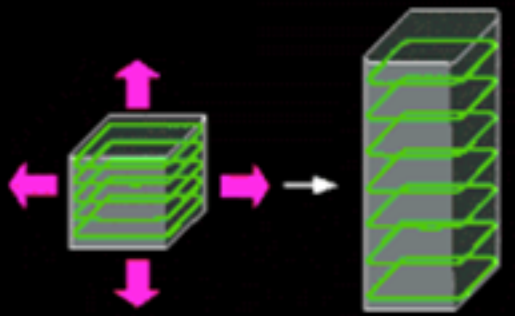
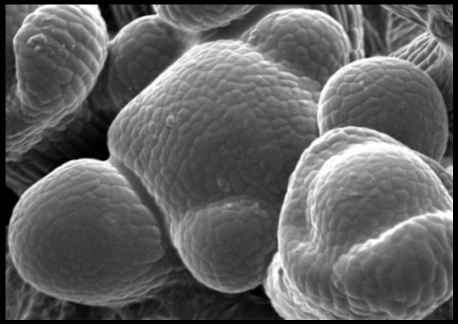
Part II. Mechanical signals in development

ICTS– April 2018

Olivier Hamant - Reproduction et Développement des Plantes, ENS Lyon, France

A mechanical feedback loop



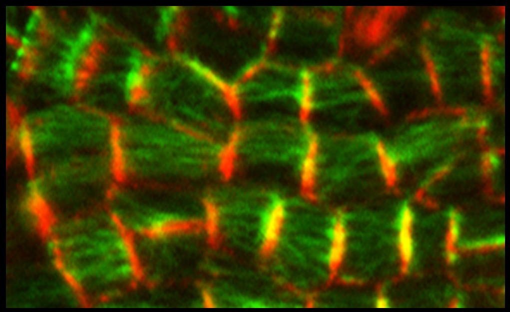


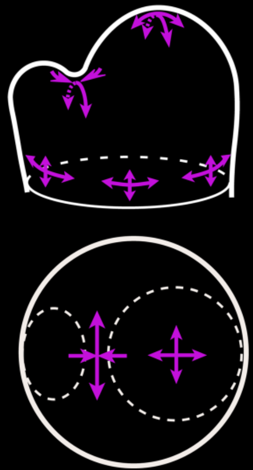
Direction
of cell growth

Shape

Microtubule
orientation

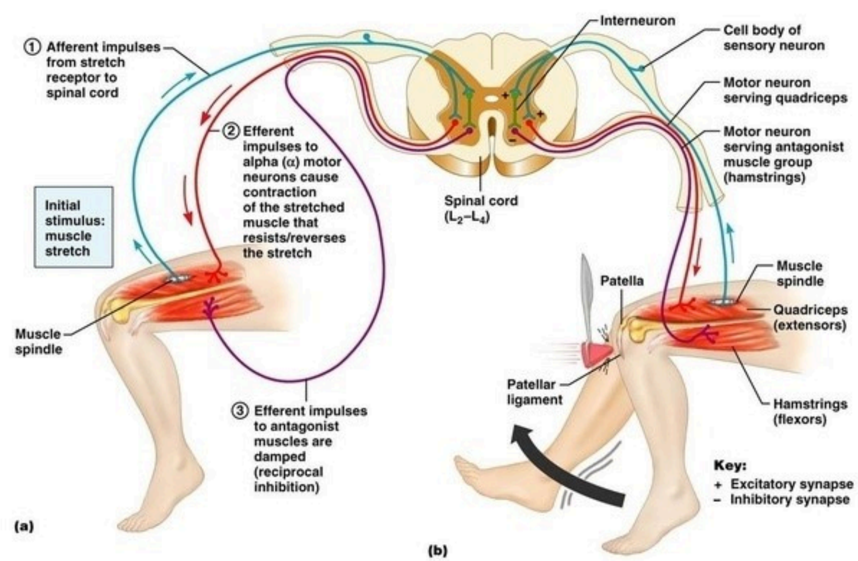
Direction
of mechanical stress



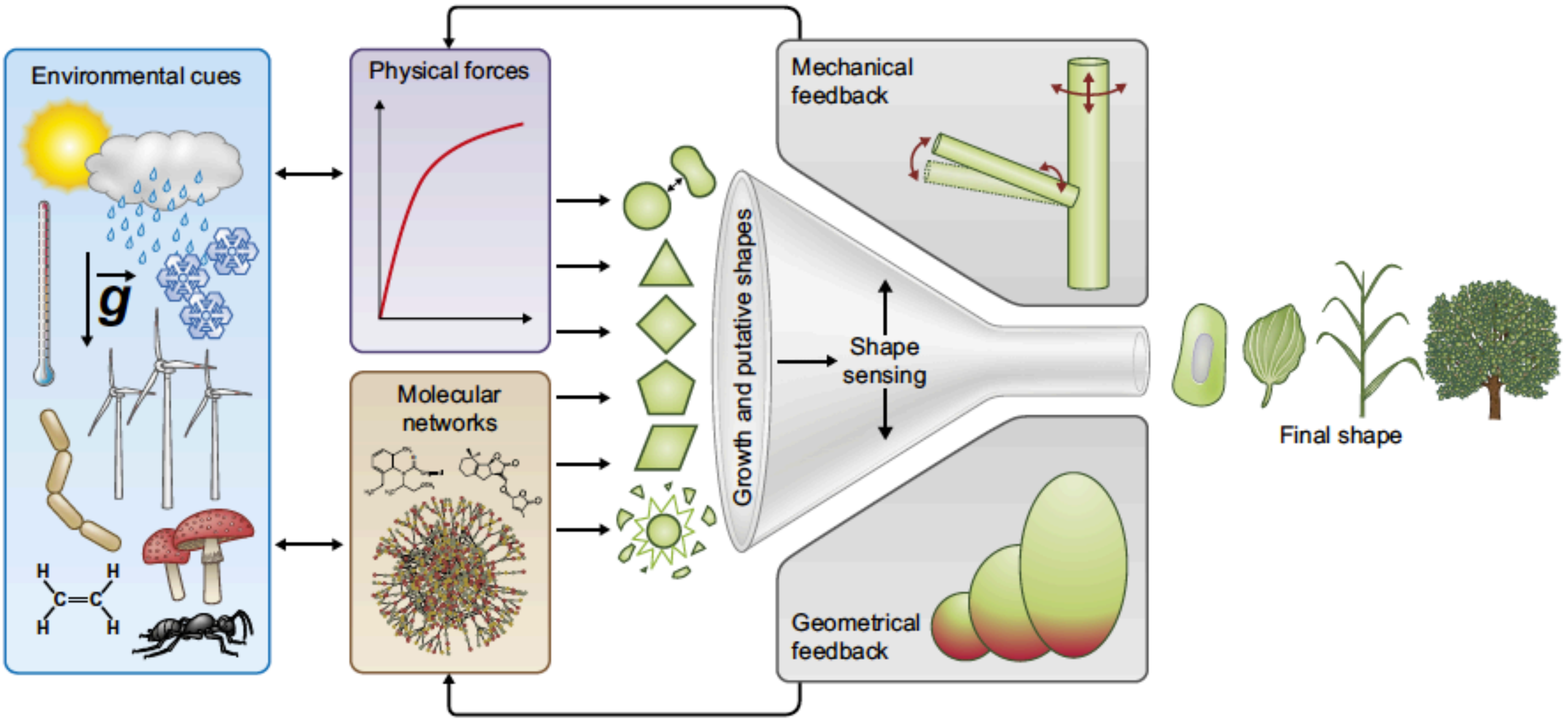


Green and King, 1966 Aust J Pl Sci.
Hejnowicz *et al.*, 2000 J Pl Growth Regul.
Hamant *et al.*, 2008 Science

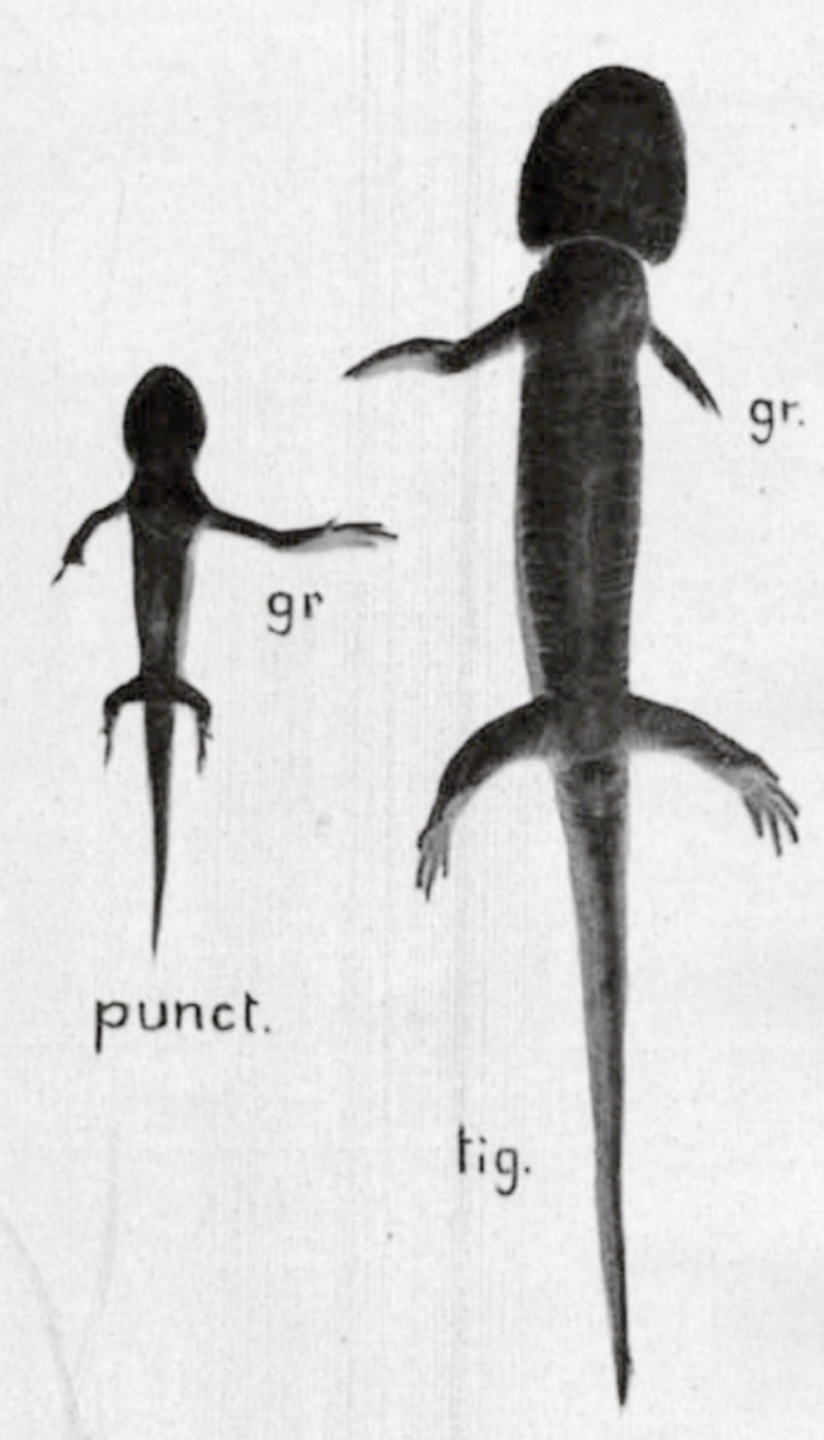
Proprioception



The « proprioceptive funnel » (in development)



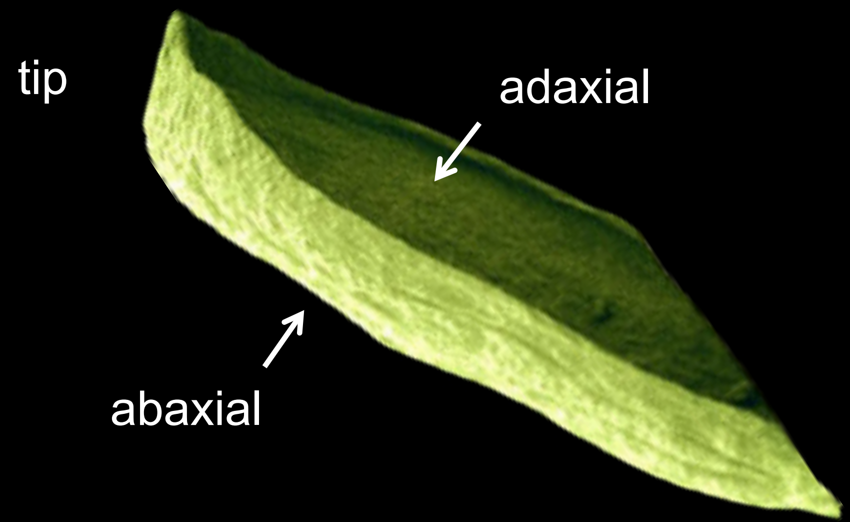
Hamant & Moulia, 2016 New Phytol.



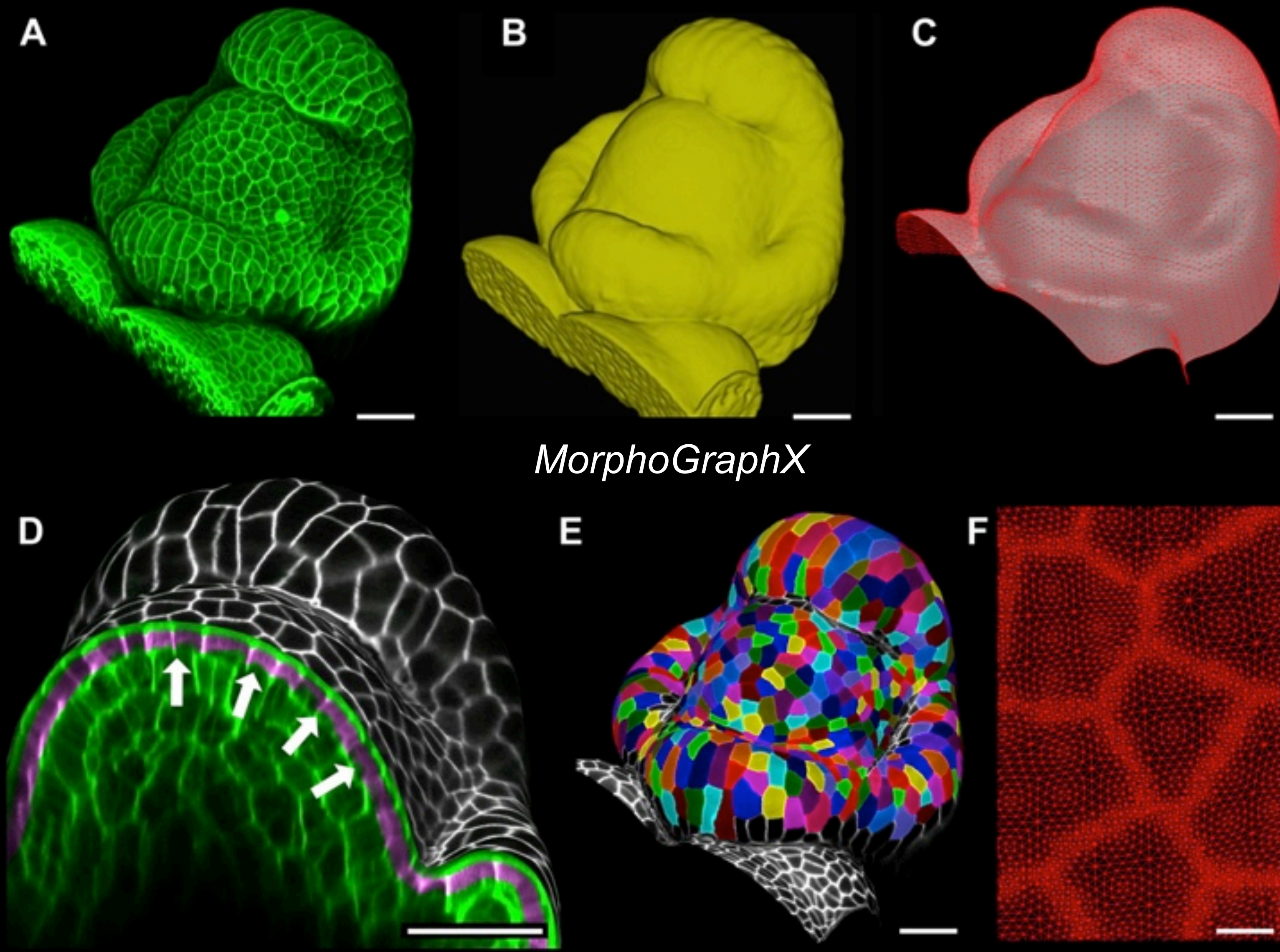
How do organs know when to stop growing?

Twitty and Schwind, 1931 J. Exp. Zool.

A contribution of mechanical signals in shaping organs?

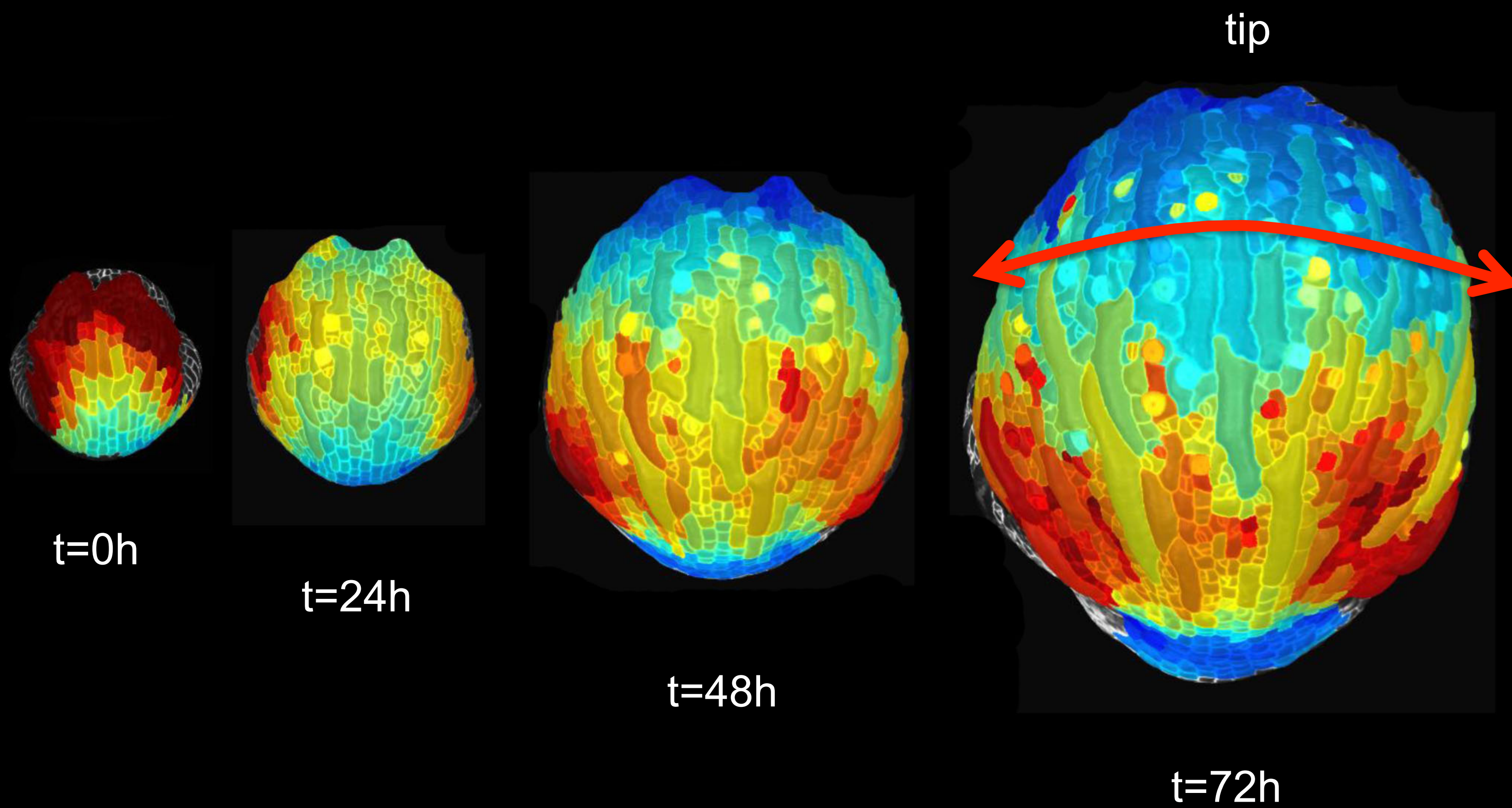


Measuring multicellular growth patterns



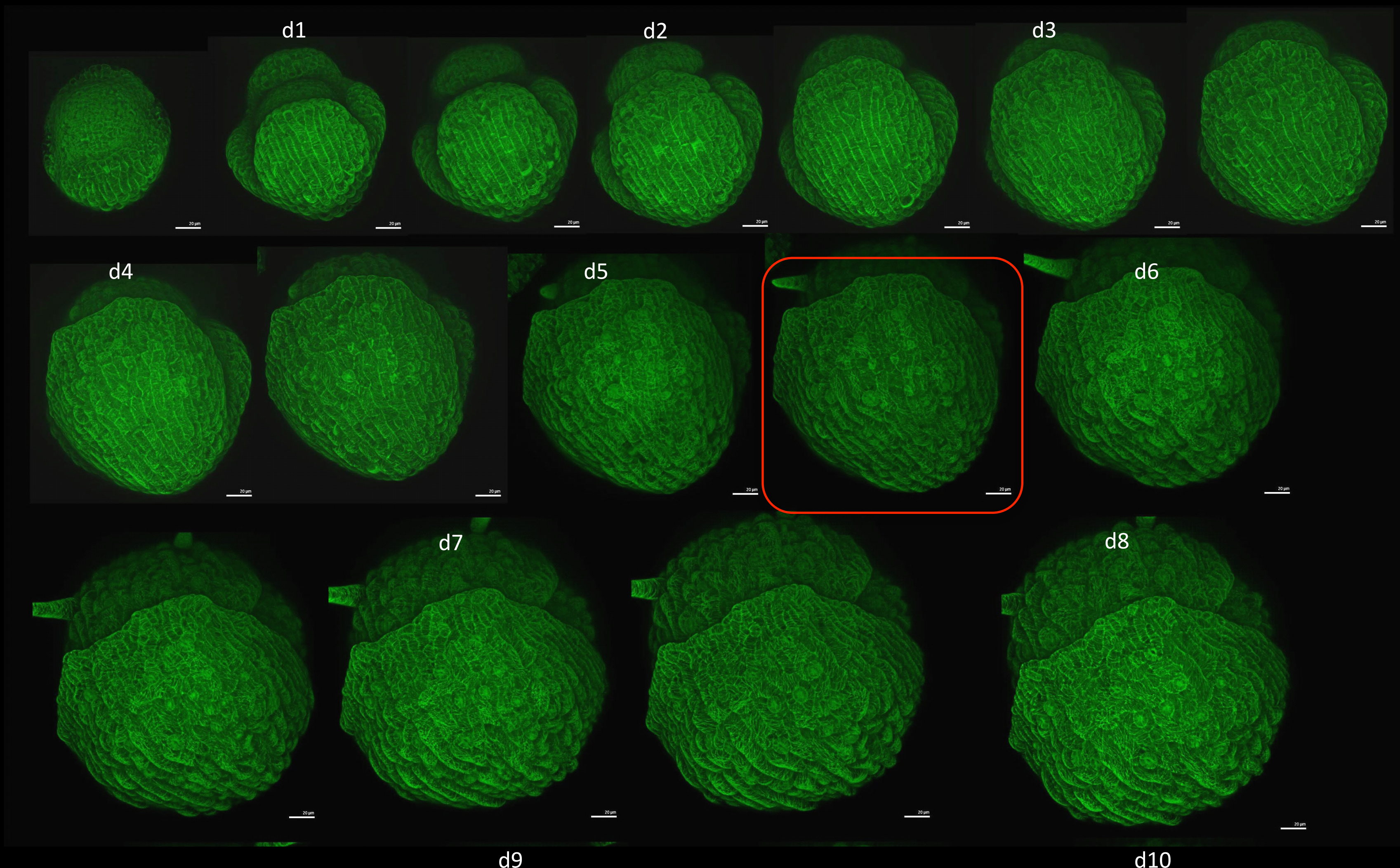
De Reuille *et al.,* 2015 eLife

Growth pattern in the sepal

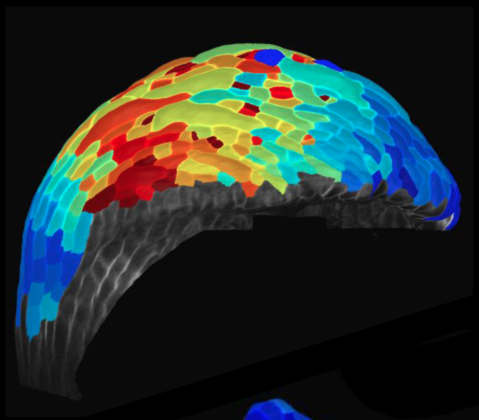


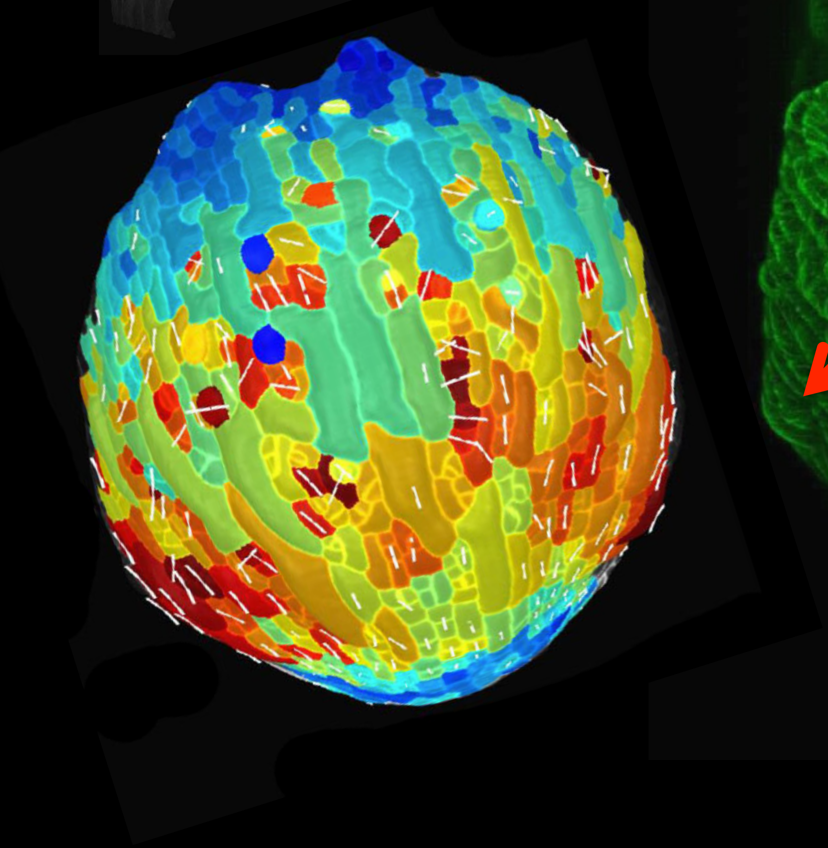
Anne-Lise Routier & Aleksandra Sapala

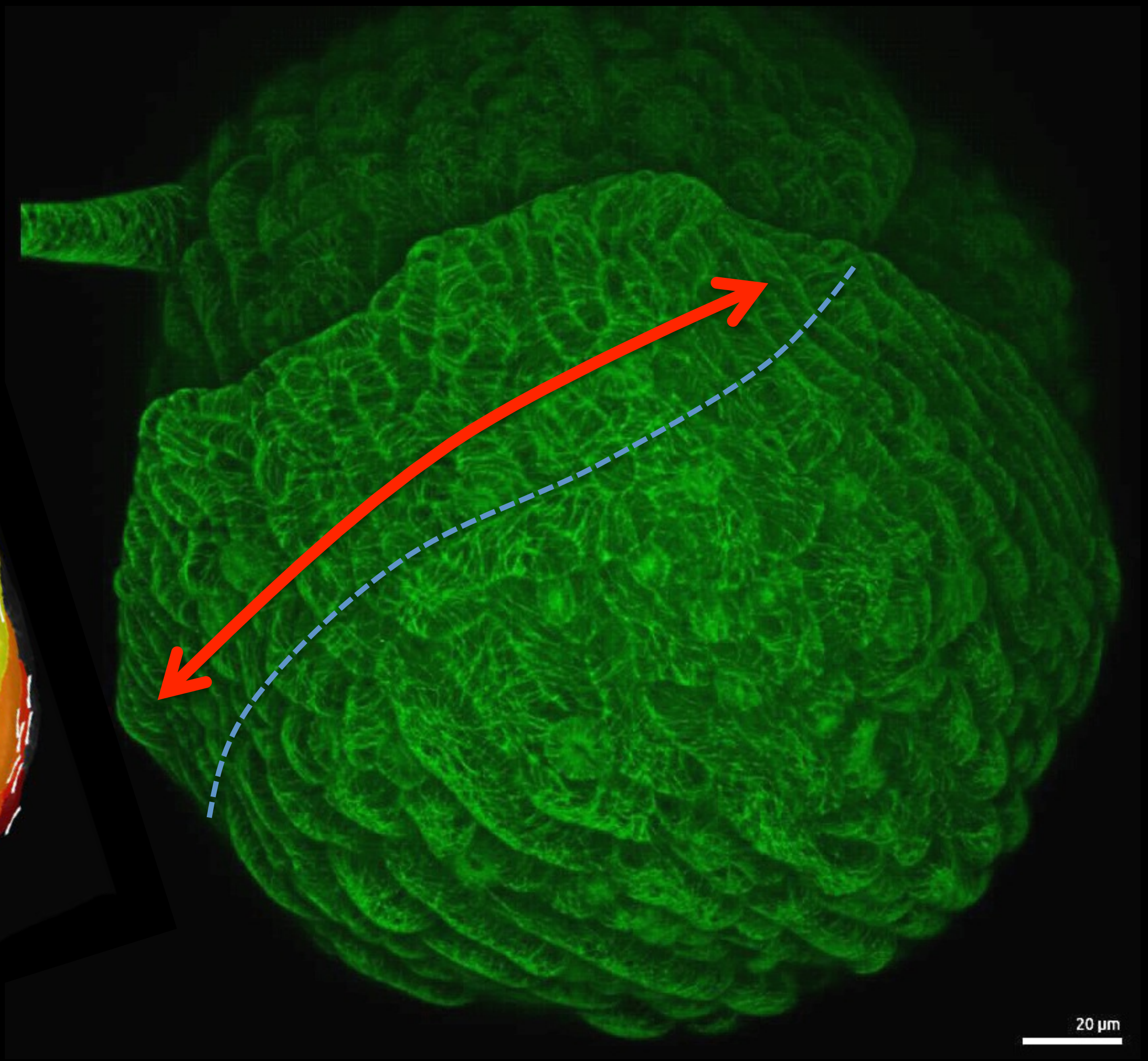
Microtubule pattern in the sepal



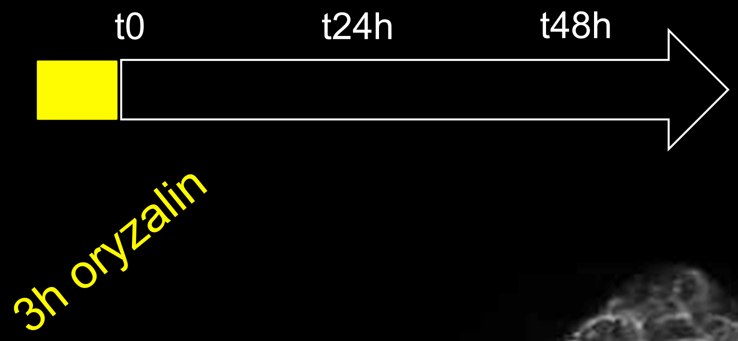
Microtubule alignment at the tip

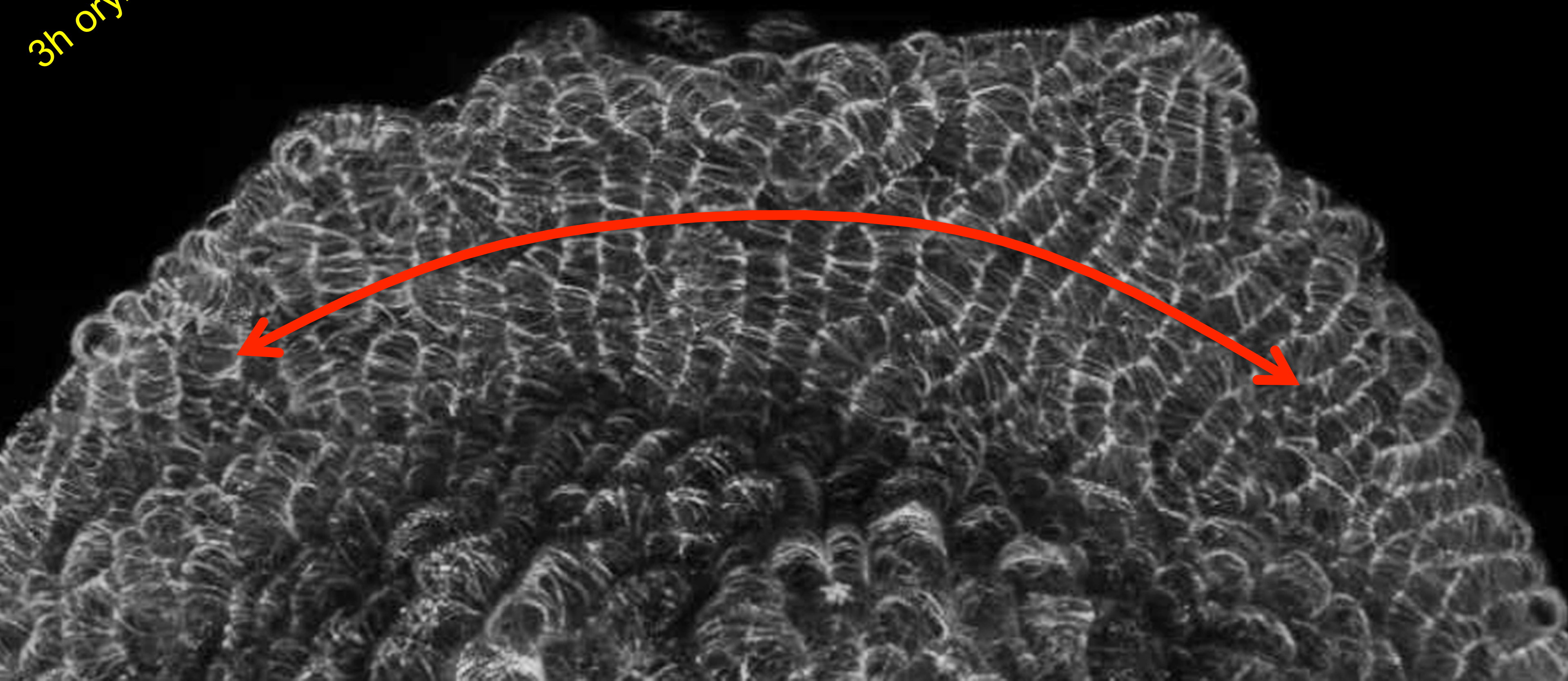




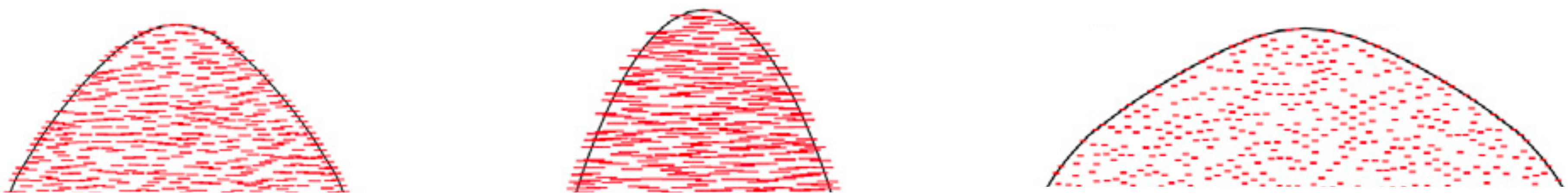


Enhanced supracellular MT alignment at the tip after oryzalin recovery

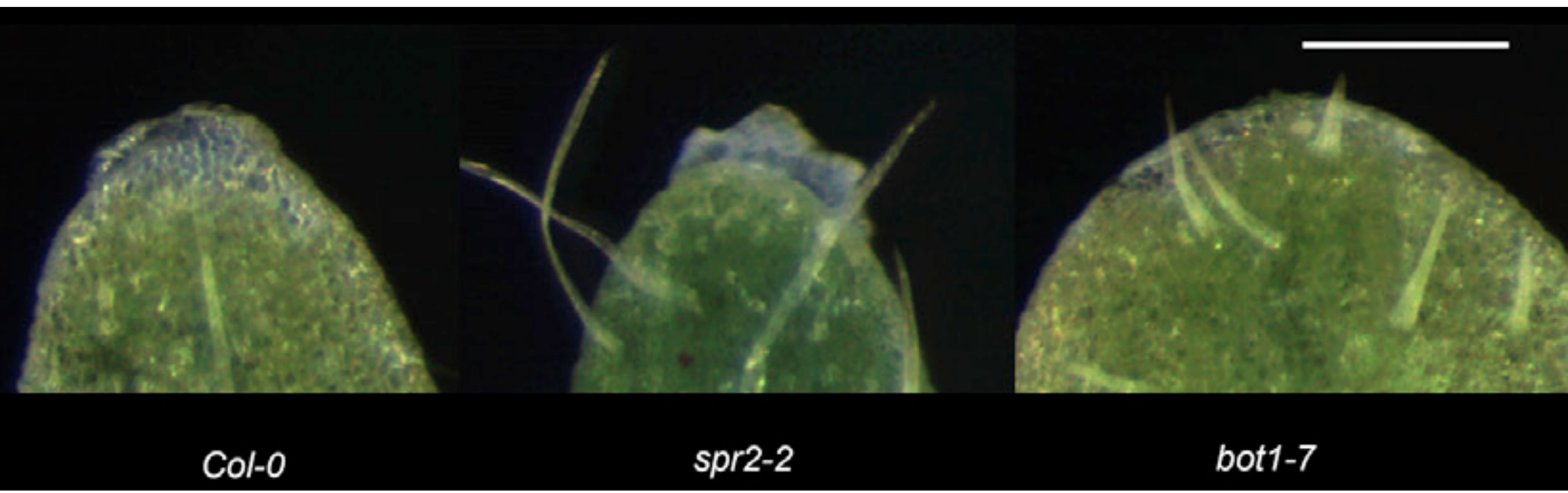




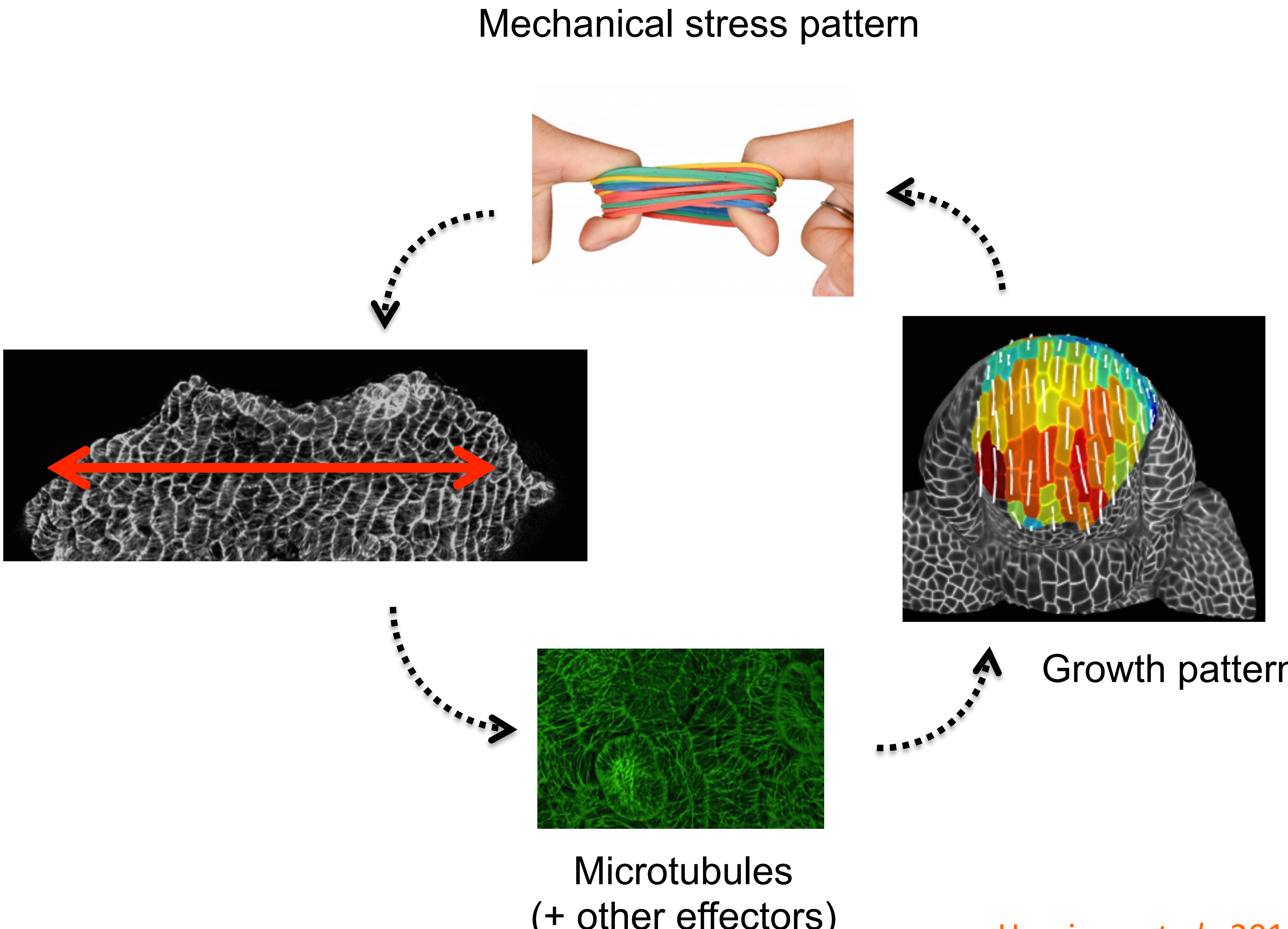
Sepal tip shape depends on feedback strength



