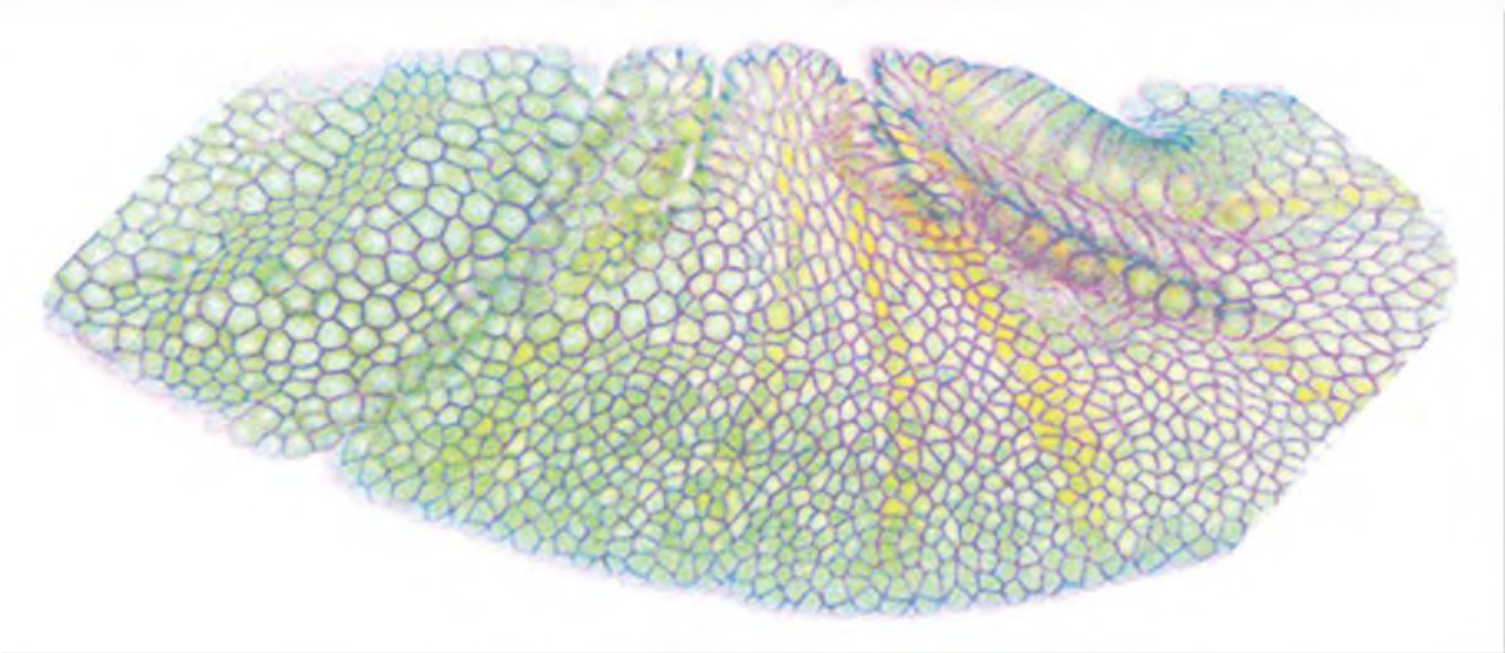
Mechanics of Morphogenesis



Lecture 6: Cell tension - cortical tension

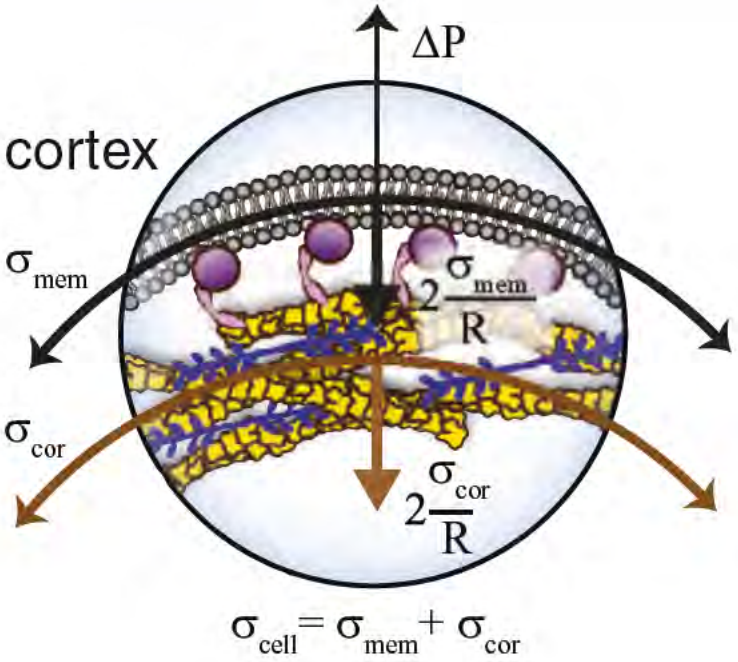
Thomas Lecuit
chaire: Dynamiques du vivant

COLLÈGE DE FRANCE
1530

Membrane Tension: Conclusions

- Membrane is a composite material comprising a lipid bilayer coupled dynamically to an actin rich cortex.
- The effective membrane tension reflects contributions of in-plane tension and adhesion between the cortex and the membrane
- The membrane is an inelastic fluid so membrane tension can, in some conditions, propagate mechanical information nearly instantly across a cell
- Coupling between membrane tension and actin turnover can determine cell shape and cell motility (keratocytes).
- Membrane availability and possibility to change surface can tune membrane tension: but this is a slow process
- In general coupling between the membrane and actin cortex is the main contributor of effective membrane tension (but not always: keratocytes…)
- Dynamic coupling between actin and membrane tunes effective viscosity associated with membrane flows.

Membrane vs Cortical Tension



- Membrane is not permeable and bears most of pressure difference

$$\Delta P = \sigma_{\mathrm{mem}} C + f_{\mathrm{link}} \rho_{\mathrm{link}}$$

ρ_{link} : density of linkers

f_{link} : force per membrane/cortex linker

- Linkers are under tension due to cortical tension

$$f_{\mathrm{link}} \rho_{\mathrm{link}} = \sigma_{\mathrm{cor}} C$$

>> Cell tension contributed by both membrane and cortex tension

$$\Delta P = (\sigma_{\mathrm{mem}} + \sigma_{\mathrm{cor}}) C$$

- Cell tension = Membrane tension + Cortical tension

Sens P and Plastino J. *J. Phys.: Condens. Matter.* 27:273103. 2015

Early description of cell cortex mechanics

- **Tension of outer layer (cortex)**
  - *viscous, contractile gel layer* characterised by WH. Lewis: plasmagel

Lewis WH, *Am. J. Cancer.* 35:408-415. 1939
Contorted mitosis and the superficial plasma gel layer.

Review:
The relation of the viscosity changes of protoplasm to ameboid locomotion and cell division.
In: *A Symposium on the Structure of Protoplasm*, ed. by W. Seifritz, pp. 163-197. 1942

Observed and characterised pinocytosis and cell locomotion: endorsed the theory developed by Samuel O. Mast that cells contain a plasmasol surrounded by plasmagel and that cells bleb by imbibition of fluid by plasmasol that stretches the plasmagel.

Proposed that gel has inherent contractility

Applied this to explain cell locomotion (ameba, ameboid, slime mold) and cell division

SUMMARY

The assumption that cells possess a superficial layer of gelated cytoplasm (plasmagel layer) which automatically exerts continuous contractile tension and that this layer undergoes various local and general changes in viscosity and thickness with corresponding variations in its contractile tension, offers a key to one of the important factors concerned in changes of cell form, in cell locomotion, and in cell division.

The viscosity of protoplasm is often readily changeable from sol to gel, gel to sol, and to various intermediate states by unknown internal factors and a few known external ones. The contractile tension which protoplasm exerts varies more or less with its viscosity. The contractile tensions exerted by protoplasm play important roles in the activities of cells and organisms, some of which are to be considered in the following pages.

  - elastic coat characterised by J. Holtfreter

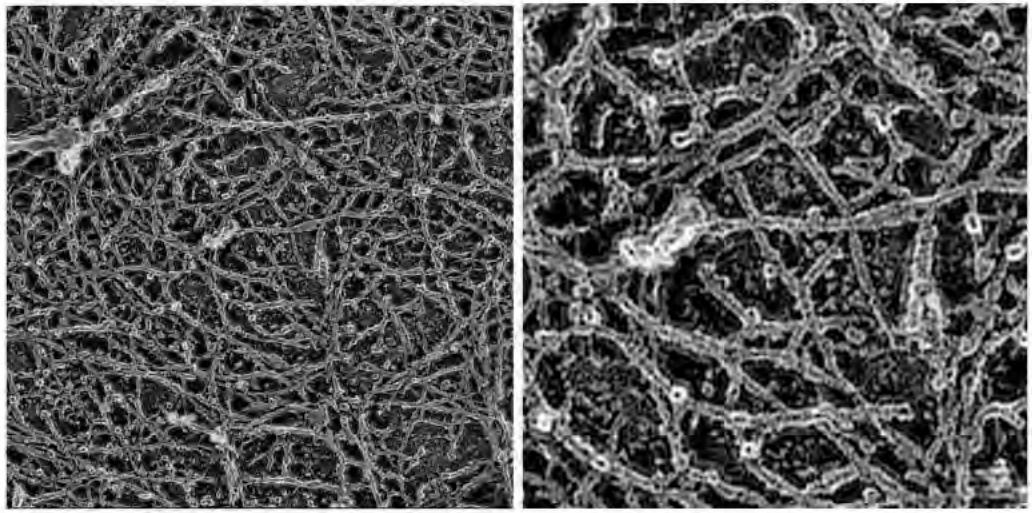
Properties and functions of the surface coat in Amphibian embryos
J. Exp. Zool., 93:251-323.

Contractile, supracellular, contains protein fibrillary structure.
Participates in cellular wound healing and multicellular wound healing.
Describes elasticity of this material.

