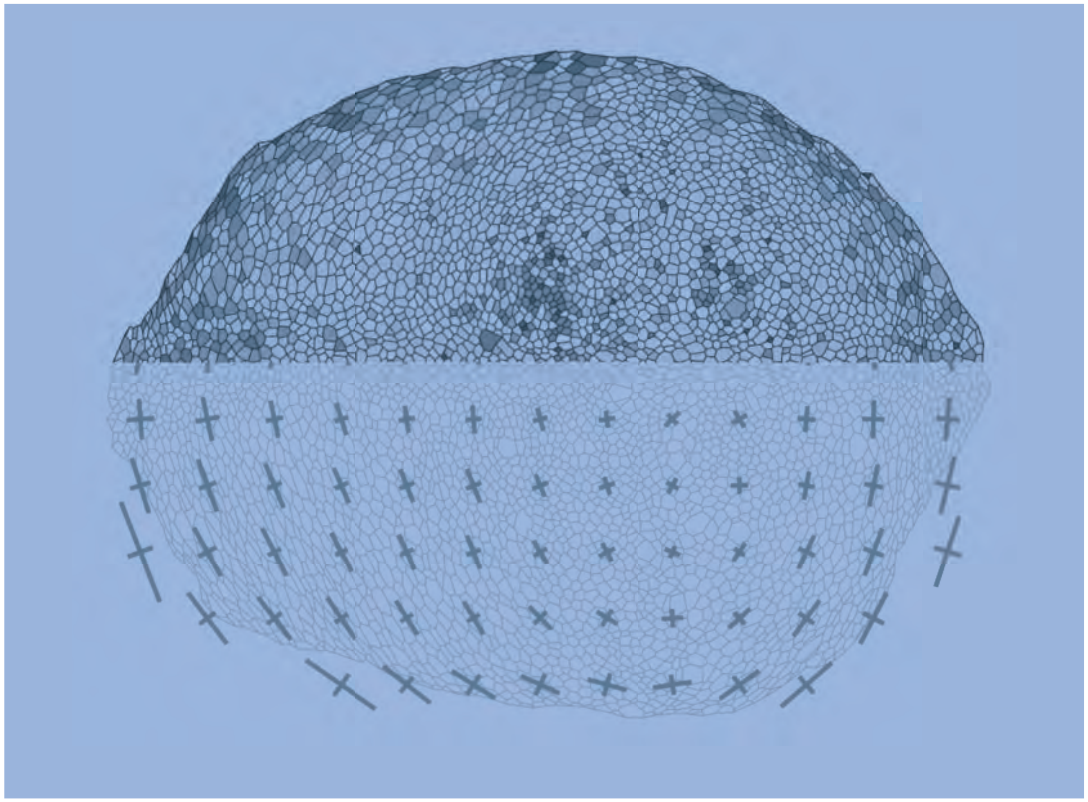
Organism and Tissue Growth



Course 5: Growth control and mechanics

Thomas Lecuit

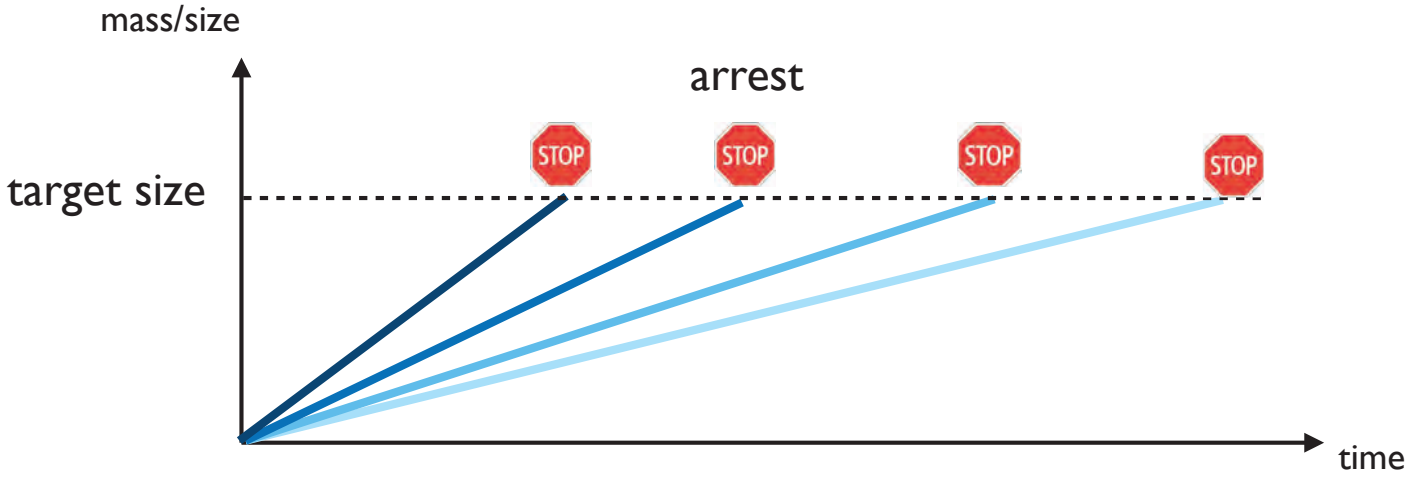
chaire: Dynamiques du vivant

COLLÈGE DE FRANCE 1530

• Control of growth arrest at target size

— Organ and organism size may be regulated by:
growth duration and/or growth rate.

— Need to regulate growth arrest at the appropriate target size:
process vs endpoint regulation.



— What is measured? Intrinsic « ruler » of growth: « size-meter »
Organ intrinsic feedback of target organ size on cell growth/proliferation

—Need to consider *scaling* between factors that promote growth and size itself

1. Energy and mass/volume
2. Pattern and mass/volume
3. Mechanics and mass/volume

—Need to consider feedback mechanisms operating across scales (organ/tissue to cell)

• Conclusions

— Cell growth is controlled intrinsically

— What are the mechanisms of organ specific tissue size sensing/measurement?

— What is measured? Intrinsic « ruler » of growth: « size-meter », scaling.

- Energy delivery/demand unbalance.
- Organ-size dependent negative feedback on cell growth/proliferation (chalone)
- « Size-meter »: scaling of chalone concentration and tissue size
  distance between activator and feedback inhibitor
  mechanical signal that scales with size
  patterning cue: morphogen gradient

- Organ-size dependent growth inducer: Morphogen and growth arrest:
  Spatial model: gradient scaling and gradient slope.
  Temporal model: gradient scaling and rate of signal increase

- Importance of considering robustness of mechanisms: e.g. integral feedback

Programmed vs Self-organised regulation of Growth

- hierarchical
- modular
- deterministic rules (ie. genetically encoded)

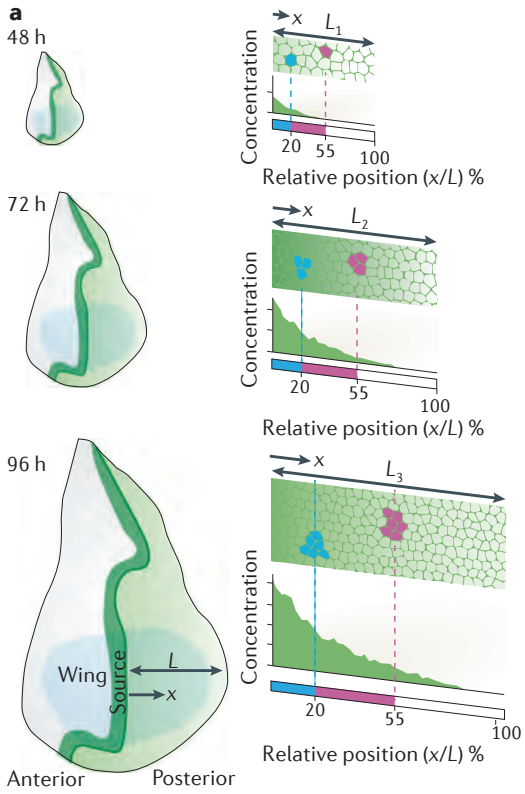
- no hierarchy
- **feedbacks**
- statistical rules

Mechanical regulation of tissue size?

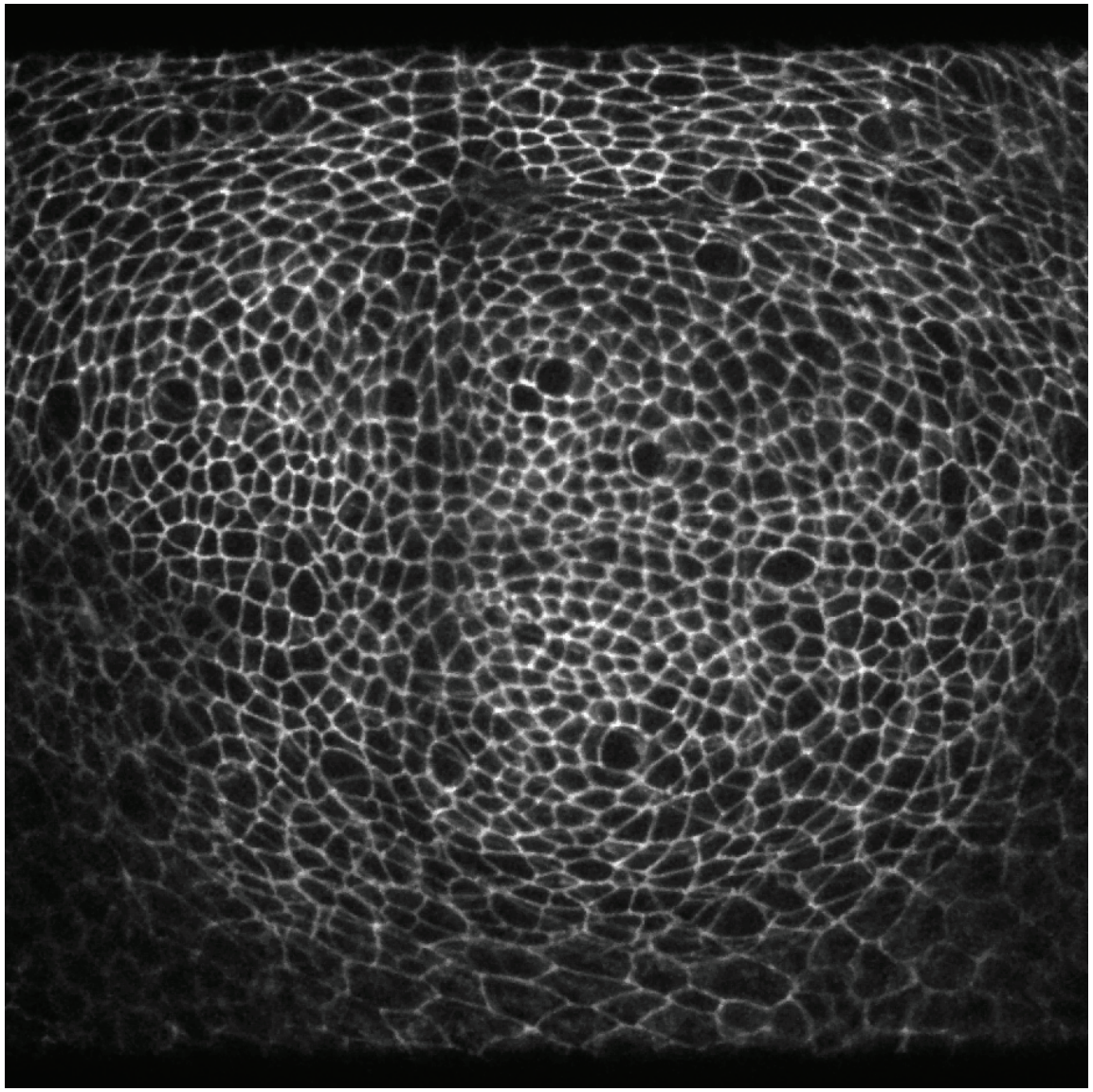
- Organs grow until they reach a fixed size characteristic of each organ/species
- Growth is often uniform in the face of non uniform growth factor distribution (morphogens)
- Tissues can compensate for growth heterogeneities and reach a normal final size arguing that cells assess their environment

—How do cells compare their growth rates?
—How is growth uniform on average in a tissue?
—How is tissue size determined?

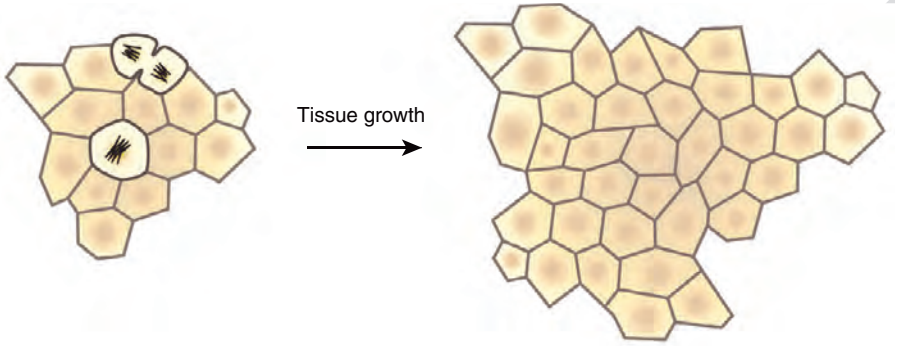
- Cell-cell mechanical interactions

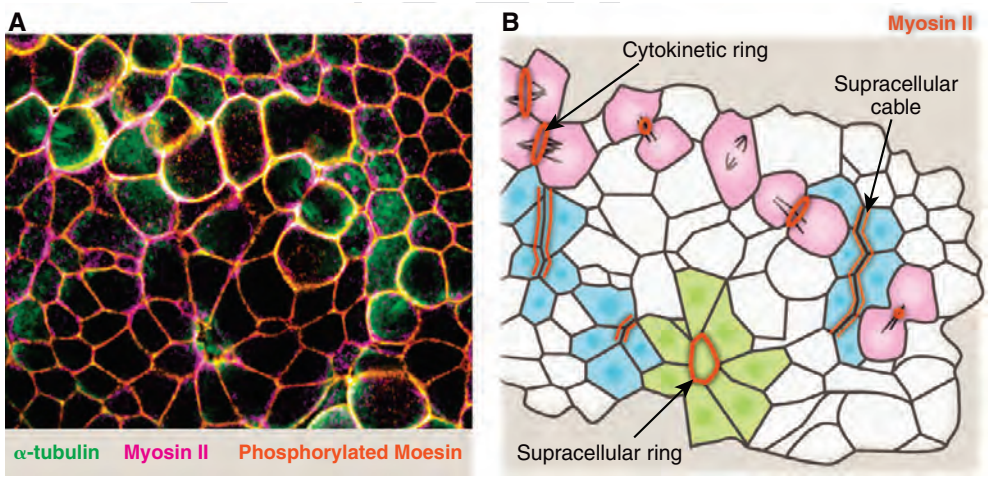


Tissue Growth and Homeostasis

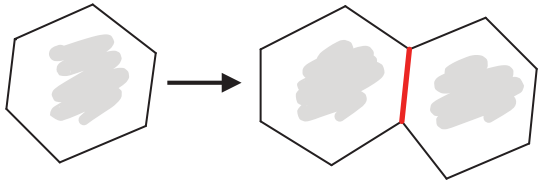


Tissue Growth and Homeostasis

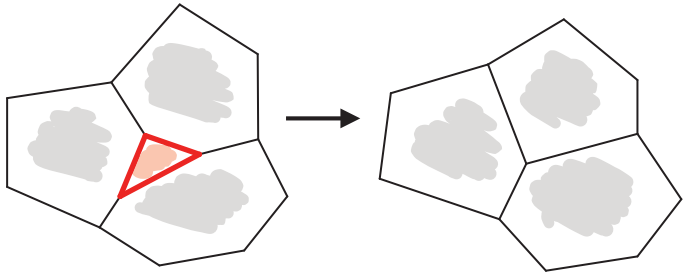




- cell division



- cell death/extrusion



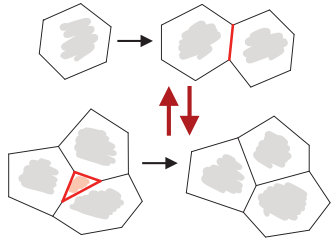
Tissue Growth and Homeostasis

—Tissue growth depends on the balance between:

- Rate of cell division
- Rate of cell death and cell extrusion

—Tissue growth leads to homeostasis at a fixed size where cell growth/division and death/extrusion balance each other (unless growth is arrested): eg. gut.

- How is such a balance achieved?
- Feedback interactions
- Does growth impact on extrusion?
- Does extrusion lead to compensatory growth?



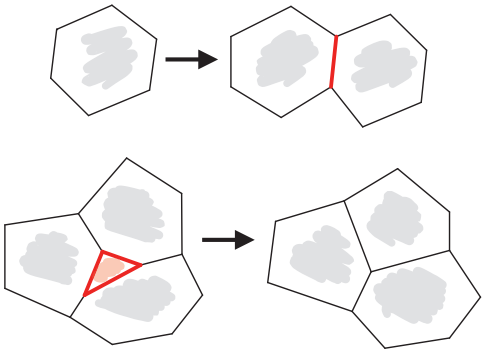
—What are the consequence of differential tissue growth rates?

- Cell and tissue competition (eg. tumours)
- Tissue buckling (morphogenesis)

Biomechanical signalling driving tissue growth

Biochemistry ⇄ Mechanics

Information
(mechano-chemistry)



Control of cell division by mechanics: tension

Cells in culture are subjected to low frequency stretching (60µm) for 1 hour

The control of cell division by tension or diffusion

The anchorage dependence of division in normal cells[11,12,15] and the fact that there is a minimum dimension of surface for this phenomenon[16] can be explained by this hypothesis if it is presumed, as Maroudas[16] first postulated, that the cells have to spread sufficiently to tense themselves, and that tension stimulates the cell cycle.

Table 1 Effects of repetitive tensing of a cell sheet on mitotic index (Expt 1) and on ^{3}H-thymidine incorporation (Expt 2)

Expt 1

Frequency (Hz)	Experiment mitotic index	Control: probe moving, not tensing cells mitotic index
0.25	4.1 ± 1.1	2.5 ± 0.4
0.5	5.1 ± 1.5	2.3 ± 0.7
1.0	3.8 ± 1.7	2.2 ± 0.4
Control, no probe inserted	2.1 ± 0.4	
Control, probe inserted, not moving	1.9 ± 0.6	

Expt 2

Frequency (Hz)	Experiment % labelled cells	Control: probe moving, not tensing cells % labelled cells
0.25	21.4 ± 5.1	14.3 ± 1.5
0.50	22.5 ± 5.6	14.1 ± 3.2
1.00	15.3 ± 2.5	13.9 ± 1.6
Control, probe not inserted	14.7 ± 2.2	
Control, probe inserted	14.3 ± 2.2	

ASG. Curtis and GM Seehar, *Nature* 274, 52-53 (1978)

Control of cell division by mechanics: geometry

- Density dependence of cell proliferation and contact inhibition are characteristic of cells in culture

- Increased cell-substrate adhesion causes reduction of cell height and increased cell diameter
- Cell proliferation increases as cells spread/adhere more to the substrate

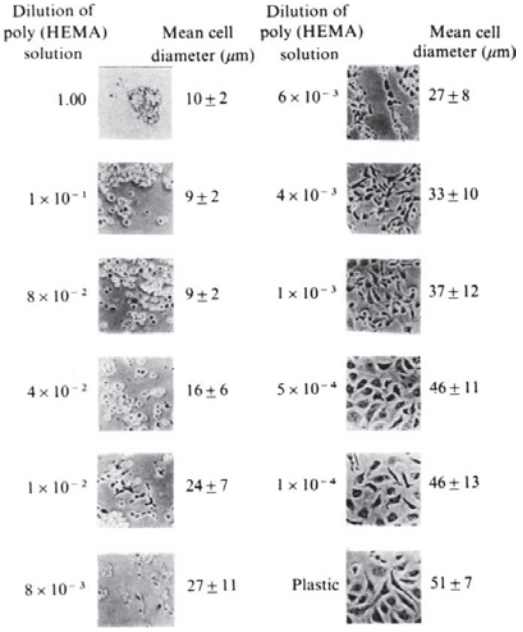
- Is it *adhesion per se or cell shape per se?*

Nature Vol. 273 1 June 1978 345

Role of cell shape in growth control

Judah Folkman & Anne Moscona

Department of Surgery, Children's Hospital Medical Center and The Harvard Medical School, Boston, Massachusetts 02115



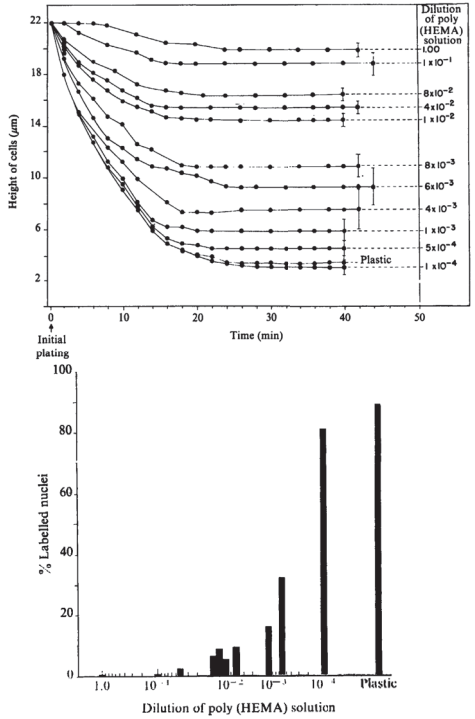


Table 1 Effect of crowding on cell shape and DNA synthesis (WI-38 cells)

Dilution of poly (HEMA)	Poly (HEMA) Cell density (cells per cm²)	³H Incorporation (c.p.m.)	Cell height (μm)	Plastic Cell density (cells per cm²)	³H Incorporation (c.p.m.)
1.00	15,000	7±5	22	250,000 (confluent)	7±1
4×10^{-2}	15,000	50±5	15	100,000 (confluent)	55±9
4×10^{-3}	15,000	250±15	6	30,000 (subconfluent)	254±23

J. M. Folkman and A. Moscona, *Nature* 273, 345 (1978)

Control of cell division by mechanics: geometry or adhesion?

