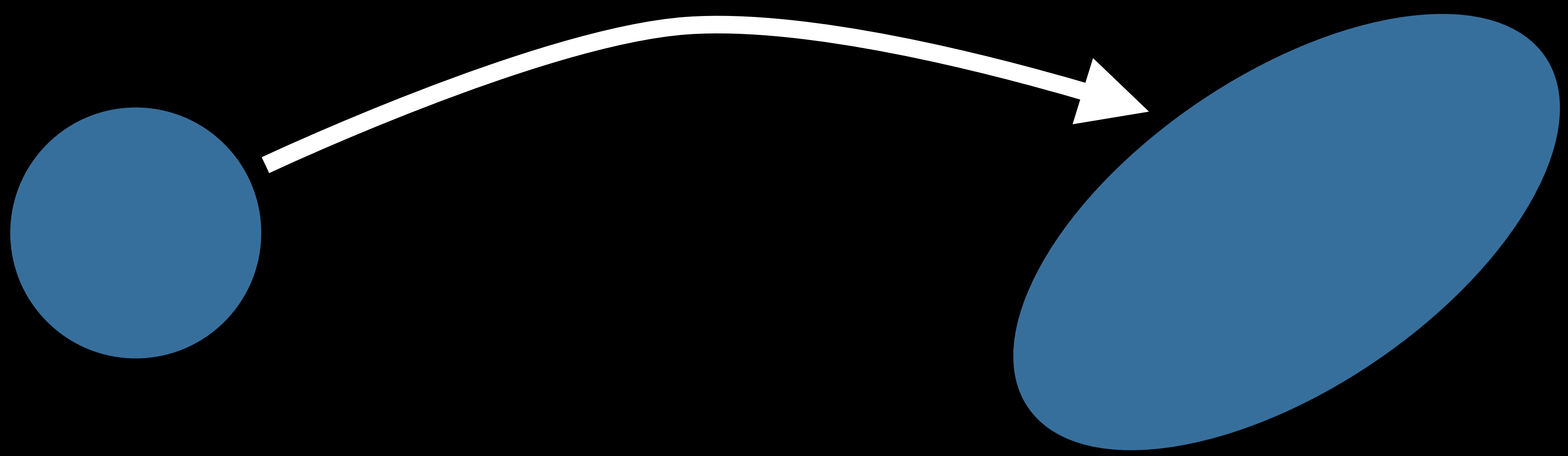
What if each cell had its own growth rate?



Shraiman PNAS 2005
Aergerter-Wilemsen et al Mech Dev 2007
Hufnagel et al PNAS 2007

Circumferential mechanical stress around fast growing cells

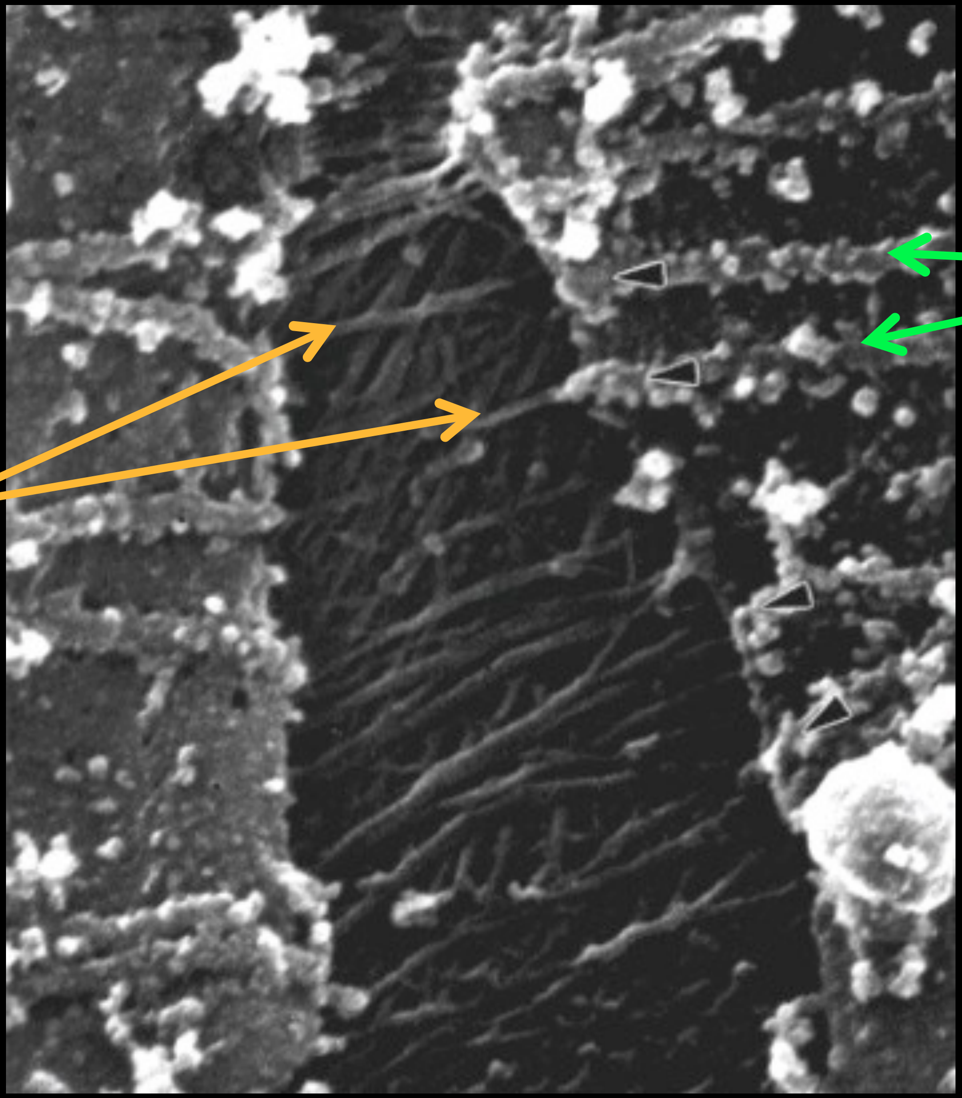
Growth homogeneity in Arabidopsis

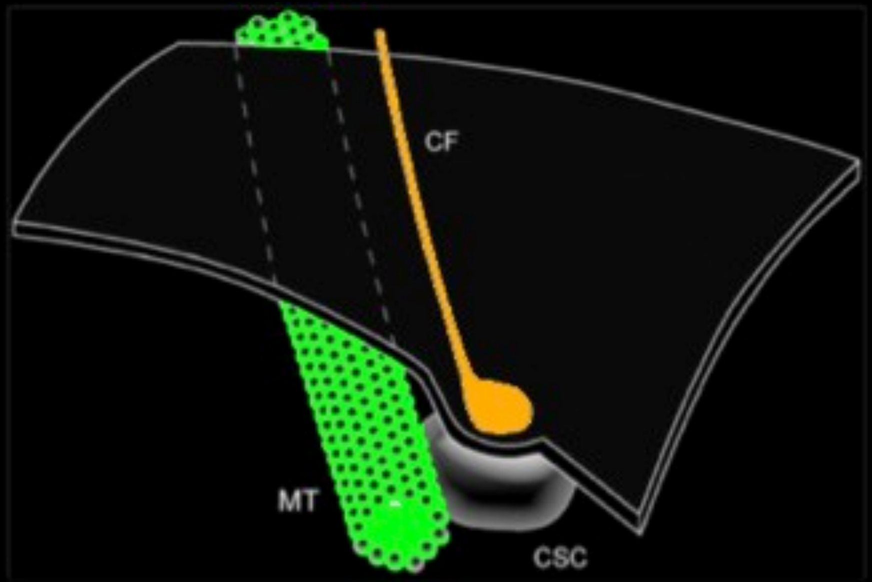


Growth rate in area (volume)
Anisotropy
Direction of maximal growth

Regulation of growth rate?
Control of anisotropy?