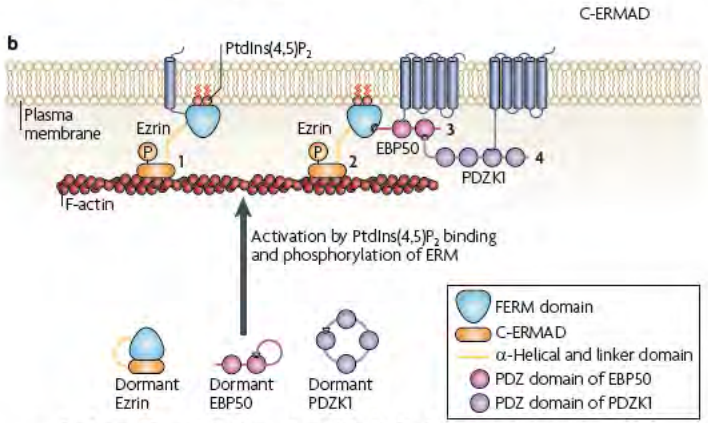
Mechanics of the cell cortex

Cortical actin network organisation

- Actin filaments in tight contact with membrane via lipid-actin binders (e.g. ERM proteins)
- 20-250nm thick cortex of isotropic meshwork



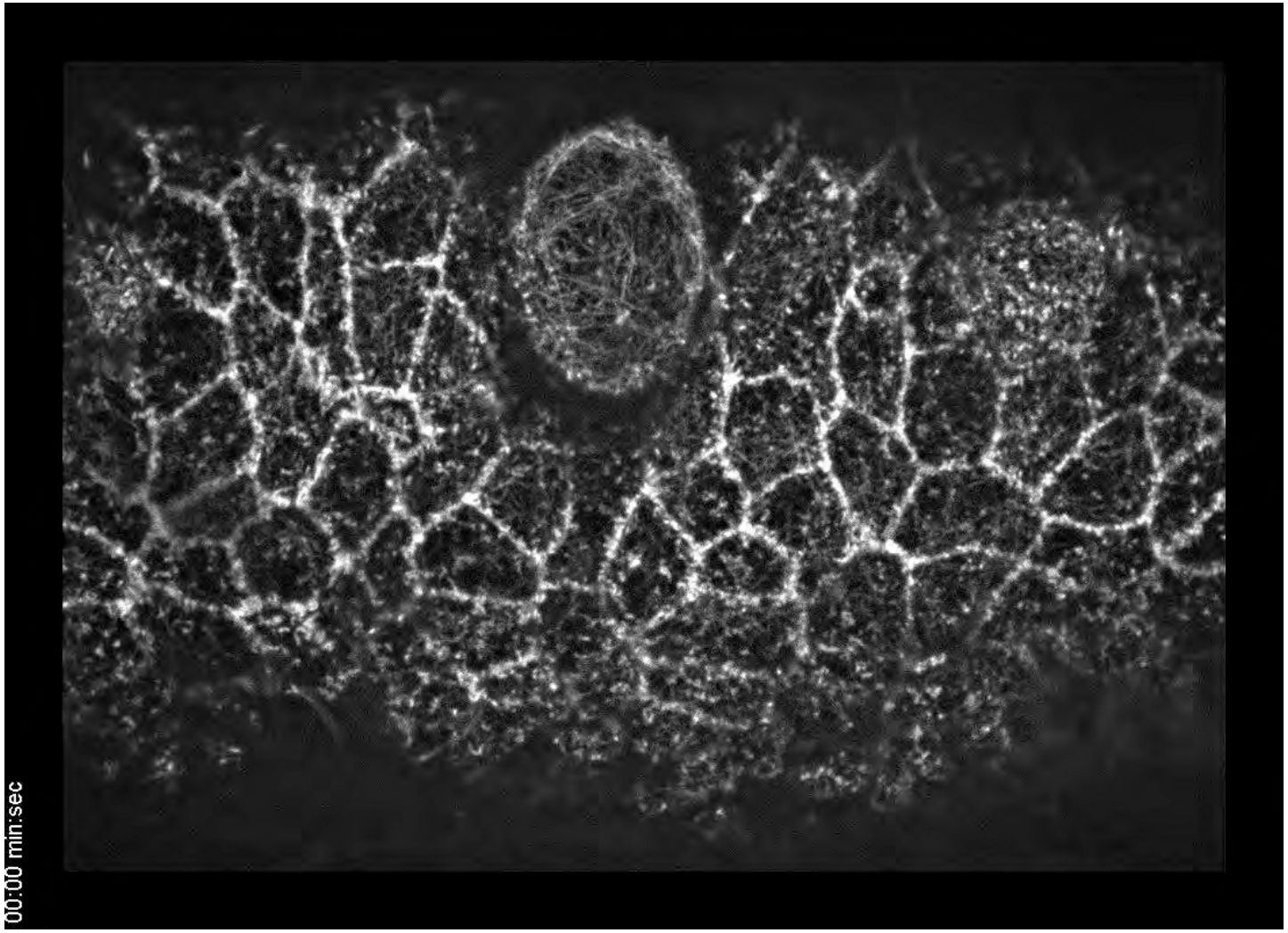
Morone N et al, and Kusumi A. *J. Cell Biol*. 174:851. 2006



Fehon R. et al *Nat Rev Mol. Cell Biol*. 11:276. 2010

Mechanics of the cell cortex

Cortical actin network dynamics



F-actin labelled with UtrophinABD::GFP (Benoit Dehapiot, T. Lecuit)

Drosophila embryo epithelial cells

Mechanics of the cell cortex

• Cortex Viscoelastic properties

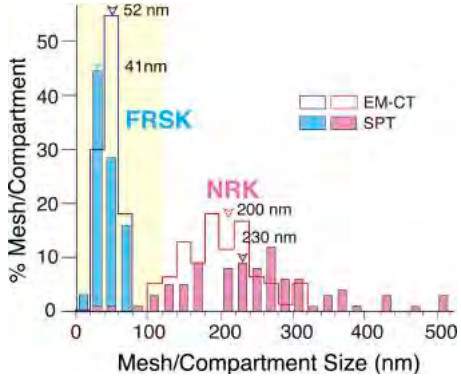
- Mechanics dependent on:
  - Single filaments mechanics : semi flexible polymers

    Persistence length $l_p = B/k_BT \approx 8\text{-}17\mu m$ so filaments are not bend strongly by thermal agitation

    Bending modulus small: contributes to filament mechanics and is modified by Cofilin

    actin: $0.040\ pN/\mu m^2$ Cofilin + actin: $0.0091\ pN/\mu m^2$

    McCullough BR, Blanchoin L, Martiel JL, De la Cruz EM. *J Mol Biol*. 381(3):550-8; 2008
  - Filaments length distribution
  - Filaments turnover: half-time 10-50s
  - Geometrical arrangement in network: mesh size 50-250nm
  - Molecular interactions kinetics (crosslinkers): half-time <1s-few s



- Contractility:
  - Molecular motors: Myosins: Active semi flexible polymer gel

Morone N et al, and Kusumi A. *J. Cell Biol*. 174:851. 2006

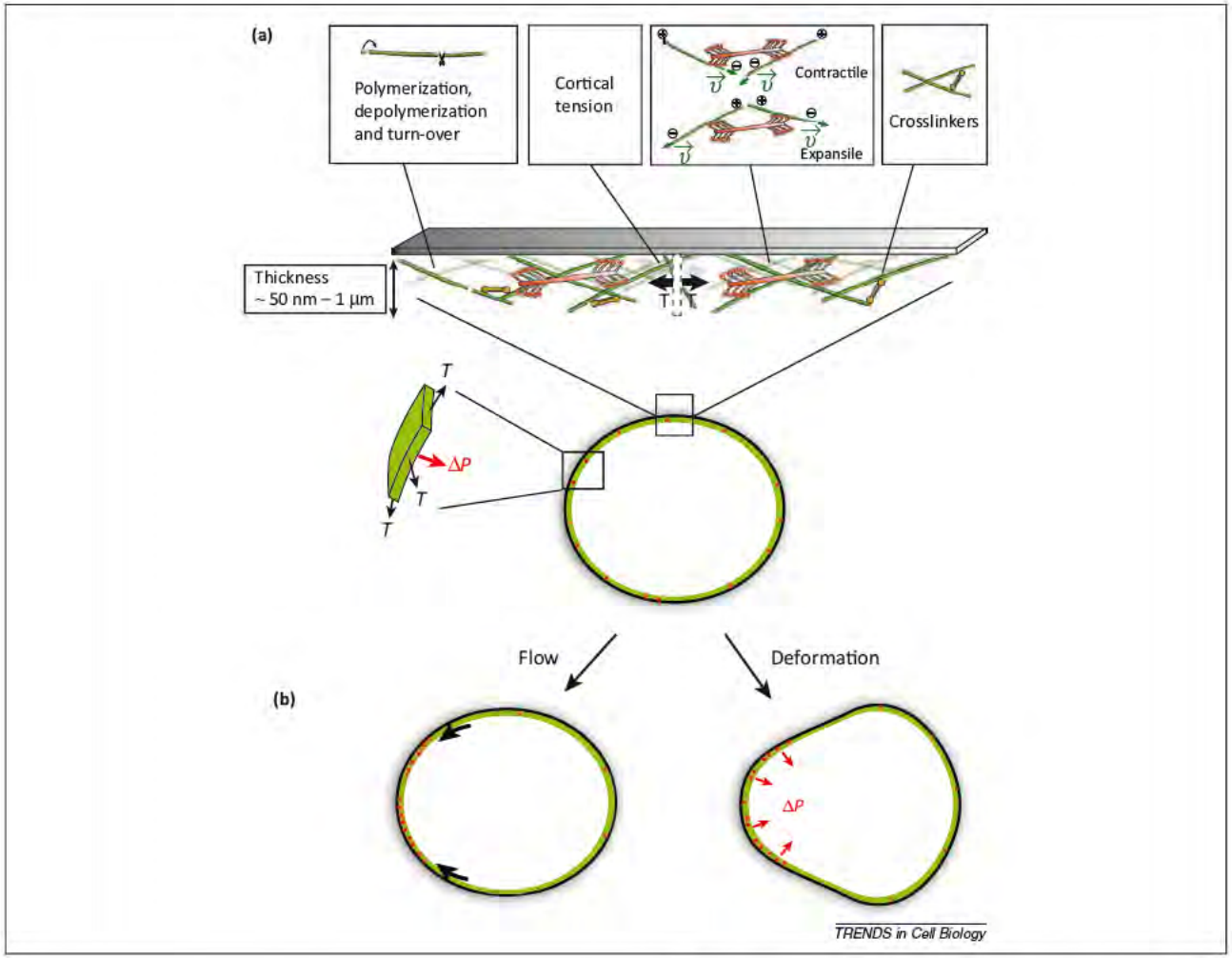
- Viscoelastic properties:
  - Elastic on short time scales (seconds) and fluid like on longer timescales (flow, creep etc)
  - Strain stiffening: in response to external sheer or internal contractile stress.
  - Fluidisation through molecular motors

