



Part I. The structure behind the architecture

ICTS– April 2018

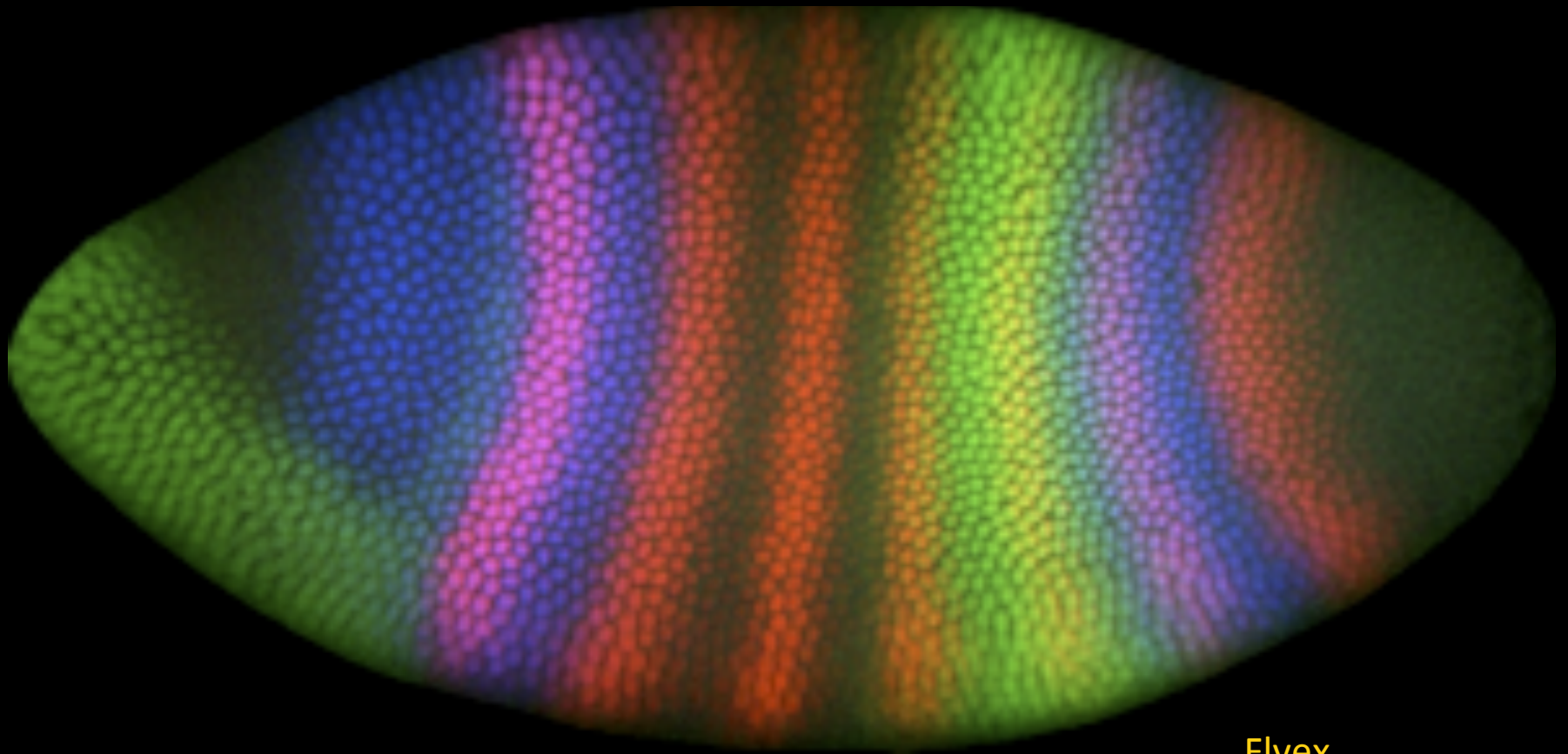
Olivier Hamant - Reproduction et Développement des Plantes, ENS Lyon, France



UNIVERSITÉ
DE LYON



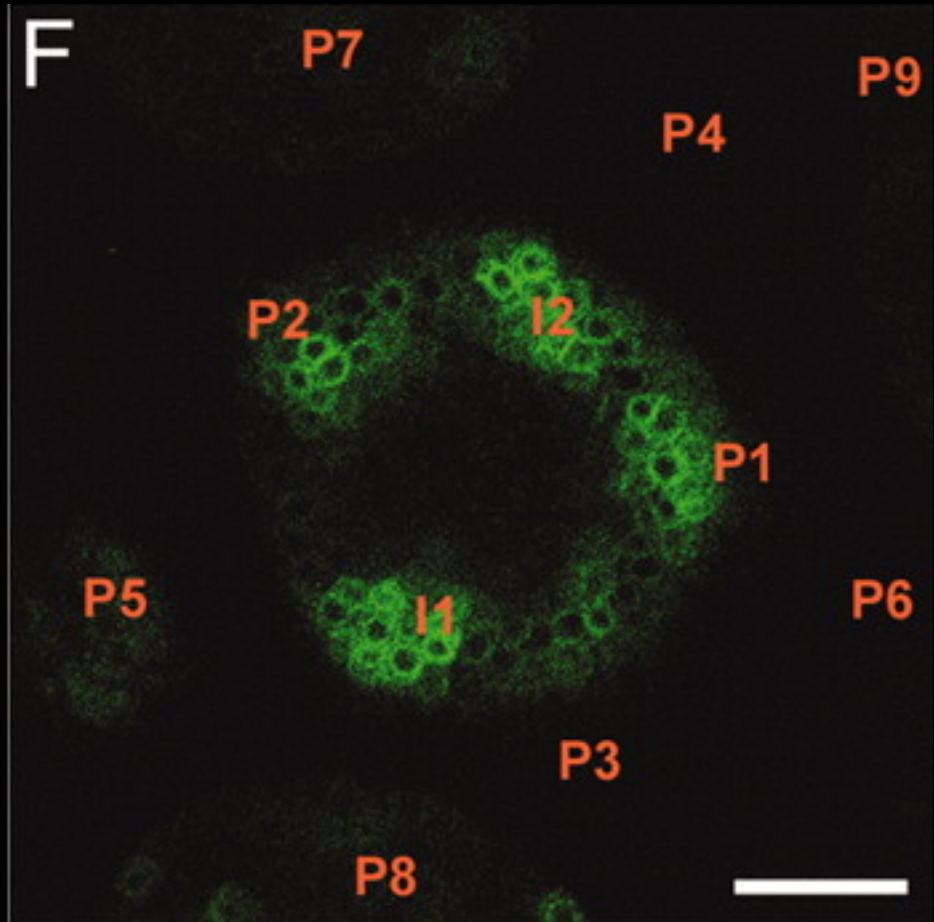
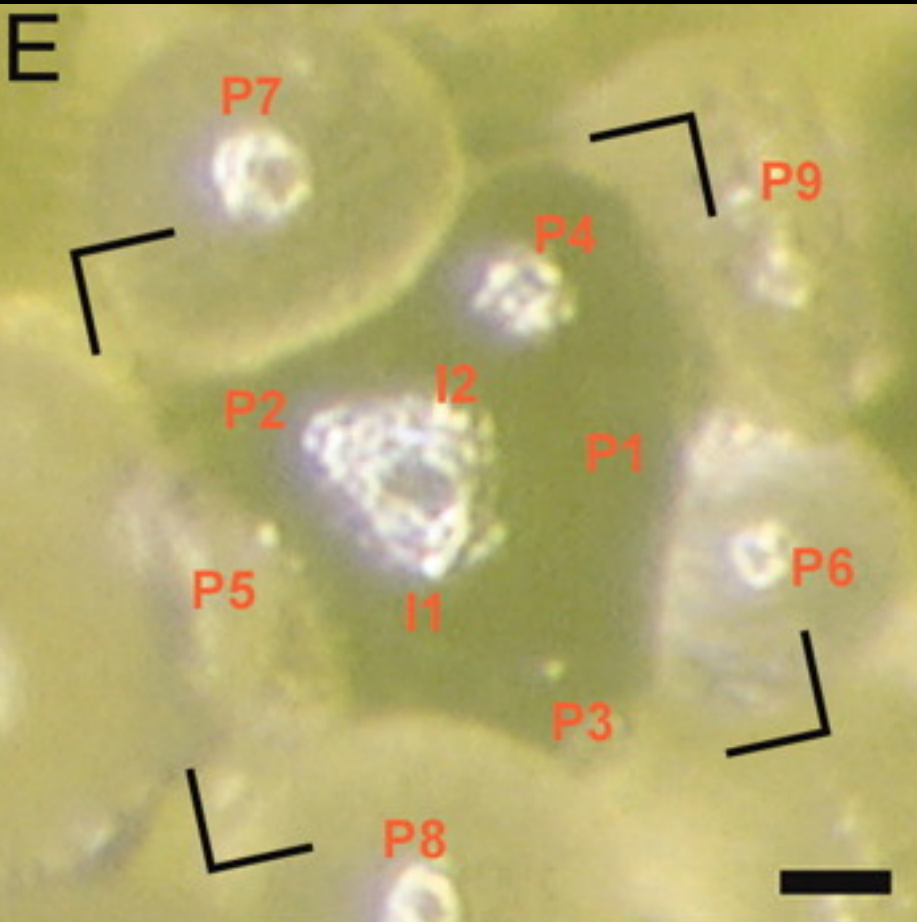
Biochemical signals drive positional information



Flyex

In Drosophila

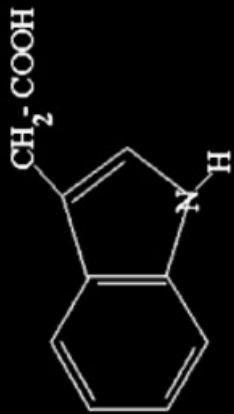
Biochemical signals drive positional information



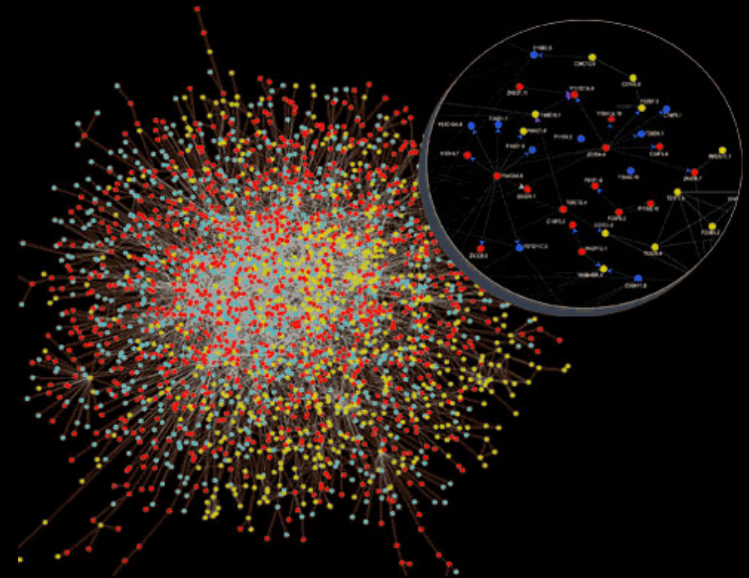
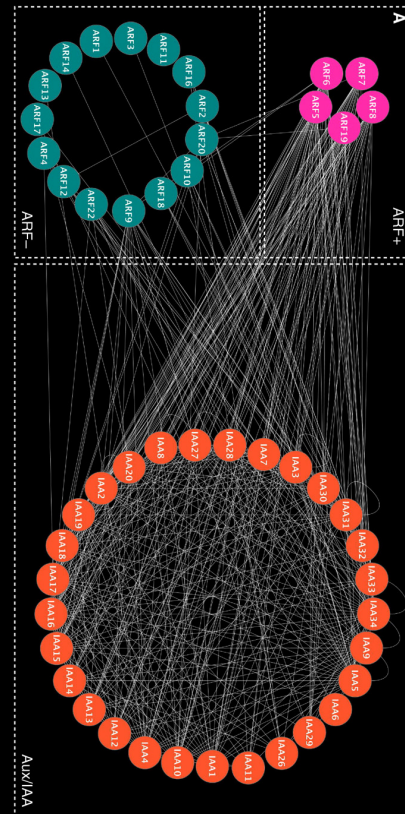
Ex: auxin and organ initiation

Smith *et al.*, 2006 PNAS

From one signal to complex networks



Auxin

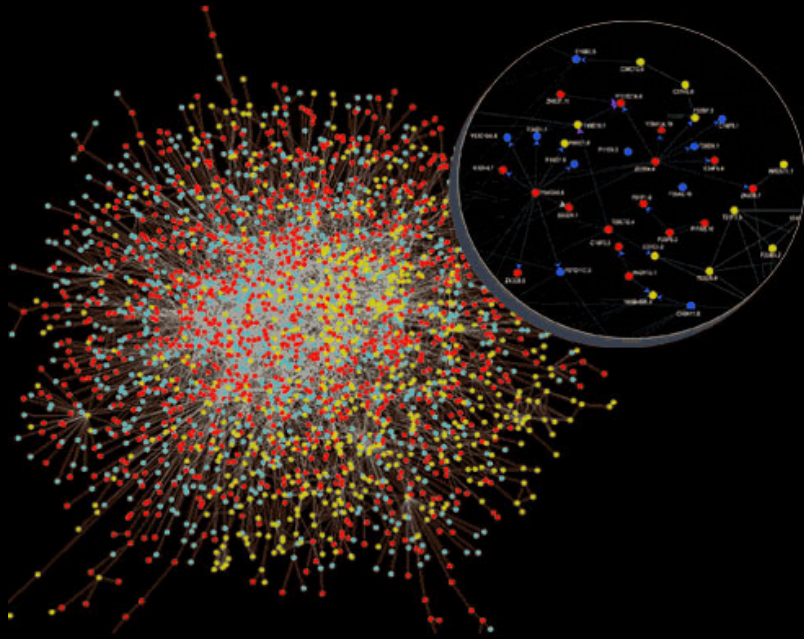


Towards larger networks...

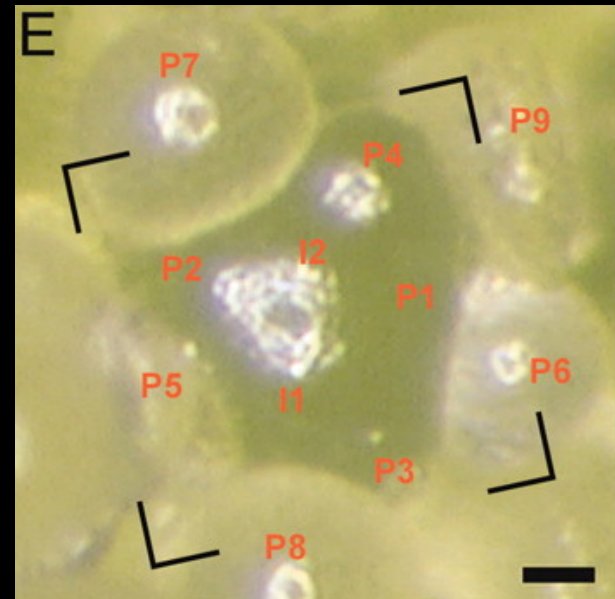
ARF/AuxIAA interactome

Vernoux *et al.*, 2011

Translating biochemistry to shape:

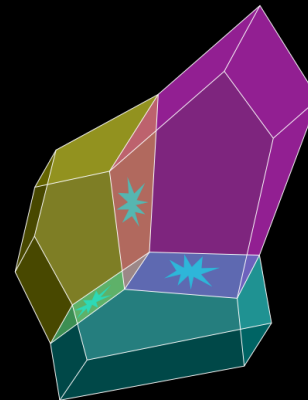
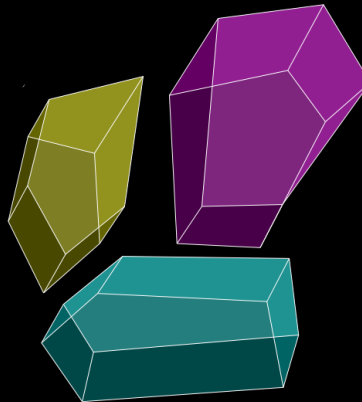
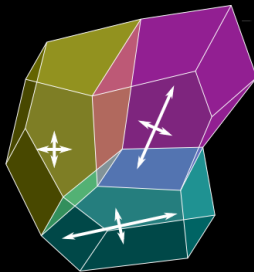
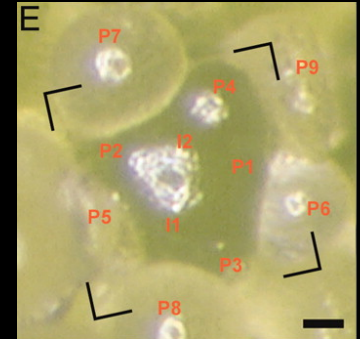
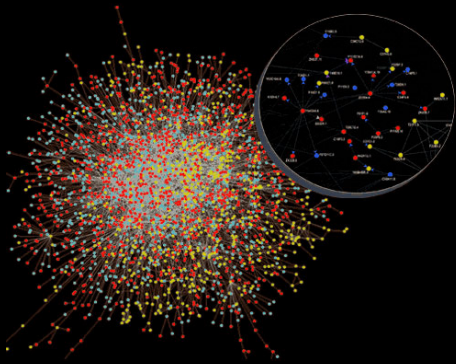


Molecular input

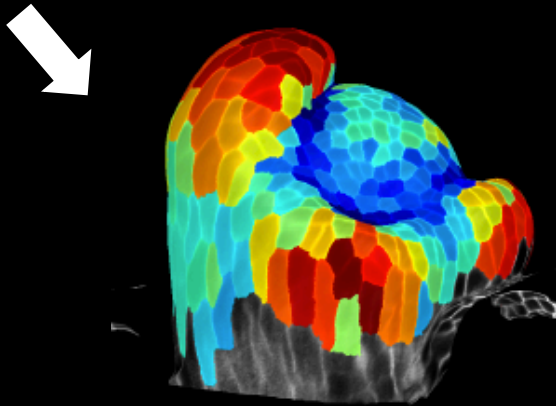
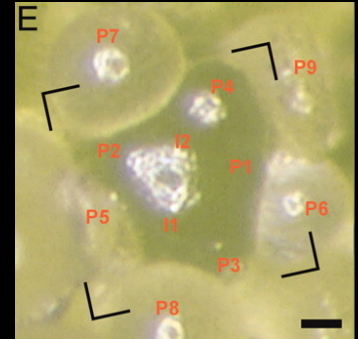
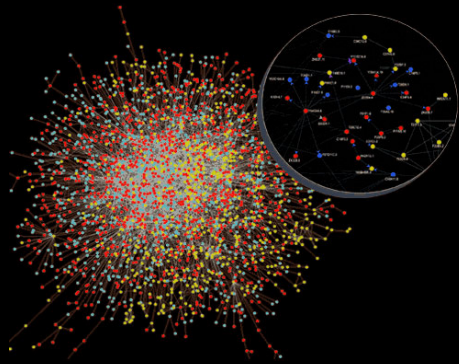


Shape

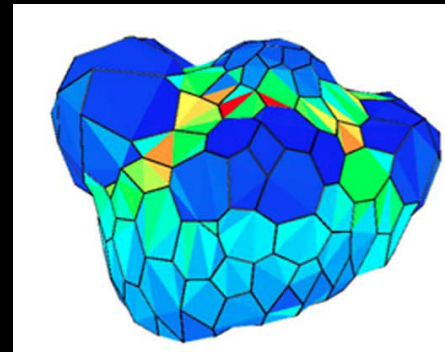
Translating biochemistry to shape: the laws of mechanics



Translating biochemistry to shape: A strategy



Quantitative imaging

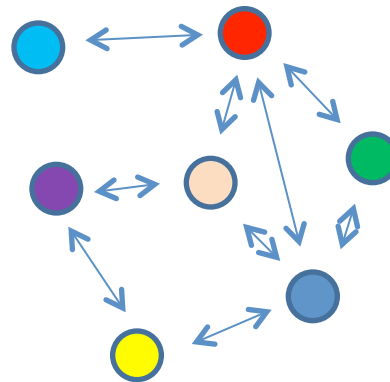


Computational modeling

A few definitions

Definition: Complex system

- A set (whole) of entities that interact according to simple local rules.
- The system shows the emergence of properties that cannot be deduced from the local properties and rules.
- Well suited to describe structures without a clear leader



Experience a complex system in action



Complex is NOT complicated



Clock : COMPLICATED
(depends on one leader)



Ant colony: COMPLEX

Ex: The shape of ant colonies can be entirely predicted based on how ants interact with each other



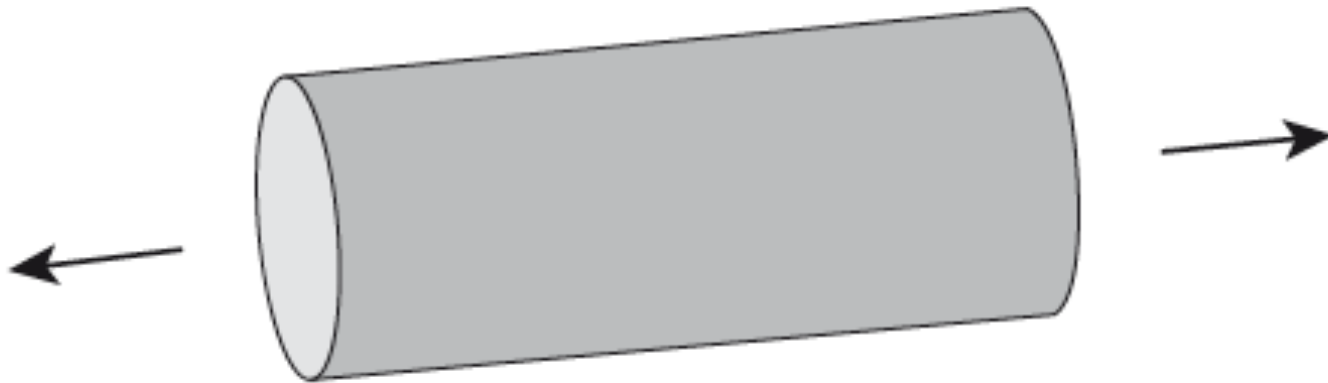
Ant colony

Application of Systems biology: examples

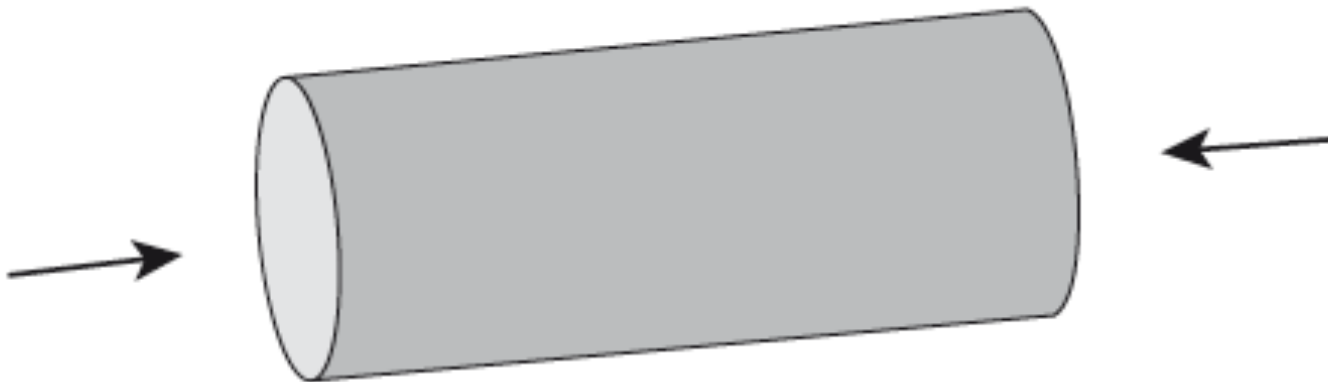
- Extracting meaningful information from **huge datasets**
- The contribution of gene network **topology** in regulation
- The role of **stochasticity**
- **Multiscale** emerging properties
- The counter-intuitive implications of **feedbacks**
- Visualizing the **invisible**, e.g. forces

Defining tension and compression

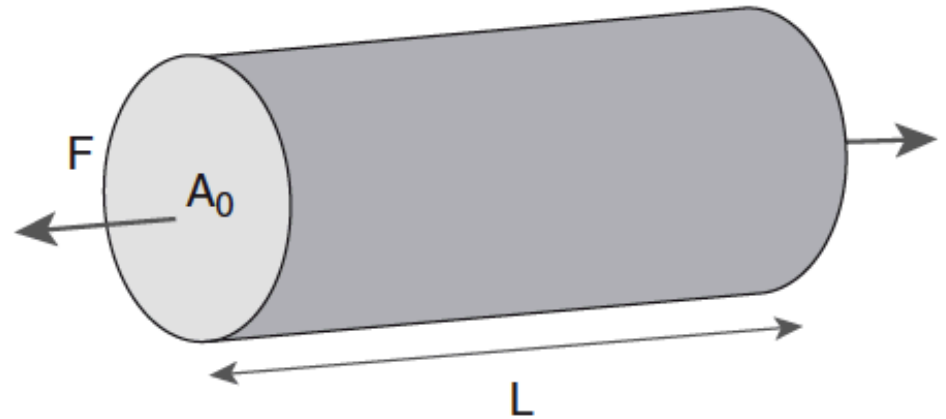
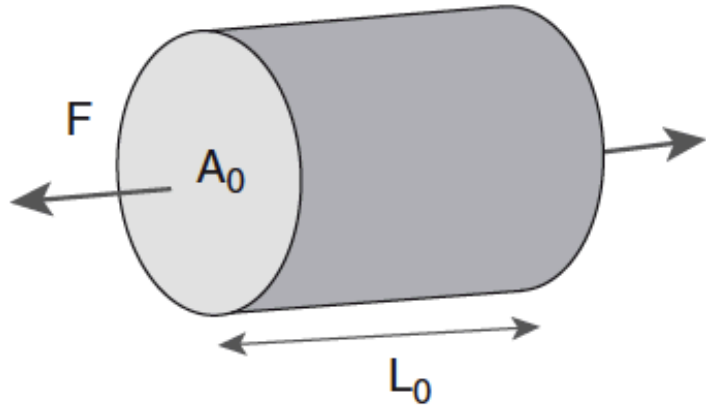
Tension: length increase



Compression: length decrease



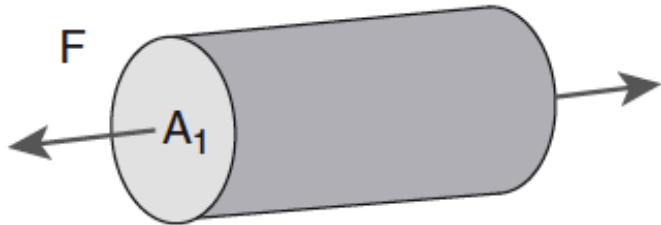
Mechanical force vs. Mechanical stress



Stress $\sigma = F/A_0$ (in N/m^2 or Pa)

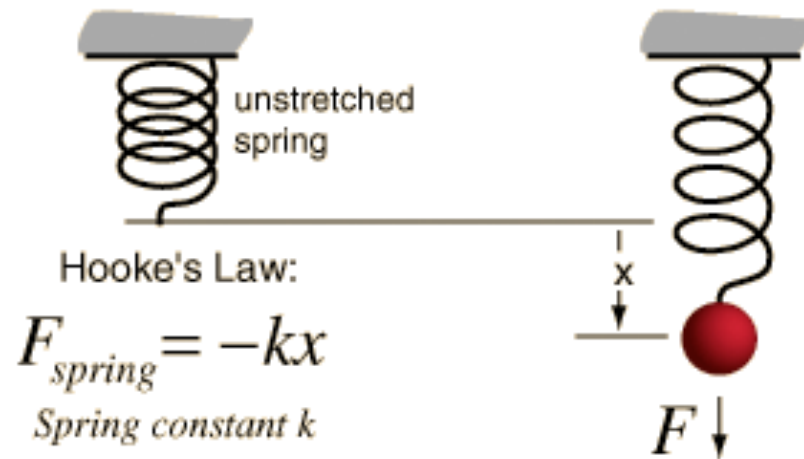
Strain $\epsilon = (L-L_0)/L_0$ (dimensionless)

Ex: $\sigma_1 > \sigma_0$ because $A_1 < A_0$

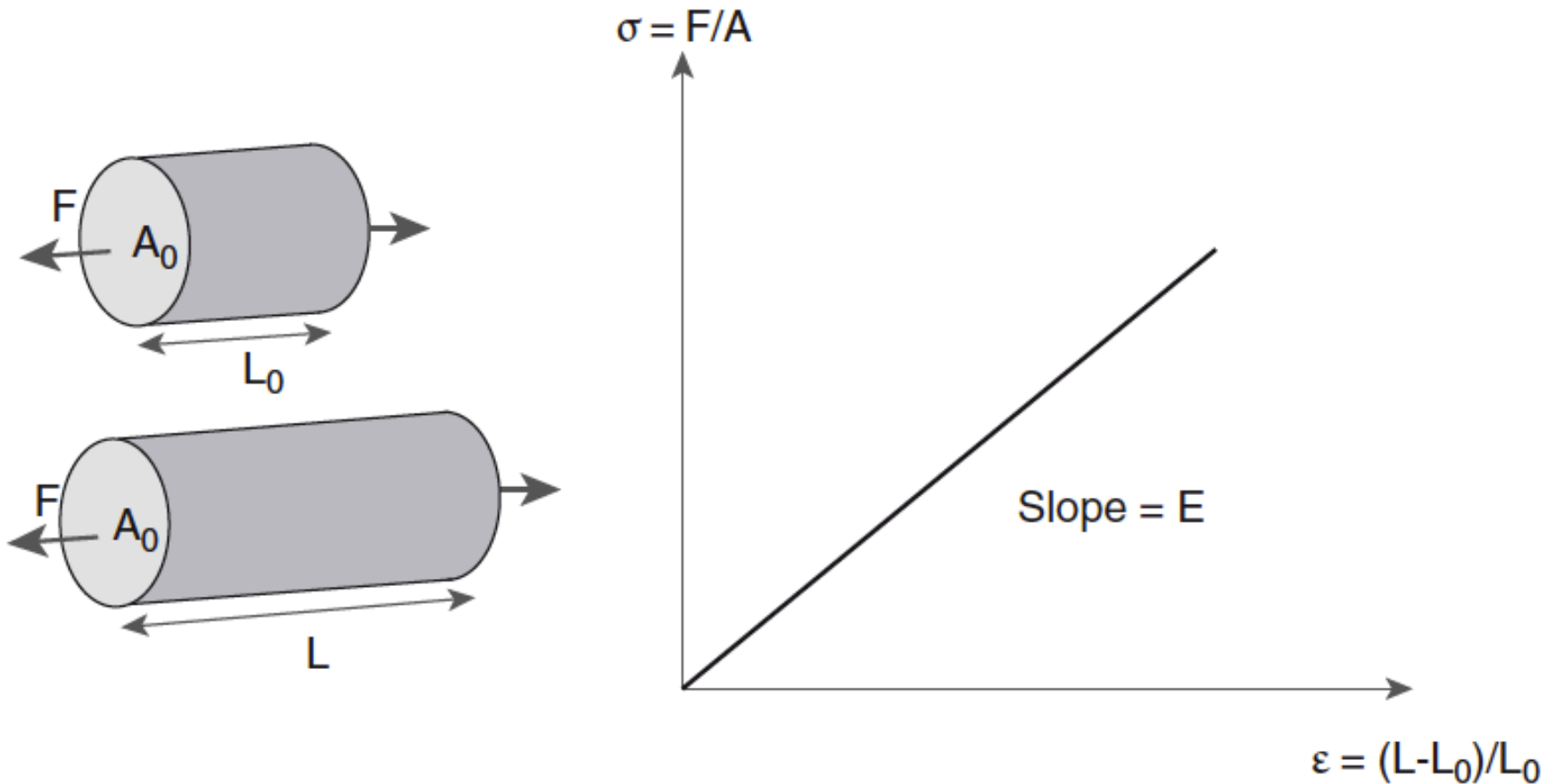


Defining stiffness

In 1D



Stiffness relates stress and strain



Hooke's law (spring):

$$F = k(L - L_0)$$

k : spring stiffness

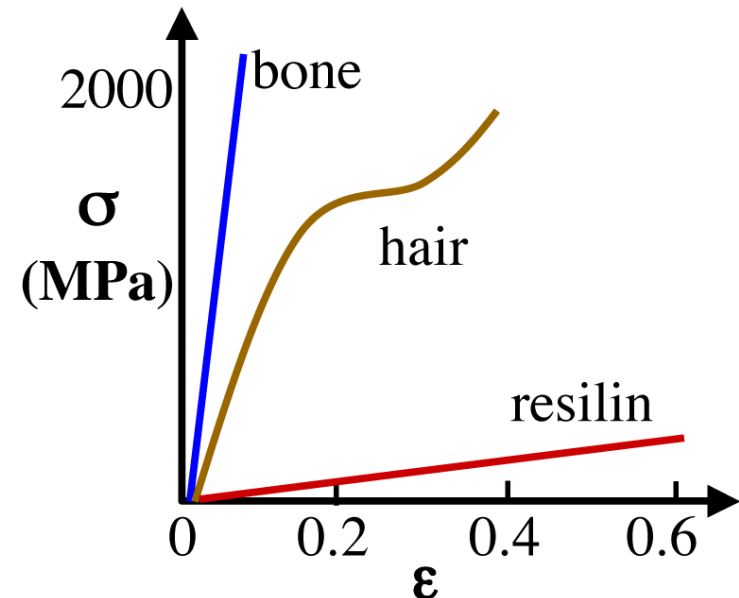
Hooke's law (material):

$$\sigma = E\epsilon$$

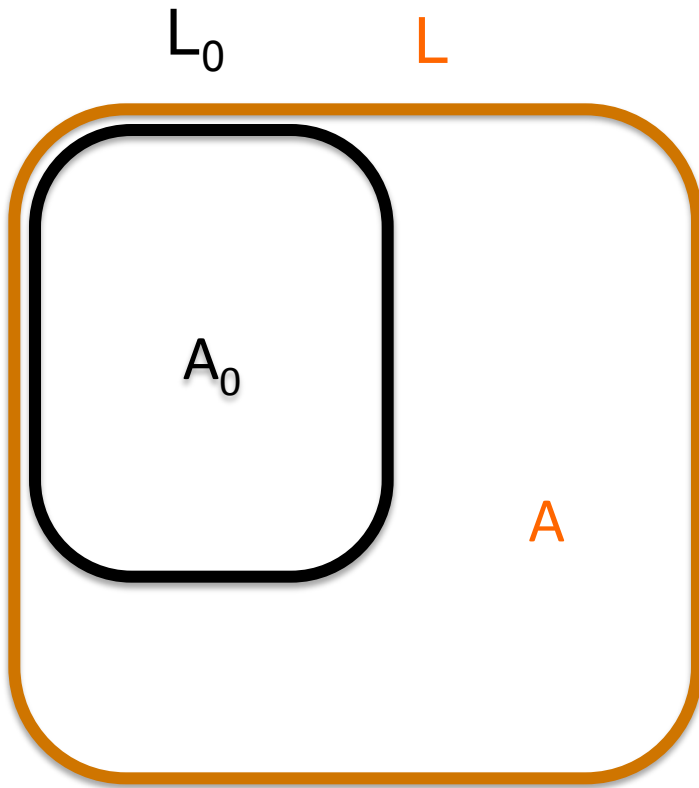
E : Young's modulus
(i.e. stiffness of a material)