Growth homogeneity in Arabidopsis





Burgert and Fratzl 2009

Growth anisotropy: Microtubules orientation

Growth homogeneity in Arabidopsis

No cortical microtubules
Isotropic growth

Francis CORSON Olivier HAMANT

F. Corson et al. PNAS 2009

Jan TRAAS
Lyon

Yves COUDER
Paris Diderot

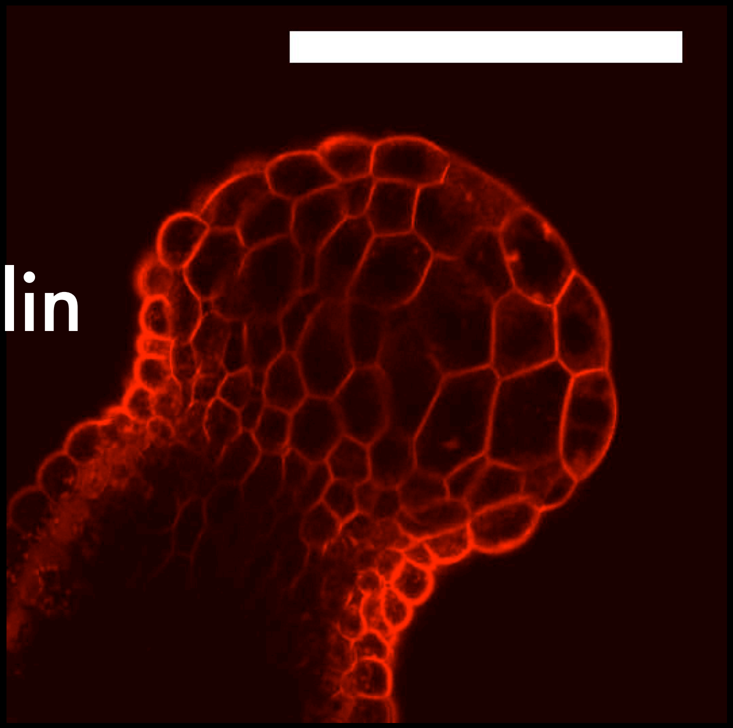
Steffen Bohn
Paris Diderot

Growth homogeneity in Arabidopsis

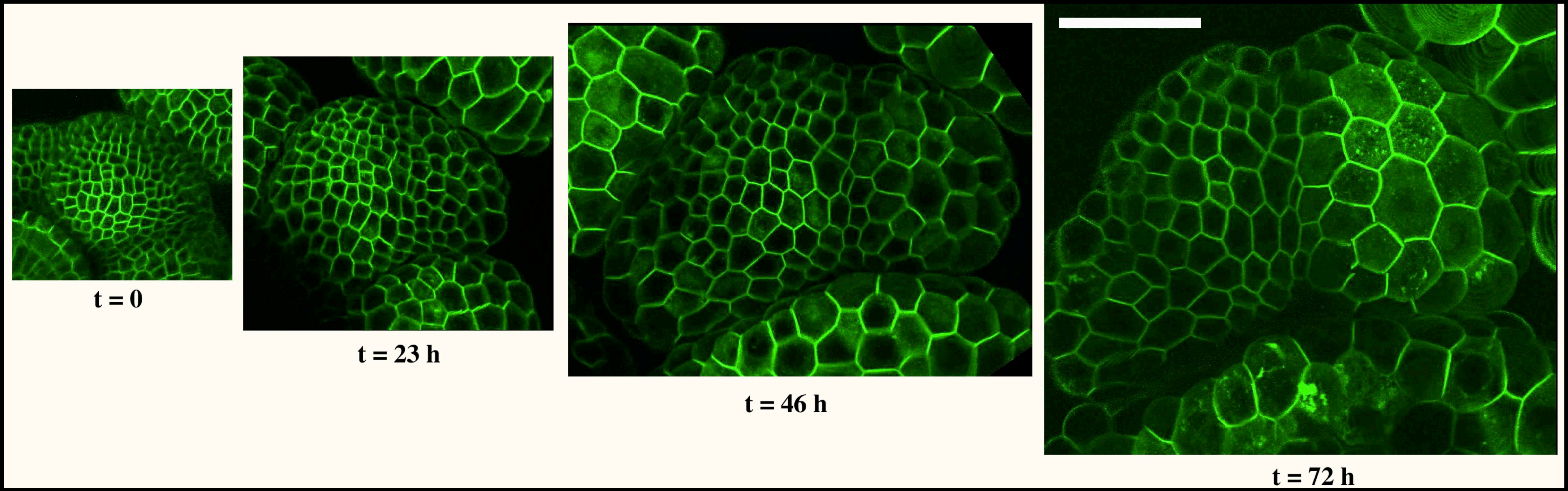
No cortical microtubules

Isotropic growth

NPA + oryzalin

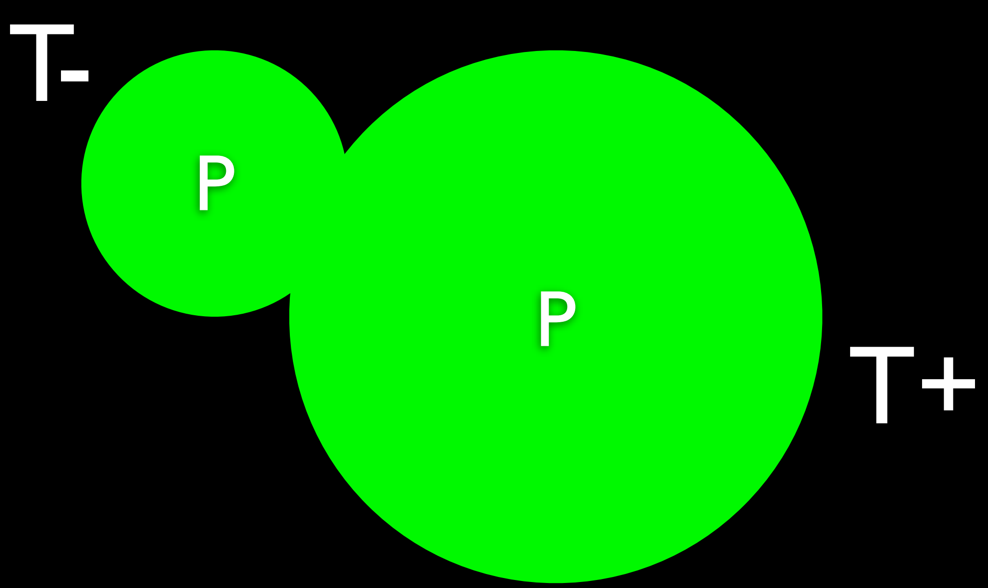


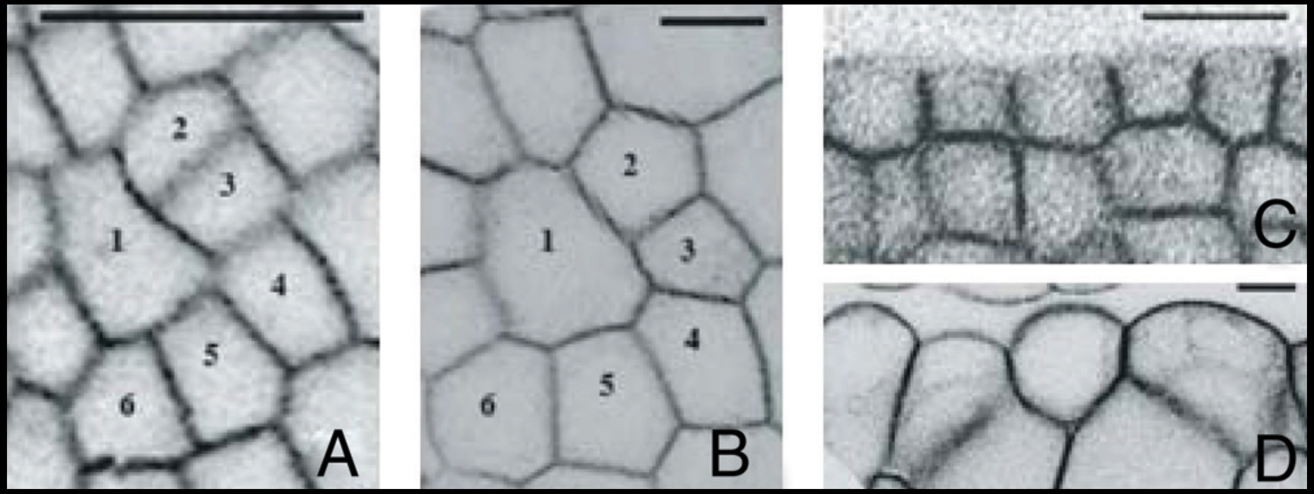
Oryzalin

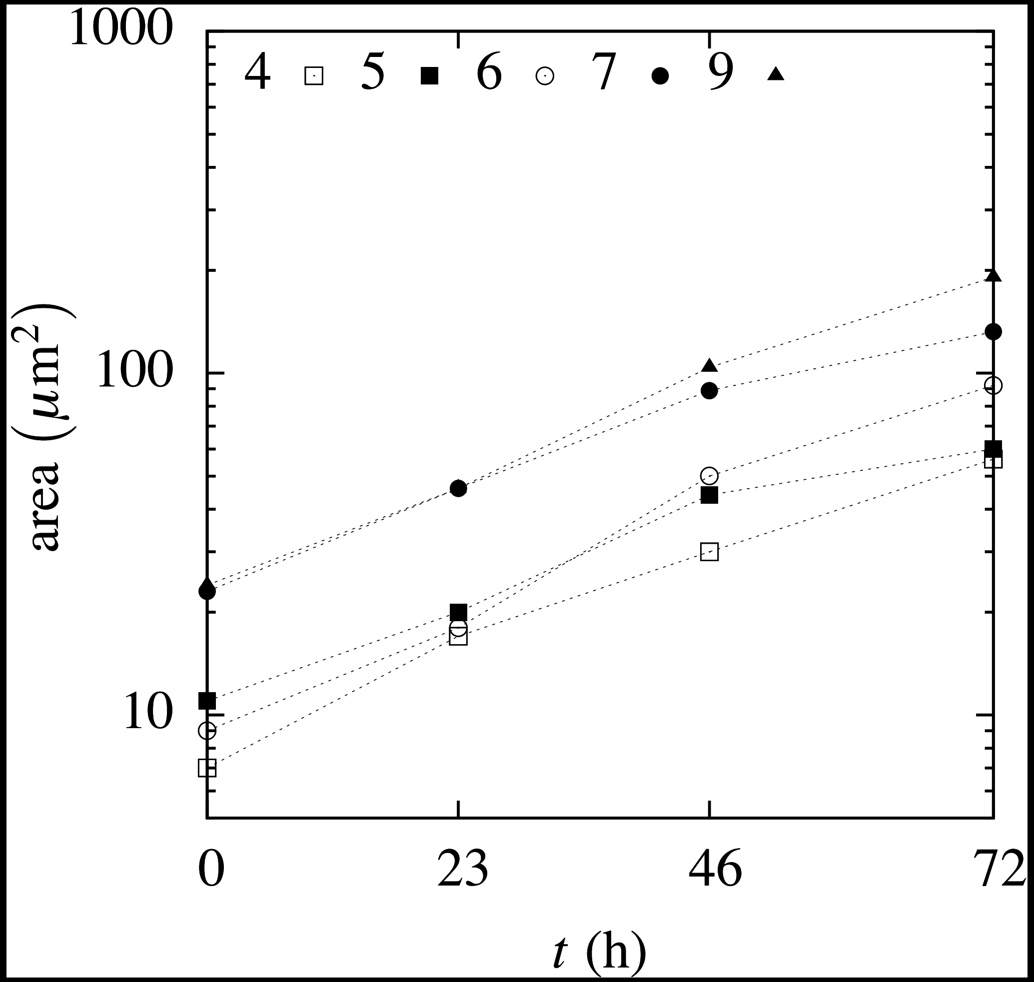


Growth homogeneity in Arabidopsis

Larger growth rate in big cells?





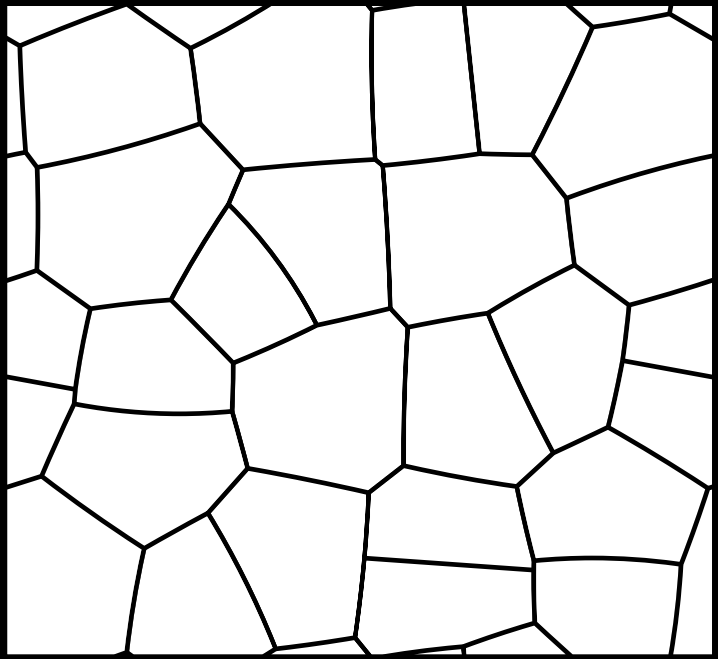


Suggests pressure differences between cells

Growth homogeneity in Arabidopsis

Model:

- Two dimensions
- Cell based
- Viscoelastic cell walls
- Growth driven by turgor
- Turgor pressure is regulated in each cell through osmolite contents



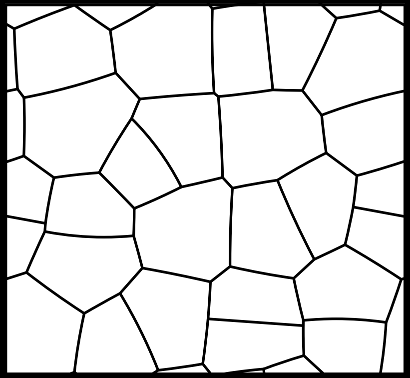
$$T_i = \mu h \left(\frac{l_i}{l_i^0} - 1 \right) = \frac{\nu h}{l_i^0} \frac{dl_i^0}{dt}$$

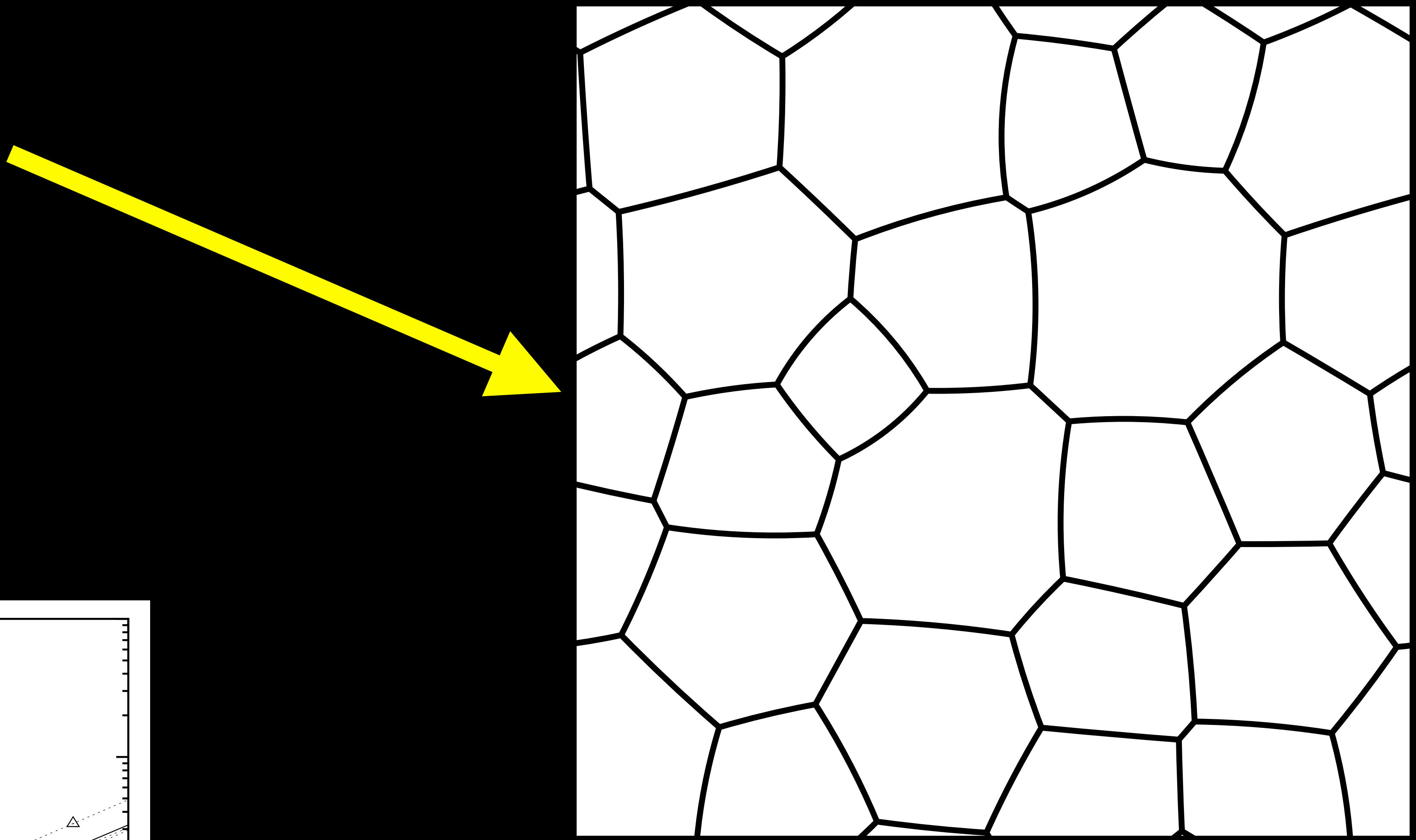
$$\frac{\mathrm{d}n}{\mathrm{d}t} = \frac{P(S)S - n}{\tau}$$