Humphrey D et al Käs J. *Nature*. 416:413. 2002

Le Goff L, Amblard F, Furst EM. *Phys Rev Lett*. 88(1):018101. 2002

Mechanics of the cell cortex

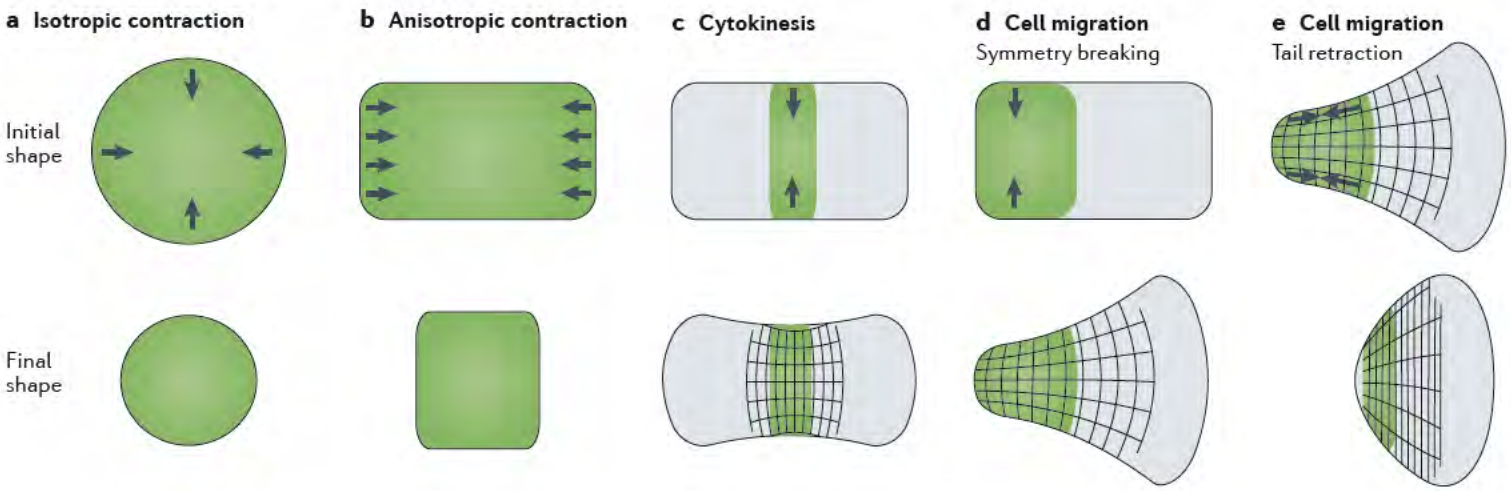
Coarse-grained descriptions of the actomyosin cortex
>>modelling of cortical actomyosin flows



Review: Salbreux G, Charras G. and Paluch E. *Trends Cell Biol*. 22:536. 2010

Actomyosin contraction drives cell dynamics

Spatial patterns of actomyosin networks contraction dictate cell shape and dynamics



Murrell M, Oakes PW, Lenz M, Gardel ML. *Nat Rev Mol Cell Biol*. 16(8):486-98. 2015.

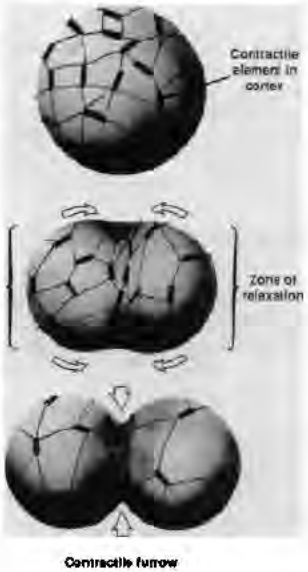
Mechanics of the cell cortex

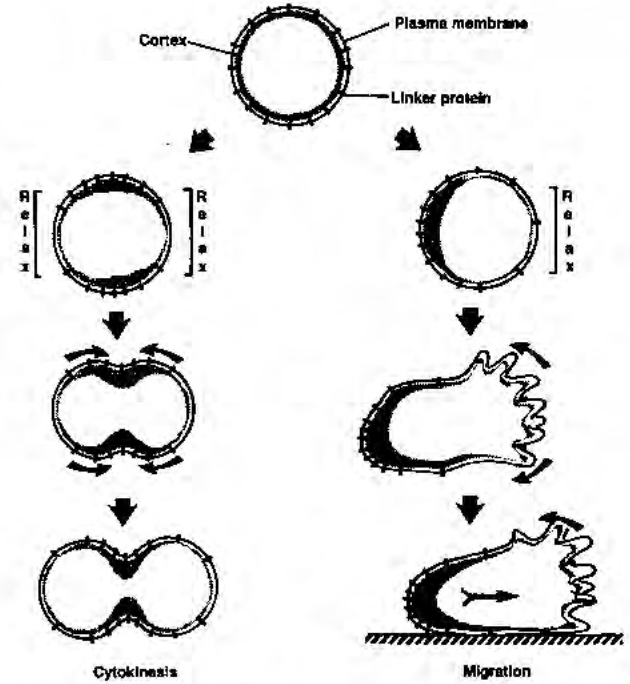
Spatial dynamics of actomyosin networks contraction >>Cortical actomyosin flows

- Hypothesis: Flows emerge from gradient of cortical tension

Cortical Flow in Animal Cells

D. BRAY AND J. G. WHITE
SCIENCE, VOL. 239 883 19 FEBRUARY 1988



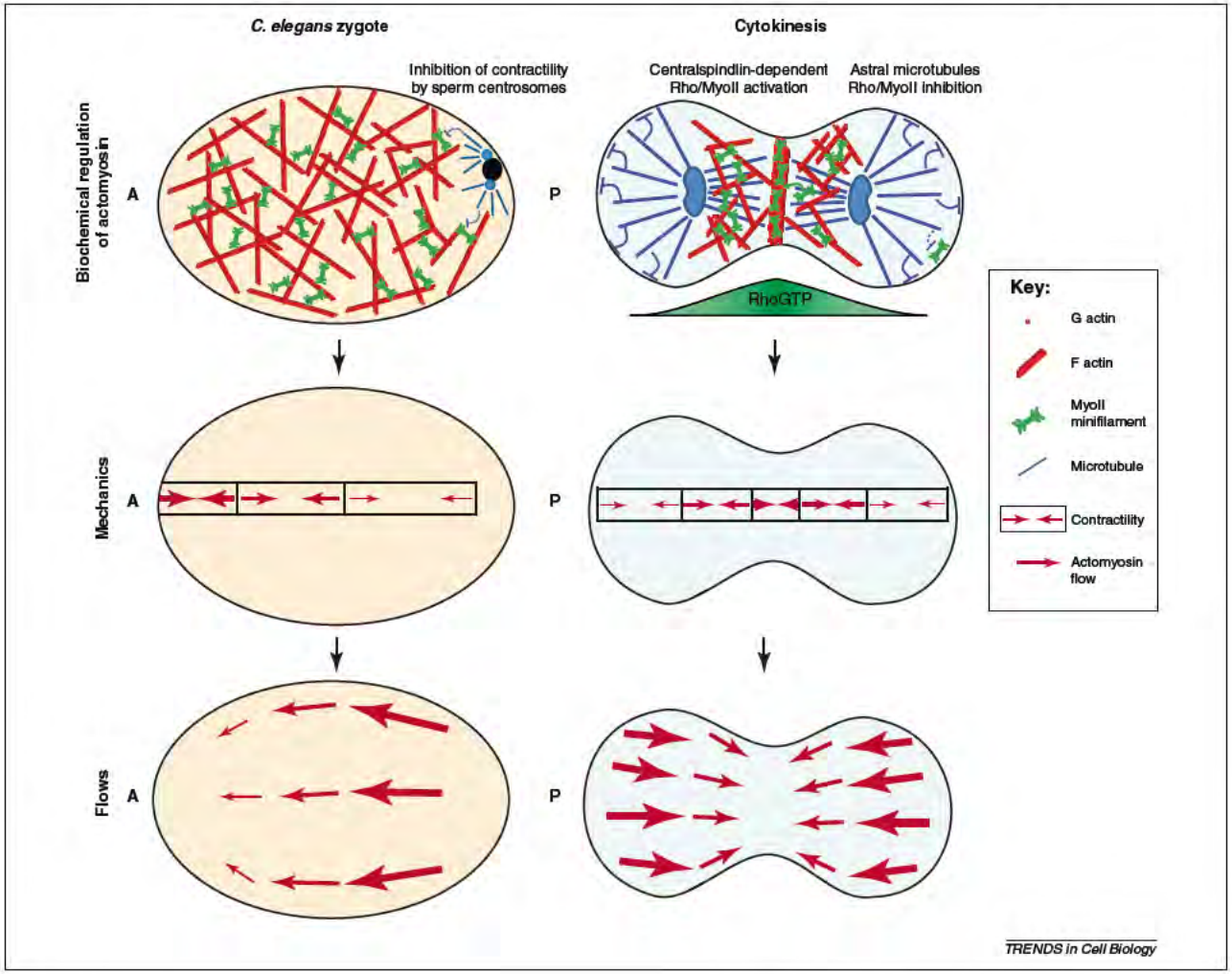


cytokinesis cell locomotion

Mechanics of the cell cortex

Cortical actomyosin flows

- gradient of cortical tension or contractility?