Examples of Young modulus values

<i>Material</i>	<i>Young's modulus (MPa)</i>
Resilin	1.8
Rubber	7
Human cartilage	24
Vertebrate tendon	1,200
Cheap plastic	1,400
Hair (keratin)	5,000 to 8,000
Locust cuticle	10,000
Plywood	14,000
Vertebrate bone	20,000
Cellulose (crystalline)	25,000
Glass	70,000
Iron	210,000
Diamond	1,200,000



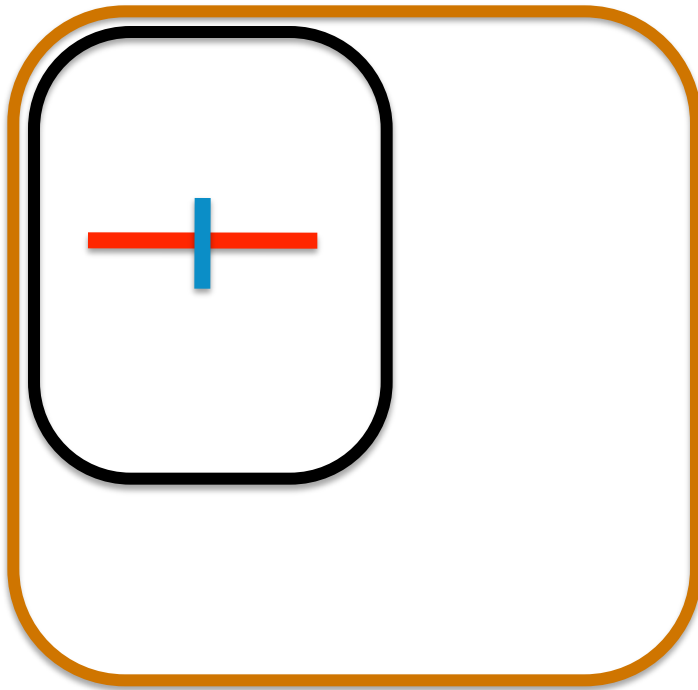
Strain rate



$$\varepsilon_L = (L - L_0) / L_0$$

$$\varepsilon_S = (A - A_0) / A_0$$

Strain rate



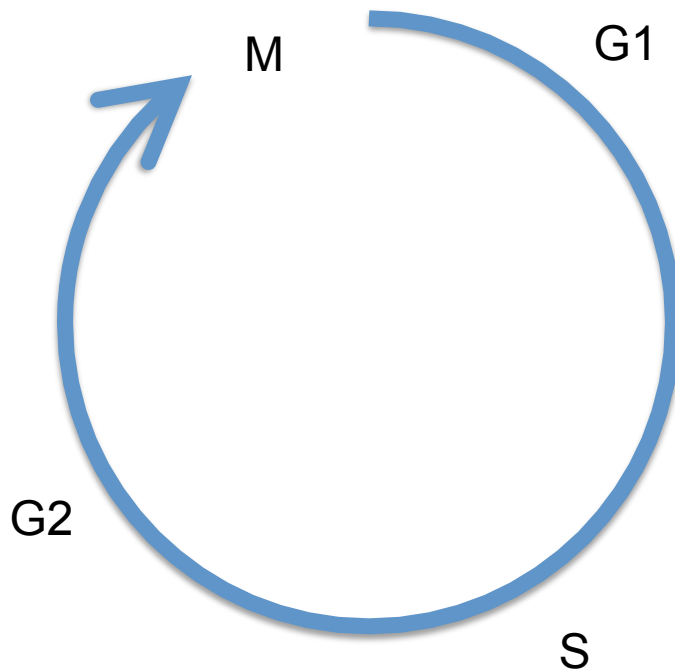
Maximal strain rate

Minimal strain rate

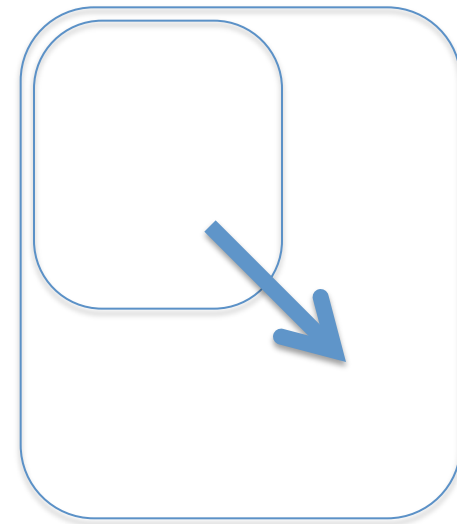
Anisotropy vs. Isotropy

Growth: irreversible increase in volume

Cell proliferation

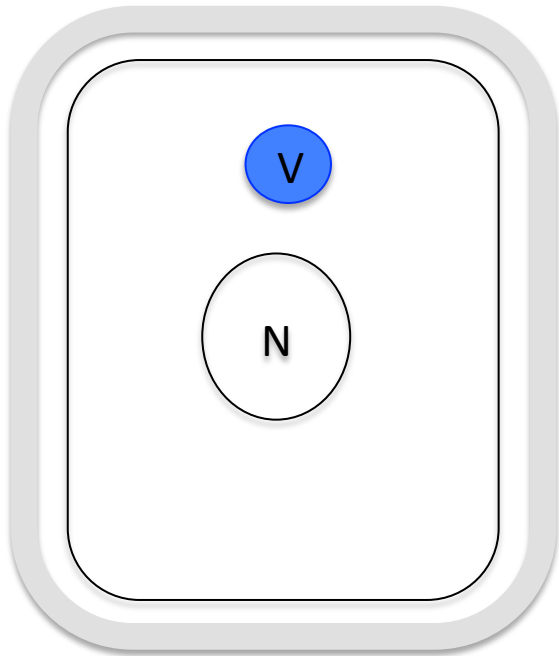


Cell expansion

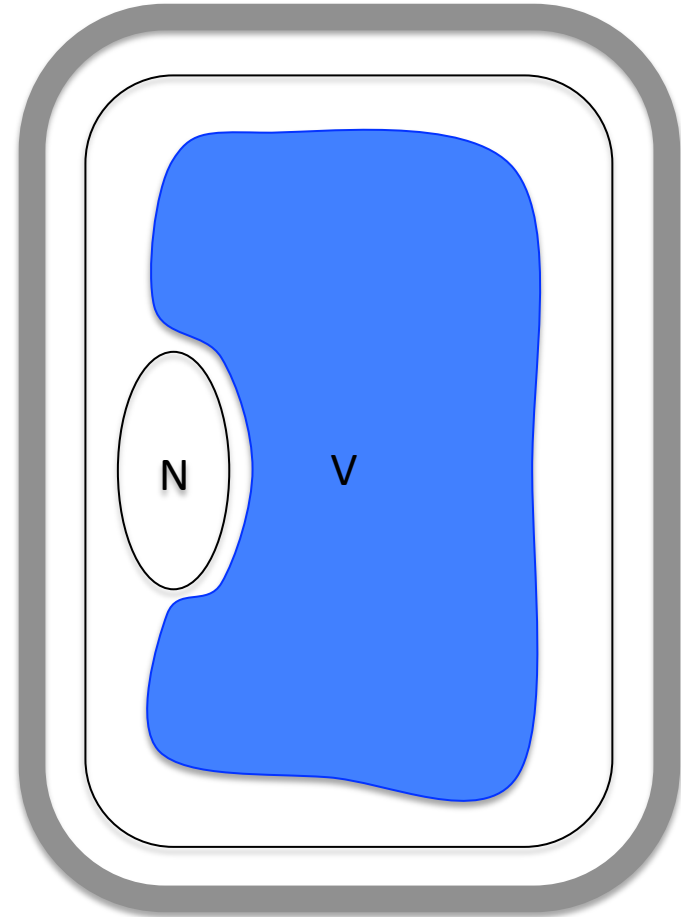


+ endoreduplication

Animal cell



Plant cell

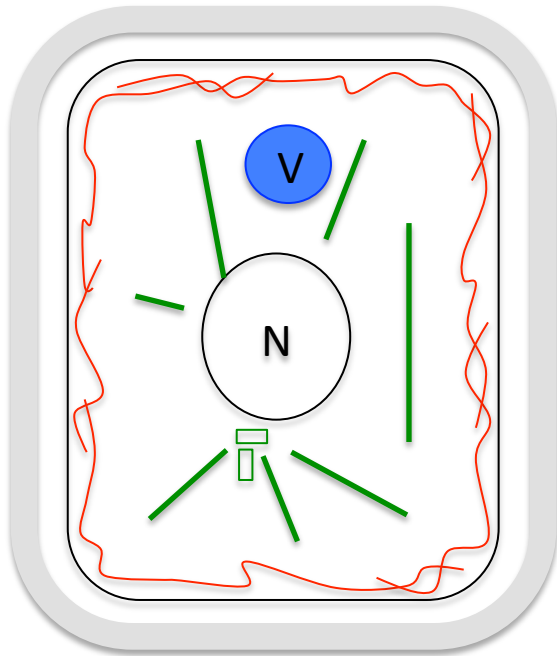


Extra Cellular Matrix

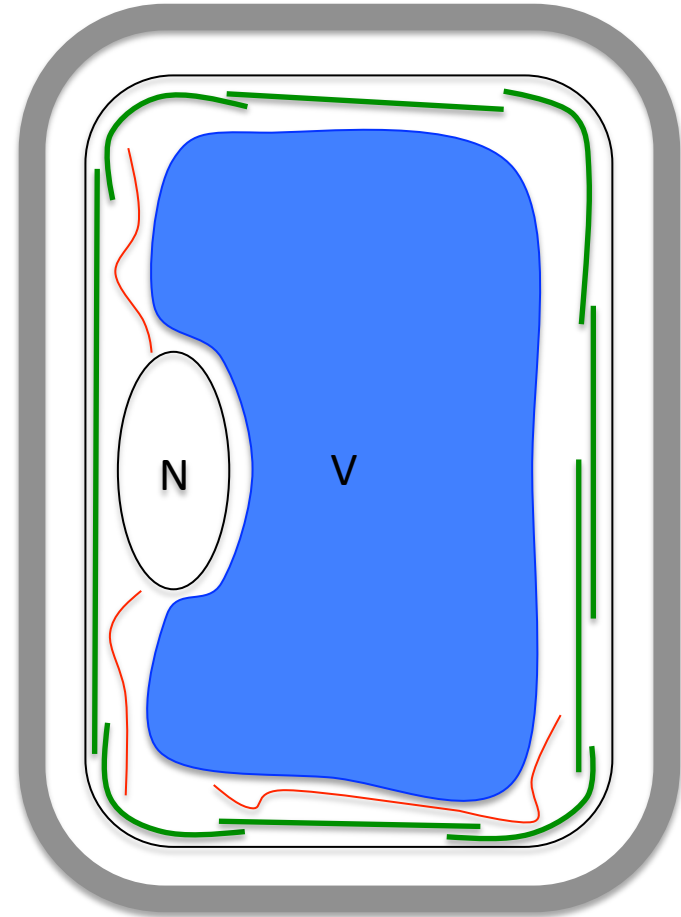
Actin

Microtubules

Animal cell



Plant cell

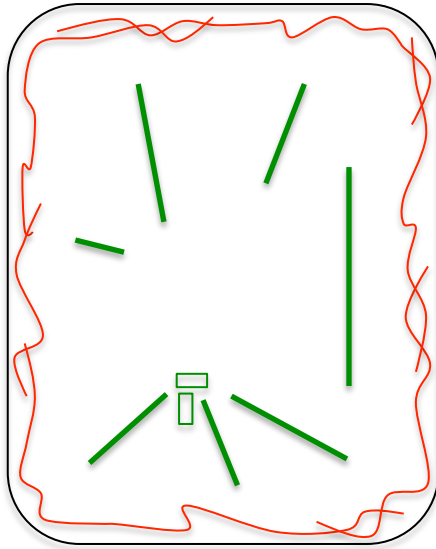


Extra Cellular Matrix

Actin

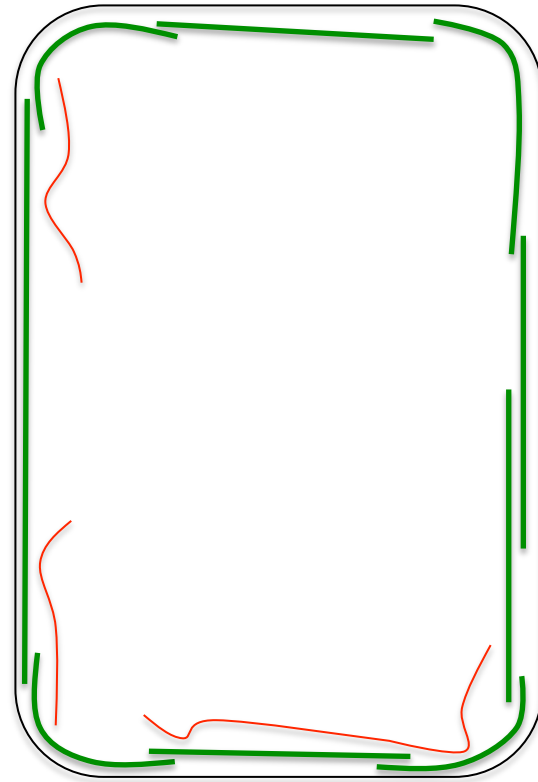
Microtubules

Animal cell



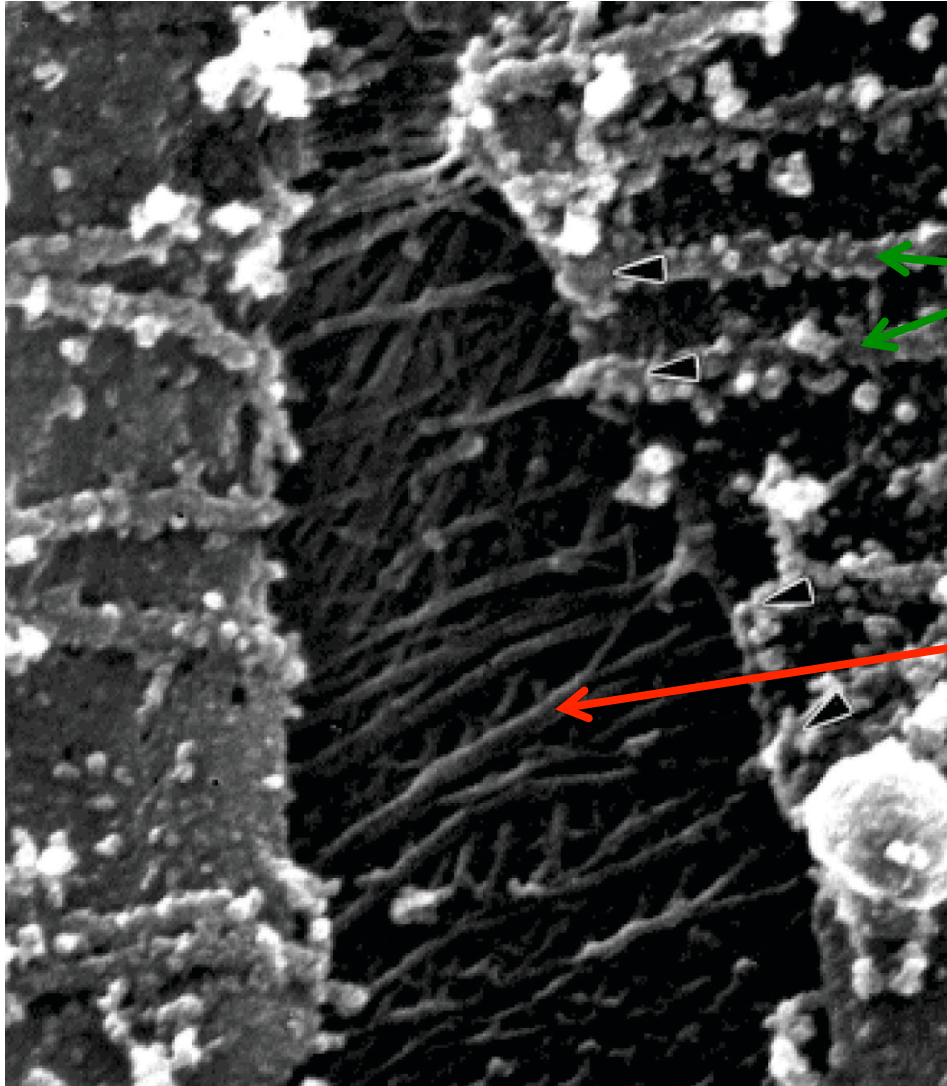
Actin: cell shape
MT: vesicle transport

Plant cell



Actin: vesicle transport
MT: cell shape

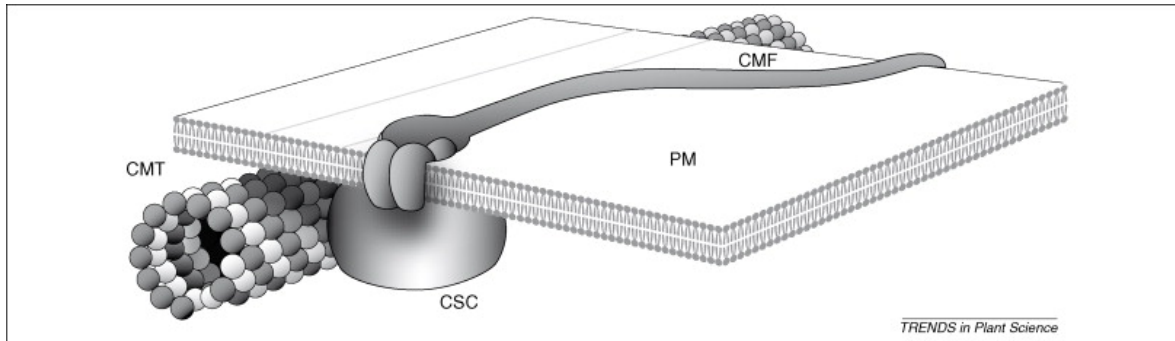
The deposition of cellulose is guided
by cortical microtubules



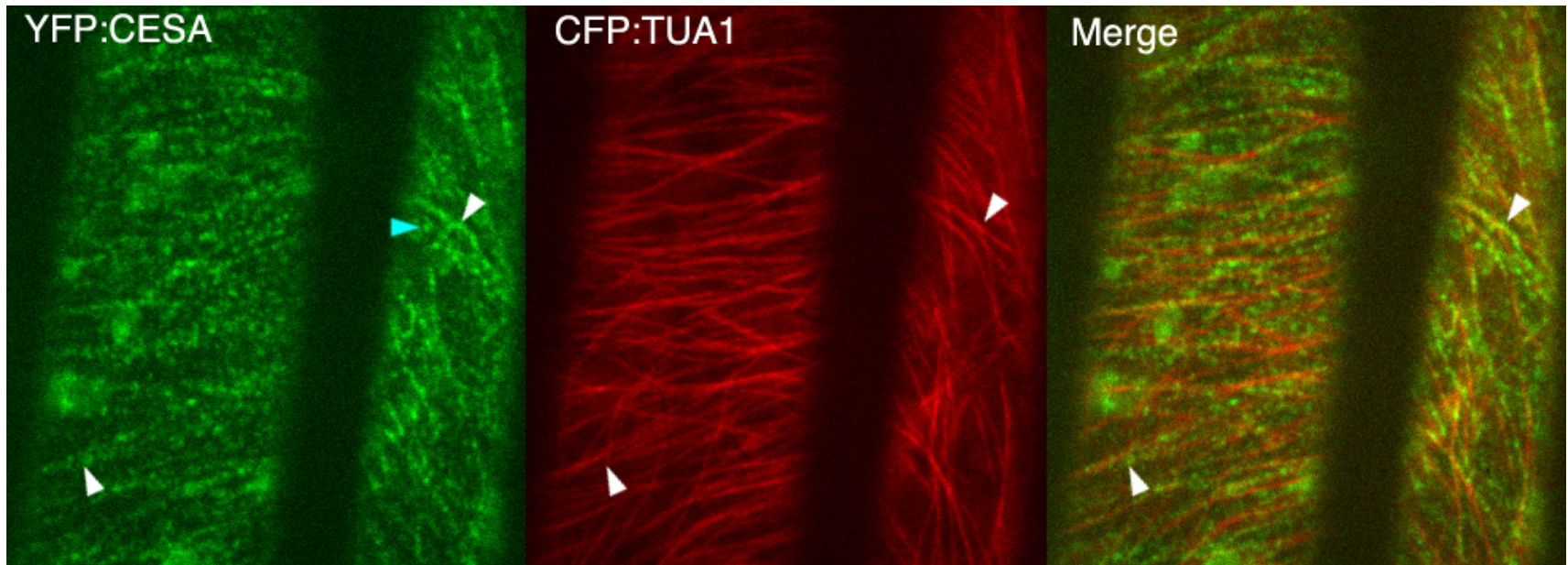
Microtubules

Cellulose
microfibrils

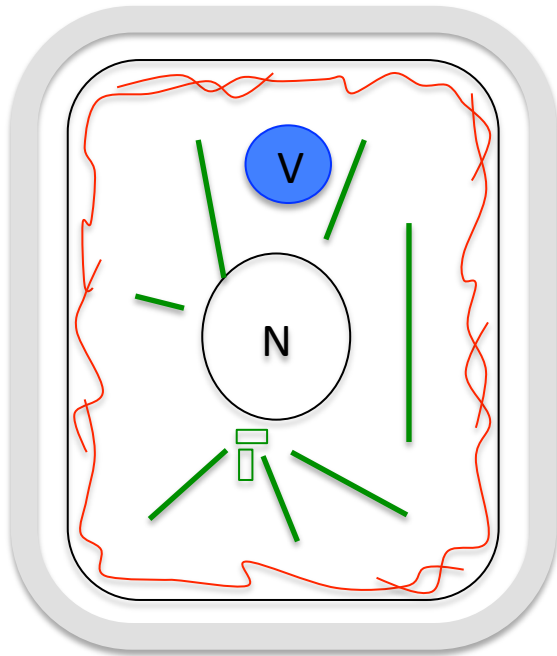
The deposition of cellulose is guided by cortical microtubules



Paredez et al., 2006
Emons 2006

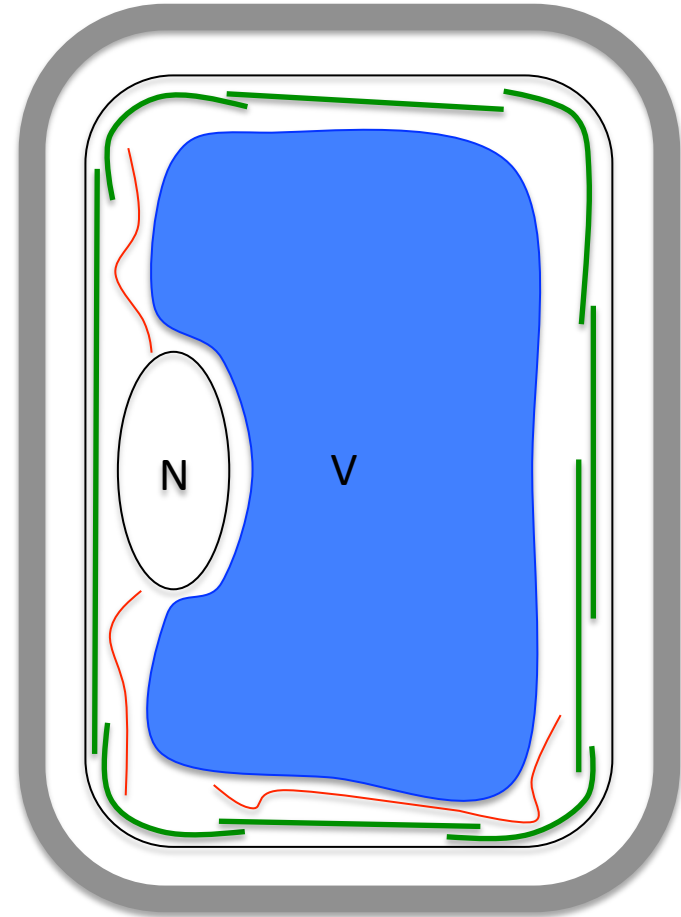


Animal cell



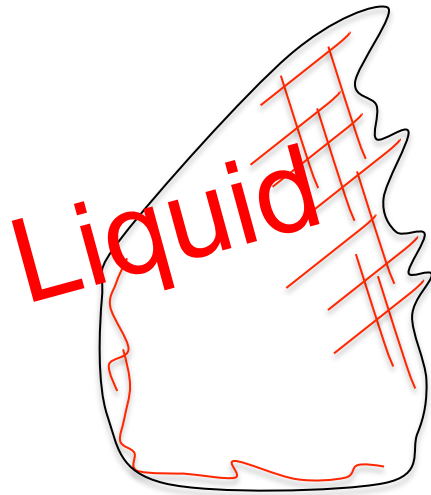
Cell stiffness: kPa range

Plant cell



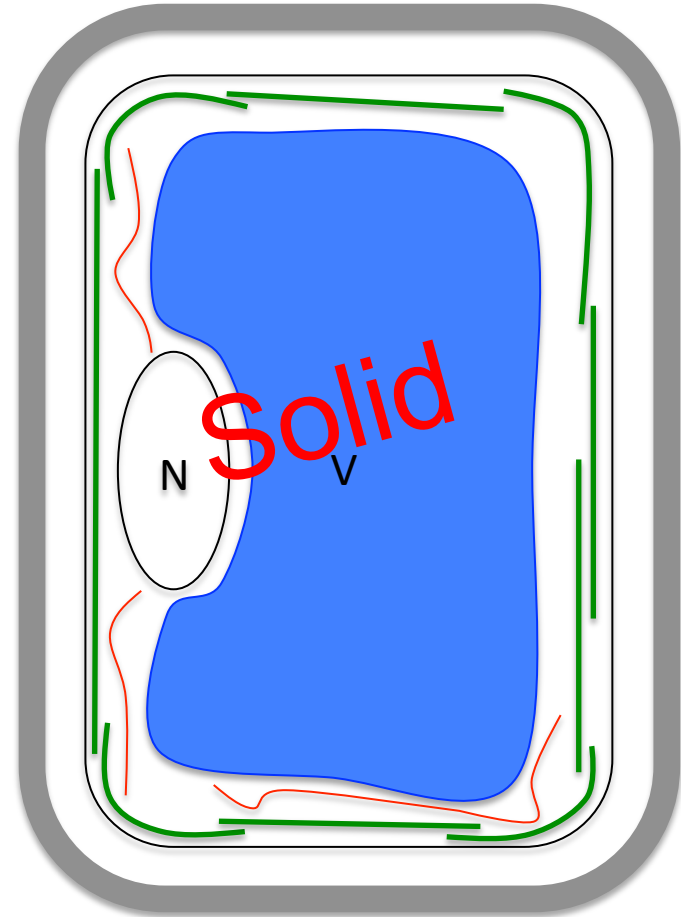
Cell stiffness: MPa range

Animal cell



Contractility
(actomyosin)

Plant cell



Stiffness
(cell wall)

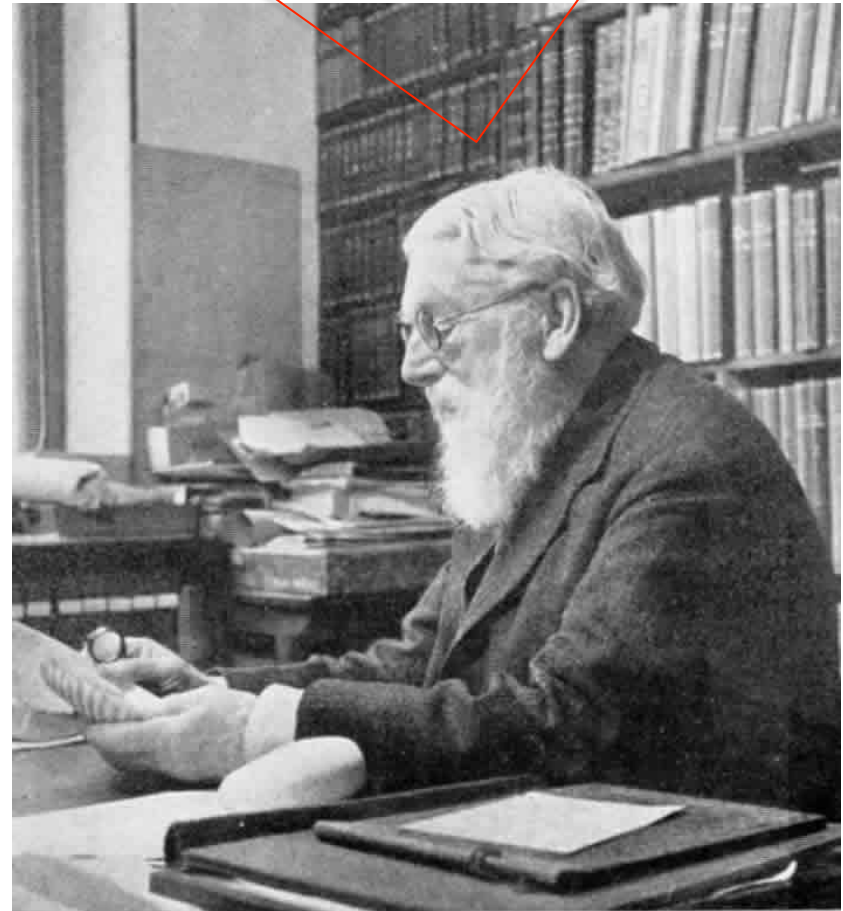
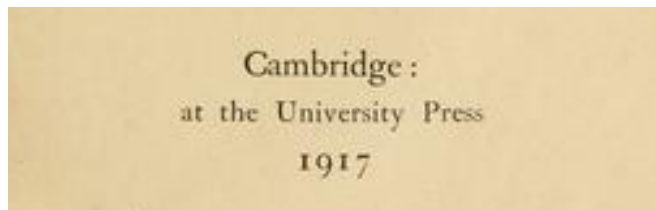
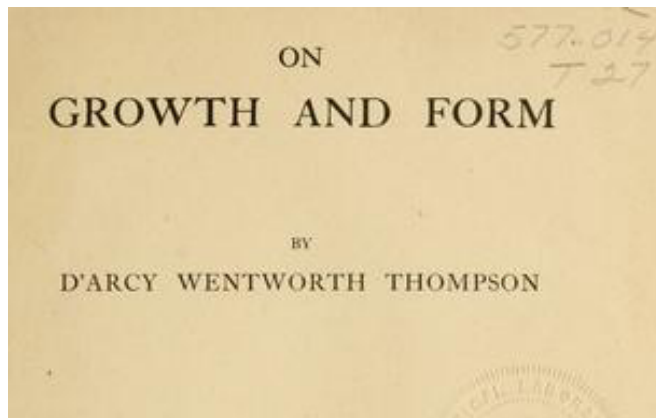
Summary