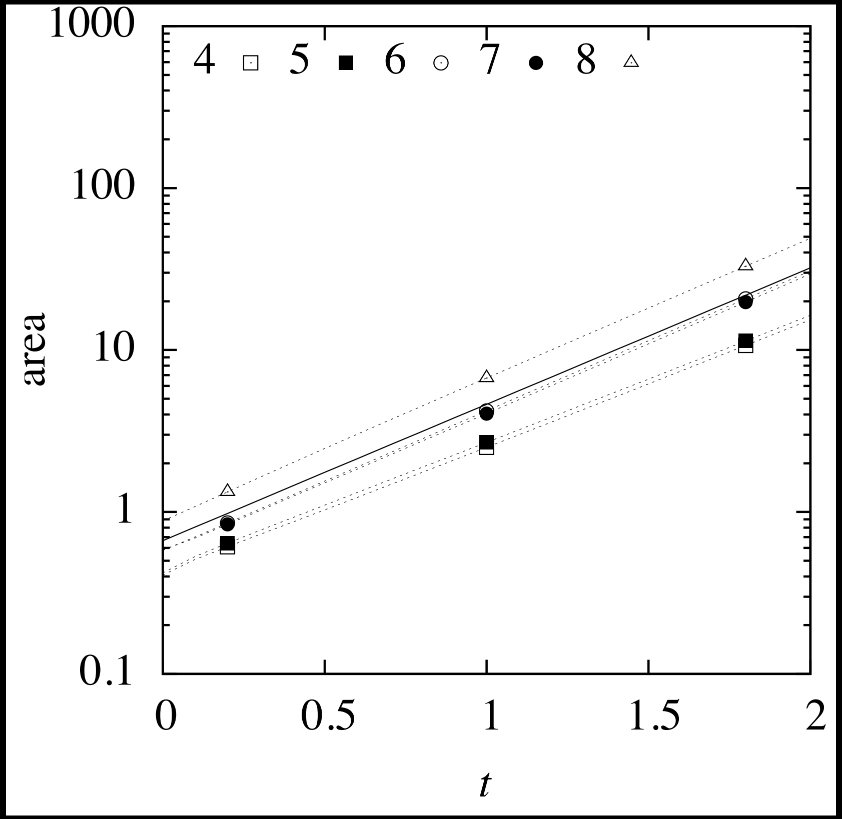
$$\kappa_i = \frac{\delta P}{T_i}$$

$$P(S) = \nu h S^{-1/2}$$

Growth homogeneity in Arabidopsis







+ retrieve experimental distributions of angles

Growth homogeneity in Arabidopsis

Suggest turgor regulation to maintain homogeneity

Now with microtubules?

Olivier HAMANT

Marcus HEISLER
now EMBL

Henrik JONSSON
now Lund and Cambridge

Elliot MEYEROWITZ
Caltech

Yves COUDER
Paris Diderot

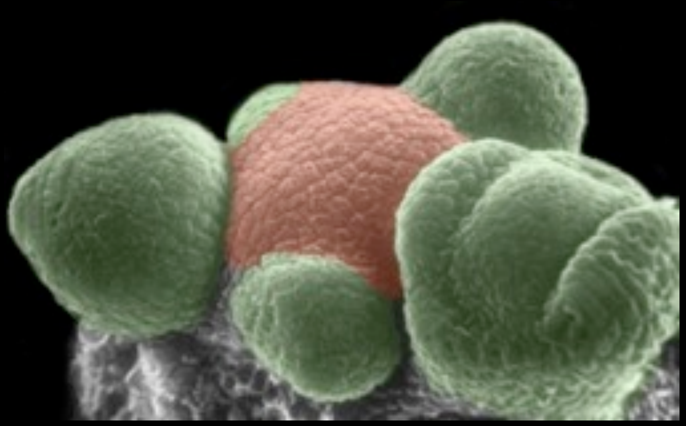
Jan TRAAS
Lyon

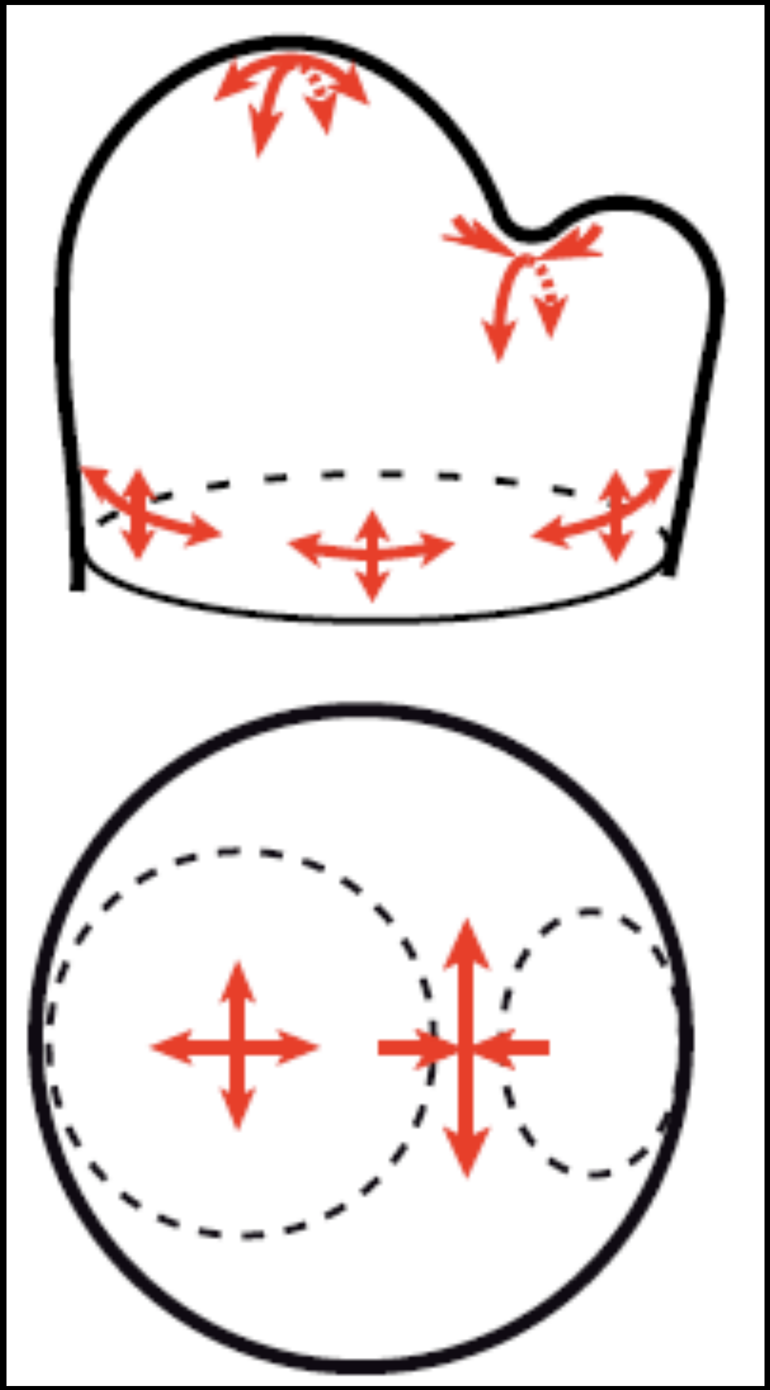
also Pawel KRUPINSKI, Magalie UYTTEWAAL, Plamen BOKOV, Francis CORSON, Patrik SAHLIN