Review: Levayer R. and Lecuit T. *Trends Cell Biol*. 22:61-81

Mechanics of the cell cortex

Cortical actomyosin flows

- Model:

T: cortical tension
C: contractility driven tension (MyoII)
v: actomyosin flow velocity

$$T = C + \eta\, \partial v / \partial x$$

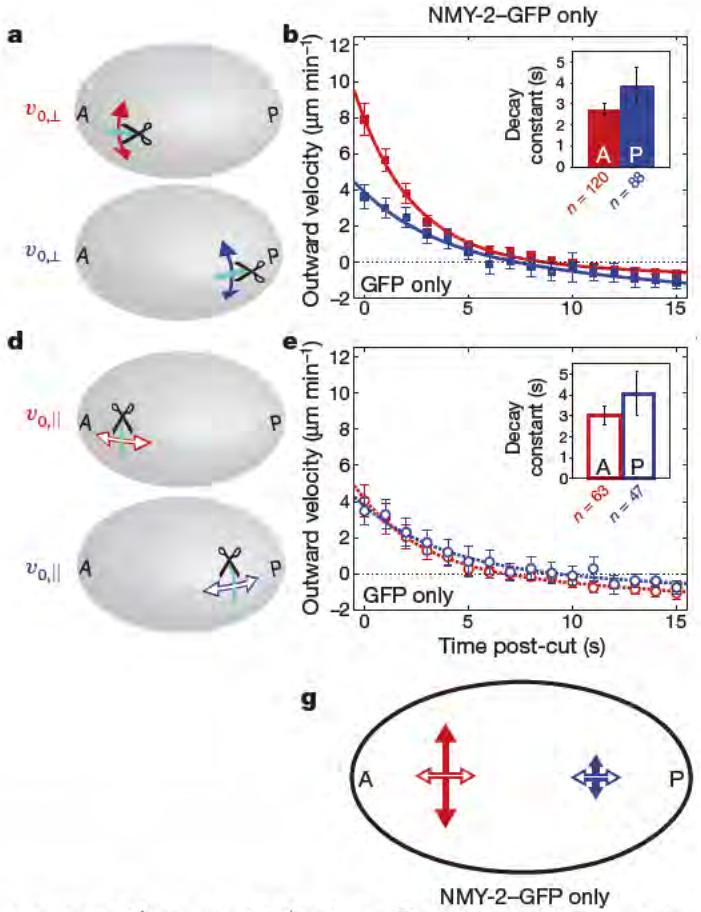
rate of compression

η :viscosity

$$\partial T / \partial x = \gamma\, v$$

γ :friction

hydrodynamic length scale $\ell = (\eta/\gamma)^{1/2}$



- Measurements

If viscosity can be neglected (cortex can be rapidly compressed/expanded), then $T = C$ and isotropic
- but tension is anisotropic, so viscosity cannot be neglected.

No gradient of cortical tension: so friction can be neglected.

>> Viscosity dominates over friction

Hydrodynamic length scale estimated by calculated flow profile from observed MyosinII gradient: ≈14μm.

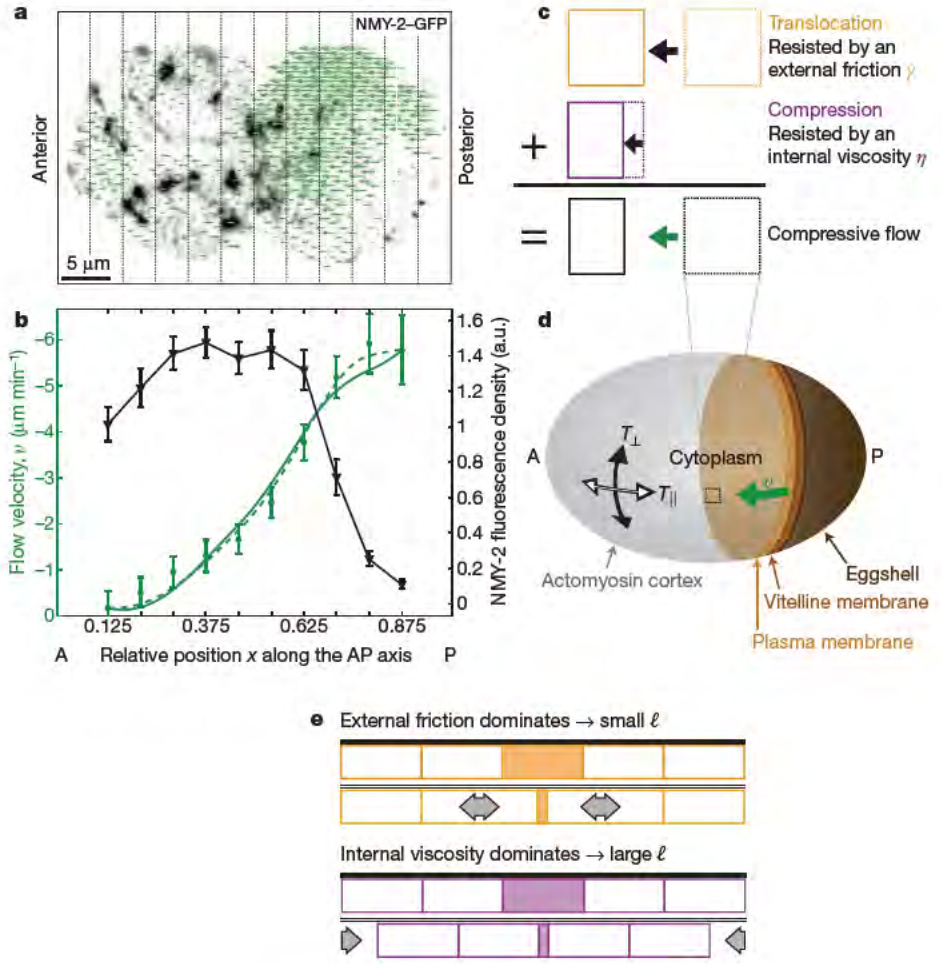
Mayer M. et al, Jülicher F. and Grill S. *Nature*. 467:617. 2010

Mechanics of the cell cortex

Cortical actomyosin flows

- emerges from gradient of contractility (Myosin-II)
- Long rang flow arises from the fact that viscosity dominates over friction

- Viscosity dominates over friction
- Hydrodynamic length scale estimated by calculated flow profile from observed MyosinII gradient: ≈14µm.