Definitions:

- Complex systems
- Tension/compression
- Stress/strain
- Stiffness
- Growth (rate, direction)
- Plant/animal cell structural elements
- In plants, cortical microtubules guide cellulose deposition

All living organisms are pre-stressed

the form of an object is a “diagram of forces”



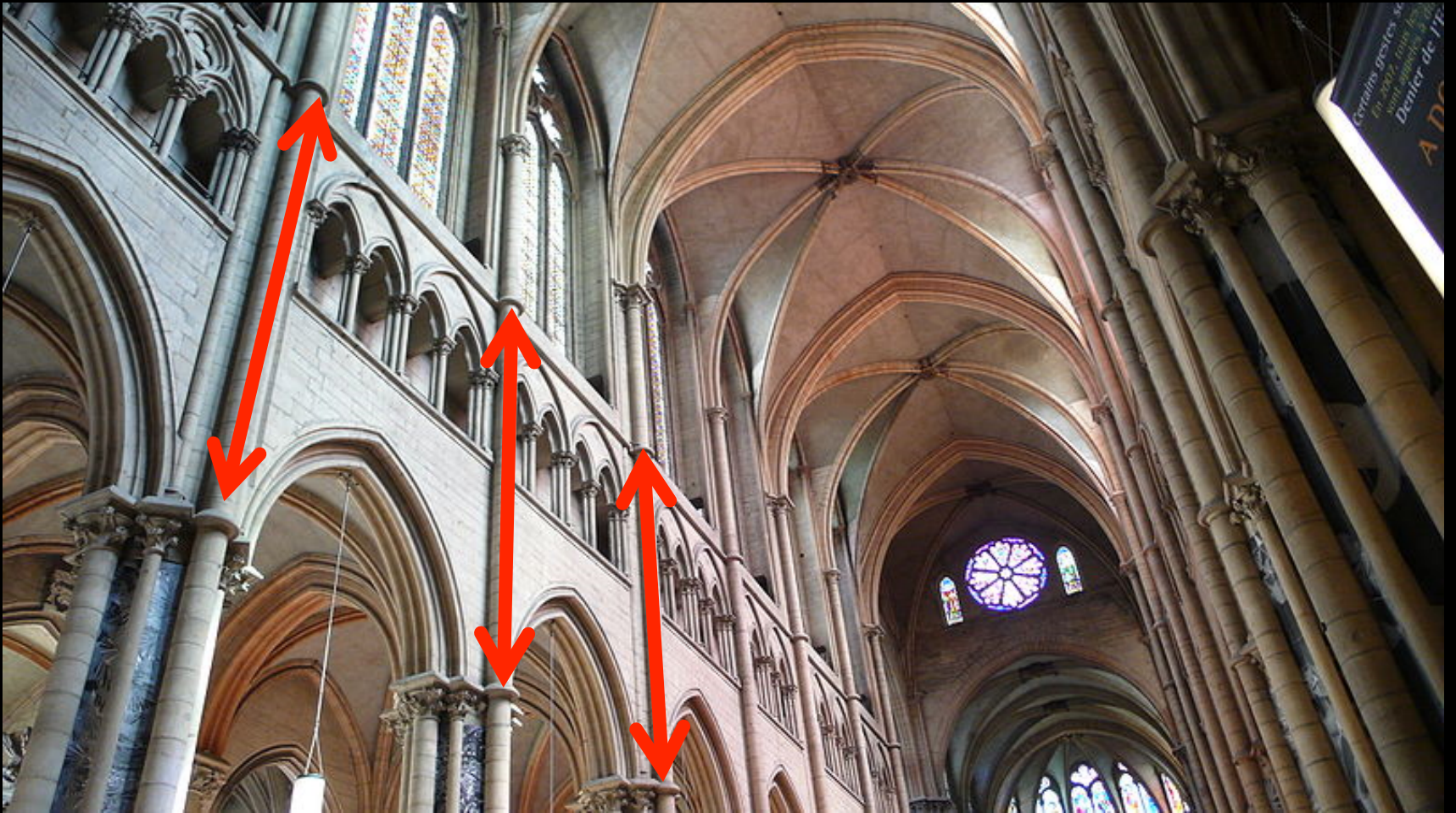
D'Arcy W. Thompson

A simple way to generate a shape: piling



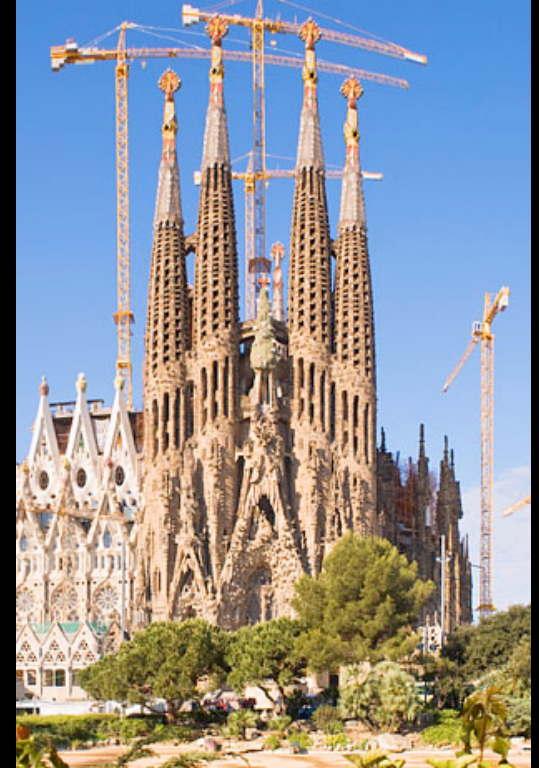
Continuous compression

the form of an object is a “diagram of forces”



Cathédrale Saint Jean, Lyon

the form of an object is a “diagram of forces”

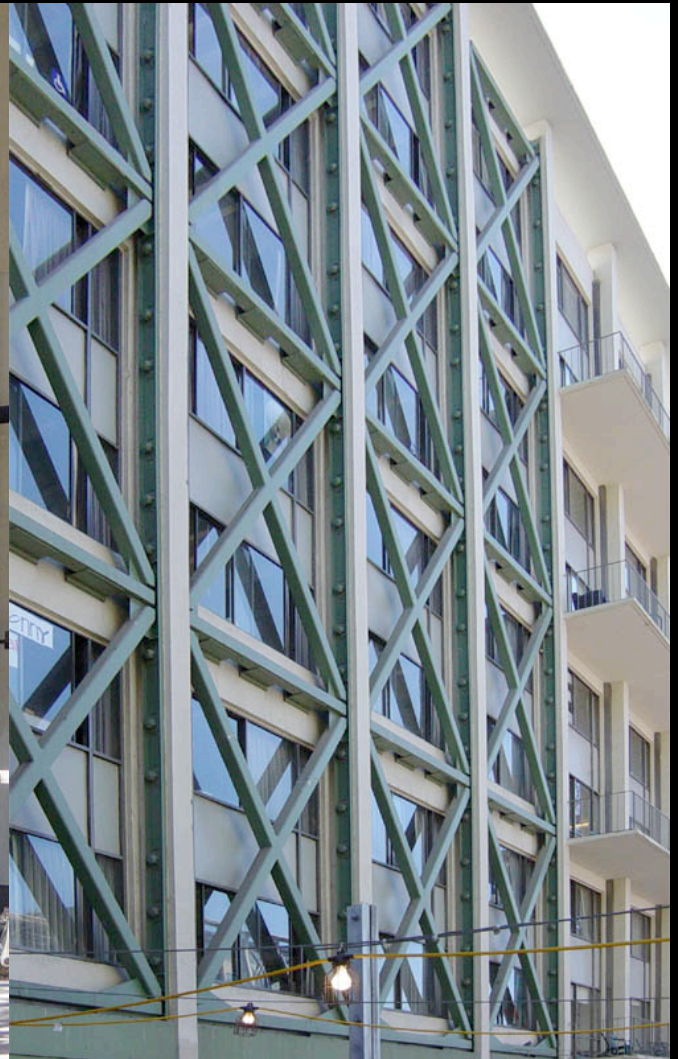


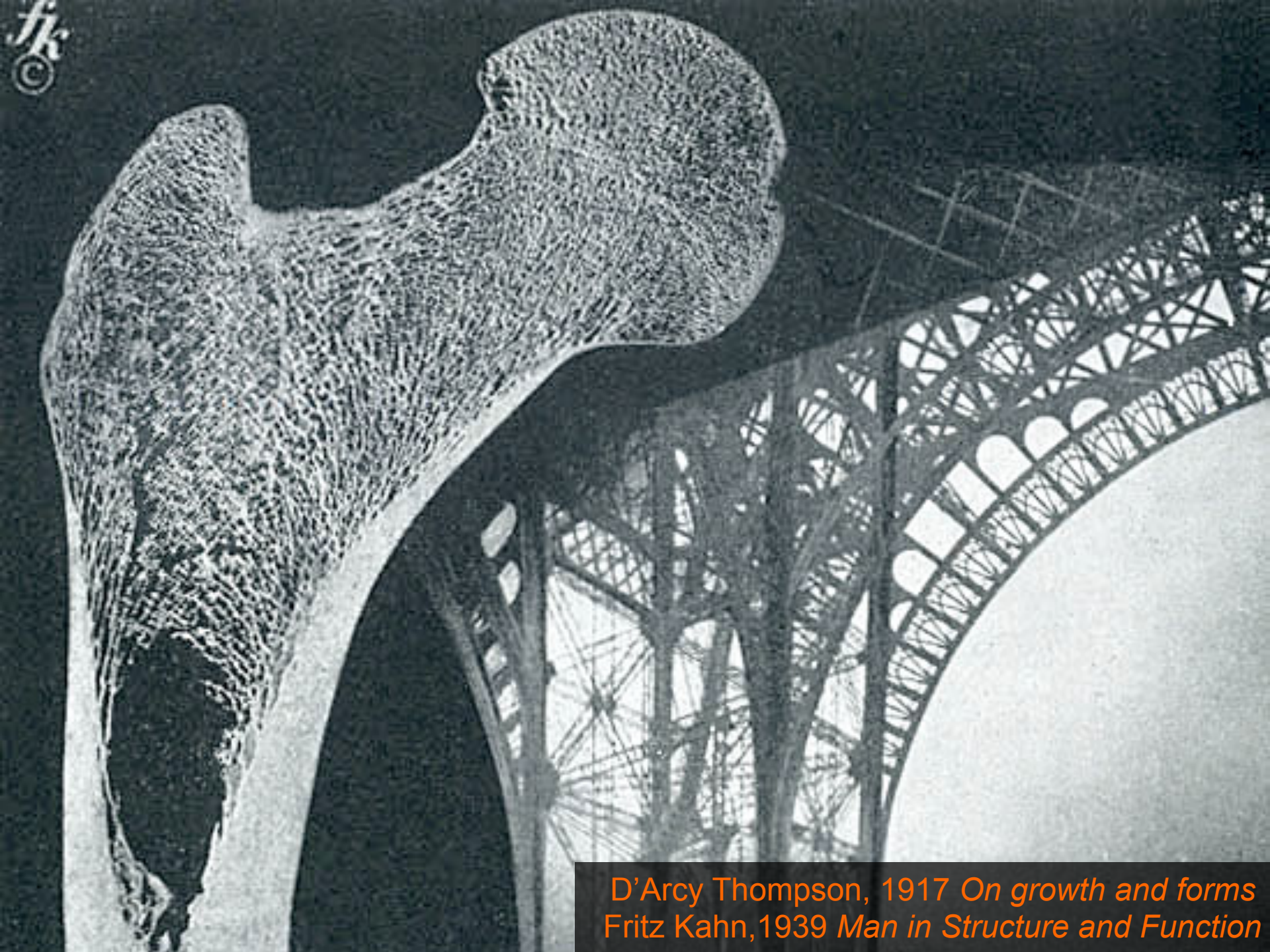
Gaudi's sand bag model

the form of an object is a “diagram of forces”



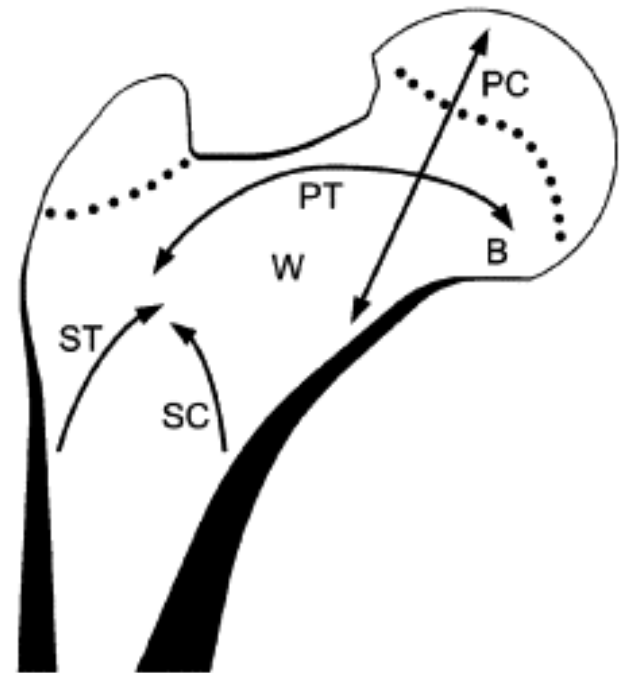
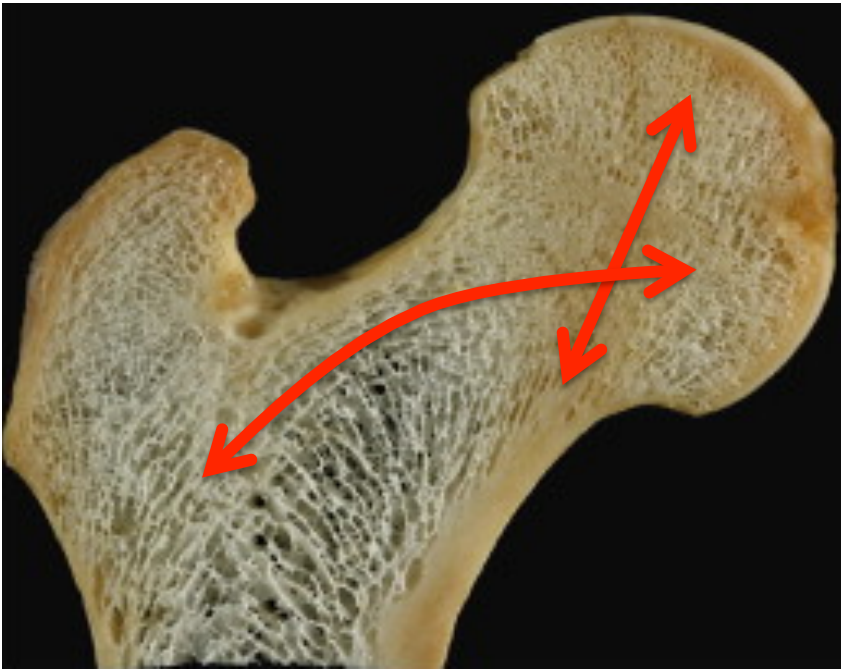
Berkeley





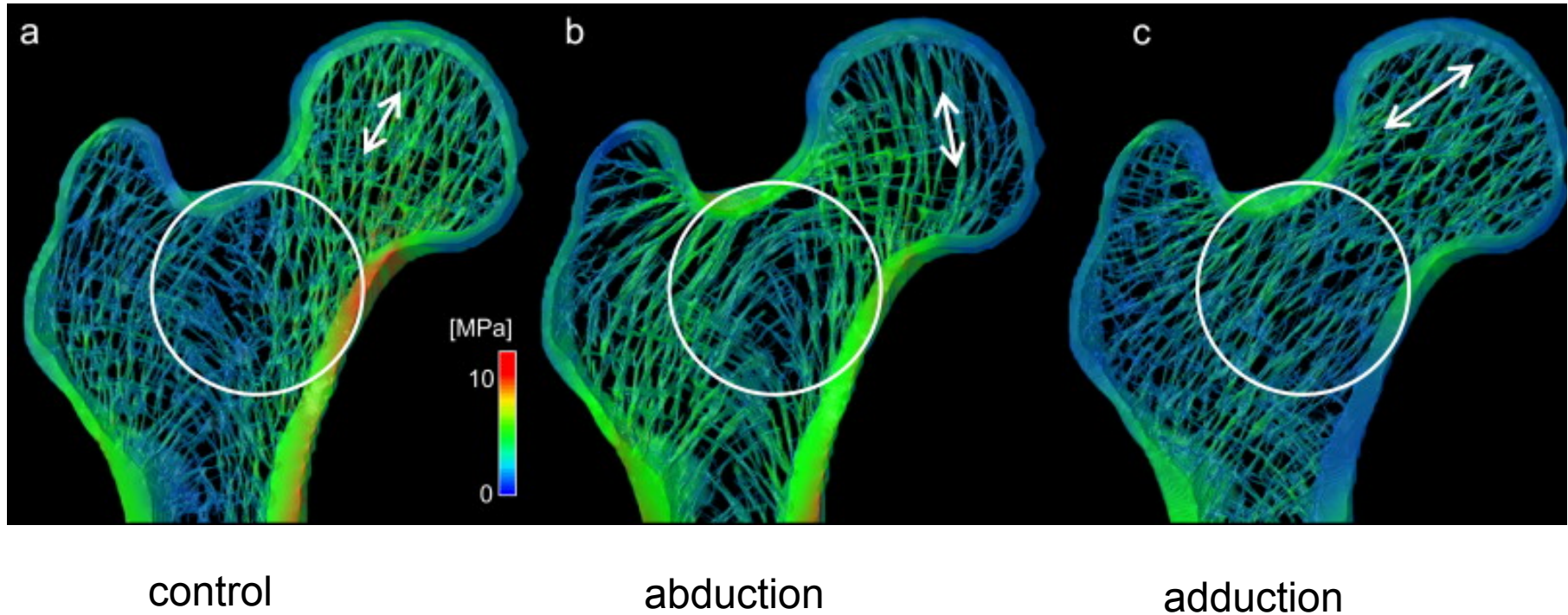
D'Arcy Thompson, 1917 *On growth and forms*
Fritz Kahn, 1939 *Man in Structure and Function*

In living organisms?



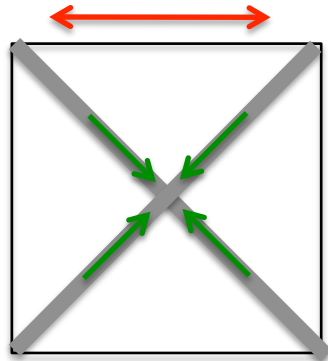
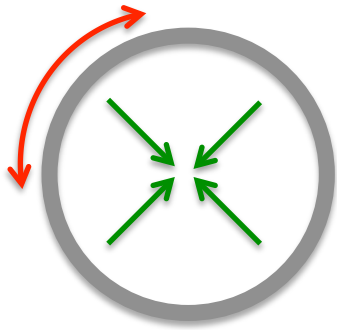
Wolff's law: bone architecture can be predicted by patterns of forces

A local response of cells to the mechanical load



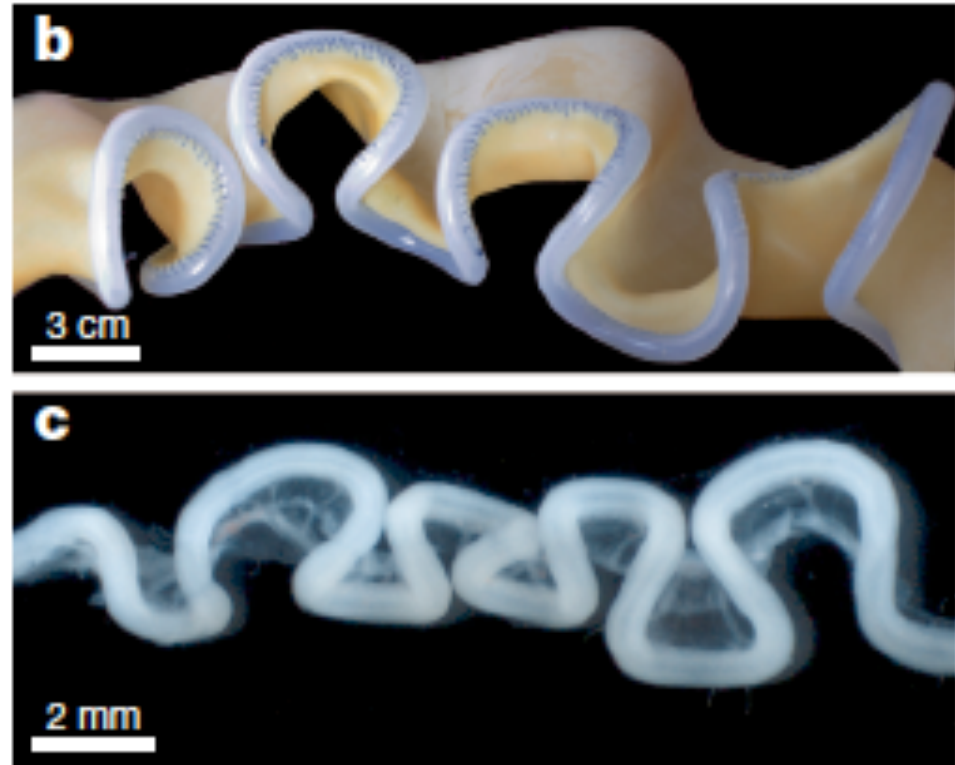
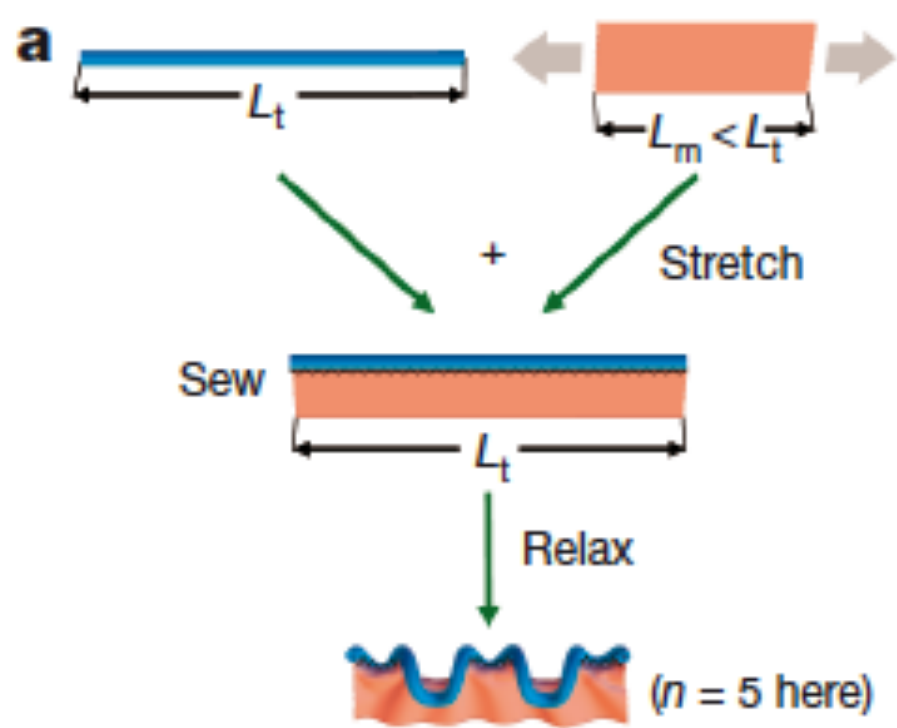
Tsubota et al., 2009

Shapes can be generated by a balance
between compression and tension



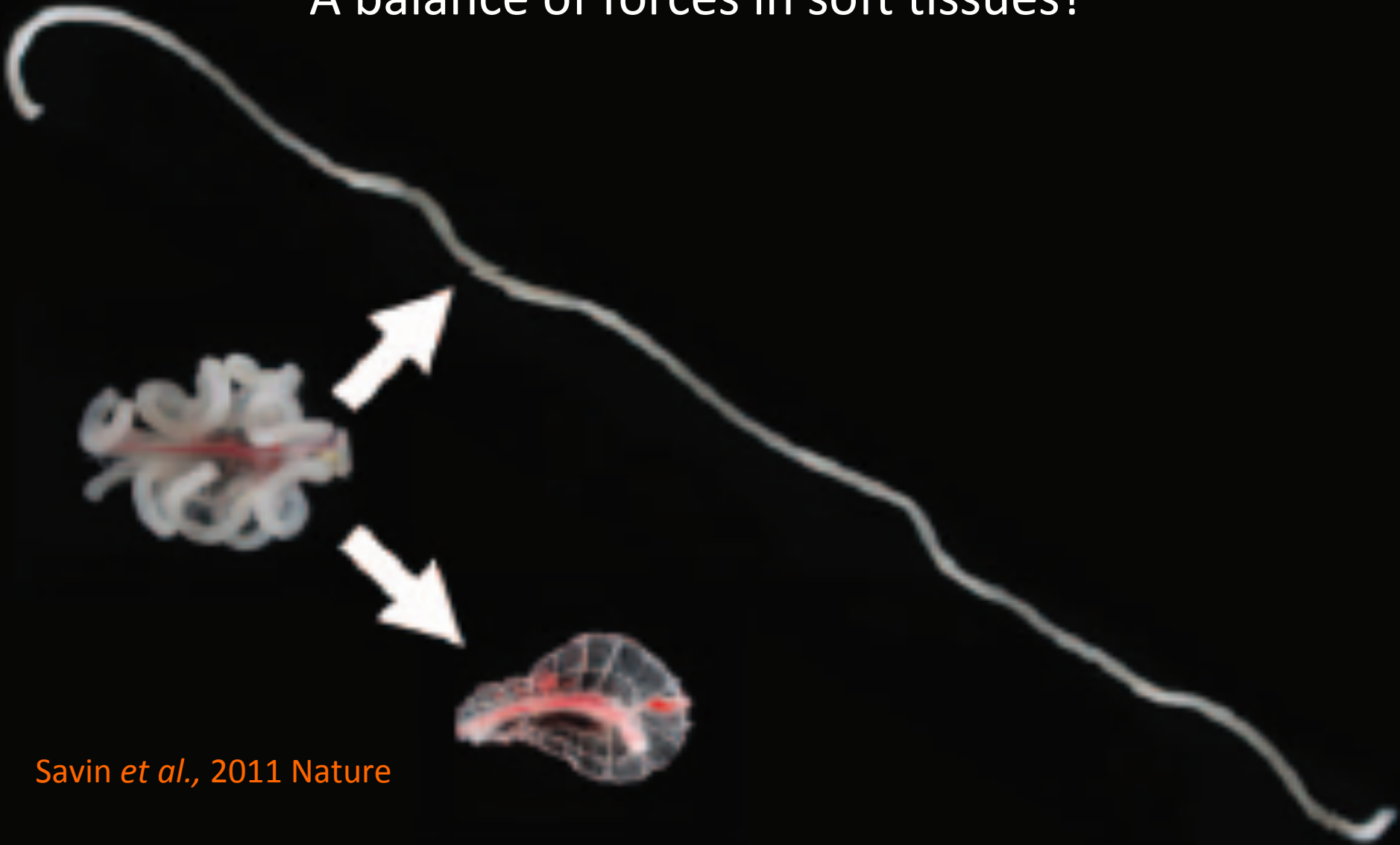


A balance of forces in soft tissues?



Savin *et al.*, 2011 Nature

A balance of forces in soft tissues?



Savin *et al.*, 2011 Nature

Turgor pressure $\sim$ bike tyre pressure



$\sim$



An analog model of a plant?