O. Hamant et al. Science 2008

Growth homogeneity in Arabidopsis

Pattern of mechanical stress at the shoot apex

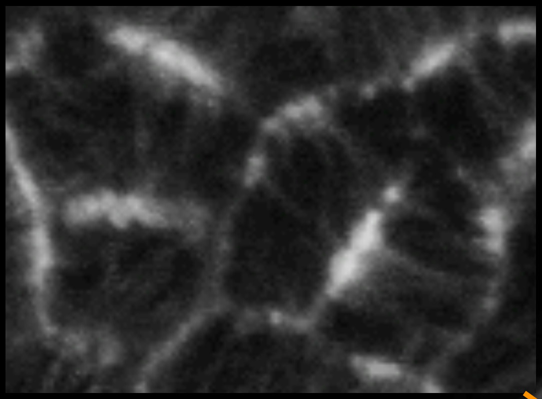


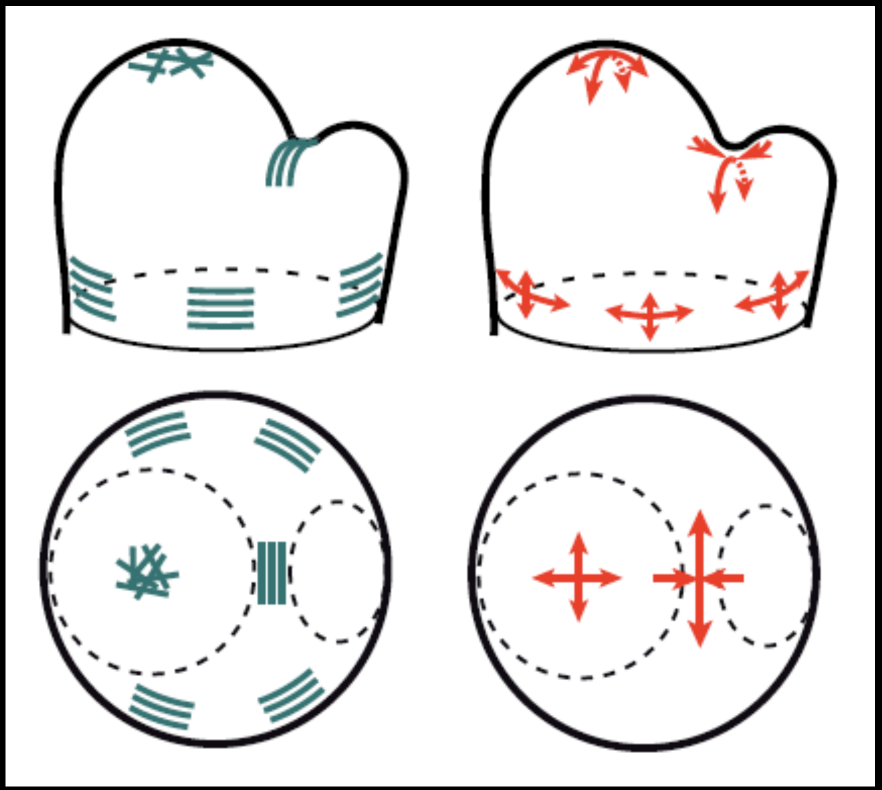


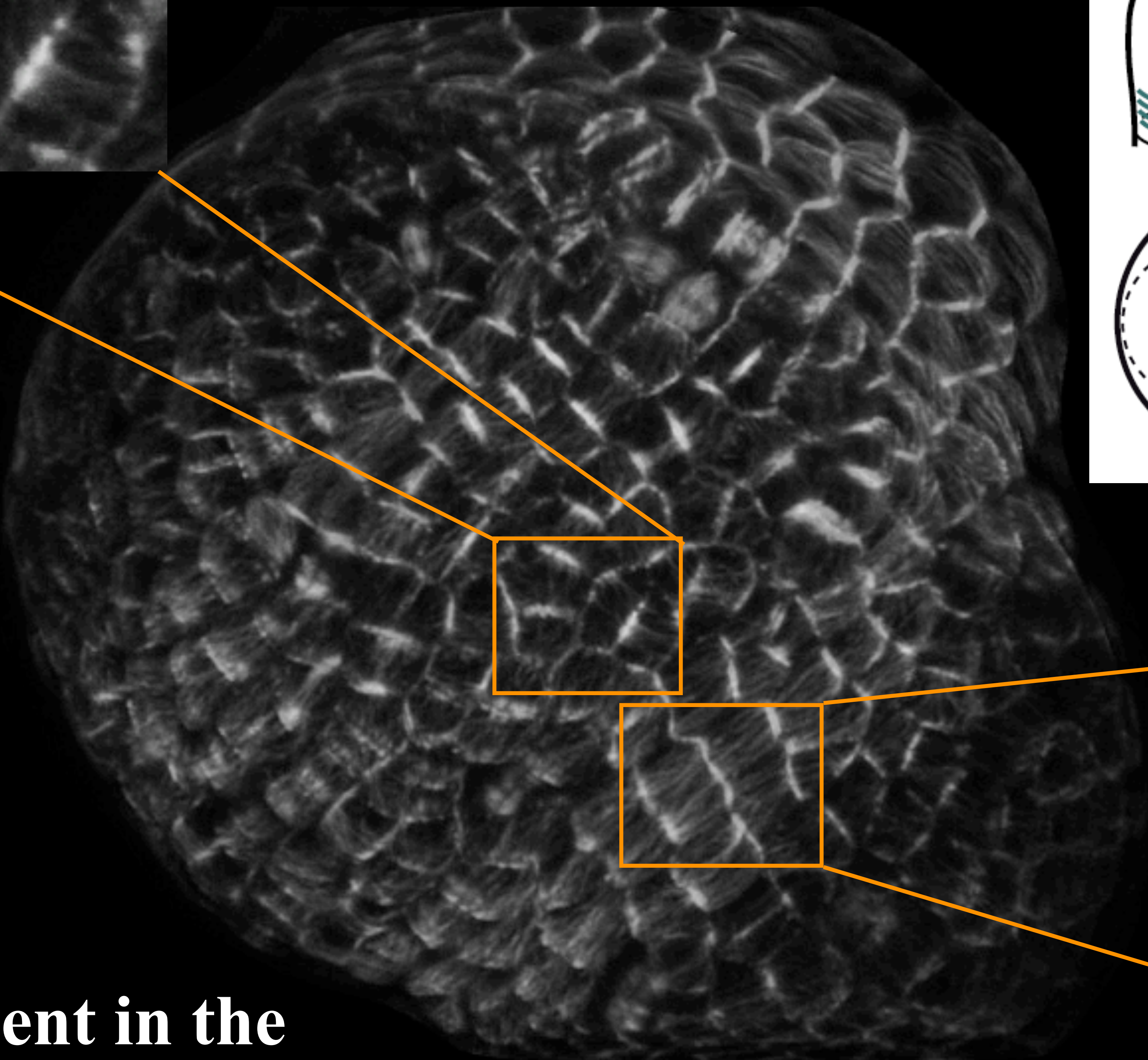
- A continuum mechanical model of the shoot apex
  - Much stiffer epidermis
  - Turgor

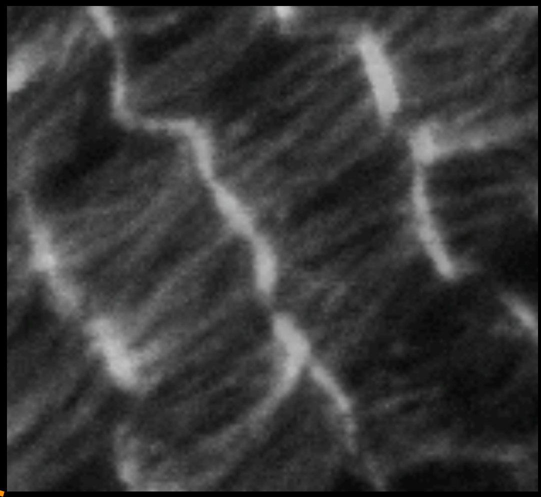
- Prediction of mechanical stress patterns
  - Link with growth?
  - with cellulose/microtubules?

Mechanical feedback



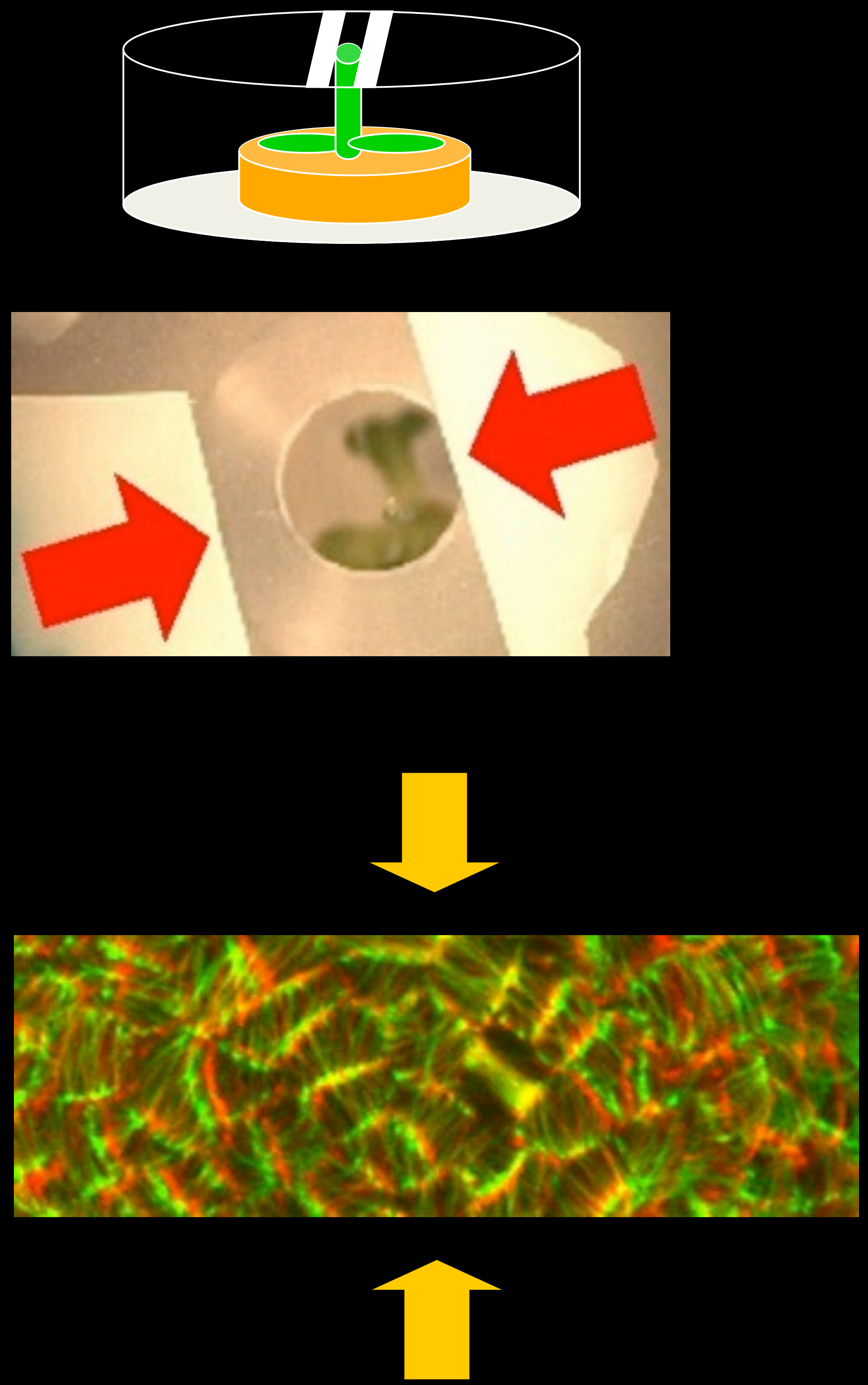




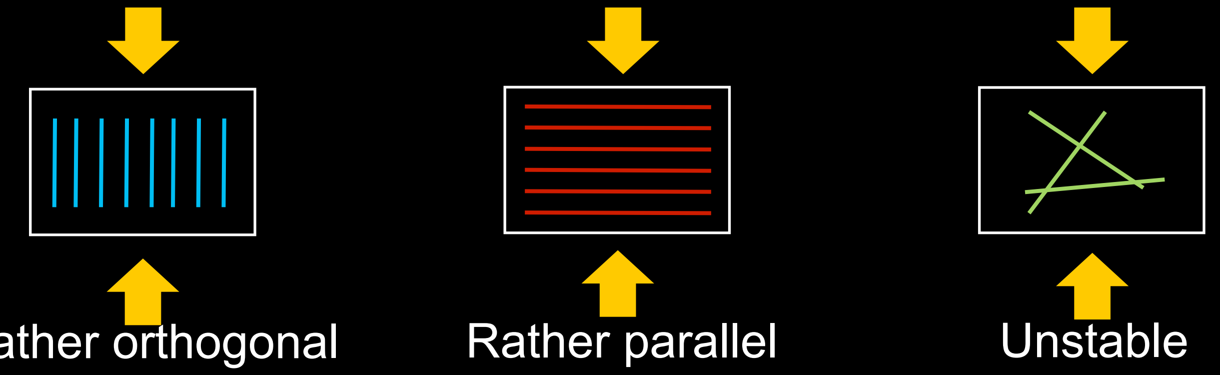


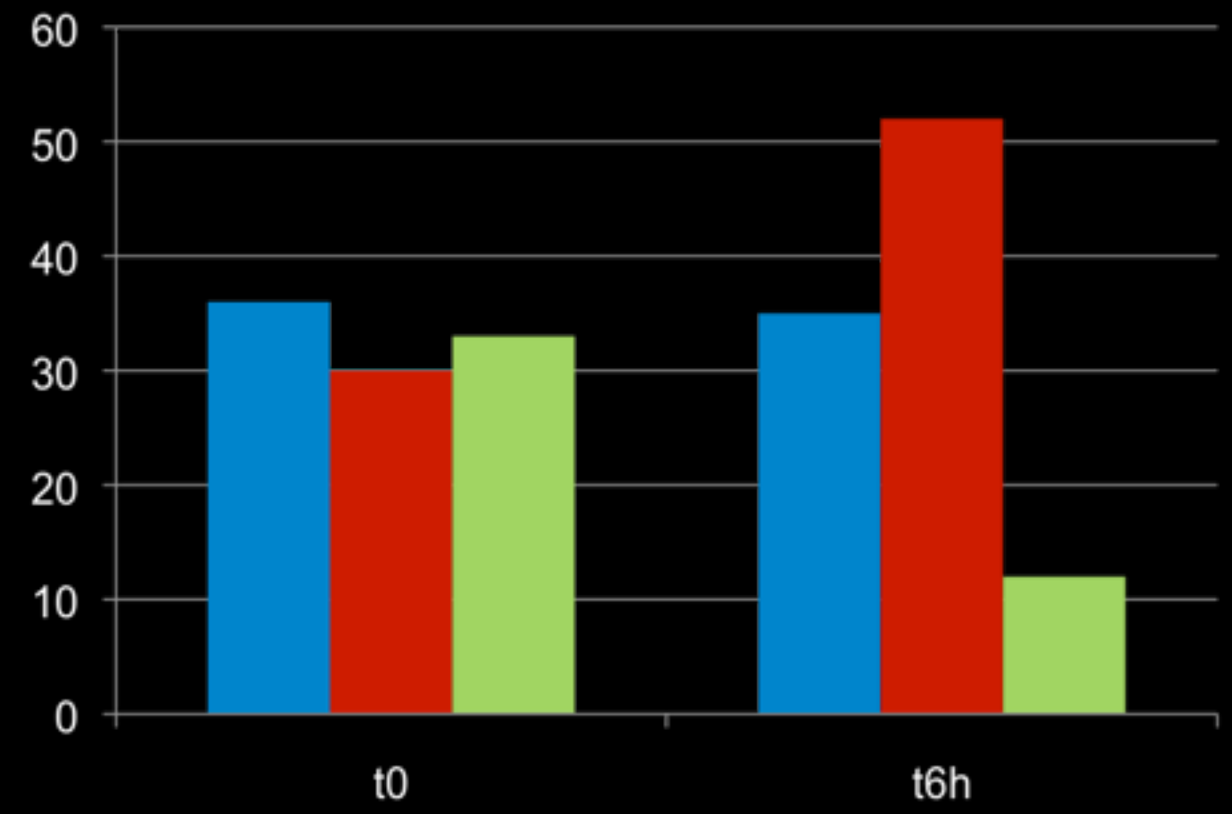
Alignment in the direction of maximal force