WT | HIGH response to stress | LOW response to stress

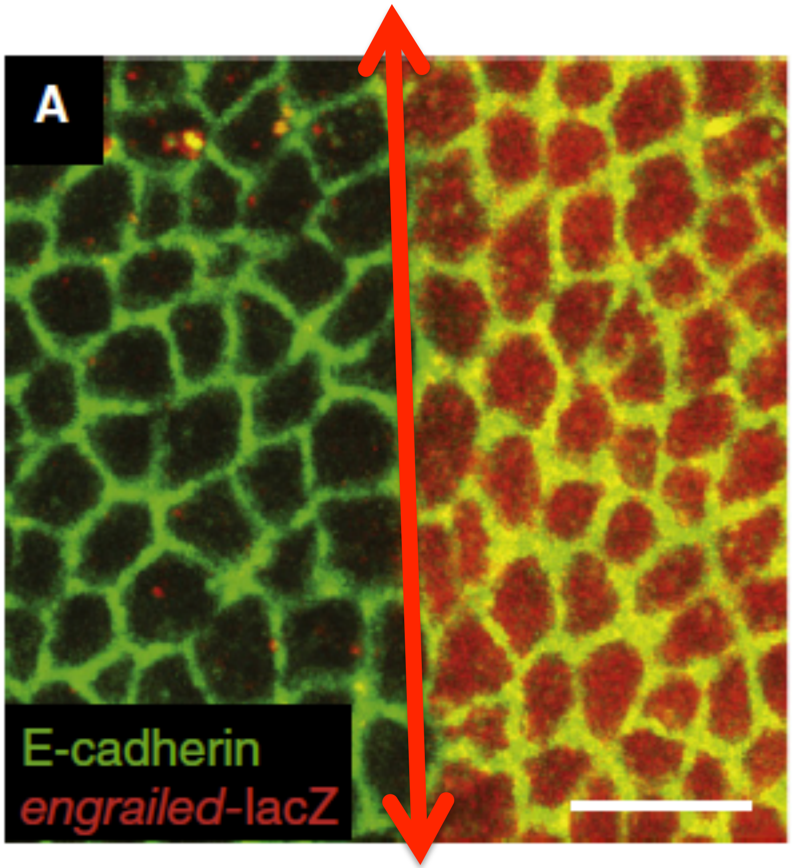


Mechanical signals channel organ shape

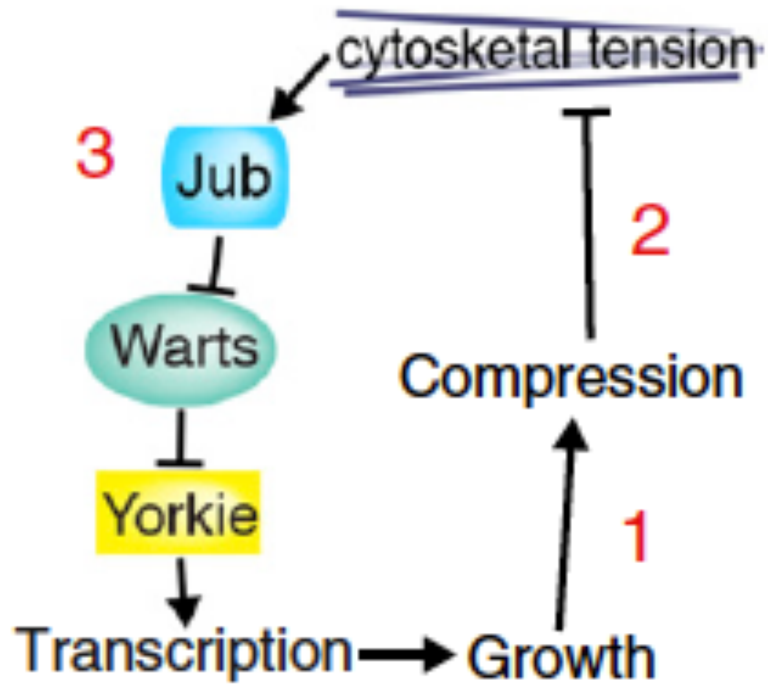


Hervieux *et al.*, 2016 Curr. Biol.

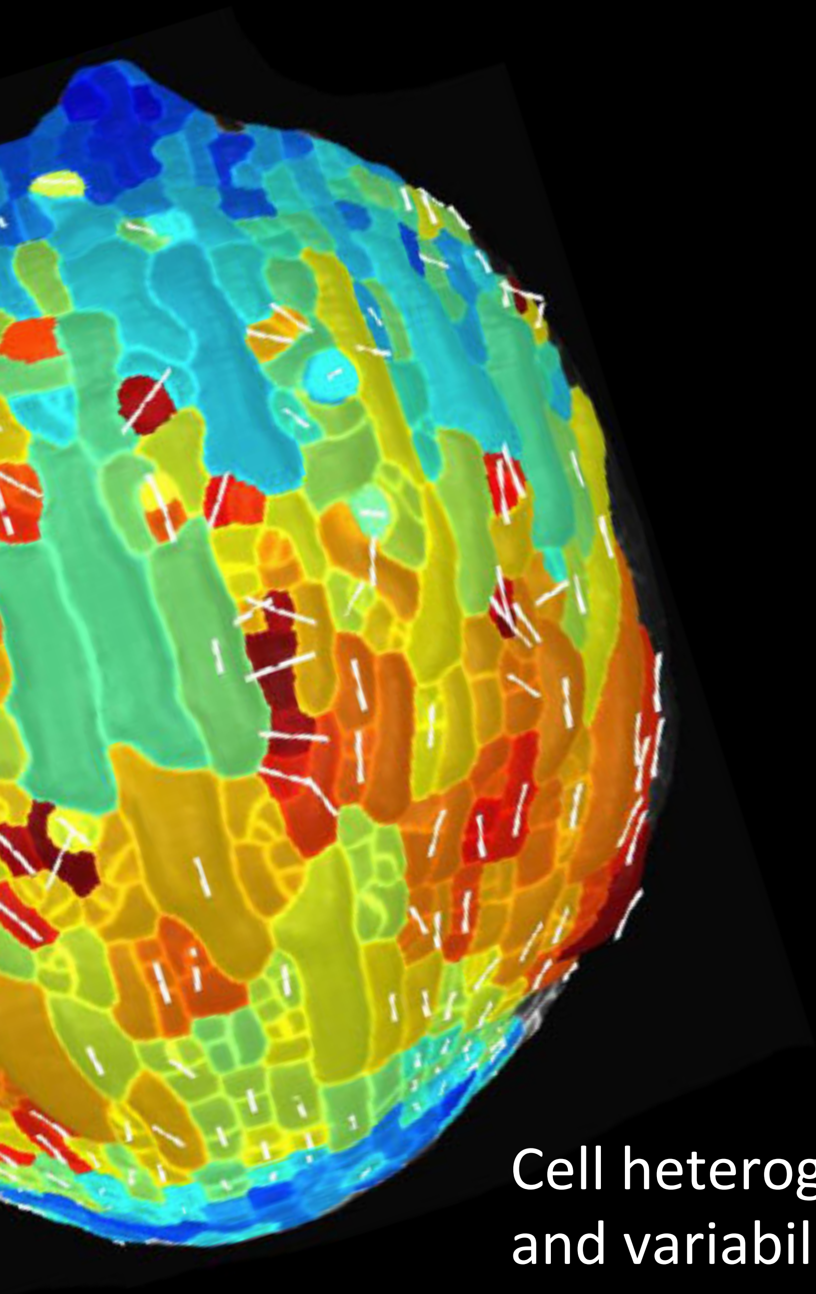
Mechanical signals channel organ shape in animals too



Landsberg *et al*., 2009 Curr. Biol.

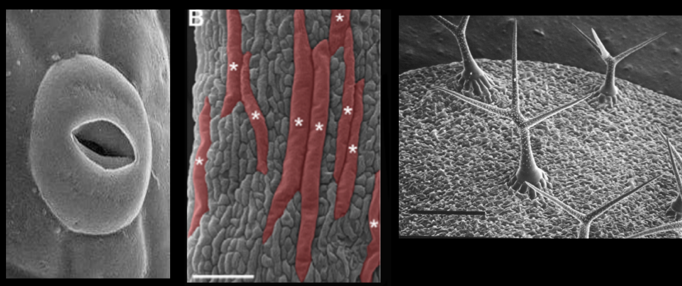


Pan *et al*., 2016 PNAS



Cell heterogeneity
and variability

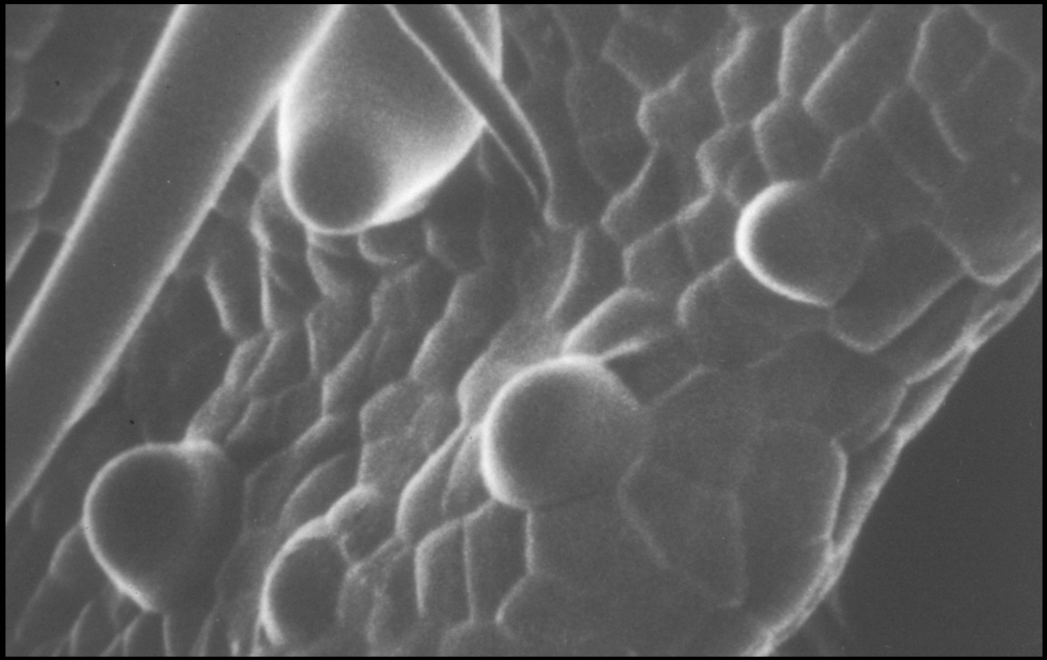
Reproducible
shapes



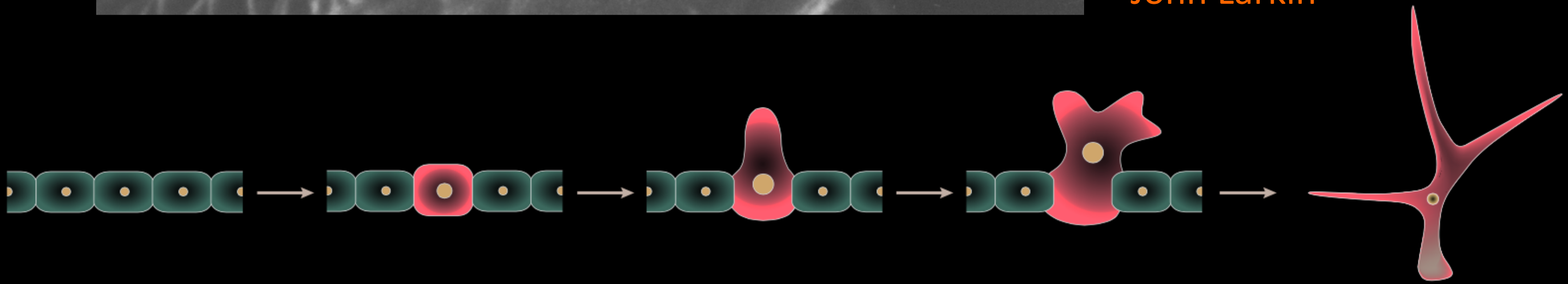
Cell heterogeneity and variability

Reproducible shapes

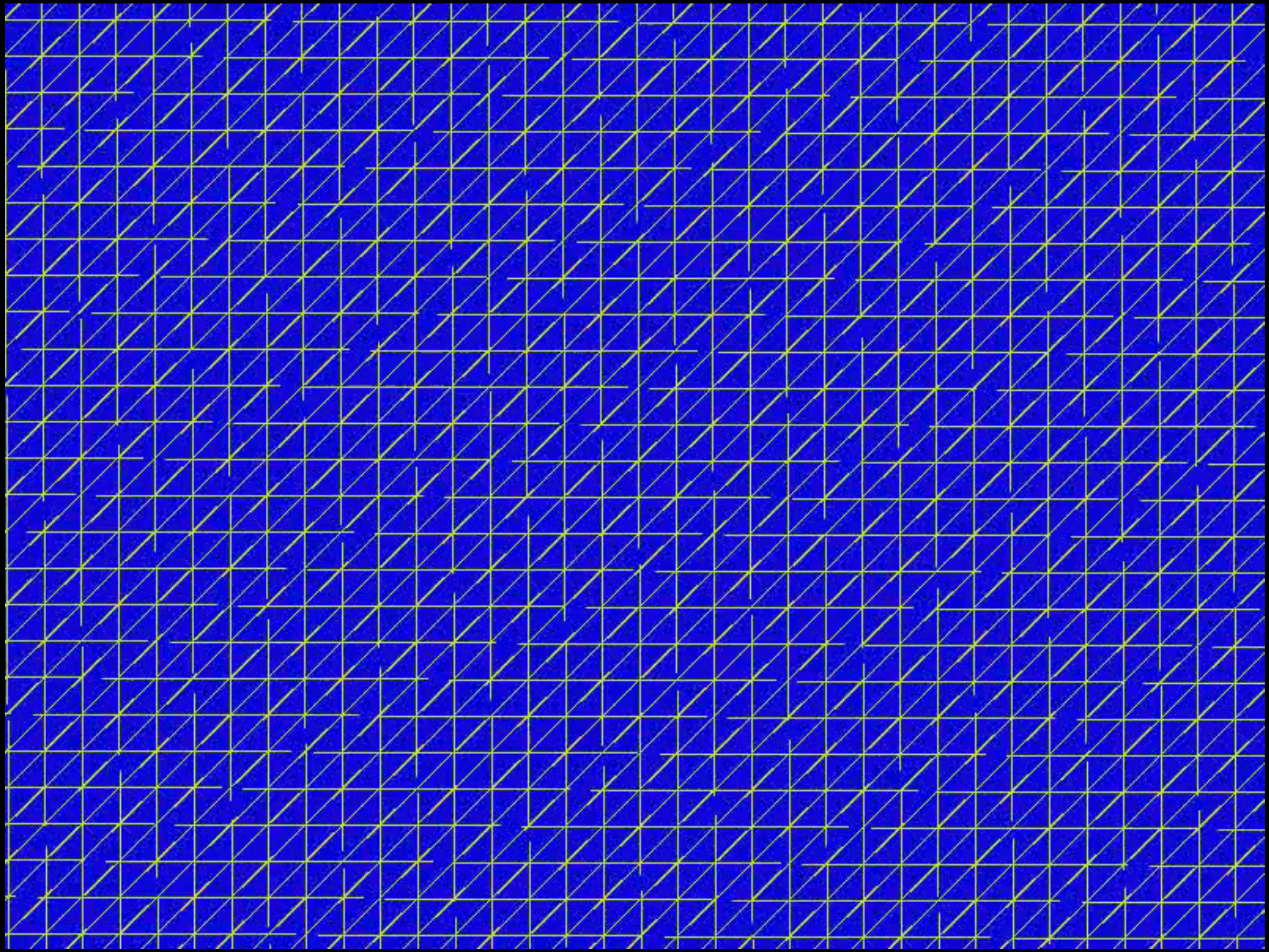
Mechanical heterogeneity around a growing trichome



John Larkin

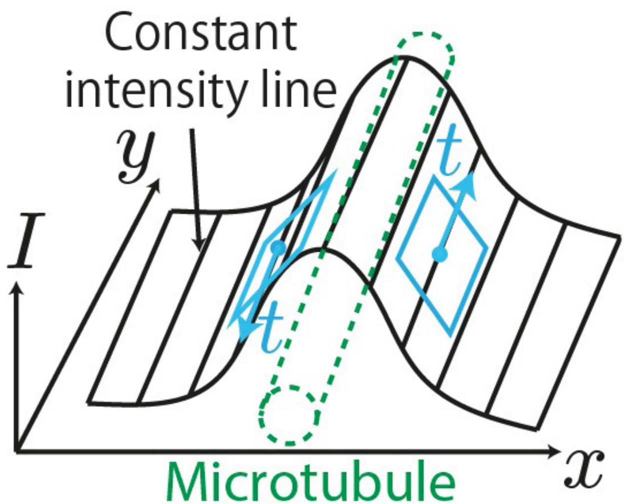


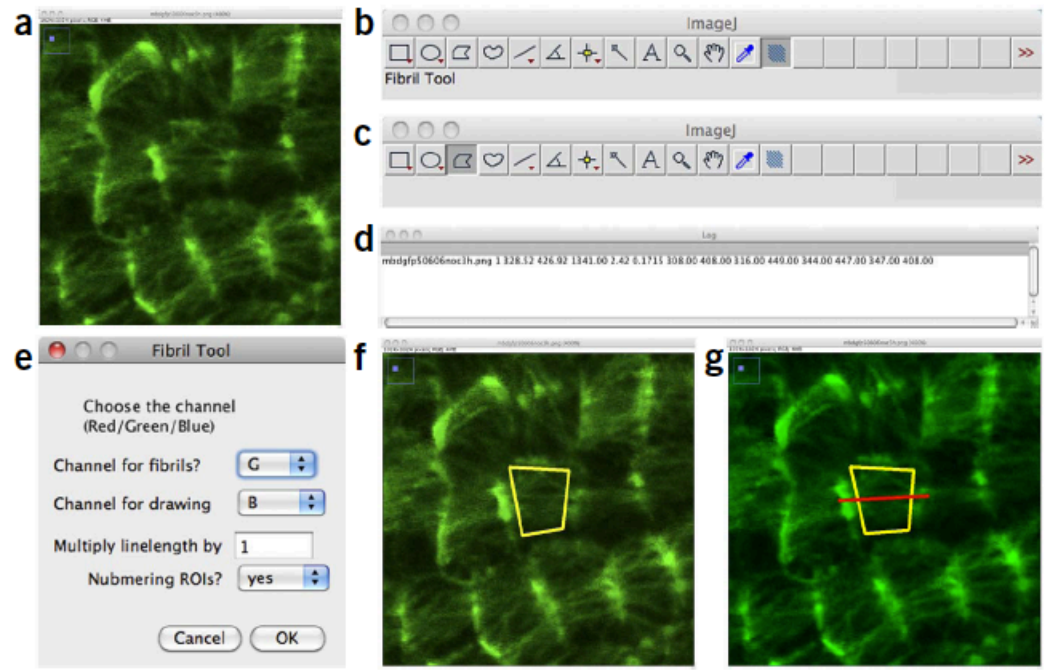
Mechanical stress pattern around a growing trichome



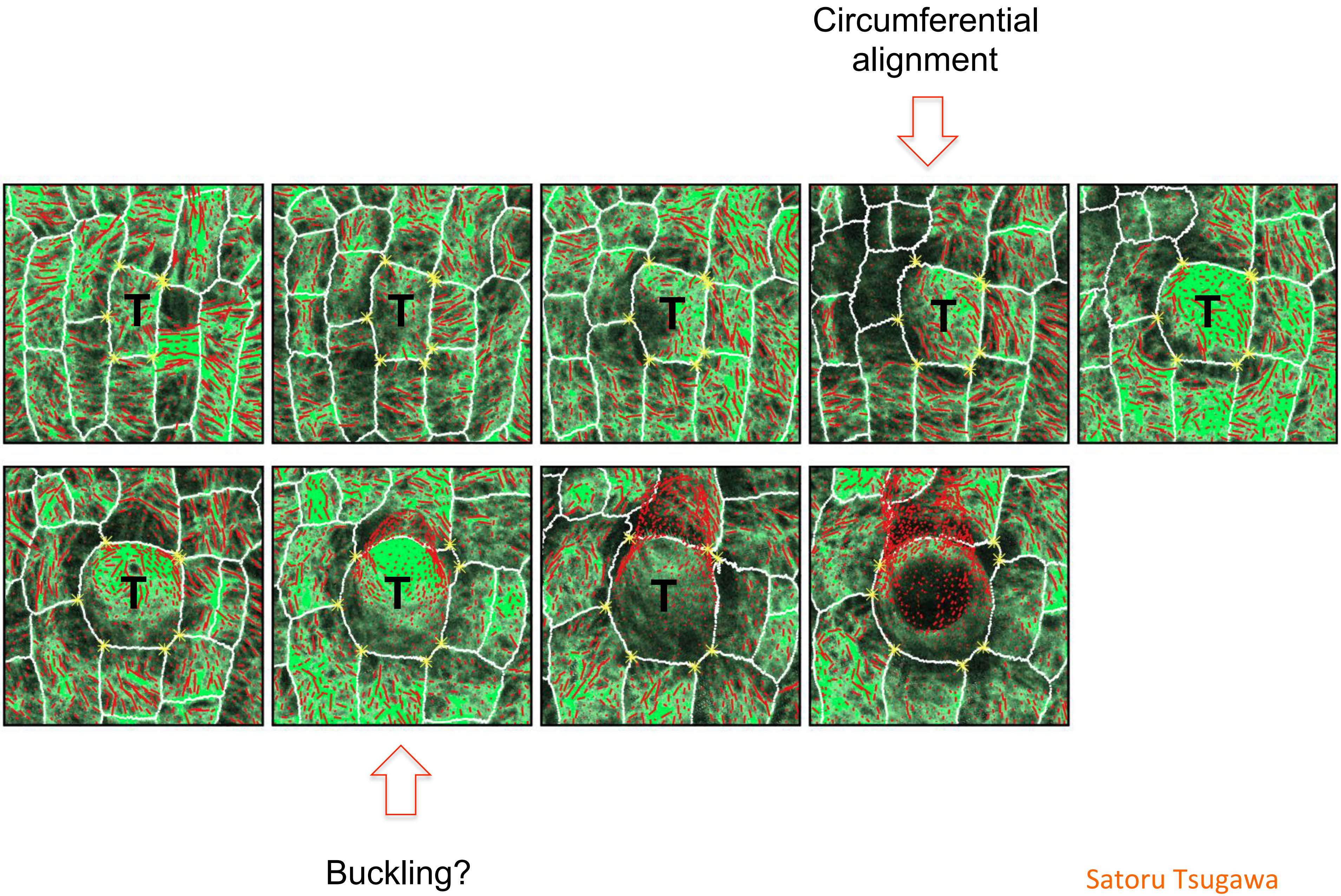
Richard Smith

Fibriltool: Quantifying the microtubule response



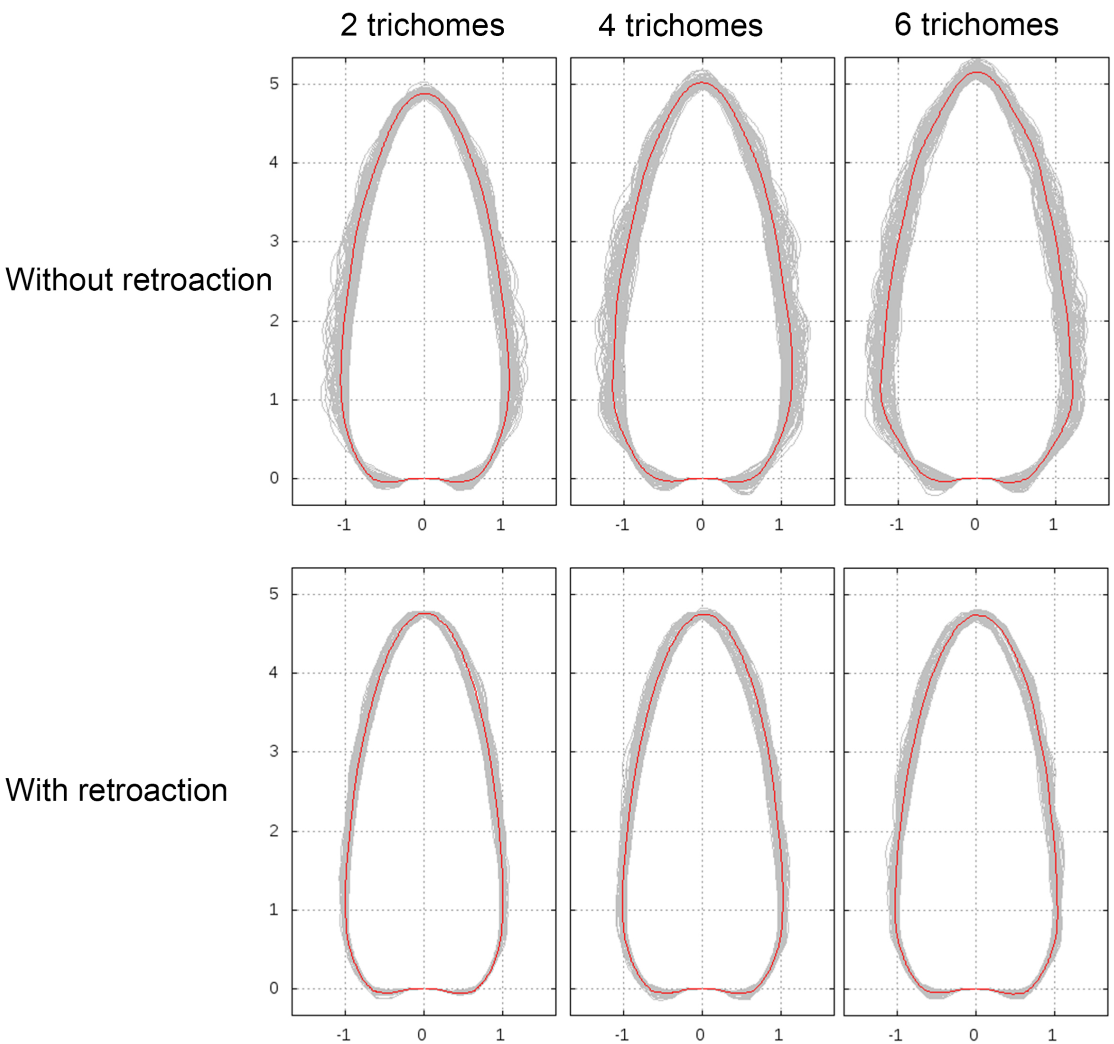


Boudaoud *et al.,* 2014 Nat. Protocol
Tsugawa *et al.*, 2016 Biophys. J.