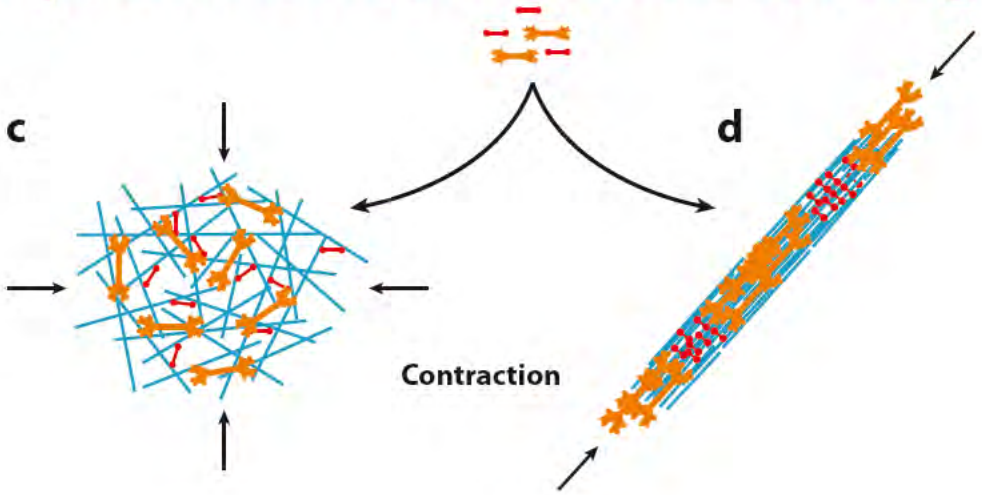


Mayer M. et al, Jülicher F. and Grill S. *Nature*. 467:617. 2010

Origin of actomyosin contraction

Single motor force generation

Actomyosin network tensile force generation

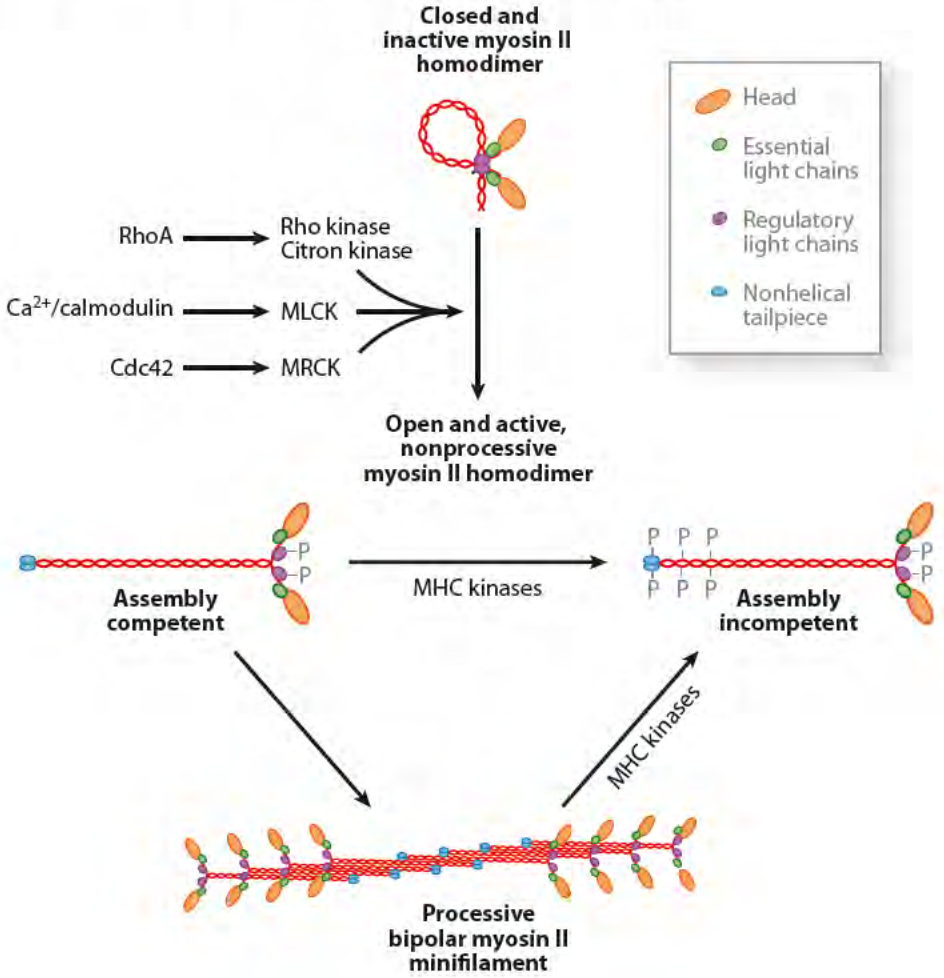


Myosin motor Mechanochemistry

Non-muscle Myosin-II Minifilament assembly

- Myosin-II is a hetero-hexameric complex
  - Heavy Chain (2)
  - Essential (2) and Regulatory (2) Light Chains.

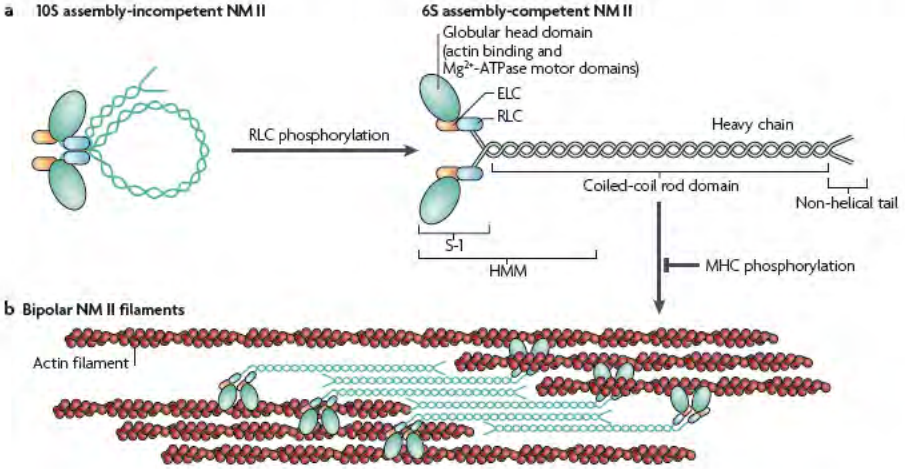
- Regulatory Light Chain Phosphorylation converts an intrinsically non processive motor into highly processive multi-molecular bipolar minifilament (14-30 Myosin-II)

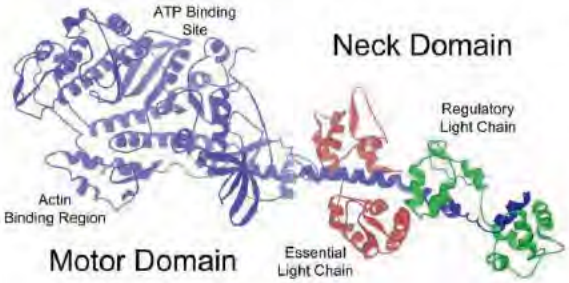


Lecuit T., Lenne PF. and Munro E. *Annu. Rev. Cell Dev. Biol.* 27:157–84. 2011

Myosin motor Mechanochemistry

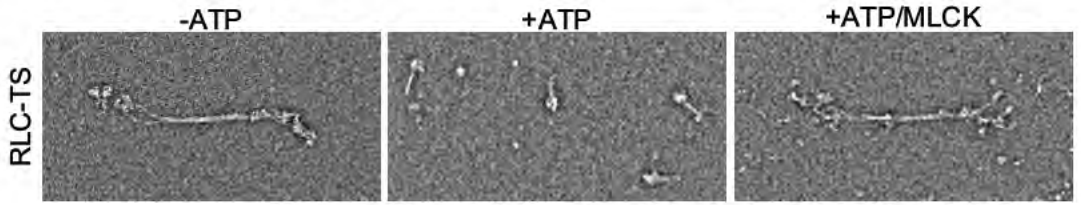
Non-muscle Myosin-II Minifilament assembly





- ATP binding disassembles miniflaments
- Phosphorylation of Myosin-II Regulatory Light Chain favours monofilament assembly in 2 ways:

- it counteracts ATP induced mini filament disassembly by inducing ATP hydrolysis
- it stabilises « open » conformation of Myosin-II



Vasquez CG. et al. Sellers J. and Martin A. *eLife* 2016. 5:e20818

Vicente-Manzanares M. et al, and Horwitz AR. *Nature Rev. Mol. Cell Biol.* 10:778. 2010

Myosin motor Mechanochemistry

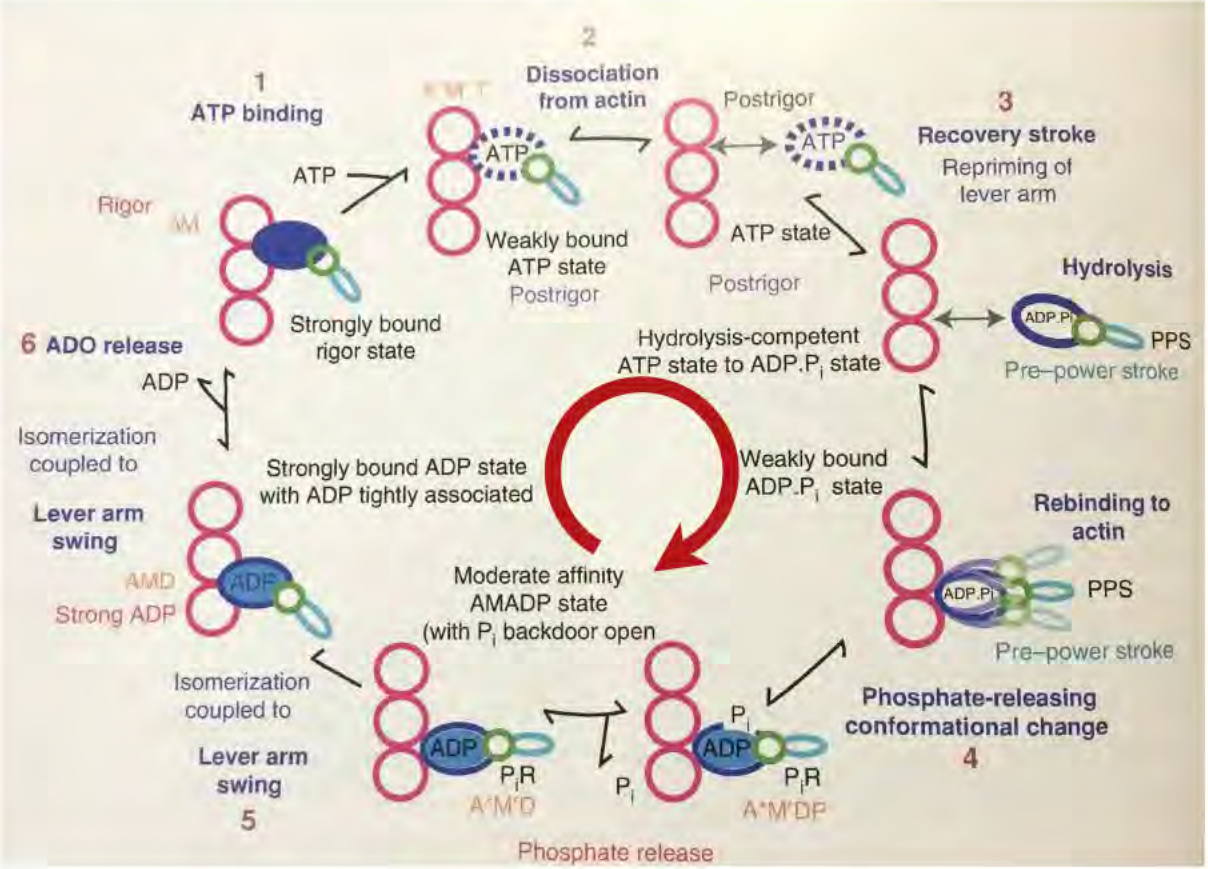
Myosin motor ATP-driven kinetic cycle

- Myosins cycle between an ATP bound state that is dissociated from Actin and an ADP+Pi bound state that is bound to Actin.

Cycle steps:

- Nucleotide free Myosin strongly bound to Actin
- ATP binding induces Myosin release from Actin.
- ATP hydrolysis occurs in Actin detached state
- ADP+Pi bound Myosin weakly binds to Actin
- ADP+Pi release is strongly increased when Myosin is bound to Actin
- ADP+Pi release shifts back Myosin into strong Actin-binding state.

- ATP cycle breaks detailed balance and brings about irreversibility.