Mechanical feedback

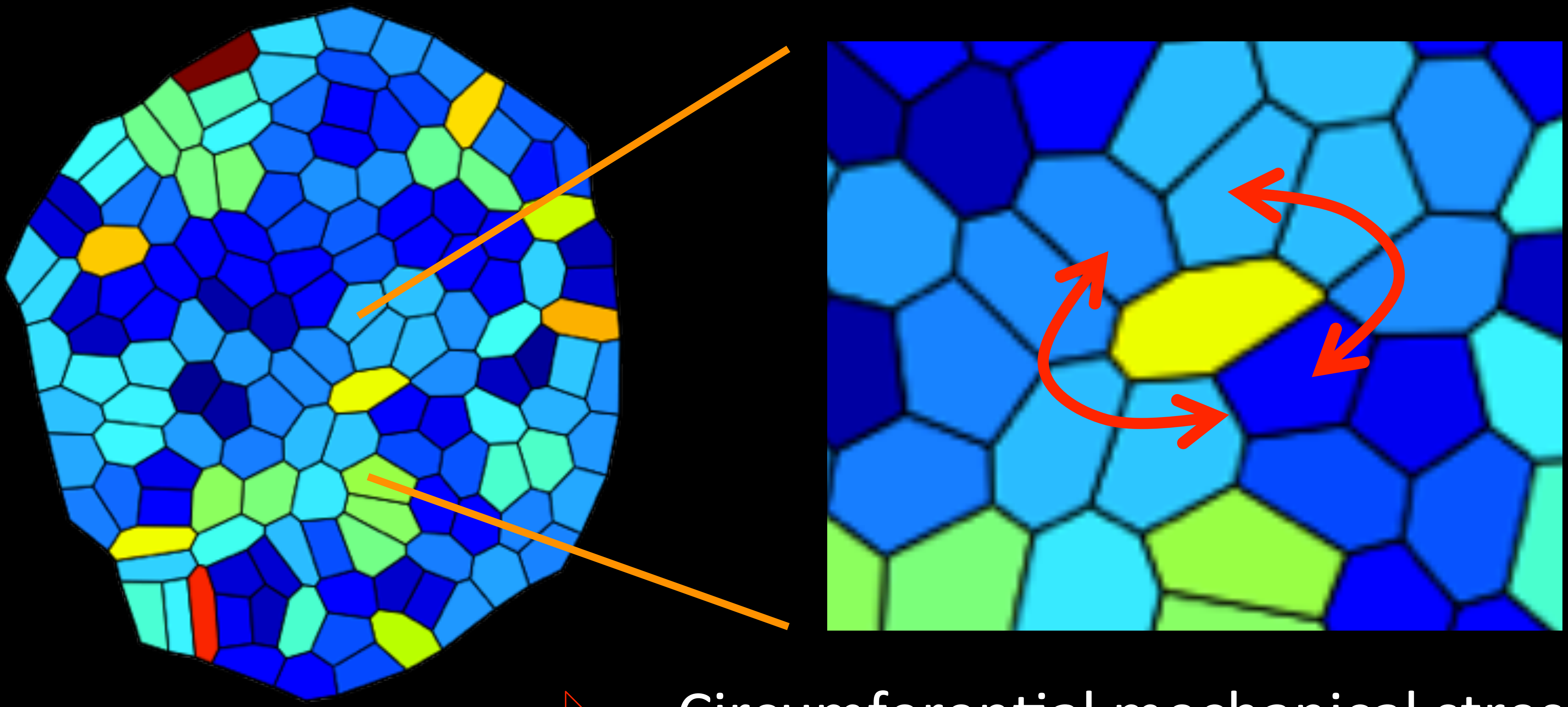


Unstable Microtubules seem to be preferentially recruited by mechanical stress





Growth homogeneity in Arabidopsis

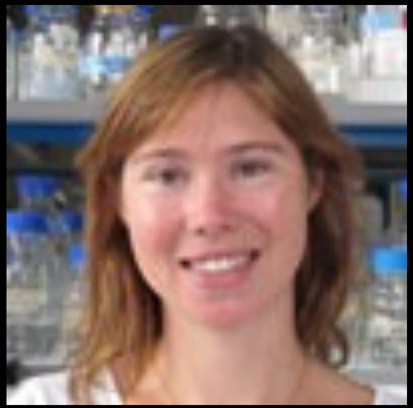


Circumferential mechanical stress around fast growing cells

Alignment in the direction of maximal stress
- Long term re-enforcement in that direction
- Reduction of growth heterogeneity?

Growth homogeneity in Arabidopsis

Suggest turgor regulation to maintain homogeneity
Now with microtubules?



Olivier HAMANT

Magalie UYTTEWAAL
now INRA Versailles

Karen ALIM
Harvard

Agata BURIAN
University of Silesia

Uyttewaal et al.
Cell 2012

Dorota KWIATKOWSKA
University of Silesia, Poland

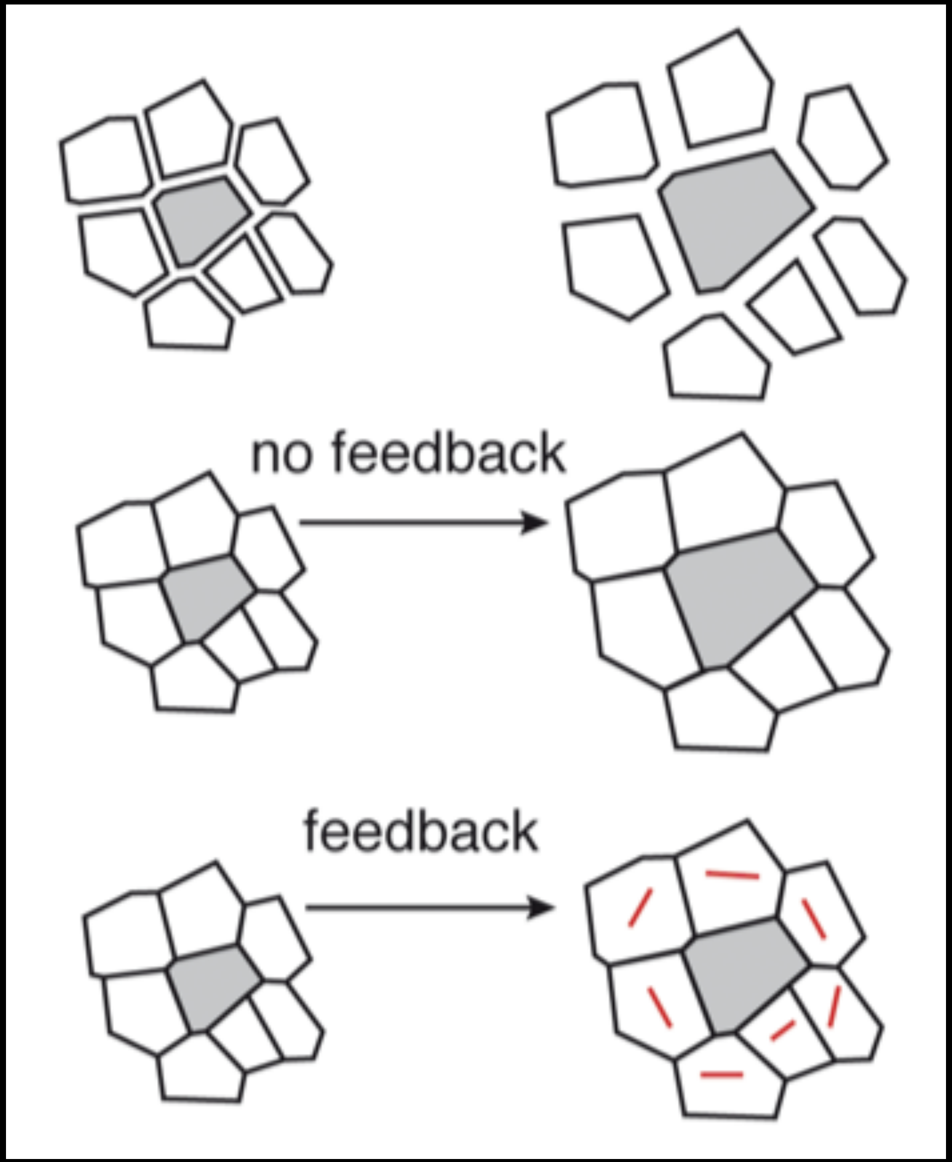
also Benoit LANDREIN, Dorota BOROVSKA-WYKRET, Annick DEDIEU, Alexis PEAUCELLE, Michal LUDYNIA, Jan TRAAS

Growth homogeneity in Arabidopsis

Does the microtubule response to stress homogenize growth?

Model: A link between mechanical forces and growth rate

Hypotheses:
i. A specified growth rate for each cell, noisy
ii. Mechanical feedback:
less growth in the direction of main stress



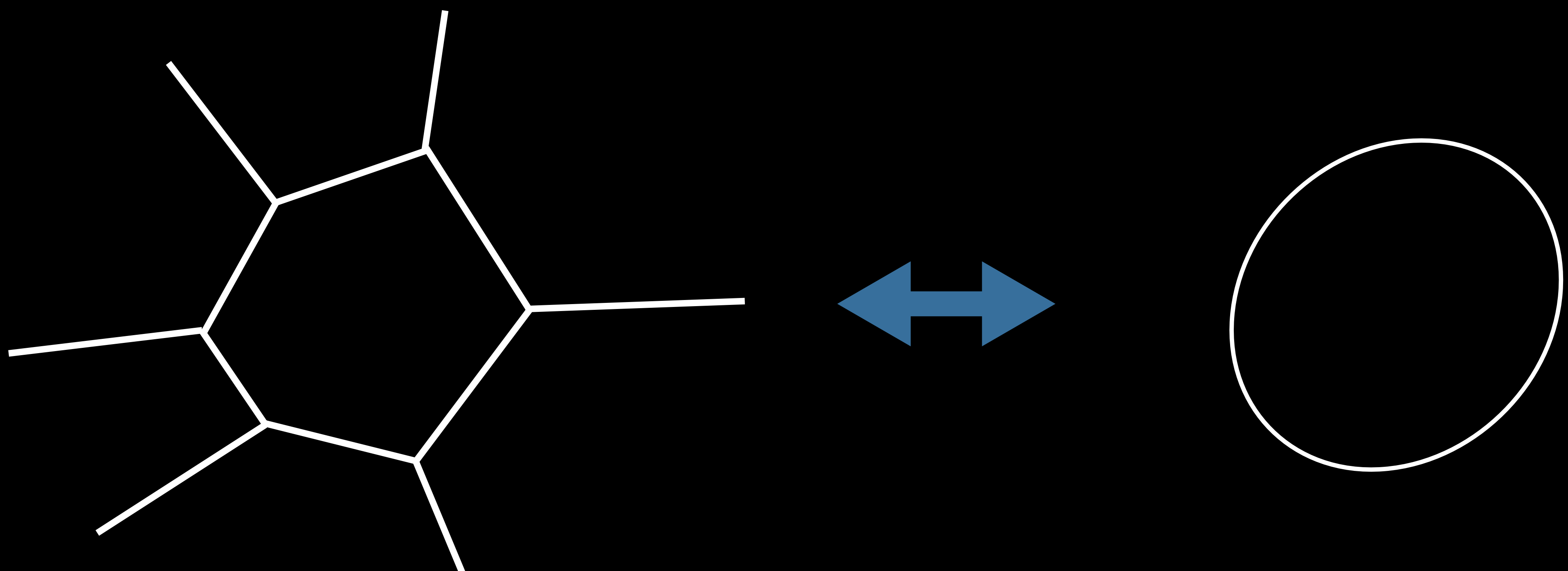
two important parameters:
noise level + feedback strength