Geometric Control of Cell Life and Death

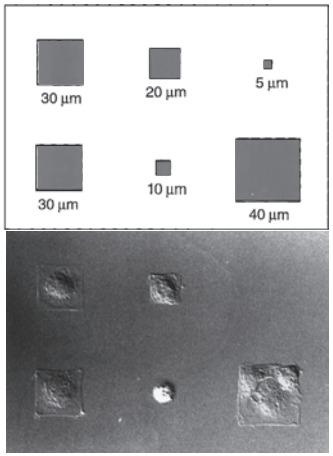
Christopher S. Chen, Milan Mrksich, Sui Huang,
George M. Whitesides, Donald E. Ingber*

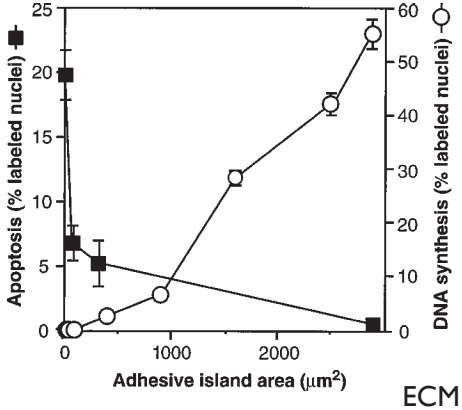
—The role of surface of adhesion:

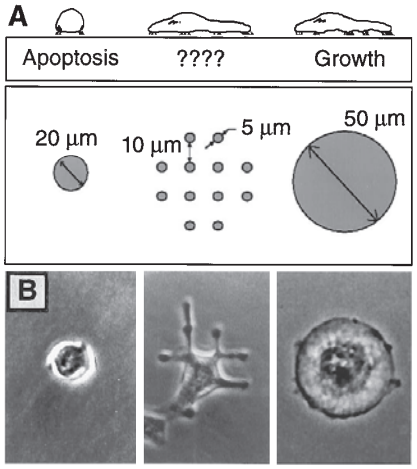
- Cell division increases as the adhesive island surface increases
- Cell death by apoptosis decreases in parallel

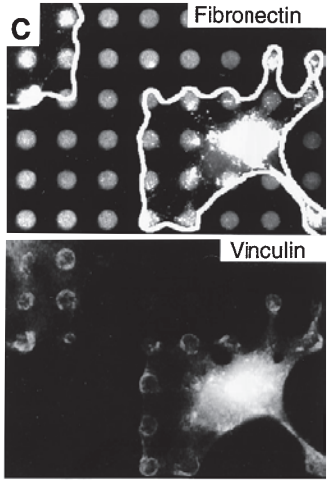
—Disentangling cell geometry and substrate adhesion using a controlled micro fabricated substrate

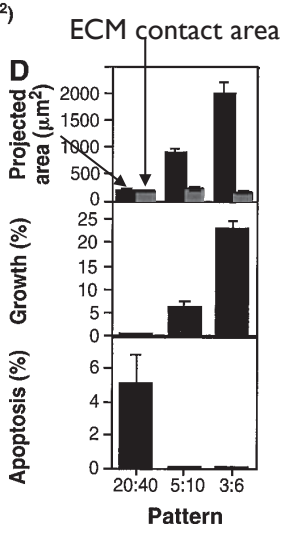
- The projected surface area, but not the surface of adhesion to the ECM impacts on cell proliferation and cell death.
- Does this reflect cell shape per se or cell stresses?











C. Chen et al., and D. Ingber. *Science* (1997):Vol. 276, Issue 5317, pp. 1425-1428
DOI: 10.1126/science.276.5317.1425

Control of cell growth by mechanics

—Disentangling the role of cell stress and cell shape

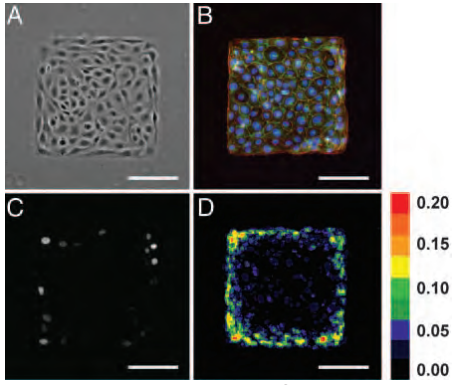
- using micro fabricated substrates on colony island of multiple cells (rather than single cells)
- Cell division patterns depend on the geometry colony islands

—Mapping stress pattern

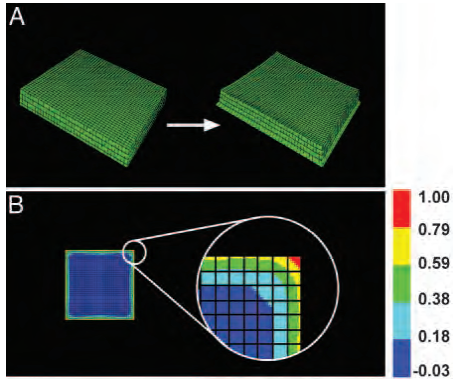
- Use of finite element modelling (FEM) to deduce stress patterns in colony islands.
- Cell division patterns correlate with stress patterns that emerge from cell contractility and connectedness between cells and tissue geometry.

- Traction Force microscopy confirms the deduced mechanical stress pattern.

endothelial cells on fibronectin

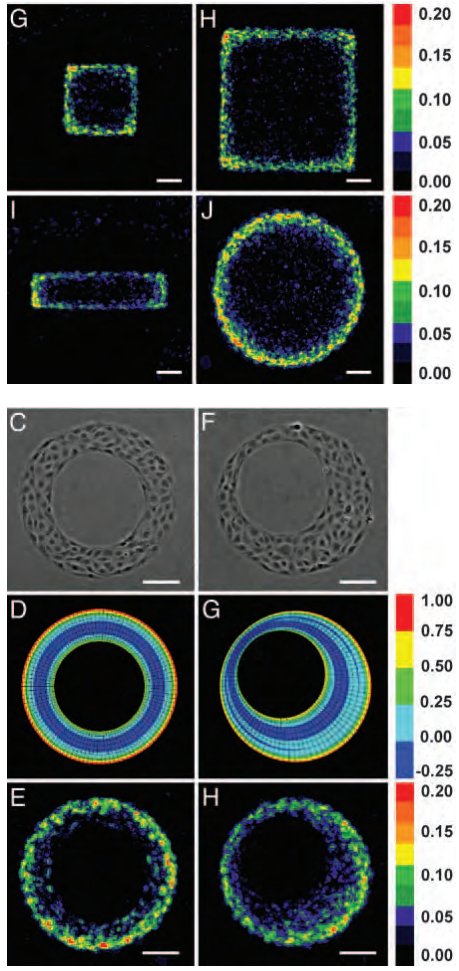


BrdU Proliferation map

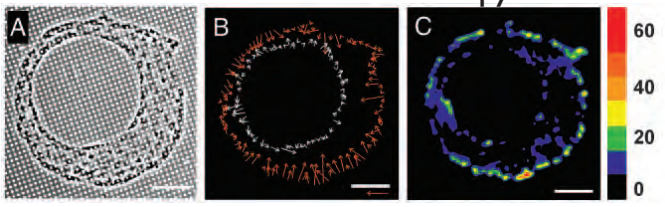


Stress from FEM

BrdU incorporation density maps



traction force microscopy



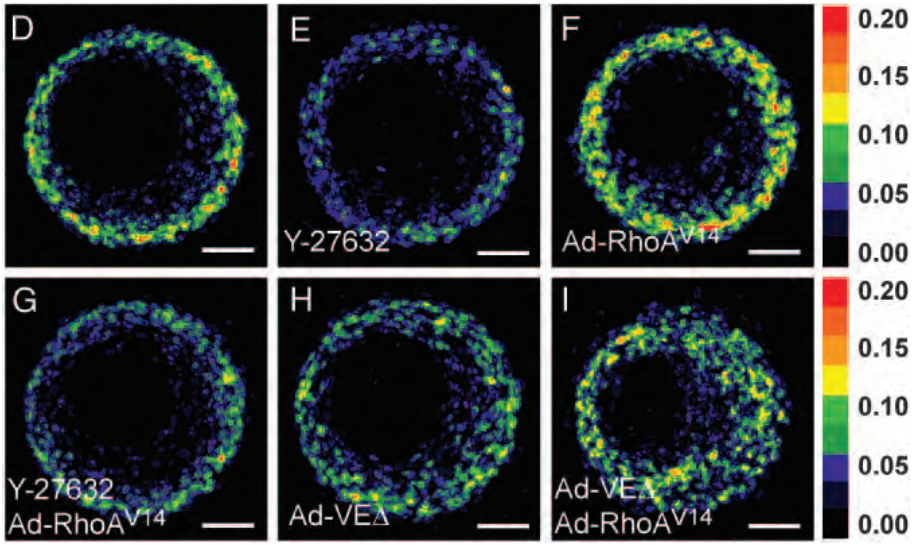
C. Nelson *et al.* C. Chen. (2005) *P.N.A.S.* 102: 11594–11599
www.pnas.orgcgidoi10.1073pnas.0502575102

Control of cell growth by mechanics

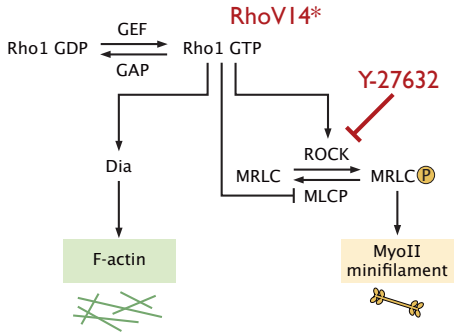
—Disentangling the role of cell stress and cell shape

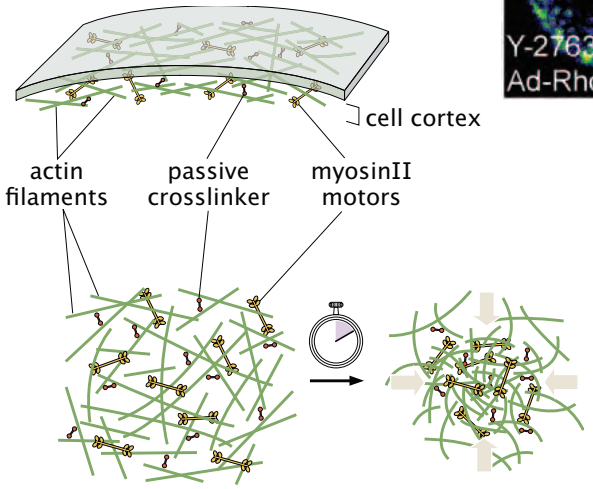
- Mechanical forces generated by the actomyosin cytoskeleton control cell proliferation patterns

BrdU density map



dominant negative VE-cadherin
(blocks mechanical coupling at cell junctions)



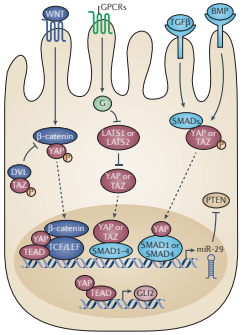


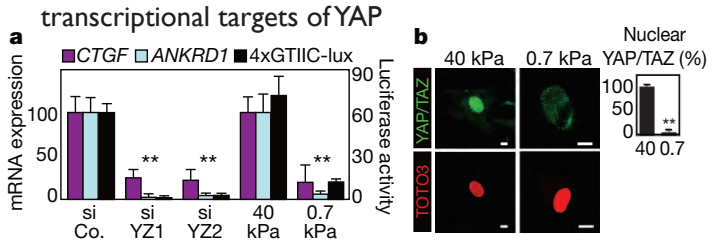
C. Nelson *et al.* C. Chen. (2005) *P.N.A.S.* 102: 11594–11599
www.pnas.orgcgidoi10.1073pnas.0502575102

Control of cell growth by mechanics

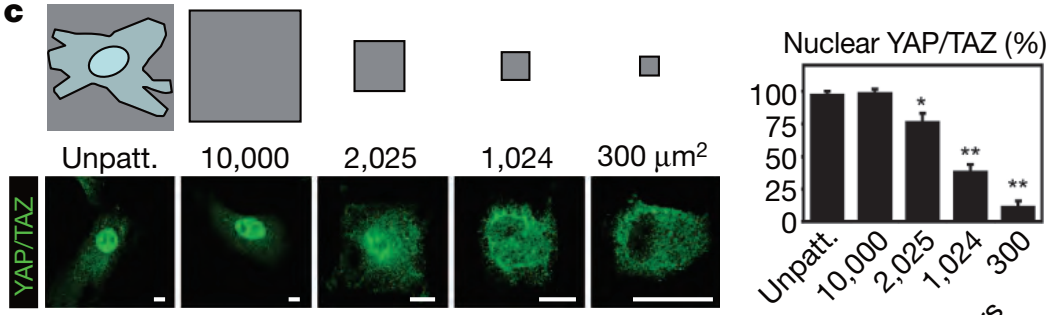
—Identification of transcription factors that mediate mechanically induced cell proliferation

- YAP/TAZ are known components of the Hippo/LATS growth pathway that translocates in the nucleus once activated.
- Substrate stiffness induces YAP activation

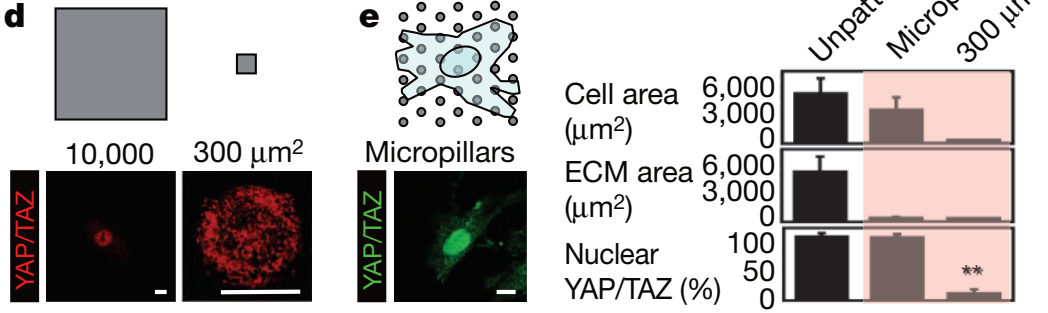




- Cell substrate adhesion/geometry affects YAP/TAZ activation



- Cell projected area, but not adhesion per se activates YAP//TAZ

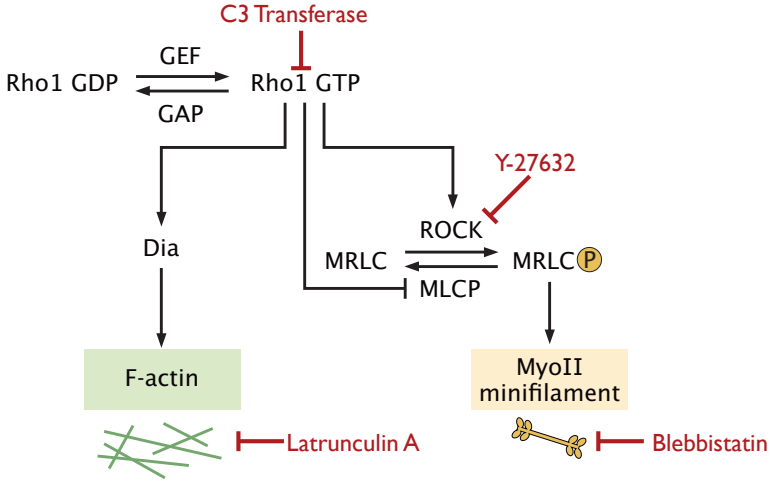


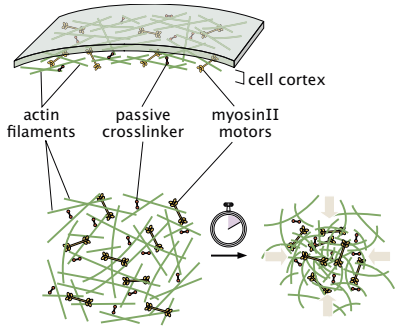
S. Dupont et al., and S. Piccolo. *Nature* (2011). 474::179-183

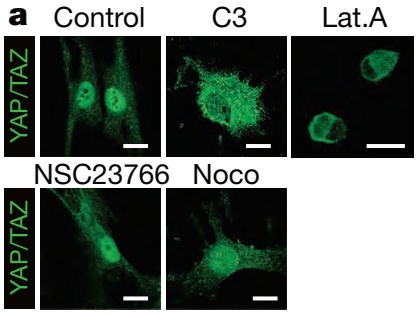
Control of cell growth by mechanics

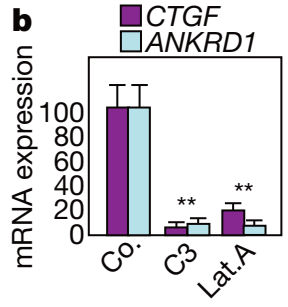
—Identification of transcription factors that mediate mechanically induced cell proliferation

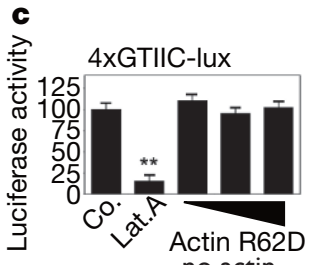
- The actomyosin cytoskeleton (but *not* the microtubule network) is required for YAP/TAZ activation.

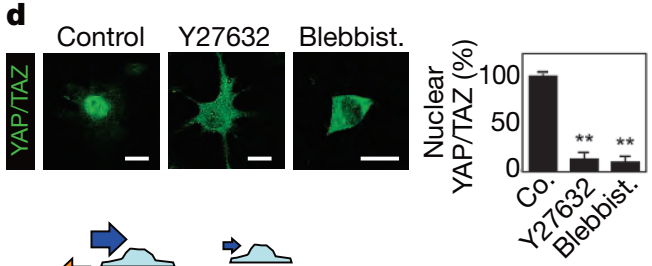


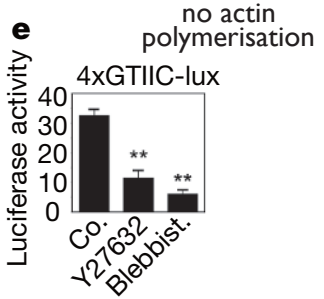


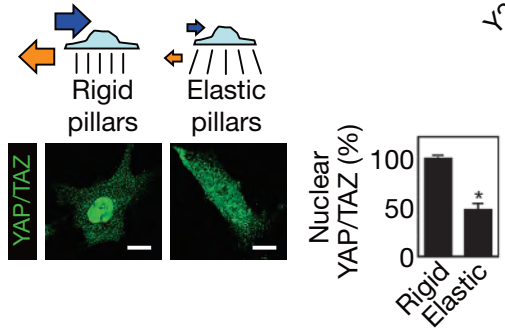










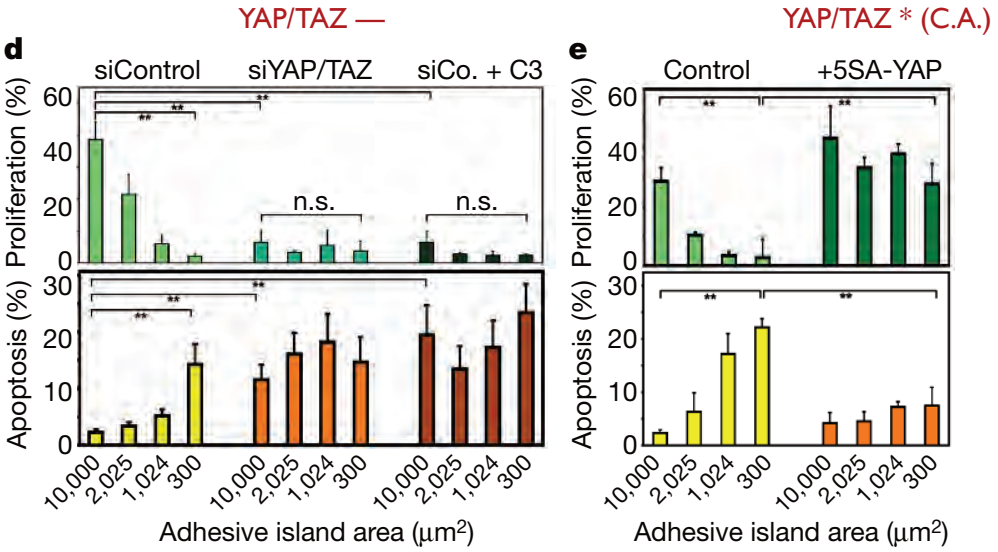


S. Dupont et al., and S. Piccolo. *Nature* (2011). 474::179-183

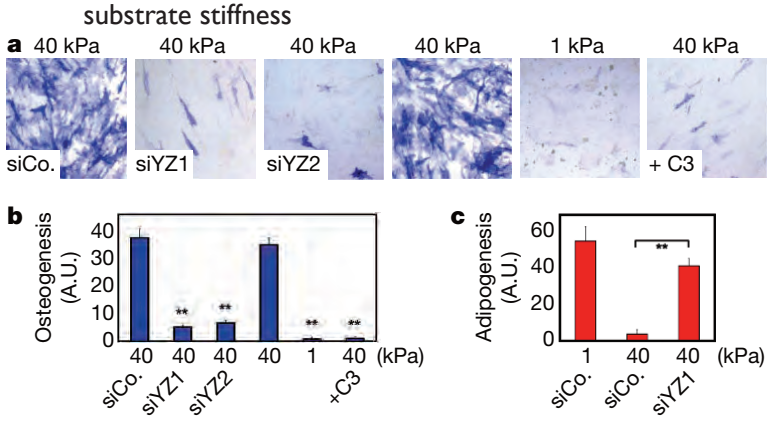
Control of cell growth by mechanics

—Identification of transcription factors that mediate mechanically induced cell proliferation

- Cell stretching induces activation of cell proliferation and inhibition of apoptosis
- YAP/TAZ are both required for this process
- Constitutively active YAP expression induces cell proliferation and inhibits apoptosis
- This effect is independent of upstream Hippo/LATS pathway activity (not shown)



- YAP/TAZ similarly mediates the cell differentiation of stem cells dependent on substrate stiffness
- On soft substrates adipocytes, on stiff substrates osteocytes.