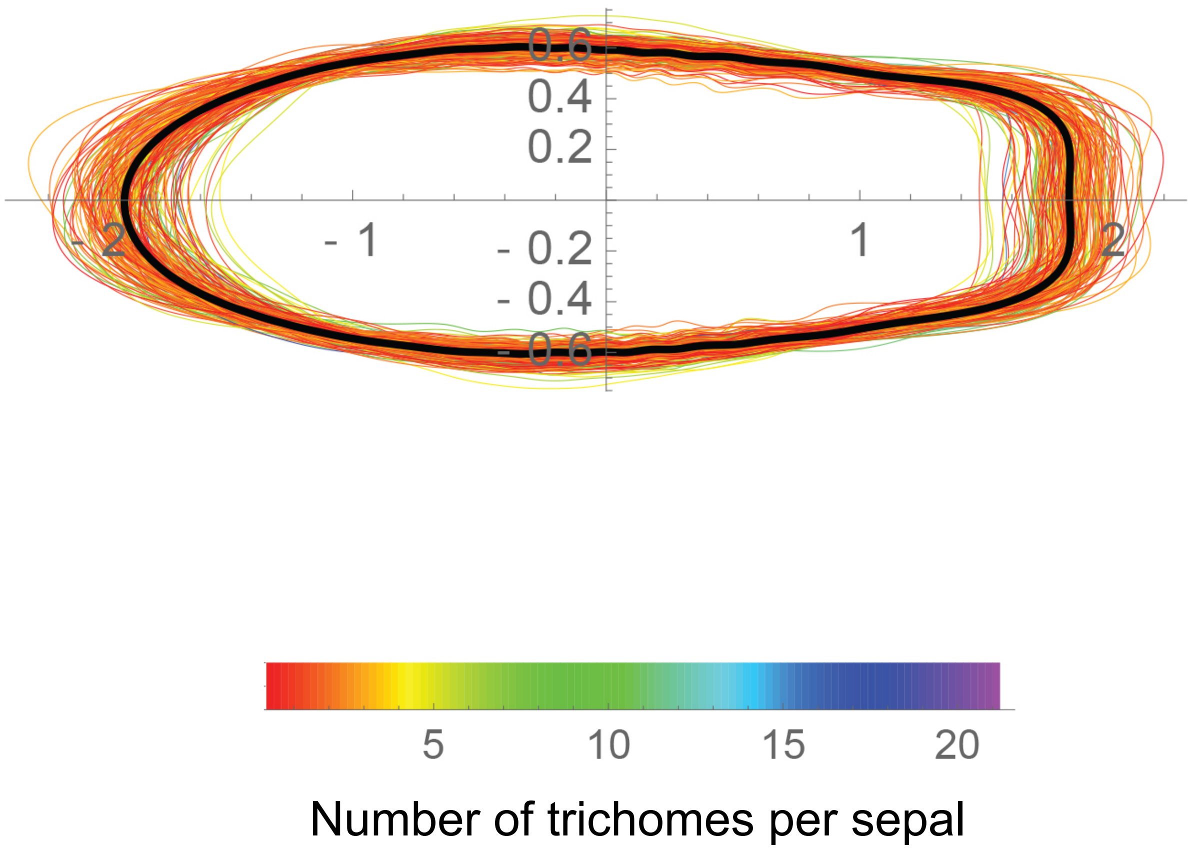


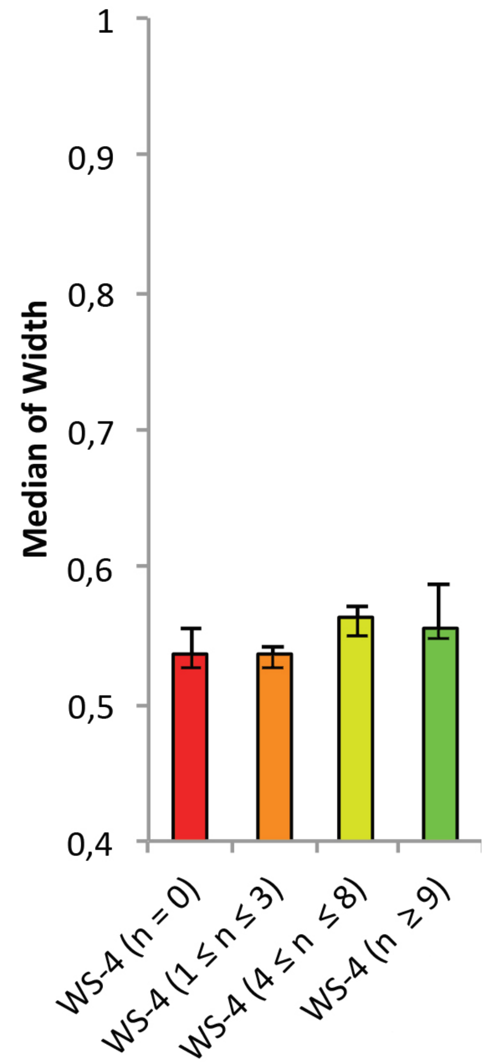
Satoru Tsugawa

Hyp: Mechanical shielding of fast growing cells buffers growth heterogeneity

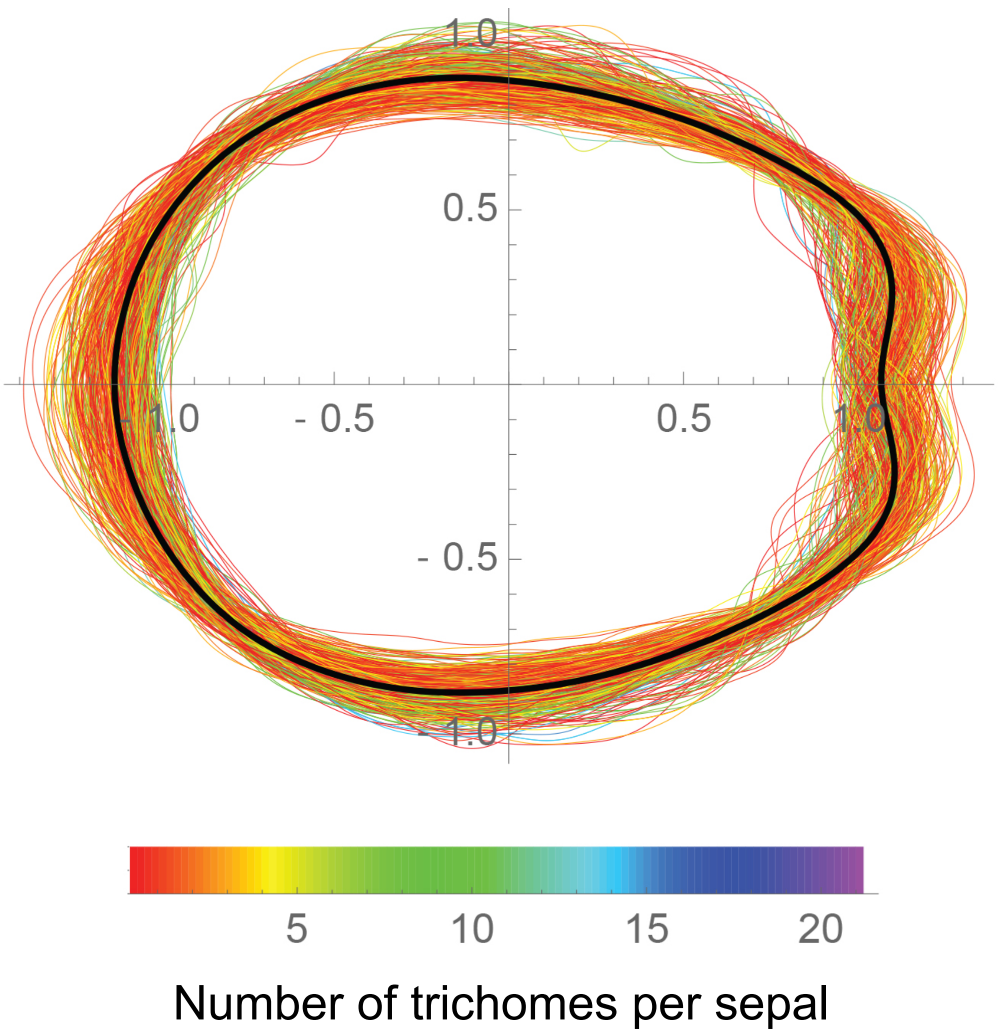


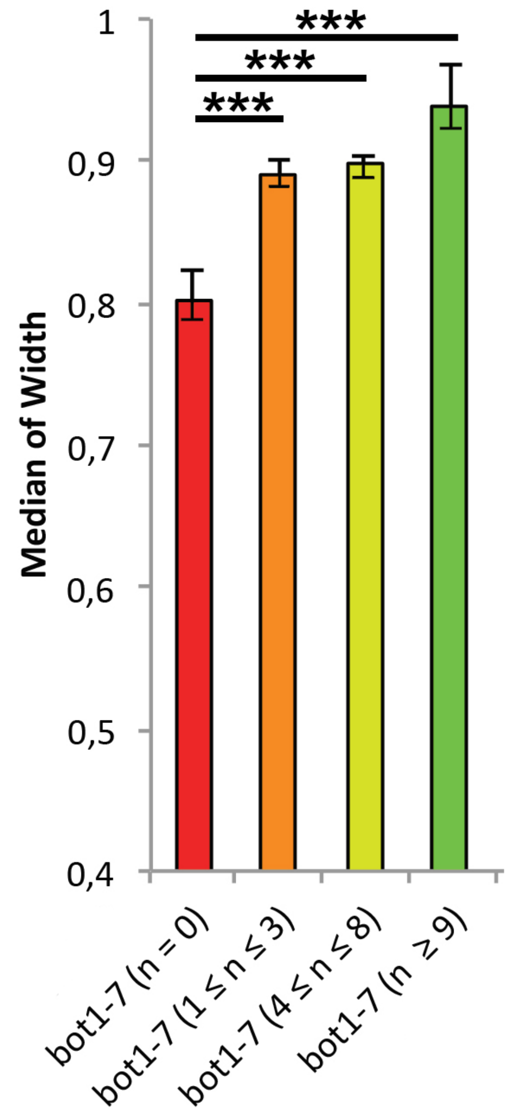
Trichome number and sepal shape are not correlated in the WT



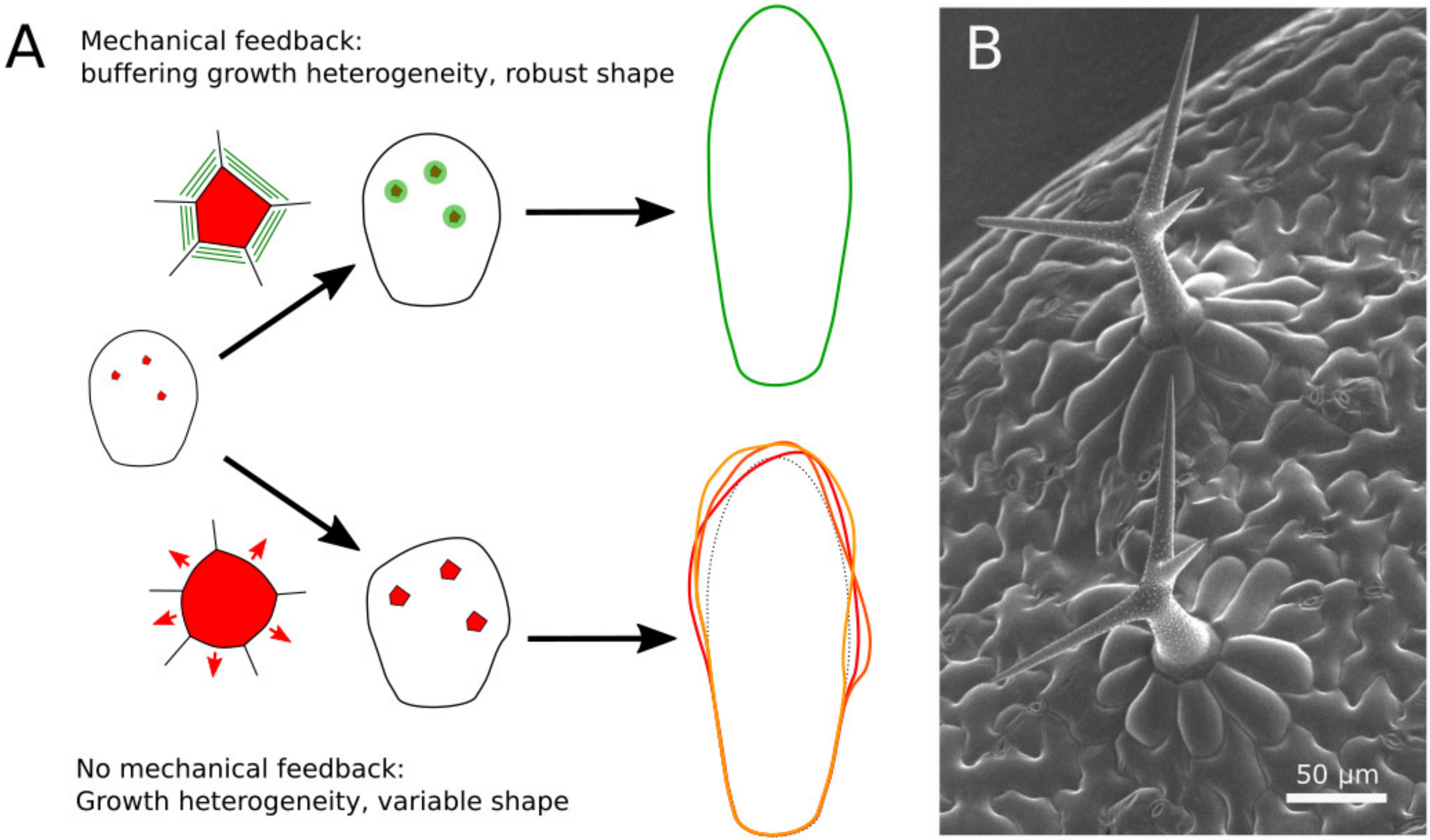


Trichome number and sepal shape are correlated in the *katanin* mutant



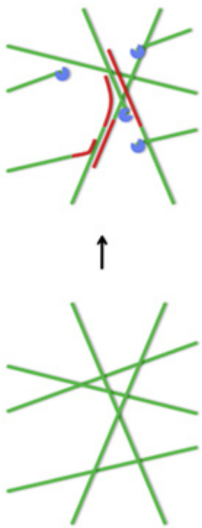


Mechanical shielding filters growth heterogeneity

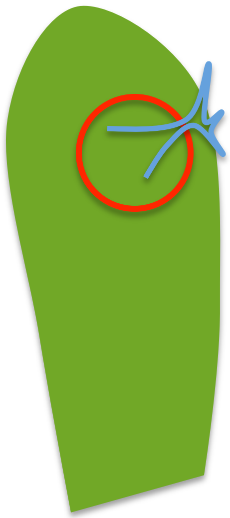


Hervieux *et al.*, 2017 Curr. Biol.

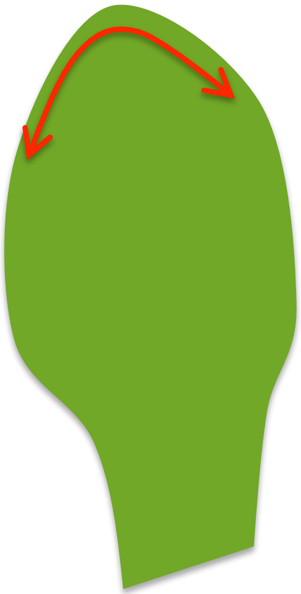
Mechanical stress channels cell and organ shapes



Microtubule dynamics under stress



Local growth heterogeneity



Growth arrest

Hervieux *et al.*, 2016, 2017 Curr. Biol.

Summary

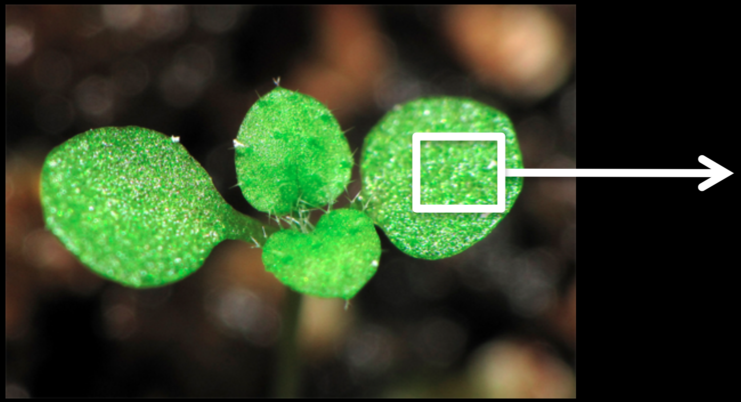
Proprioception through mechanical signals

Differential growth can be perceived and serve as a cue to channel growth / trigger growth arrest

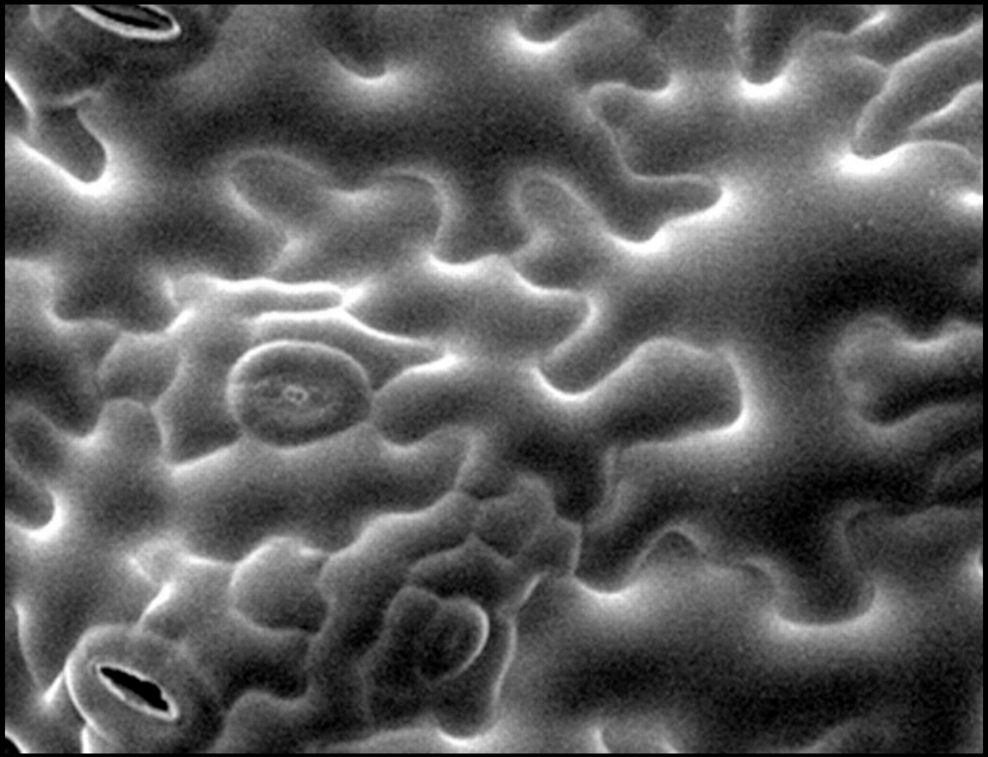
Mechanical shielding: mechanical signals help filtering growth heterogeneity to increase the reproducibility of organ shapes

The mechanical control of cell shape

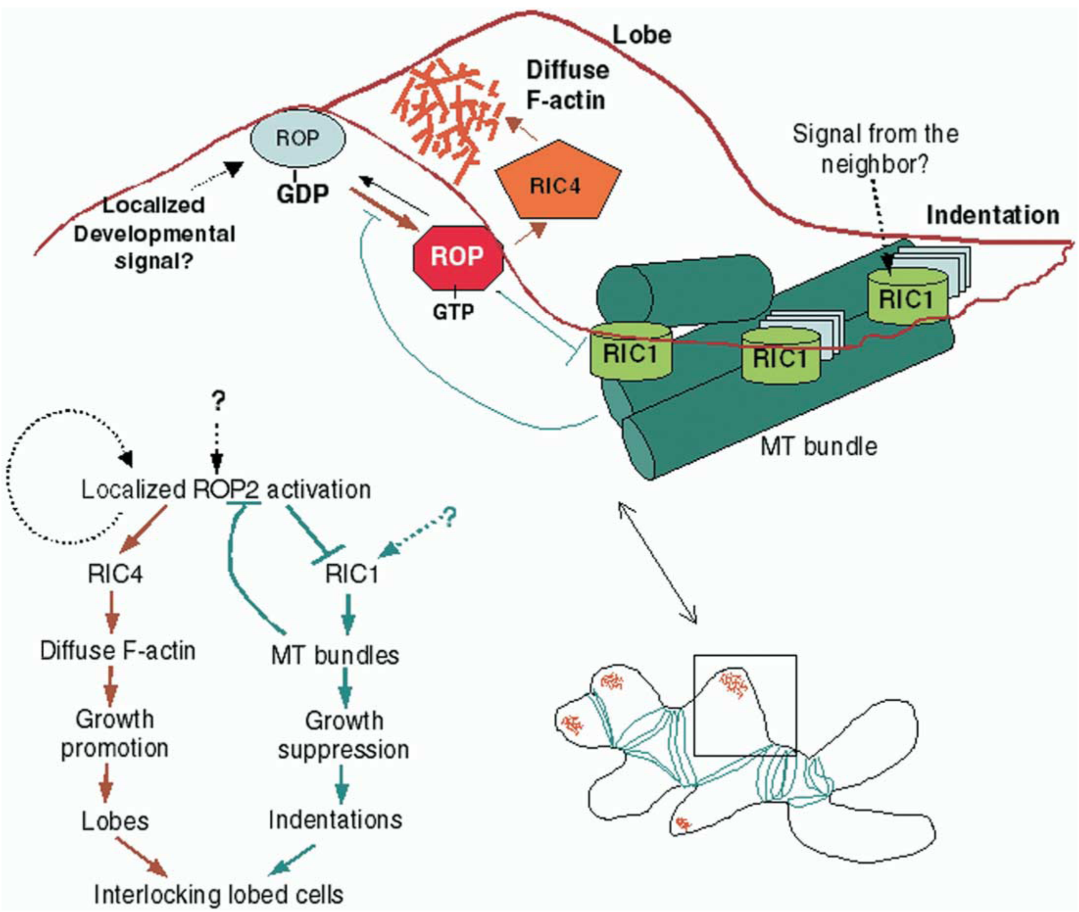
The jigsaw puzzle shaped pavement cells



Cotyledons (embryonic leaves)



Pavement cell morphogenesis is well described molecularly



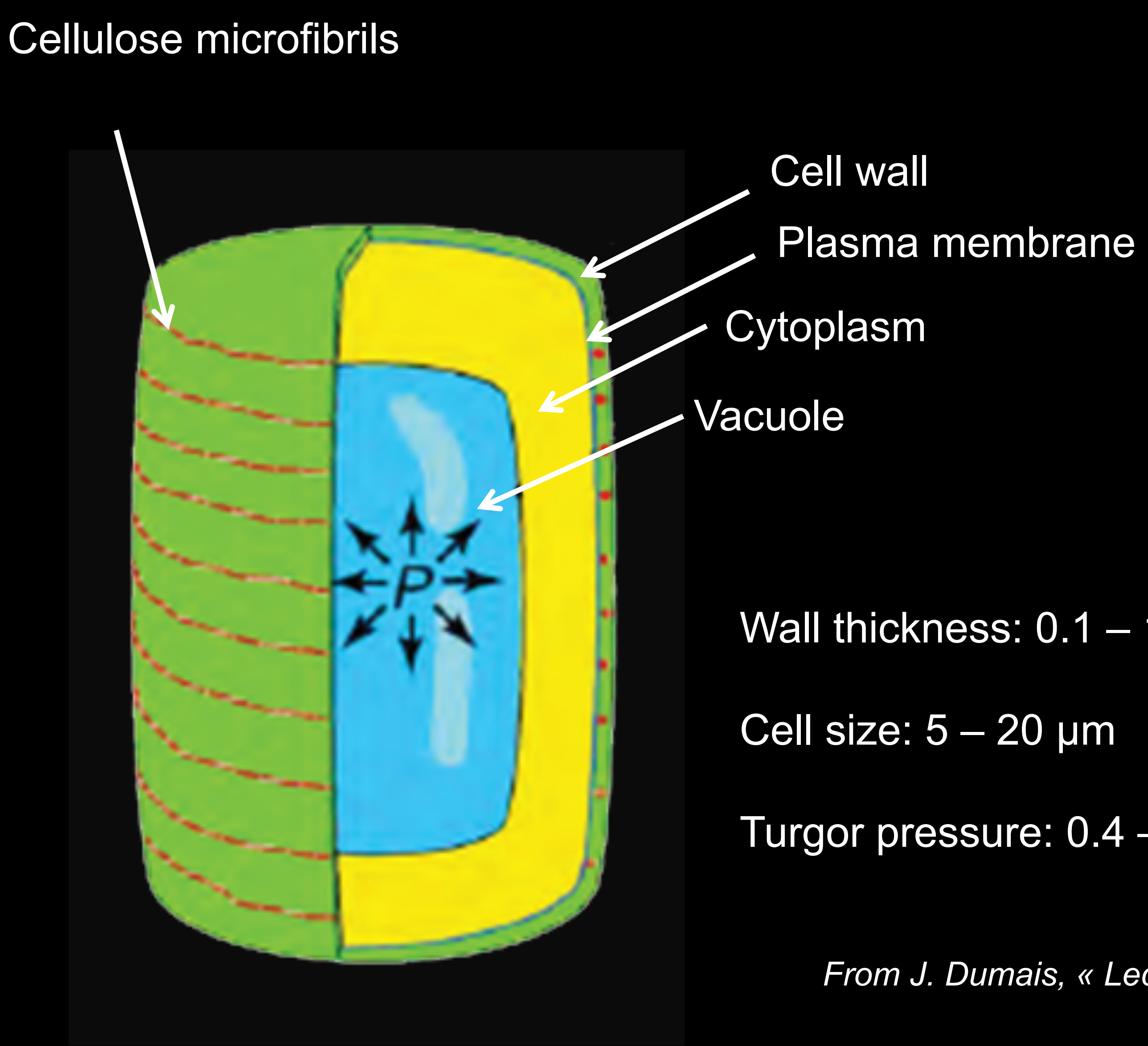
Underlined mechanical assumptions:

- Lobes grow into neighboring cell

- MT stiffen walls and restrict growth in necks

- Function: increasing interaction surface

Pavement cell mechanics?



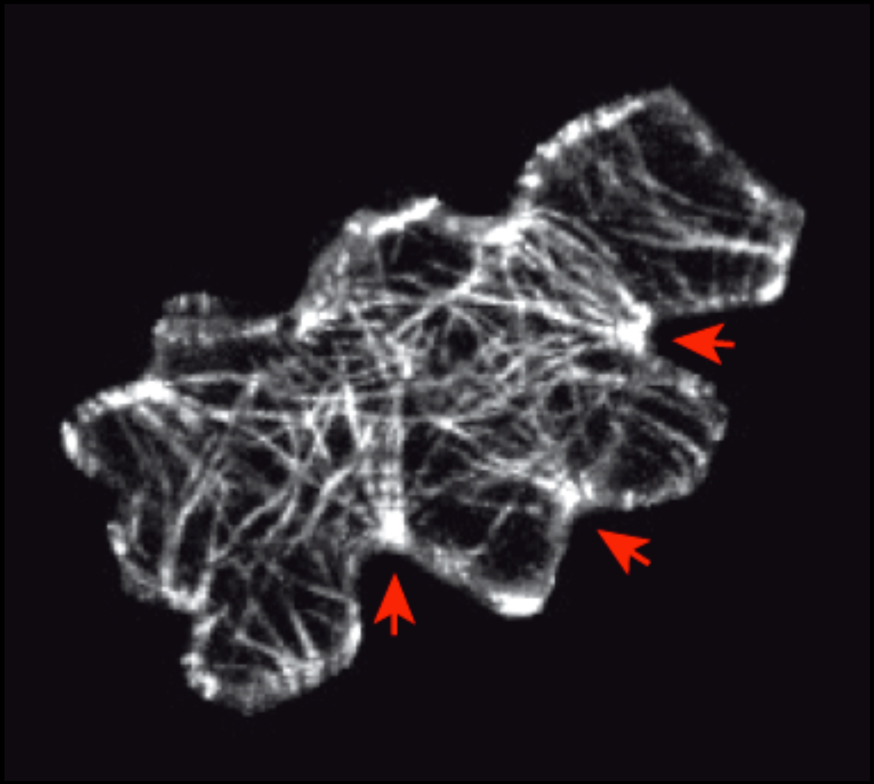
Wall thickness: 0.1 – 1 μm

Cell size: 5 – 20 μm

Turgor pressure: 0.4 – 0.8 MPa

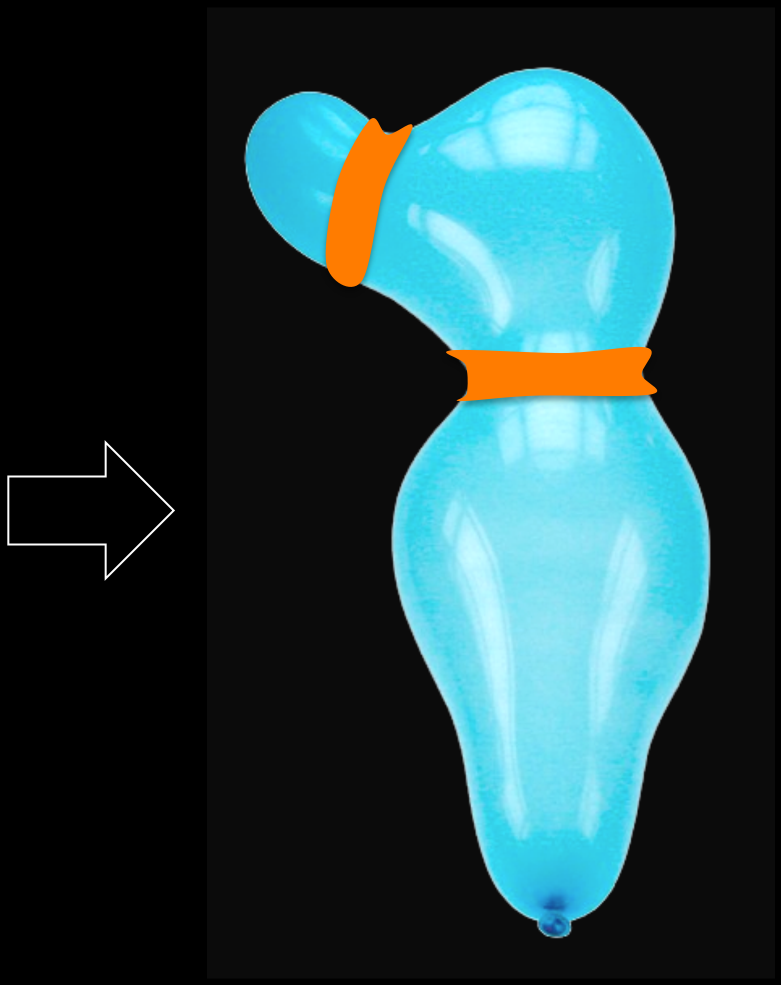
From J. Dumais, « Lectures on biomechanics »

Correlating microtubule distribution with cell shape

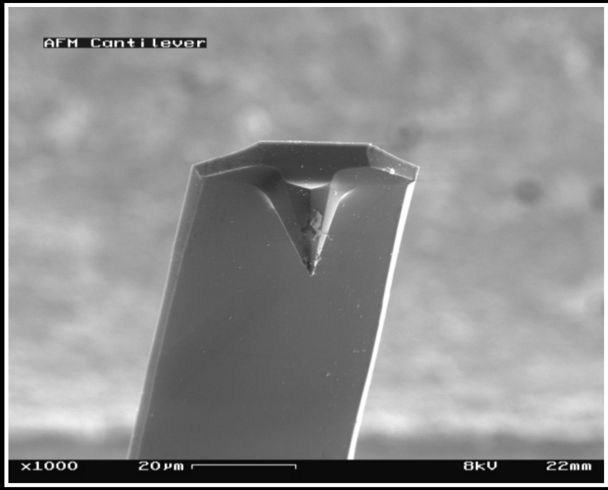


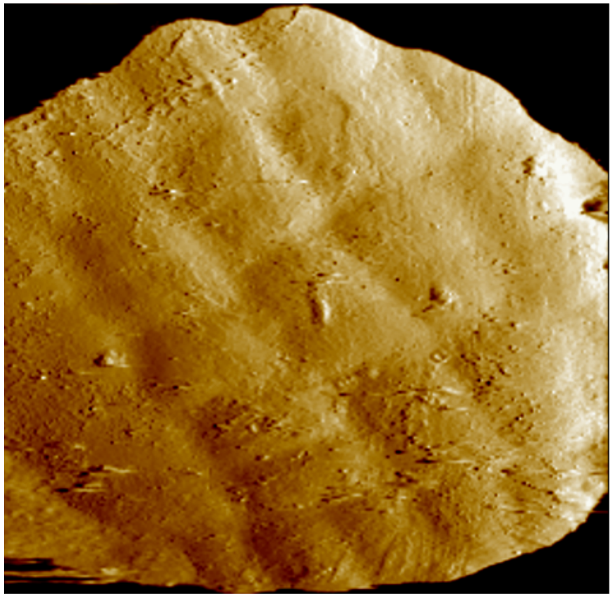
Microtubules

A local wall reinforcement to explain the puzzle shape of pavement cells?

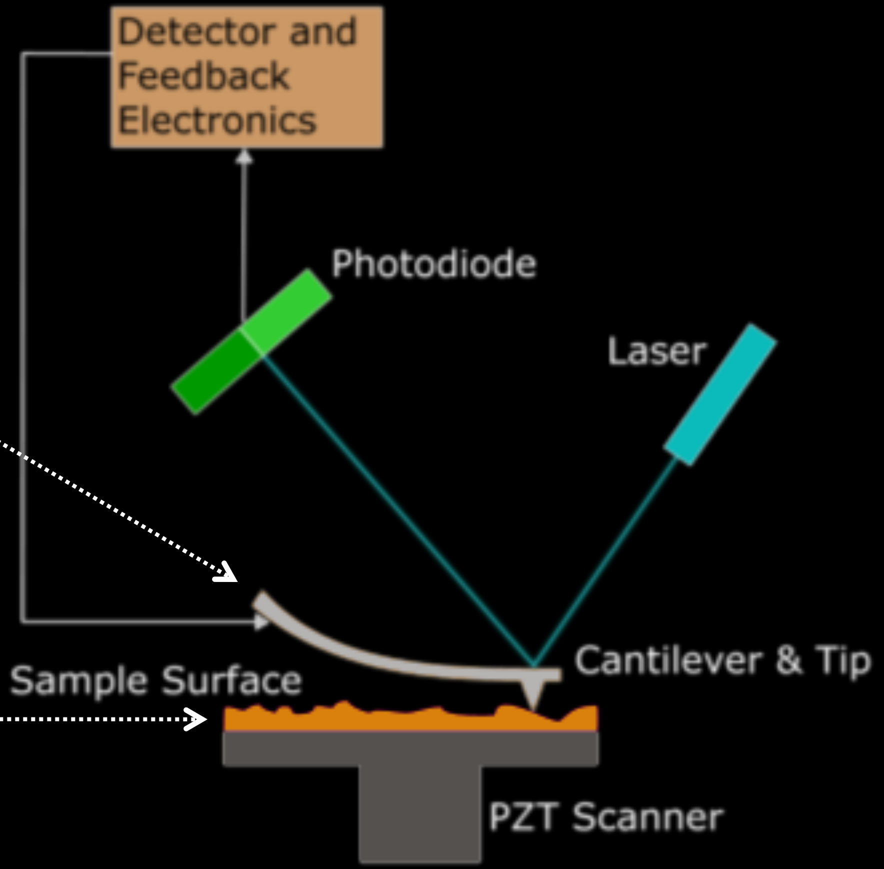


Quantifying local mechanical properties with AFM



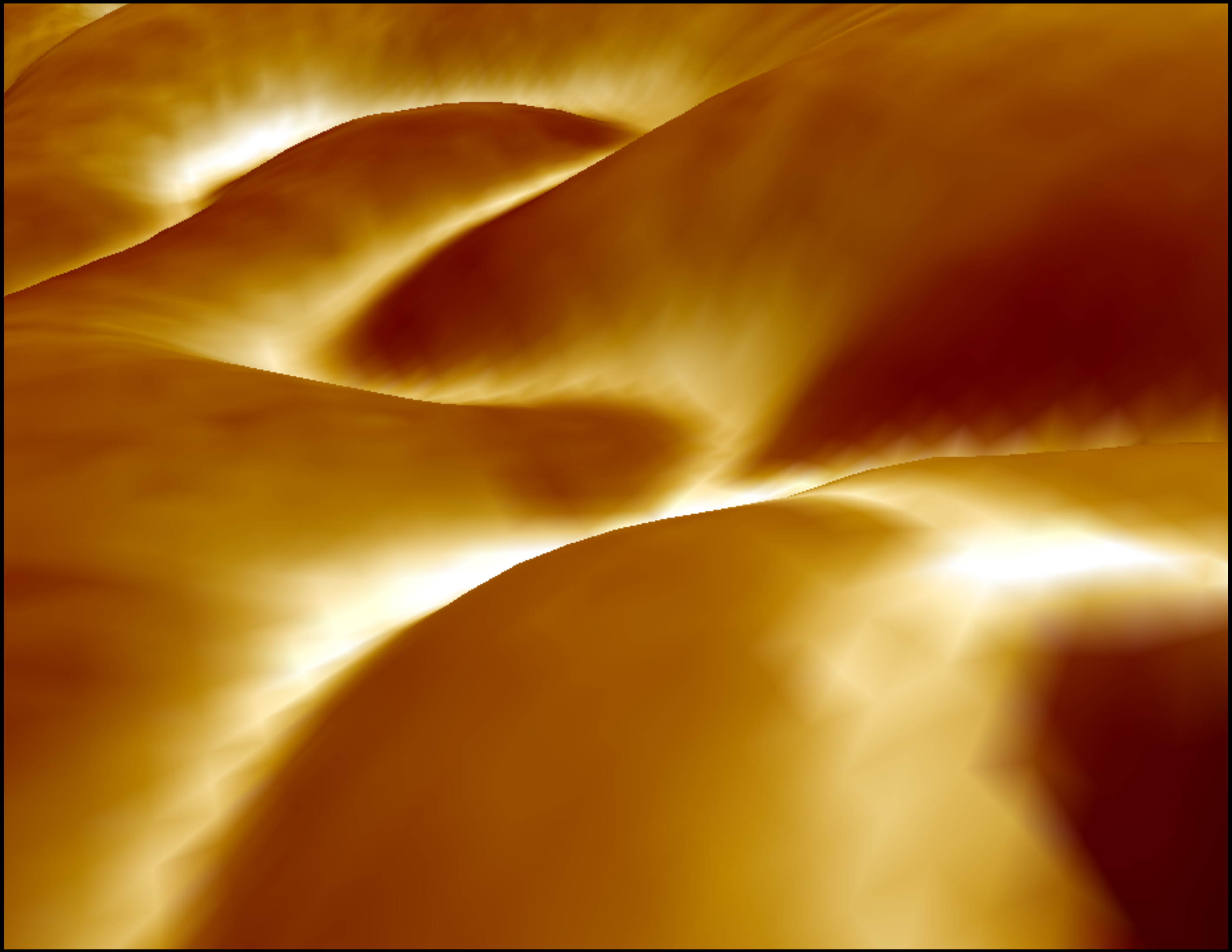


Atomic Force Microscopy

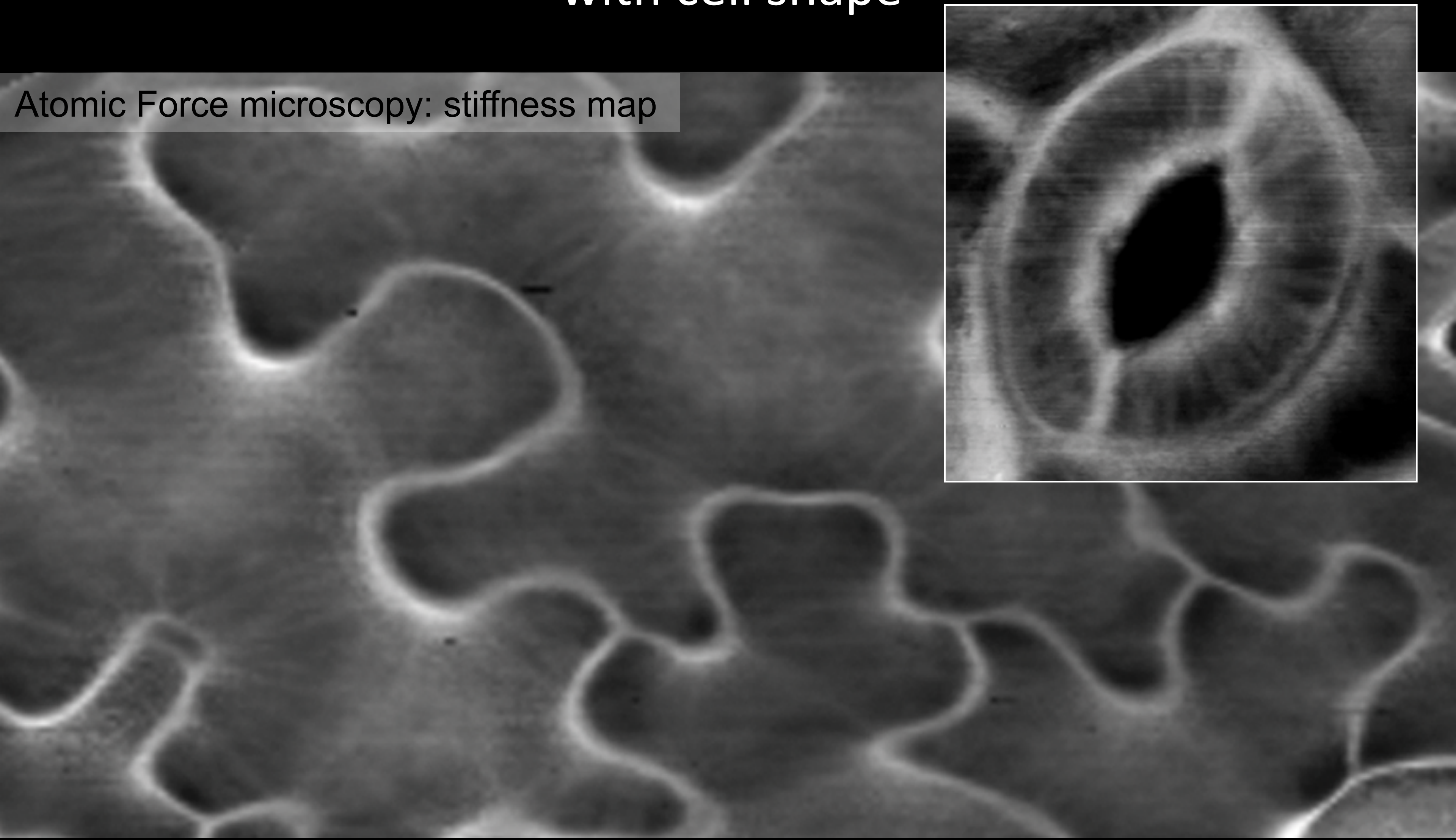


Milani et al., 2011 Plant J

Pavement cell topography with AFM

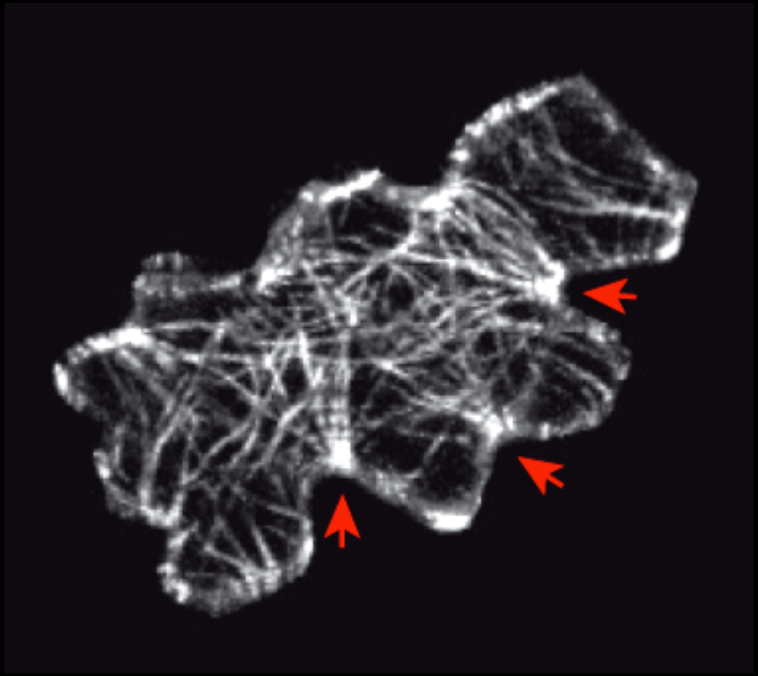


Correlating local wall stiffening with cell shape



Pascale Milani & Alexandre Berquand

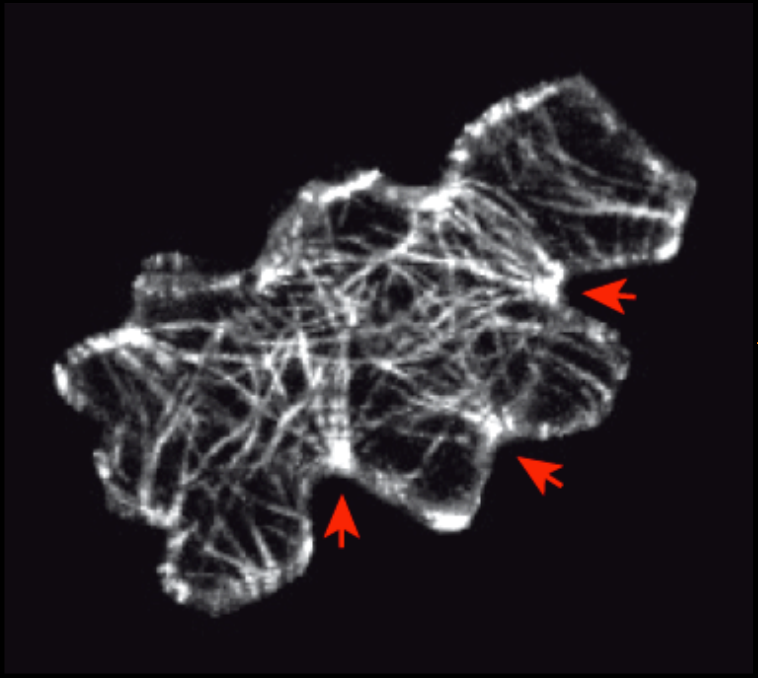
Microtubules control the formation of necks and lobes