Growth homogeneity in Arabidopsis

Generalization (anisotropy) of a model used for animal epithelia

Elastic energy
Quasi-static equilibrium
Mechanical stress computed from equilibrium state

$$\mathcal{E}(\mathbf{r_m}) = \Sigma_i ||M_i - M_i^{(0)}||^2$$



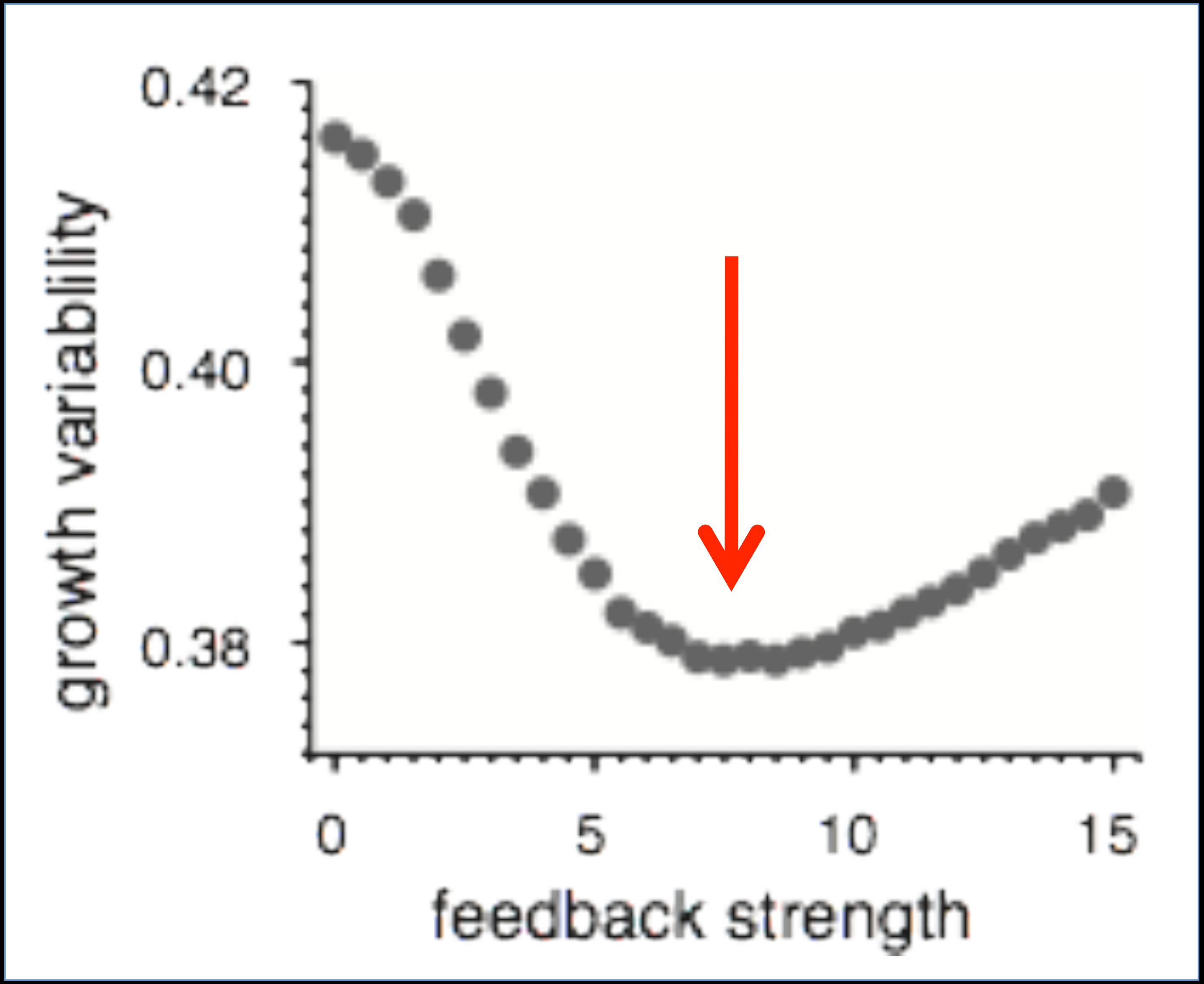
specified growth with noisy source + mechanical feedback

$$\frac{d}{dt} M_i^{(0)} = \gamma_0 M_i^{(0)} - \gamma_1 \frac{M_i^{(0)} D_i + D_i M_i^{(0)}}{2}$$

two important parameters: noise level + feedback strength

Growth homogeneity in Arabidopsis

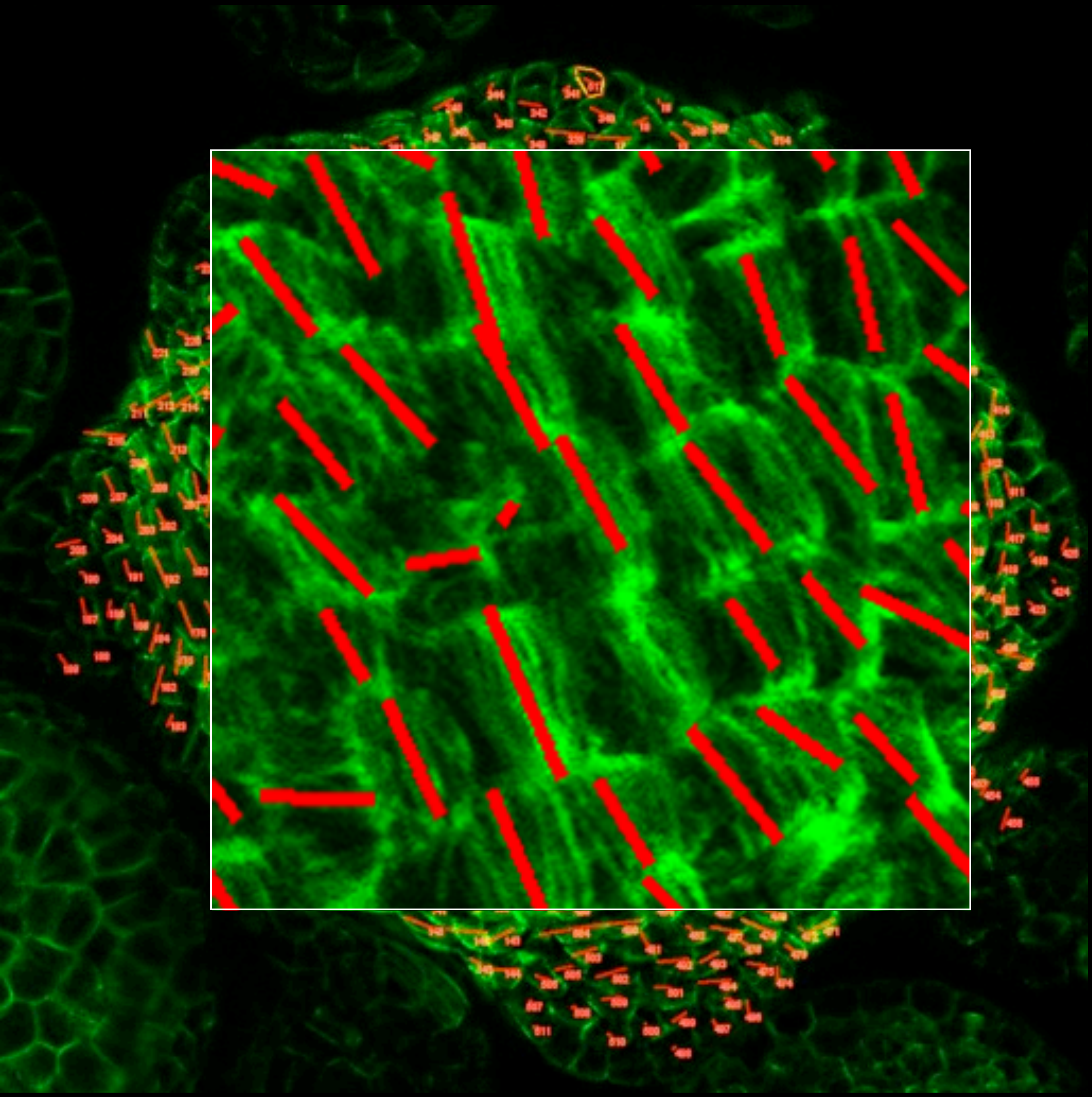
Optimum of growth homeostasis?



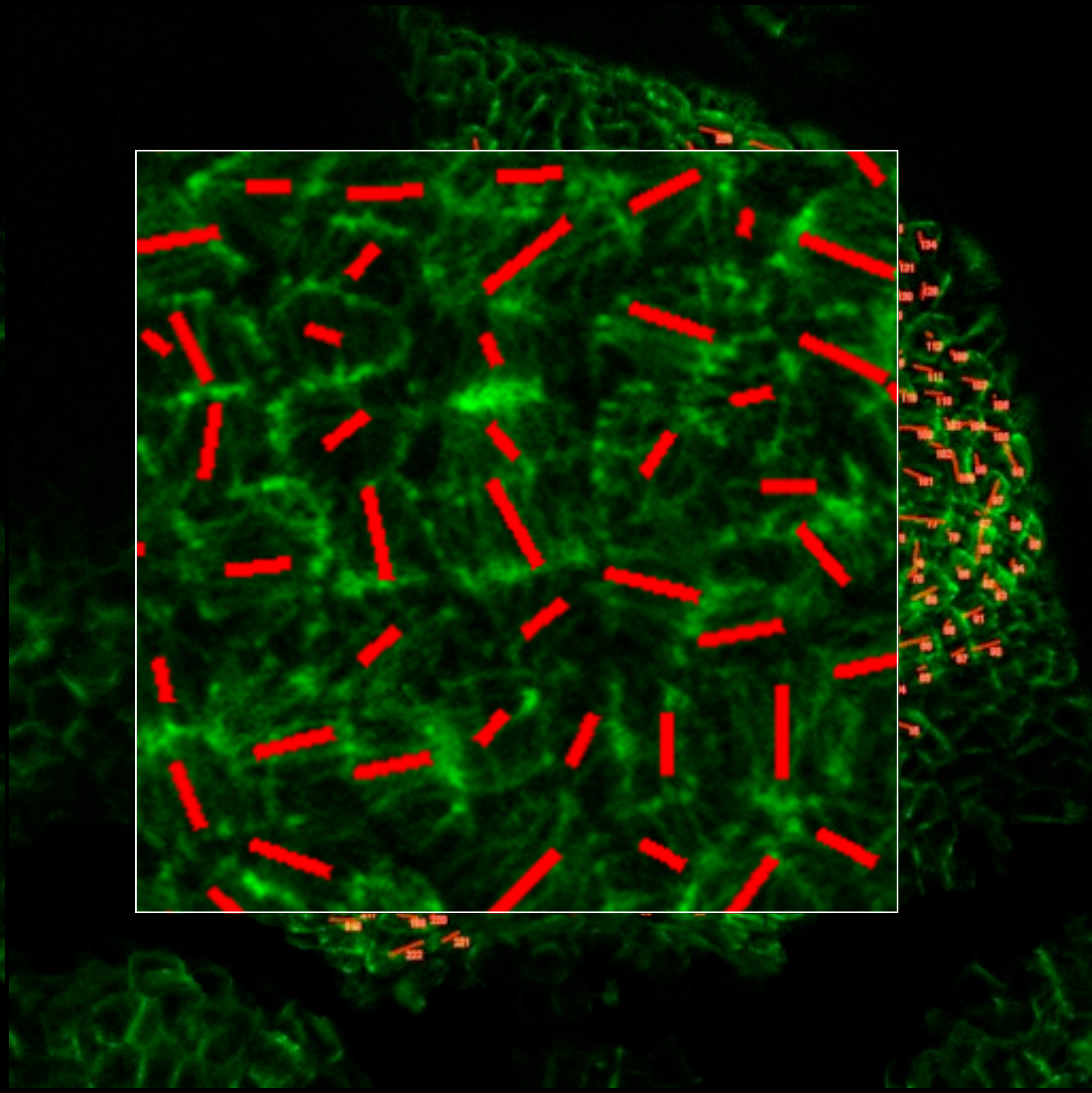
Test: a mutant with a decreased reponse to mechanical stress