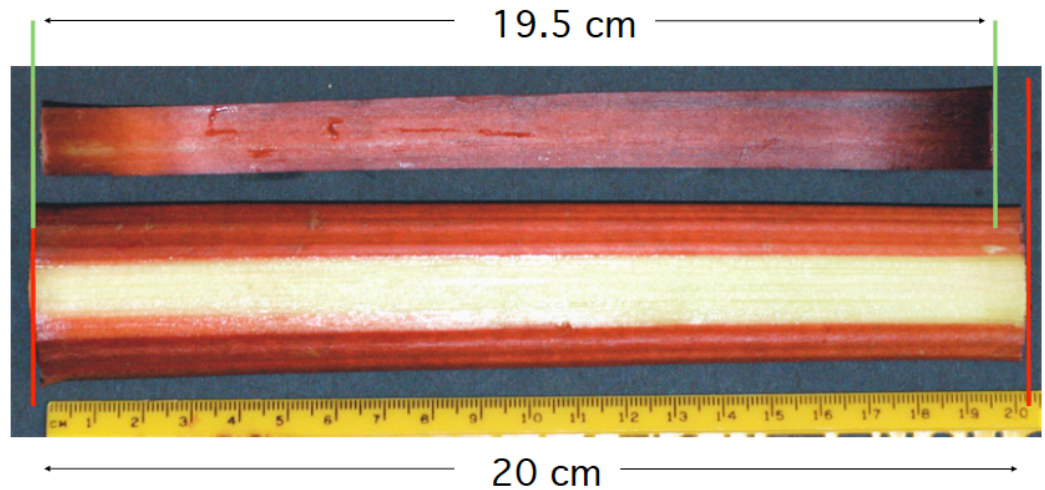
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An epidermis under tension in plants

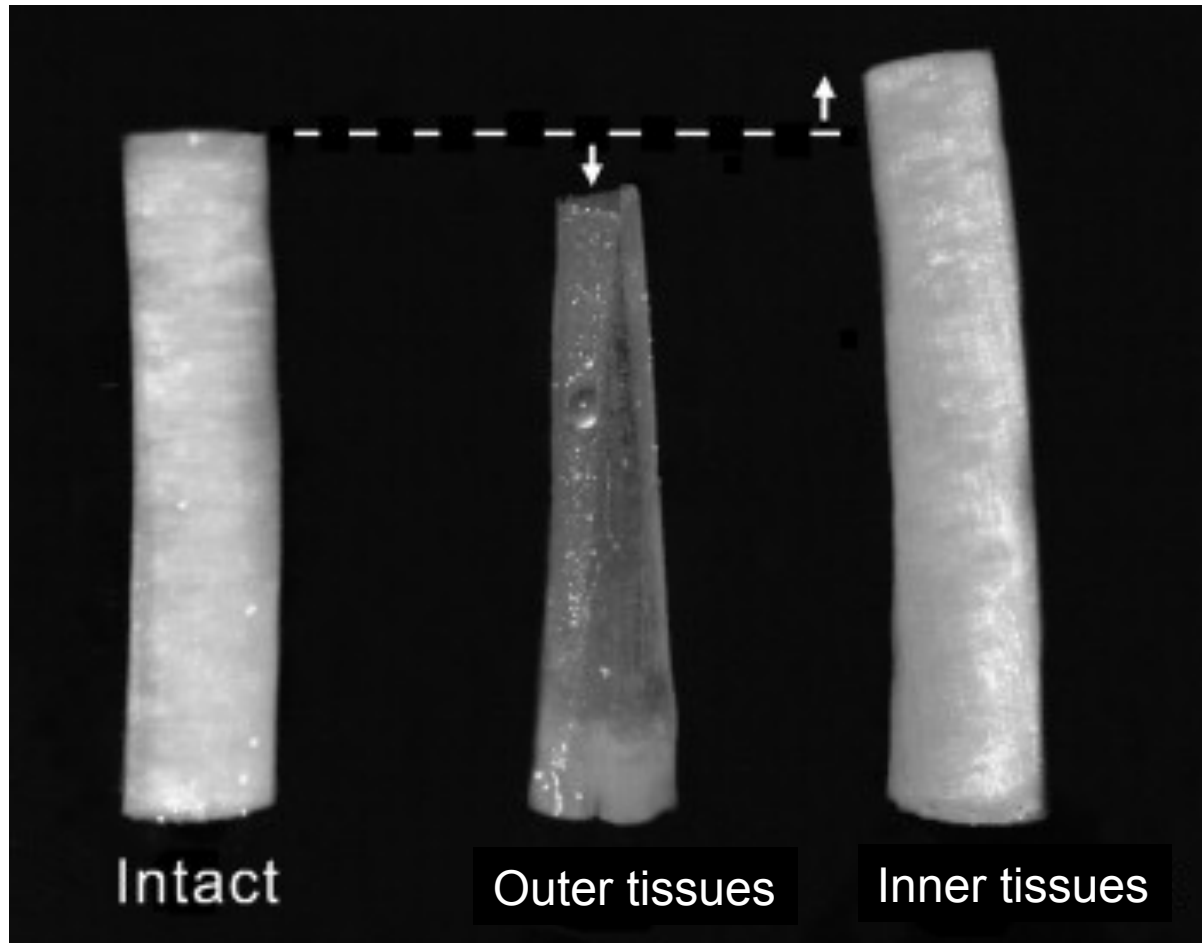


Radishes « open »



A peeled epidermis contracts

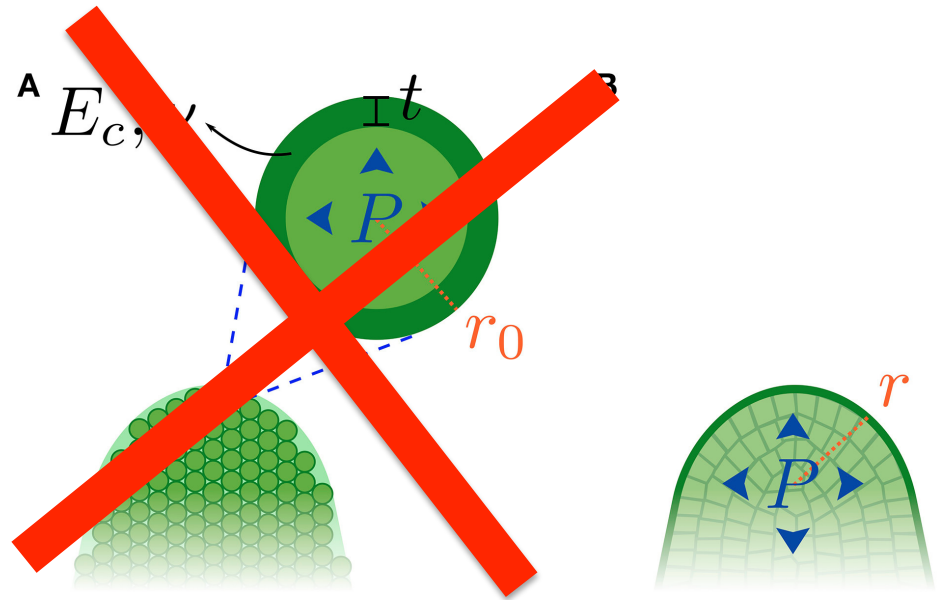
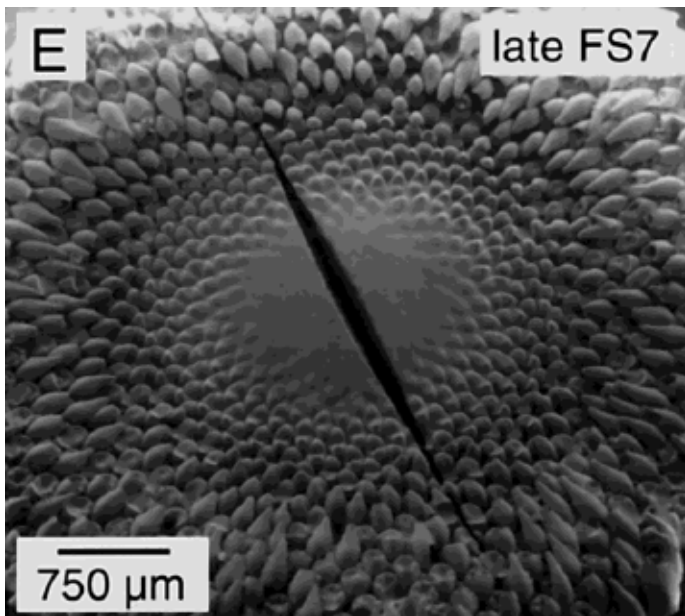
An epidermis under tension in plants



Peeling experiments with excised hypocotyl segments of 4-day-old etiolated sunflower seedlings

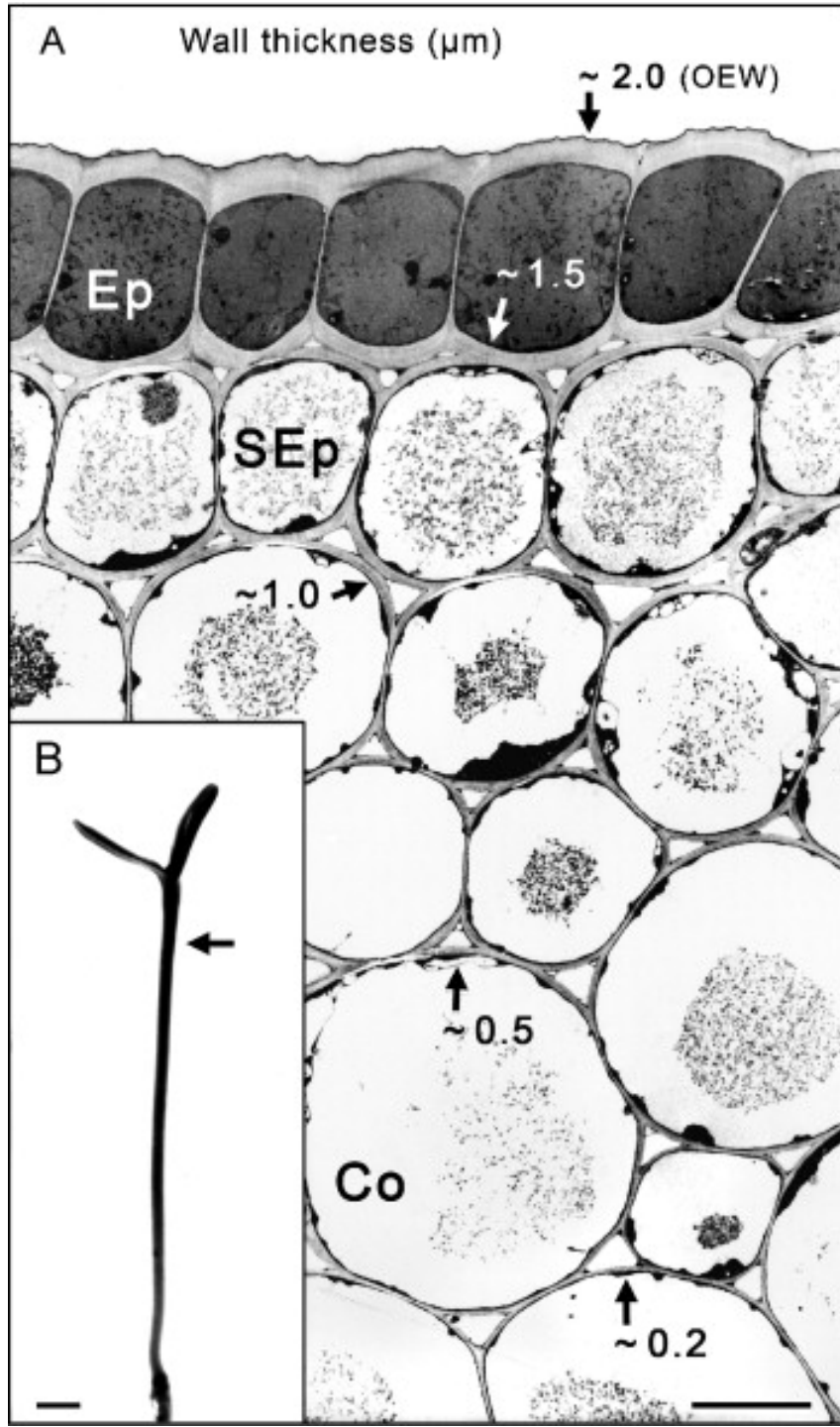
Kutschera and Niklas 2007

Mechanically, aerial plant tissues
behaves like a pressure vessel



Dumais and Steele, 2000 J.P. Gr. Regul.

Beauzamy *et al.*, 2015 Fr. Pl. Sci.



In plants,
the epidermis
is limiting for growth

Thickness of the cell walls in the
sunflower hypocotyl (TEM)

Co=cortex,
Ep=epidermis,
OEW=outer epidermal wall,
SEp=subepidermis.

In plants, the epidermis is limiting for growth



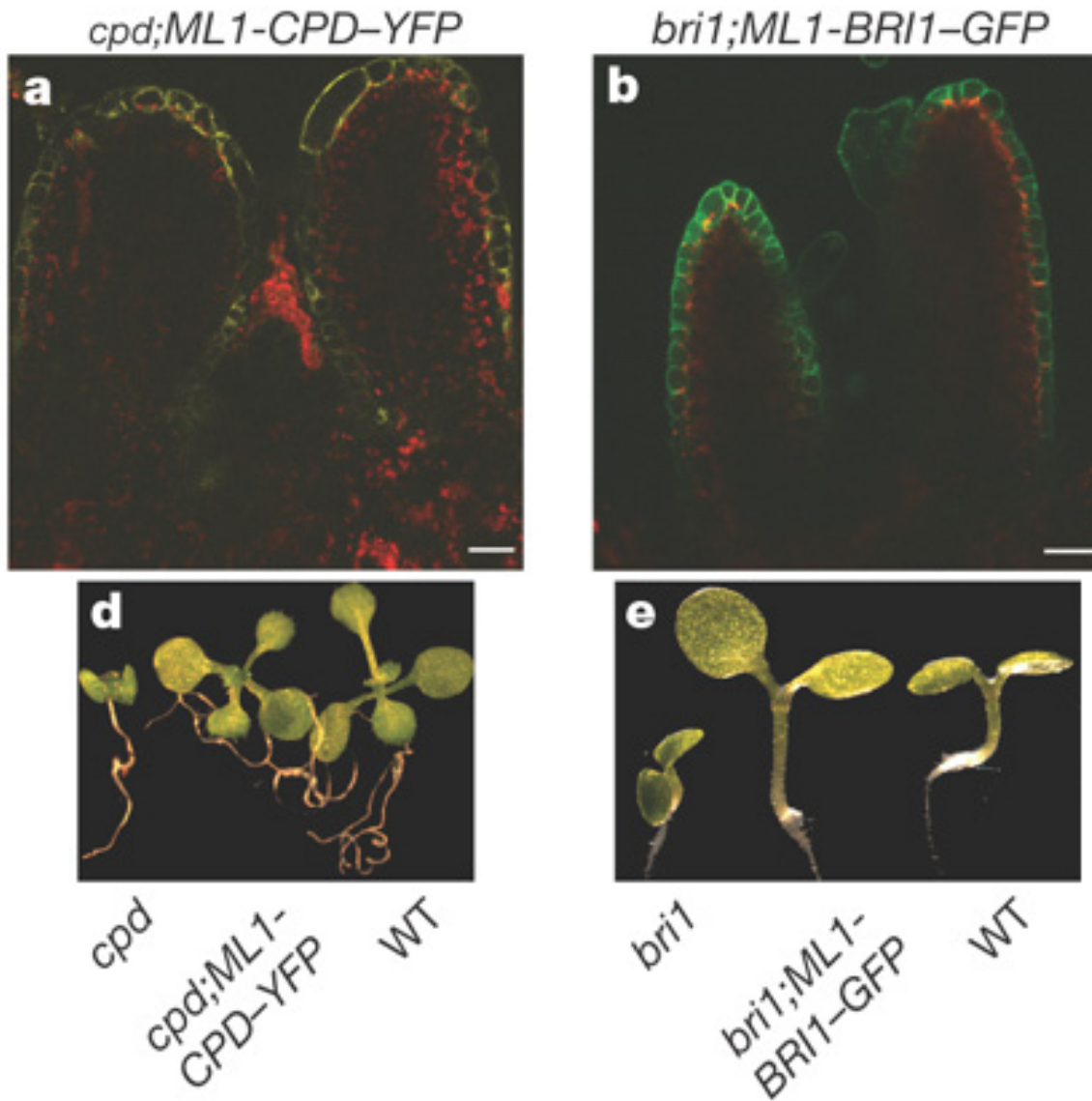
WT



bri1-5

Brassinosteroids
promote growth

In plants, the epidermis is limiting for growth



An analog model of a plant



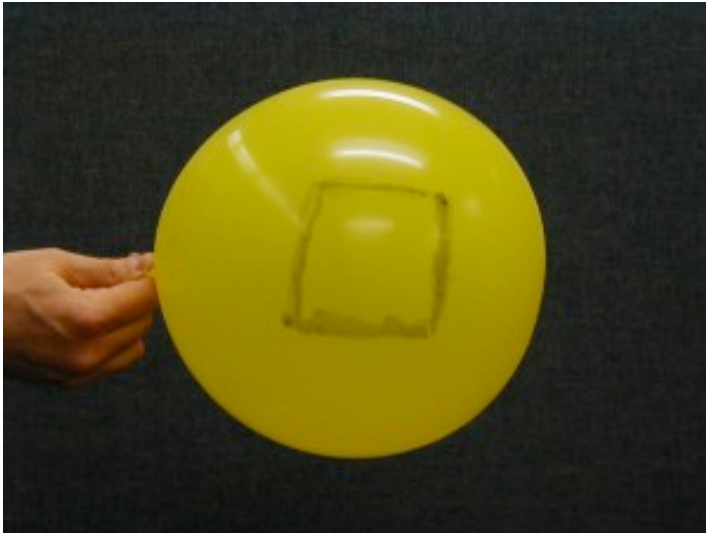
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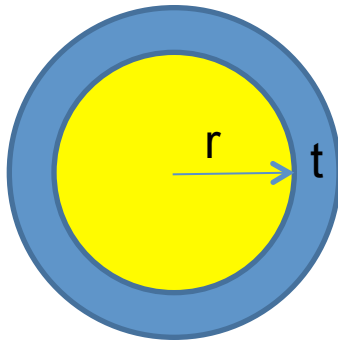
Calculating a stress pattern

“If you can’t demonstrate it with balloons, it’s probably not important anyway”

Stephen A. Wainwright



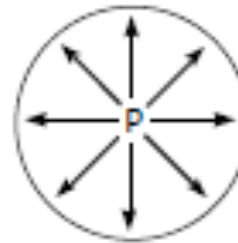
Axial Stress



$r \gg t$

Area for a ring
 $A = \pi((r+t)^2 - r^2)$
 $= \pi(2rt + t^2)$
 $\sim 2\pi rt$

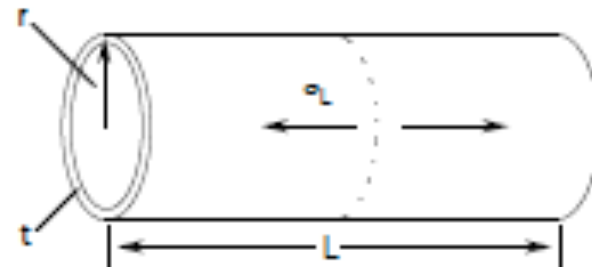
For a sphere



Applied force at a given cross section: $F = \pi r^2 P$ (pressure $\times$ projected area)

Area of material resisting: $A = 2\pi r t$

$$\sigma = F/A = Pr/2t$$



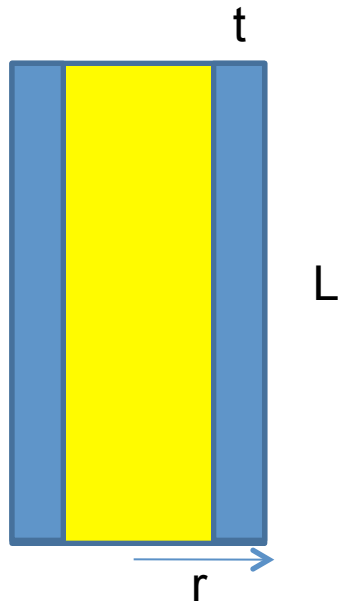
For a cylinder (axial stress)

Applied force at a given cross section: $F = \pi r^2 P$

Area of material resisting: $A = 2\pi r t$

$$\sigma_L = F/A = Pr/2t \quad (\text{same as sphere})$$

$$\sigma_L = Pr/2t$$



Circumferential Stress

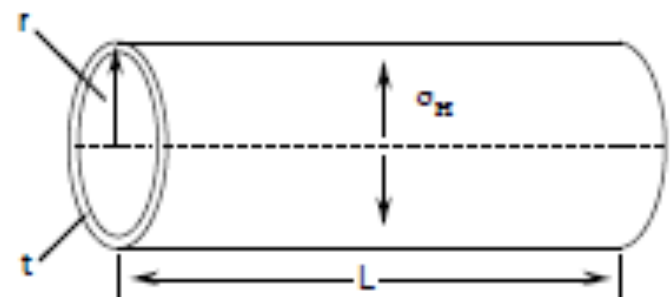
For a cylinder (circumferential stresses)

Applied force at a given cross section: $F = 2r \times L \times P$

Area of material resisting: $A = 2tL$

$$\sigma_H = F/A = Pr/t = 2\sigma_L$$

For any pressurized cylinder: circumferential stresses are 2x longitudinal stresses.



How to reveal the stress pattern?



qua1 mutants

Cell separation depends on turgor pressure: PEG experiments

Control medium

WT



qua1-1



Cell separation depends on turgor pressure: PEG experiments

Control medium

+50 mOsm

+100 mOsm

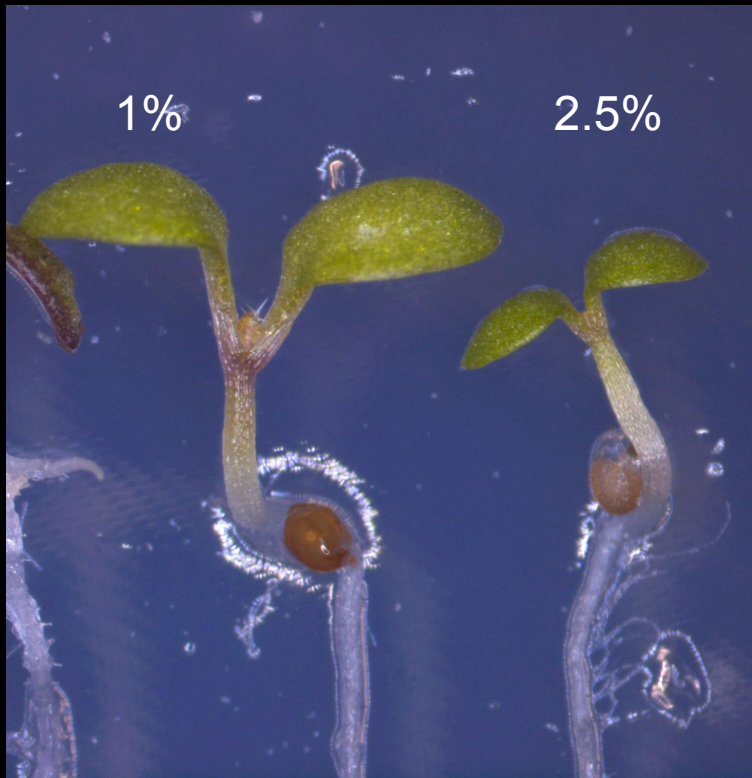
WT



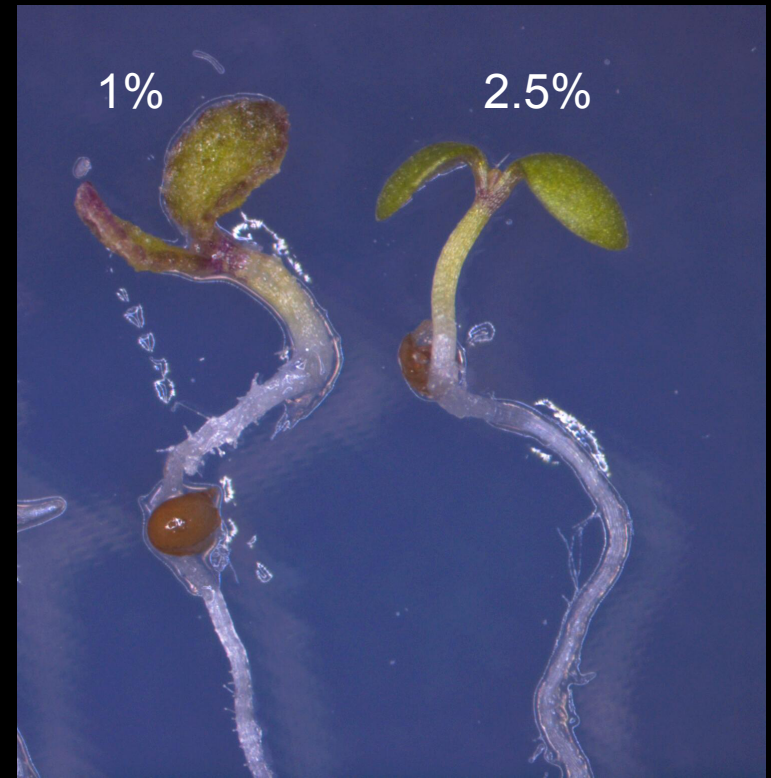
qua1-1



Cell separation depends on turgor pressure: Agar concentration

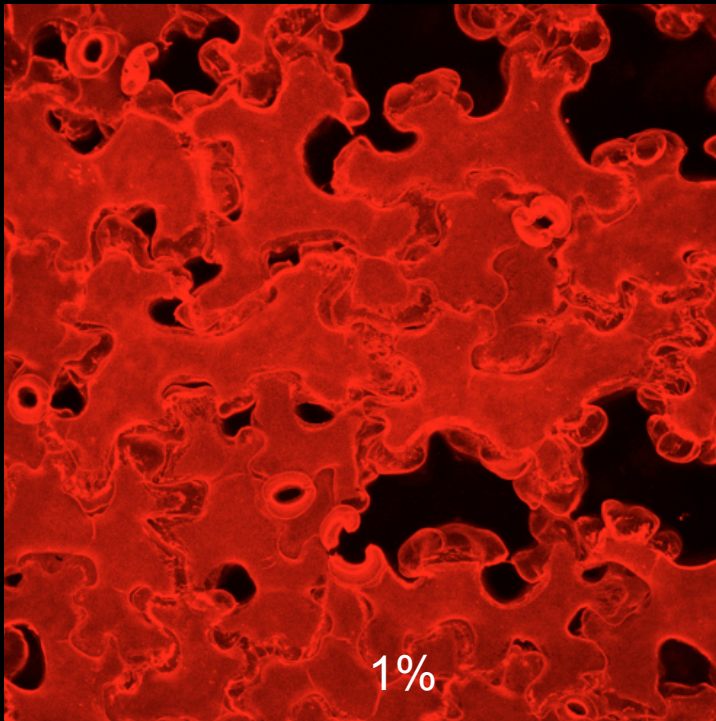


WT

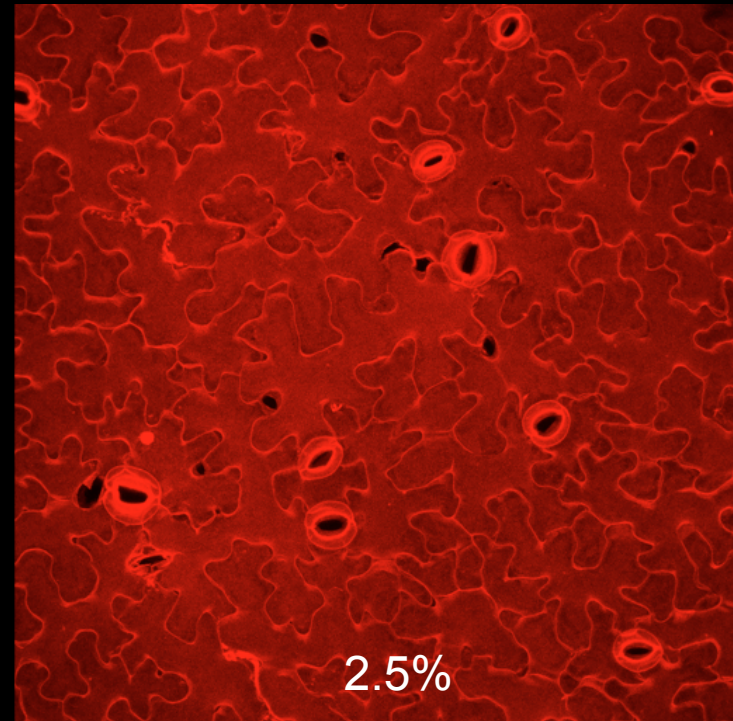


qua1-1

Cell separation depends on turgor pressure: Agar concentration

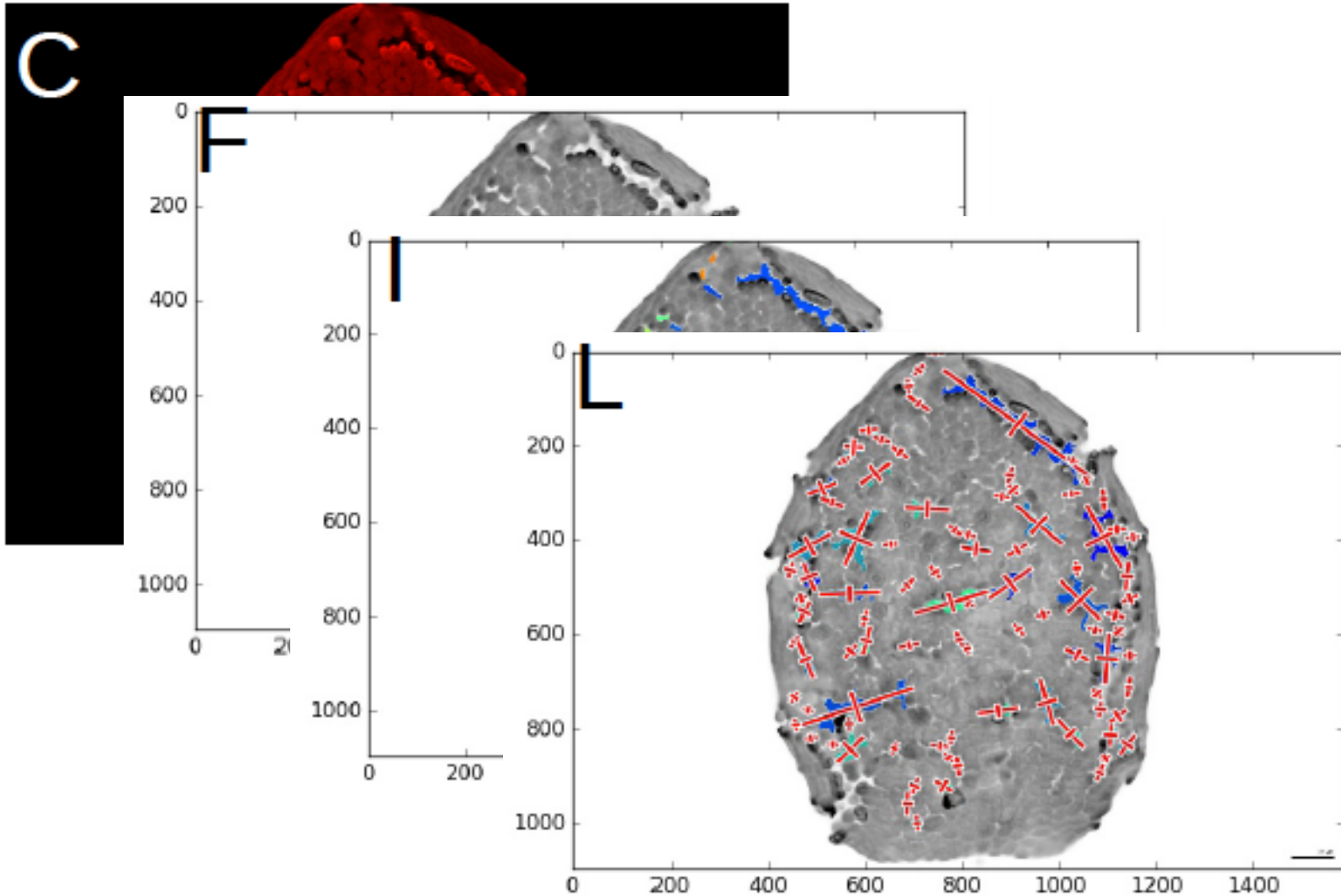


qua1-1

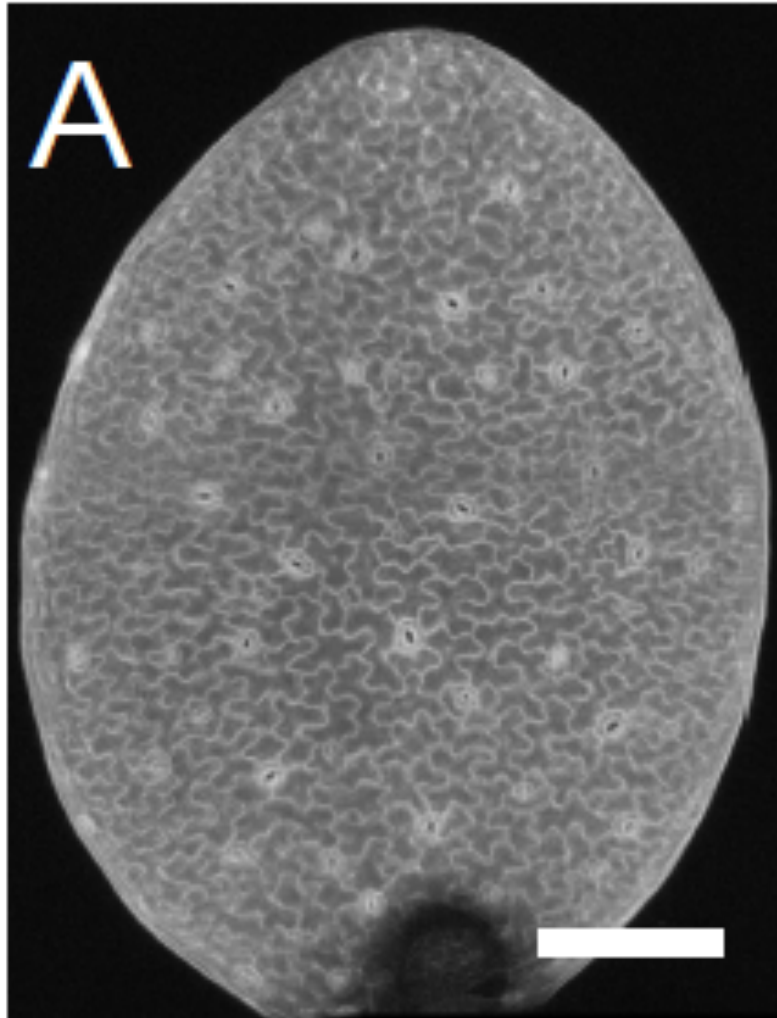


qua1-1

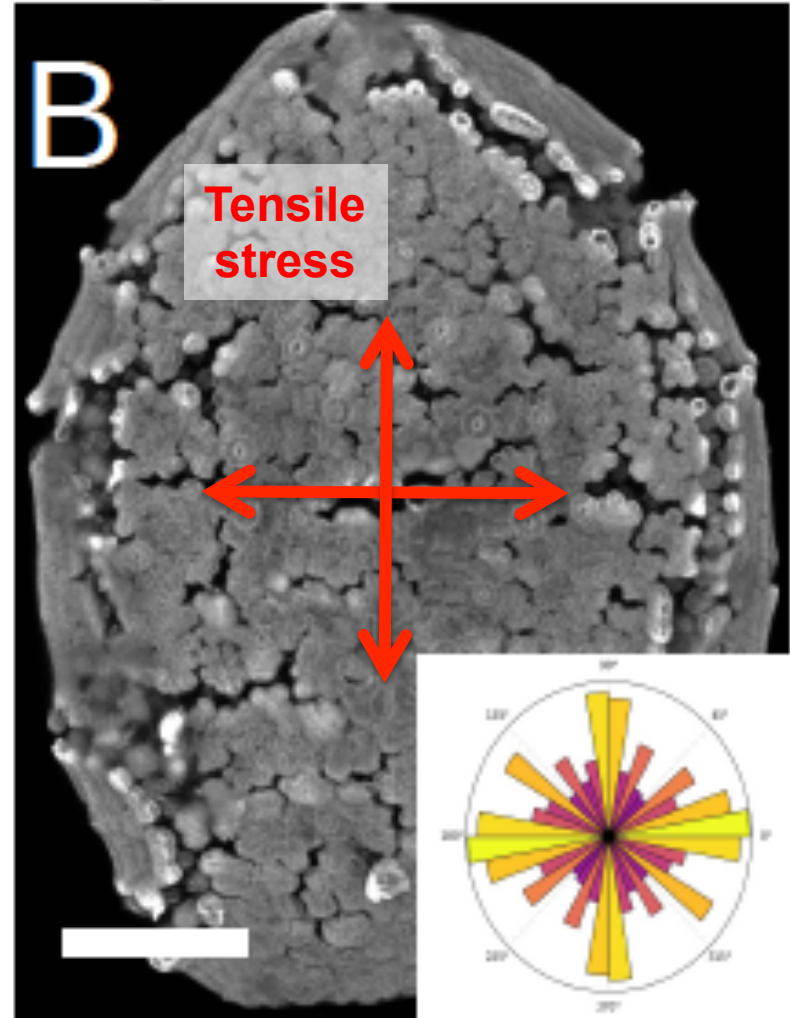
Extracting tensile stress directions from gaping patterns