Microtubules

Local wall reinforcements
via cellulose deposition

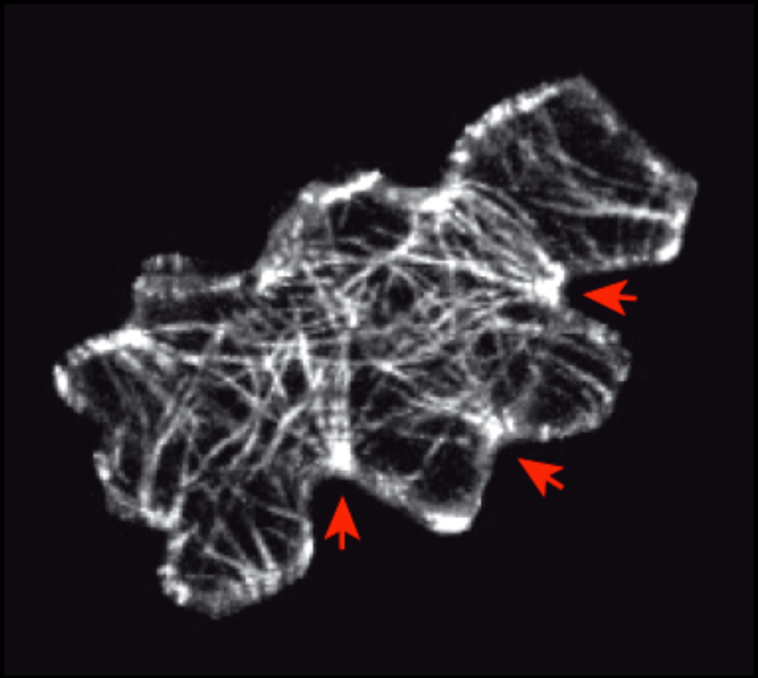
What is orienting the cortical microtubules?



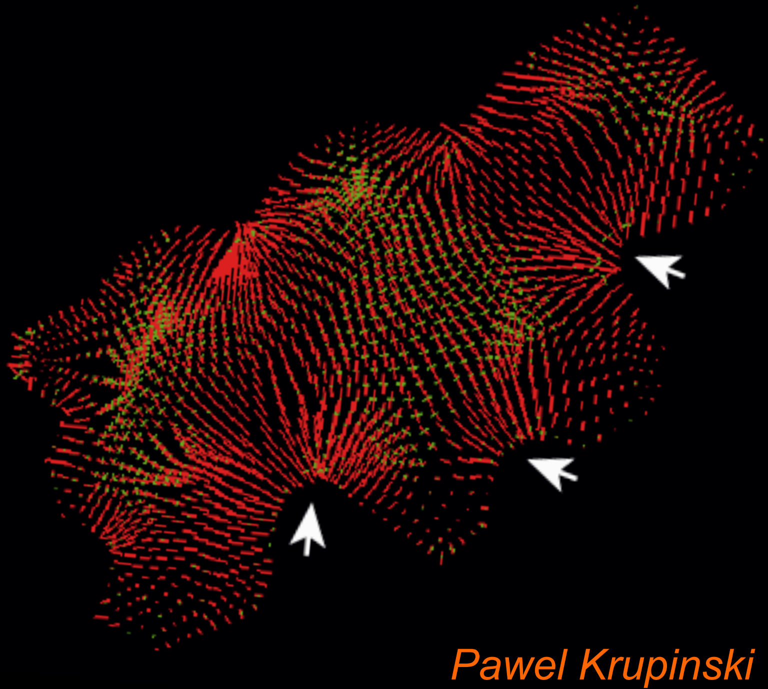
Microtubules

?

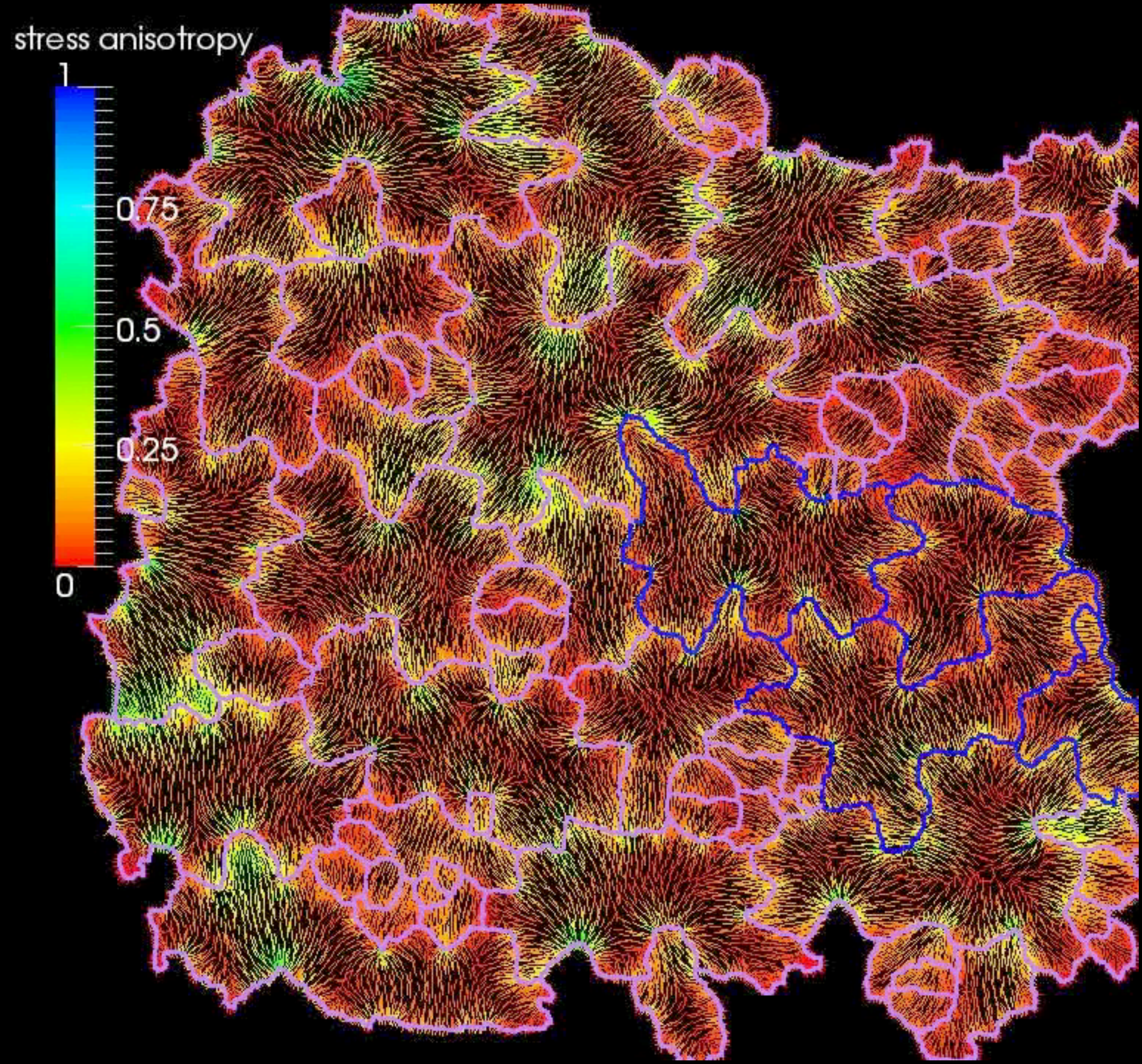
A contribution of mechanical stress?



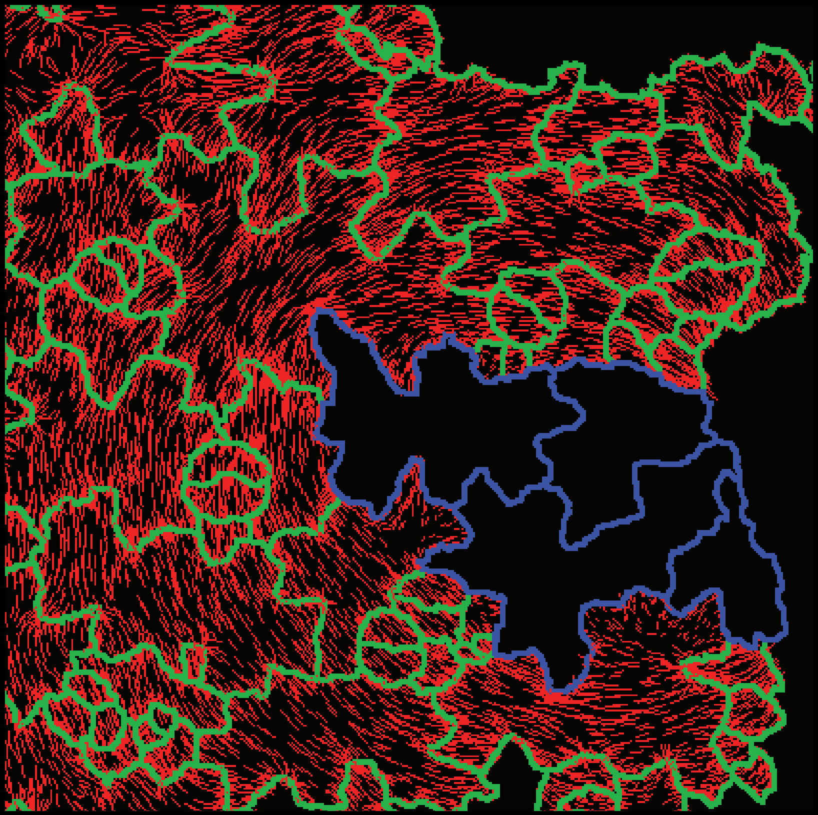
Microtubules



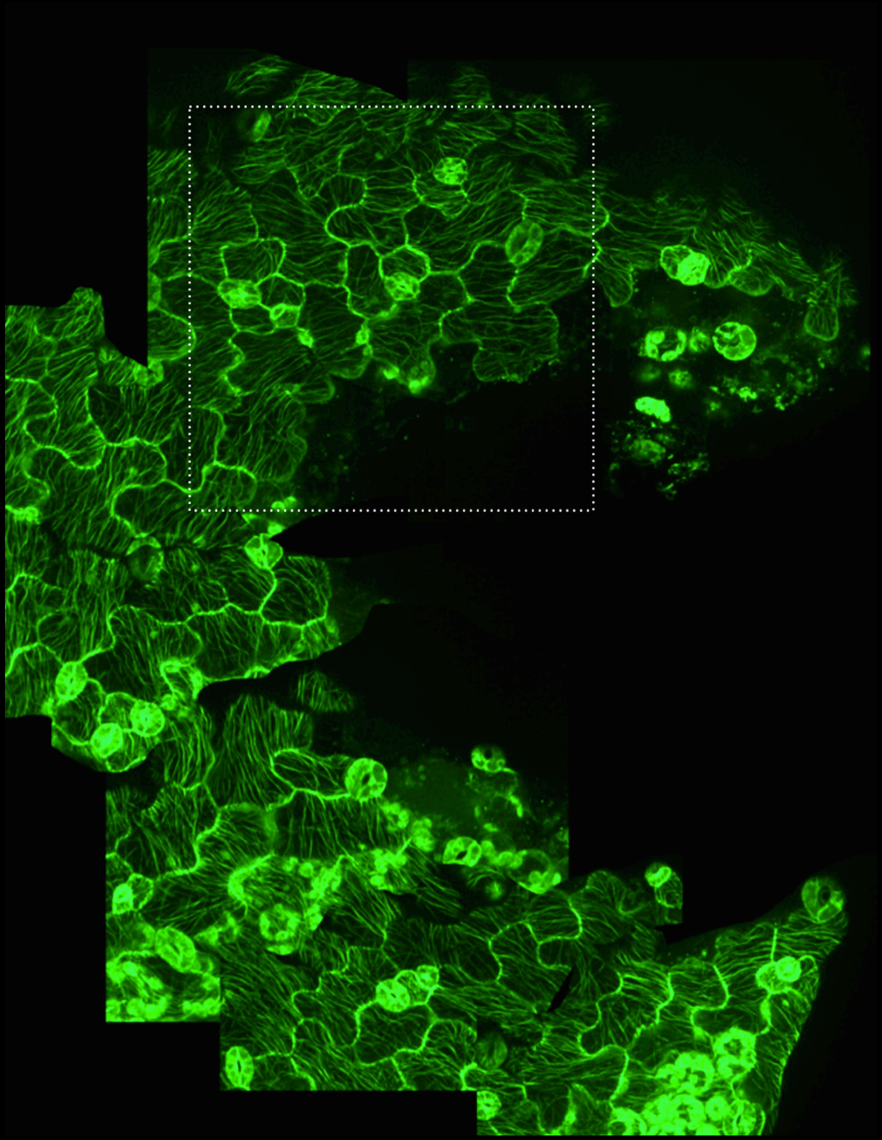
Mechanical stress pattern


stress anisotropy
1
0.75
0.5
0.25
0

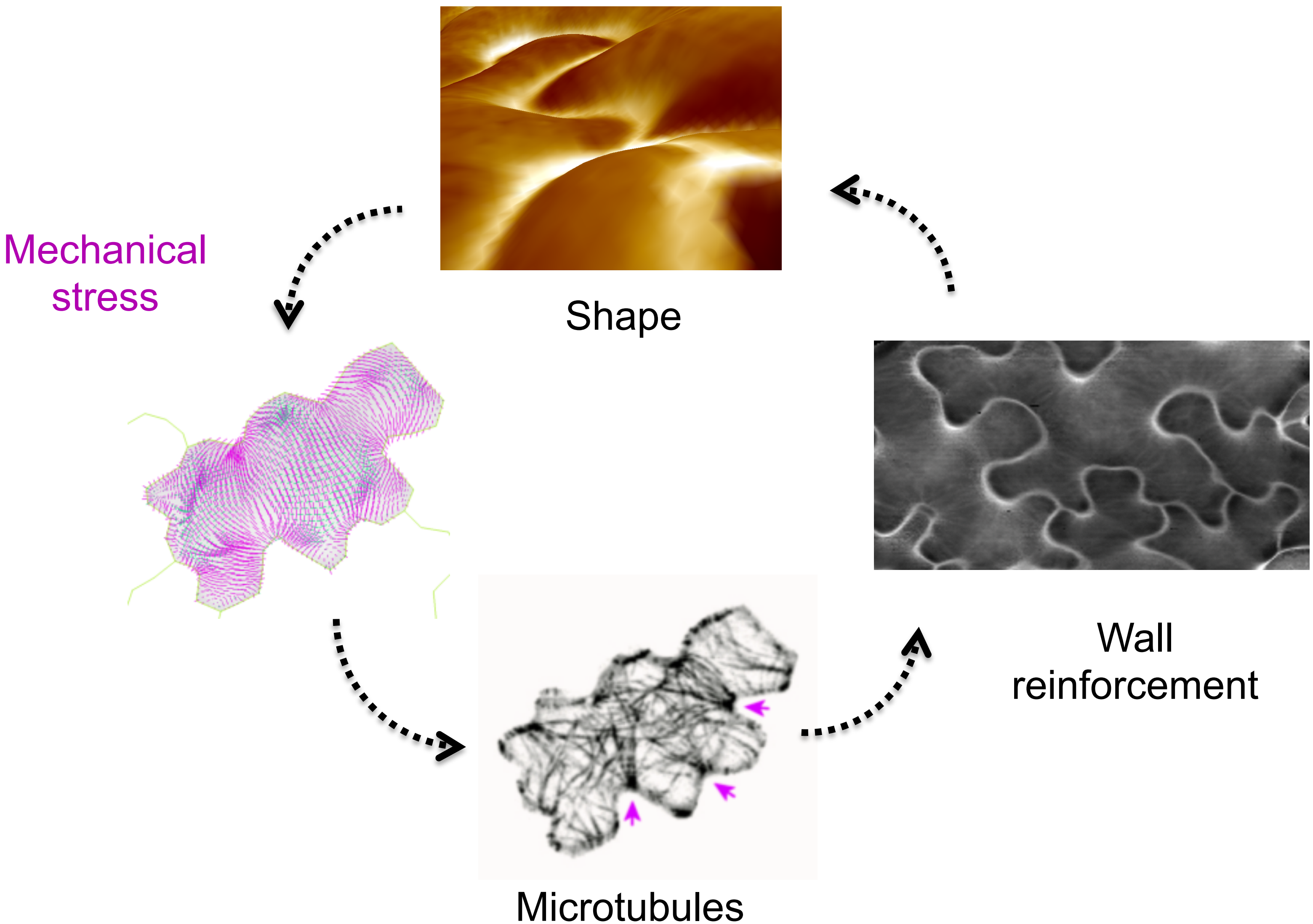
Test: Induce a local modification of the stress pattern



Circumferential stress pattern
after ablation

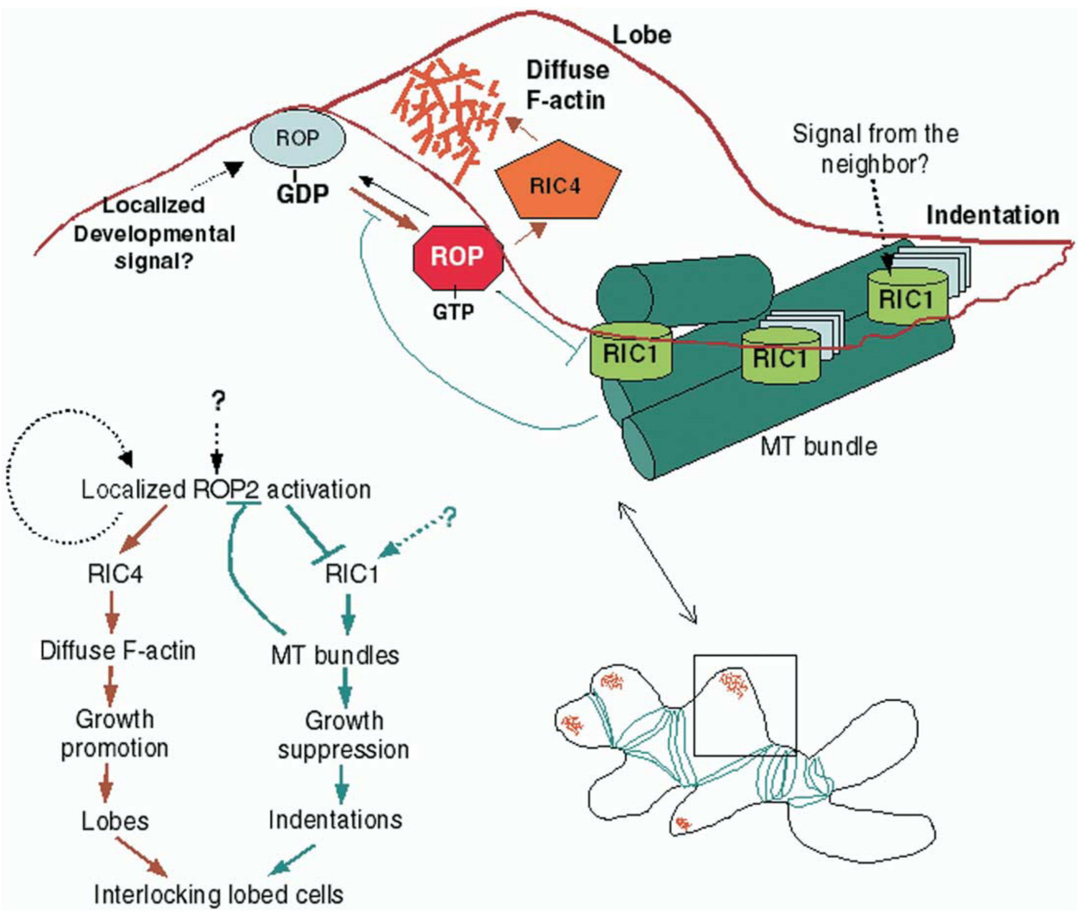


The microtubule response to tension maintains cell shapes



Sampathkumar *et al.,* eLife 2014

Pavement cell morphogenesis is well described molecularly



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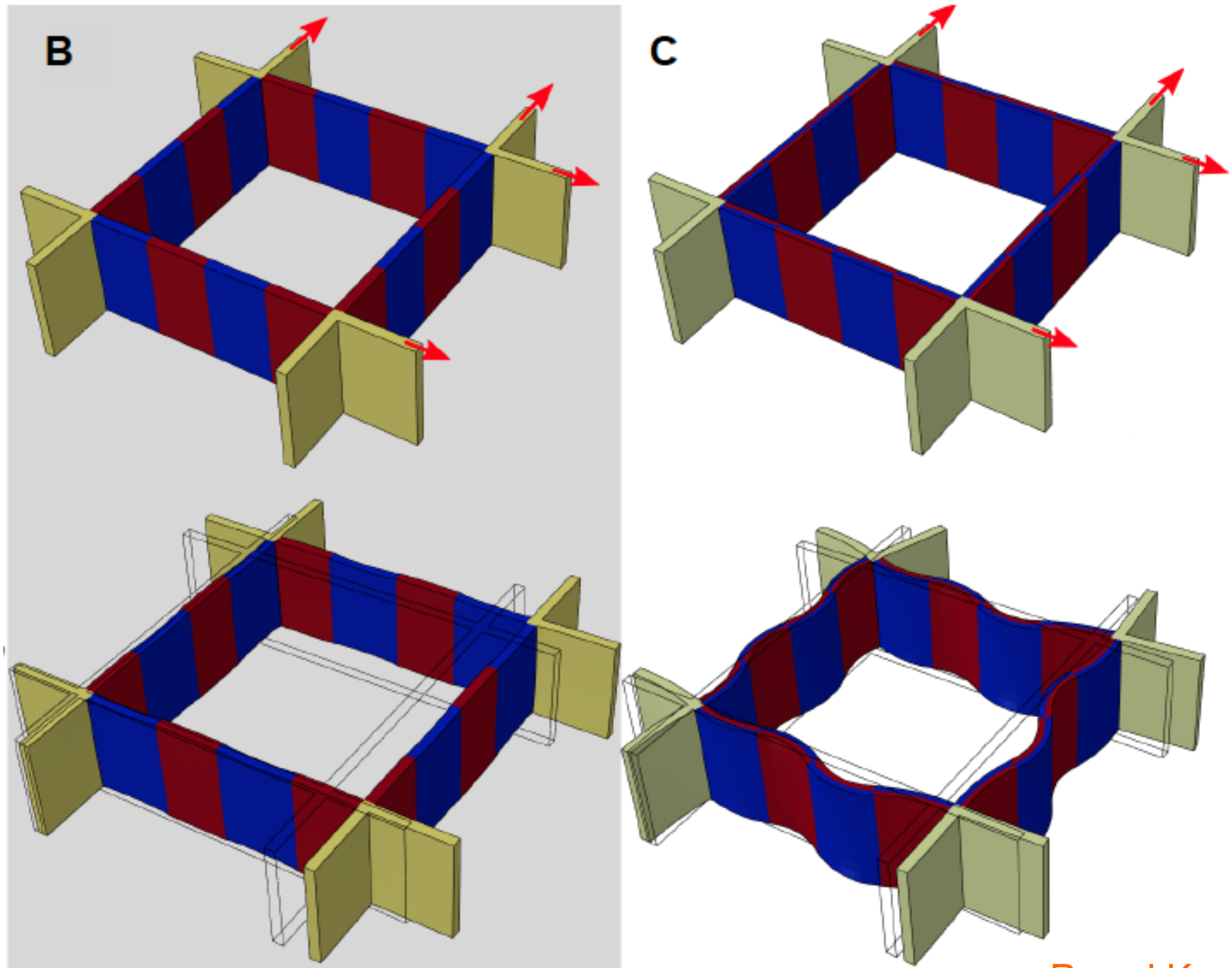
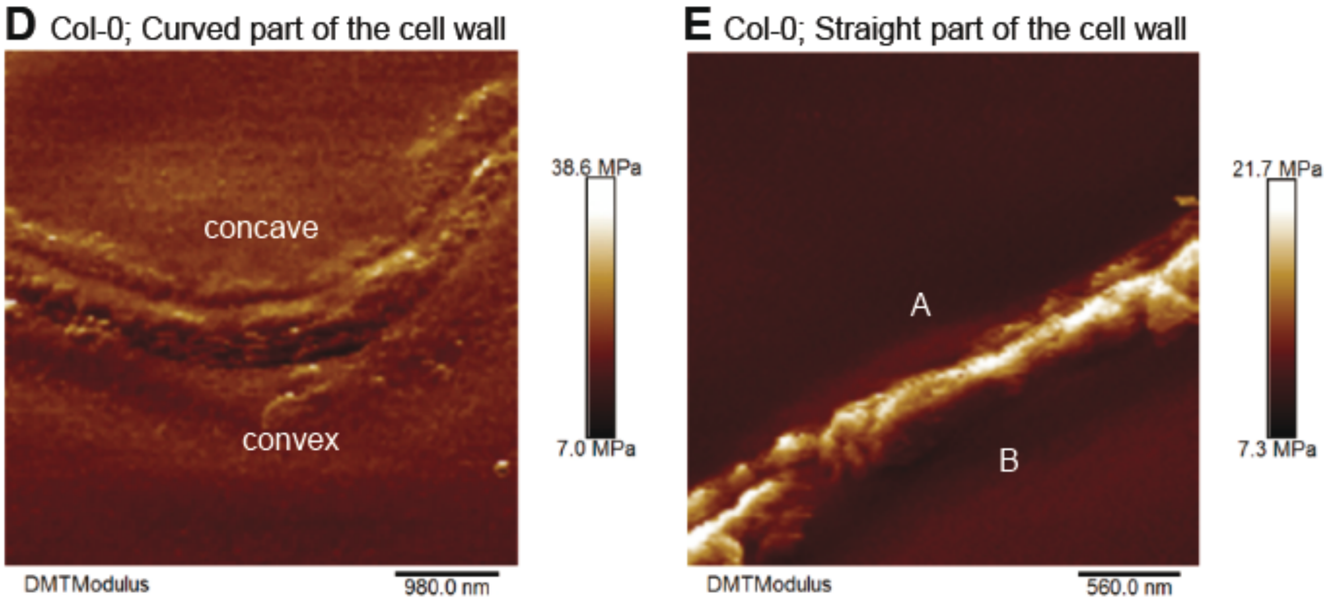
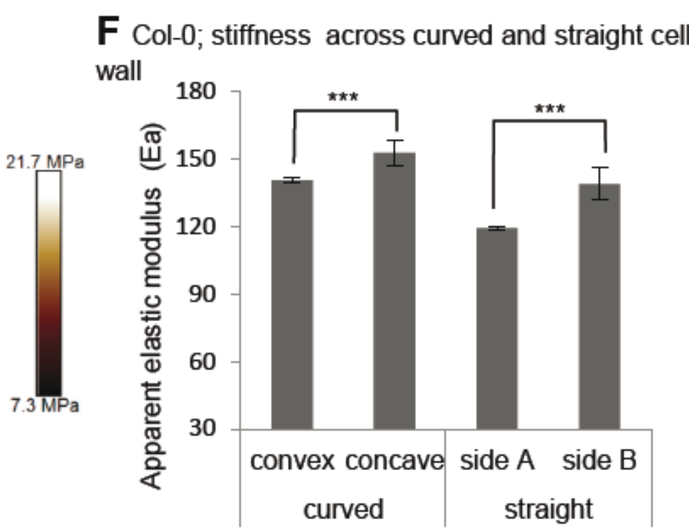
Anticlinal walls: the "buckling under tension" paradox



Pawel Krupinski

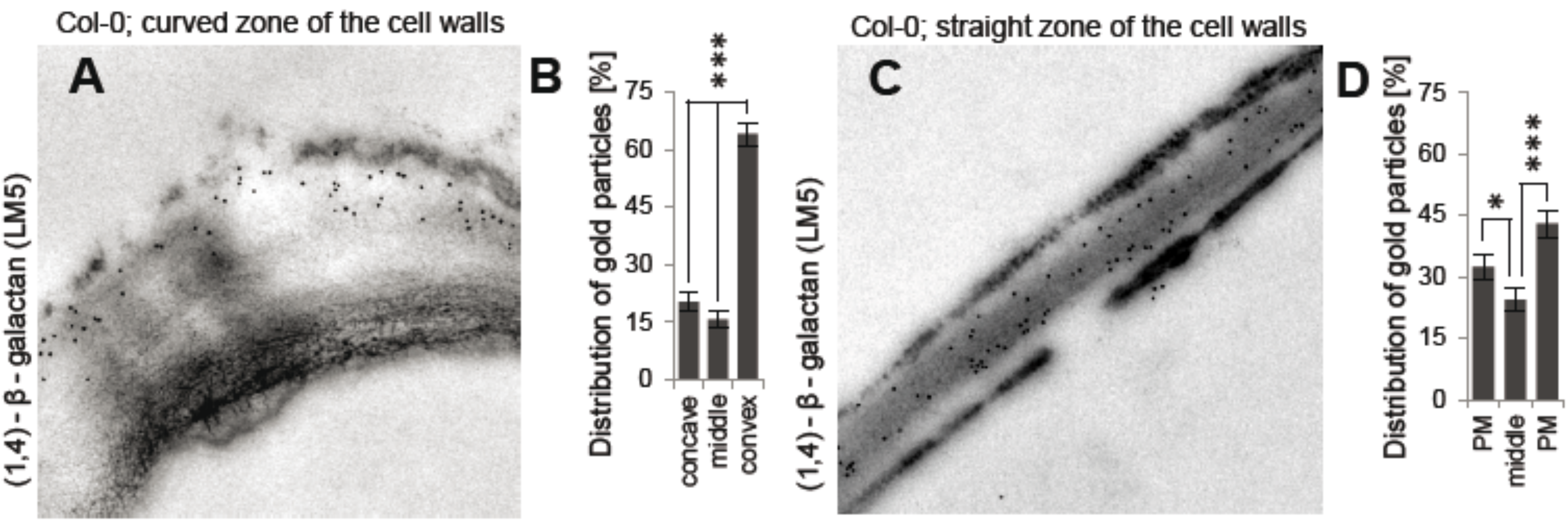
Probing anticlinal wall stiffness with AFM (on sections)

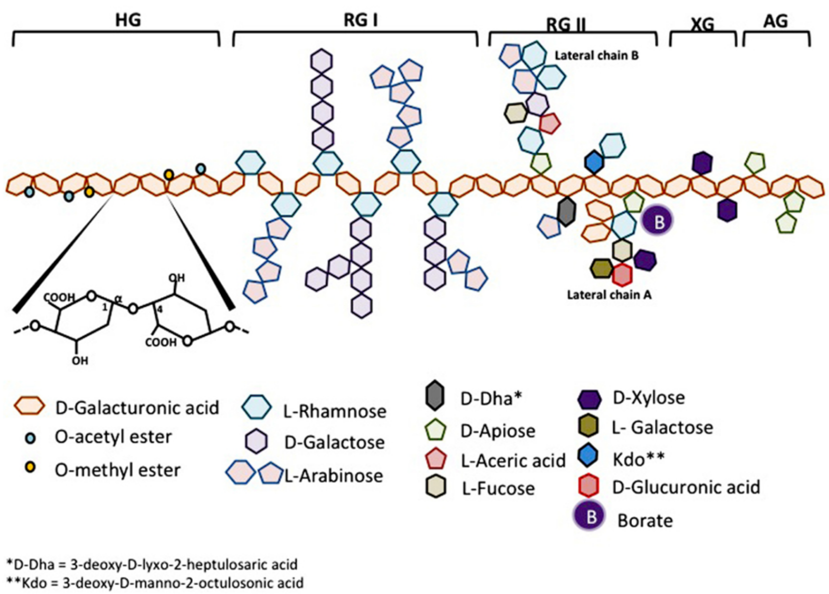




Pascale Minali and Mateusz Majda

A matching biochemical pattern

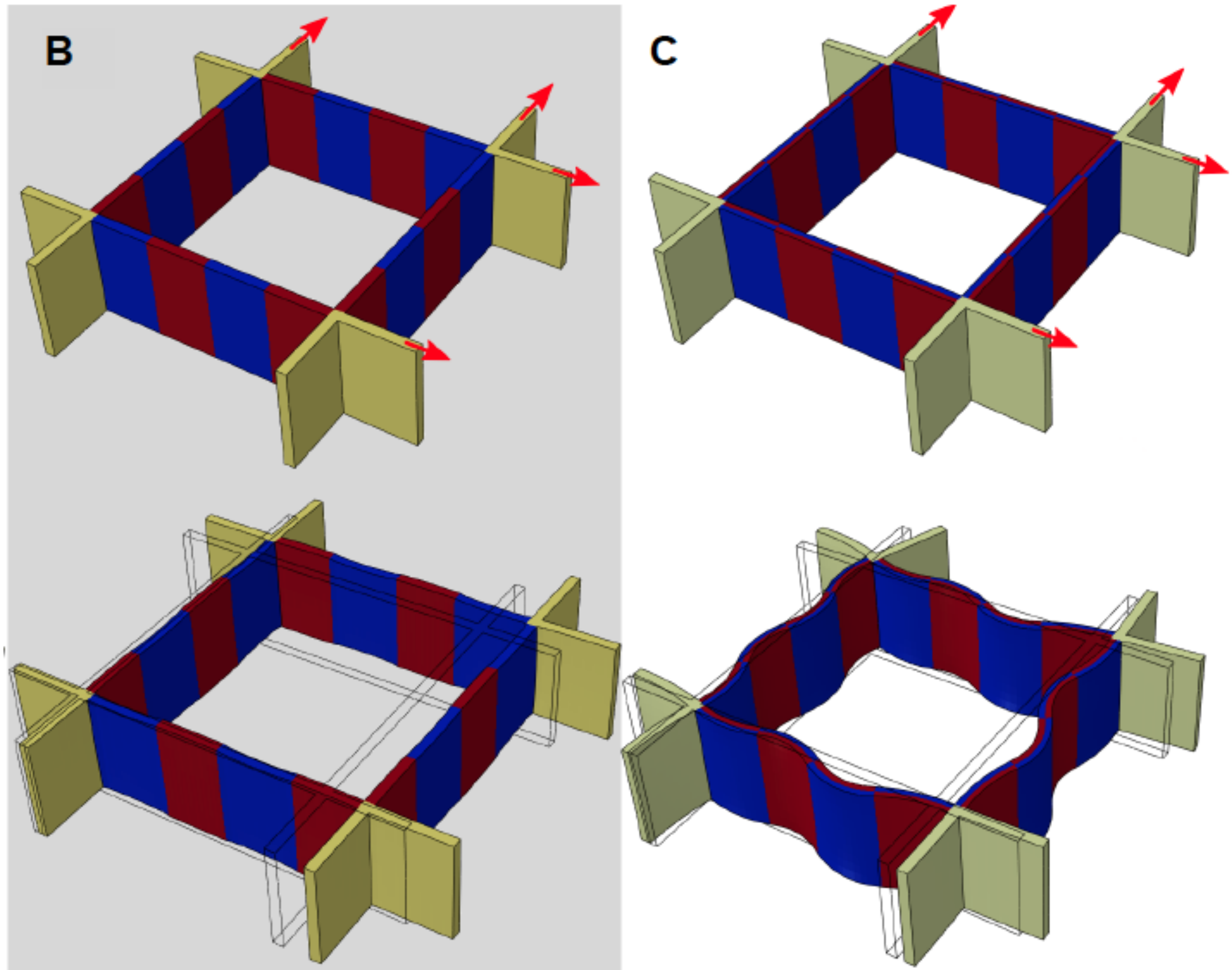




(1-4)-β-D-galactan is often associated with the pectic polymer rhamnogalacturonan-I