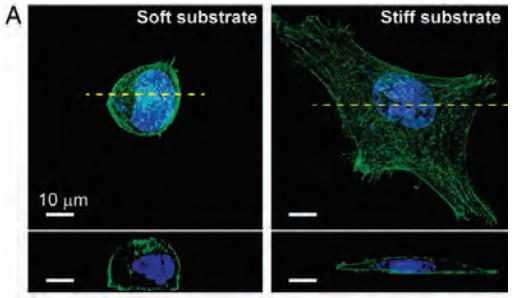
[see also A. Engler et al, D. Discher. (2006) *Cell* 126, 677–689]

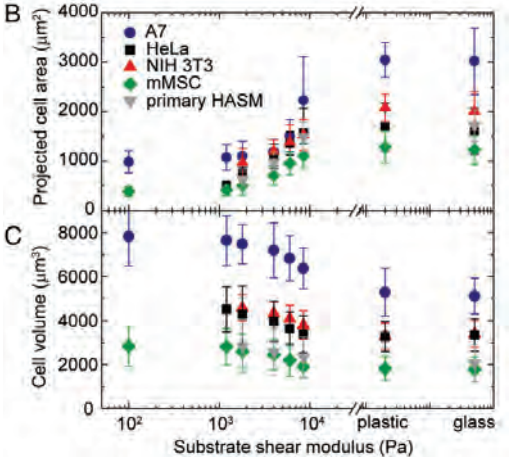
S. Dupont et al., and S. Piccolo. *Nature* (2011). 474::179-183

Control of cell growth by mechanics

—Cell spreading affects cell volume via water efflux

- Cells spread more on stiffer substrates and reduce concomitantly their volume

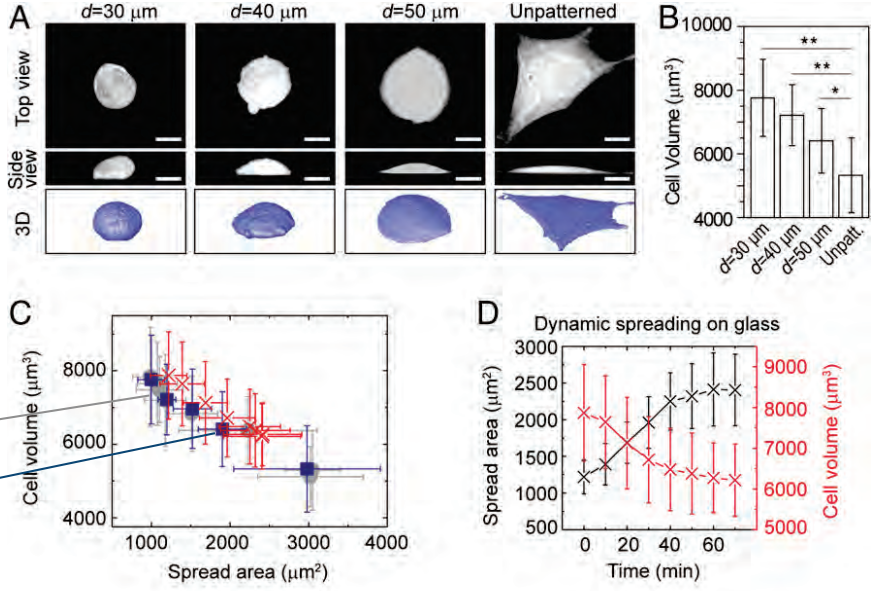




- Restricting cell area using smaller adhesive islands, increases cell volume
- Dynamic cell spreading is associated with dynamic change in cell volume

- The volume dependence on spread area is the same irrespective of how area is attained (substrate stiffness, adhesion area, dynamics of spreading)

- Volume could be changed by protein concentration changes or water efflux: no [protein] change
- Volume change caused by water efflux



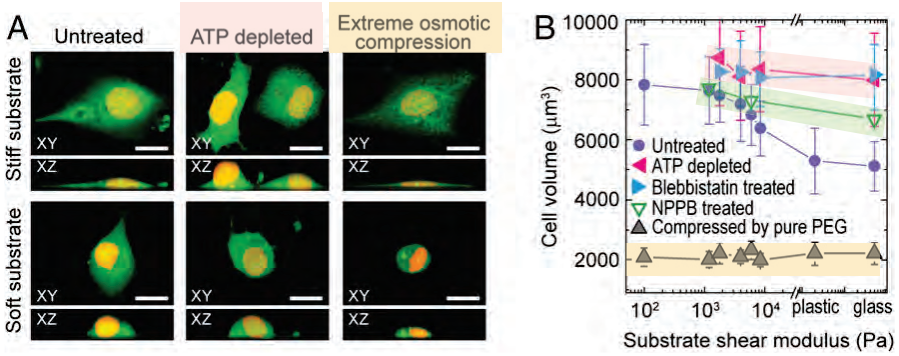
M. Guo et al. J. Lippincott-Schwartz and D. Weitz. PNAS. (2017) E8618–E8627
www.pnas.org/cgi/doi/10.1073/pnas.1705179114

Control of cell growth by mechanics

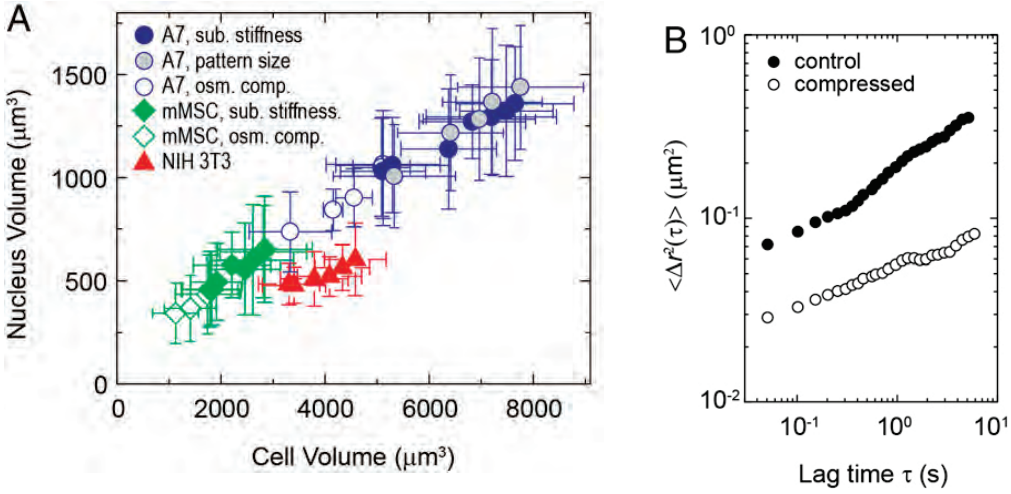
—Volume changes induced by cell spreading or osmotic stress affect nuclear volume and molecular crowding in the nucleus.

- Hyper-osmotic stress (with high PEG concentration in the medium) causes a reduction in cell volume through water efflux irrespective of substrate stiffness.
- Blocking Cl ion channels (NPPB) blocks volume change as a function of substrate stiffness so volume adjustment involves changes in osmolytes.
- Blocking cell contractility also affects stiffness dependent volume change.
- Consistent with tension dependent regulation of mechanosensitive ion channels, affecting osmolyte concentration and water flux.

reviewed in R. Phillips, T. Ursell, P. Wiggins and P. Sens (2009) *Nature* 459: 379-385



- In all conditions nuclear volume adjusts to cell volume
- Histine H2::GFP mean square displacement is lower in compressed cells consistent with increased molecular crowding



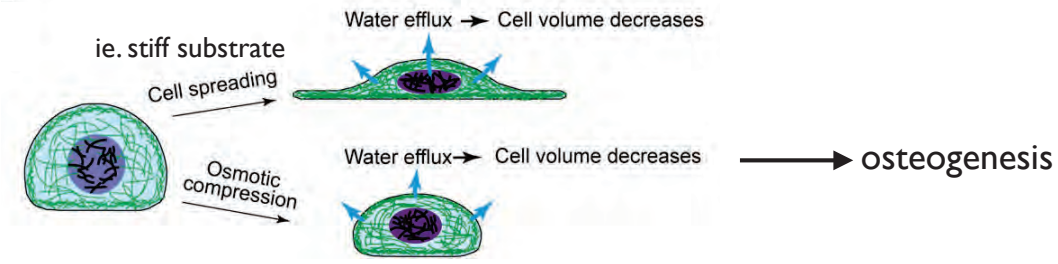
M. Guo et al. J. Lippincott-Schwartz and D. Weitz. PNAS. (2017) E8618–E8627
www.pnas.org/cgi/doi/10.1073/pnas.1705179114

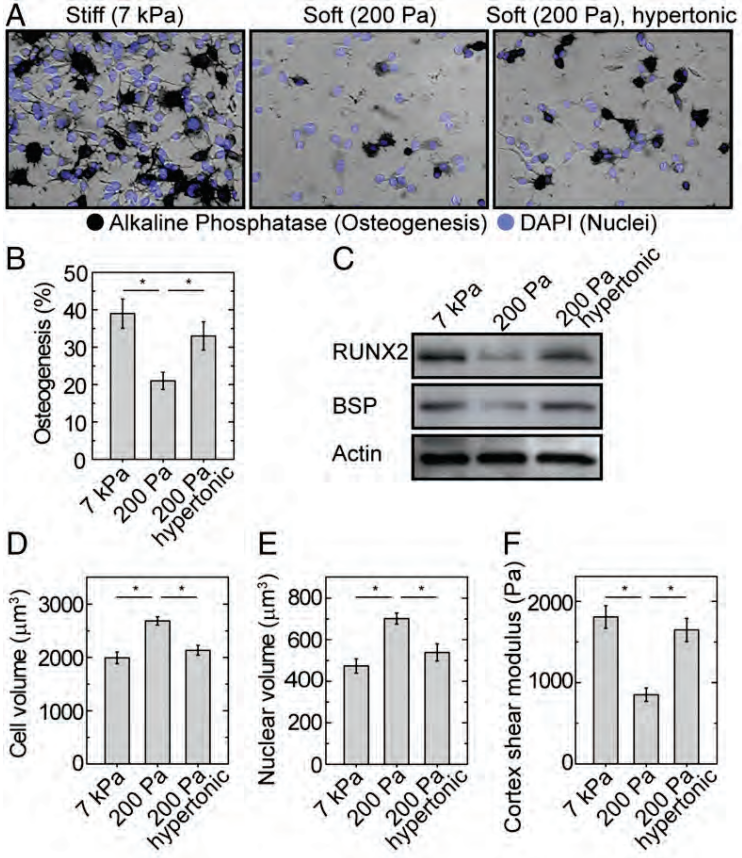
Control of cell growth by mechanics

—Volume changed induced by osmotic stress affects differentiation of stem cells

- Differentiation of stem cells into osteocytes on a stiff substrate
- Hypertonic medium rescues differentiation on soft substrate.
- This is associated with volume reduction of cell and nucleus and increased cell stiffness

- On soft substrates, conversely cells differentiate into adipocytes
- Hypotonic medium rescues adipogenesis if on stiff substrate





M. Guo et al. J. Lippincott-Schwartz and D. Weitz. PNAS. (2017) E8618–E8627
www.pnas.org/cgi/doi/10.1073/pnas.1705179114

#YAP forms liquid like nuclear condensates in response to molecular crowding induced by hyperosmotic stress

D. Cai et al. and J. Lippincott-Schwartz. bioRxiv. http://dx.doi.org/10.1101/438416. (2019)

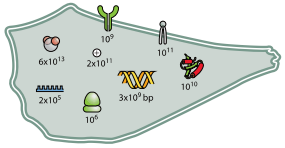
now in press in *Nature Cell Biology*

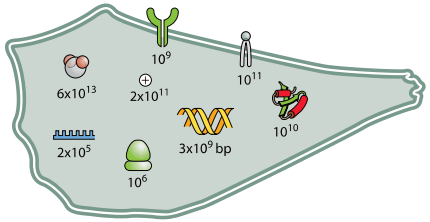
Nat Cell Biol. 2019 Dec;21(12):1578-1589. doi: 10.1038/s41556-019-0433-z.

Control of cell growth/division & death by mechanics

- Summary

No cell division
Apoptosis





Cell division

Geometry: spreading, volume reduction (but not adhesion per se)

Mechanics: tension

compression

Mechanochemical signalling: ion channels, YAP, etc.

Mechanics of Tissue Growth and Homeostasis

—Tissue growth depends on the balance between:

- Rate of cell division
- Rate of cell death and cell extrusion

—Tissue growth leads to homeostasis at a fixed size where cell growth/division and death/extrusion balance each other (unless growth is arrested): eg. gut.

- How is such a balance achieved?
- Feedback interactions?
- Does growth impact on extrusion?
- Does extrusion lead to compensatory growth?

—What are the consequence of differential tissue growth rates?

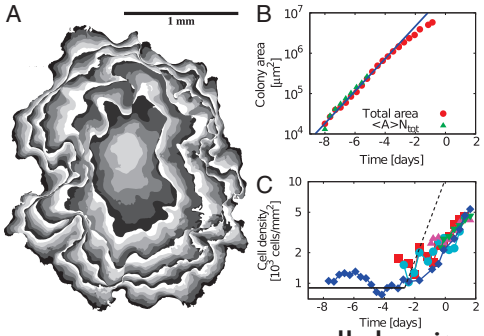
- Cell and tissue competition (eg. tumours)
- Tissue buckling (morphogenesis)

Control of cell growth/division by mechanics

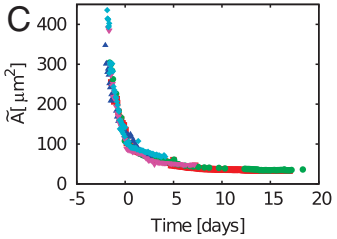
—Changes in cell behaviour during collective tissue growth *in vitro*: contact inhibition

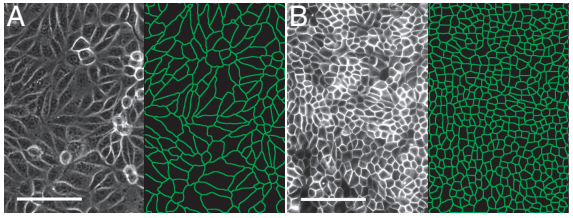
- Growth of isolated colonies to disentangle cell contact from mechanics
- « free growth » regime is associated with exponential growth while cells are in contact

- Colony growth slows down, limited by constrained velocity of edge cells (ie. friction).
- This is associated with a transition to increased cell density and slower motility which are mechanically induced

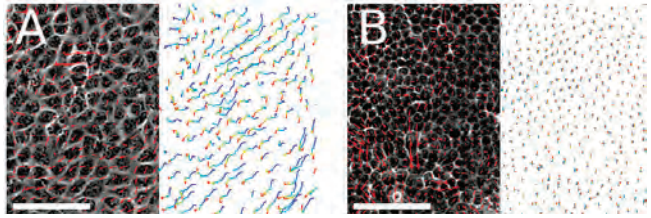


cell density



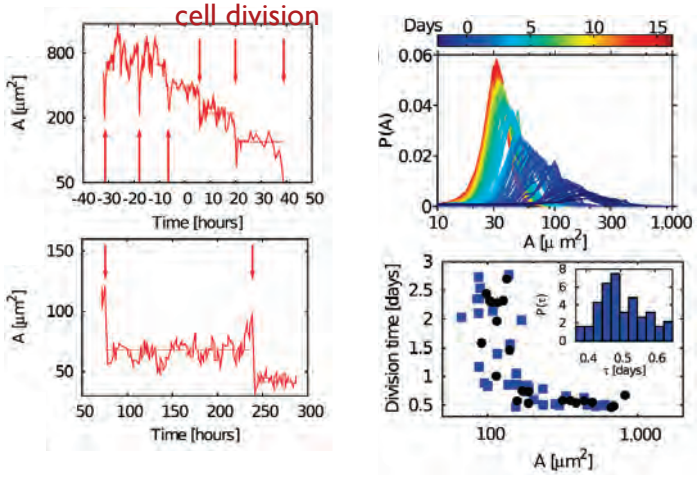


cell density and size



cell motility

- Increased cell density is associated with gradual increase of division time



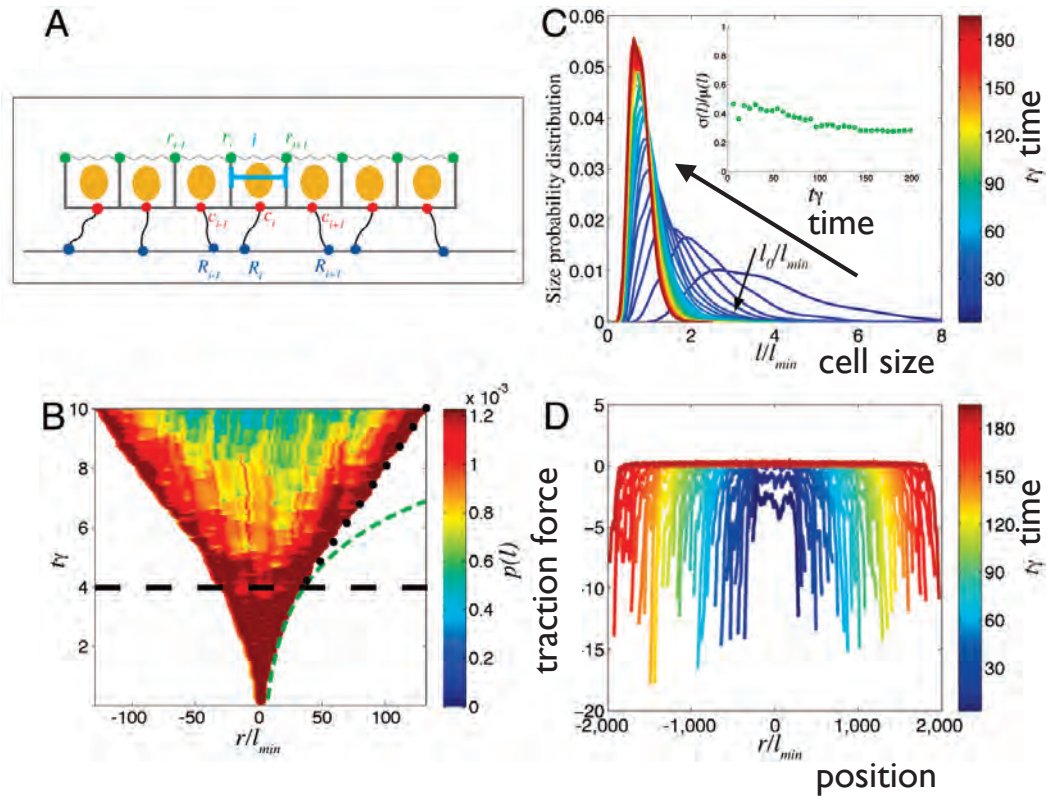
A. Puliafito et al. and B. Shraiman. (2012) *PNAS* 109:739–744

Control of cell growth/division by mechanics

—Changes in cell behaviour during collective tissue growth *in vitro*: contact inhibition

—1D vertex mechanical model of self-limiting growth

- Short time elastic response of cells
- Friction coupling to the substrate (elastic restoring force)
- Active random cell motion (Langevin driving force) averages to zero in colony bulk but not at colony edges
- Cell growth is modelled by increasing the rest length of a cell edge when neighbouring cells stretch it.
- Cell division halves rest length
- Cell division rate depends on cell size



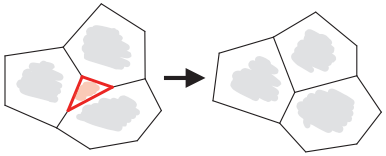
—Simulation results

- 2 phases: exponential and linear growth
- Cell division in the bulk and gradual arrests due to increased cell density
- Cell division persists at colony edges as cells are stretched.