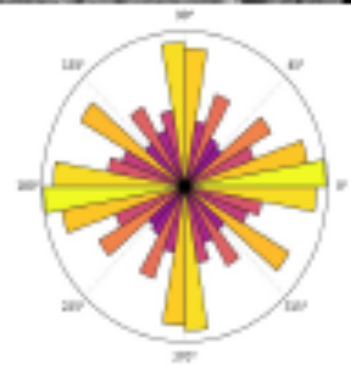
Isotropic stress pattern in cotyledon blade



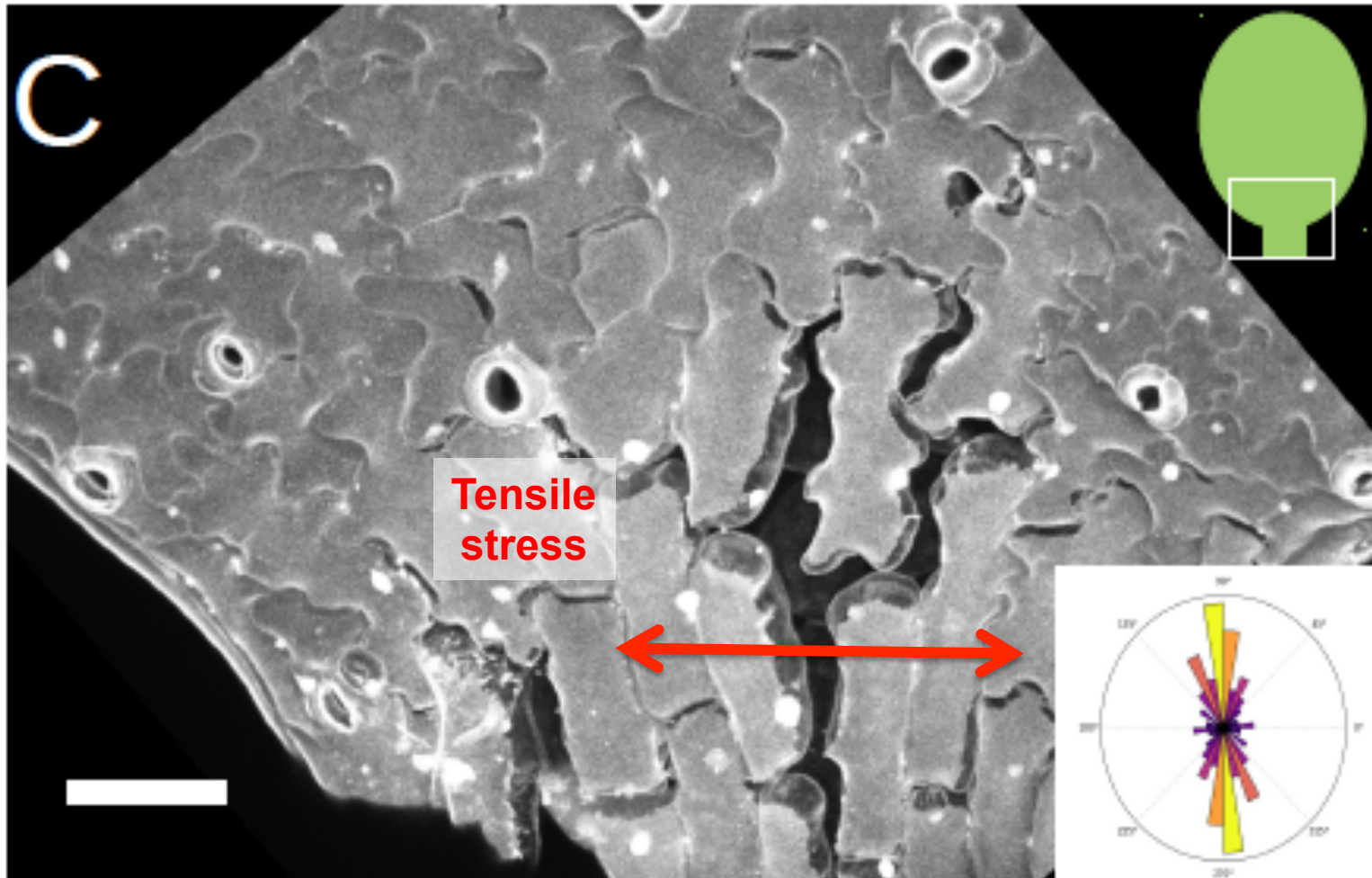
WT



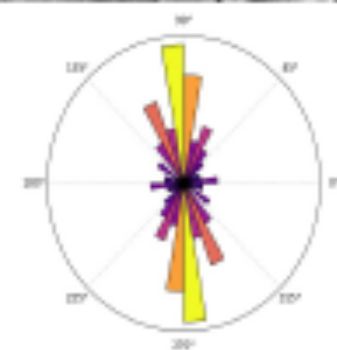
qua1 (Agar 1%)



Growth-induced directional stress at the petiole-blade junction

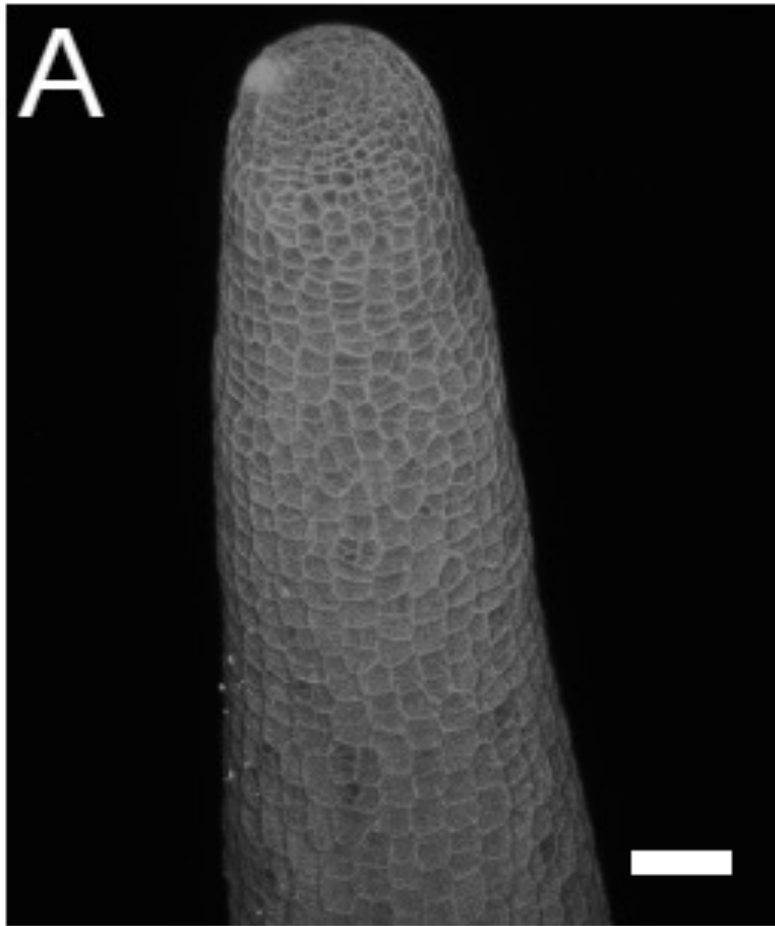


qua1 (Agar 2.5%)

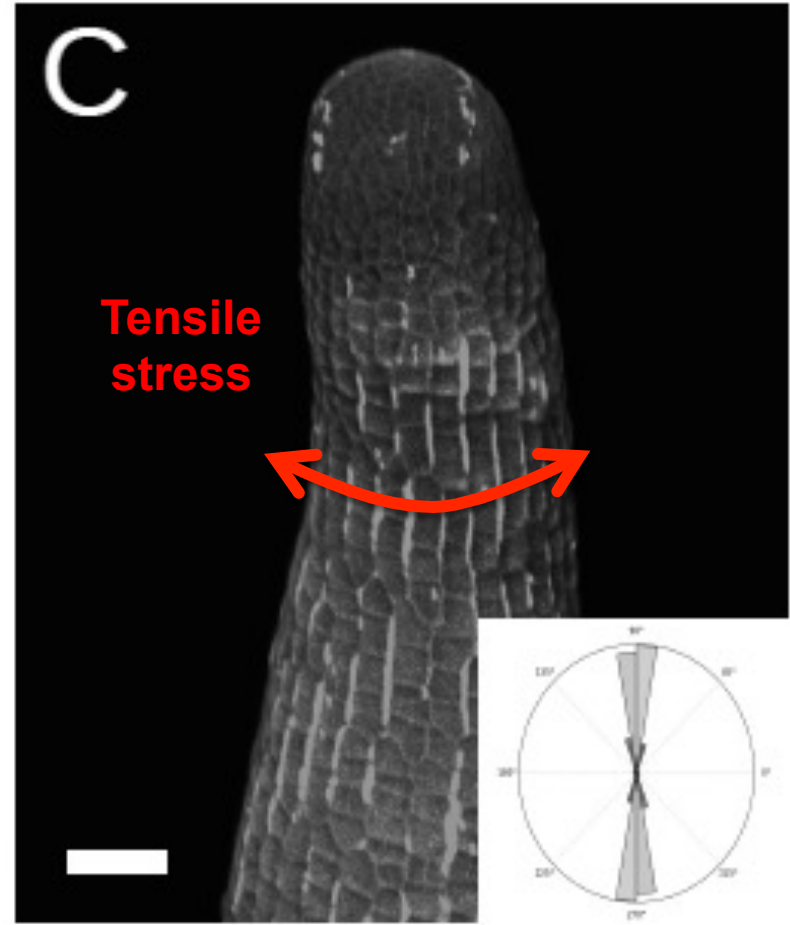


Verger *et al.*, eLife in press

Shape-induced stress in cylindrical stem

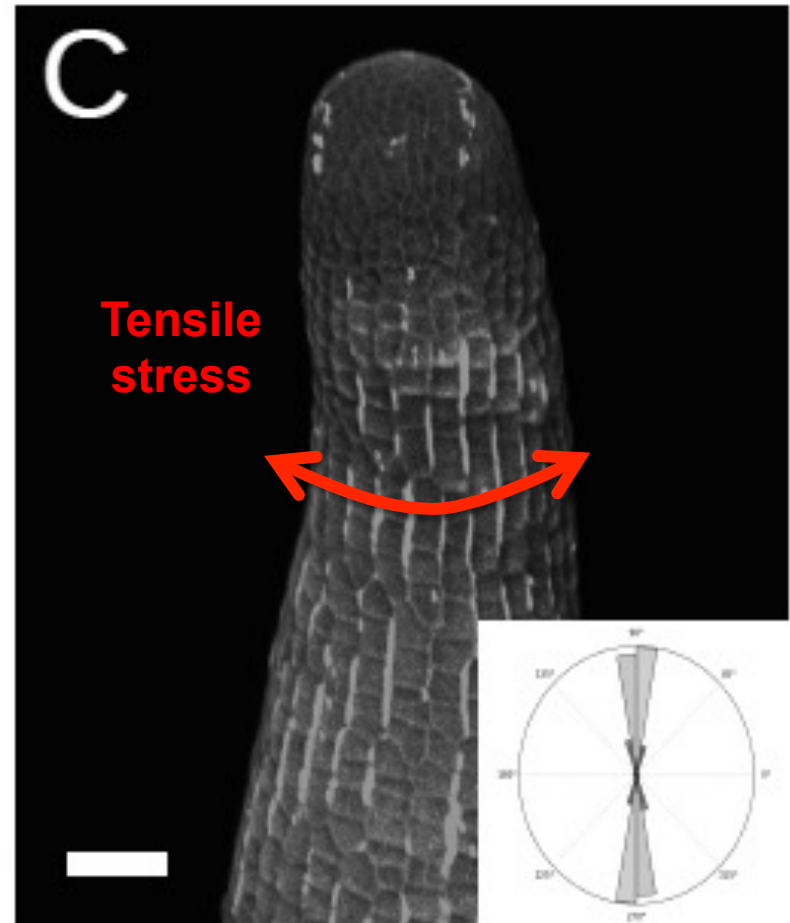


WT

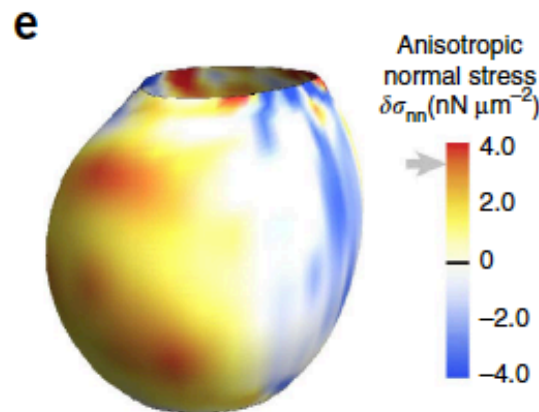
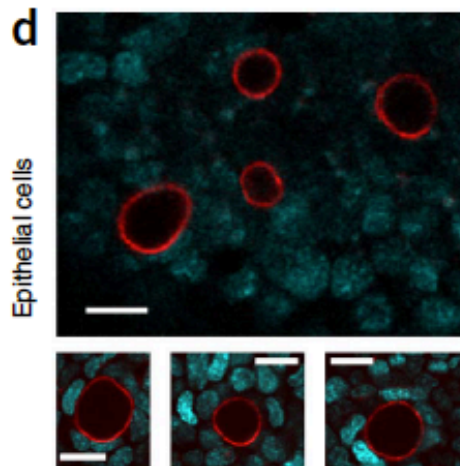
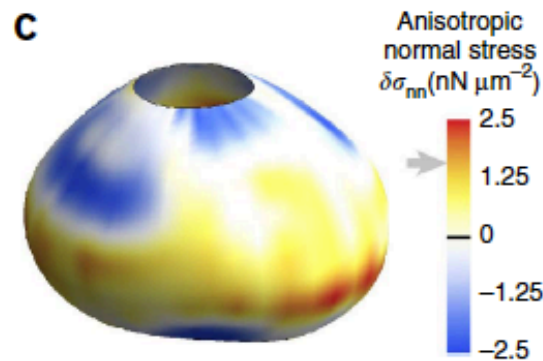
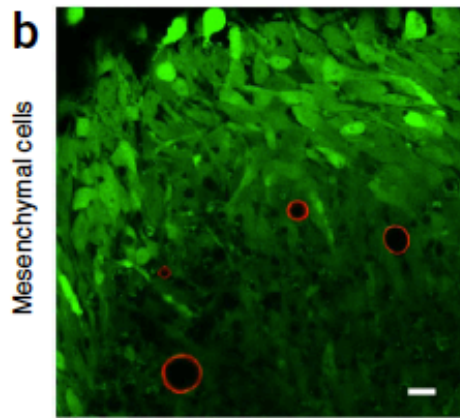
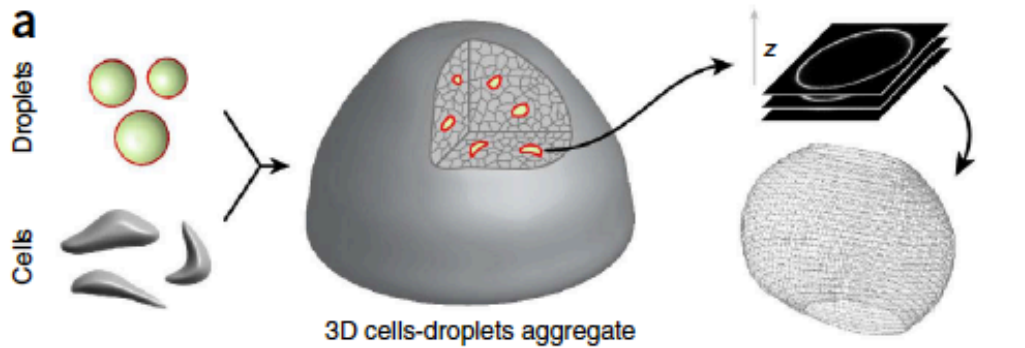


qua1 (Agar 2.5%)

Shape-induced stress in cylindrical stem



qua1 (Agar 2.5%)



Quantifying mechanical stresses in living embryonic tissues, using fluorescent, cell-sized oil microdroplets

Summary

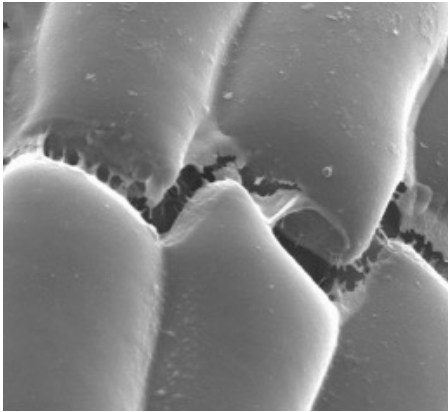
A shape prescribes a pattern of stress (and vice versa) at all scales, in all objects and living organisms

Inferring a stress pattern using calculus, analogous models or experiments

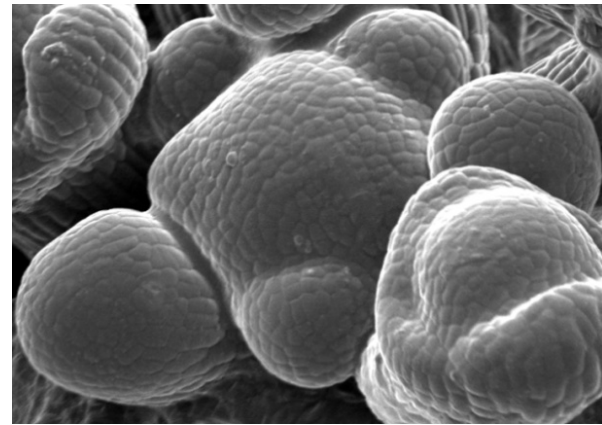
Epidermis is under tension in the aerial parts of plants

Tissue shape and growth prescribe a stress pattern.
In turn, can mechanical stress control cell behavior?

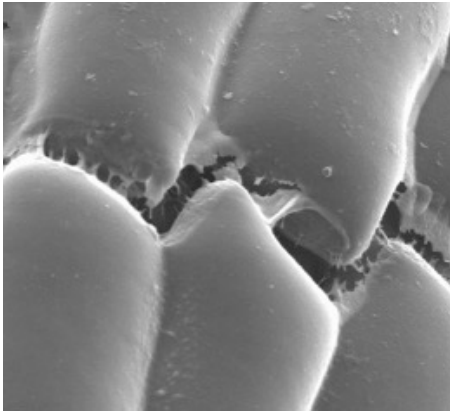
**Mechanical
stress pattern**



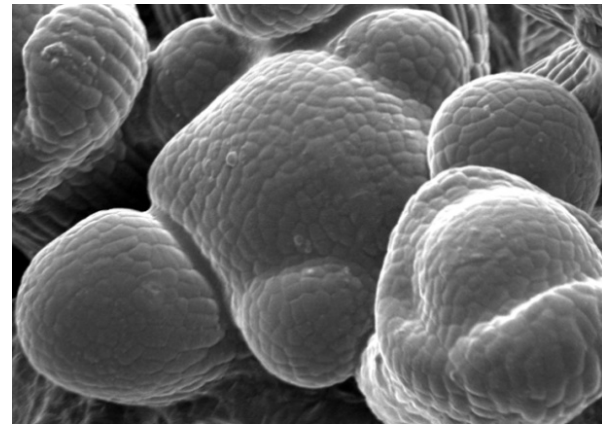
**Shape
and growth**



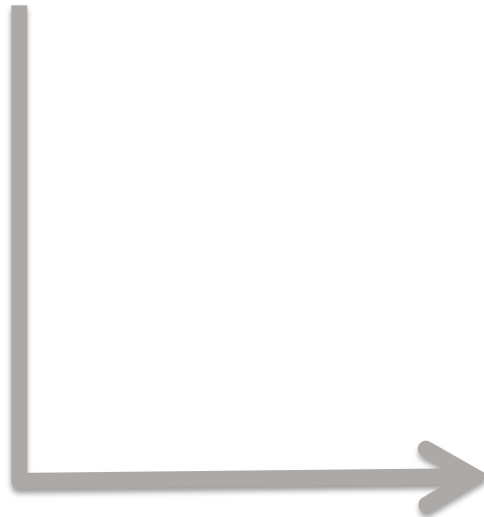
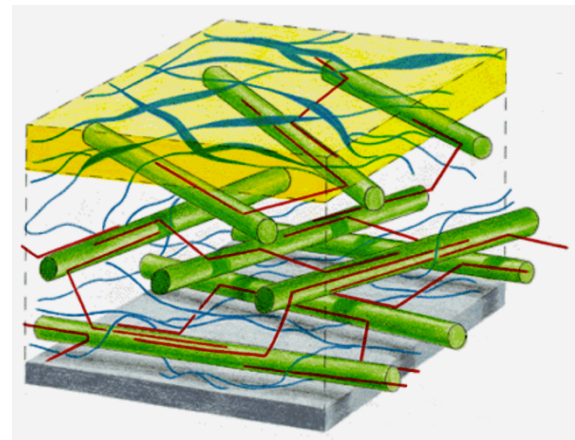
Mechanical stress pattern

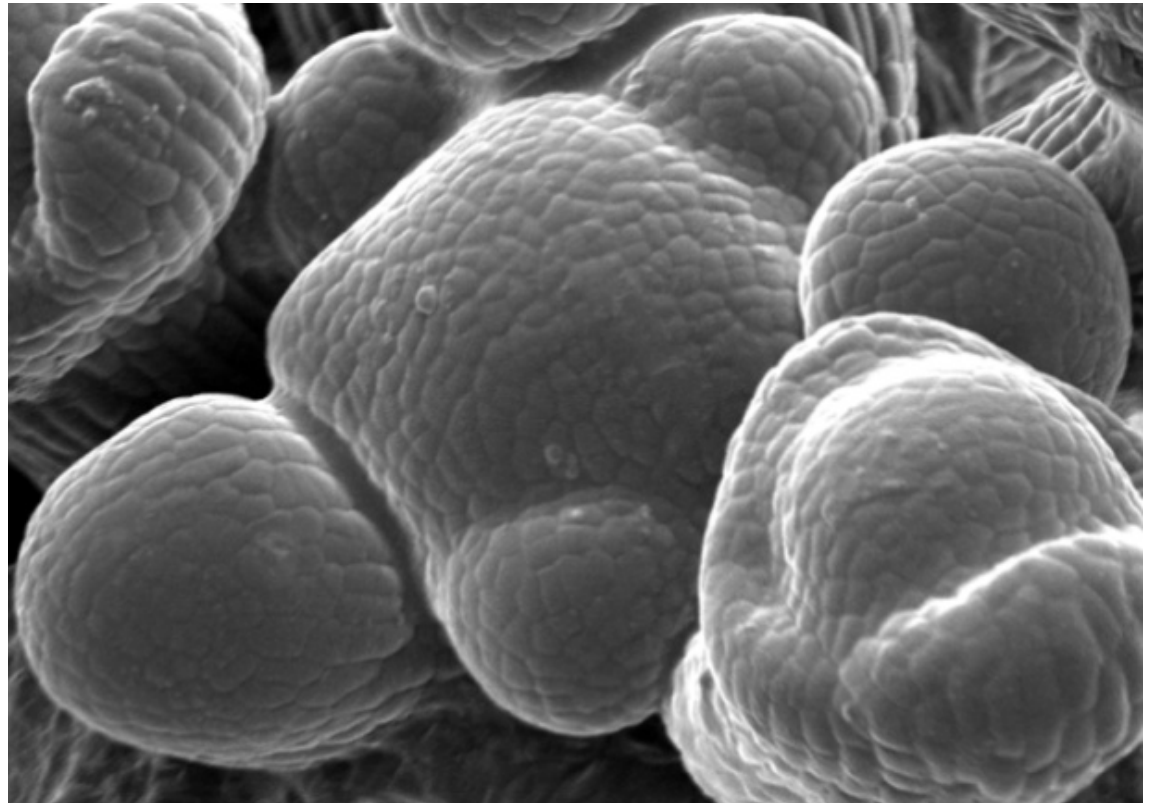


Shape and growth



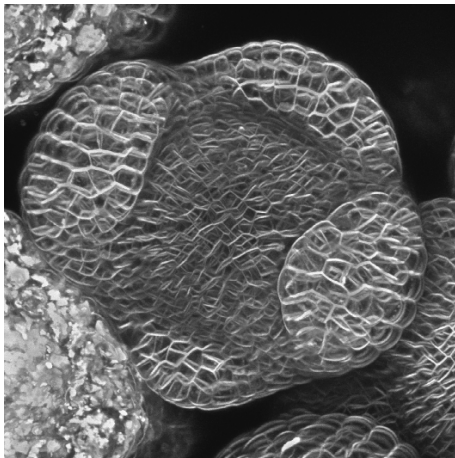
Cell targets?



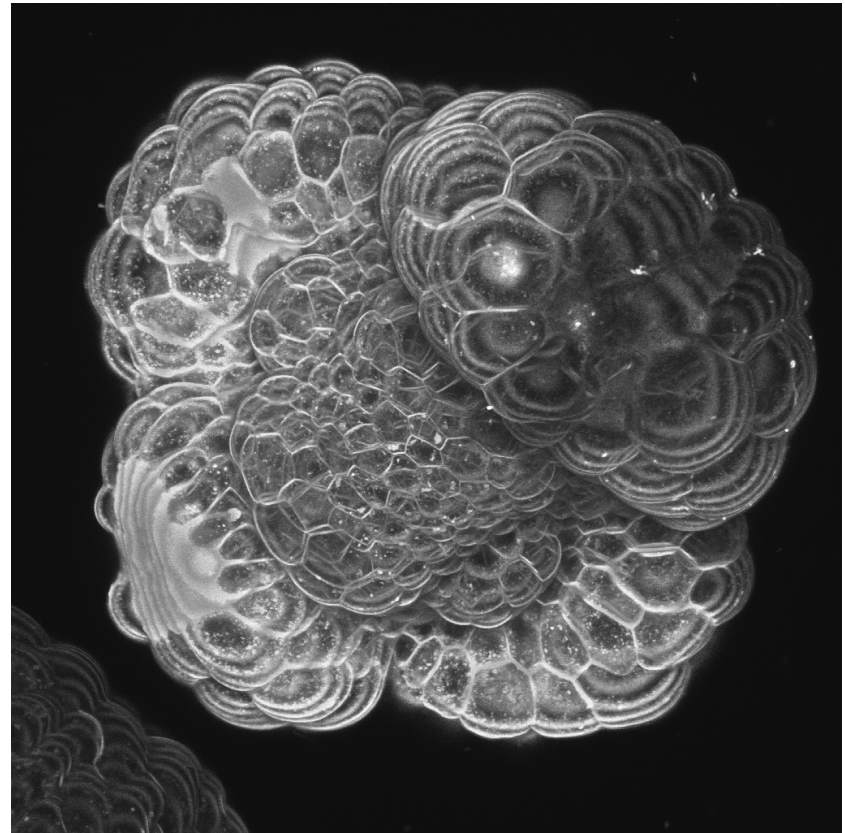


The shoot apical meristem

Microtubules depolymerisation leads to isotropic growth

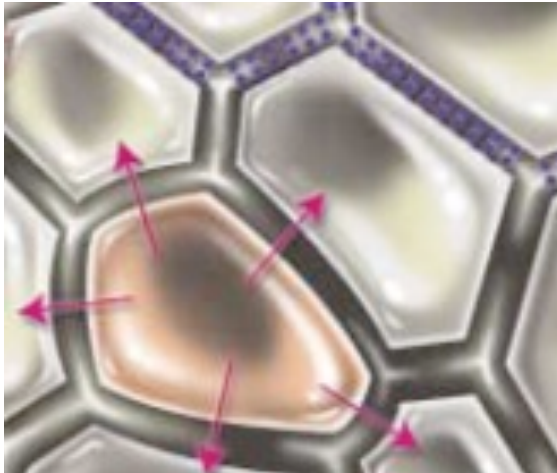


48h



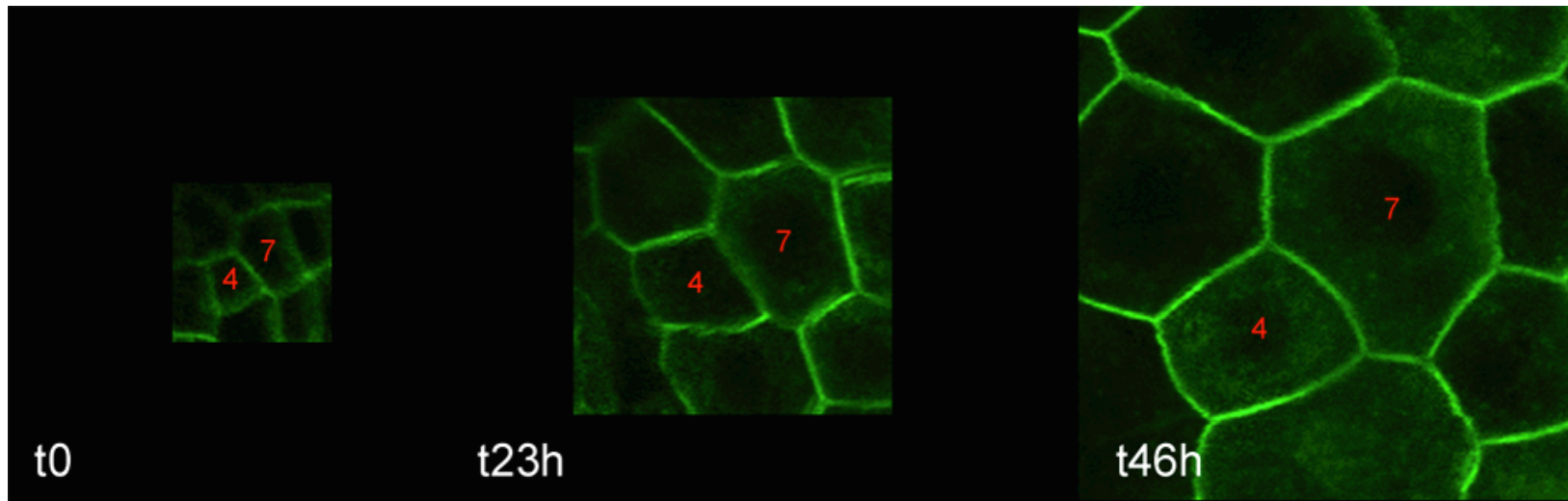
A foam-like behavior

In 2D foams:

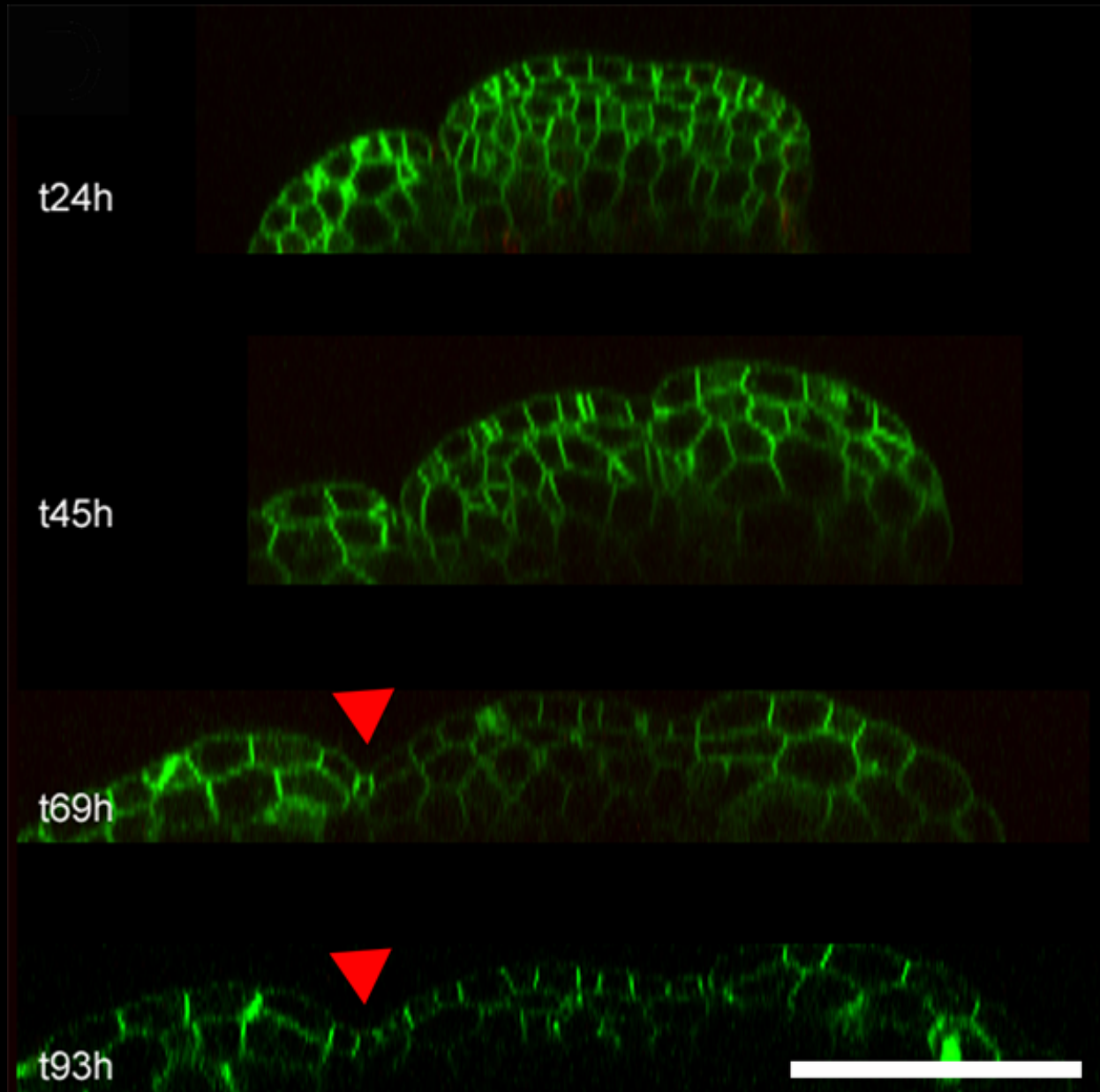


- Convex walls when the number of vertices is < 6
- Concave walls when the number of vertices is > 6

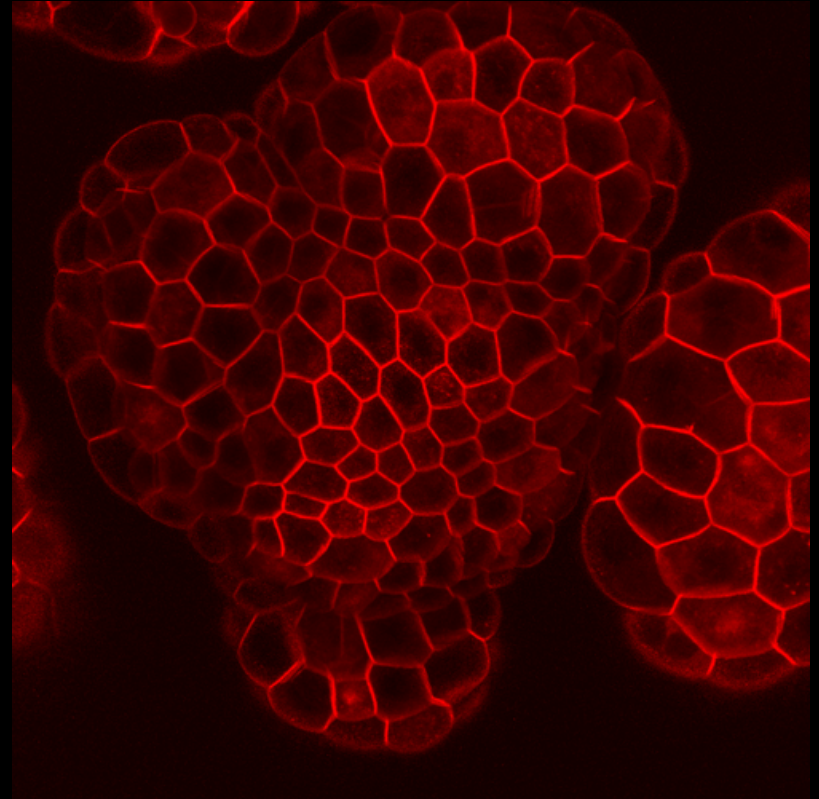
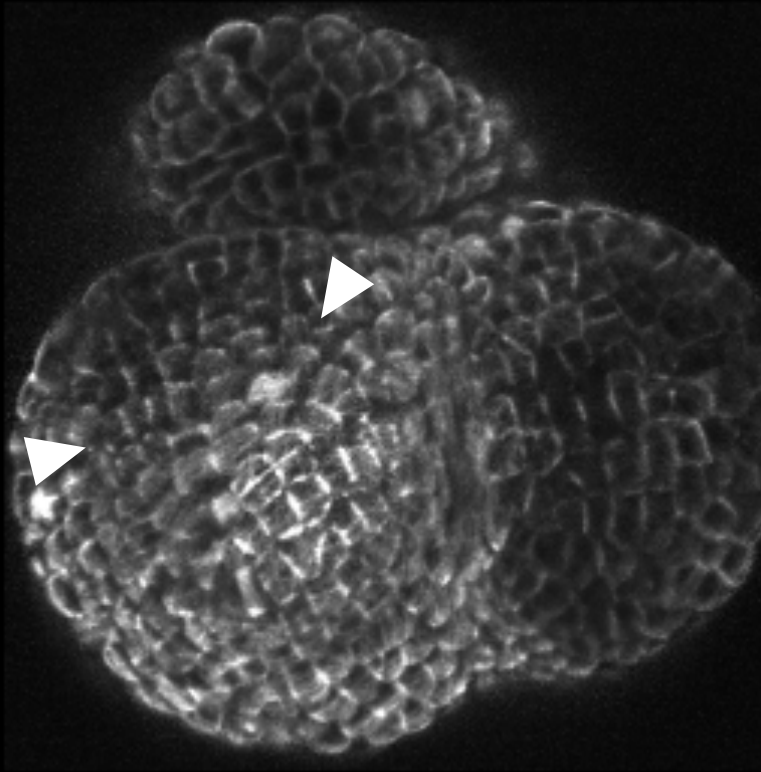
At the meristem surface treated with oryzalin:



Microtubule depolymerisation prevents crease formation

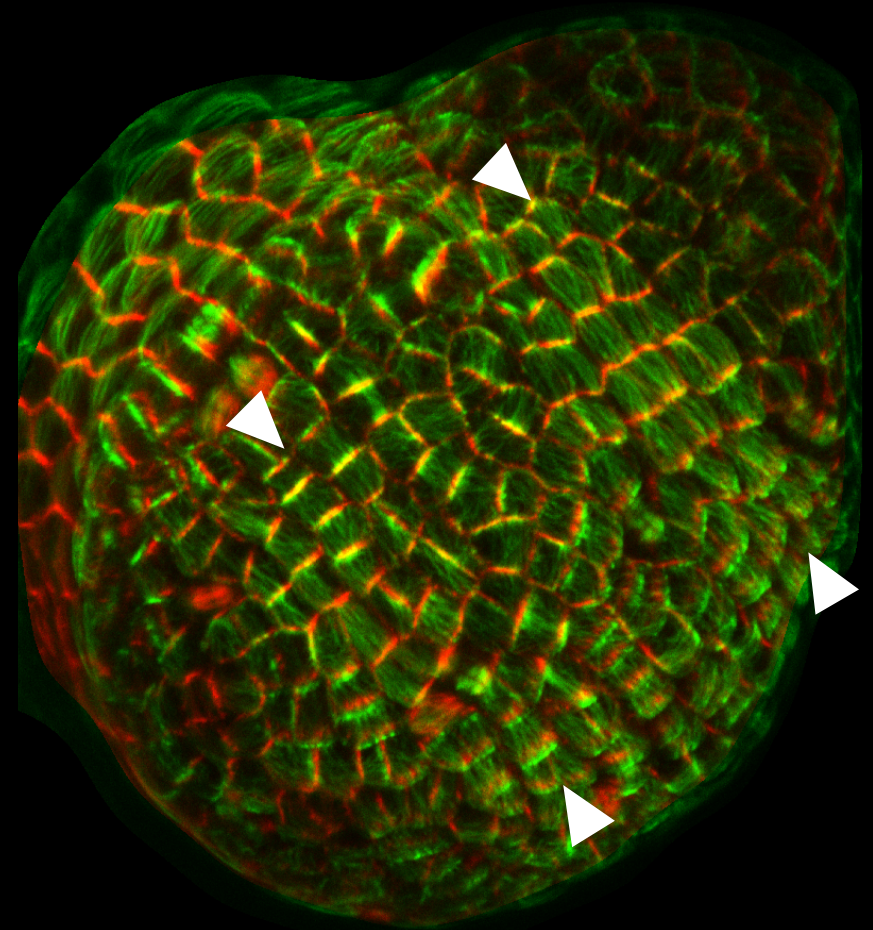
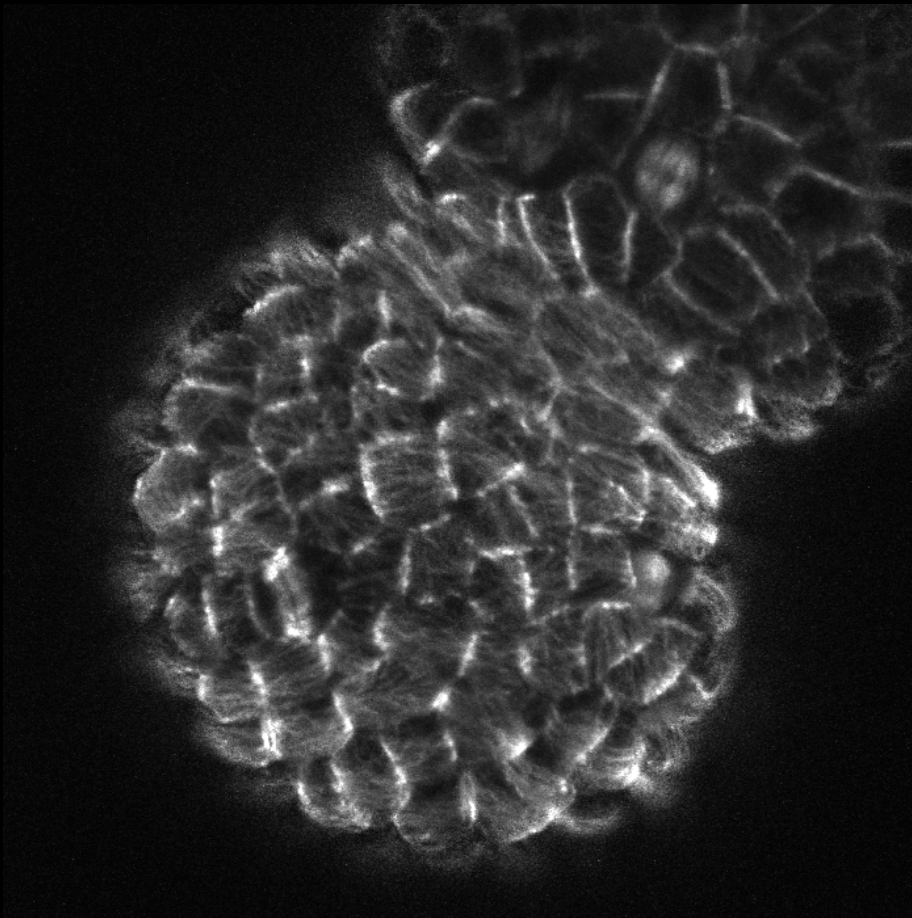


Patterns of microtubule orientations mark morphogenetic events

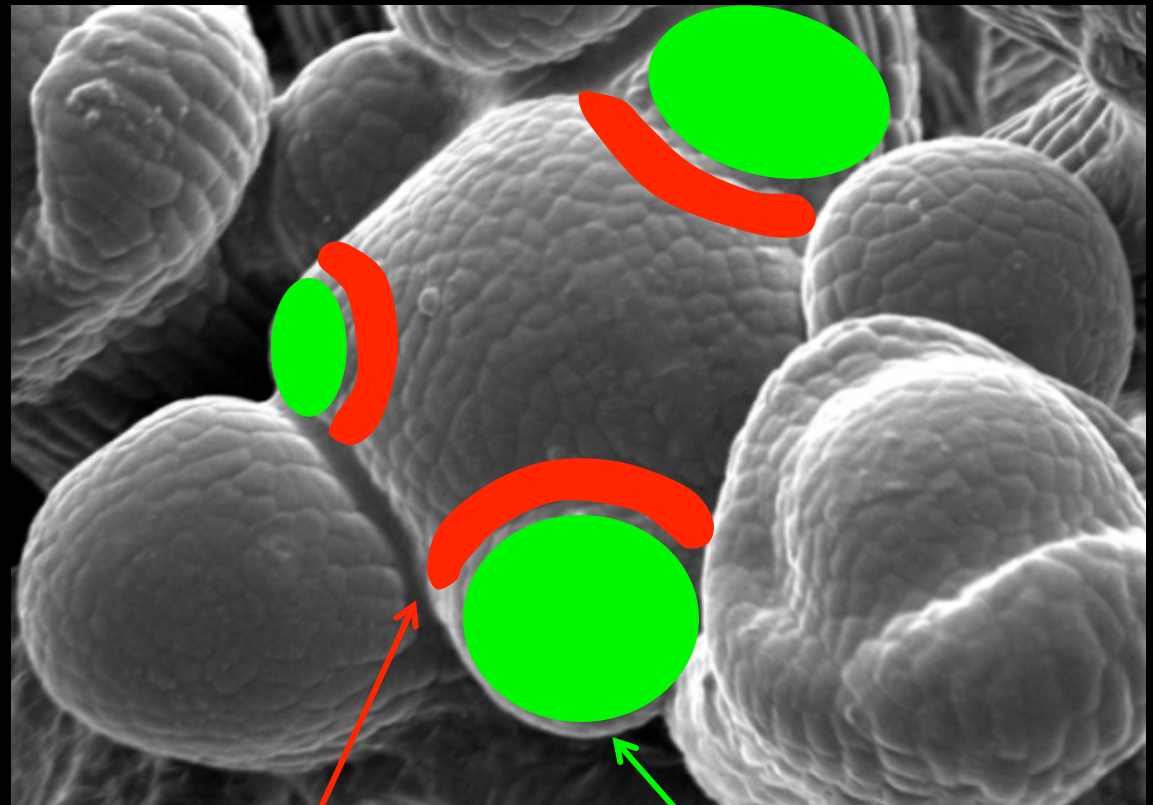


After MT depolymerization:
no crease

Patterns of microtubule orientations mark morphogenetic events



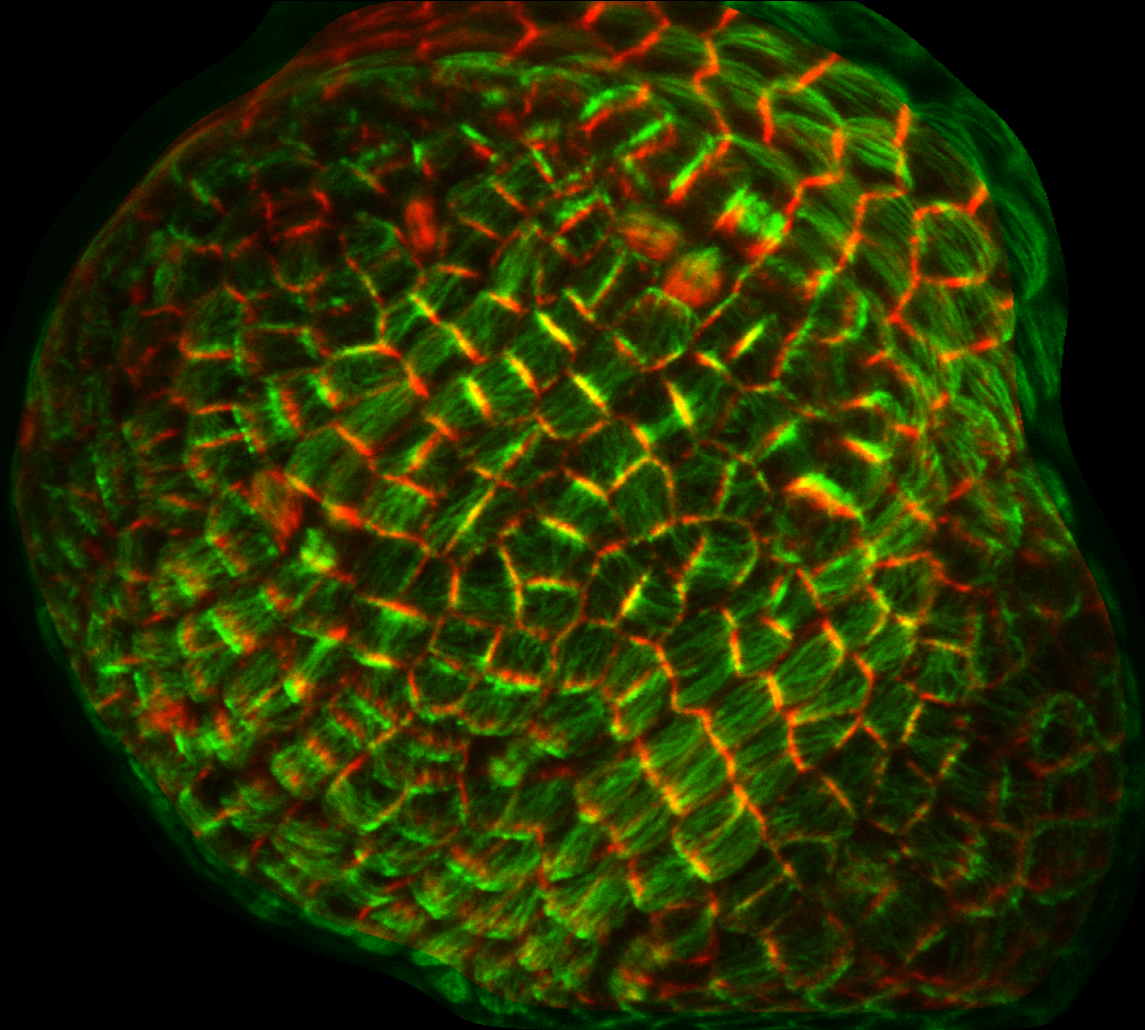
- Cell shape
- Microtubules



Crease:
Anisotropic walls
(microtubules)

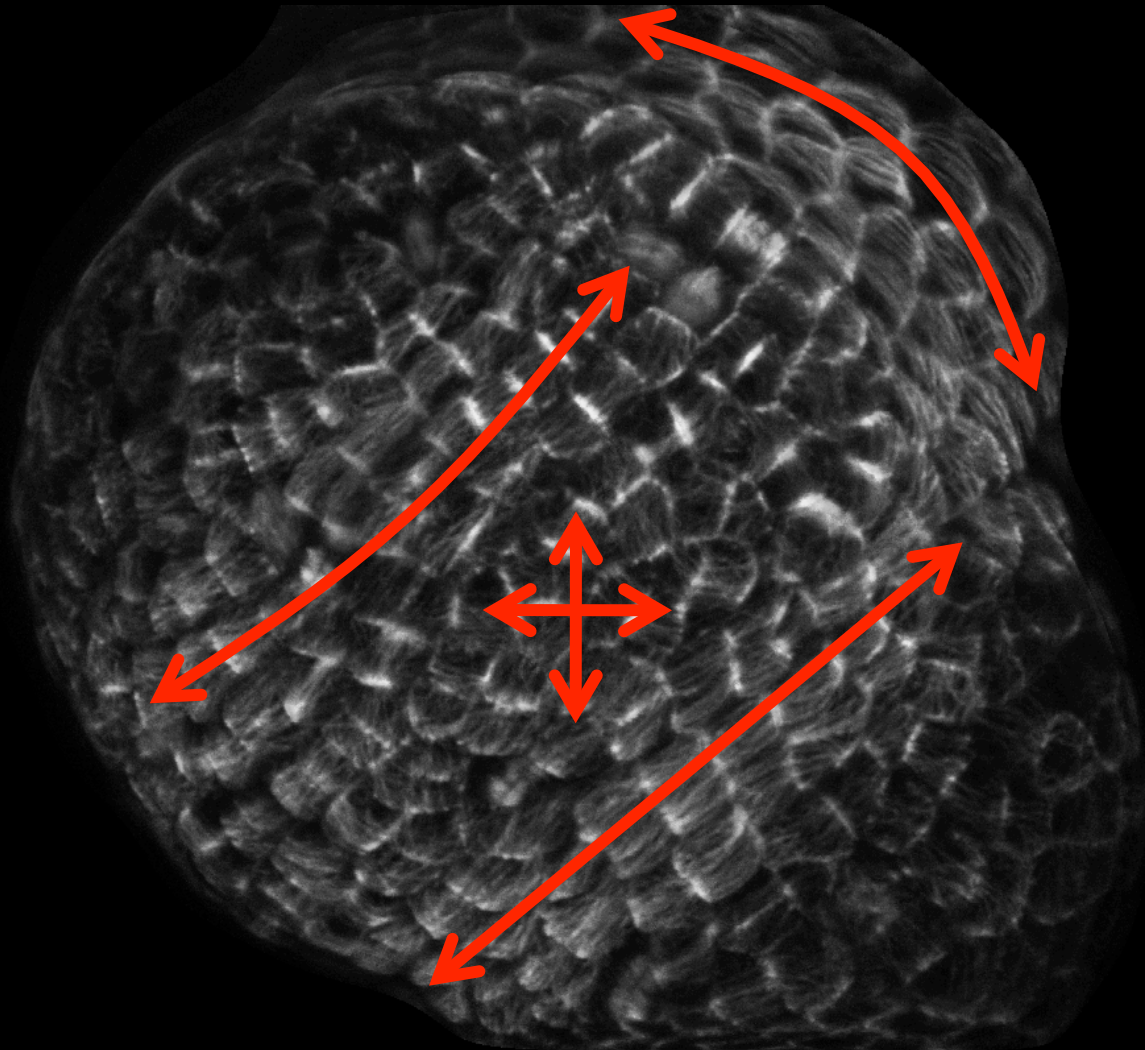
Bump:
Local wall loosening
(auxin)

What is orienting the microtubules?

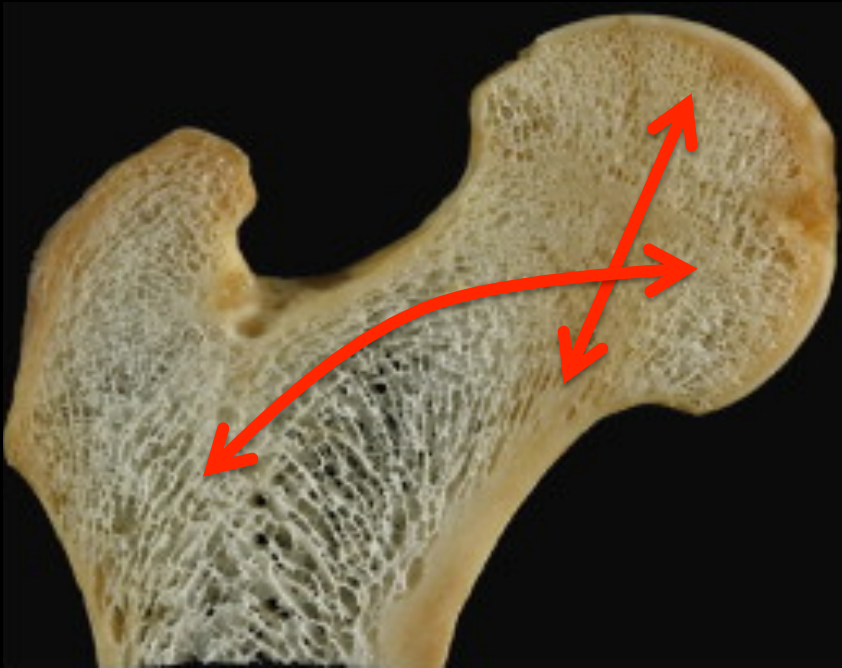


- Cell shape
- Microtubule orientation

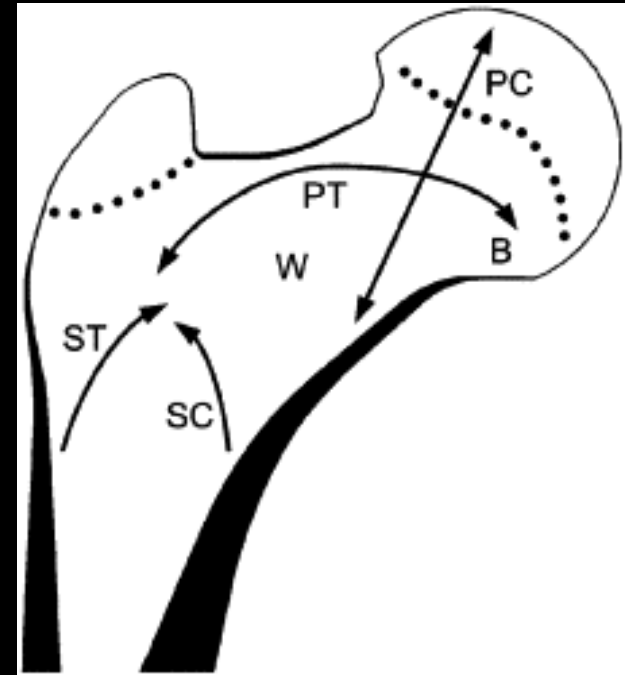
Microtubule orientation matches
pattern of maximal tensile stresses



Analogous to Wolff's law in plants?

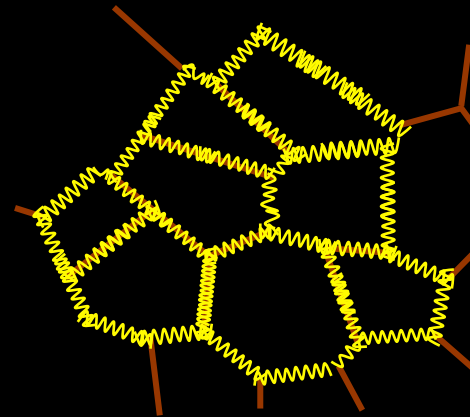
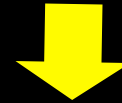
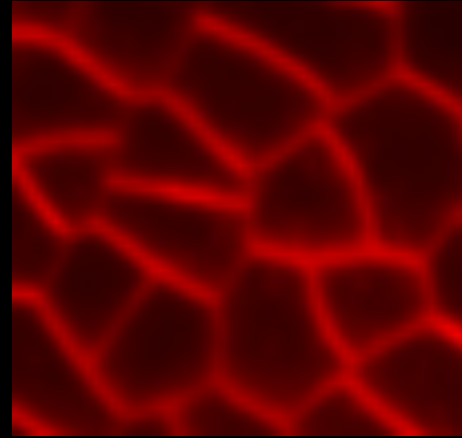
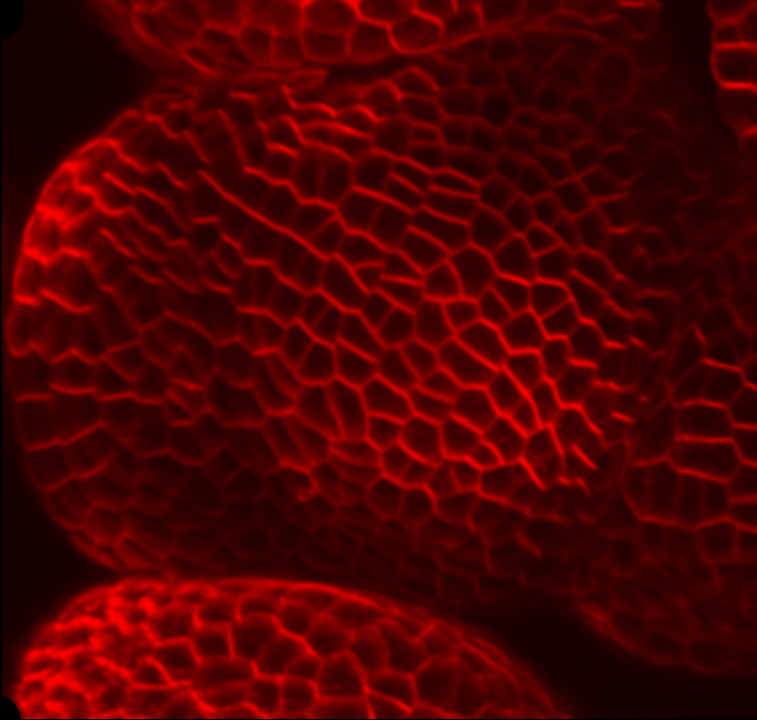


Wolff's law: bone architecture can be predicted by patterns of forces



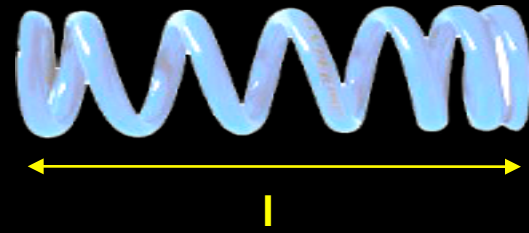
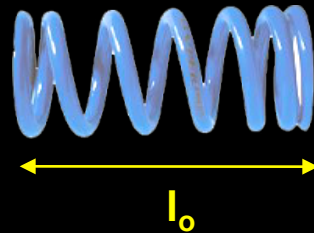
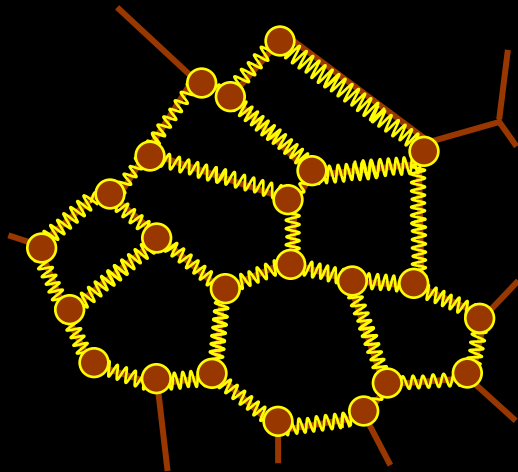
Pattern of forces