Majda *et al.*, 2017 Dev. Cell

Anticlinal walls: the “buckling under tension” paradox



Majda *et al.*, 2017 Dev. Cell

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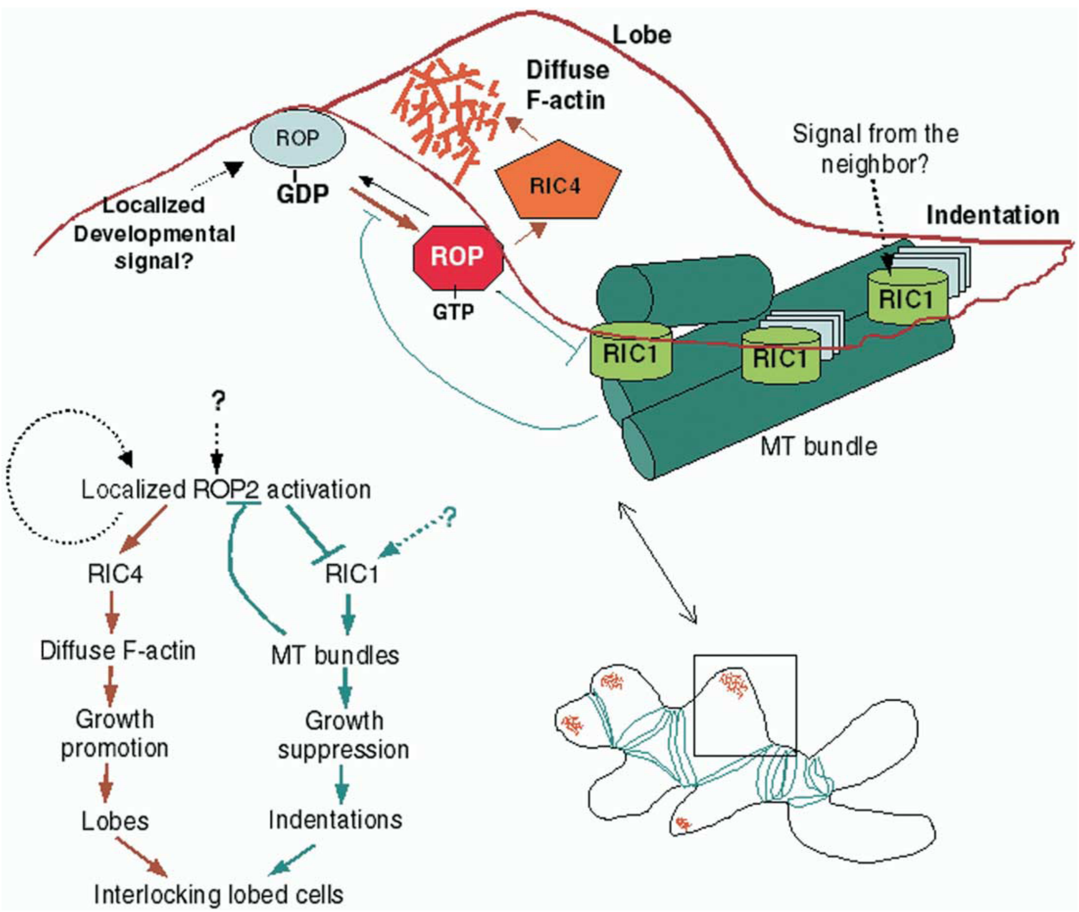

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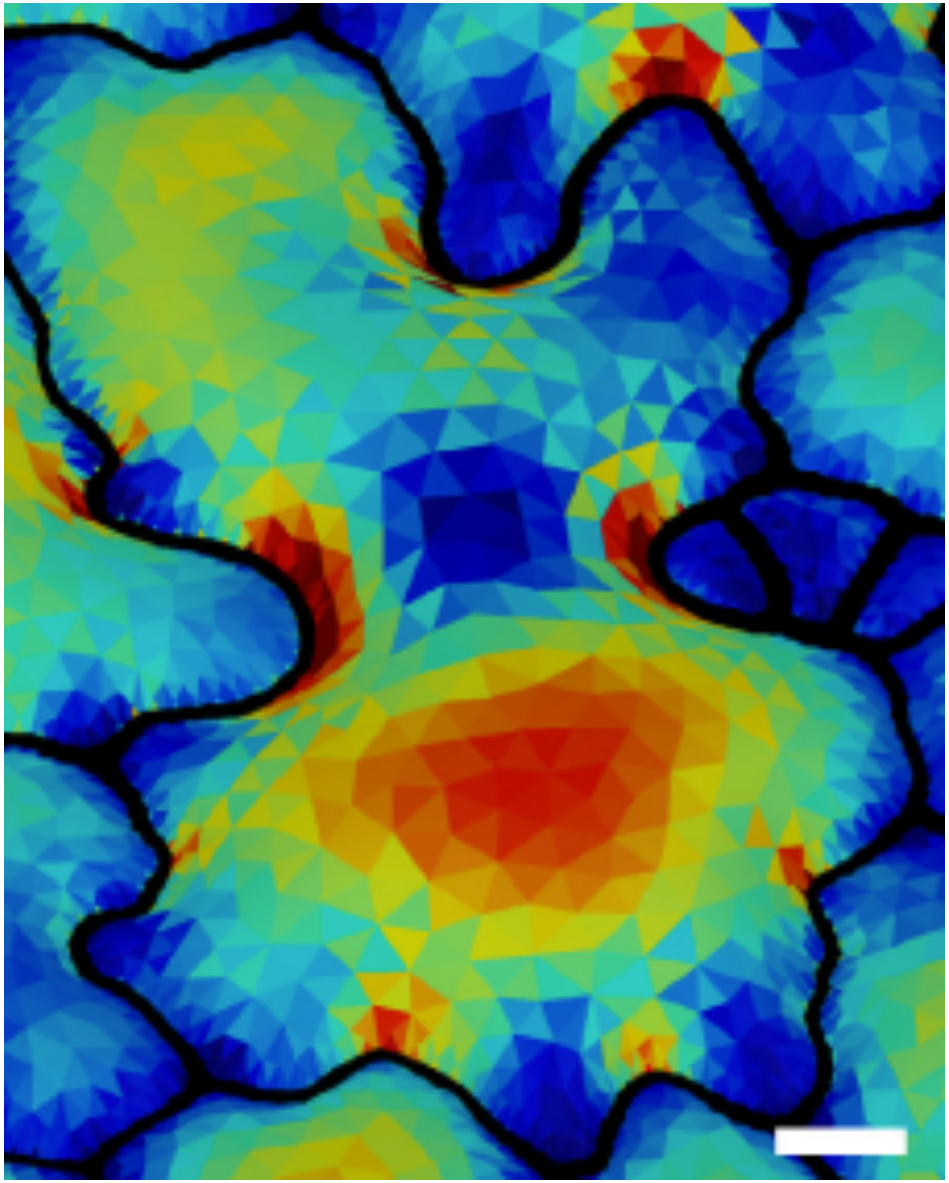
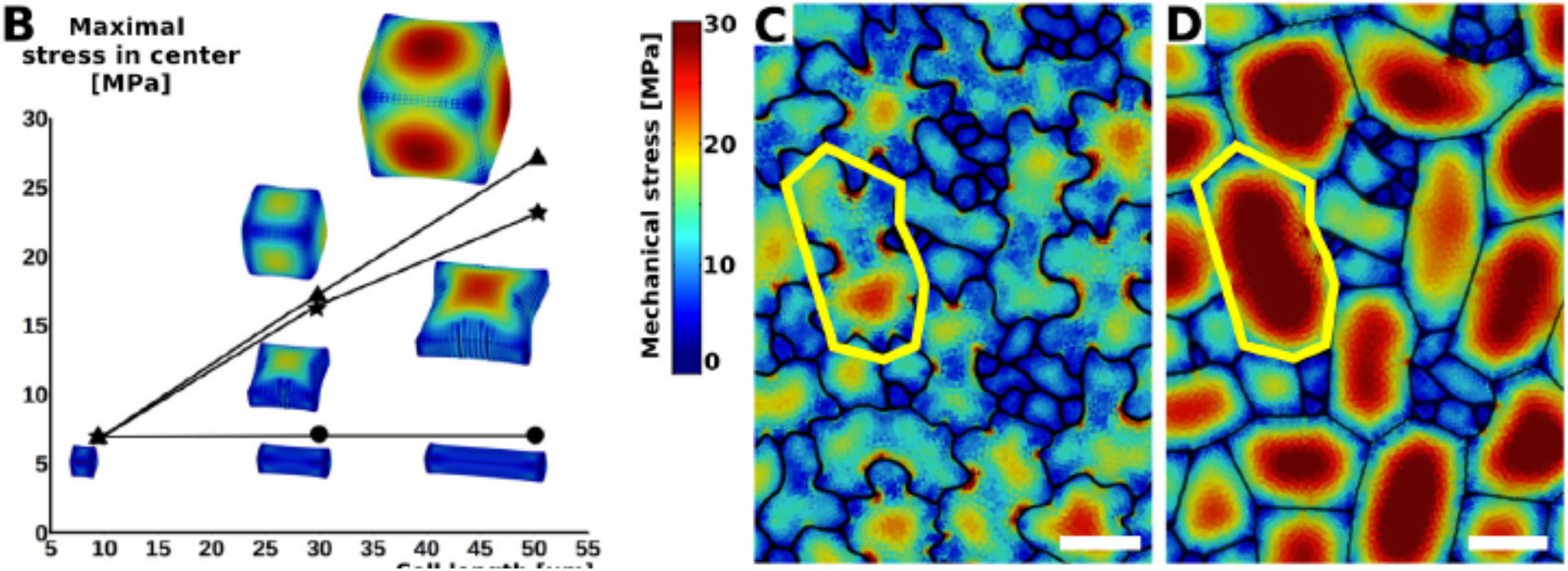
Why a jigsaw puzzle shape?

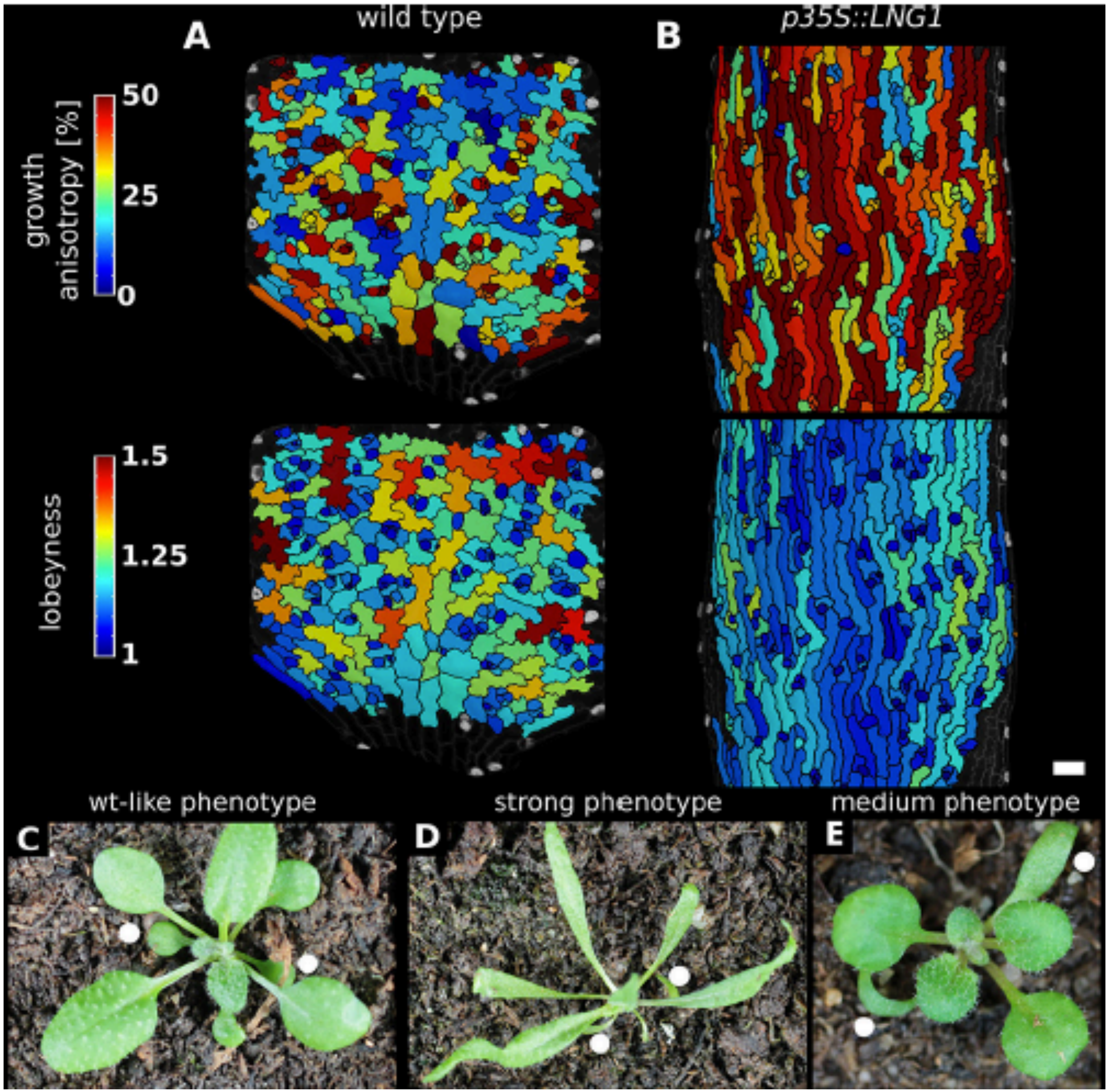


Why a jigsaw puzzle shape?



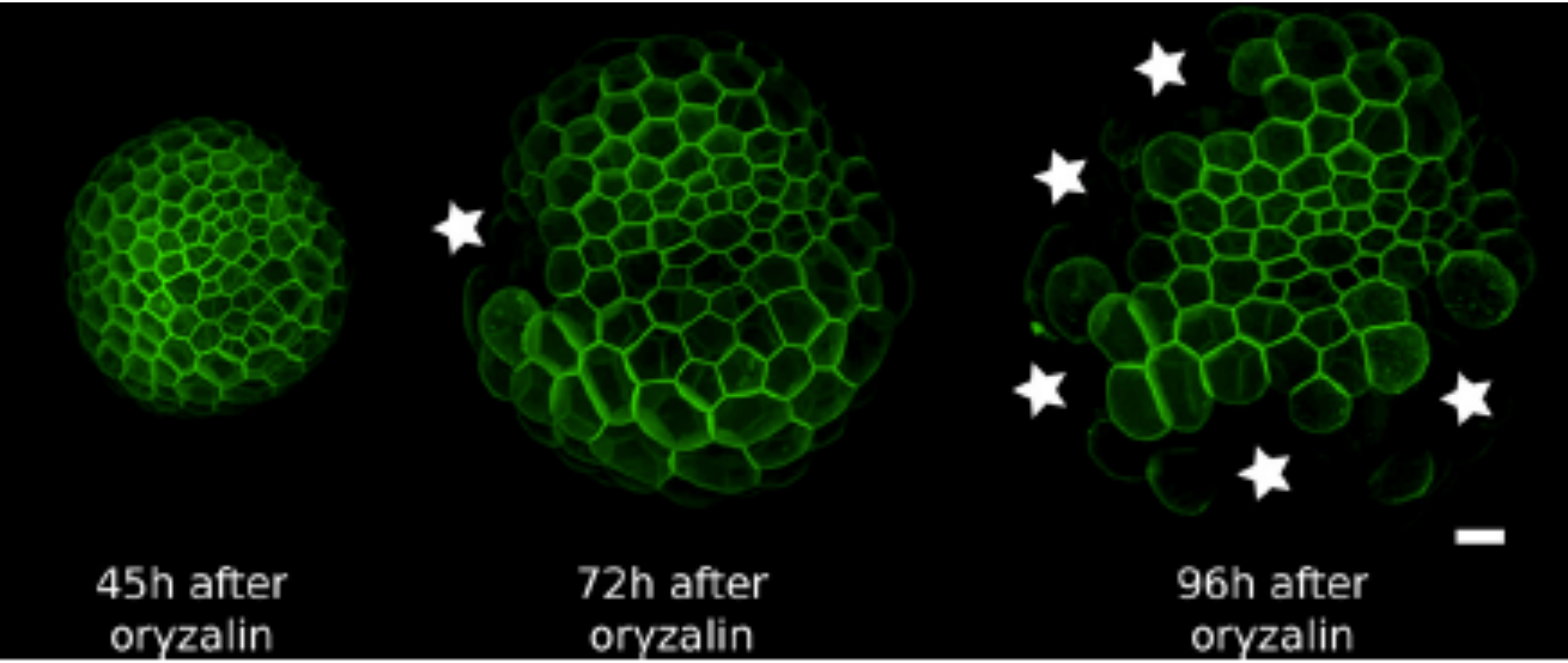
Sapala *et al.*, 2018 eLife

Growth isotropy positively correlates with lobeyness



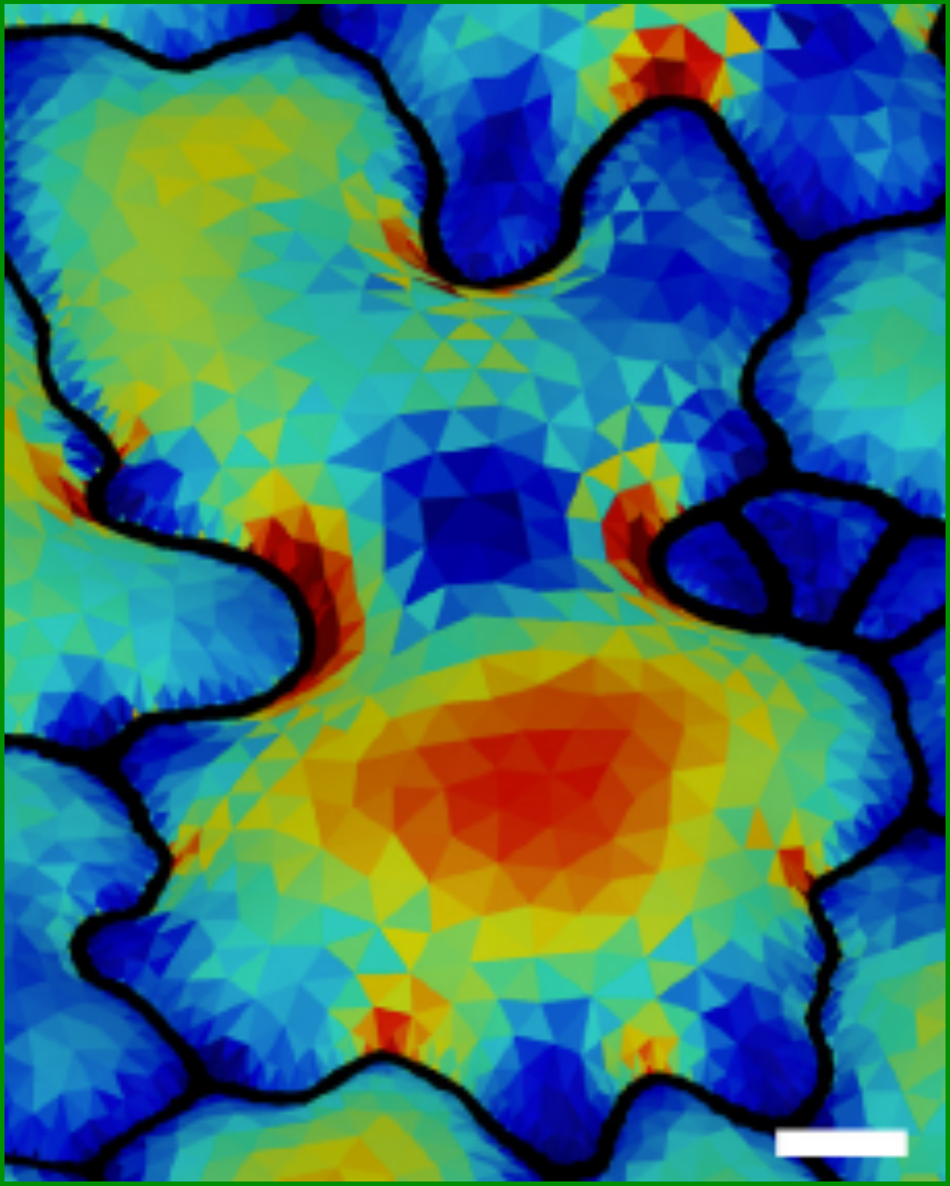
Sapala *et al.*, 2018 eLife

Prolonged isotropic growth leads to (big) cell bursting



Sapala *et al.*, 2018 eLife

Summary: Pavement cells revisited through mechanics



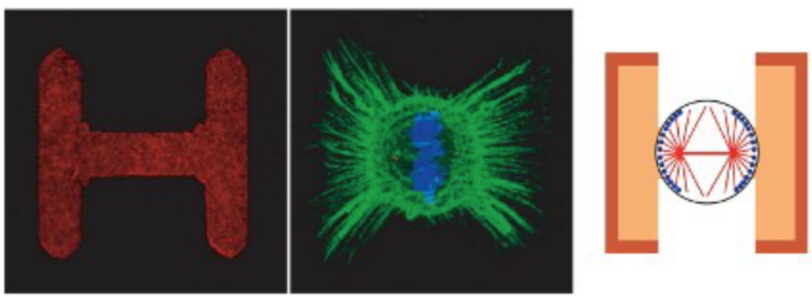
- MT stiffen walls and restrict growth in necks

- Lobes result from buckling between regions of different stiffness in a tissue under tension

- Function: managing stress levels

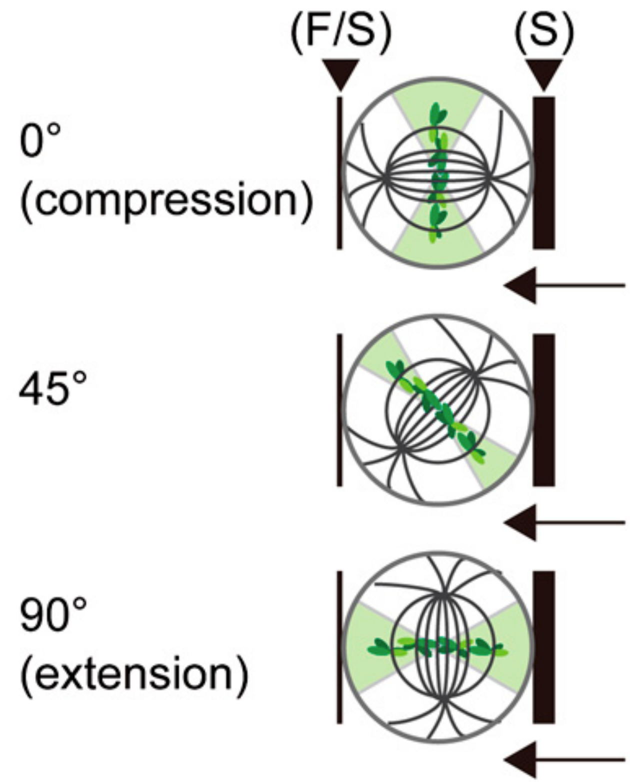
Conserved mechanical signals in plants and animals?

Mechanical cues control cell division profiles



Mitotic spindle orientation depends on pattern of stress

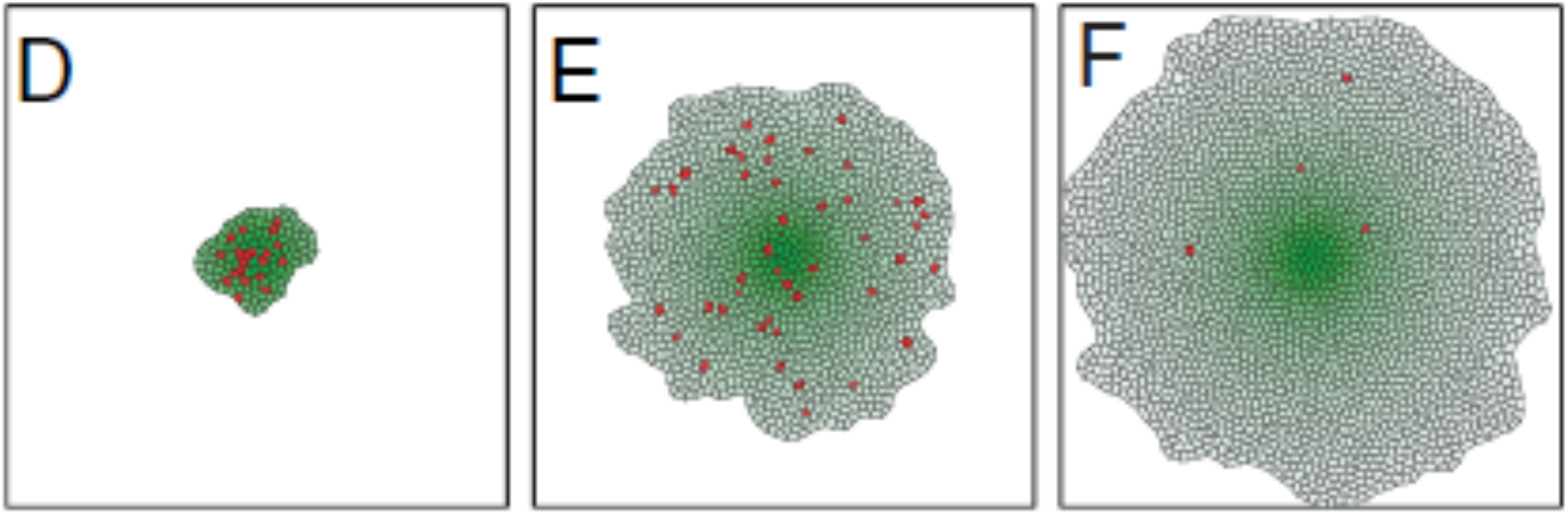
Théry et al., 2007 Nature



Extra tension in the spindle accelerates anaphase progression

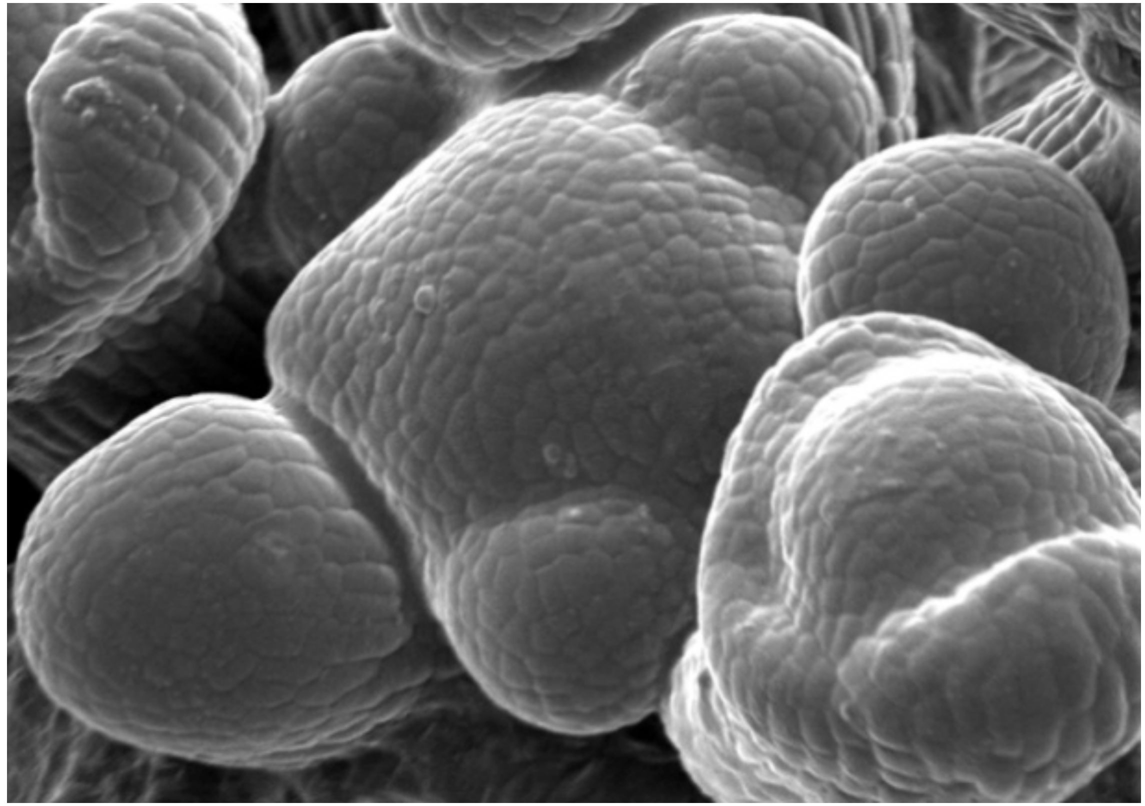
Itabashi et al,. 2012 PNAS

A role of mechanical forces in growth homeostasis?



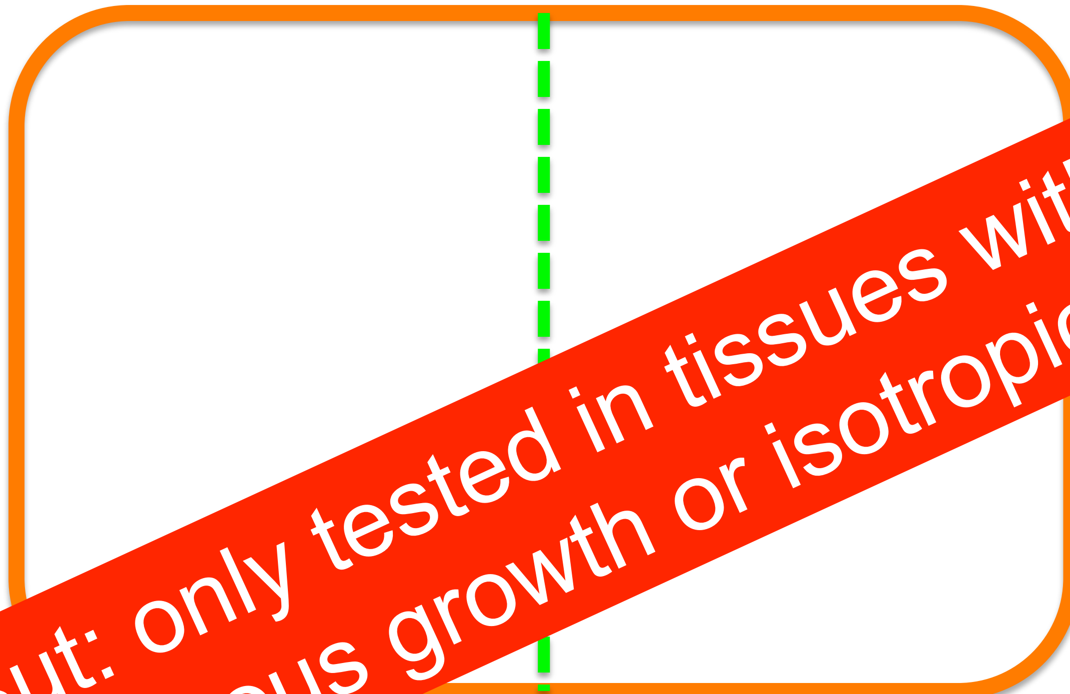
- **Dpp** induces cell proliferation (growth)
- Differential growth compress the tissue and reduces **cell proliferation**

Sraiman, 2005 PNAS; Hufnagel *et al.*, 2007 PNAS



Cell division under mechanical control in plants too?