A. Puliafito et al. and B. Shraiman. (2012) *PNAS* 109:739–744

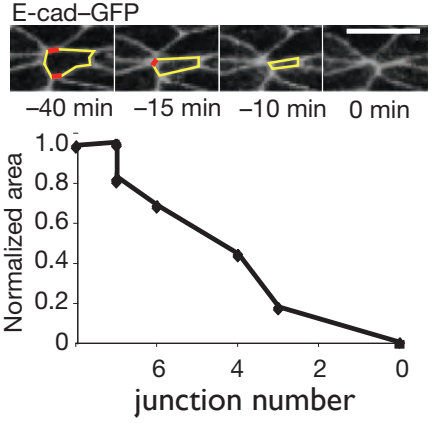
Balancing cell growth and extrusion by mechanics

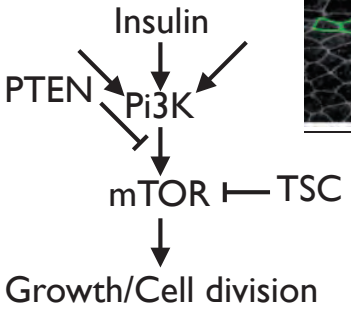
—Compression induced cell extrusion

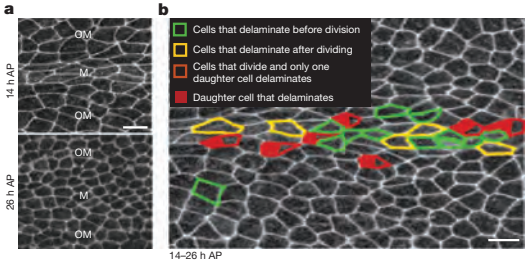


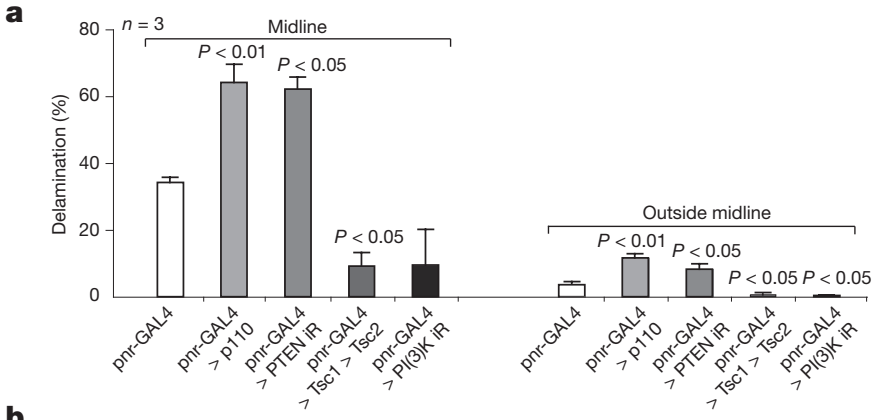
- Cells in the midline of a growing tissue delaminate.
- Tissue overgrowth (induced by activation of the Pi3K pathway) increases cell delamination
- Reducing tissue growth reduces cell delamination/ extrusion
- Tissue crowding induces cell delamination

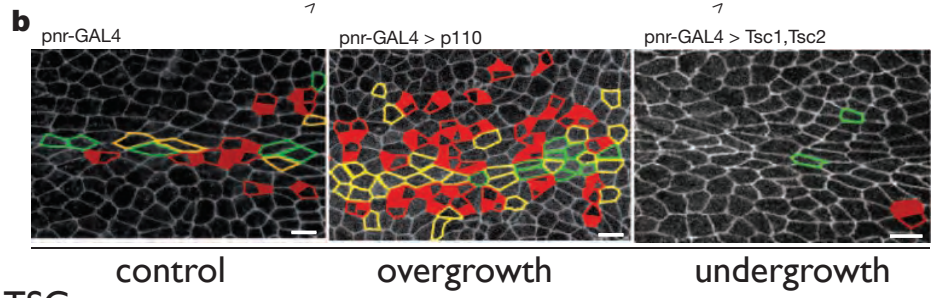
- Cell delamination is associated with apical area reduction and junction remodelling (loss).







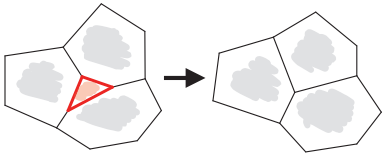




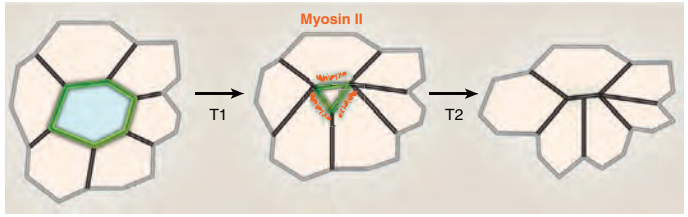
Marinari, E., et al, and Baum, B. (2012). *Nature 484*, 542–545

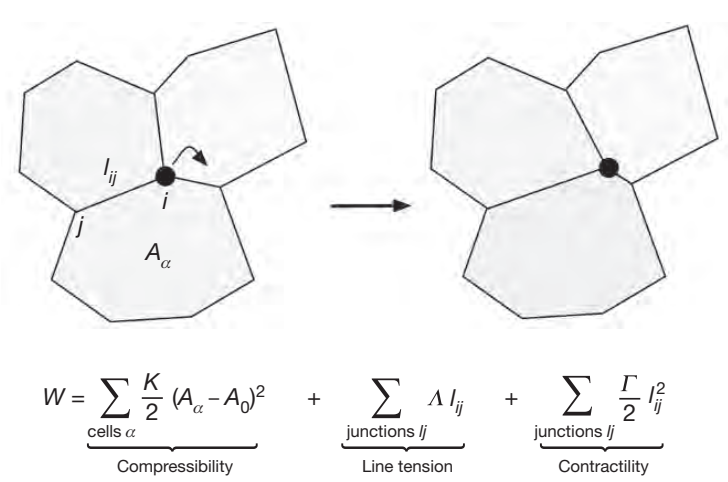
Balancing cell growth and extrusion by mechanics

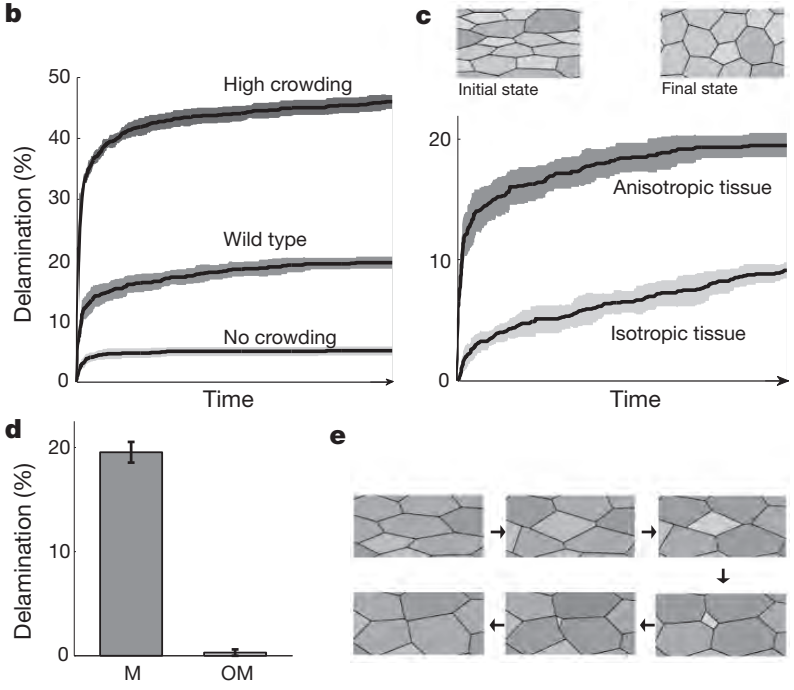
—Compression induced cell extrusion



- Mechanical vertex model of junction remodelling underlying compression induced cell delamination.



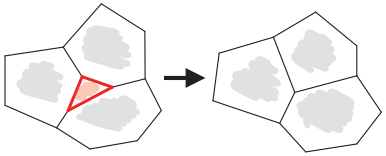




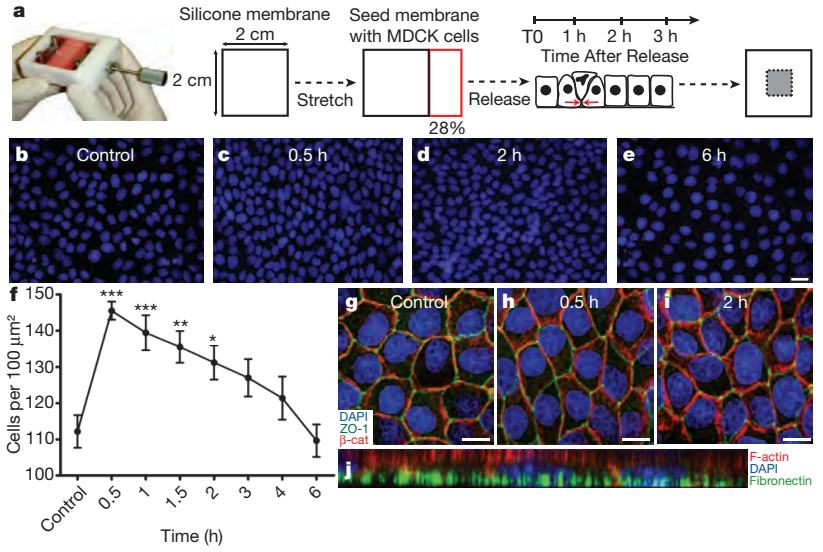
Marinari, E., et al, and Baum, B. (2012). *Nature 484*, 542–545

Balancing cell growth and extrusion by mechanics

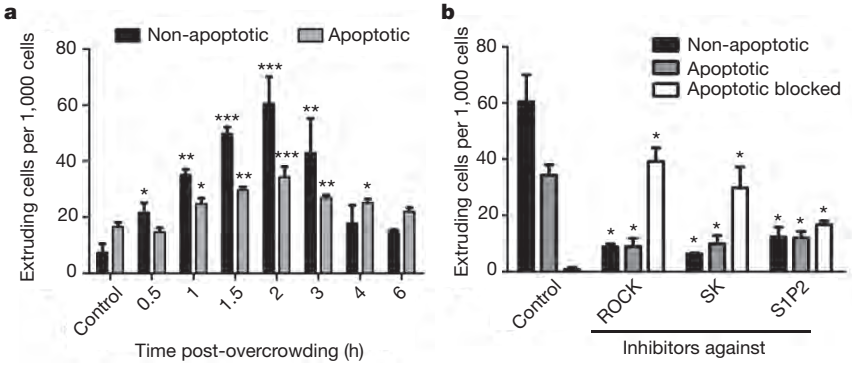
—Compression induced cell extrusion



- A tissue monolayer under compression adjusts its cell density in two phases:
- Phase 1: Density first increases rapidly due to compression.
- Phase 2: Cell density then slowly decreases until reaching initial steady state.



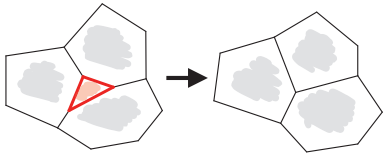
- Adjustment of cell density is associated with cell extrusion by apoptosis and independent of apoptosis.
- Cell extrusion requires ROCK, the sphingosine kinase SK and sphingosine phosphate (SIP2).



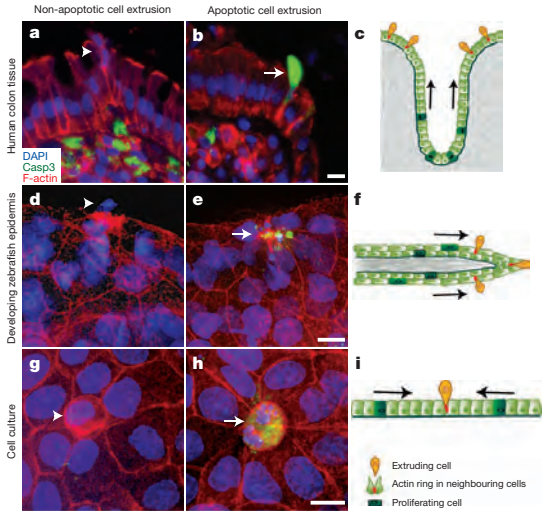
Eisenhoffer, G.T., et al., and Rosenblatt, J. (2012). *Nature 484*, 546–549.

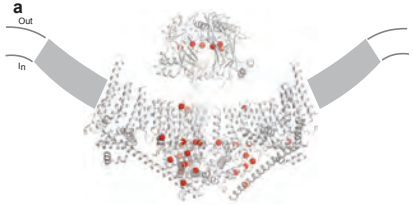
Balancing cell growth and extrusion by mechanics

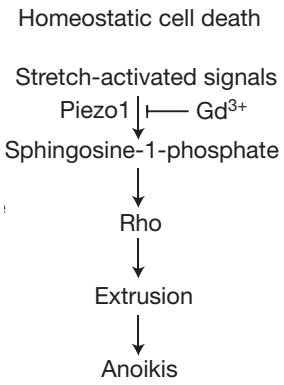
—Compression induced cell extrusion

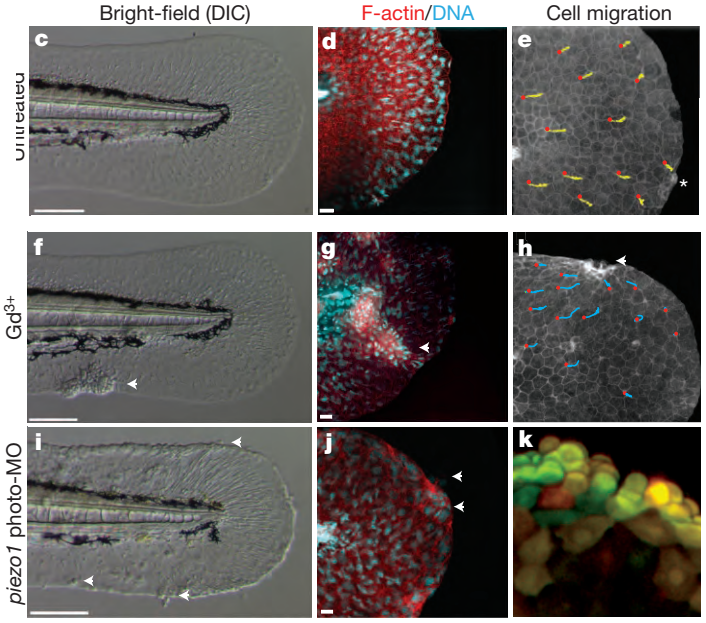


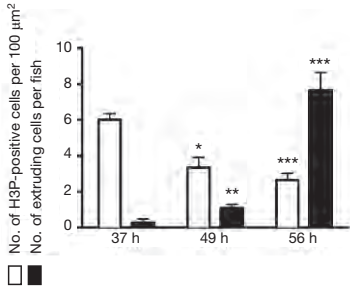
- In whole organisms: gut villi and fins in zebrafish, cell crowding causes cell extrusion.
- This requires the mechanonosensitive receptors Piezo.

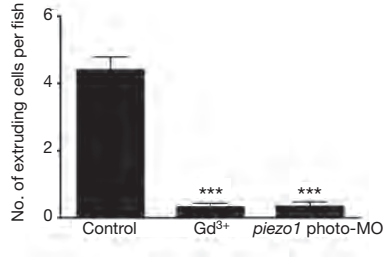












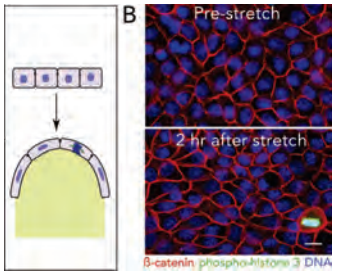
Eisenhoffer, G.T., et al., and Rosenblatt, J. (2012). *Nature 484*, 546–549.

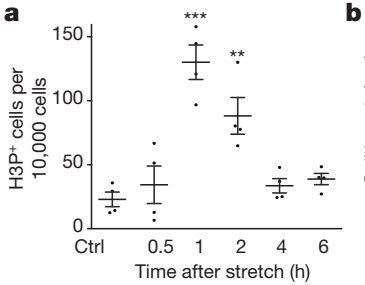
Balancing cell growth and extrusion by mechanics

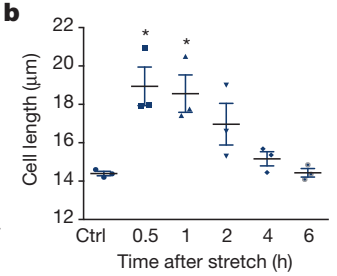
—Stretch induced cell divisions

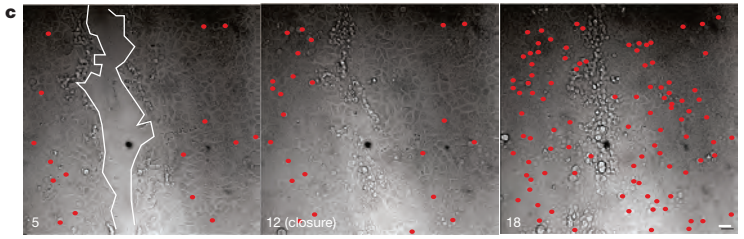
- Stretching of cell cultures (MDCK cells) induces cell division.
- Wound healing in a cell monolayer causes stretching of cells after closure and induces a burst of cell division after closure

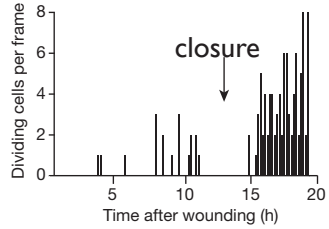
- The mechanosensitive receptor Piezo 1 is required for cell division in MDCK culture cells and in zebrafish fins
- Piezo activation gives rise to calcium signalling, ERK activation and Cyclin B induction

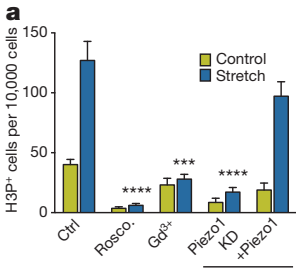


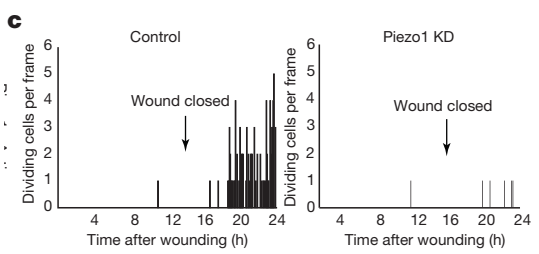


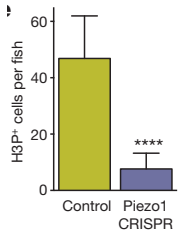


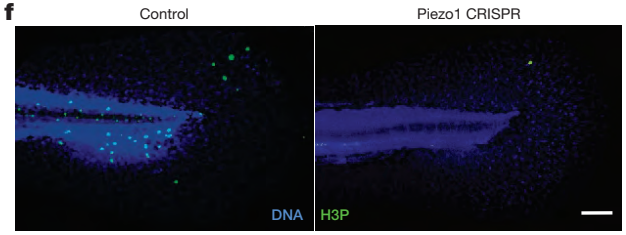


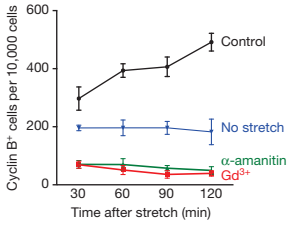


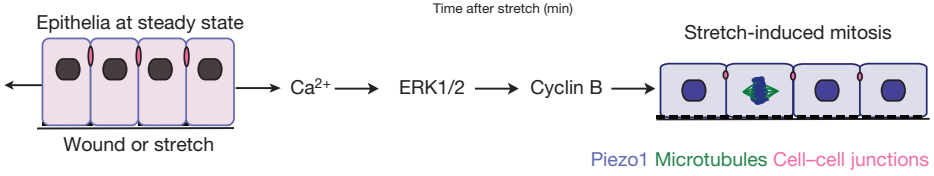












Gudipaty, S.A., et al., and Rosenblatt, J. (2017). *Nature 543*, 118–121.

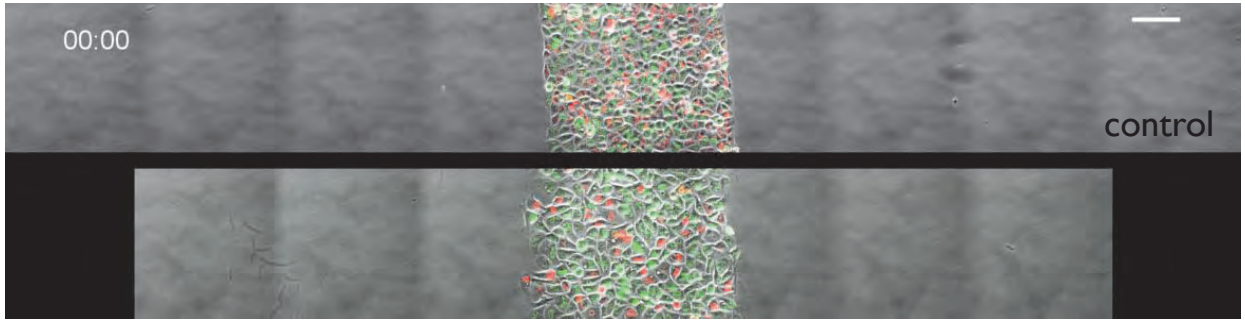
Balancing cell growth and extrusion by mechanics

—Stretch induced cell divisions

Regulation of cell cycle progression by cell–cell and cell–matrix forces

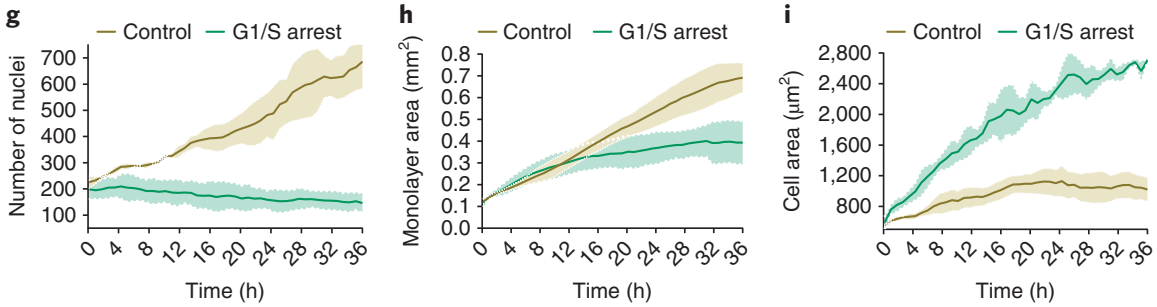
Cdt1::RFP: G1-S
Geminin::GFP: S-G2-M

- MDCK cell culture expand at a constant cell density
- When cell division is blocked (G1/S arrest), cell area increases



G1/S arrest

- Cell division is sustained while the colony expands and thereby maintains a constant density.