Sweeney LH. and Holzbaur E. *Motor proteins. 2017 CSHL Perspect Biol doc 10.1101/cshperspect.a021931* in *The Cytoskeleton* ed. Thomas Pollard & Robert Goldman

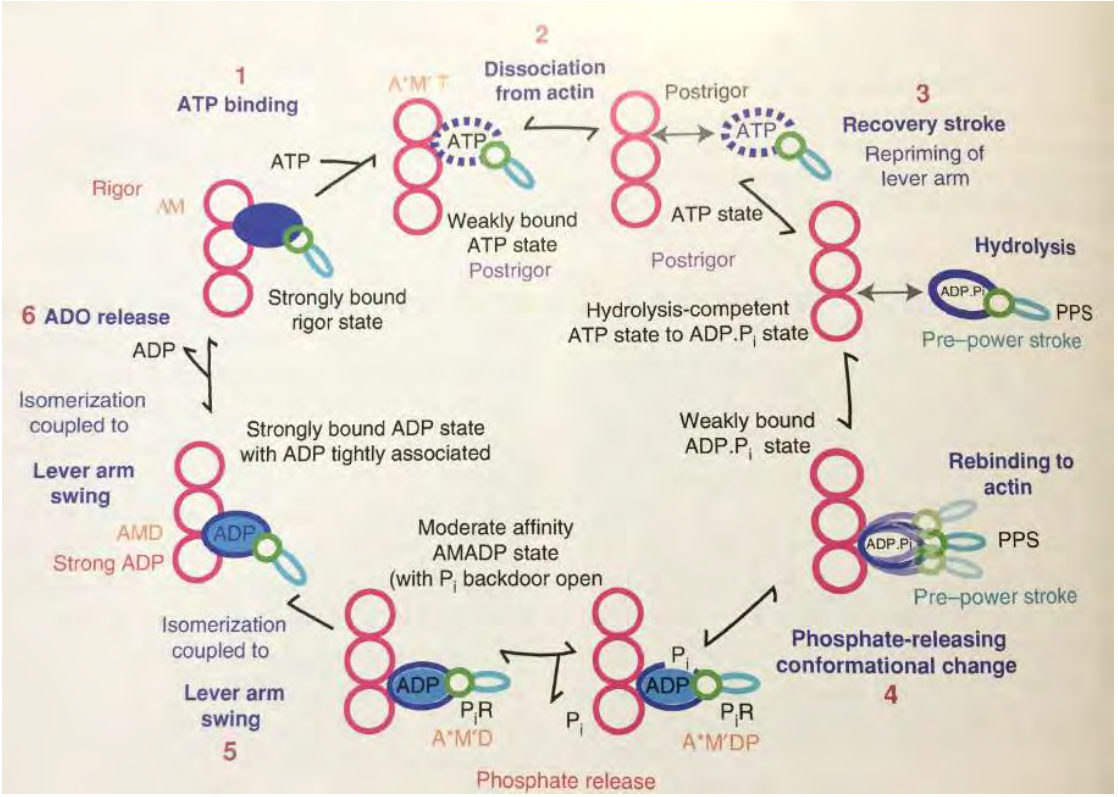
Myosin motor Mechanochemistry

Mechanoenzymatic coupling

- **Free-Energy consumption associated with ATP Binding, ATP hydrolysis and ADP+Pi dissociation**
- ATP binding resets the cycle: escape from *rigor* state
- ADP+Pi release have 2 effects:
  - -subtle change in conformation of head domain that is allosterically communicated to and amplified by the lever arm. This causes power stroke and force generation
  - -increase in affinity of binding to Actin that increases load and force generation state.
- ATP hydrolysis associated with recovery stroke

- **Motors are kinetically tuned** so as to change the speed at which different phases of the cycle occur.

- **Motor duty ratio:** fraction of the cycle that motor spends in strong actin bound state (*i.e.* fraction of cycle that is force generating).



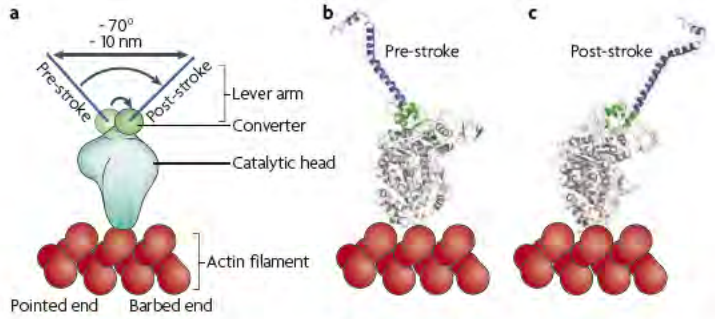
Sweeney LH. and Holzbaur E. *Motor proteins. 2017 CSHL Perspect Biol doc 10.1101/cshperspect.a021931*
in *The Cytoskeleton* ed. Thomas Pollard & Robert Goldman

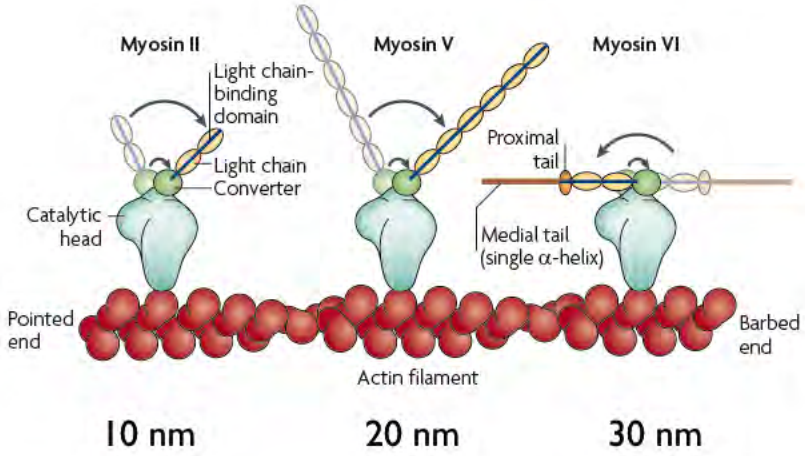
Myosin motor Mechanochemistry

Myosin motor lever arm swing

- Lever arm length and angle of swing determine power stroke.

- Force per head ~2-5pN

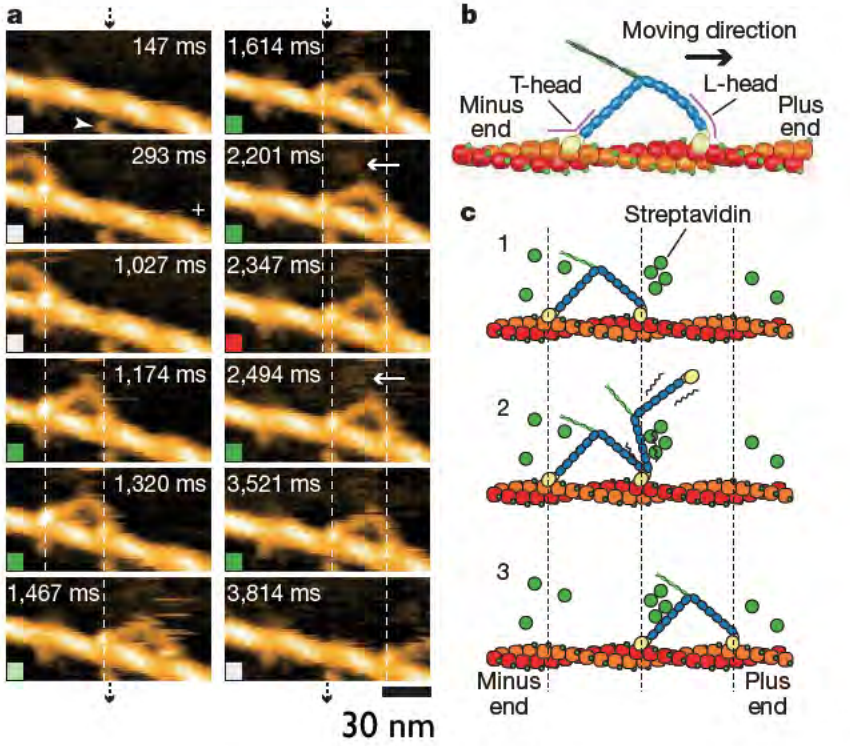




Spudich J and Sivaramakrishnan S. *Nature Rev. Mol. Cell Biol.* 11:128-137. 2010

Myosin motor lever arm swing

- High speed Atomic Force Microscopy visualises lever arm swing of Myosin-V



Kodera N. Yamamoto D., Ishikawa R. and Ando T. *Nature* 468:72-77. 2010

Myosin Duty Ratio

- **Motor duty ratio:** fraction of the cycle that motor spends in strong actin bound state (*i.e.* fraction of cycle that is force generating).
- The duty ratio varies between Myosins and has different functions.
  - Low duty ratio: e.g. in fast-muscle contraction. It increases the speed of movement on actin filaments. The power per motor is low, but this is compensated by the tight, high density organisation of motors on actin.
  - High duty ratio: e.g. Myosin-V allows high processivity of 2-headed motor and cargo transport.

  (when 1 motor in ATP bound state, detached from actin, the other head is strongly bound to actin).

- **The kinetics of ADP release tunes the duty ratio:** Pi versus ADP release is rate-limiting step.

- **Regulation:**

  -Varies between different Myosin motors or Myosin-II isoforms (IIA versus IIB)

  Wang F. et al., and Sellers J. *J. Biol. Chemistry*. 278:27439-27448. 2003

  -Duty ratio scales inversely with number of motors in complex: small minifilaments/higher duty ratio. Collective duty ratio in mini filaments.

  Ex: duty ratio of NMII: ~0.1-0.35 ; 14-30 motors in small filaments.
  duty ratio of smooth muscle MyoII: ~0.04 ; 100s of motors in thick filaments

  -Strain on Myosin-Actin cross-bridge can slow ADP release and increase duty ratio: force dependent increase in force generation.

Review: Heissler S. and Sellers J. *Traffic*. 17(8):839-59. 2016
Bloemink MJ. and Geeves MA. *Sem. Cell & Dev. Biol*. 22:961-967. 2011

Myosin motor Mechanochemistry

Mechanoenzymatic coupling: spectrum of behaviours

- Minifilament assembly may increase duty ratio:
  - *e.g.* Regulation of auto inhibition of NMII by phosphorylation of RLC. Autoinhibited state reduces Pi release ~1000 fold (becomes rate limiting, hence low duty ratio)
  - Collective duty ratio of minifilament.