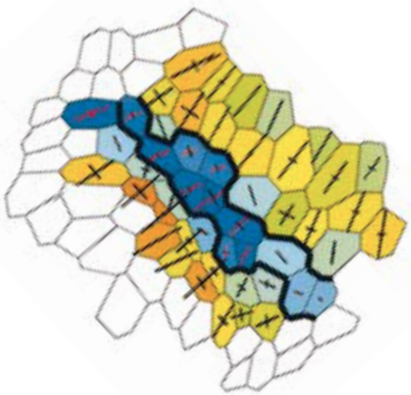
Cell division plane orientation depends on cell geometry



But: only tested in tissues with
homogeneous growth or isotropic shape

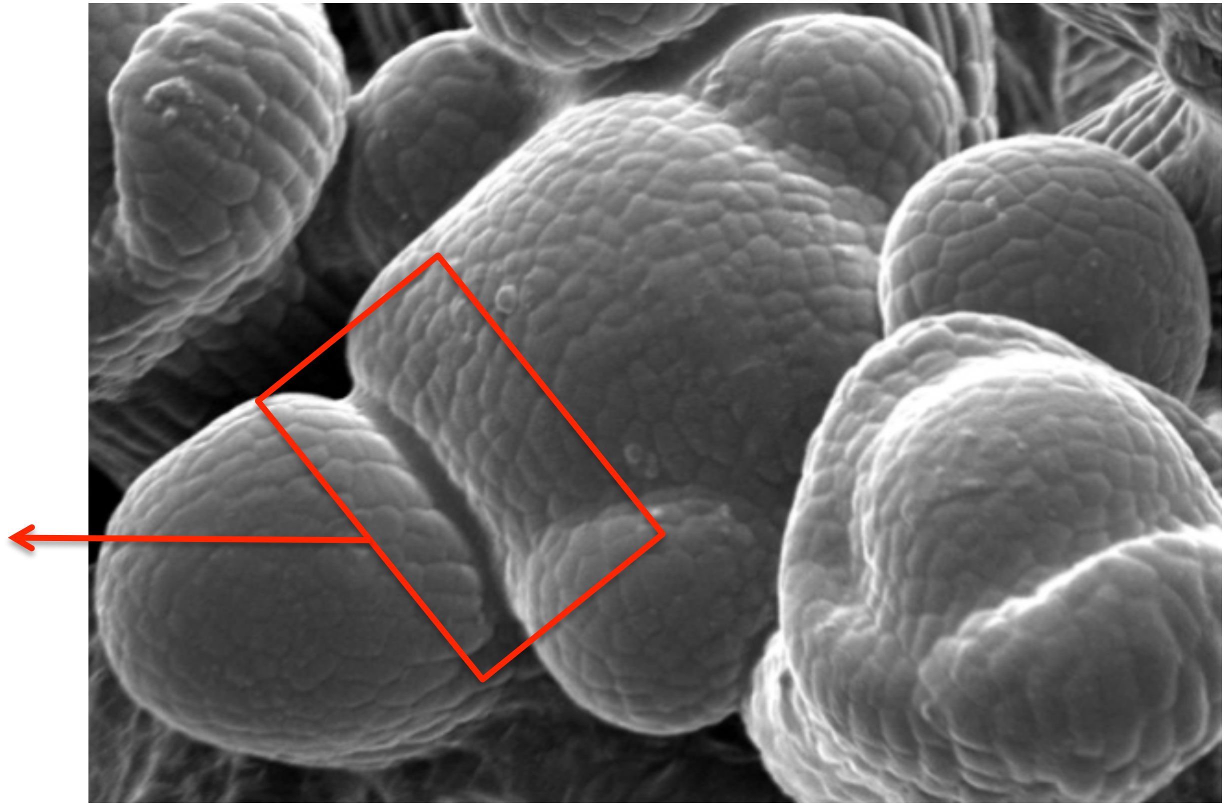
Errera's rule

Errera 1898 CRAS
Dupuy *et al.*, 2010 PNAS
Besson and Dumais, 2011 PNAS



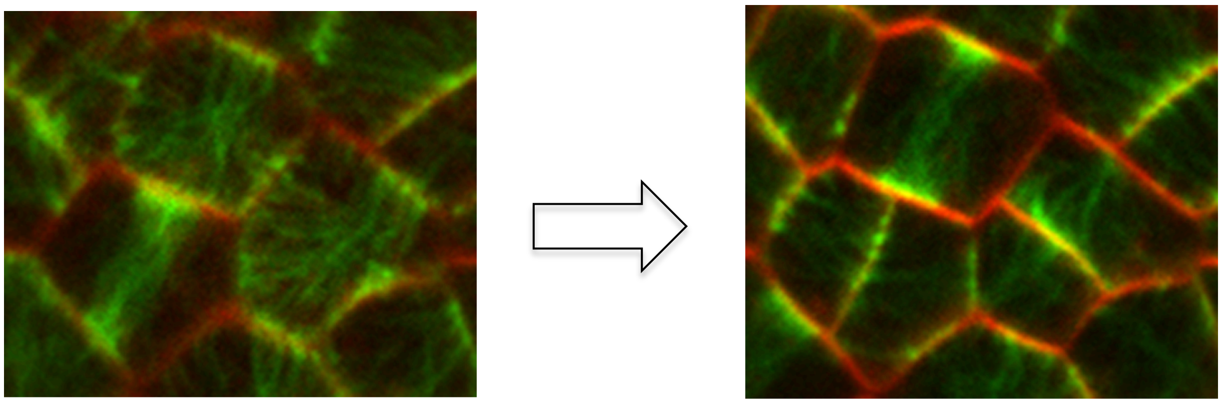
Strain rate

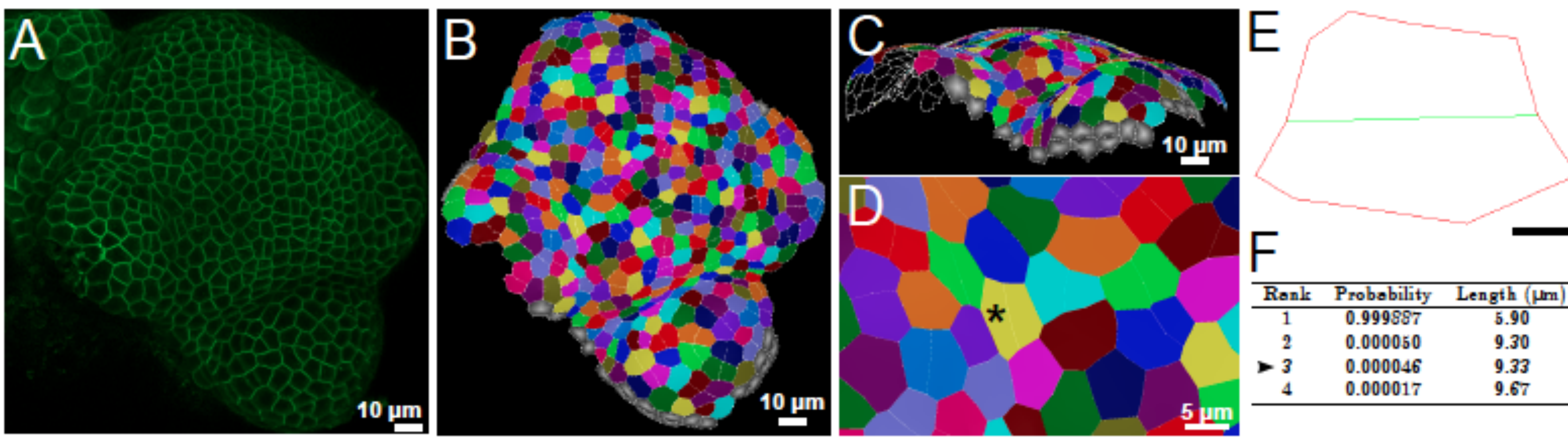
Kwiatkowska and Dumais, 2003 J. Exp Bot



Differential growth at the boundary
and
Cell division

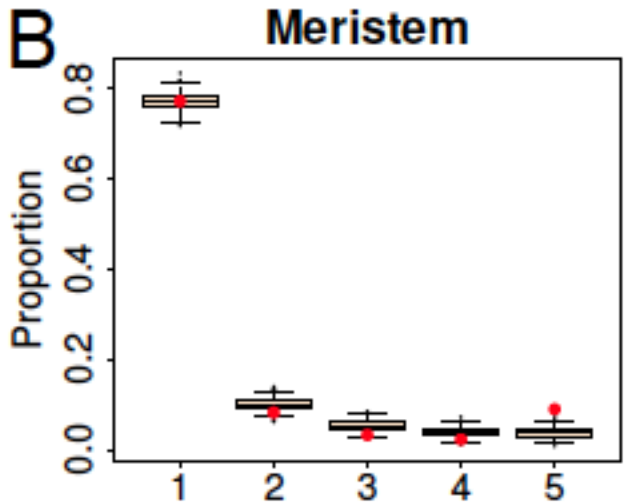
Testing Errera's rule in the shoot apical meristem

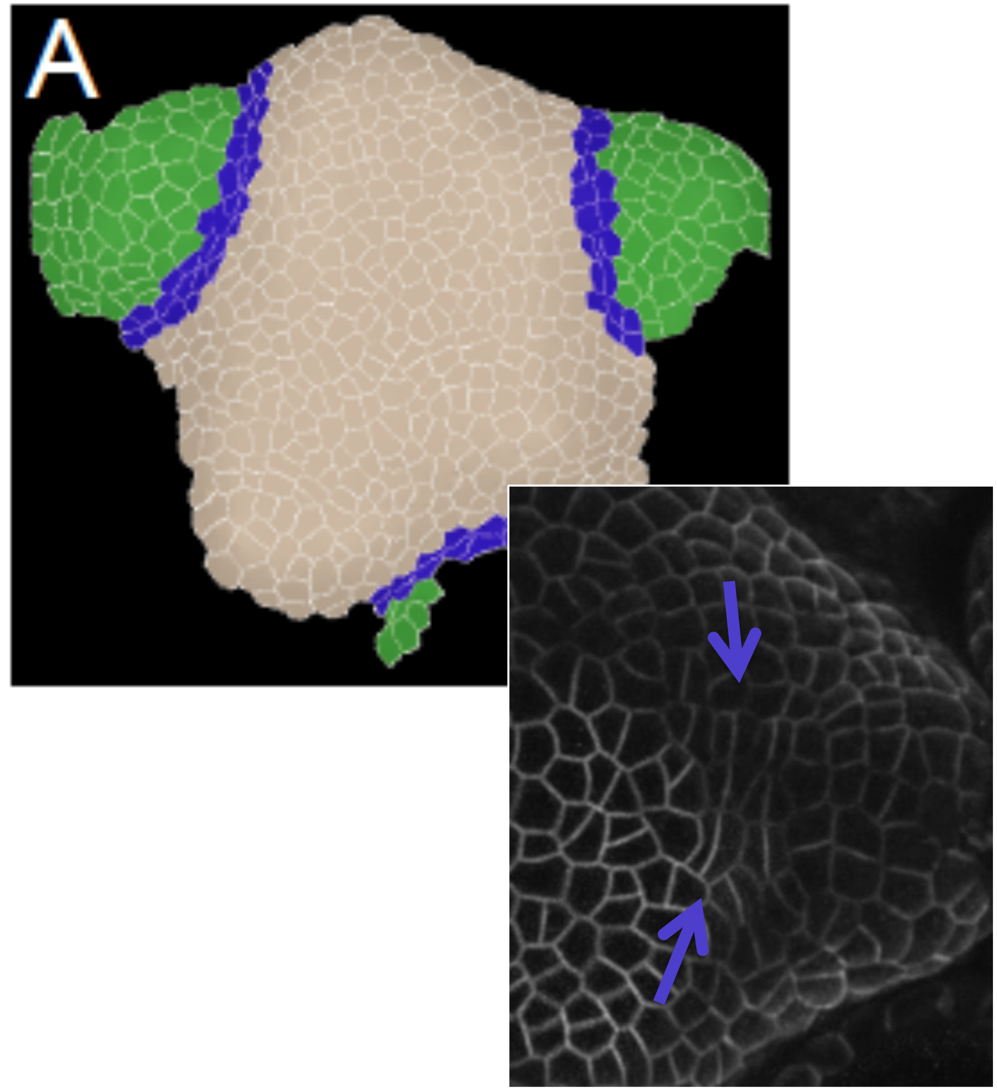




Rank	Probability	Length (µm)
1	0.999887	5.90
2	0.000050	9.30
➤ 3	0.000046	9.33
4	0.000017	9.67

A bias towards longer planes in the boundary domain

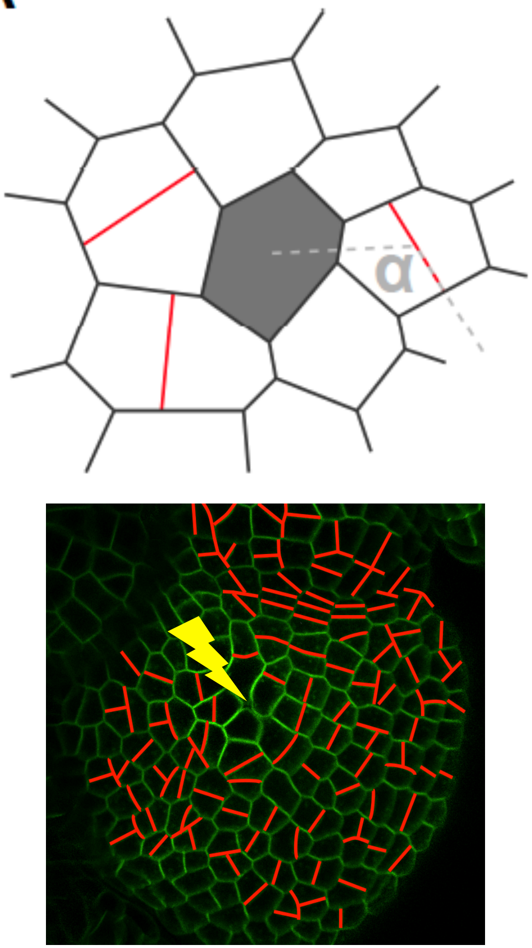




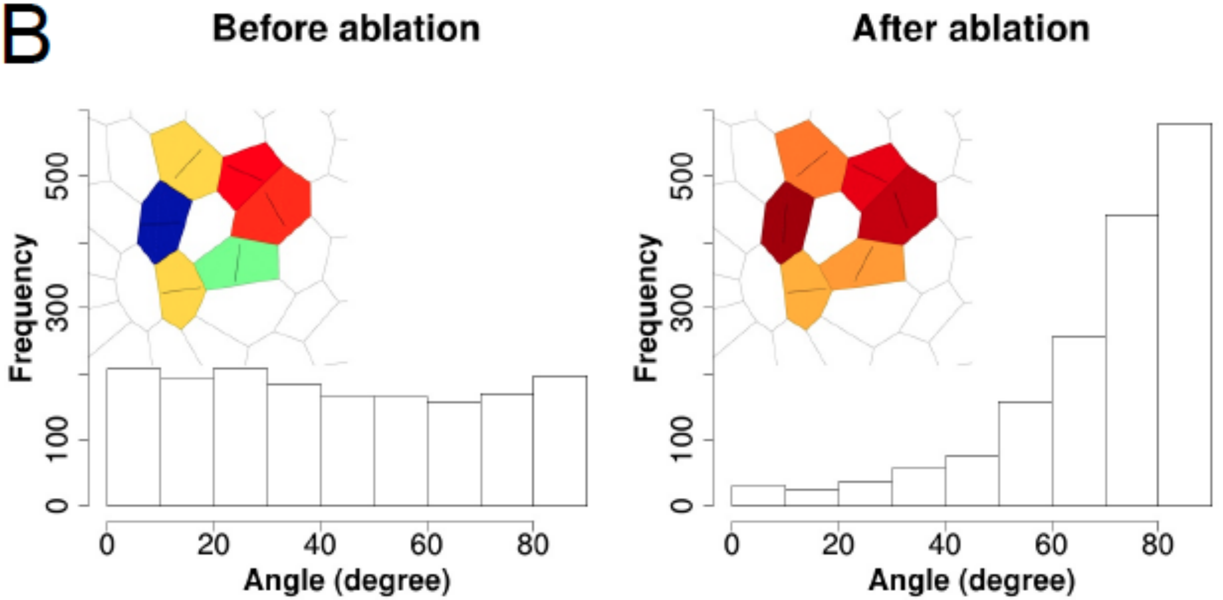
Longer planes

Changing mechanical stress locally affects the new division plane orientations

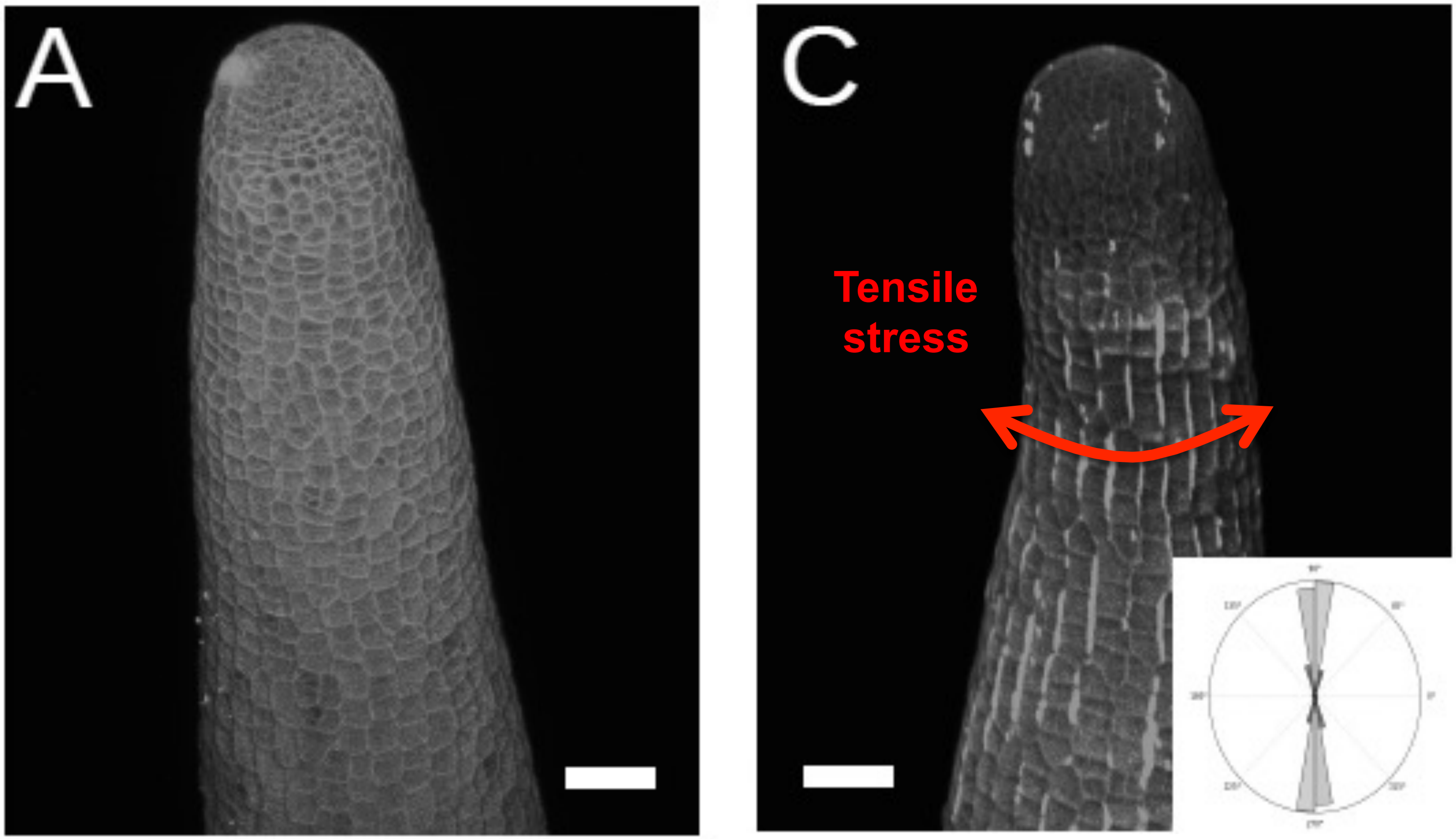
A



B



Shape-induced stress in cylindrical stem

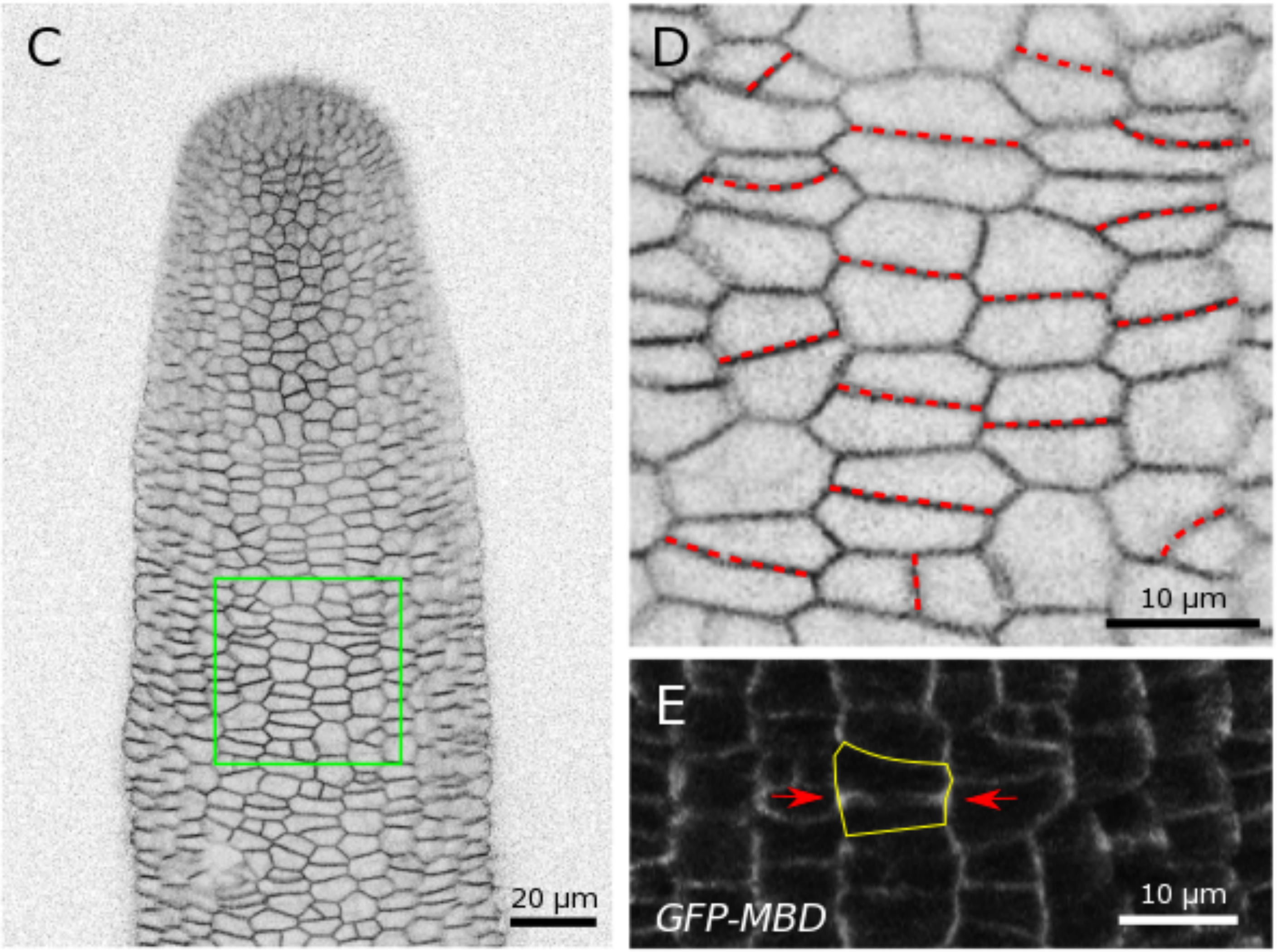


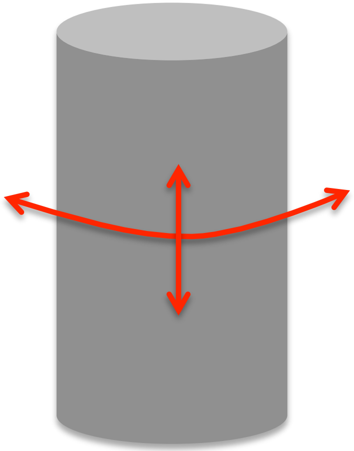
WT

qua1 (Agar 2.5%)

Verger *et al.*,, eLife *in press*

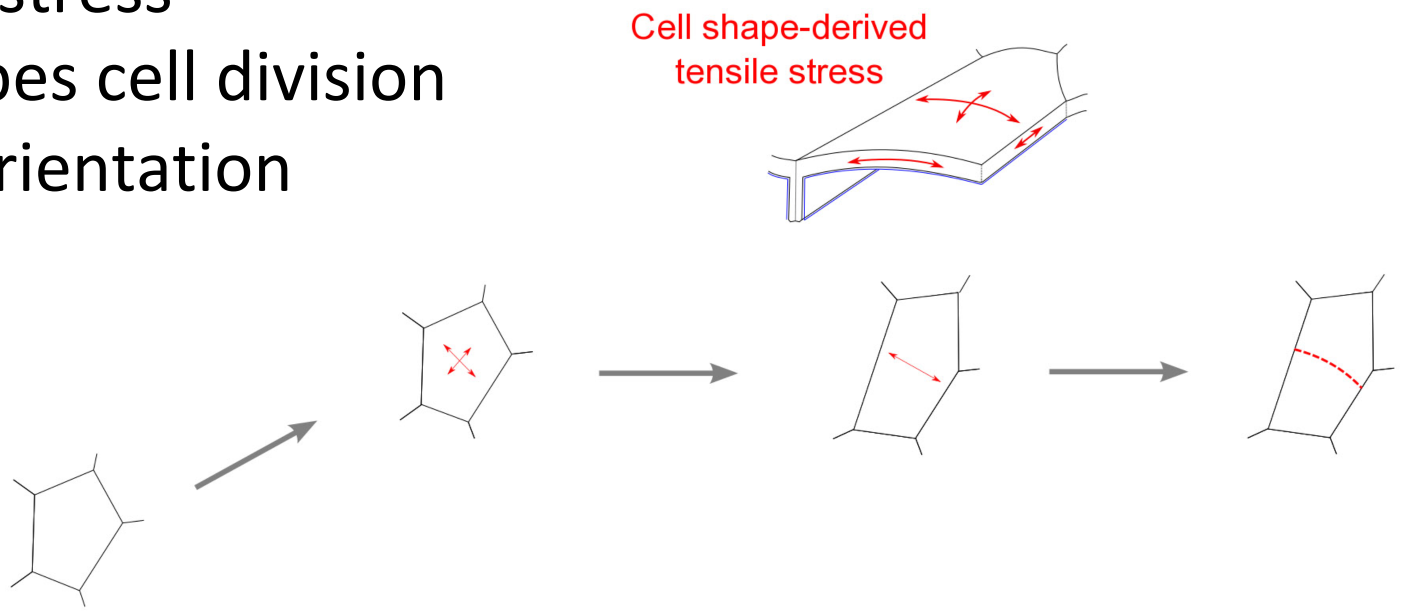
Tensile stress prescribes cell division plane orientation





Louveaux *et al.* 2016 PNAS

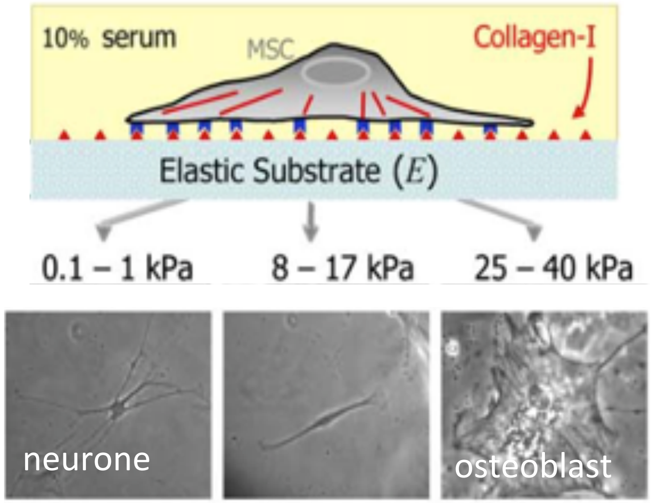
Tensile stress prescribes cell division plane orientation



Louveaux *et al.* 2016 PNAS

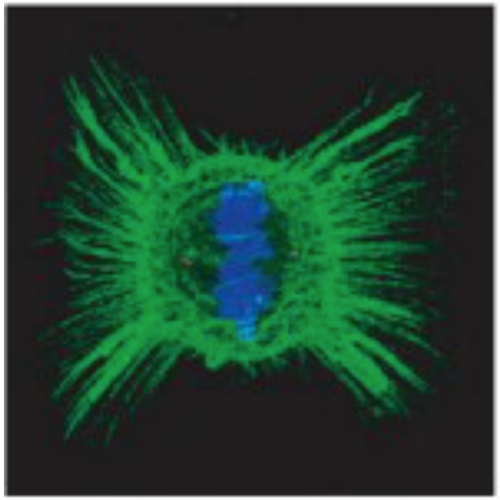
Cells sense their mechanical environment and adapt their behavior accordingly

Cell fate



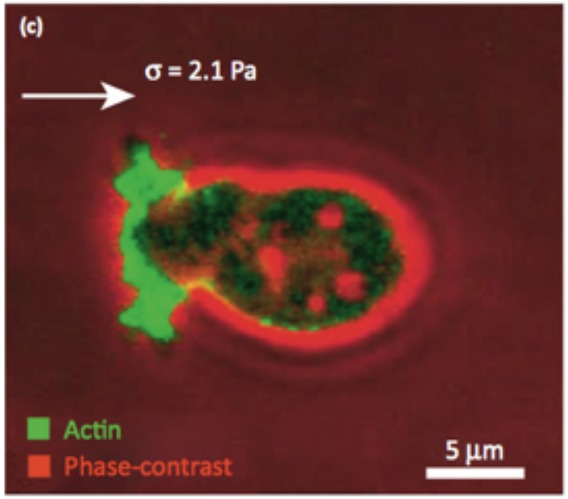
Engler *et al*, Cell, 2006

Cell division



Théry *et al*, Nature, 2007

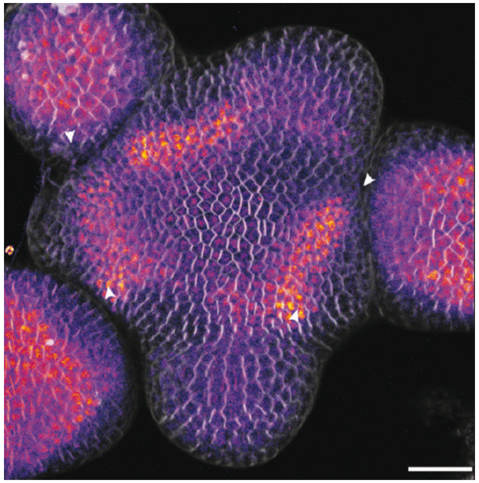
Cell polarity



Dalous, Biophys J, 2008

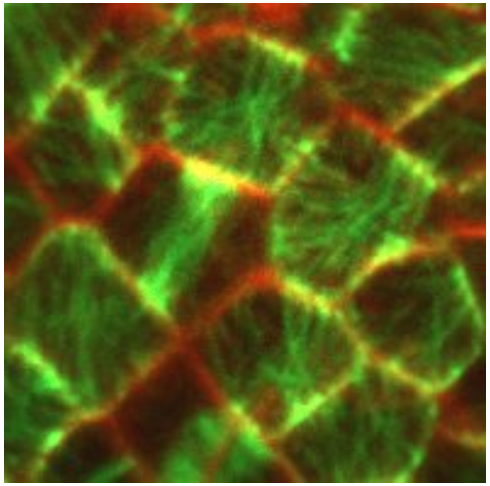
Cells sense their mechanical environment and adapt their behavior accordingly

Cell fate



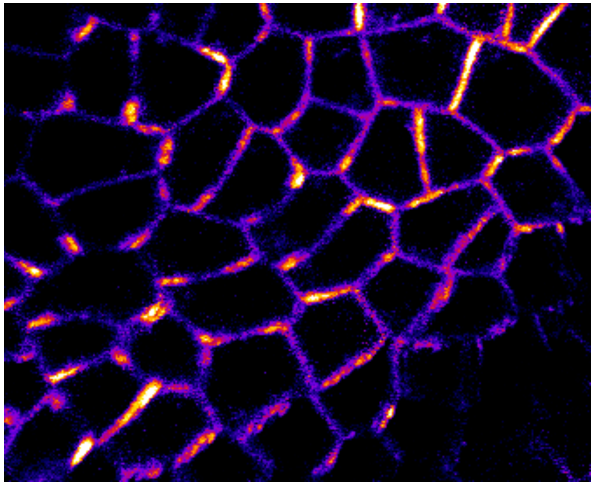
Coutand *et al.*, 2009 Plant physiol.
Landrein *et al.*, 2015 eLife
Fal *et al.*, 2016 PSB

Cell division



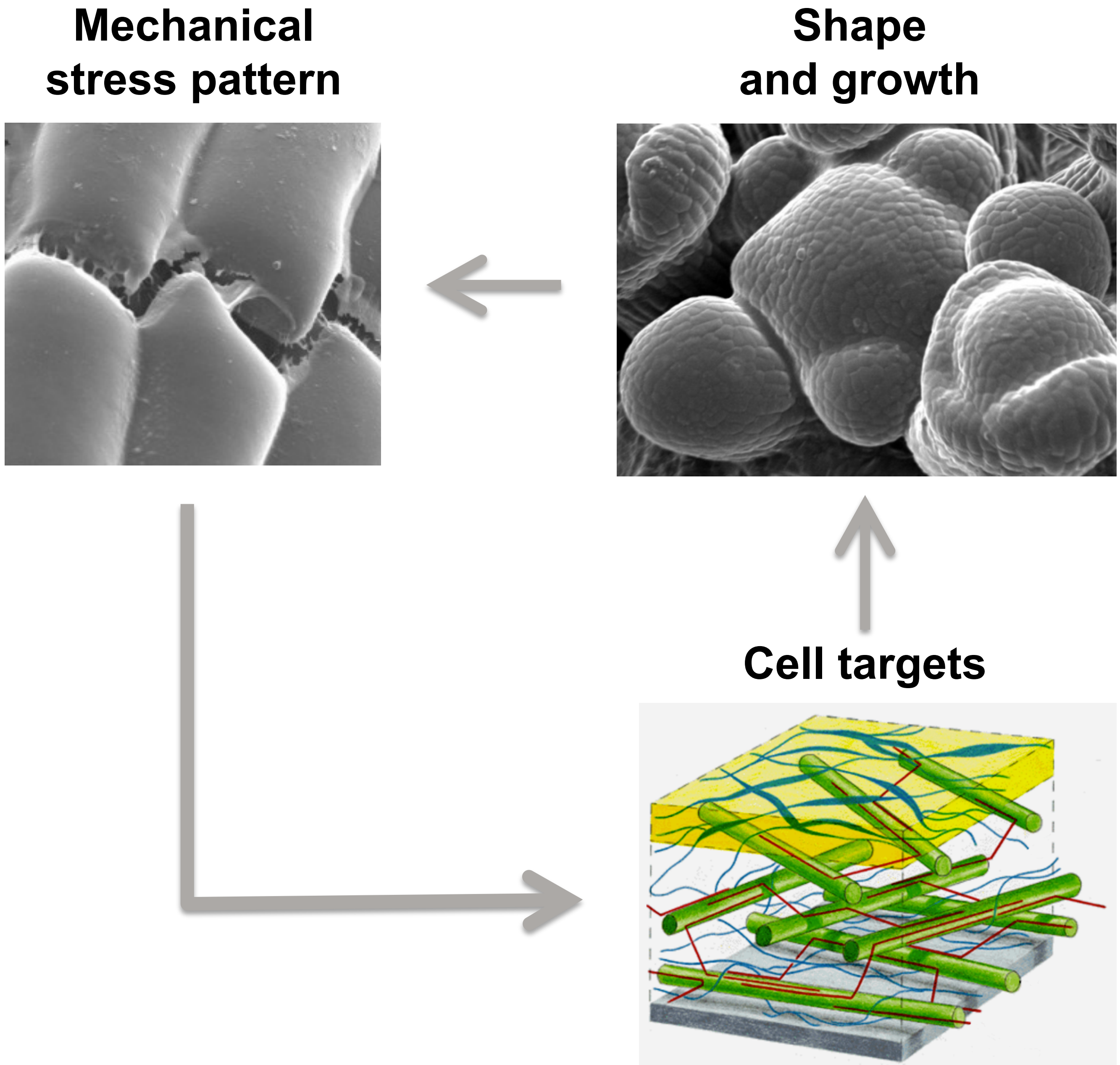
Lintilhac *et al.*, 1984 Nature
Louveaux *et al.*, 2016 PNAS

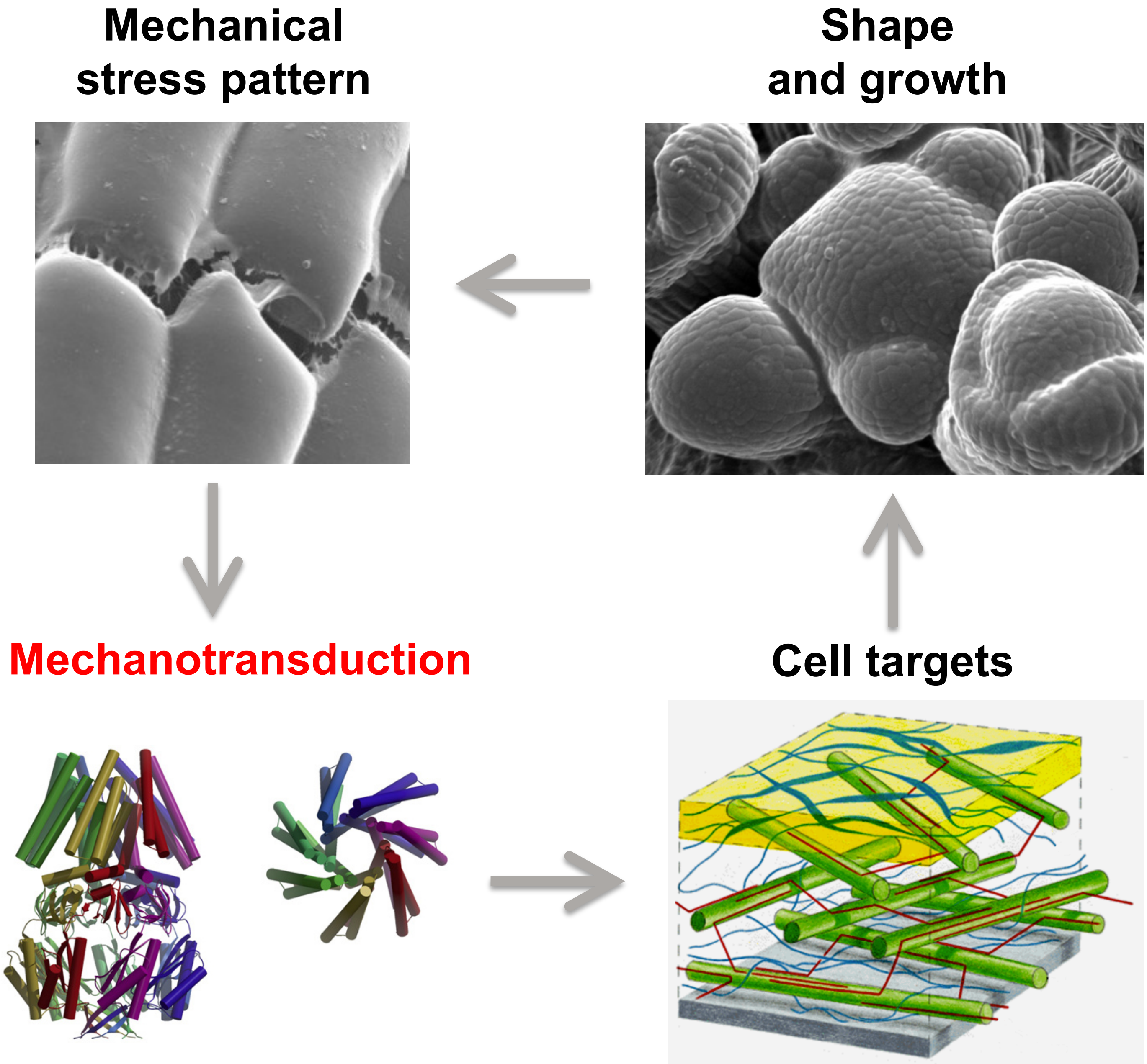
Cell polarity



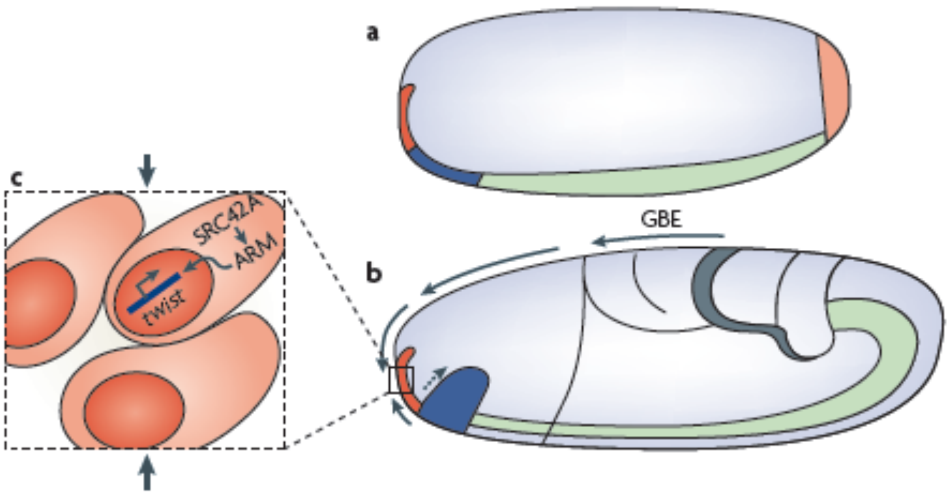
Heisler *et al.*, 2010 Plos Biol.
Nakayama *et al.*, 2012 Curr Biol.
Bringmann *et al.*, 2017 Curr Biol.