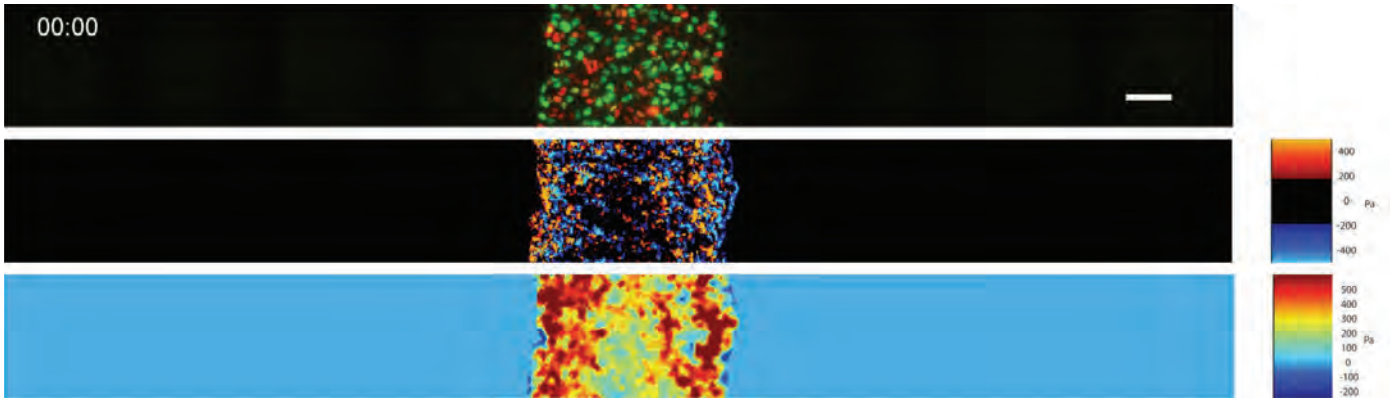


M. Uroz et al. and X. Trepat. (2018) *Nature Cell Biology* 20:646–654

Balancing cell growth and extrusion by mechanics

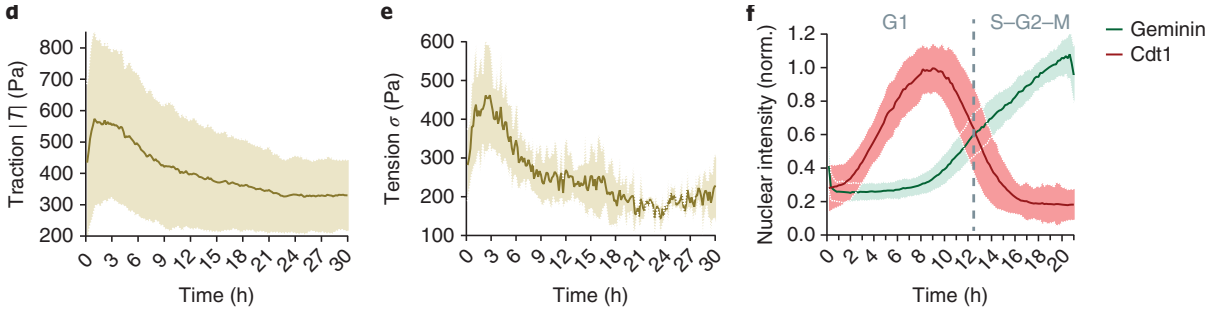
—Stretch induced cell divisions

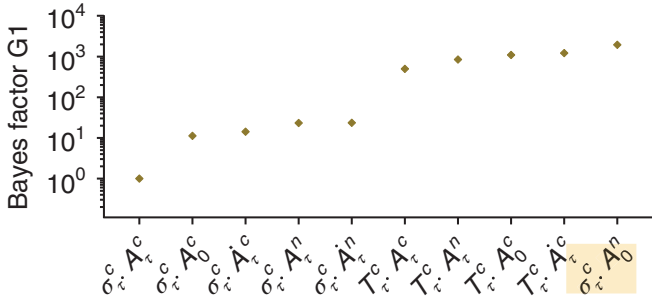
Cdt1::RFP: G1-S
Geminin::GFP: S-G2-M

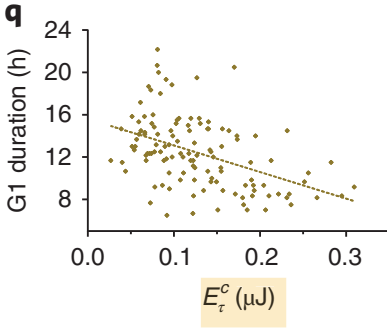


Large scale cross-correlation analysis

- Cell tension and surface energy $E(\sigma . A)$ are the best predictors of cell cycle arrest (over cell geometry).
- High surface energy correlates with a short G1 duration: cells with same surface should enter G1 earlier if tension is higher.



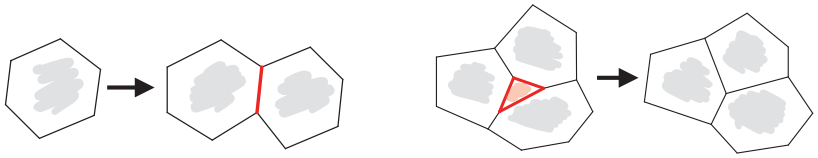




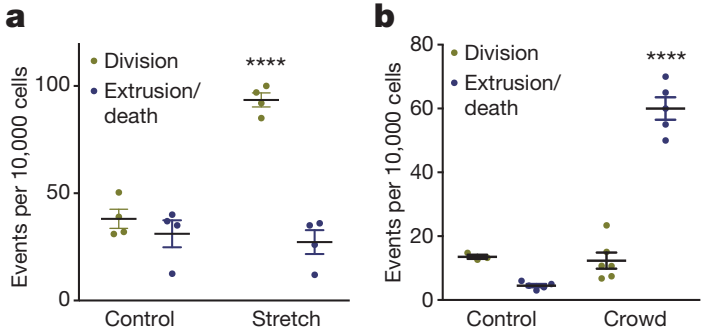
M. Uroz et al. and X. Trepat. (2018) *Nature Cell Biology* 20:646–654

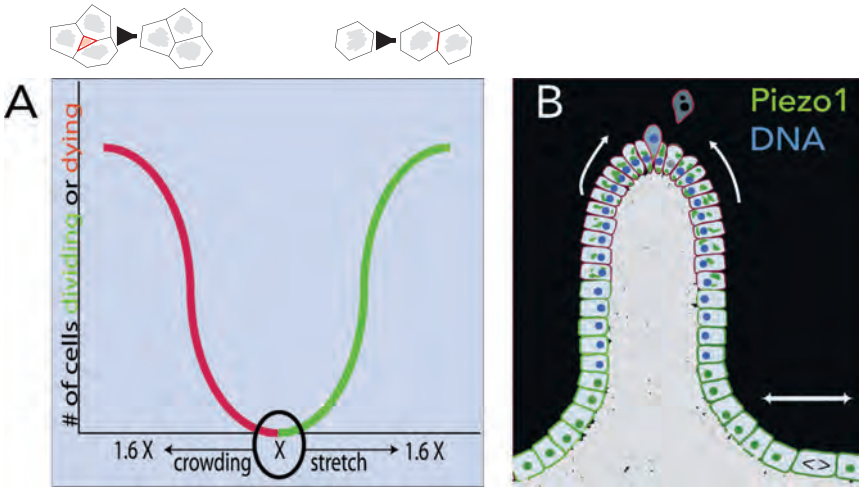
Balancing cell growth and extrusion by mechanics

—in 2D, balance between Stretch induced cell divisions and compression induced cell extrusion



- Cells sense compression and stretch via the same mechanosensitive receptor Piezo and adjust their density depending on their mechanical state.
- Cell density evolves until the population reaches a steady state X where cell death/extrusion and cell division balance each other





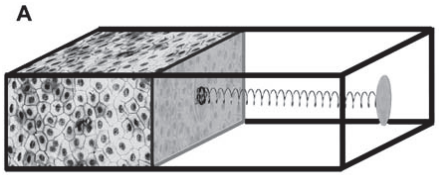
X: steady-state density where cell death/extrusion and cell division balance each other

Gudipaty, S.A., et al., and Rosenblatt, J. (2017). *Nature 543*, 118–121.

Balancing cell growth and death by mechanics: tumours

—In 3D, homeostatic pressure: a tissue property where cell growth/division balances apoptosis

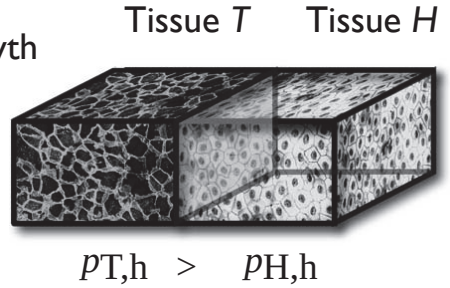
- Homeostatic pressure



Tissue grows against wall resisted by a spring. Growth proceeds until internal pressure $p = p_h$ where growth and apoptosis balance each other.

The spring position is stable, since further growth increases the pressure above this value p_h and induces apoptosis, whereas recession of the piston decreases the pressure and causes cell division.

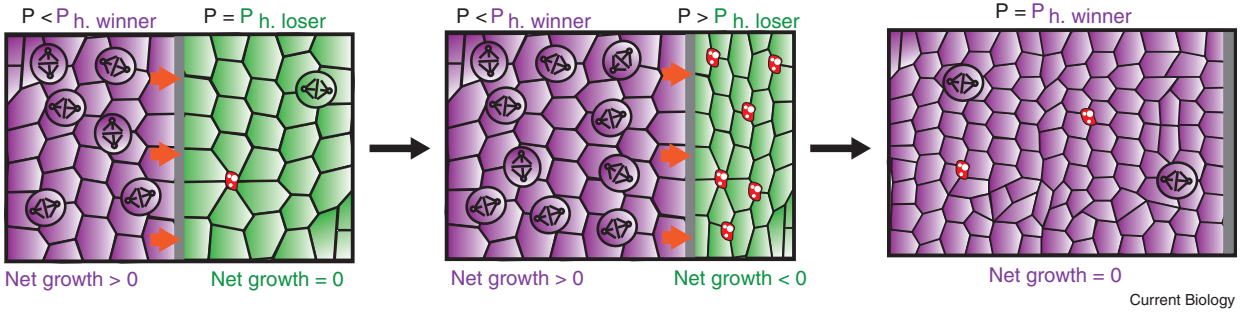
- Tumour growth



As cells divide, the pressure rises and first reaches the homeostatic pressure $p_{H,h}$. At this point, tissue *H* stops growing while tissue *T* continues to proliferate and drives the pressure above $p_{H,h}$. As a result, the apoptosis rate of *H* becomes larger than its division rate, resulting in its recession. The process continues until tissue *H* completely disappears

- Cell competition

(see 26 Nov 2019)



A. Matamoro-Vidal and R. Levayer *Current Biology* (2019) *29*, R762–R774,

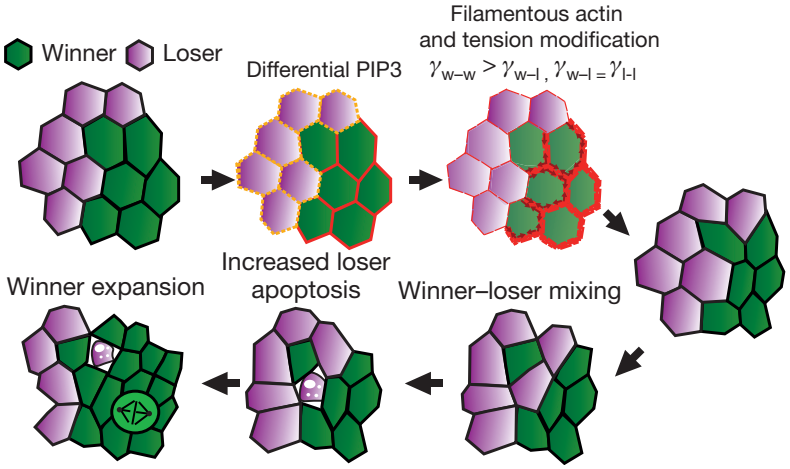
Markus Basan , Thomas Risler , Jean−François Joanny , Xavier Sastre−Garau & Jacques Prost (2009) *HFSP Journal*, 3:4, 265-272, DOI: 10.2976/1.3086732

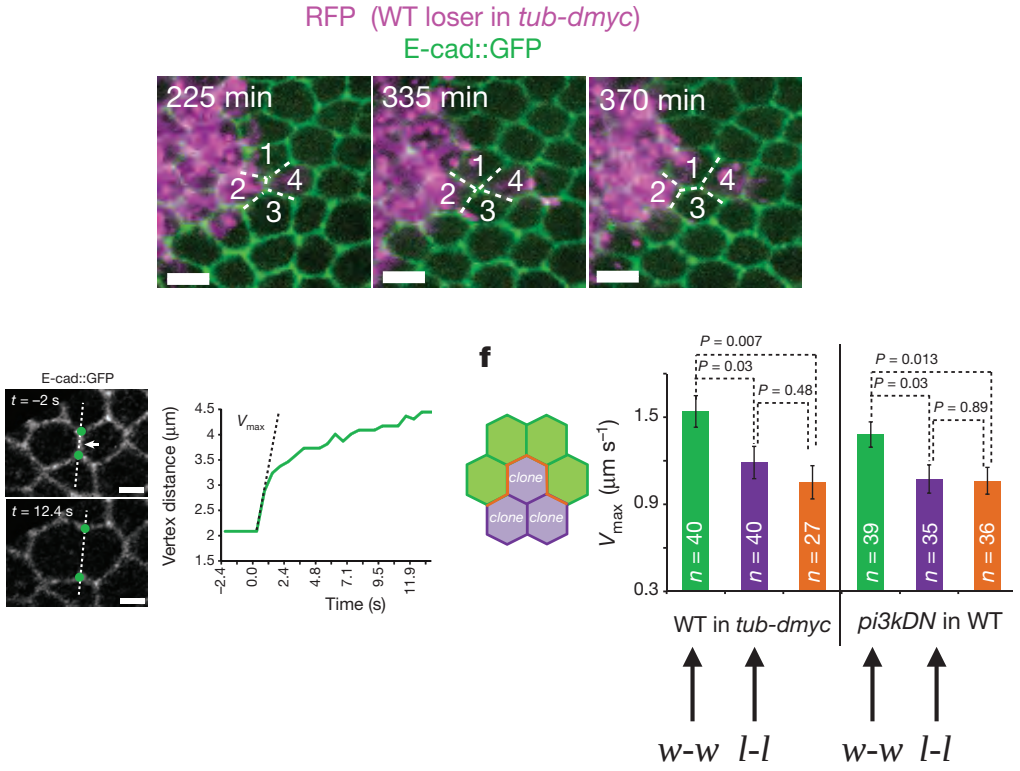
Balancing cell growth vs death by mechanics: competition

—Differential growth by cell competition requires cell intercalation

- *Differential cortical tension* between winner-winner cell interfaces and loser-loser cell interfaces causes cell intercalation at the winner/loser interface, cell isolation and cell death.

- Mechanics (differential cortical tension) drives cell competition and differential growth.

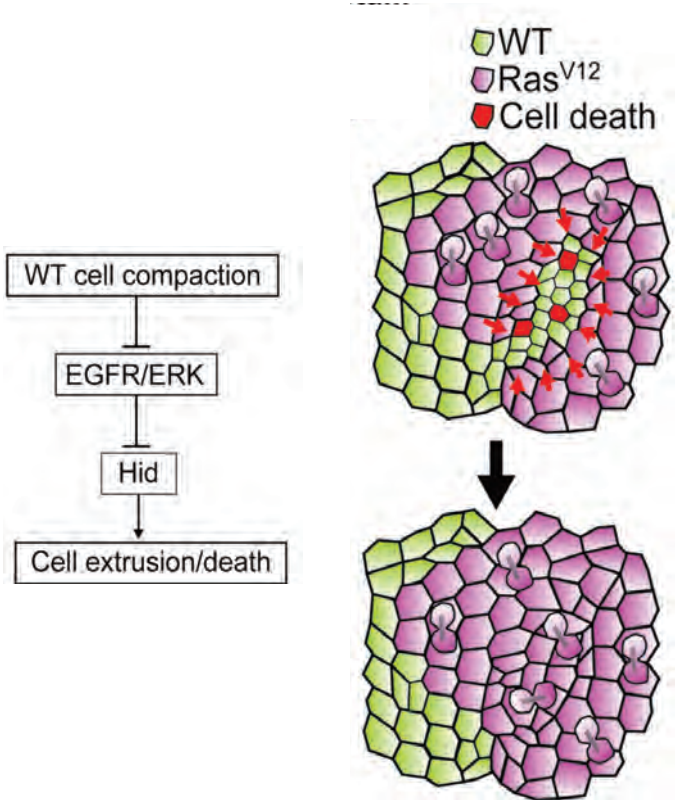




Levayer, R., Hauert, B., and Moreno, E. (2015). *Nature 524*, 476–480.

Balancing cell growth vs death by mechanics: competition

—Differential growth by cell competition requires tissue compaction induced cell delamination

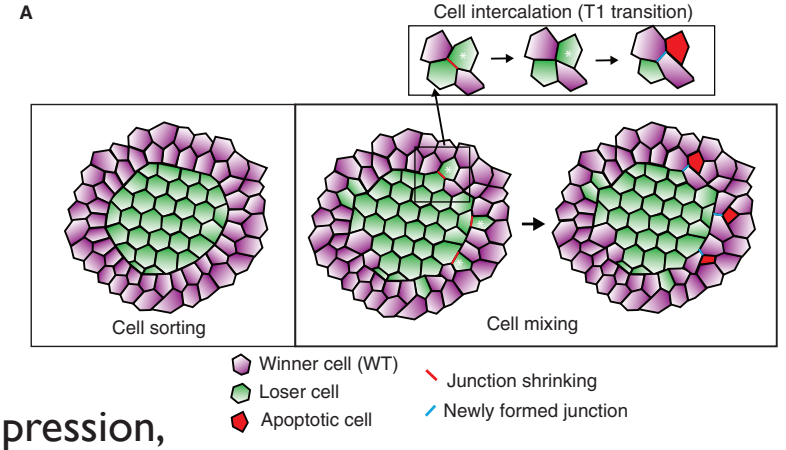


Moreno, E. et al and Levayer, R. (2019). *Current Biology* 29, 23–34

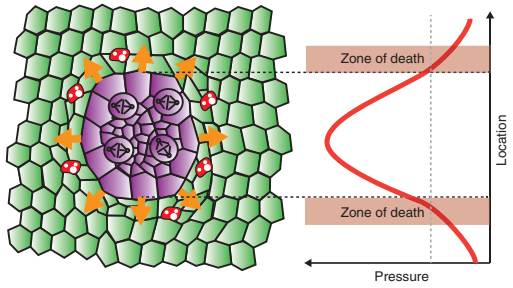
Balancing cell growth vs death by mechanics: competition

Mechanical cues affects cell competition in 3 ways:

- Modulating the geometry of cell signalling leading to loser cell elimination,

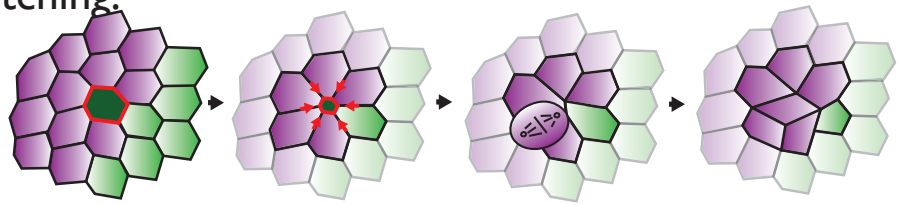


- Inducing cell death by compression,



see B. Shraiman PNAS, (2005) 102:3318–3323

- Stimulating the compensatory growth of winner cells around extruded/dying loser cells via cell stretching.

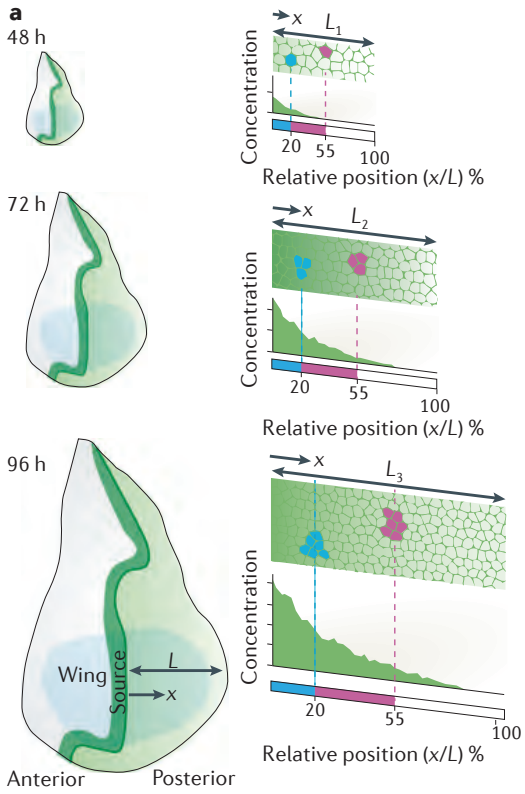


Reviewed in:
A. Matamoro-Vidal and R. Levayer
Current Biology (2019) *29*, R762–R774,
https://doi.org/10.1016/j.cub.2019.06.030

Mechanical regulation of tissue size

- Organs grow until they reach a fixed size characteristic of each organ/species
- Growth is often uniform in the face of non uniform growth factor distribution (morphogens)
- Tissues can compensate for growth heterogeneities and reach a normal final size arguing that cells assess their environment

—How do cells compare their growth rates?
—How is growth uniform on average in a tissue?
—How is tissue size determined?



Mechanical regulation of tissue size

—How do cells compare their growth rates?
—How is growth uniform on average in a tissue?