Growth homogeneity in Arabidopsis

atktn1 = Katanin mutant

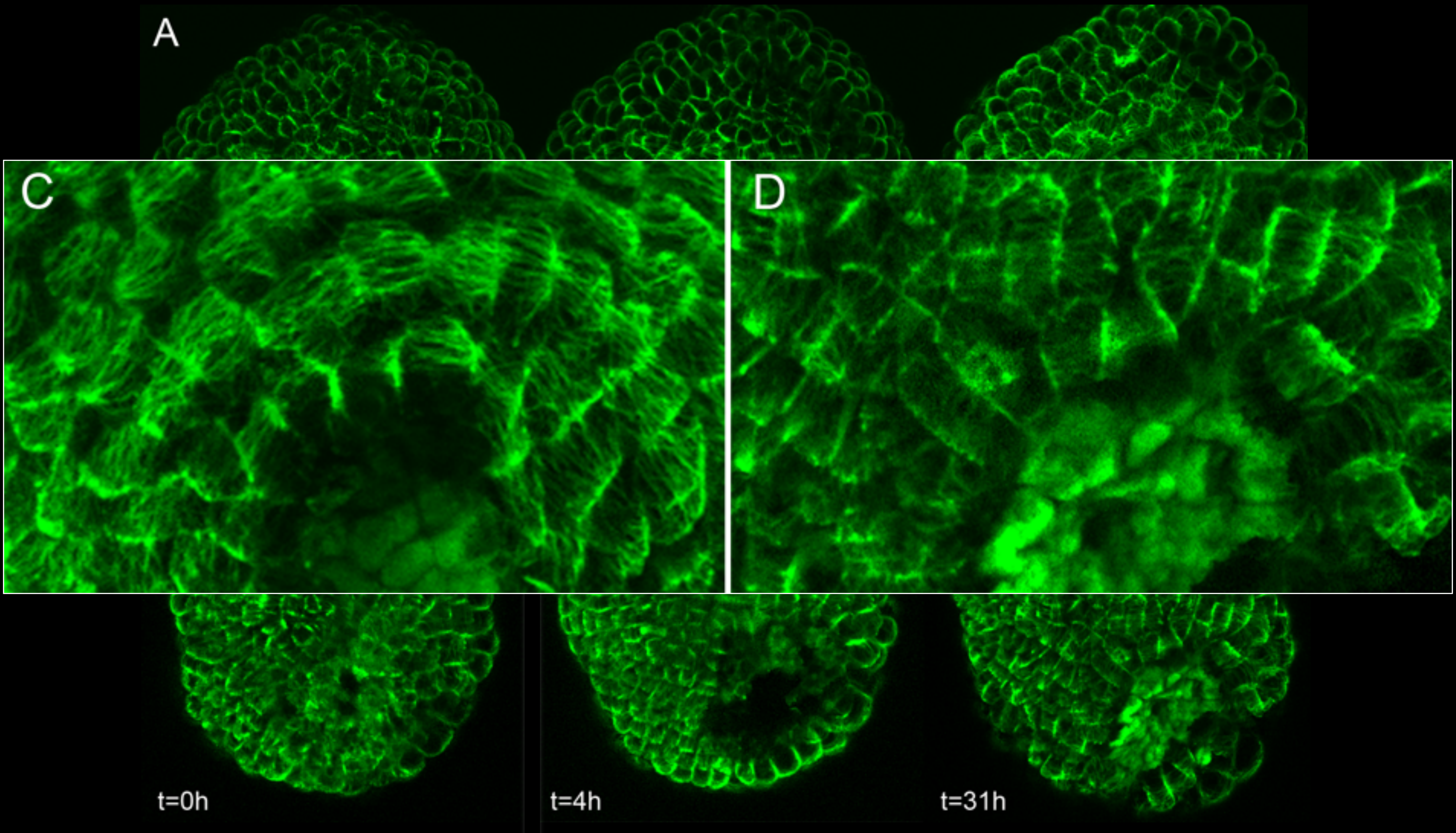


GFP-MBD



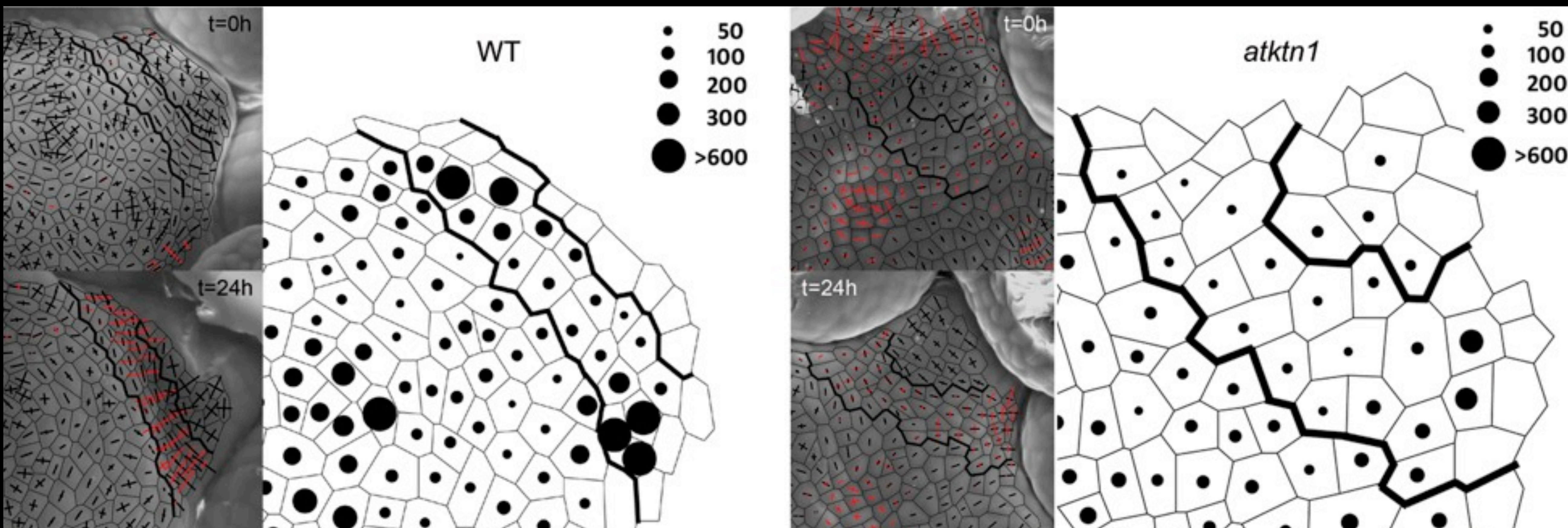
atktn1 GFP-MBD

Growth homogeneity in Arabidopsis



Weaker response to mechanical forces in *atktn1*

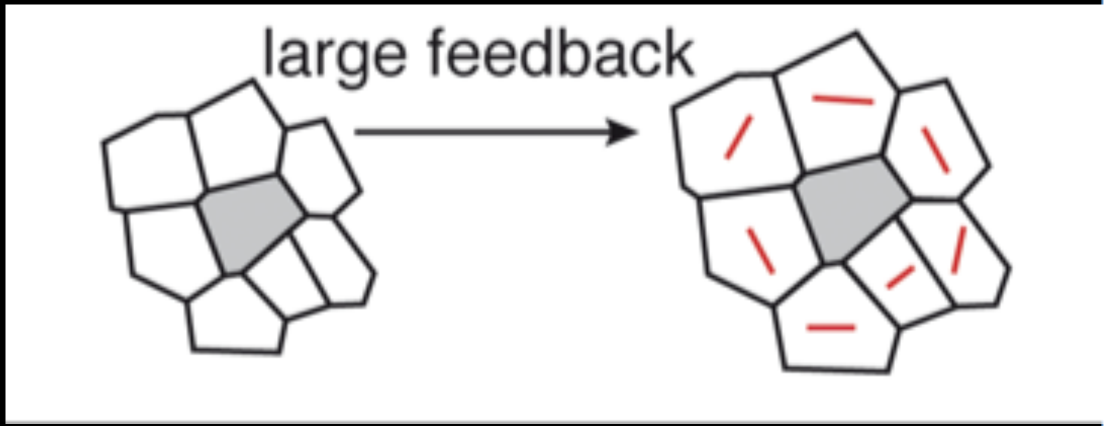
Growth heterogeneity in Arabidopsis

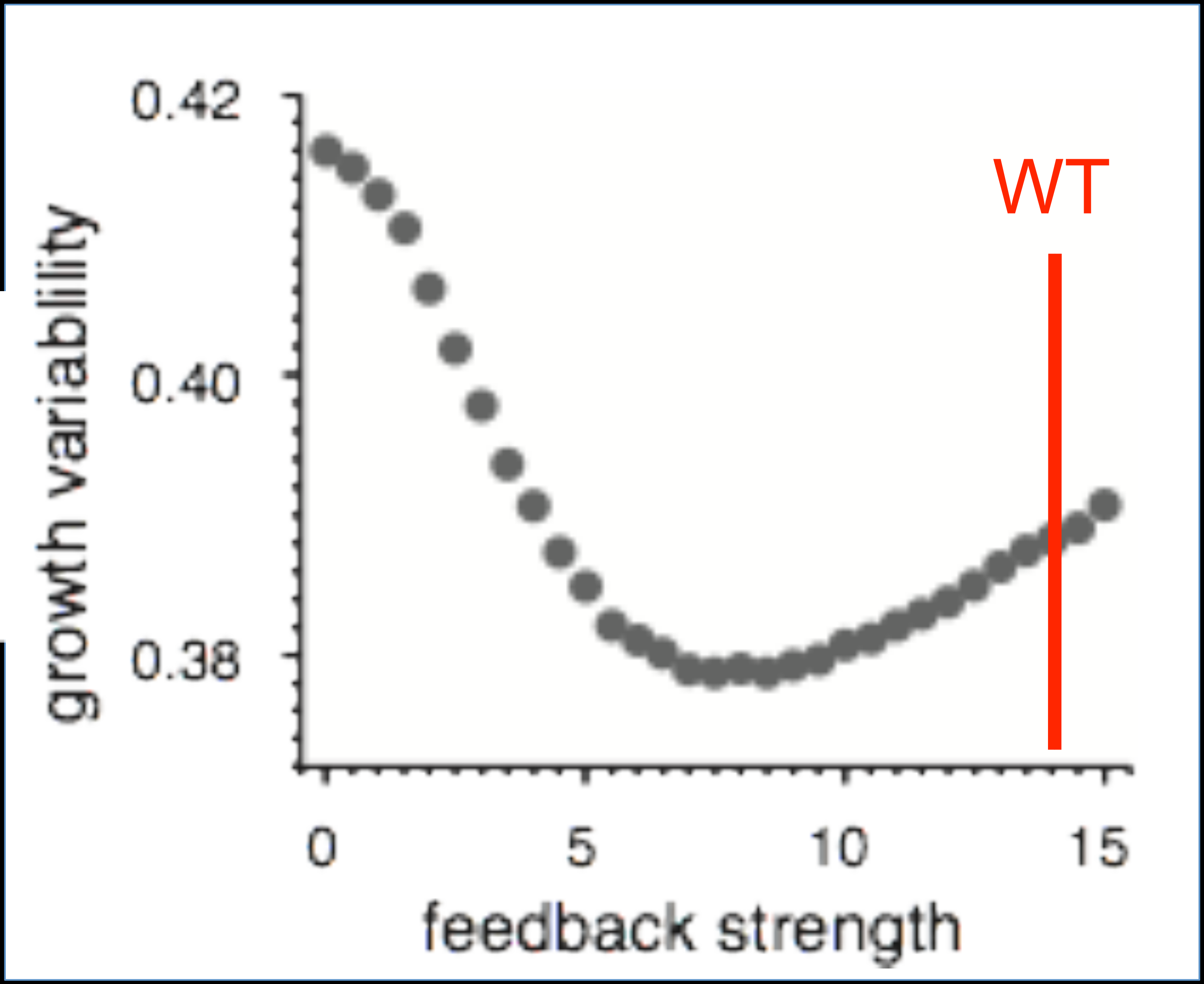


Heterogeneous growth Homogeneous growth

Growth homogeneity in Arabidopsis

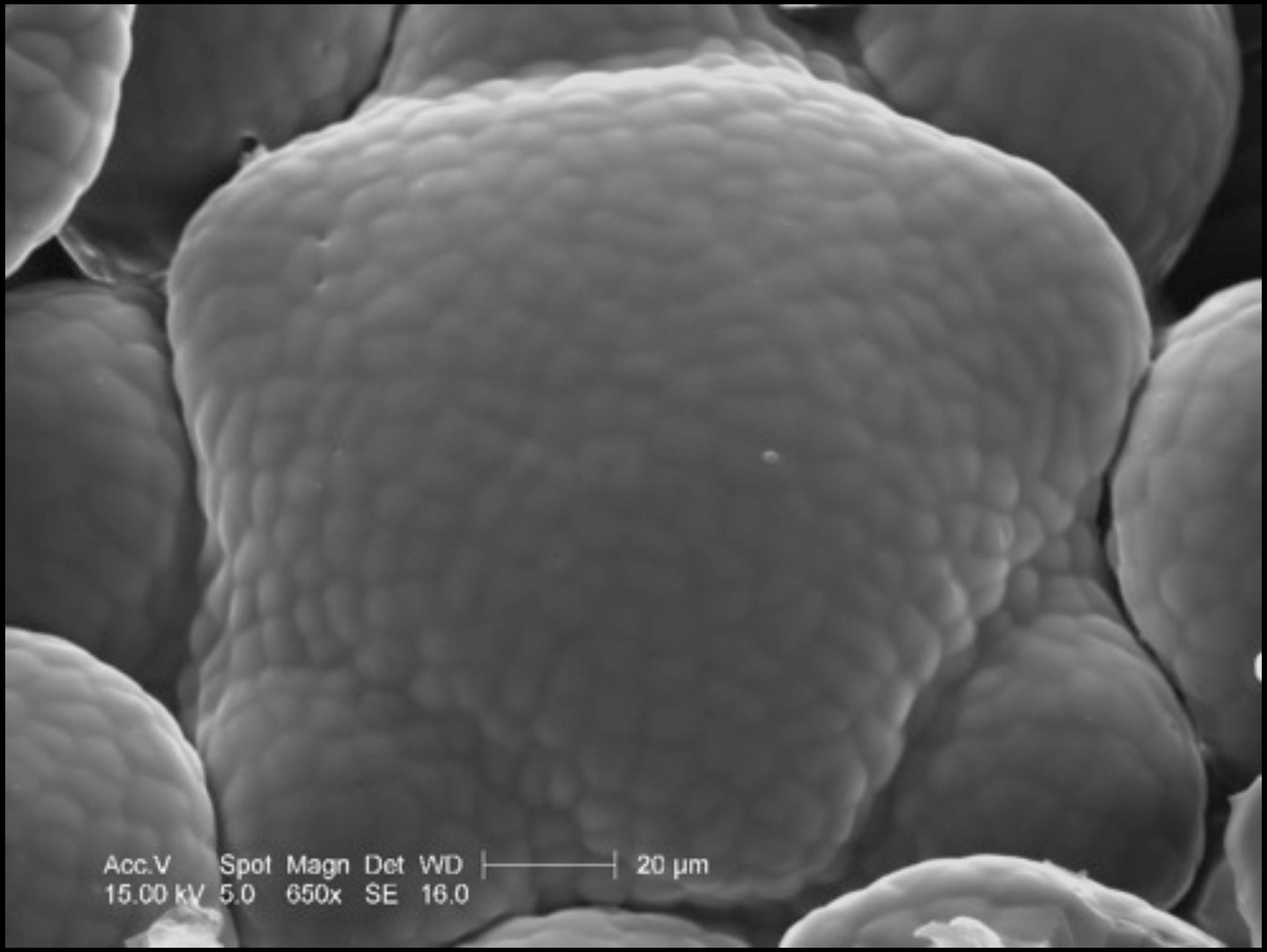
Mechanical stress can increase growth heterogeneity



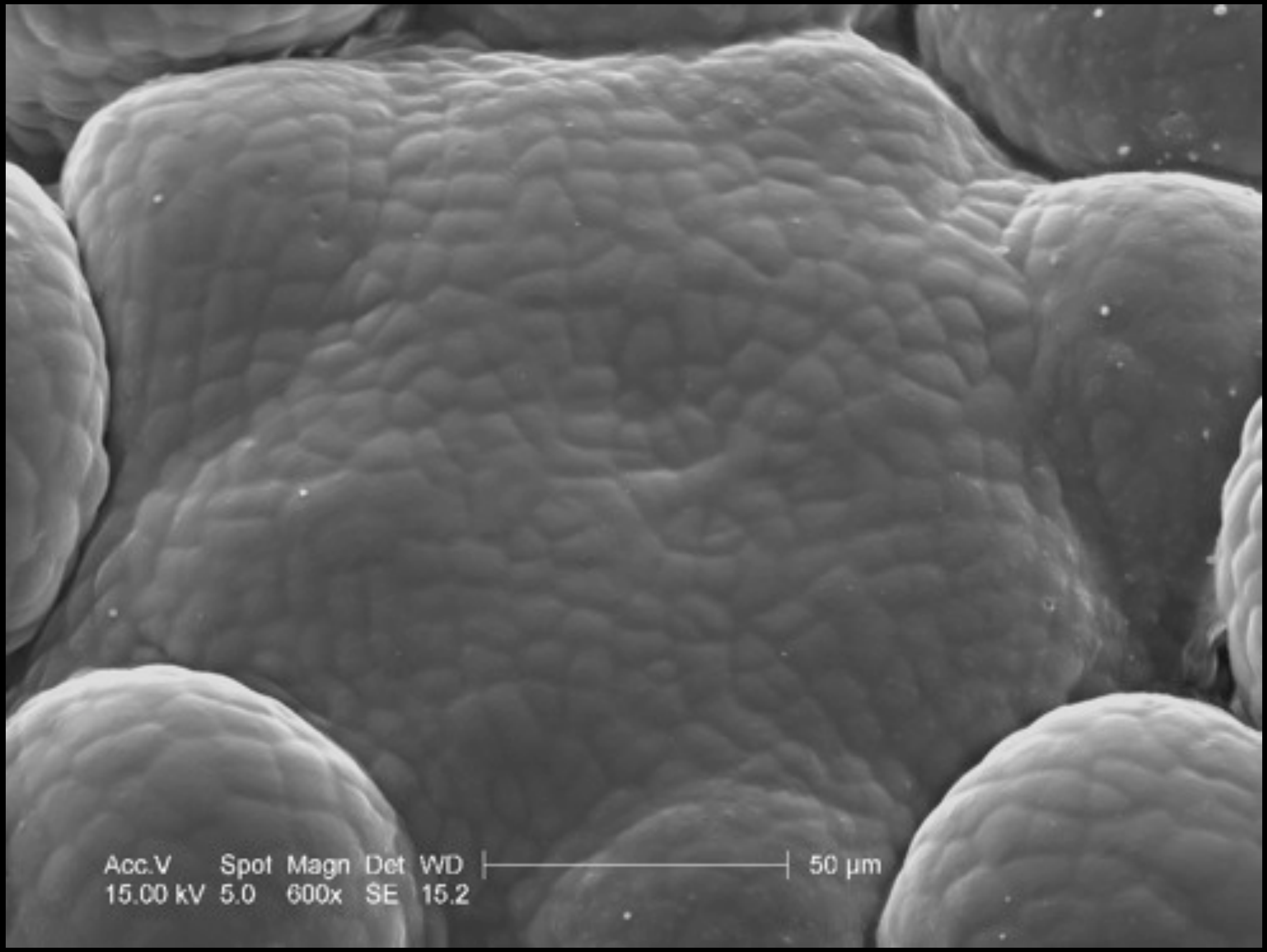


Growth heterogeneity

The shape of the SAM is altered in *atktn1*



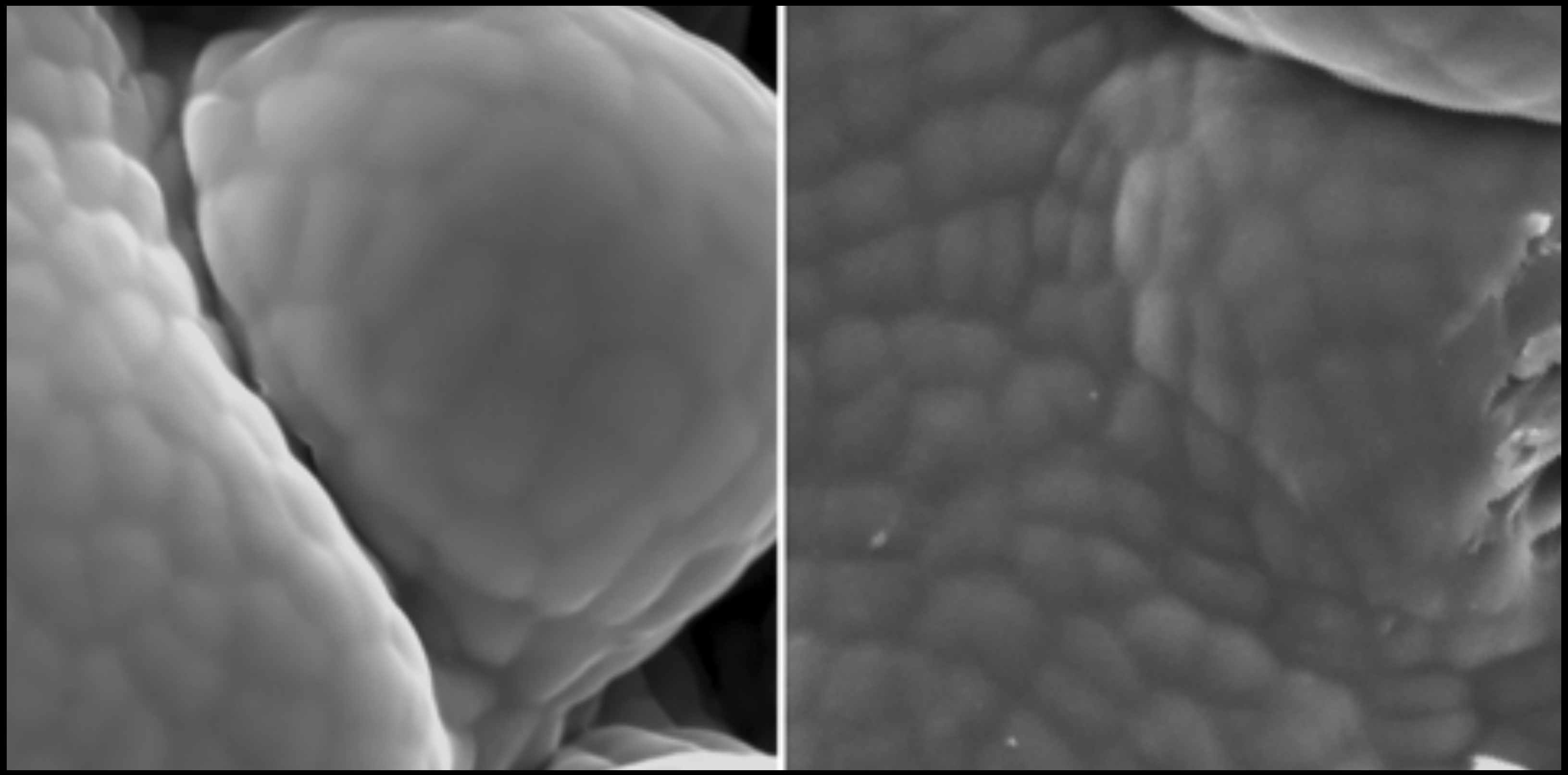
WT



atktn1

Growth heterogeneity

Organs of comparable rank



WT *atktn1*

Over-reaction to forces => organ emergence

Consequences on architecture?

Conclusions

- Morphogenesis in walled cells
  - Regulation of cell wall and turgor
  - Links with cell identity?
- Mechanical feedbacks
  - Stabilising and destabilising!
- Questions:
  - Role of variability

AND

Acknowledgements

Biophysics and Development

Maryam Aliee
Léna Beauzamy
Arezki Boudaoud
Aurélie Chauvet
Sam Collaudin
Pradeep Das
Mathilde Dumond
Olivier Hamant
Nathan Hervieux
Jean-Daniel Julien
Annamaria Kiss
Benoit Landrein
Jonathan Legrand
Marion Louveaux
Pascale Milani
Vincent Mirabet
Naomi Nakayama

SUPPORTED BY ANR
RhôneAlpes Région
erc
ENS DE LYON
INRA
cnrs
UNIVERSITÉ DE LYON

AND

The force side of plant morphogenesis