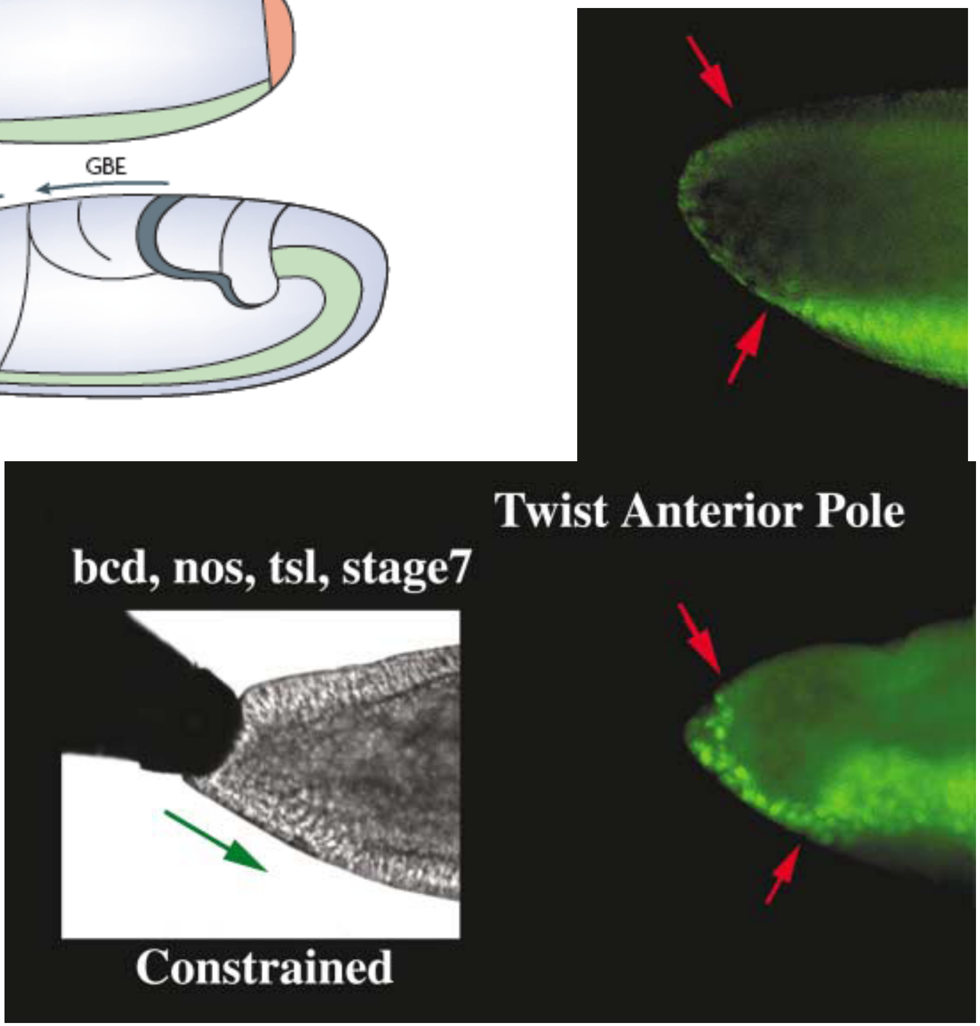
Mechanotransduction pathways


Mechanical stress pattern
Shape and growth
Cell targets


Mechanical
stress pattern
Shape
and growth
Mechanotransduction
Cell targets

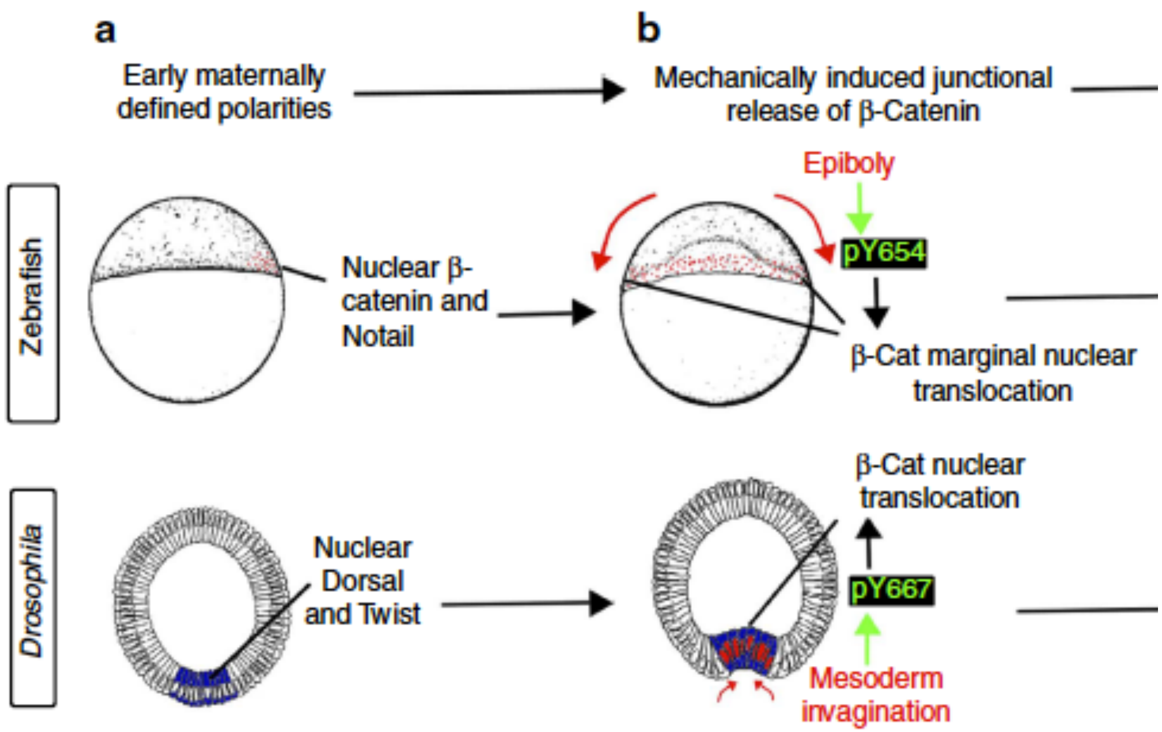
Mechanical forces as patterning signals





Farge, 2003 Curr Biol

Mechanical forces as patterning signals



Mechanical stress (gastrulation/epiboly)

↓

β-catenin phosphorylation

↓

β-catenin enters nucleus

↓

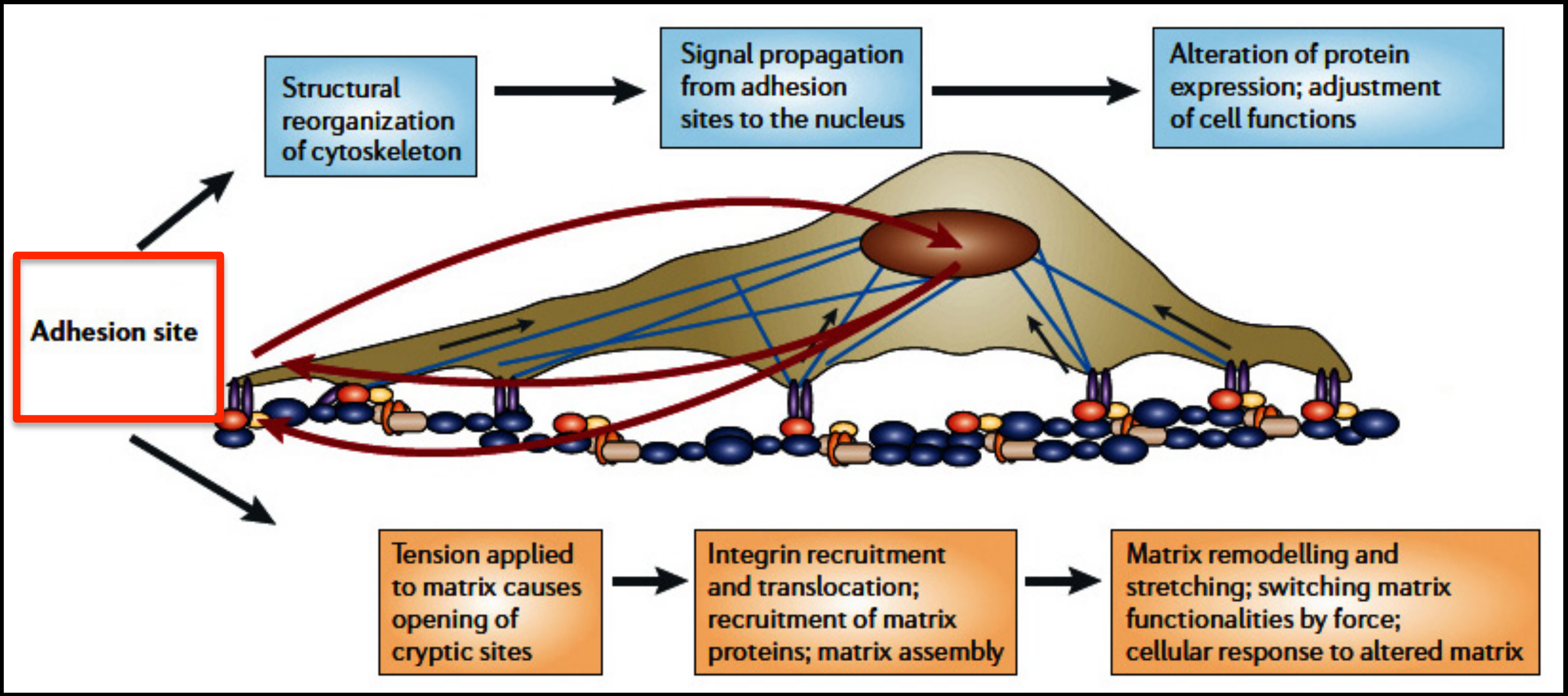
Transcription of Twist/ Notail

↓

Mesoderm identity

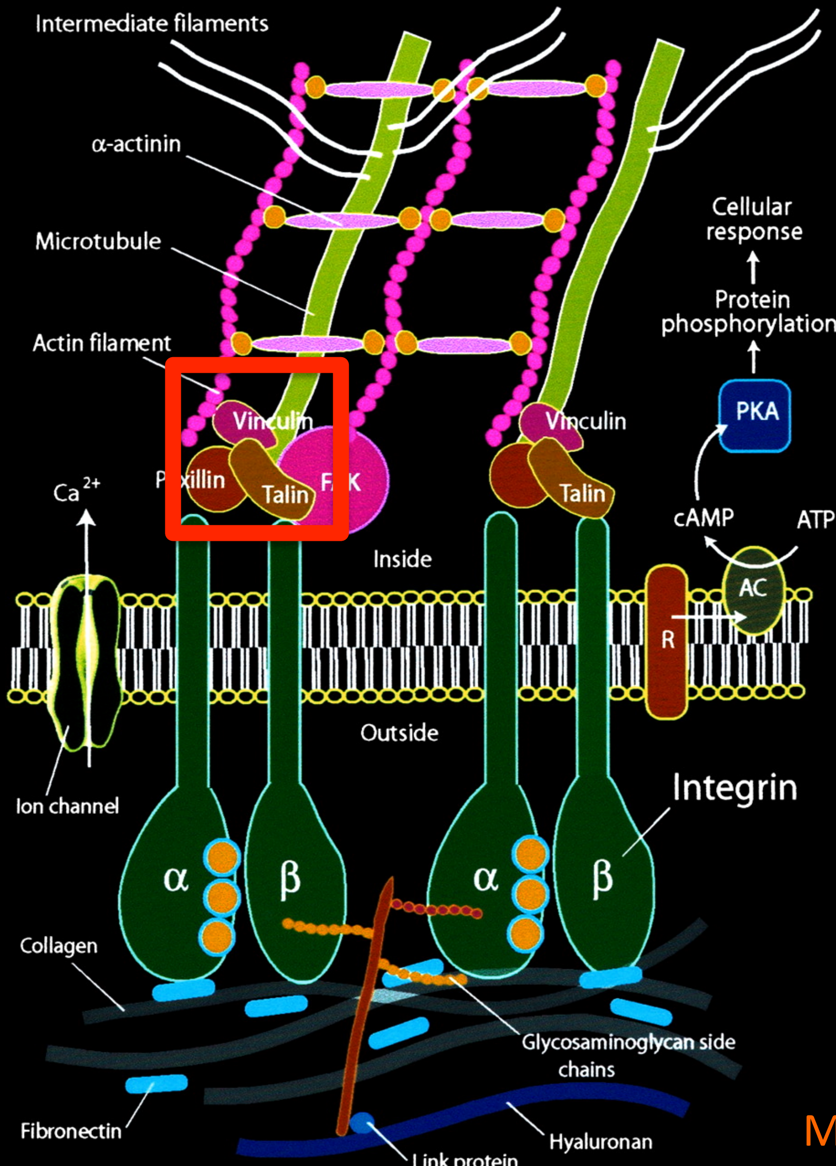
Brunet *et al.*, 2014 Nat Com.

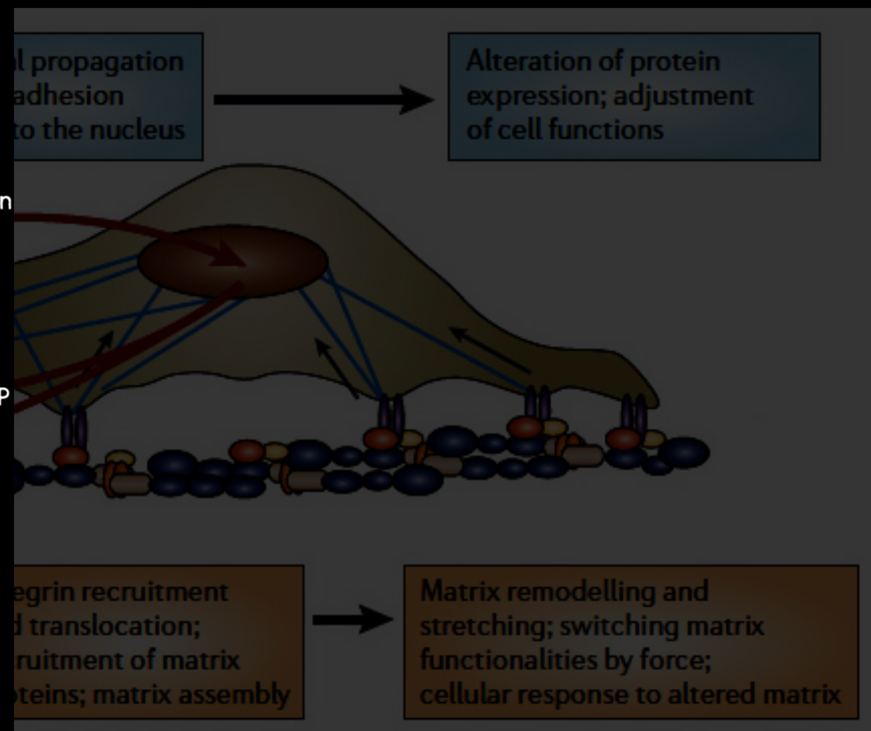
Focal adhesion points



Vogel and Sheetz, 2006

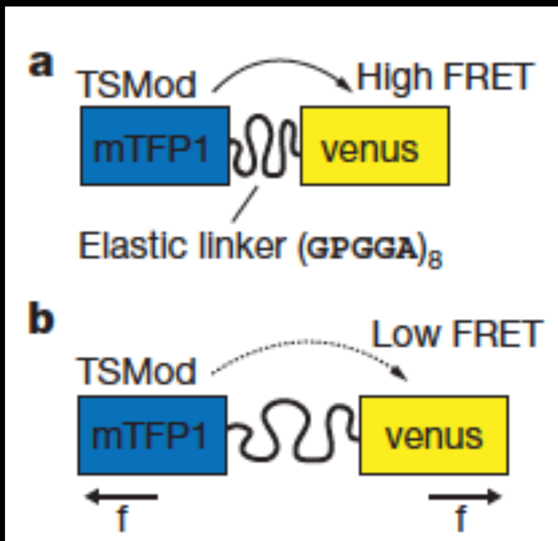
Mechanical cues upstream of a signaling cascade

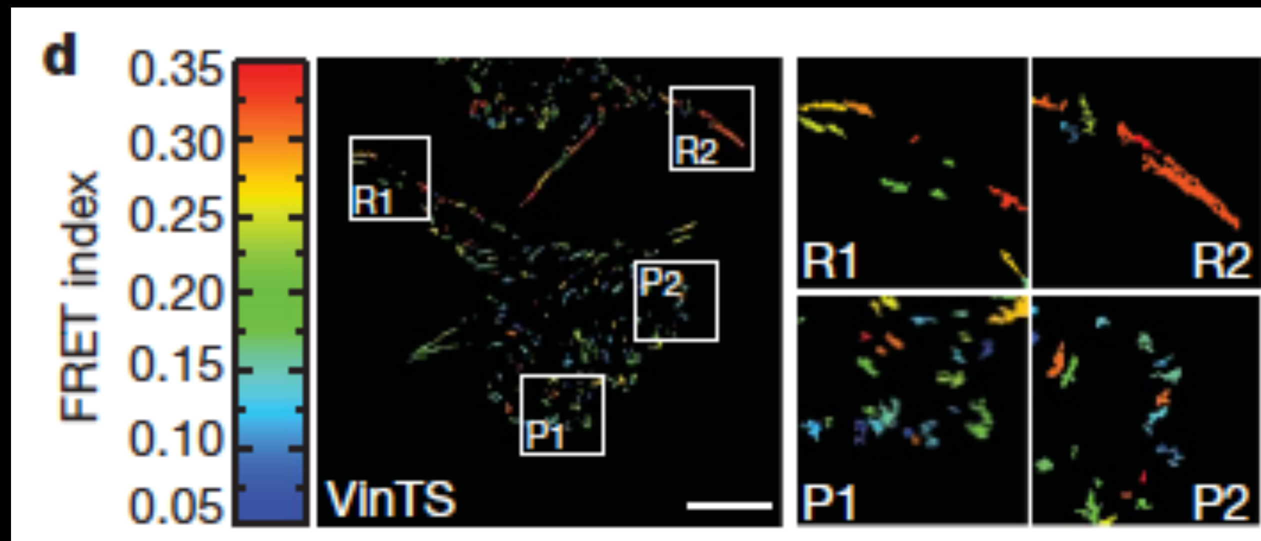




Meikle, 2006

Mechanical cues control protein conformation

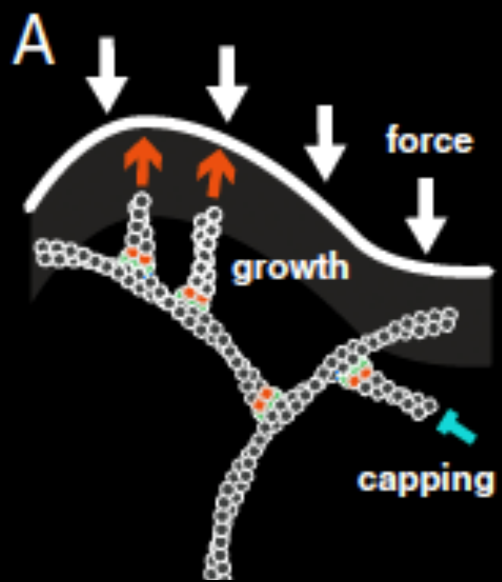




Since FRET is highly sensitive to the distance between the fluorophores, FRET decreases under tension

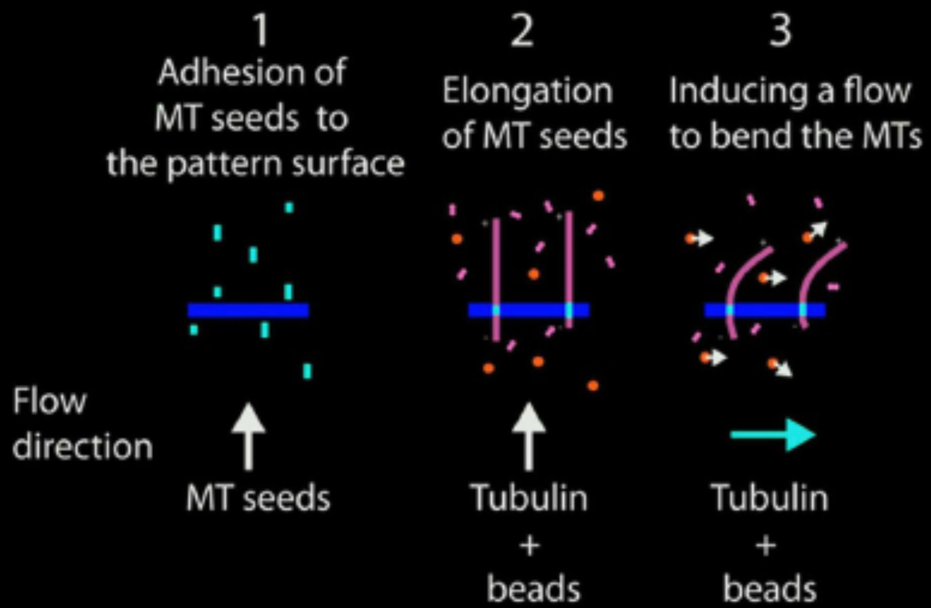
Grashoff *et al.*, 2010

A role of the cytoskeleton mechanics in mechanosensing ?



1. Compression bends filaments
2. Bending induces branching on convex side
3. Extra filament resist compressive force

Risca et al., 2012 PNAS



Modulation of microtubule flexural rigidity with MAP65

Portran et al., 2013 Mol Biol. Cell

Dynamics creates organization

Hyp: microtubule interactions

Dixit and Cyr 2004

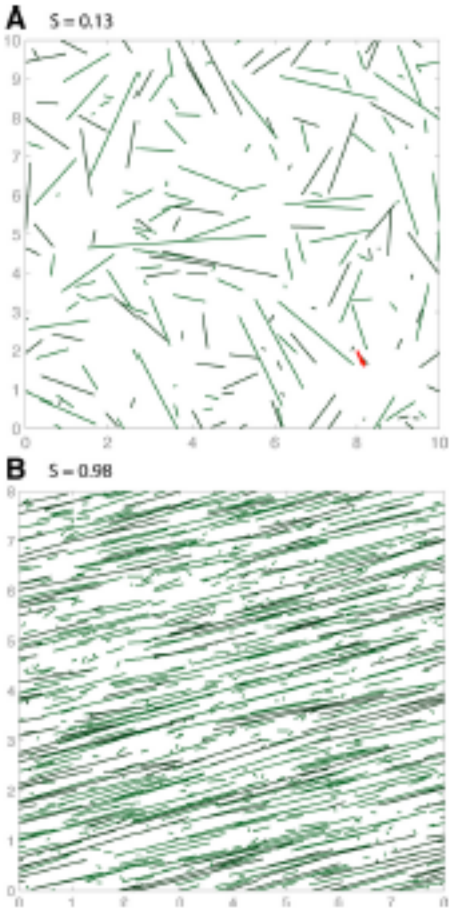
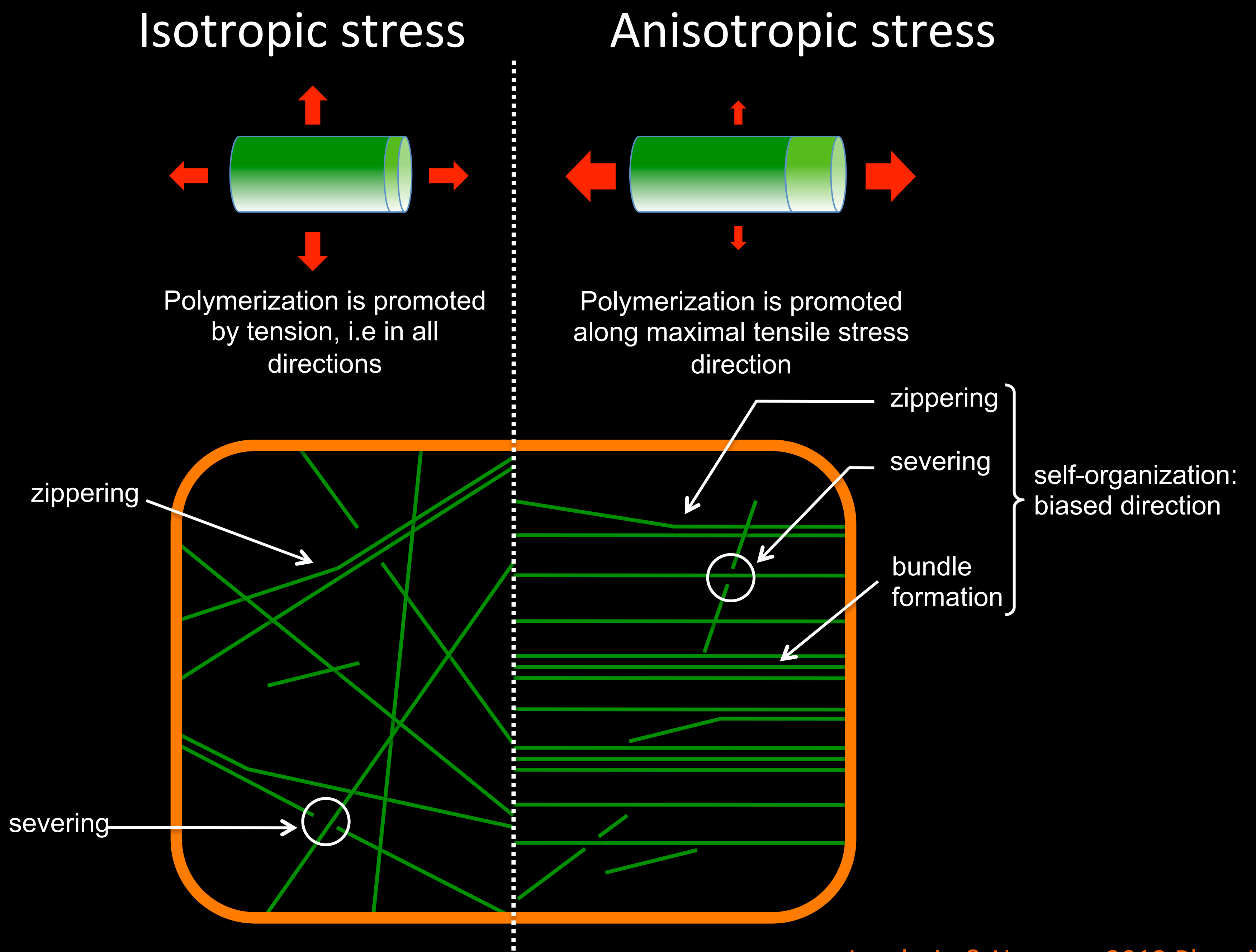


Figure 1. Simulation snapshots at t = 60 min with collision-induced catastrophe only, using parameters from wild type at 31°C (Kawamura and Wasteneys 2008) (A) and collision-induced pauses, using the single-state model of Baulin *et al.* (2007) (B).

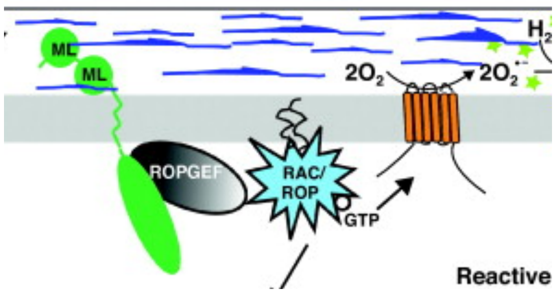
Allard et al., 2010



Landrein & Hamant, 2013 Plant J.

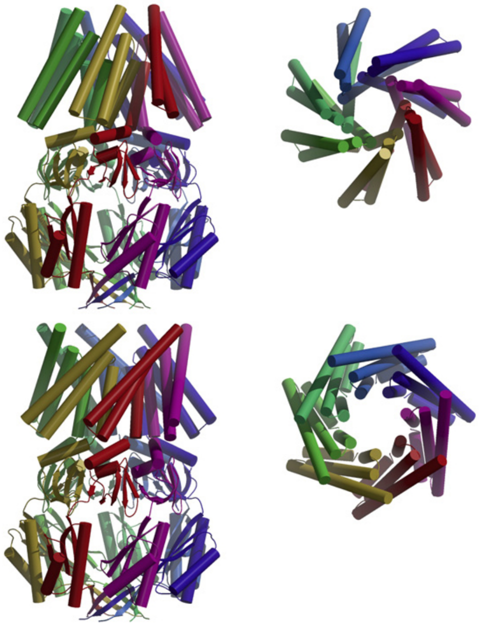
Known mechanotransduction factors in plants

Wall receptors
FER



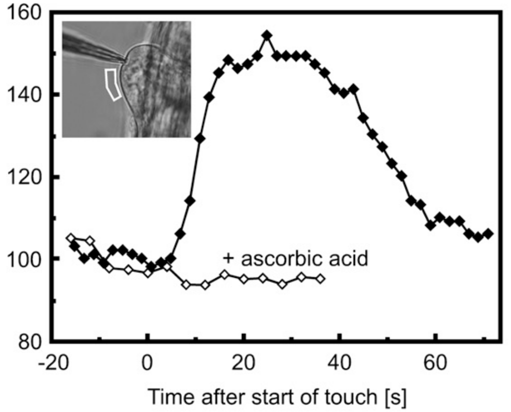
Cheung *et al.*, 2011 COPB
Shih *et al.,* 2014 Curr Biol.

Channels:
MCA1, MSL, OSCA1



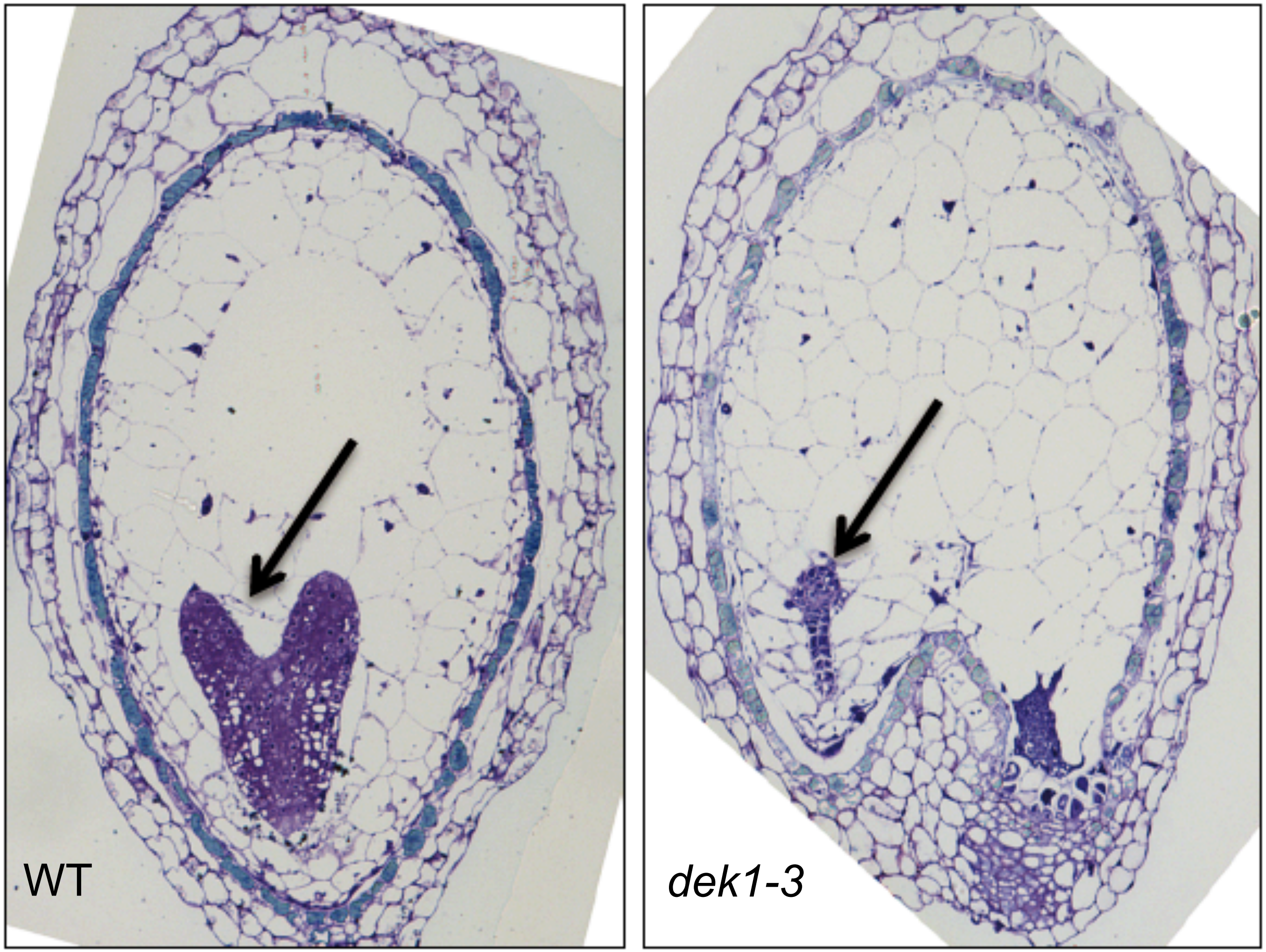
Haswell *et al.,* 2011 Structure

Secondary messengers:
Ca^{2+}, ROS, Jasmonate



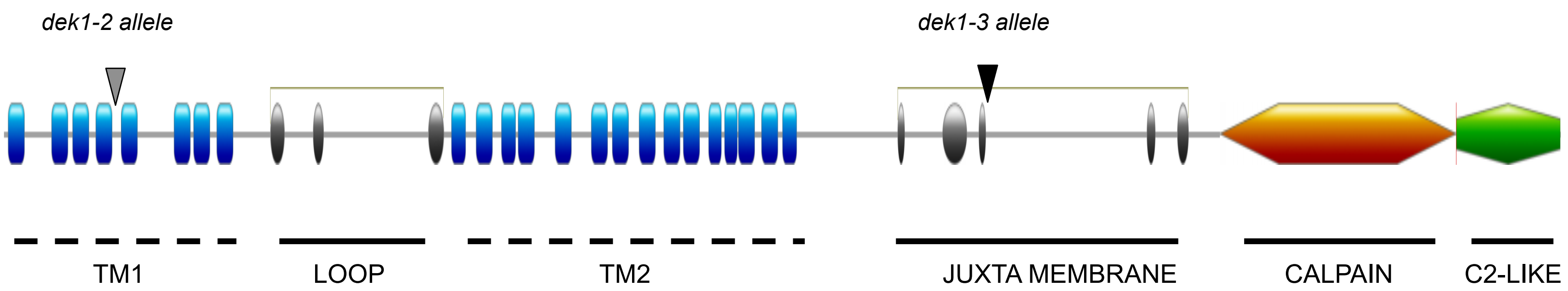
Monshausen *et al.*, 2009 Plant Cell

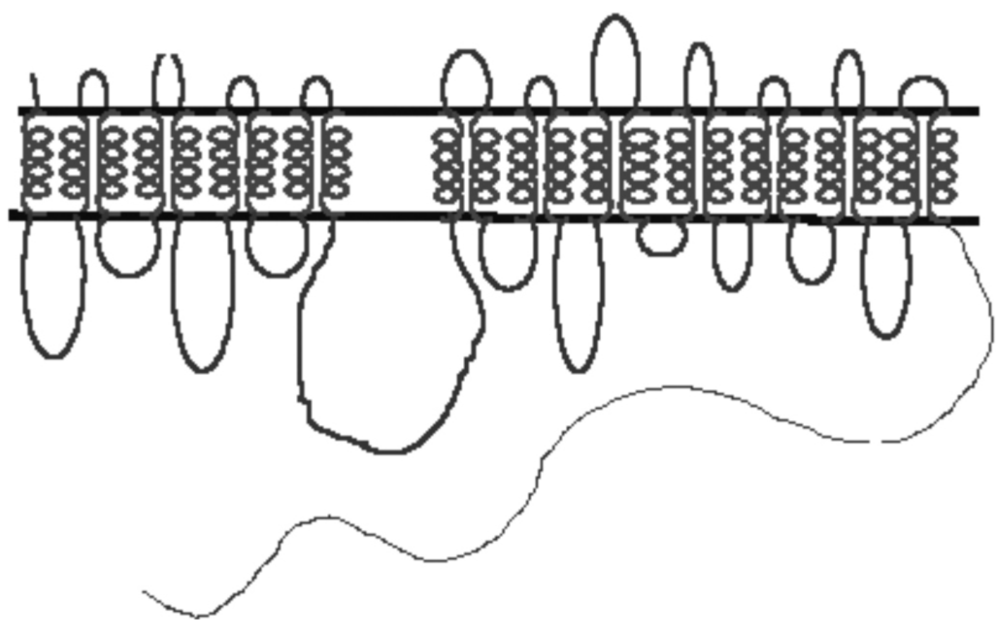
DEK1 is required for plant development



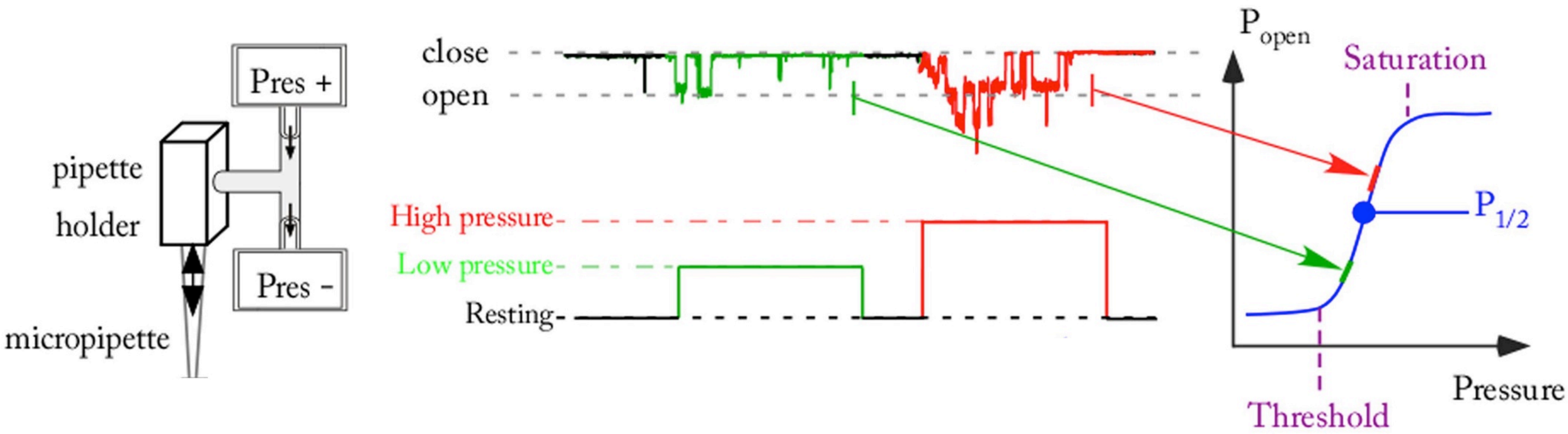
with
Gwyneth Ingram
Jean-Marie Frachisse