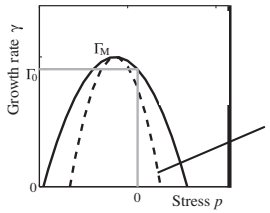
- Mechanical Feedback

- Mechanics of inhomogeneous growth

$$\frac{dp(r,t)}{dt} = \frac{\mu K}{1+\mu}\left[\gamma(r,t) - \langle\gamma(t)\rangle\right]$$

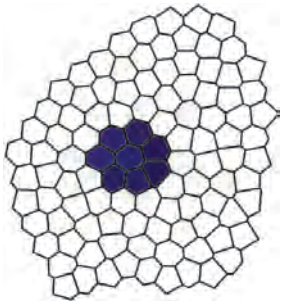
pressure; bulk modulus; growth rate; shear-rigidity

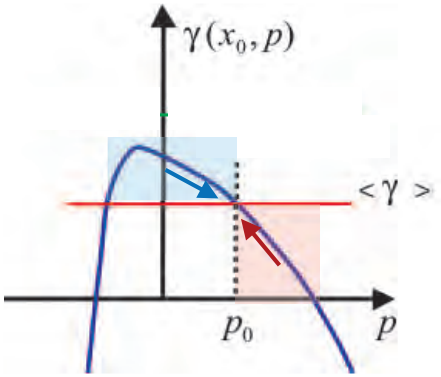
- Negative feedback of pressure on growth

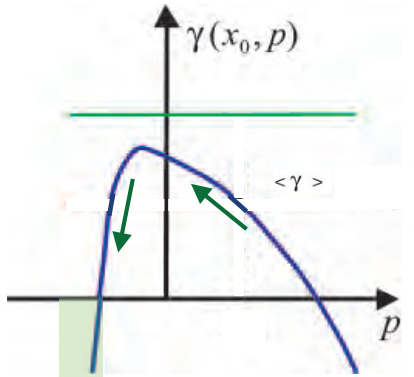


stronger feedback

- Inhomogeneous growth is smoothened through the mechanical negative feedback







If $\gamma(r,t) - \langle\gamma(t)\rangle > 0$, then p increases until p_0 where $\gamma(r,t) - \langle\gamma(t)\rangle = 0$

If $\gamma(r,t) - \langle\gamma(t)\rangle < 0$, then p decreases until p_0 where $\gamma(r,t) - \langle\gamma(t)\rangle = 0$

or until cells die ($\gamma(r,t) < 0$)

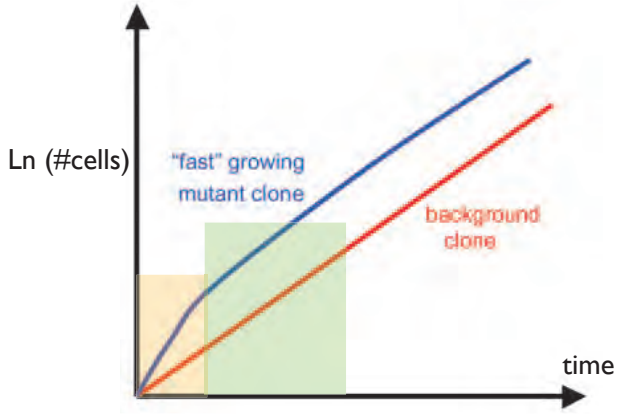
B. Shraiman PNAS, (2005) 102:3318–3323

Mechanical regulation of tissue size

— Regulation as an integral feedback:

$$\frac{dp(r,t)}{dt} = \frac{\mu K}{1+\mu}\left[\gamma(r,t) - \langle\gamma(t)\rangle\right] - \frac{1}{\tau}p(r,t),$$

- Compression or stretching of a group of cells is dependent on integral over the history of the clone of its growth rate with respect to average surrounding growth.
- Cell rearrangements limits the « memory » of this feedback so adaptation of growth rate is not perfect.



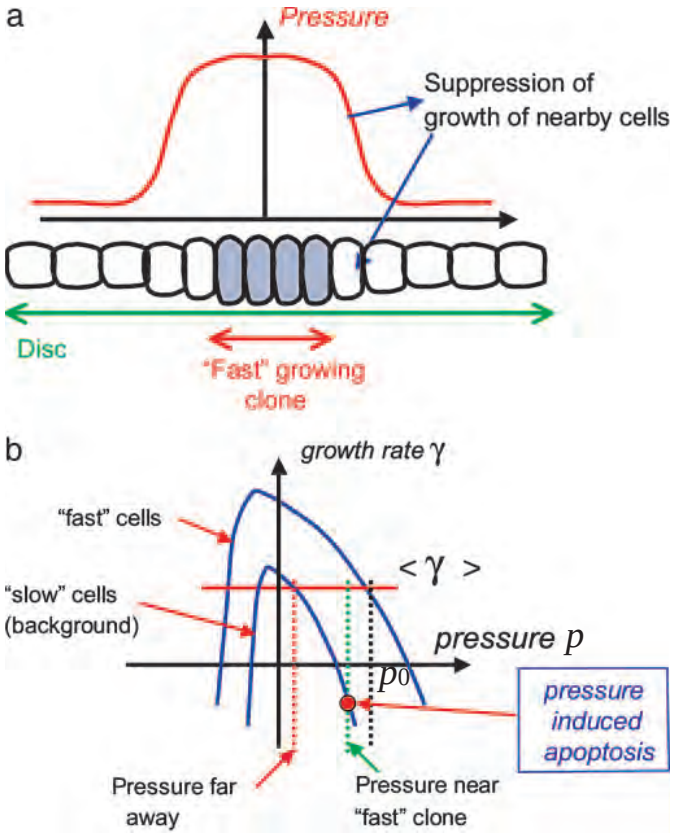
- Initial rate of growth is faster than that of a background clone.
- At later stages, the rate of growth is reduced to match that of the background, resulting in a ratio of mutant to background clone sizes (the overgrowth ratio) independent of time

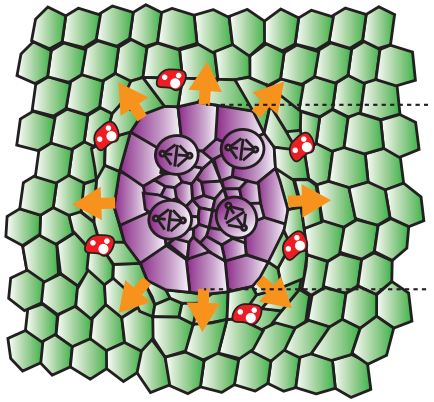
B. Shraiman PNAS, (2005) 102:3318–3323

Mechanical regulation of tissue size

— Non-autonomous effect:
The mechanical feedback as a possible mechanisms underlying cell competition

- Dynamics of a fast-growing clone in a background of slow growing cells
- Fast growing cells increase their pressure p to p_0 and reduce their growth rate until they reach the growth rate of the surrounding
- Neighbouring slow growing cells may die as a consequence of high pressure
- Non-autonomous cell death around fast growing clone



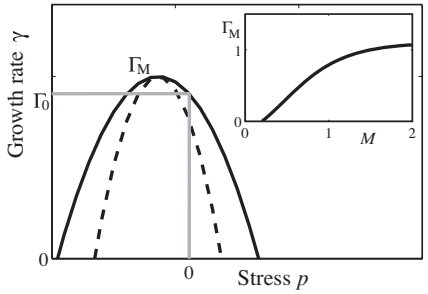


B. Shraiman PNAS, (2005) 102:3318–3323

Mechanical regulation of tissue size

— Integration of morphogen and mechanical feedback

$$\gamma(r,t) = \Gamma(M(r,t), p(r,t))$$



$$M(r) = me^{-r/\lambda}$$

1) growth rate goes down with increasing stress
   ie. negative mechanical feedback.
2) growth rate has a maximum
3) the maximum depends on morphogen concentration above a threshold value M_0
4) the morphogen M does not scale

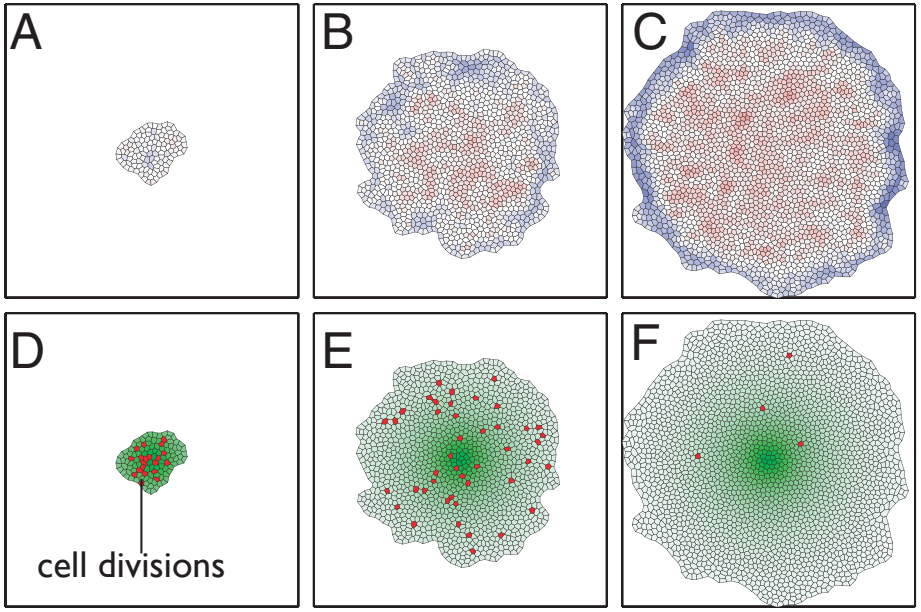
line tension — deviation from rest state — cost for cell height mismatch — deviation from rest state

$$E(r_i, \xi_\alpha) = \sum_\alpha \left[\rho_\alpha + a(V_\alpha - V_0)^2 + b \sum_{\beta=\nu(\alpha)} (\xi_\alpha - \xi_\beta)^2 + c(\xi_\alpha - 1)^2 \right]$$

perimeter — volume — height

Increased pressure reduces perimeter and increases height
Stress p is proportional to $\xi_\alpha - 1$

$\xi_\alpha - 1 > 0$:compression
$\xi_\alpha - 1 < 0$:stretch



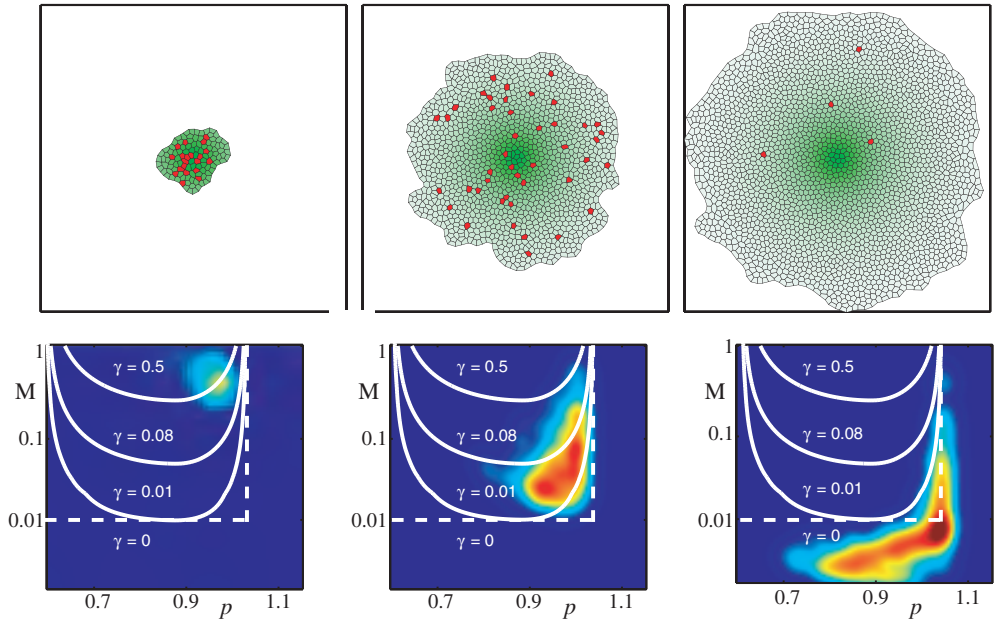
L. Hufnagel et al. and B. Shraiman (2007). *PNAS*. 104:3835–3840
www.pnas.orgcgidoi10.1073pnas.0607134104

Mechanical regulation of tissue size

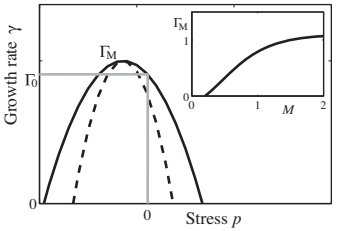
— Integration of morphogen and mechanical feedback

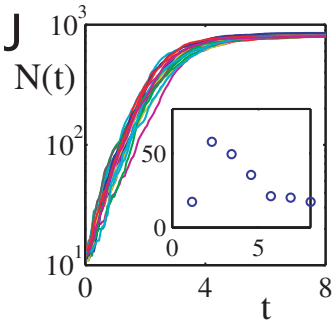
- Growth homogeneity: The mechanical feedback suppresses the effect of the high concentration of the morphogen at the center of the tissue and ensures uniform growth

- Growth arrest: when M falls below threshold at edges, growth arrests there and pressure increases in the bulk thereby causing growth arrest.

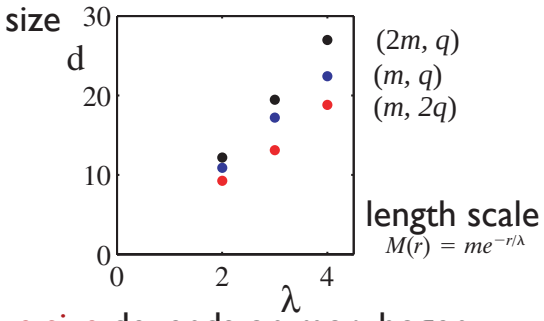


Division follows iso-growth lines in the M, p phase plane





Growth arrests at fixed size



- Tissue size depends on morphogen levels m, and length scale λ but also on feedback strength q

L. Hufnagel et al. and B. Shraiman (2007). *PNAS*. 104:3835–3840
www.pnas.orgcgidoi10.1073pnas.0607134104

Mechanical regulation of tissue size

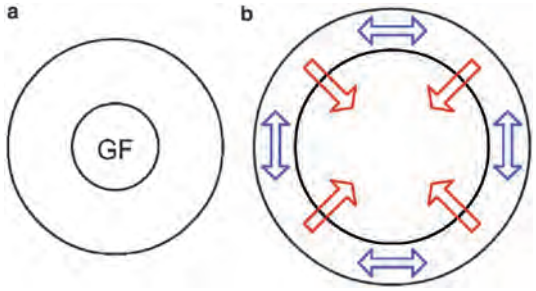
— Integration of morphogen and mechanical feedback

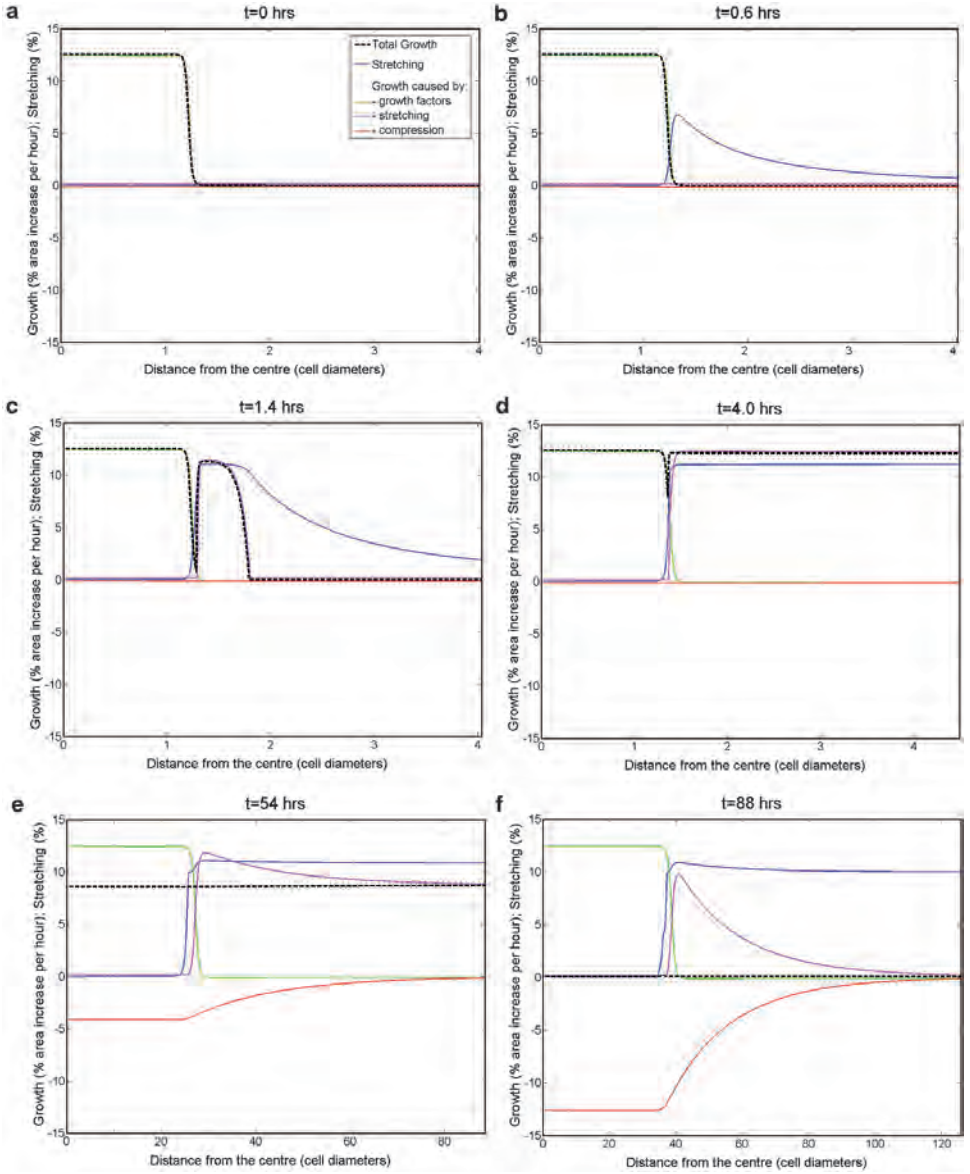
Assumptions:

- Two morphogens that scale with tissue size
- Cell stretching induces growth above a threshold
- No growth if both stretch and morphogen are too low

Results:

- *Initially growth higher in center* due to higher growth factors GF
- As a result *cells are stretched at periphery*, which promotes growth.
- Growth lowers stretching but *cells remain stretched and compress the center*, thereby *reducing growth*.
- The wider the periphery the greater the compression.
- Growth arrests when compression overcomes the effect of growth factors



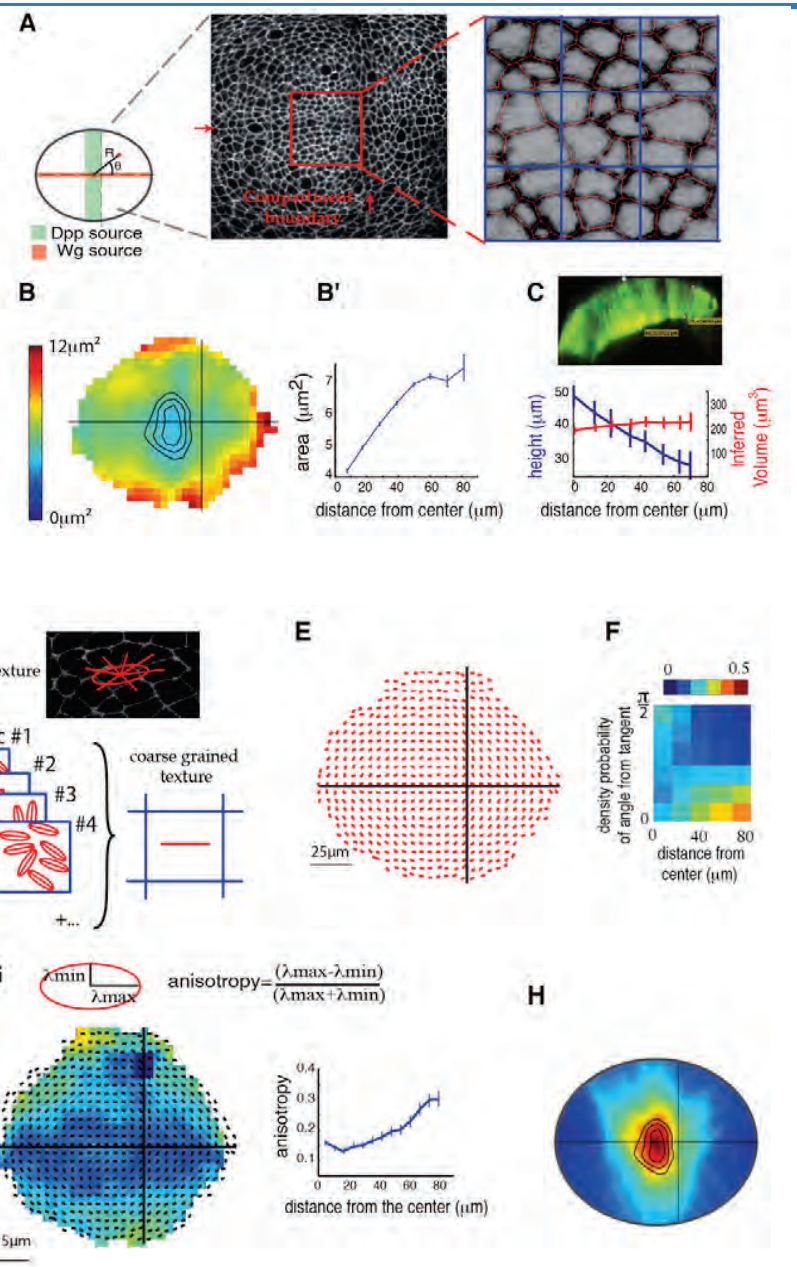


T. Aegerter-Wilmsen et al. *Mechanisms of Development* 124 (2007) 318–326

Mechanical regulation of tissue size

— Pattern of tissue deformations in growing imaginal discs

- Gradient of cell area consistent with compression at the centre.
- Evidence of tissue anisotropy in a growing epithelium at tissue periphery



Texture tensor $M_i = \frac{1}{N_i}\sum_{j=1}^{N_i}\begin{pmatrix} \Delta x_{ij}{}^2 & \Delta x_{ij}\Delta y_{ij} \\ \Delta x_{ij}\Delta y_{ij} & \Delta y_{ij}{}^2 \end{pmatrix}$

Graner, F., et al. (2008). *Eur. Phys. J. E* 25, 349-369.