Building a cell-centered mechanical model



Cell walls represented by springs

Building a cell-centered mechanical model

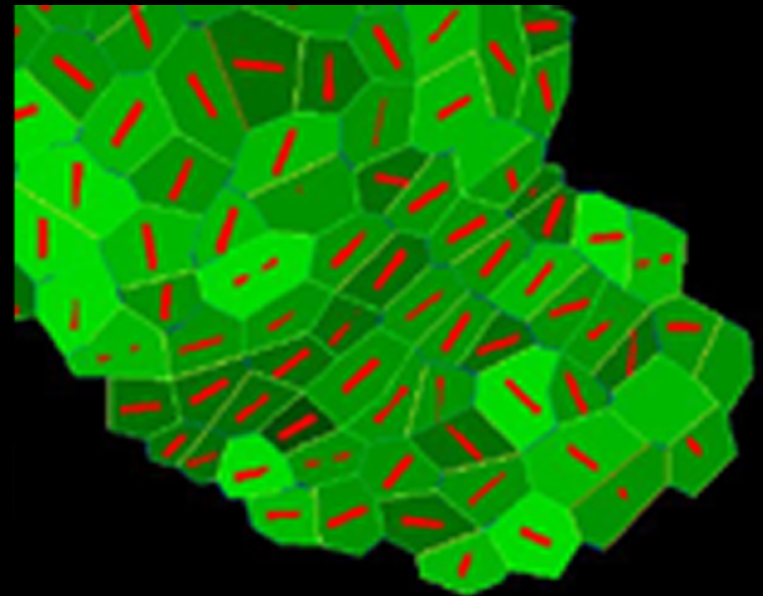
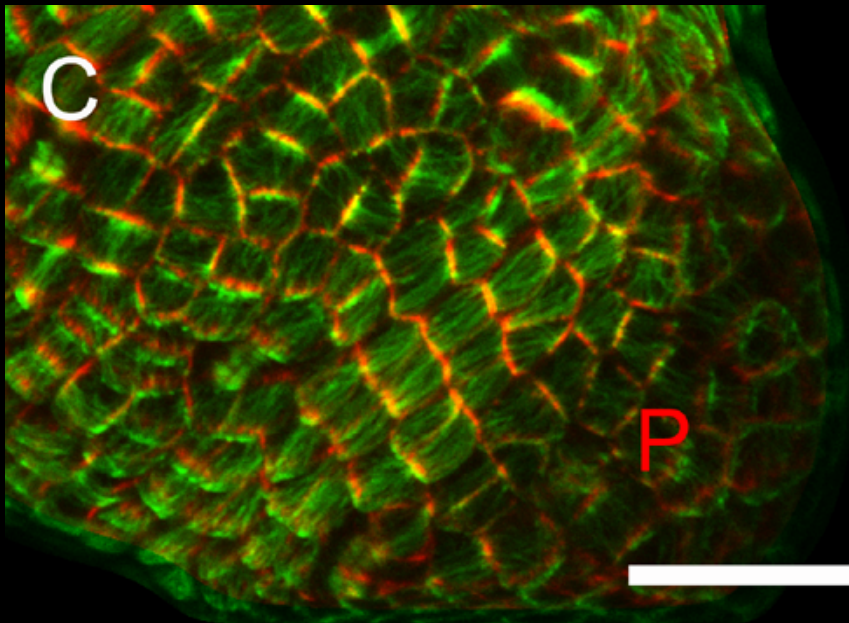


$$F = -k(l - l_0) - \beta v$$

- Cell walls represented by springs
- Growth by increasing relaxed length (l_0)
- Resistance to stress by increasing k

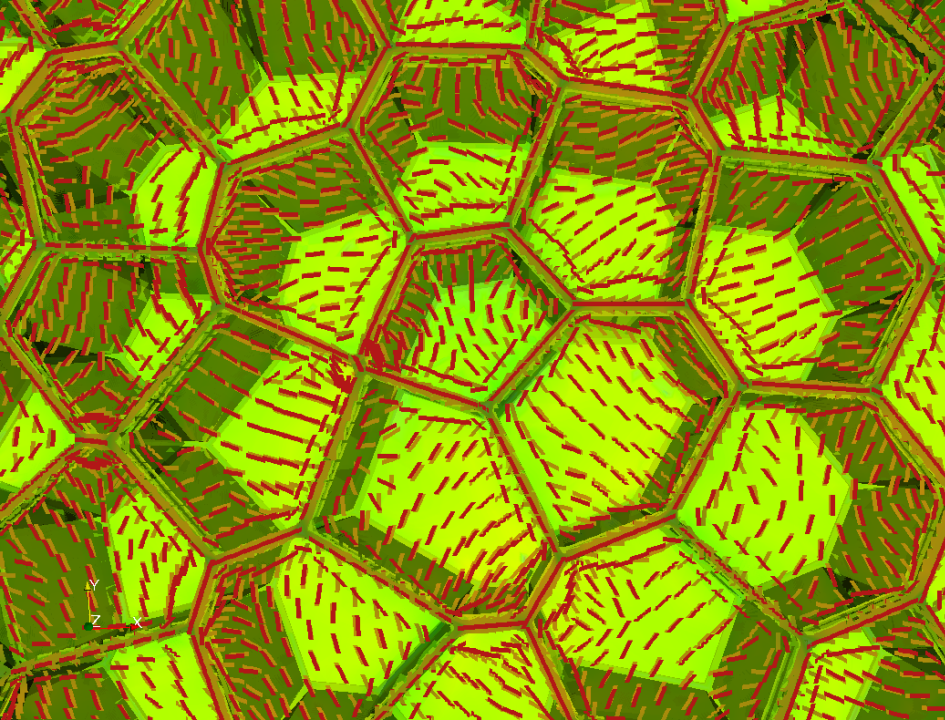
Microtubules

Hypothesis: MTs align along maximal tensile stress direction

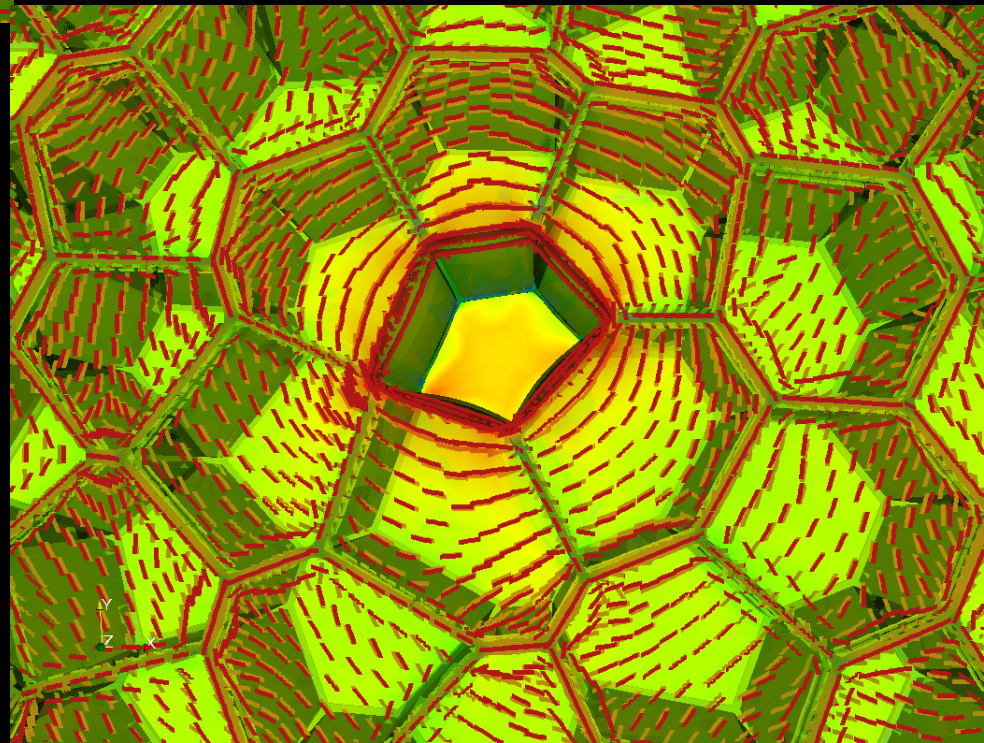


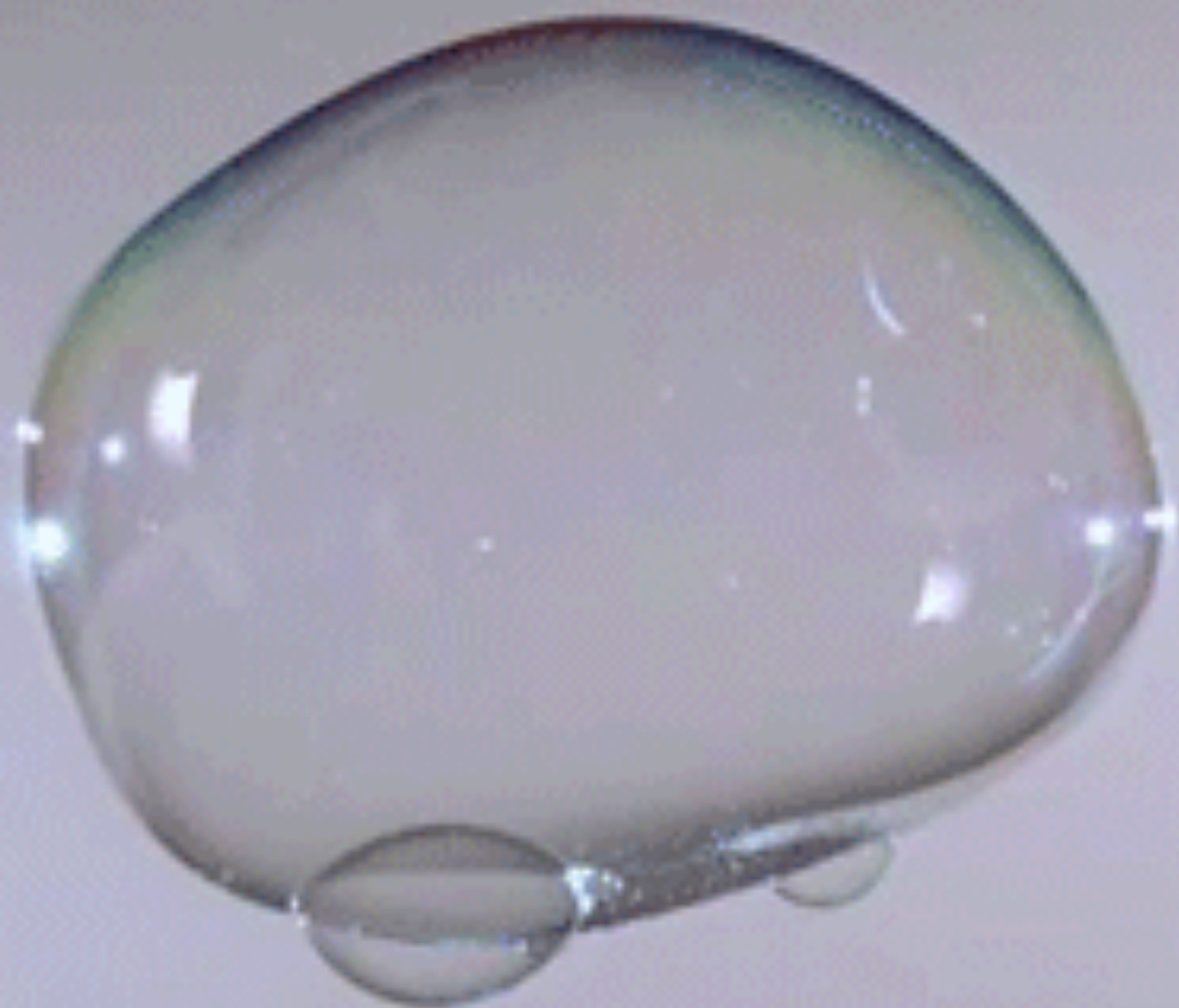
The mechanical model faithfully reproduces the Mts pattern in the meristem

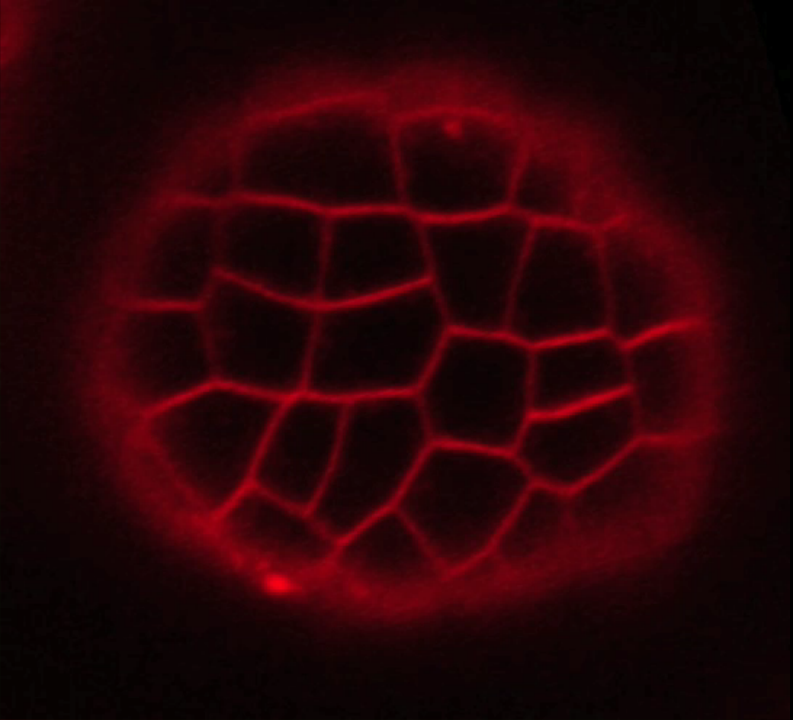
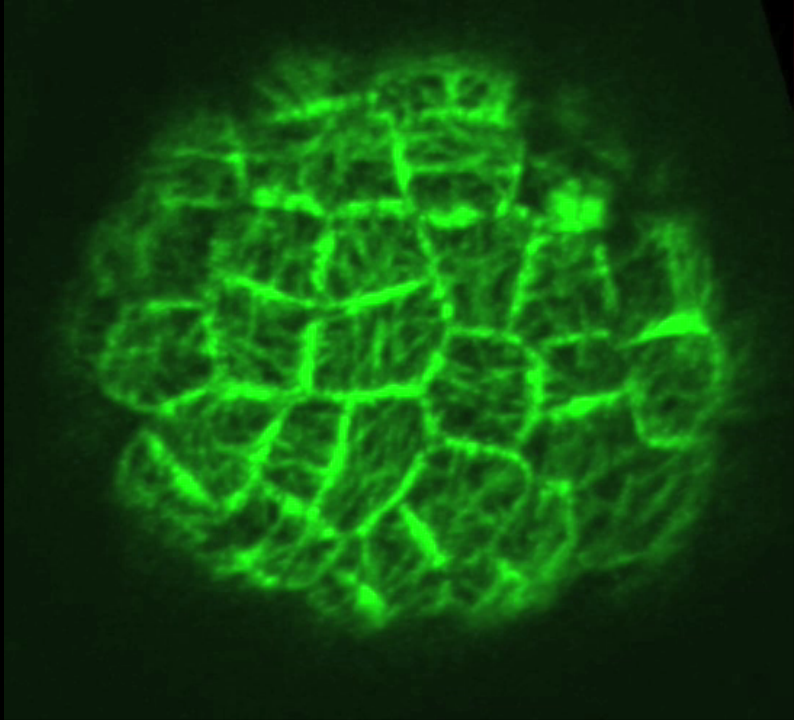
Test: Laser induced cell ablation



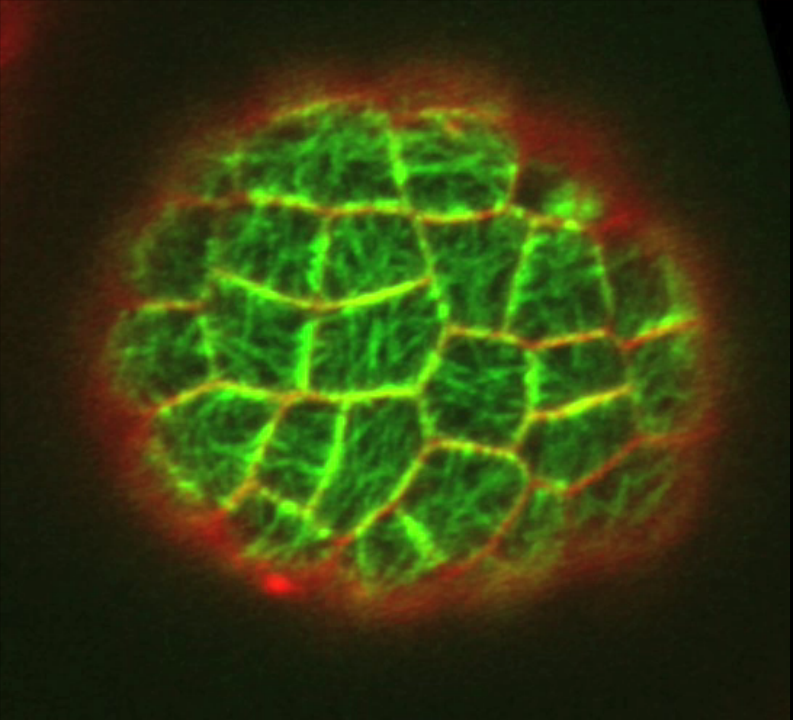
ablation

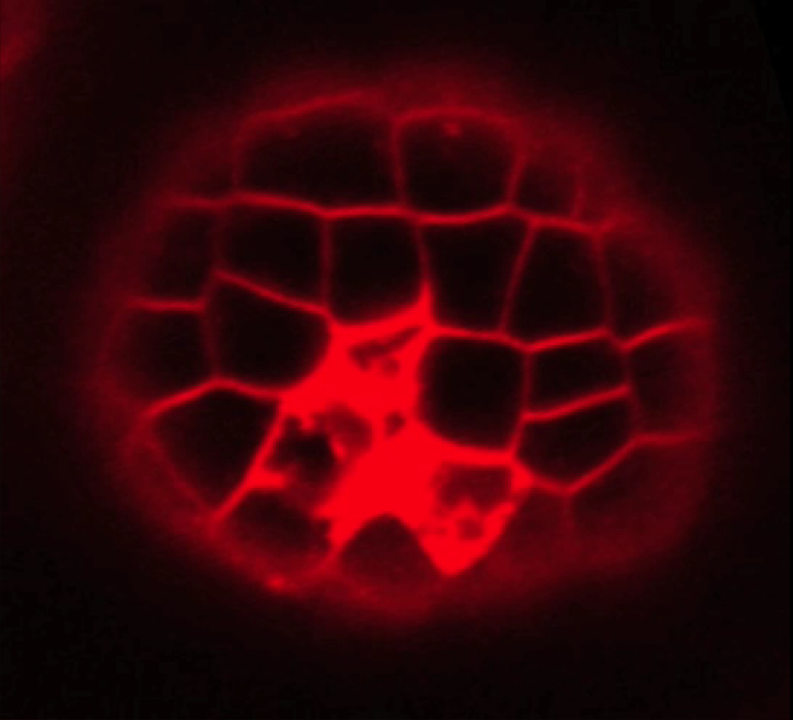
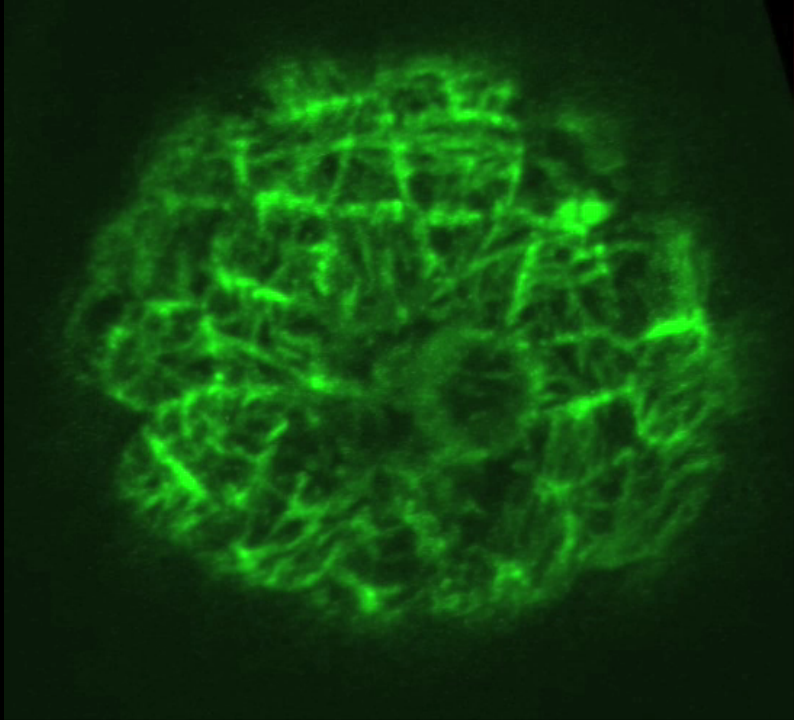




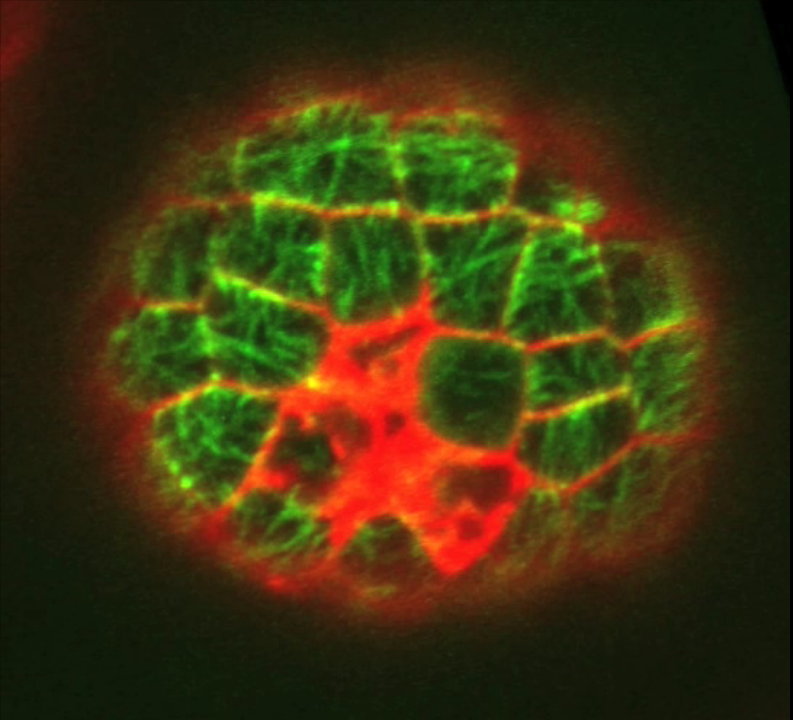


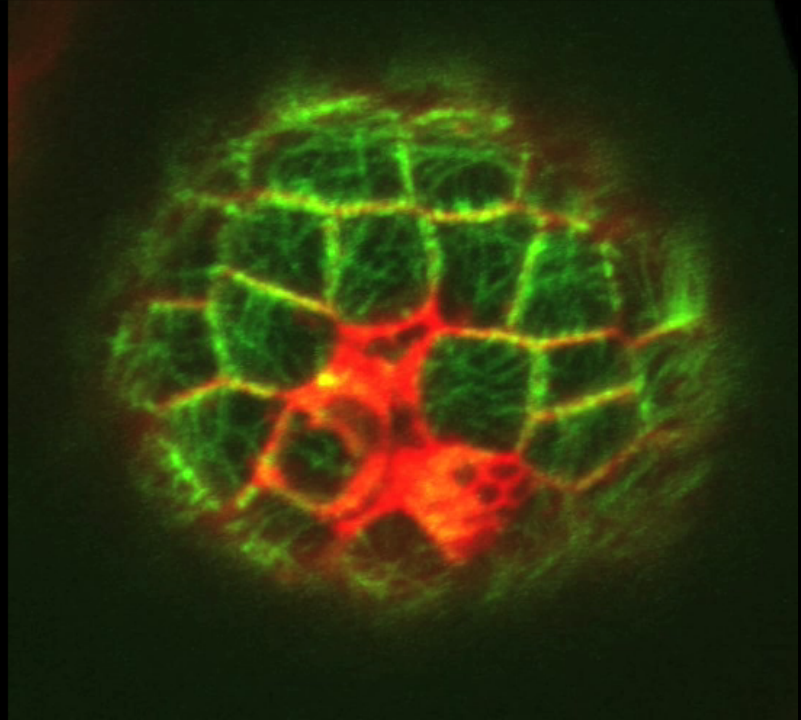
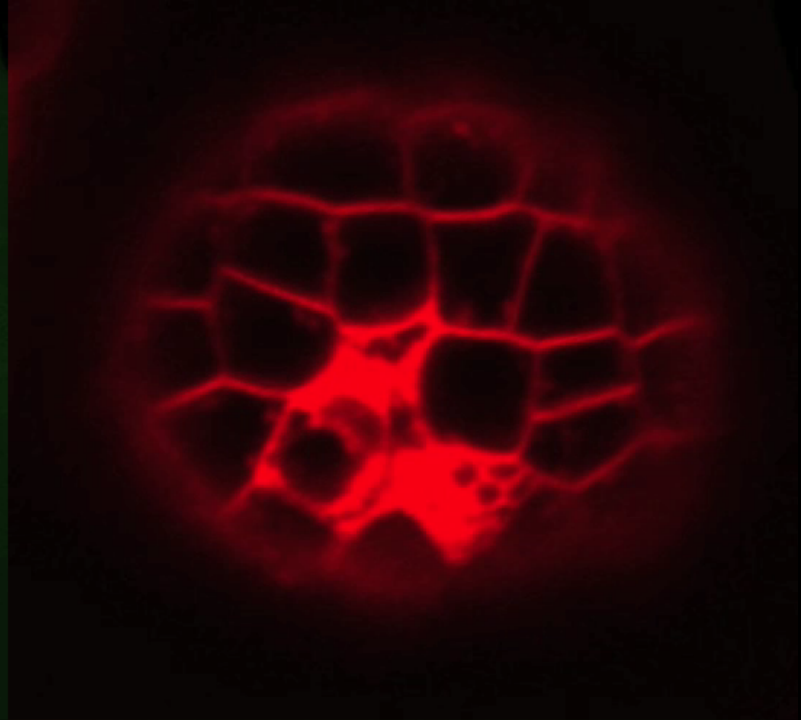
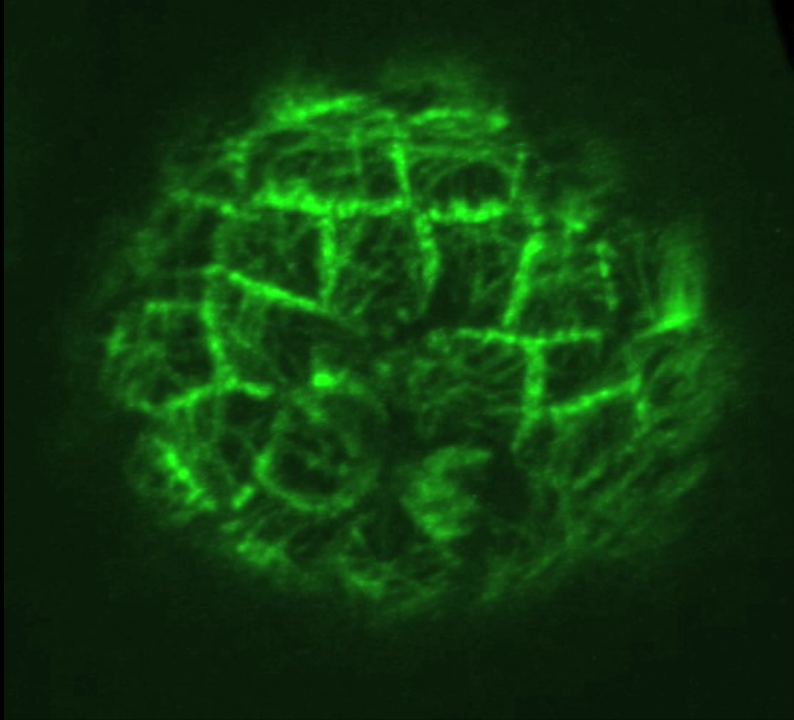
Test the stress hypothesis:
Ablations



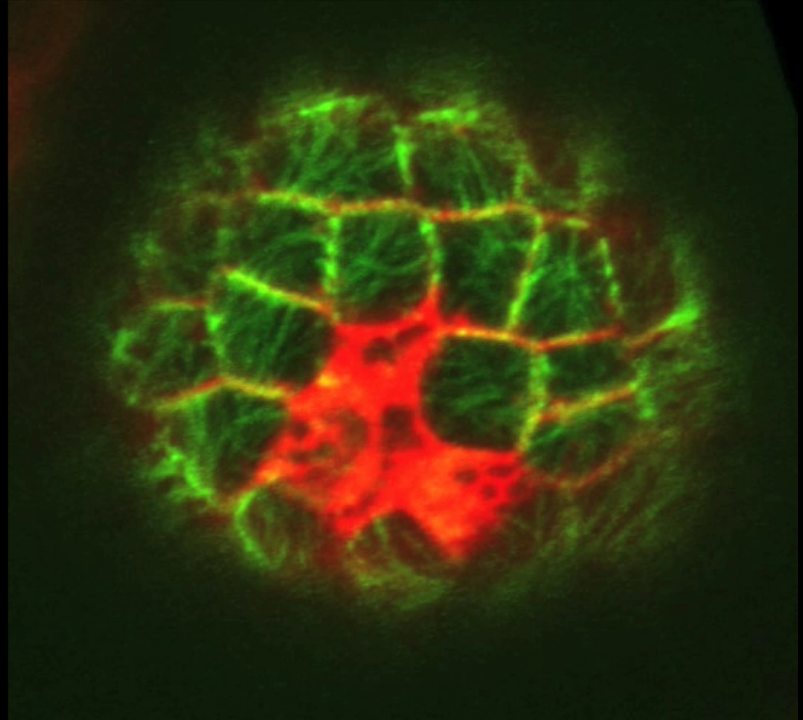
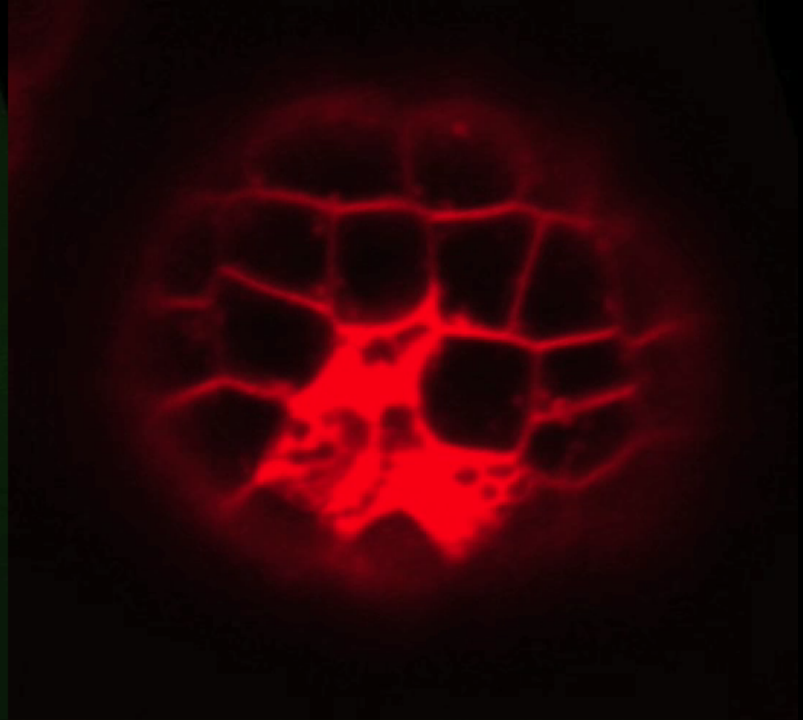
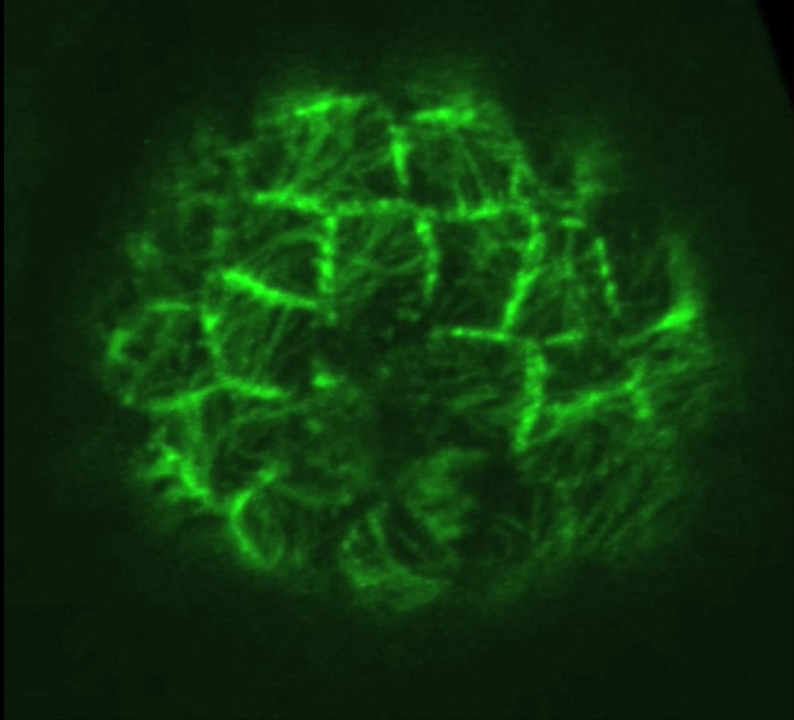