DEK1 encodes a phytocalpain with >20 TM domains



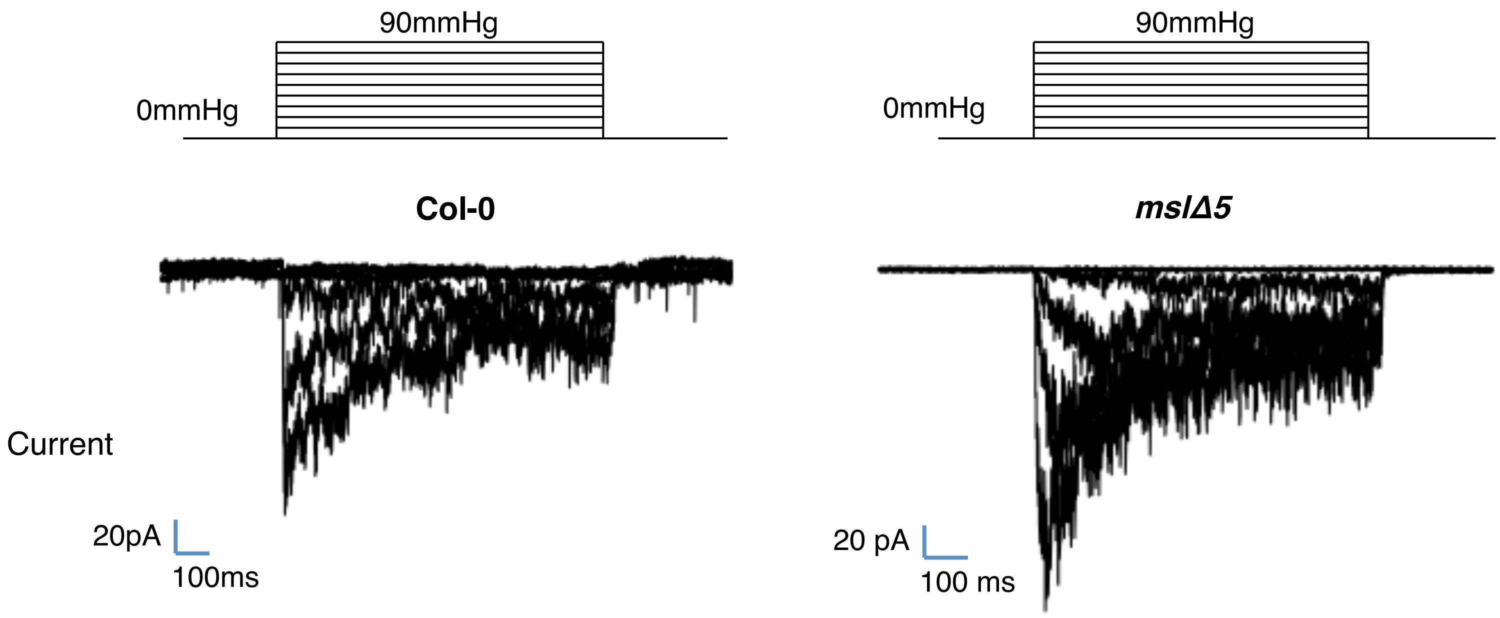


Patch clamp combined with fast speed pressure stimulation



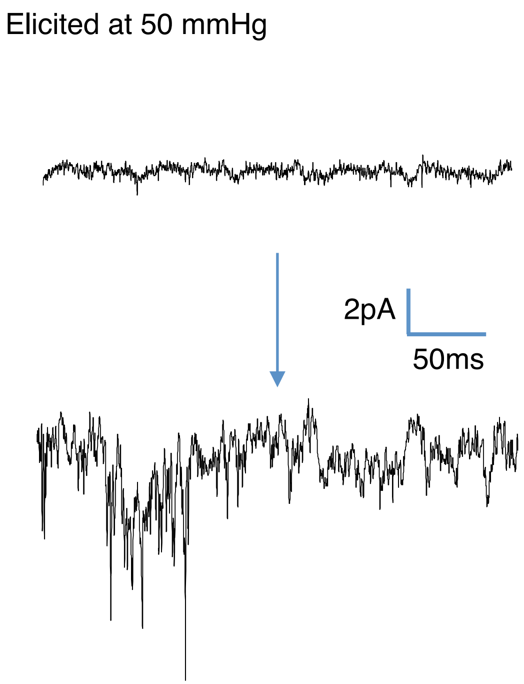
Peyronnet *et al.,* 2014 Front Pl. Sci.

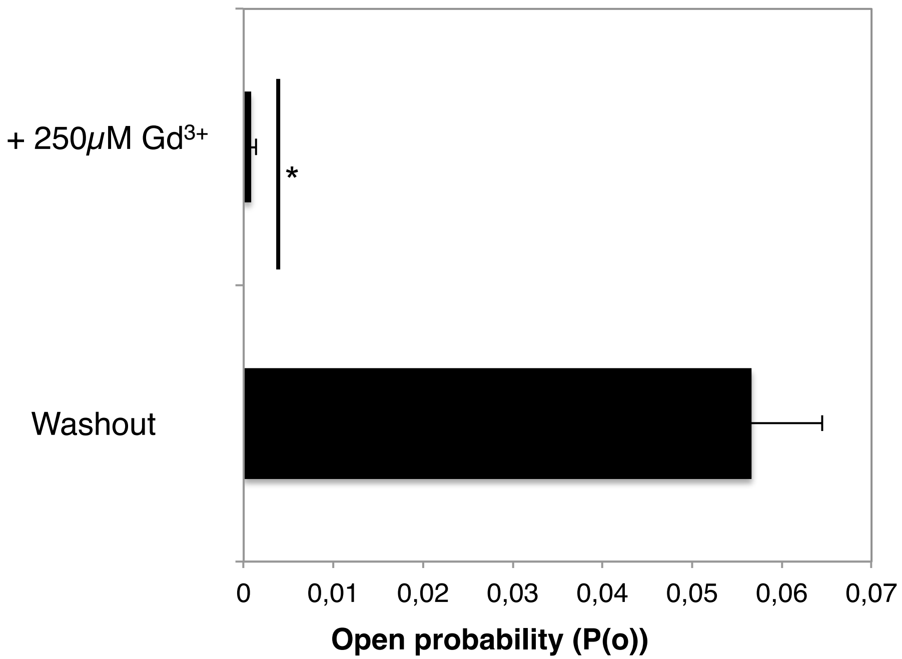
A mechanically-activated current permeable to Ca^{2+} at the plasma membrane



Daniel Tran

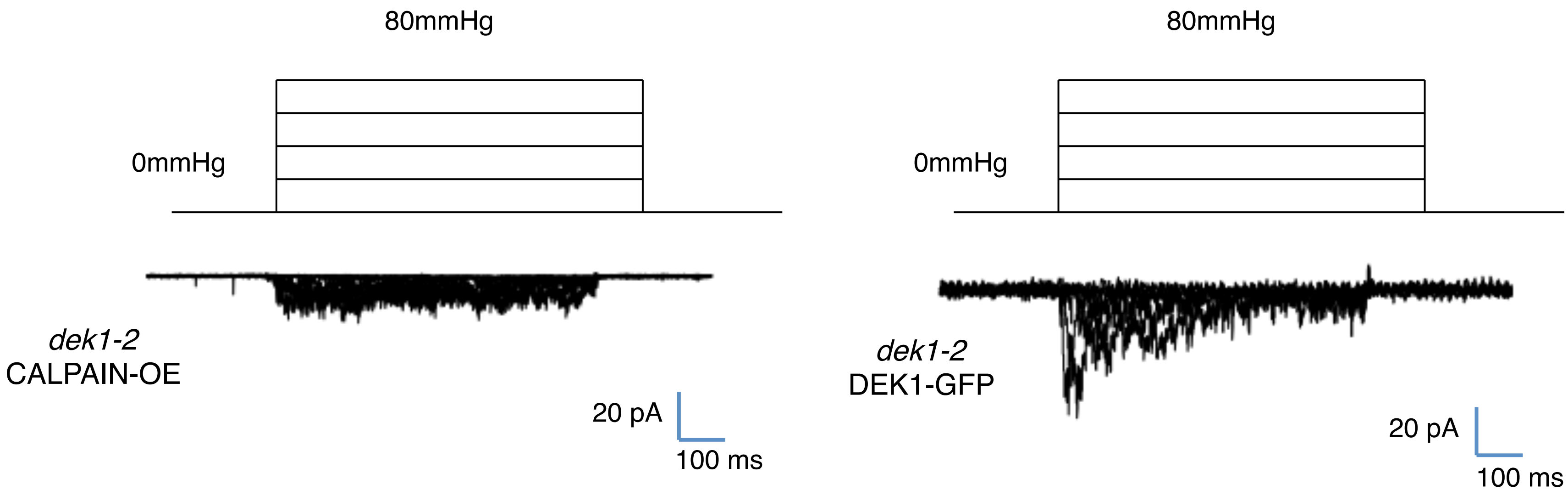
The mechanically activated Ca^{2+} current is affected by the mechanosensitive channel inhibitor Gd^{3+}





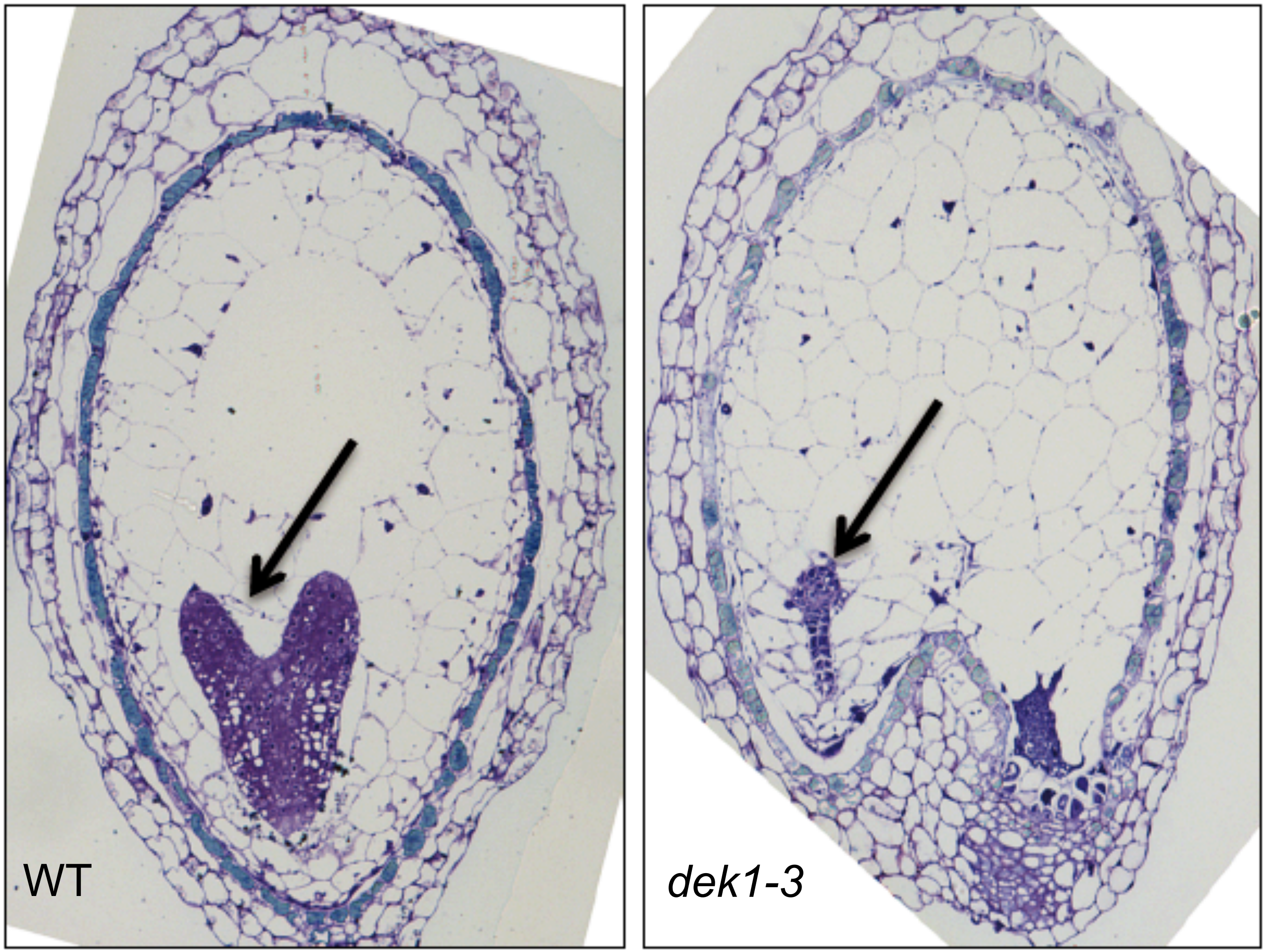
Daniel Tran

Mechanically activated Ca^{2+} current depends on the transmembrane region of DEK1



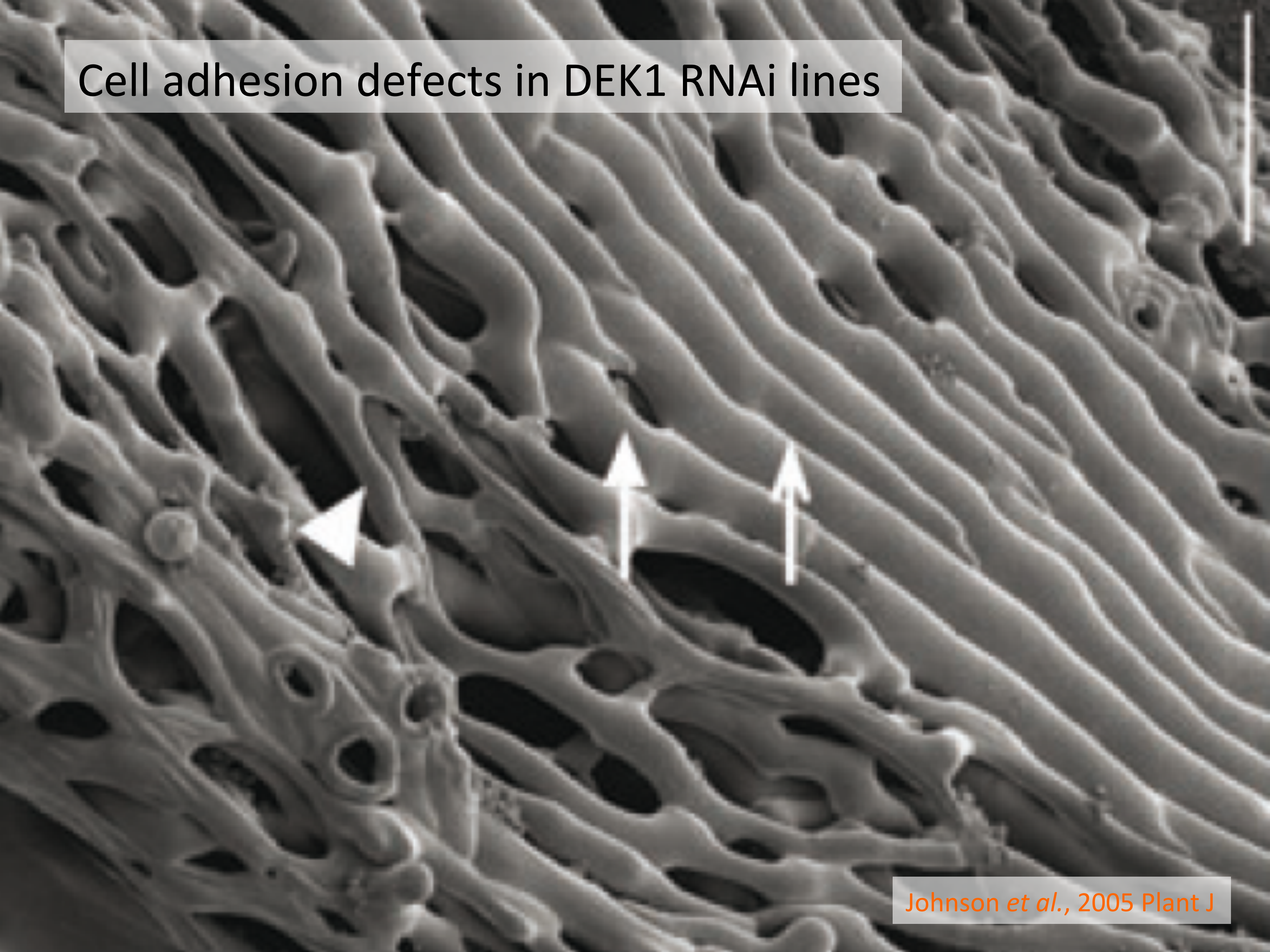
Daniel Tran

DEK1 is required for triggering a mechanically activated Ca^{2+} channel



Tran *et al.*, 2017 Nat. Com.

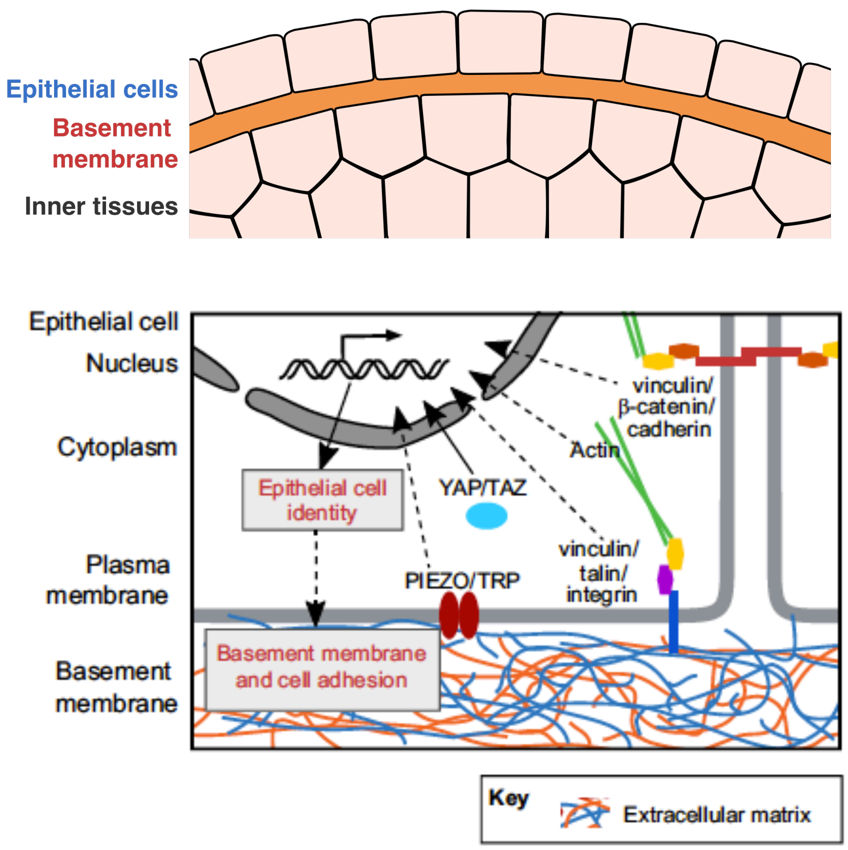
Cell adhesion defects in DEK1 RNAi lines



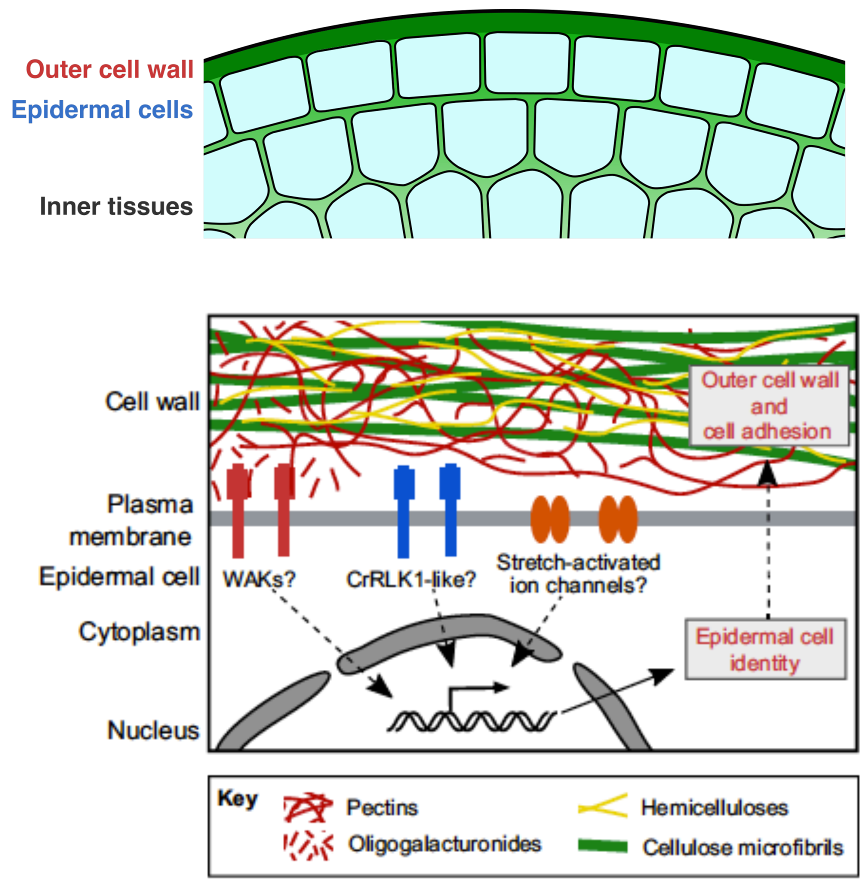
Johnson *et al.*, 2005 Plant J

The adhesion-tension nexus

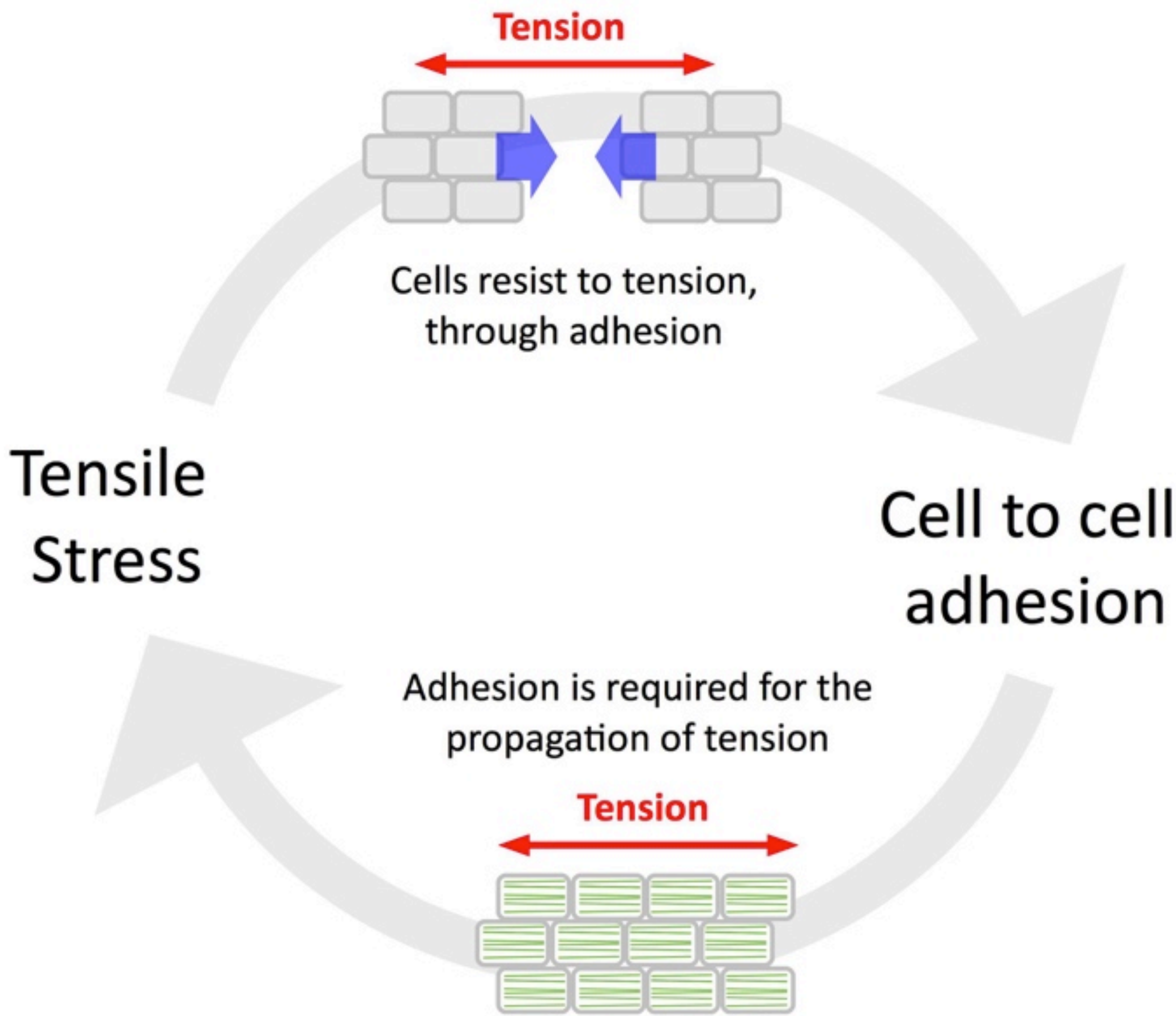
Animal epithelium



Plant epidermis



Galletti *et al.*, 2016 Development



Verger *et al.*, eLife (in press)

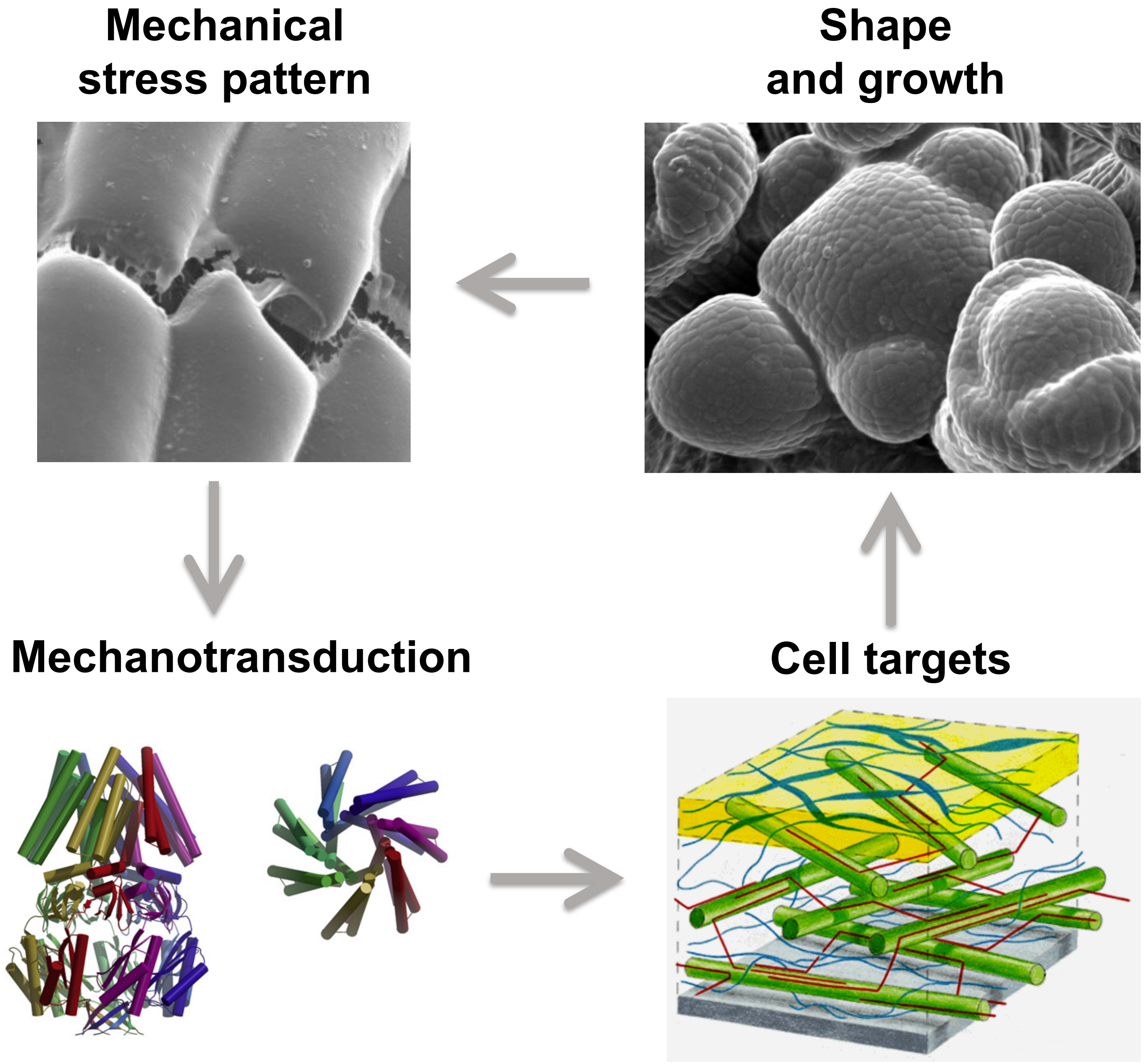
Summary

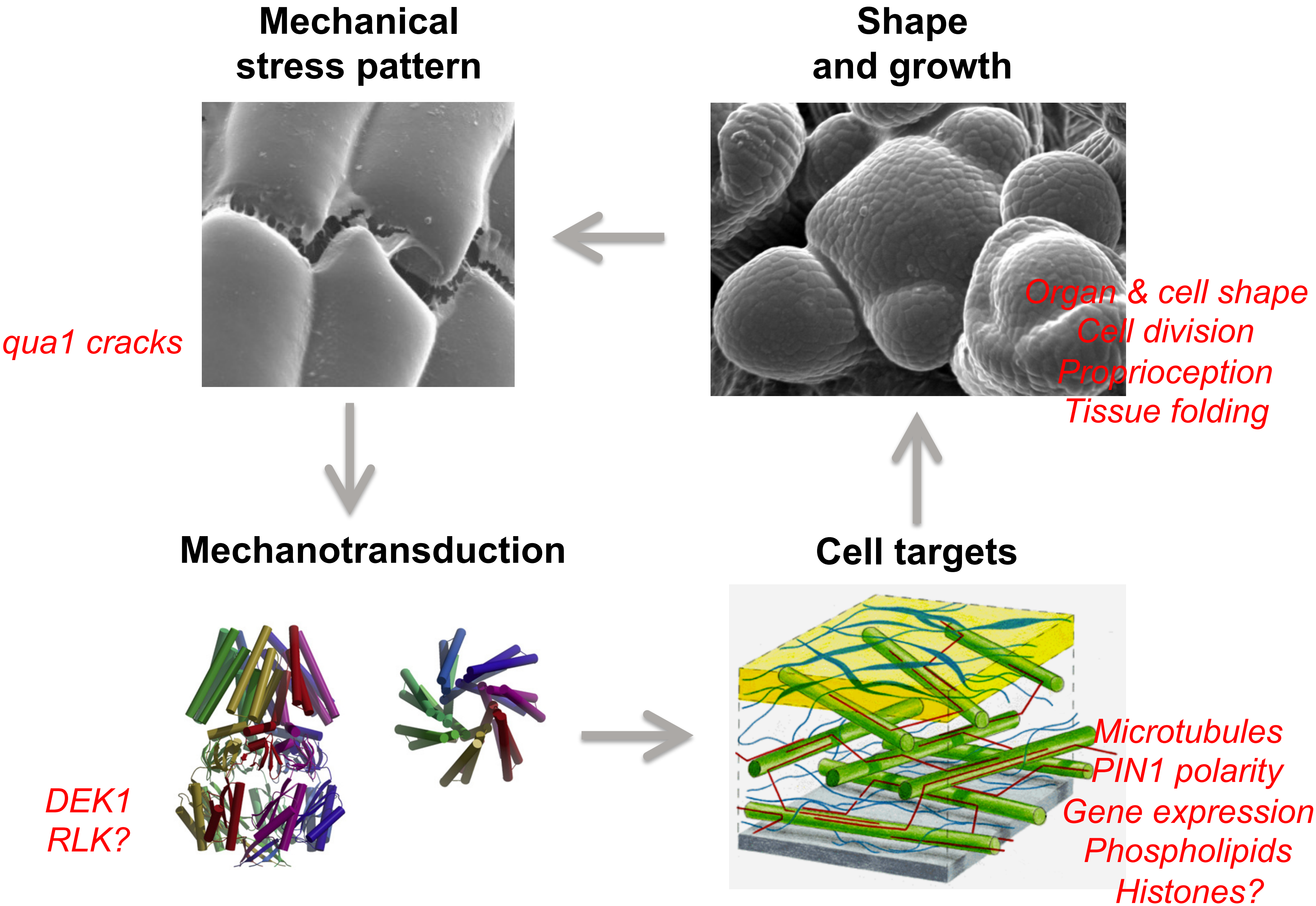
Mechanotrasnduction, like biochemical signaling, relies on protein conformation change

Mechanosensors are usually not specific to mechanical signals

Tension defines the epidermis in plants

Adhesion and tension are coupled


Mechanical stress pattern
Shape and growth
Mechanotransduction
Cell targets


Mechanical stress pattern
Shape and growth
qua1 cracks
Organ & cell shape
Cell division
Proprioception
Tissue folding
Mechanotransduction
Cell targets
DEK1
RLK?
Microtubules
PIN1 polarity
Gene expression
Phospholipids
Histones?

RDP lab

Arezki Boudaoud
Kateryna Fal
Antoine Fruleux
Roberta Galletti
Gwyneth Ingram
Yvon Jaillais
Annamaria Kiss
Mengying Liu
Yuchen Long
Thomas Stanislas
Jan Traas
Christophe Tréhin
Stéphane Verger
Yi Zhang

Past members

Léna Beauzamy
Mathilde Dumond
Nathan Hervieux
Jean-Daniel Julien
Benoit Landrein
Marion Louveaux
Pascale Milani
Vincent Mirabet
Naomi Nakayama

Sepal

Tamiki Komatsuzaki
Chun-Biu Li
Adrienne Roeder
Satoru Tsugawa

DEK1

Jean-Marie Frachisse
Daniel Tran

VIP3

Liz Haswell
Gregory Jensen

Mts and stress

Yves Couder
Henrik Jönsson
Elliot Meyerowitz

Chromatin

Atef Asnacios
Marie-Edith Chabouté
Henrik Jönsson
Yassin Réfahi
José Teles

Pavement cells

Mateusz Majda
Stéphanie Robert
Aleksandra Sappala
Richard Smith