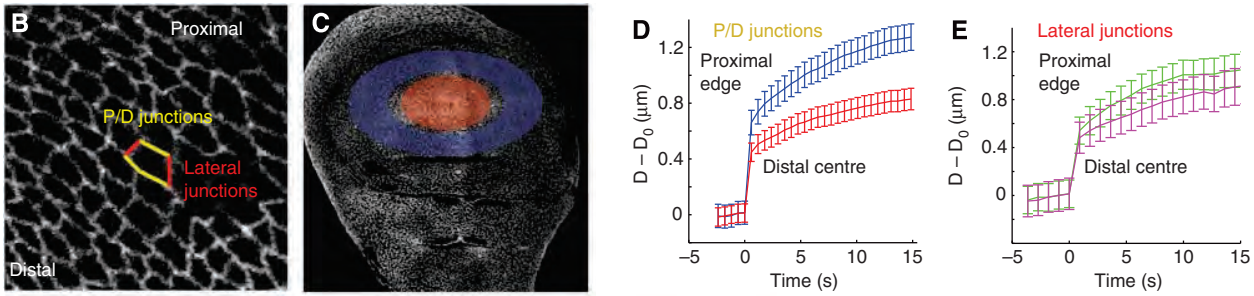
L. Le Goff et al . and T. Lecuit *Development* 140, 4051-4059 (2013) doi:10.1242/dev.090878

Y. Mao et al. and N. Tapon. *The EMBO Journal* (2013) 32, 2790–2803. doi:10.1038/ emboj.2013.197

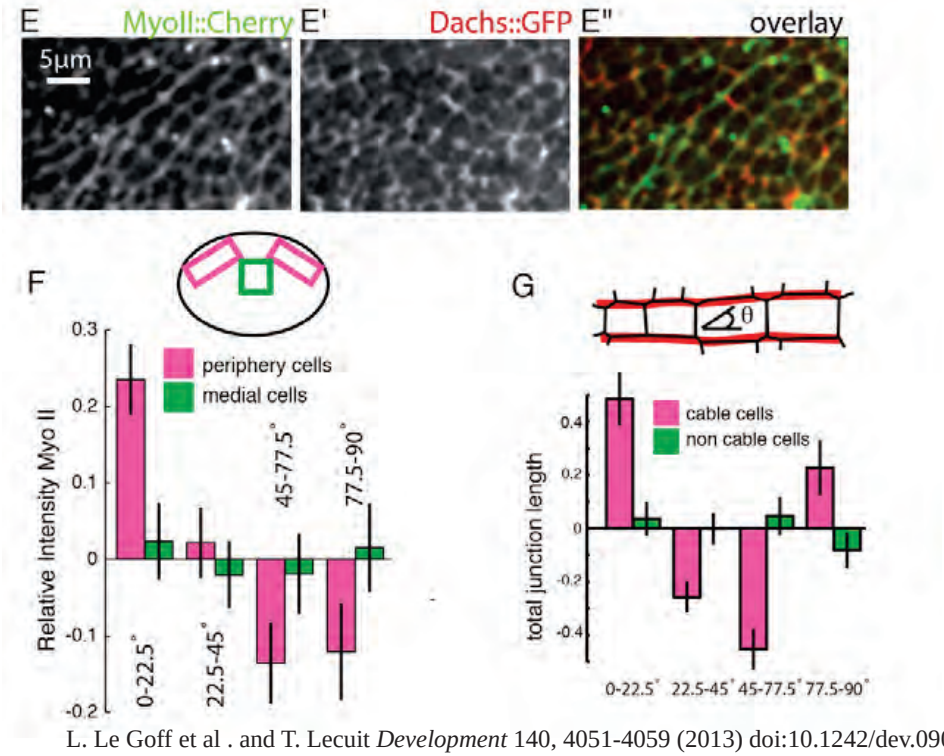
Mechanical regulation of tissue size

— Stress patterns in growing imaginal discs

- Cells are stretched tangentially in the growing tissue
  -laser ablation experiments and relaxation kinetics (related to tension over friction)



- Planar polarised distribution of actomyosin contractile network



L. Le Goff et al . and T. Lecuit *Development* 140, 4051-4059 (2013) doi:10.1242/dev.090878

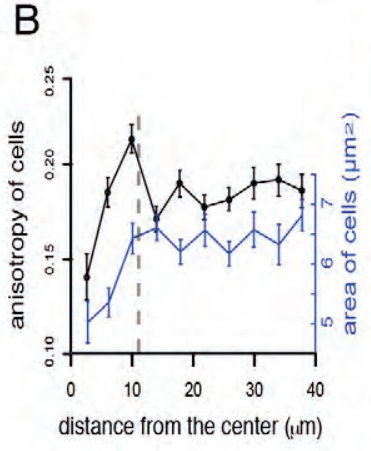
Y. Mao et al. and N. Tapon. *The EMBO Journal* (2013) 32, 2790–2803. doi:10.1038/ emboj.2013.197

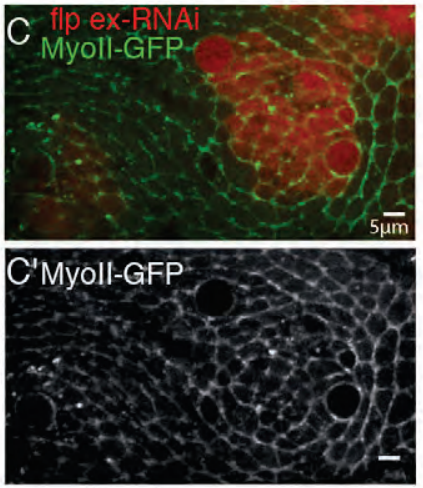
Mechanical regulation of tissue size

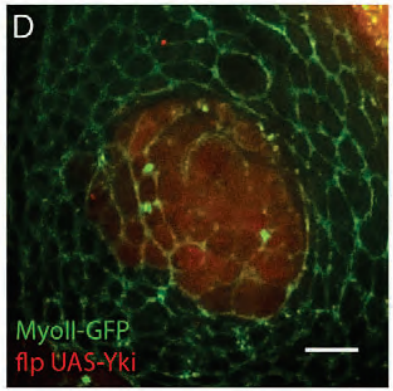
— Stress patterns in growing imaginal discs

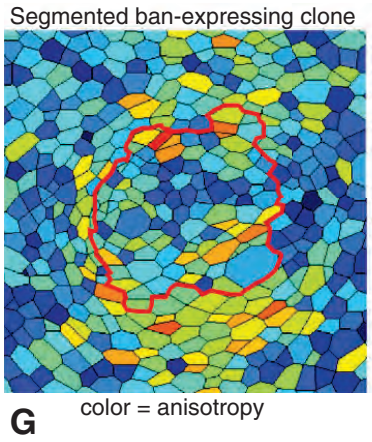
- The induction of a fast-growing clone causes non-autonomous tissue deformation (anisotropy)

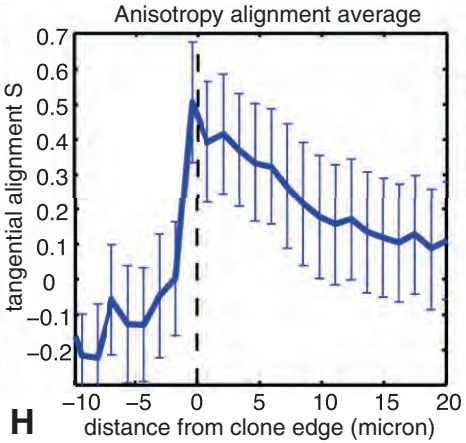
e.g. activation of the Hippo pathway (Yorkie++ or Expanded mutant) or of targets of Hippo pathway (mRNA bantam)











L. Le Goff et al . and T. Lecuit *Development* 140, 4051-4059 (2013) doi:10.1242/dev.090878

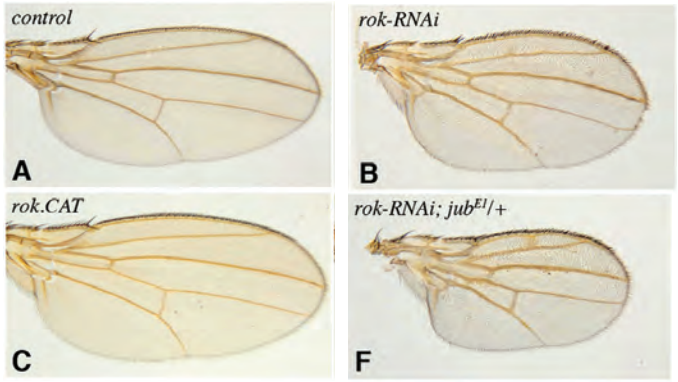
Y. Mao et al. and N. Tapon. *The EMBO Journal* (2013) 32, 2790–2803. doi:10.1038/ emboj.2013.197

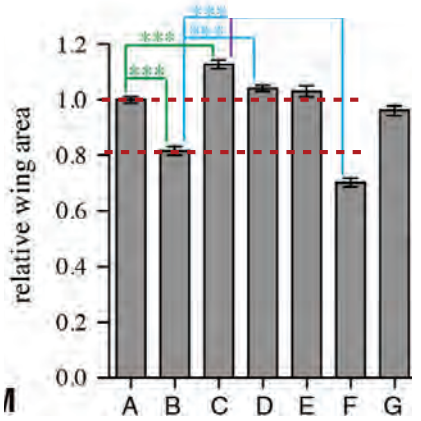
Y. Pan et al and B. Shraiman and K. Irvine. (2016) PNAS www.pnas.org/cgi/doi/10.1073/pnas.1615012113

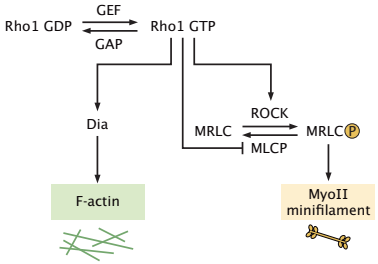
Mechanical regulation of tissue size

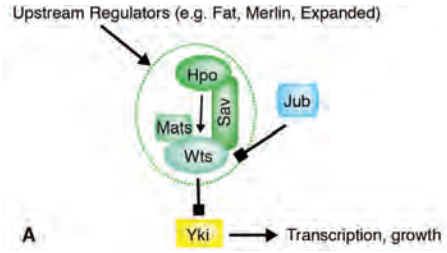
— Cytoskeletal actomyosin tension modulates tissue size

- Activation of Rho1/Rok affects wing size
- Modulates Yorkie (YAP) pathway activity (Expanded expression)
- and Adjuba recruitment at cell junctions

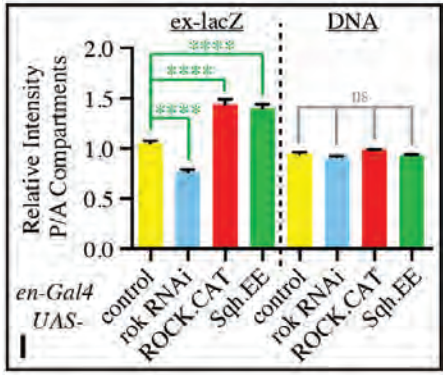


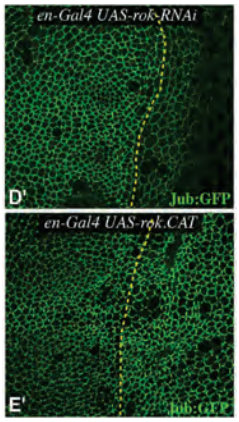


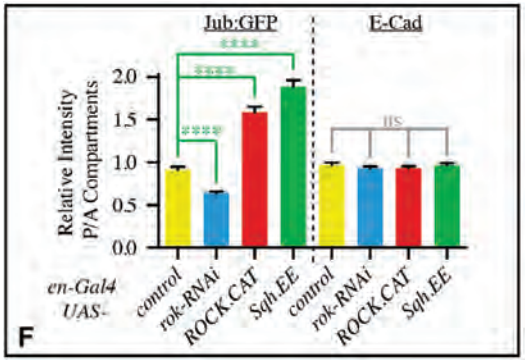




Expanded
Bantam





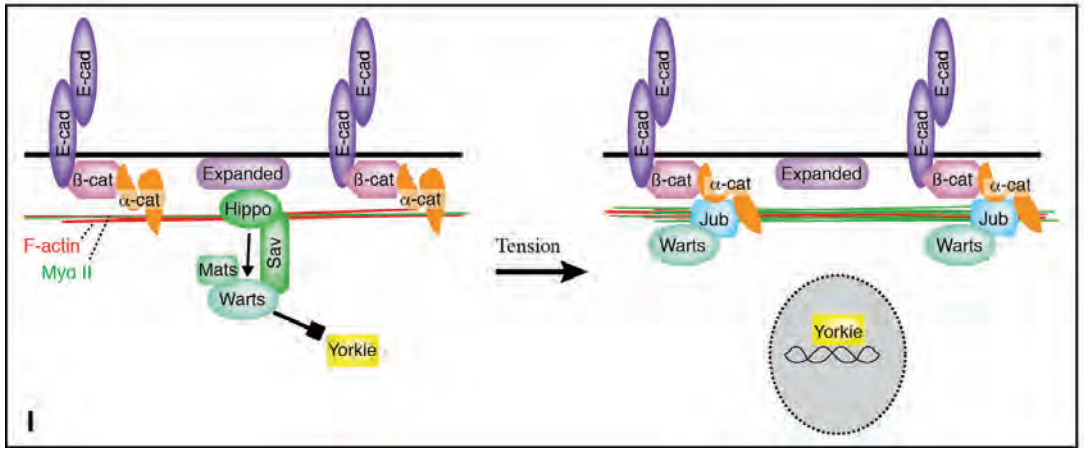


C. Rauskolb et al and K. Irvine. (2014) *Cell 158*, 143–156

Mechanical regulation of tissue size

— Cytoskeletal actomyosin tension increases tissue size via activation of the Hippo growth pathway

— Tension at E-cadherin complexes recruits Adjuba and the kinase Warts at cell junctions and inhibits its activity via « sequestration » at cell cortex.
—This releaves Yorkie repression by Warts and causes Yorkie nuclear translocation, hence activation of the pathway.



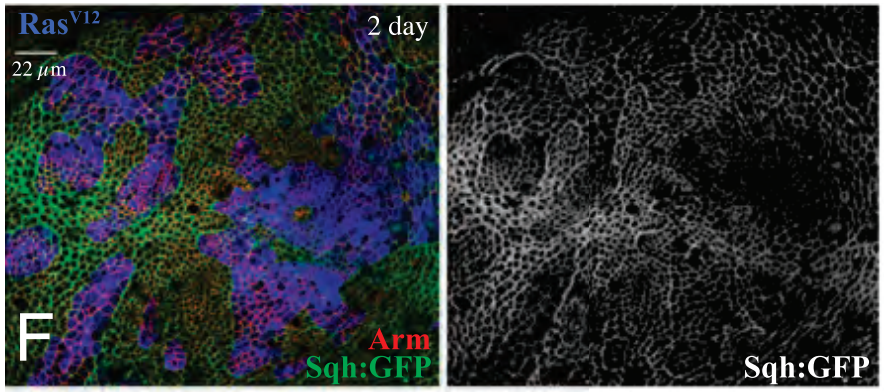
C. Rauskolb et al and K. Irvine. (2014) *Cell 158*, 143–156

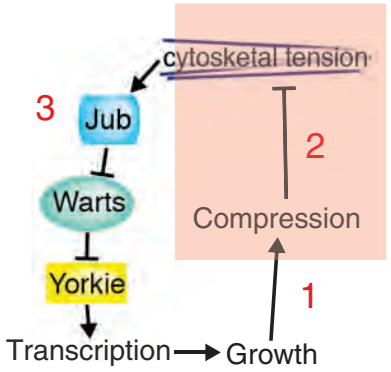
Mechanical regulation of tissue size

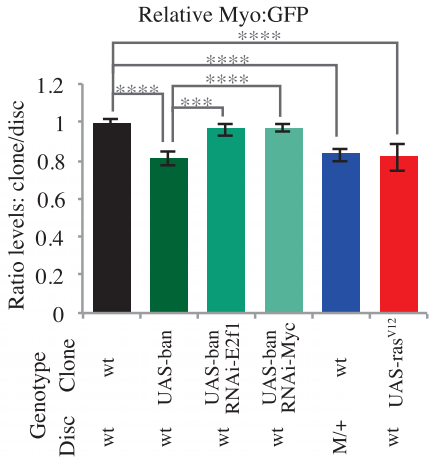
— Tension is reduced in fast growing clones

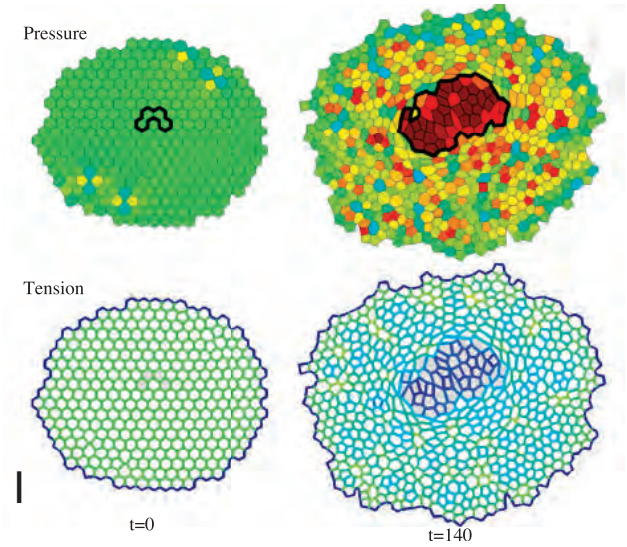
- Mechanical Feedback

1. overgrowth compresses cells;
2. compression decreases cytoskeletal tension (conversely, stretching increases actomyosin network and tension)
3. decrease in cytoskeletal tension triggers reduction in the cortical recruitment of Jub and Wts, and in Yki activity hence on growth.









— This emerges from the mechanical feedback model where mechanical cell response adapts to external mechanical stress pattern.

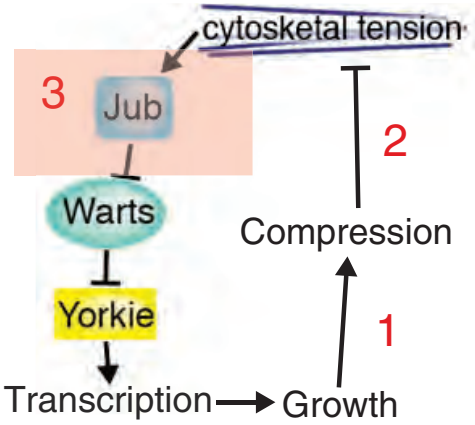
Cell shape reflects balance between edge tension and internal pressure

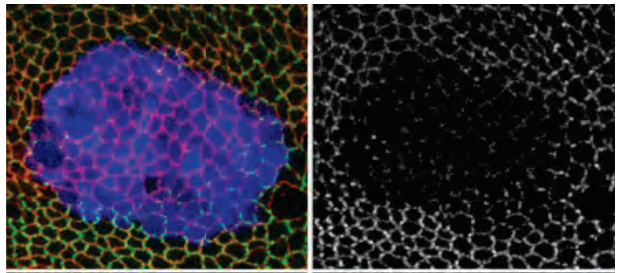
Y. Pan et al and B. Shraiman and K. Irvine. (2016) PNAS www.pnas.org/cgi/doi/10.1073/pnas.1615012113

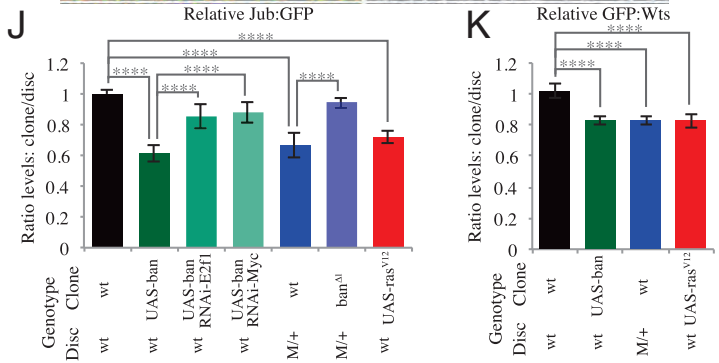
Mechanical regulation of tissue size

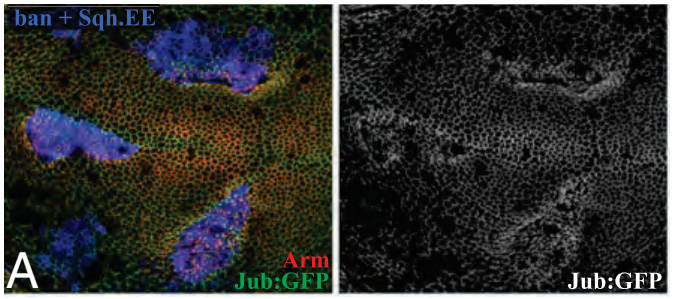
— Adjuba and Warts localization at cell junction are decreased in overgrowing clone of cells

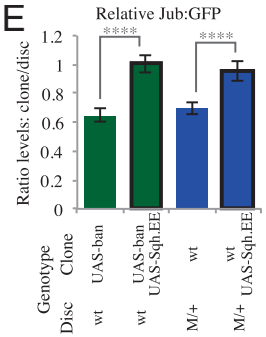
and rescued when Myosin2 is constitutively active (and tension increased in these overgrowing cells)