Review: Heissler S. and Sellers J. *Traffic*. 17(8):839-59. 2016
Bloemink MJ. and Geeves MA. *Sem. Cell & Dev. Biol*. 22:961-967. 2011

Mechanoenzymatic coupling: Load sensitivity

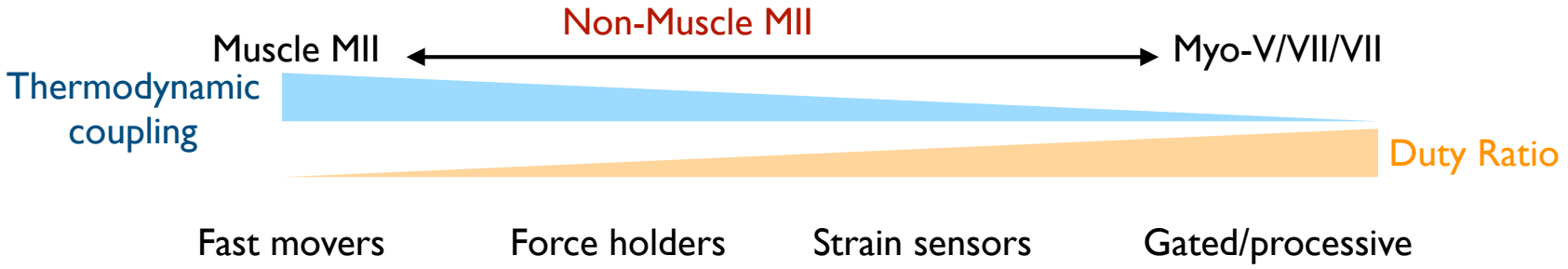
- Optical trapping experiments to study the effect of load on motor duty ratio.
- Determination of:
  - displacement of myosin on actin (power stroke size)
  - the number of steps before motor detaches (processivity)
  - amount of force a motor exerts on actin
  - mechanoenzymatic features of kinetic cycle

Review: Spudich J et al. *Cold Spring Harbor Protoc*. 2011:1305-1318. 2011

- Load sensitivity present in all motors tested.

Ex: low resisting forces >0.5pN slow down ADP release for Myo-1B, which becomes rate limiting and increases duty ratio
higher forces >1pN block detachment from actin (s-min range)

Laasko JM et al, Ostap EM. *Science* 321:133-136. 2011

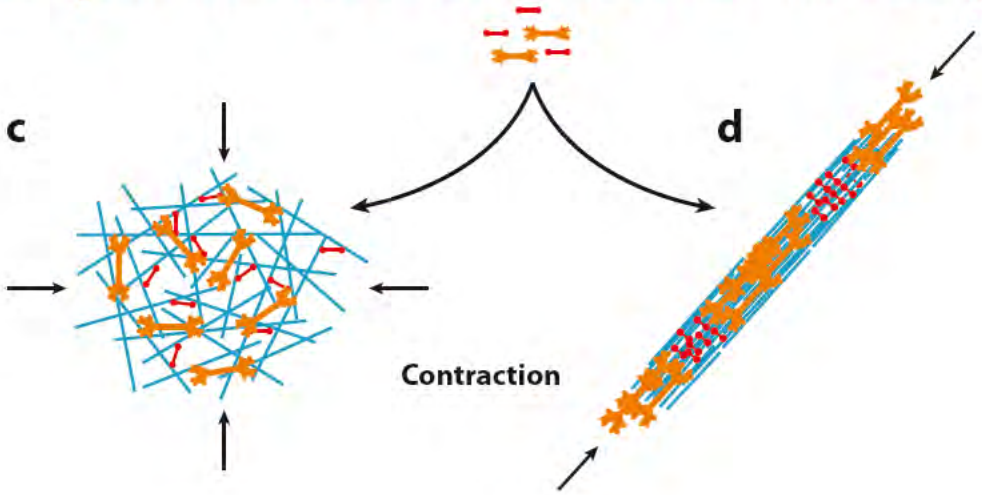


Review: Heissler S. and Sellers J. *Traffic*. 17(8):839-59. 2016
Bloemink MJ. and Geeves MA. *Sem. Cell & Dev. Biol*. 22:961-967. 2011

Origin of actomyosin contraction

Single motor force generation

Aactomyosin network tensile force generation

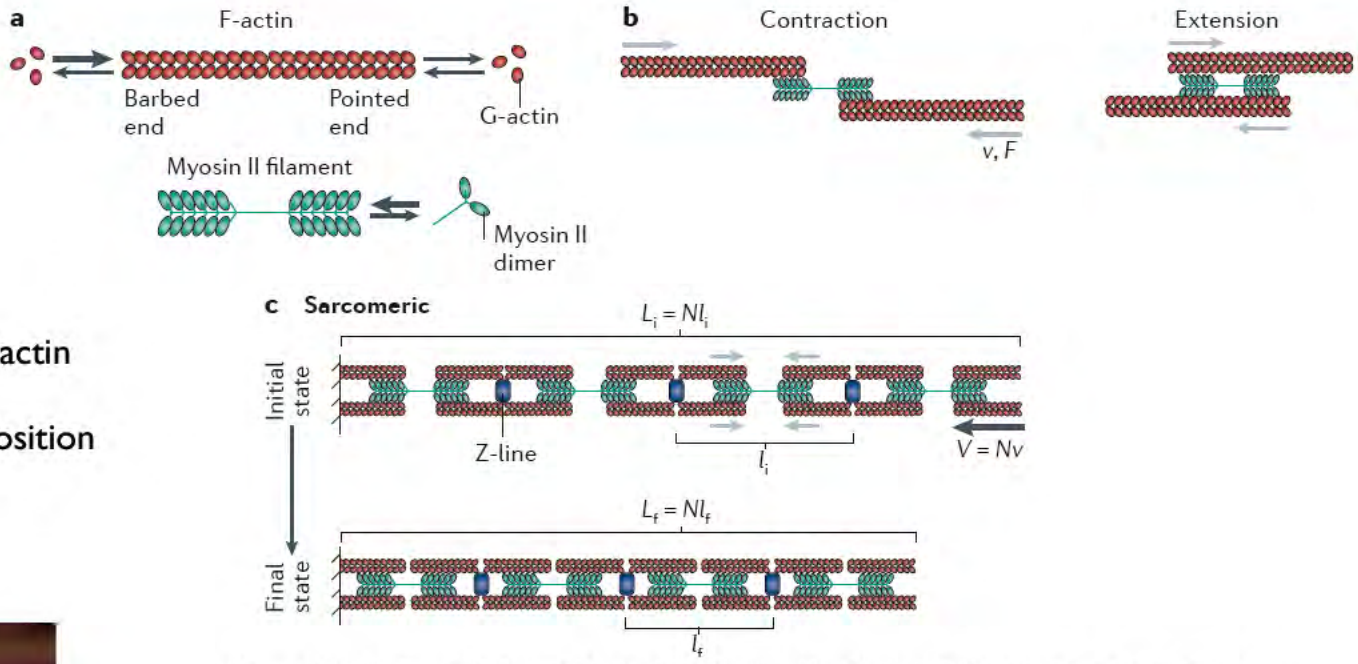


Origin of actomyosin contraction

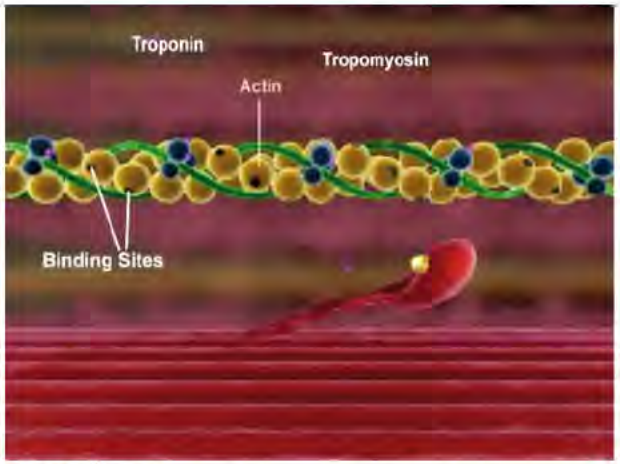
Ordered contraction

> Sarcomeric organisation in muscles

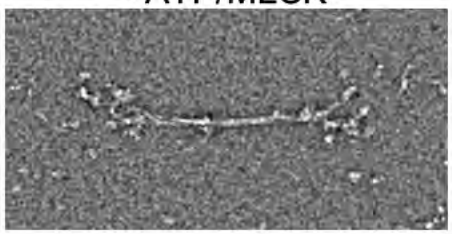
- F-actin polymerisation from barbed end
- Myosin-II moves towards barbed end of F-actin
- Contraction or Extension depending on position with respect to middle of actin filament



Murrell M, Oakes PW, Lenz M, Gardel ML. *Nat Rev Mol Cell Biol*. 16(8):486-98. 2015.