Test the stress hypothesis:
Ablations

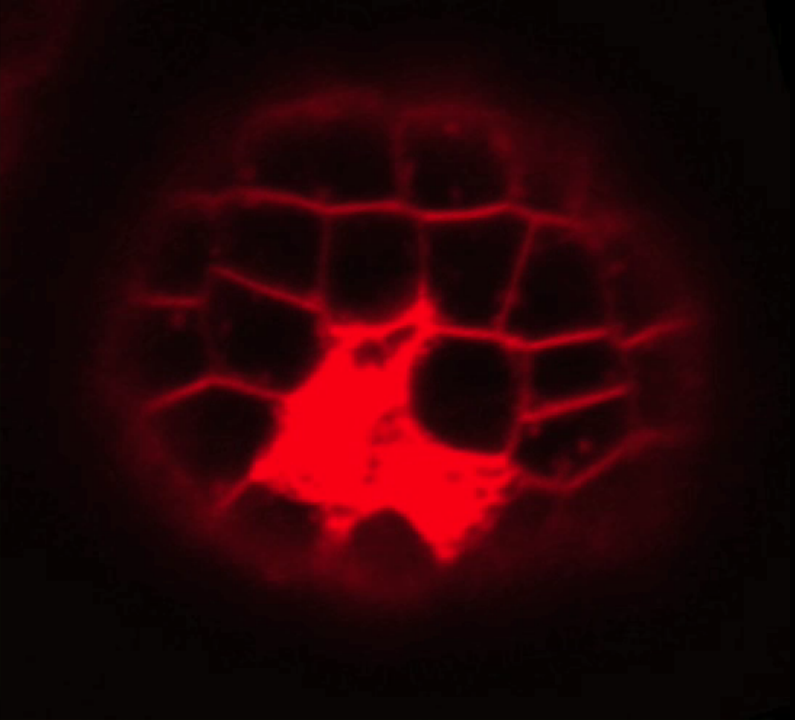
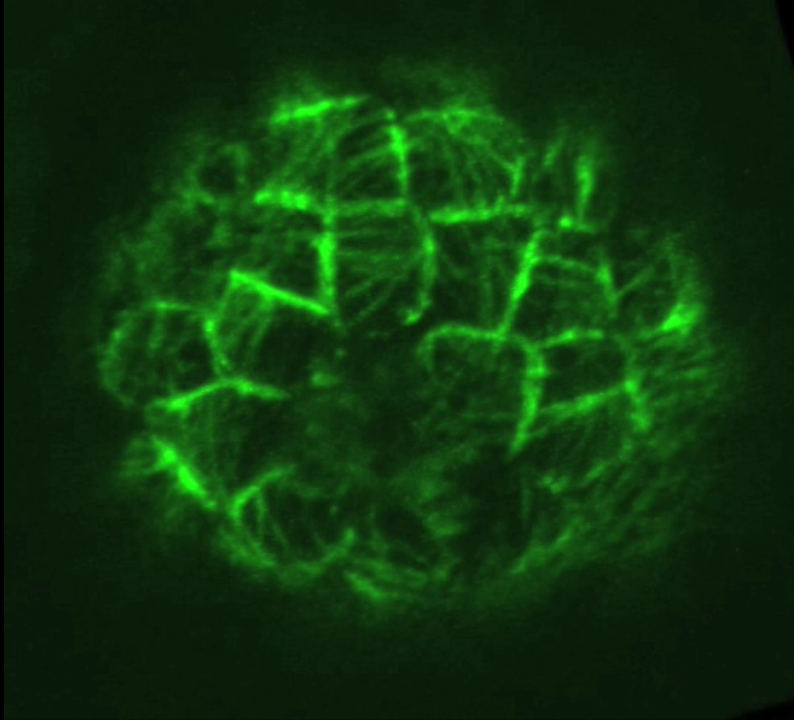




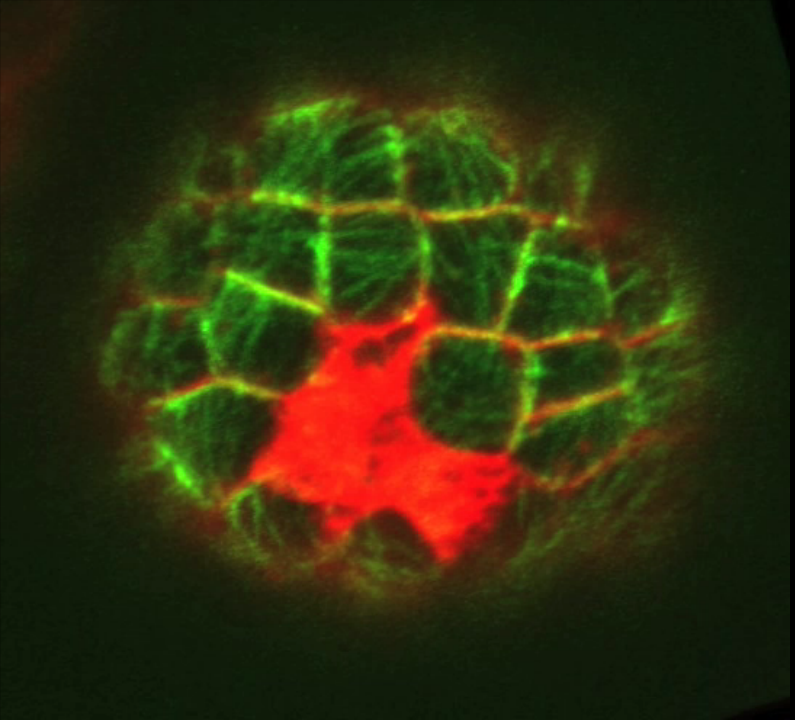
Test the stress hypothesis:
Ablations

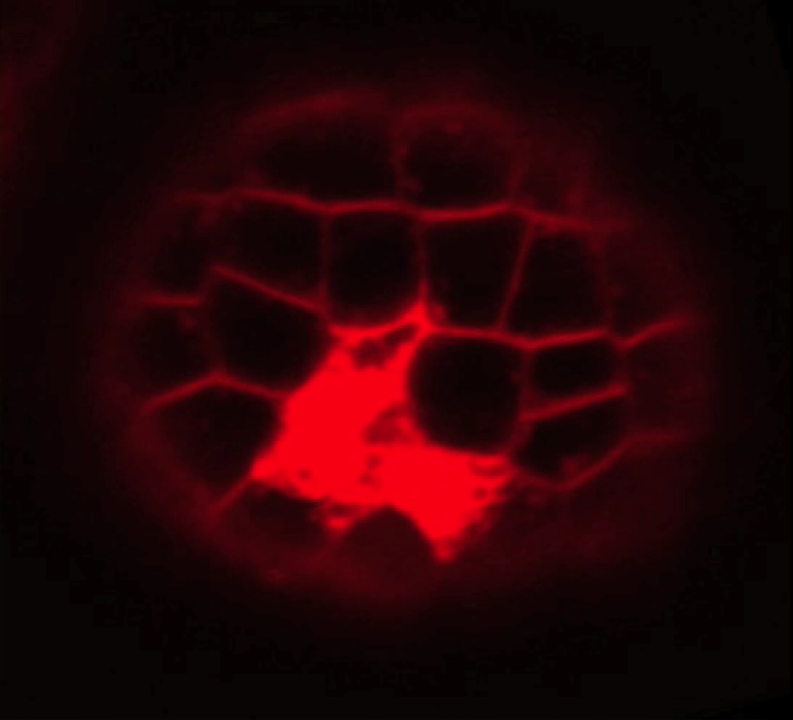
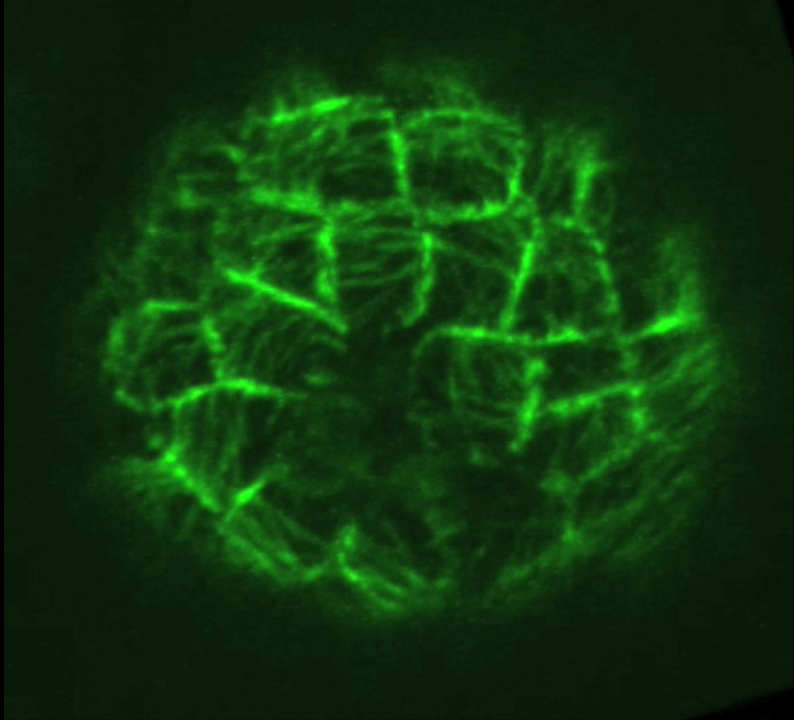


Test the stress hypothesis:
Ablations

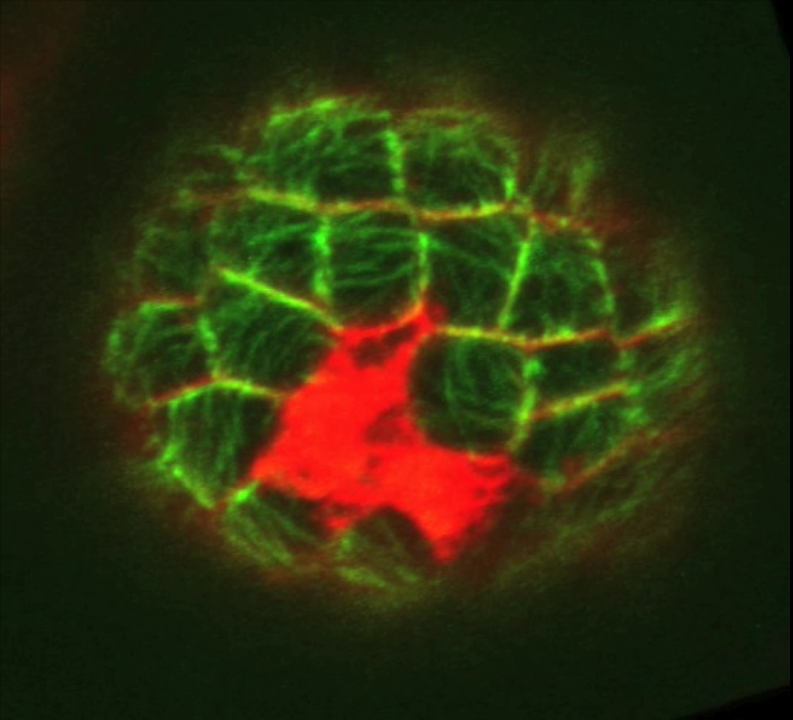


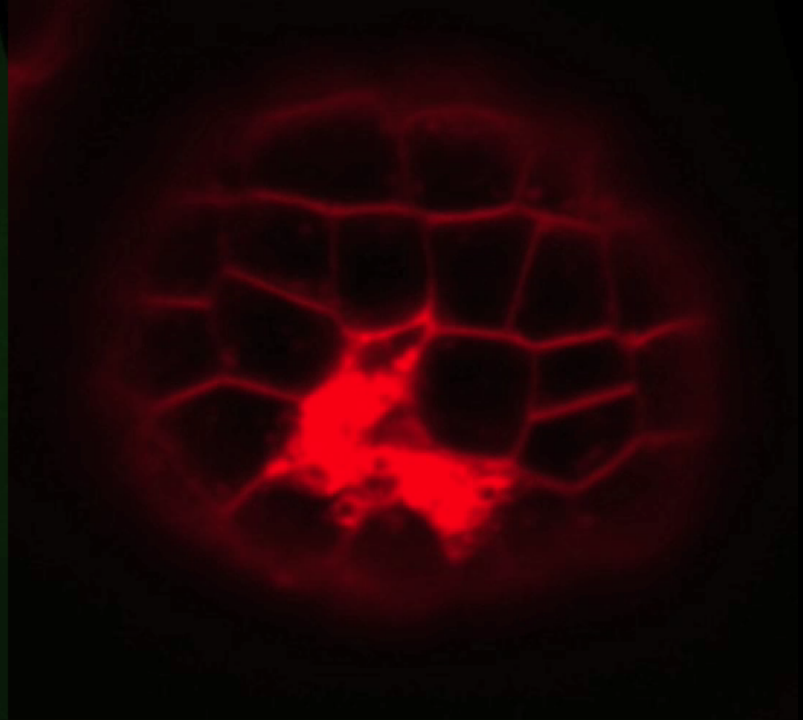
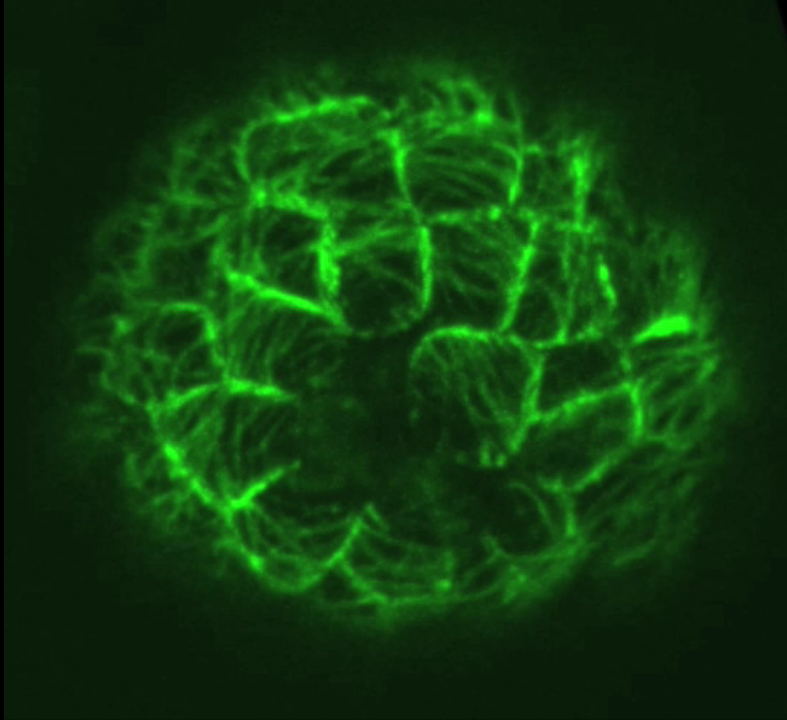
Test the stress hypothesis:
Ablations



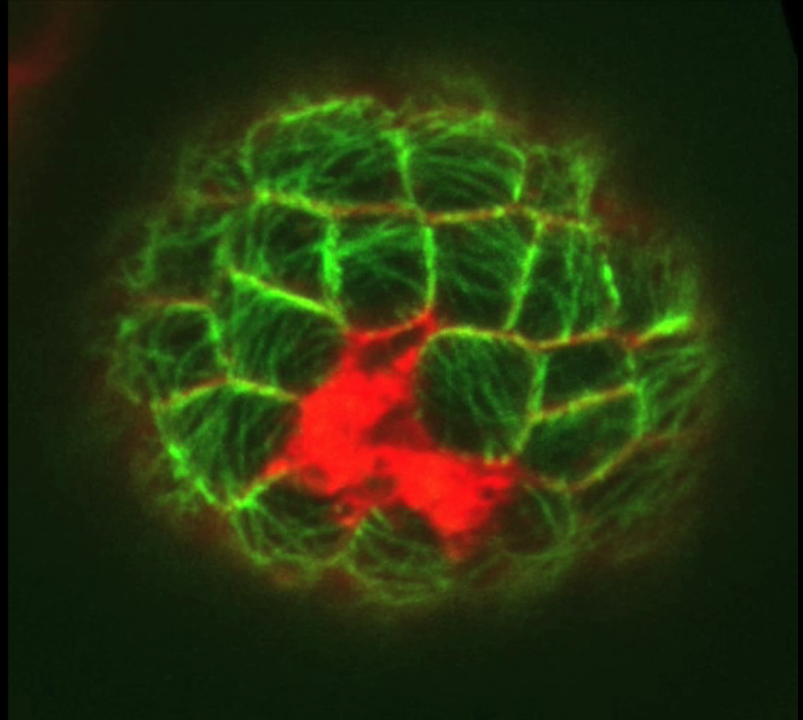


Test the stress hypothesis:
Ablations



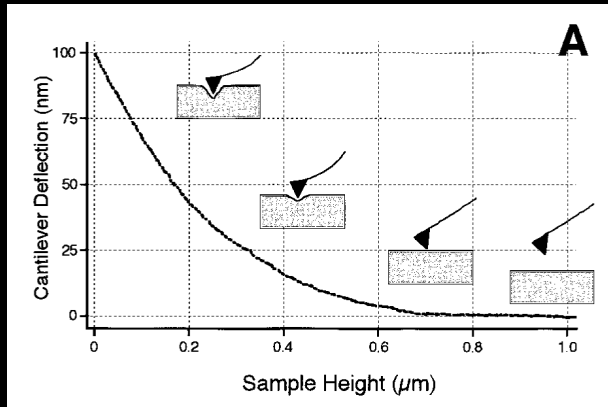


Test the stress hypothesis:
Ablations

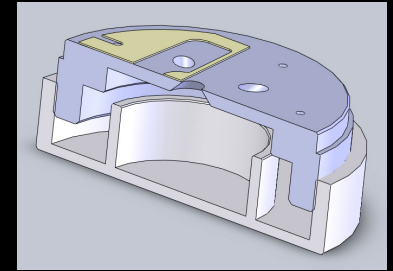
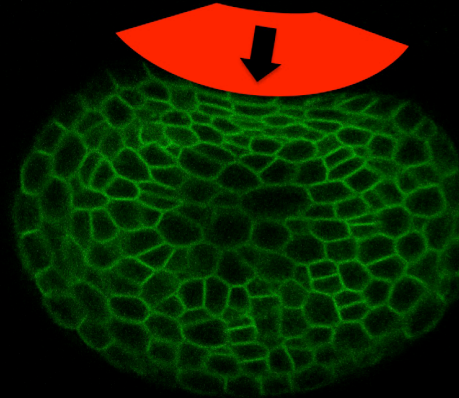


Mechanical tests on living meristems

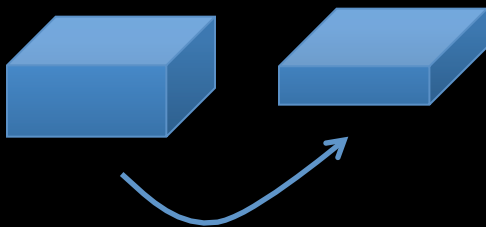
Nanoindentation



Compressions

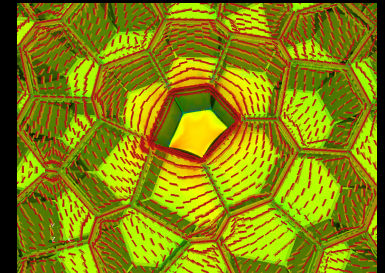
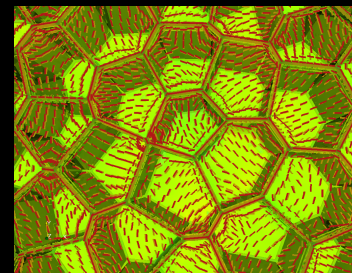


Pharmacological tests

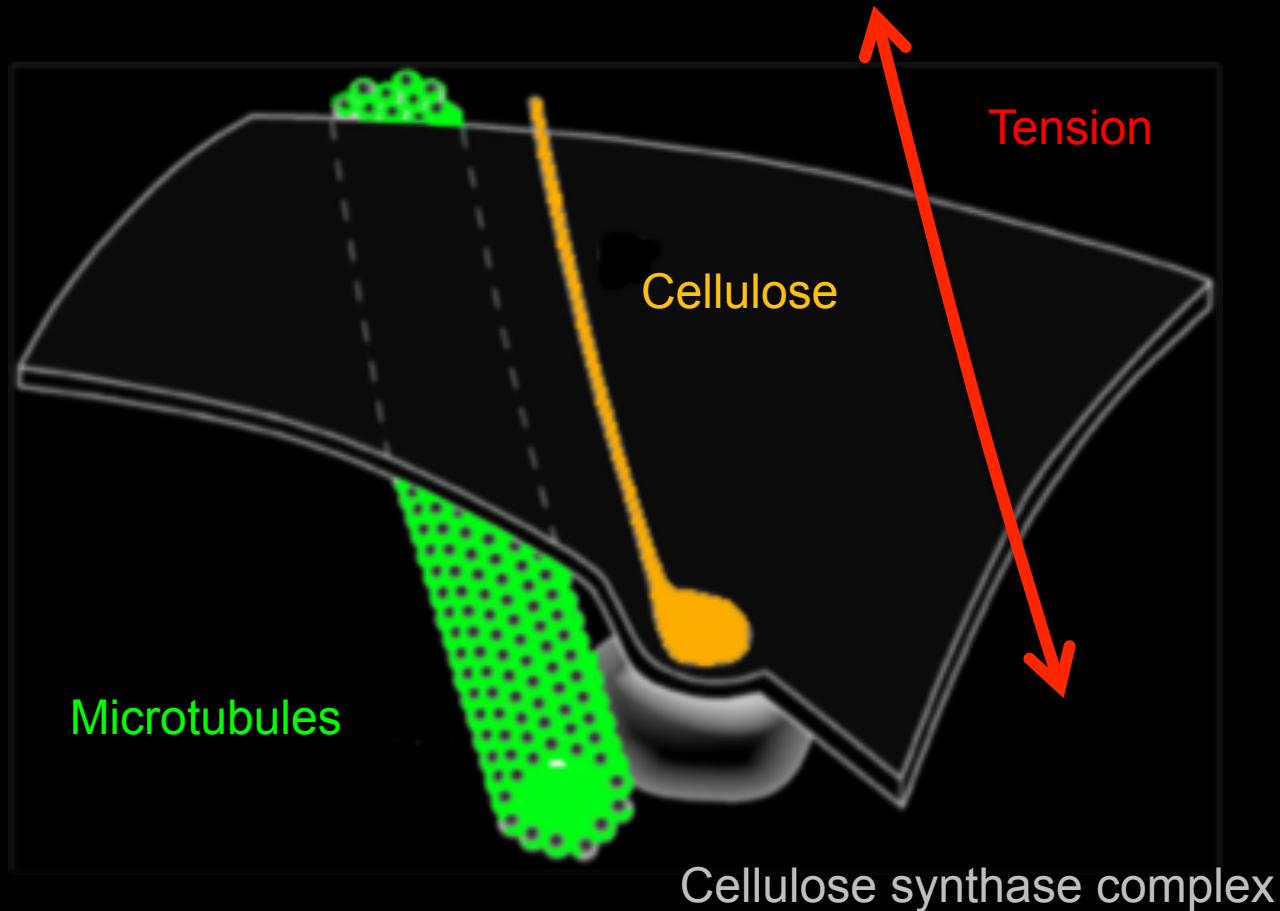


isoxaben

Ablations

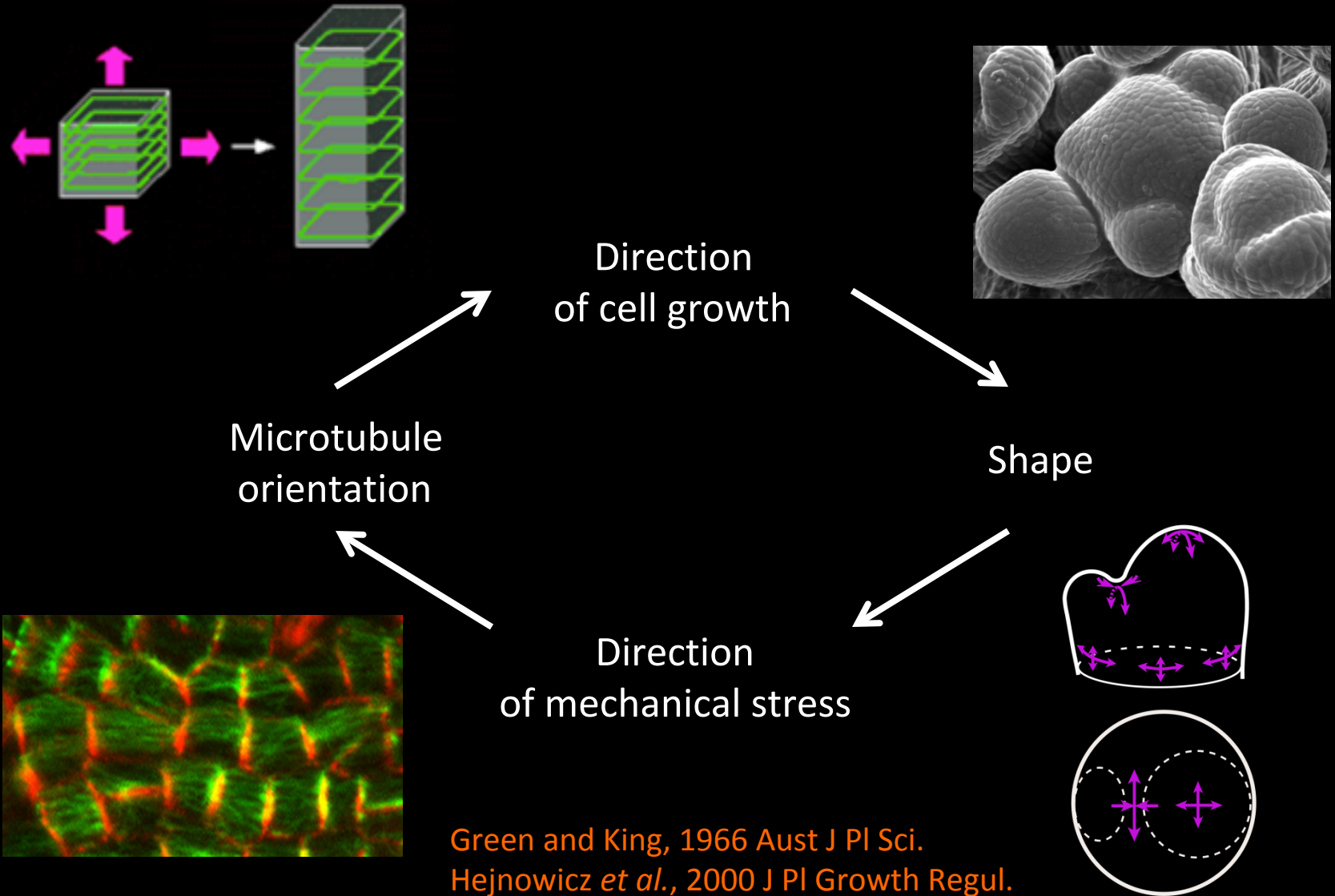


Cortical microtubules align along maximal tension



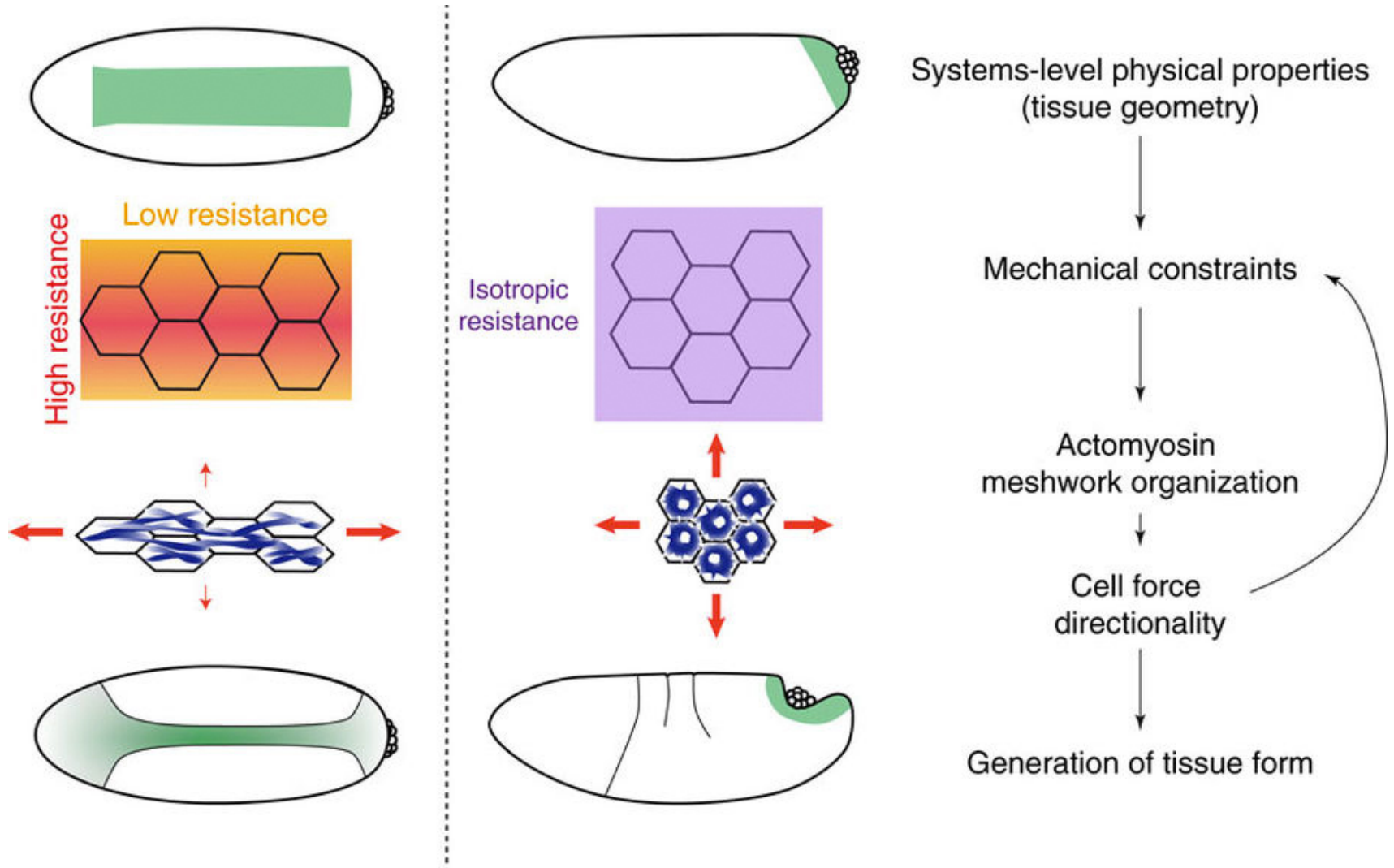
Green and King, 1966 Aust J Pl Sci.
Hejnowicz *et al.*, 2000 J Pl Growth Regul.
Hamant *et al.*, 2008 Science

A mechanical feedback loop



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

Comparable mechanical feedback loops in animals



Summary

Microtubules guide the deposition cellulose, and thus the mechanical anisotropy of cell walls

Microtubule align along maximal tensile stress directions

This mechanical feedback loop contributes to plant morphogenesis

Comparable feedback loops exist in animals