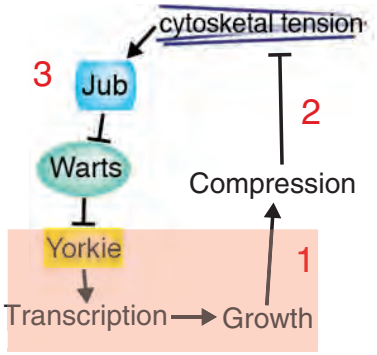


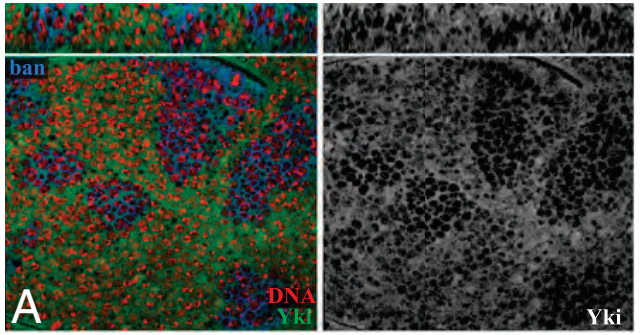


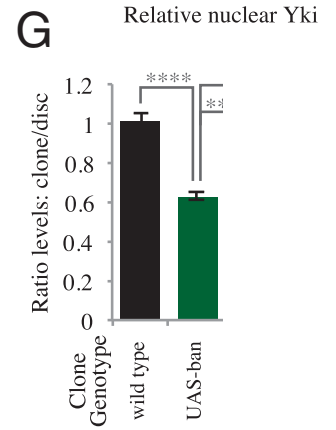
Y. Pan et al and B. Shraiman and K. Irvine. (2016) PNAS www.pnas.org/cgi/doi/10.1073/pnas.1615012113

Mechanical regulation of tissue size

— Yorkie induced overgrowth downregulates Yorkie activity (evidence of negative feedback)

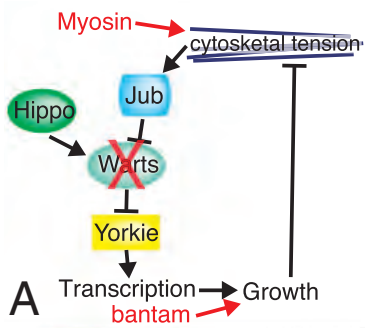


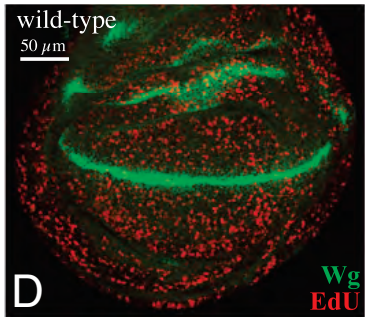


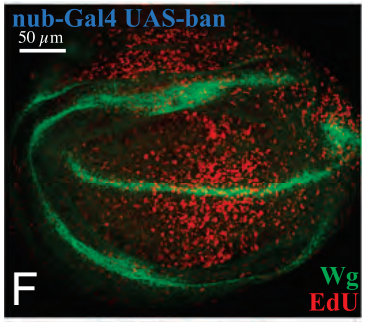


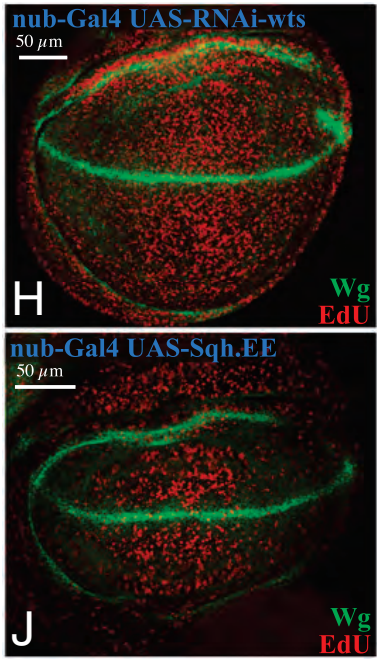
— Blocking the feedback affects causes growth heterogeneity

- Forcing Bantam expression independent of mechanical feedback
- Removing Warts so it cannot be subjected to feedback regulation
- Forcing Myosin2 constitutively









Y. Pan et al and B. Shraiman and K. Irvine. (2016) PNAS www.pnas.org/cgi/doi/10.1073/pnas.1615012113

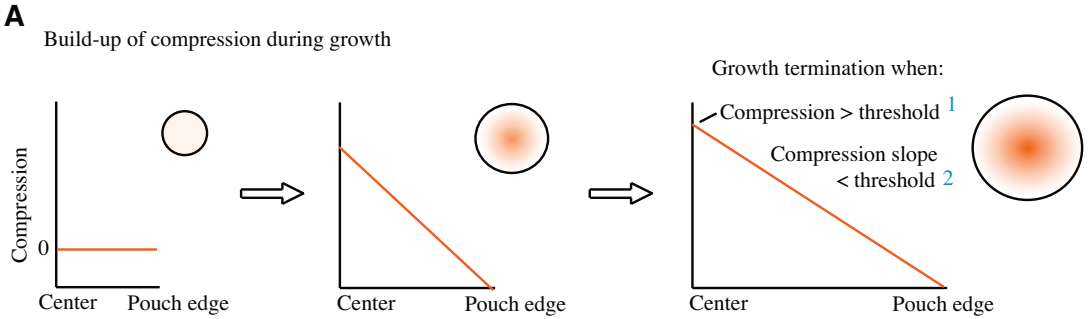
Mechanical regulation of tissue size

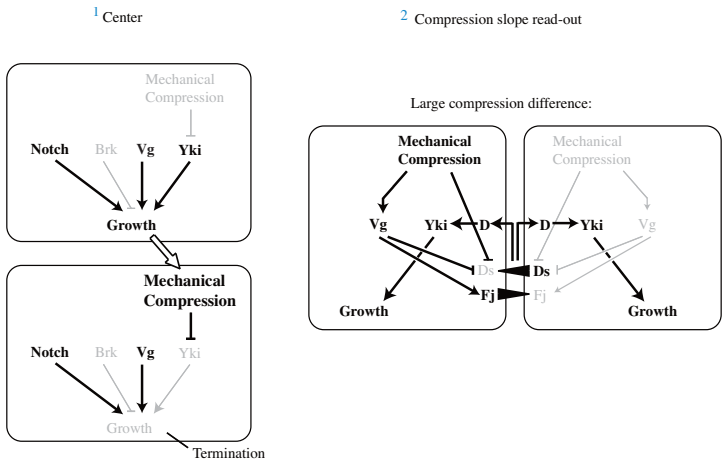
— Integration of chemical and mechanical signalling

Feedback mechanisms responsible for uniform growth:

-increased growth leads to increased compression, which decreases growth,

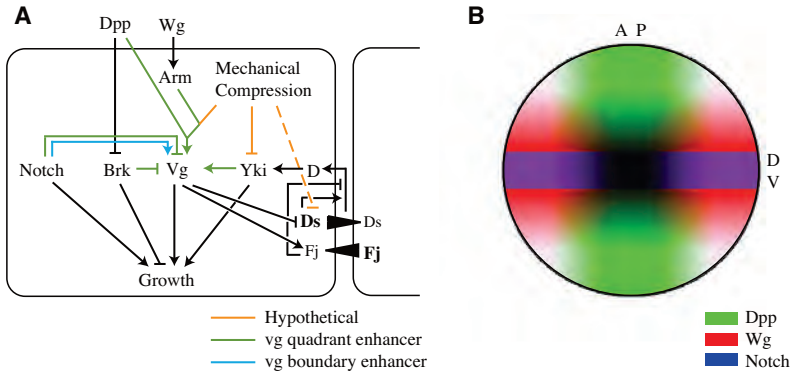
-it leads to a compression gradient that induces growth in regions with lower growth rates





- vertex model with cell shape governed by

$$E(R_i) = \sum_{\alpha} \frac{K\alpha}{2} * (A_\alpha - A_\alpha^{(0)})^2 + \sum_{<i,j>} \Lambda_{ij} l_{ij} + \sum_{\alpha} \frac{\Gamma\alpha}{2} * L_\alpha^2 \ .$$



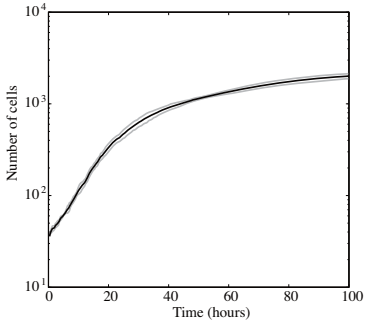
- Growth rate $G_\alpha = (cg1 + cg2*[Yki]_\alpha + cg3*[Vg]_\alpha + cg4*[N]_\alpha - cg5*[Brk]_\alpha)*cg6$

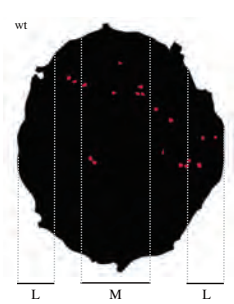
Tinri Aegerter-Wilmsen et al. and C. Aegerter and K. Basler. *Development* 139, 3221-3231 (2012) doi:10.1242/dev.082800

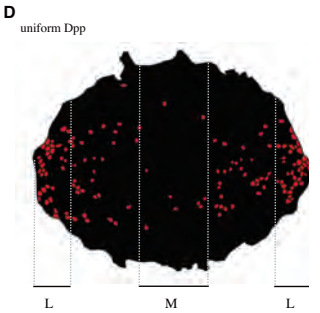
Mechanical regulation of tissue size

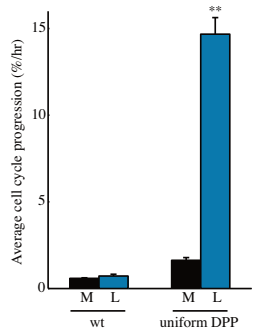
— Integration of chemical and mechanical signalling

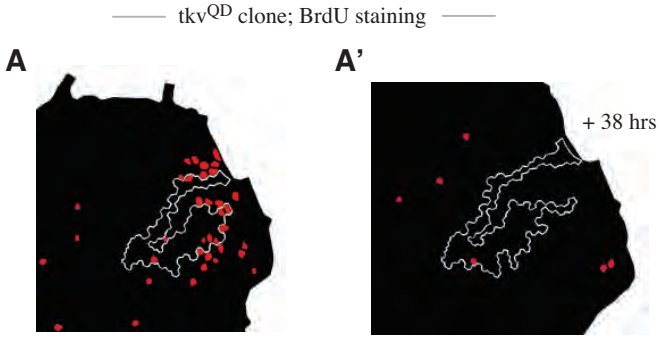
Non-trivial model predictions:











Tinri Aegerter-Wilmsen et al. and C. Aegerter and K. Basler. *Development* 139, 3221-3231 (2012) doi:10.1242/dev.082800

Mechanical regulation of tissue size

Mechanical feedback:

- corrects the consequence of non uniform growth rates, and smoothens out heterogeneities: growth homogeneity.
- may be involved in compensatory mechanisms (eg. cell competition) in the context of tissue growth and homeostasis.
- part of growth arrest mechanisms at the scale of organs.

Yet, tissue growth can be highly heterogeneous.

- normal development: growth zone (limb bud, beak morphogenesis), tissue folding.
- disease: tumour evolution.

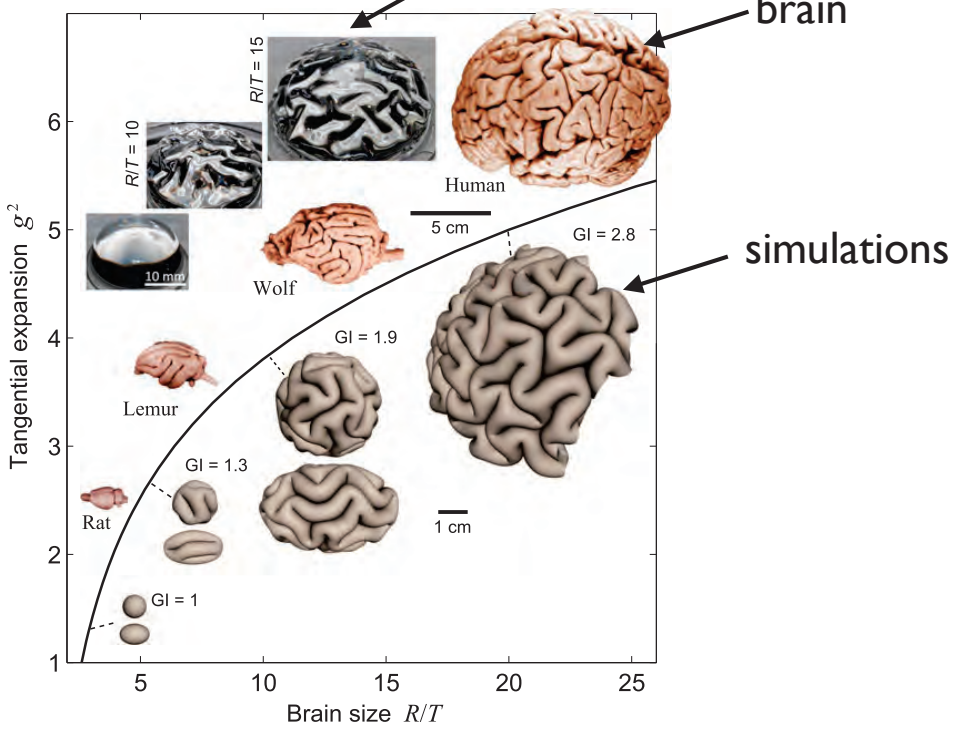
Differential growth and tissue folding

- Cortex convolutions

physical mimic of brain

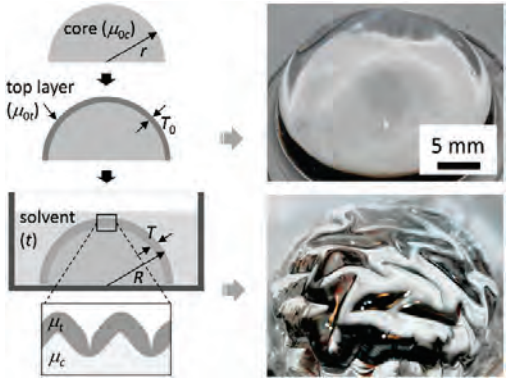
brain

simulations



GI: gyration index (total area/exposed area)

physical model of brain like instability



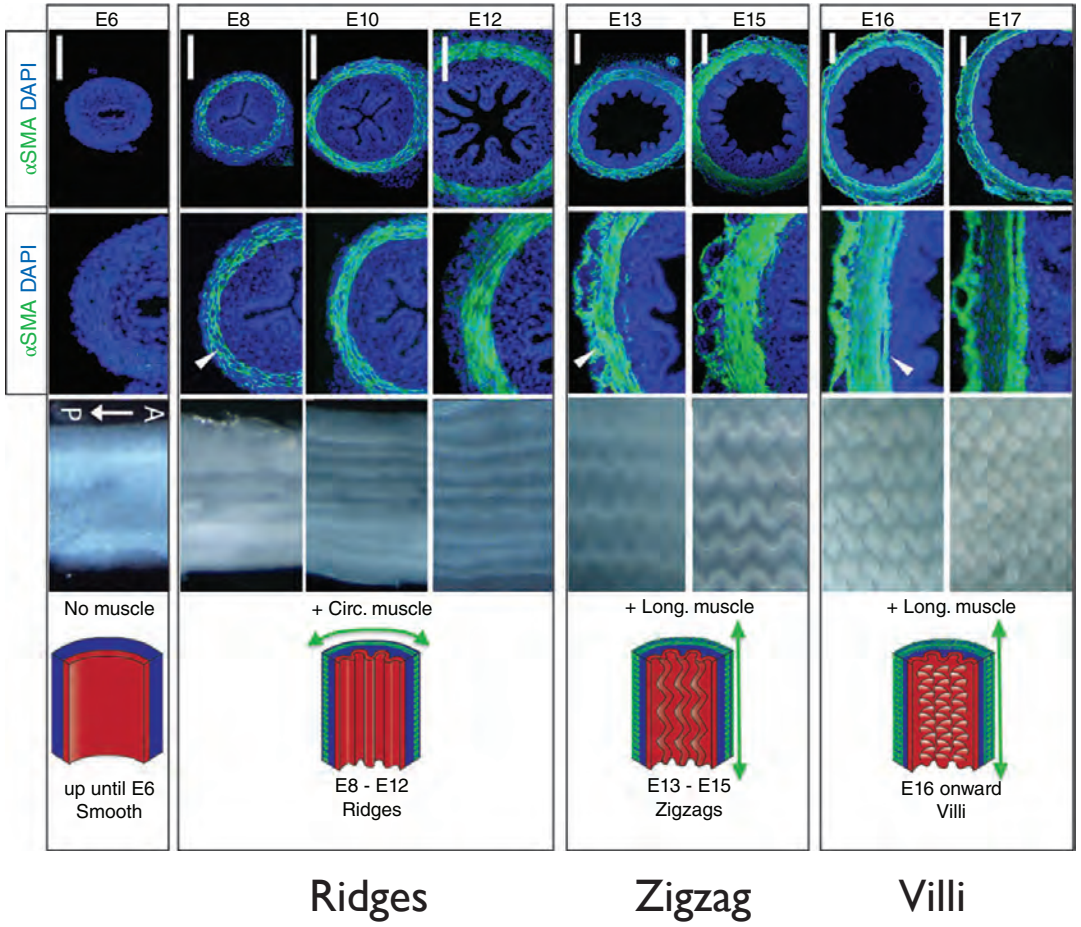
swelling of outer layer (growth)

T. Tallinen et al and L. Mahadevan. *PNAS* (2014) 111:12667

Differential growth and tissue folding

- Gut vilification:

- Epithelium and mesenchyme form annealed surfaces ensheathed by sequential layers of smooth muscles
- Formation of **Ridges** in epithelium and mesenchyme parallel formation of circumferential smooth muscles
- Formation of **Zigzags** parallels formation of longitudinal muscles
- Formation of **Villi** formation of longitudinal muscles



A. Shyer et al, C. Tabin and L. Mahadevan. *Science* 342: 212-218 (2013)

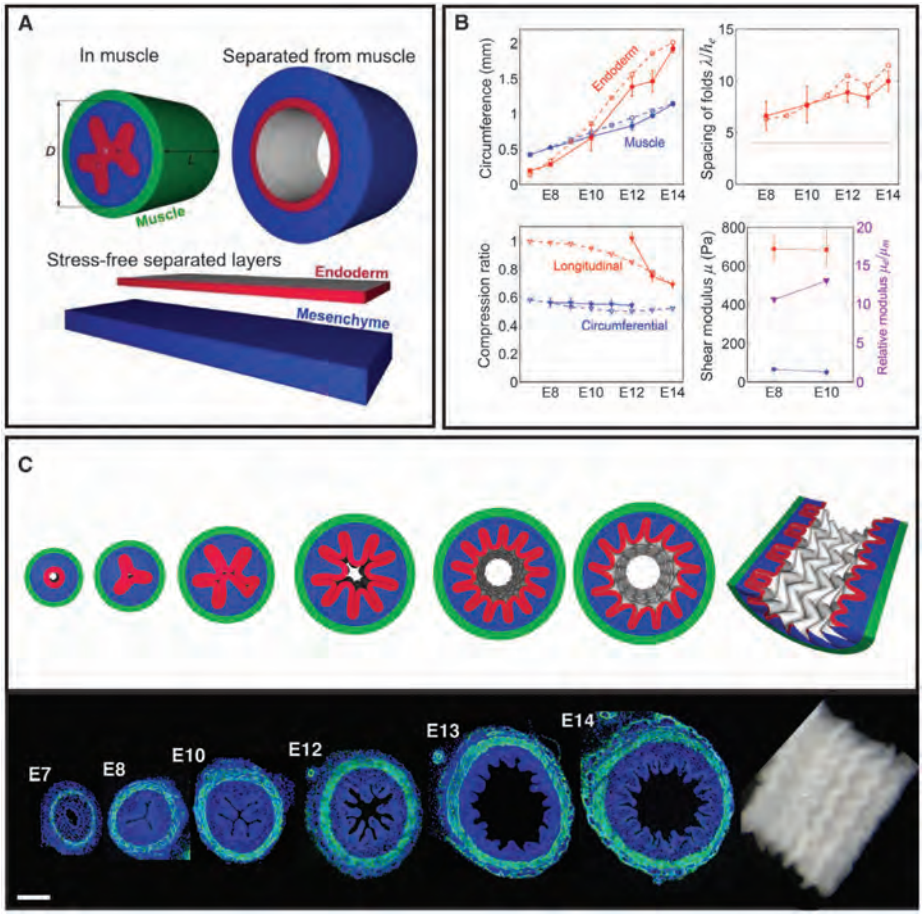
Growth induced mechanical instabilities: Gut vilification

- Gut vilification

—Theoretical studies: E. Hannezo J. Prost and J-F. Joanny *PRL* 107, 078104 (2011)

M. Ben Amar and F. Jia. *PNAS* 110: 10525–10530 (2013)

—Computation model A. Shyer et al, C. Tabin and L. Mahadevan. *Science* 342: 212-218 (2013)



Summary

Mechanical regulation of cell division and cell death/extrusion

- Cells respond differently to compressive and tensile stresses:
- Cell growth, cell division, cell extrusion or simple cell shape changes, cell intercalation.
- These processes underlie viscoelastic properties of tissues: energy dissipation in homeostatic conditions
- Cell mechanics underlies tissue homeostatic pressure.
- Not well understood what factors determine these properties

- Sensing: mechanosensitive receptors (Piezo), cortical tension and YAP pathway
- Cellular response: mechanics of junction remodelling (see Courses 2018)

Tissue growth and mechanical feedback:

- corrects the consequence of non uniform growth rates, and smoothens out heterogeneities: growth homogeneity.
- may be involved in compensatory mechanisms (eg. cell competition) in the context of tissue growth and homeostasis.
- required for growth arrest at the scale of organs.