+ATP/MLCK

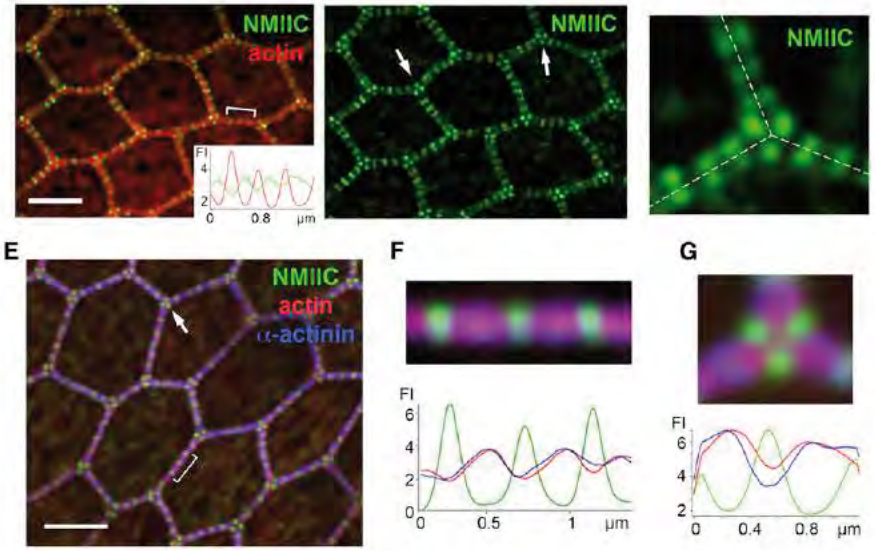


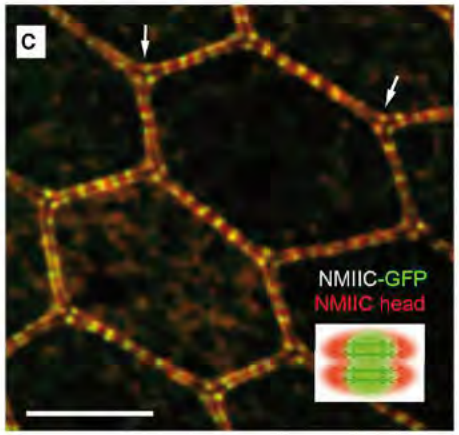
Vasquez CG. et al. Sellers J. and Martin A. *eLife* 2016. 5:e20818

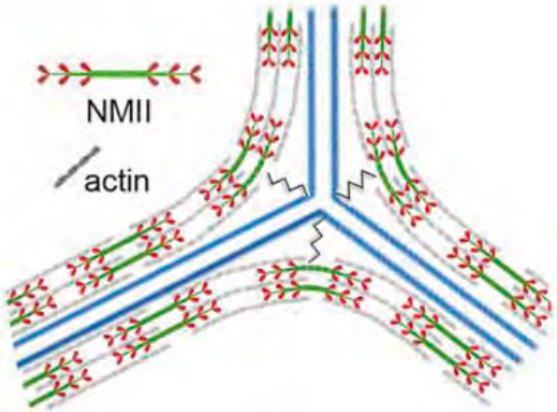
Origin of actomyosin contraction

Ordered contraction

> Sarcomeric-like organisation in epithelial cells (organ of Corti)







Ebrahim S. et al, Kachar B. *Current Biology* 23, 731–736, 2013

Origin of actomyosin contraction

Disordered contraction

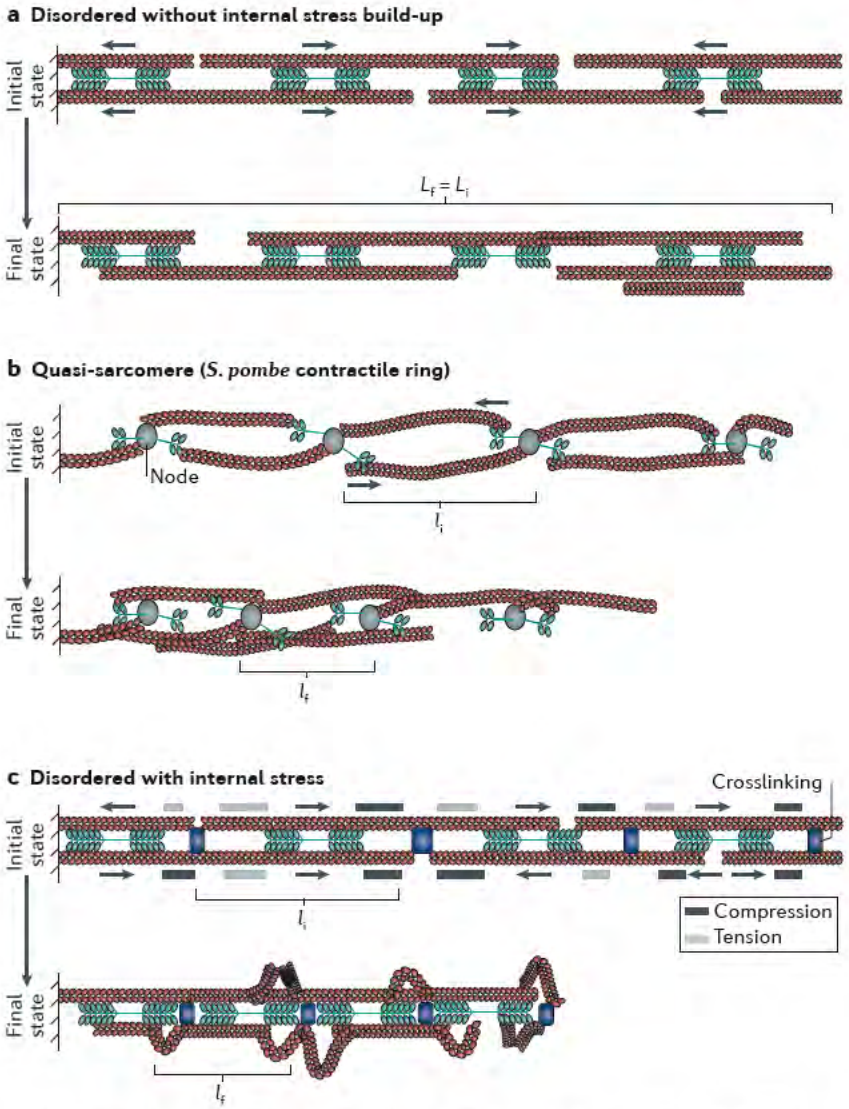
No net contraction

Net contraction

Nodes contain Formins (barbed ends)
Capture of surrounding pointed ends
Inter-node contraction

Net contraction

Crosslinkers allow build up of internal stress
Buckling instability of actin filaments under stress
Relaxation of internal stress

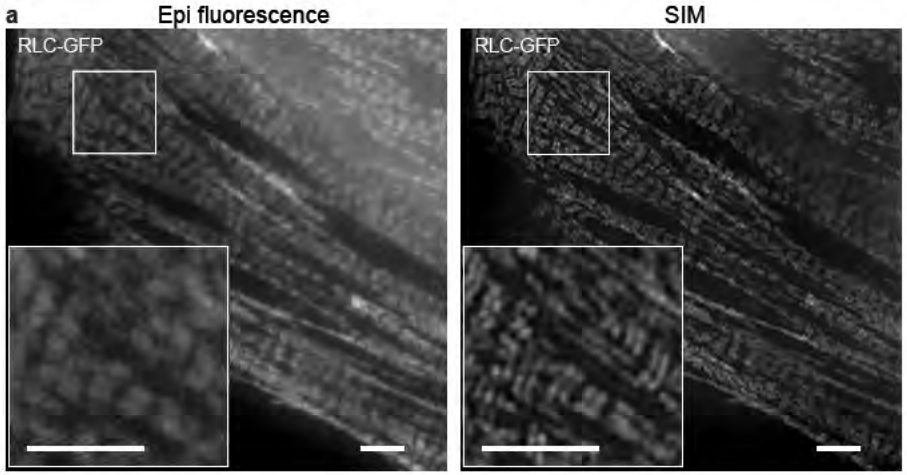


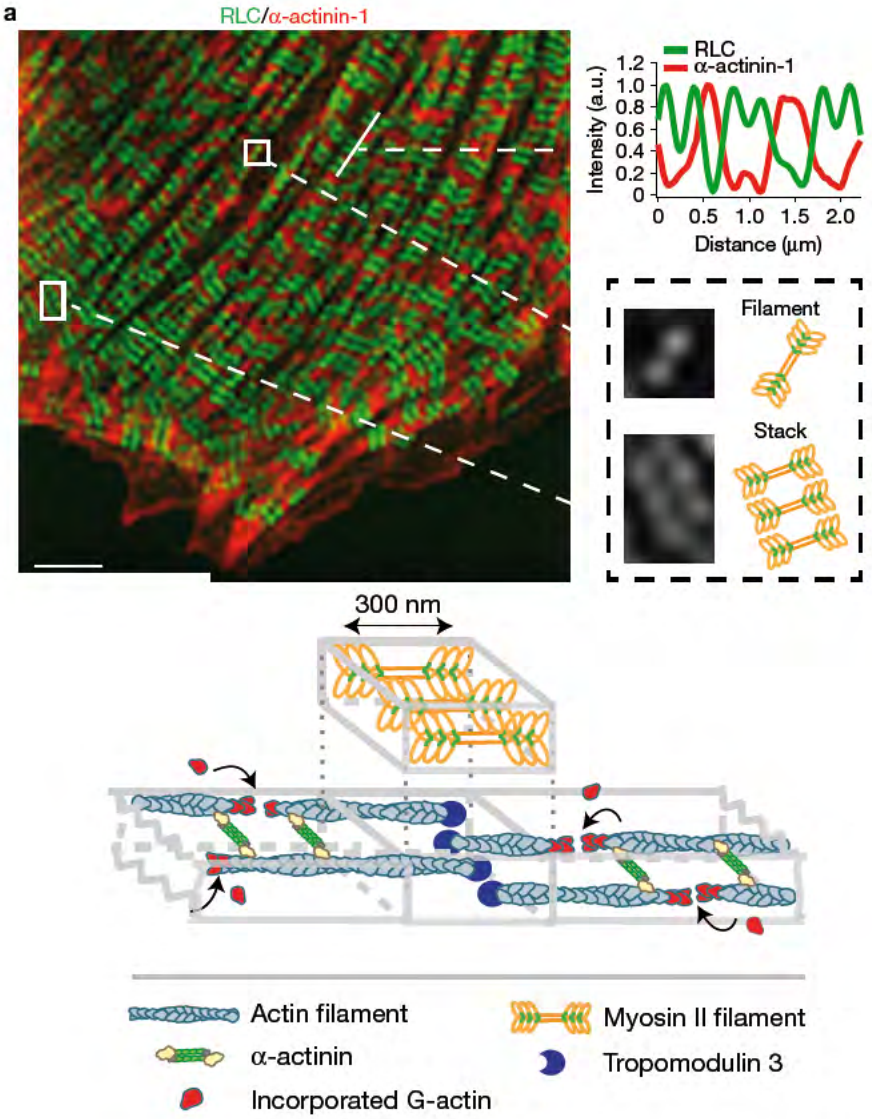
Murrell M, Oakes PW, Lenz M, Gardel ML. *Nat Rev Mol Cell Biol*. 16(8):486-98. 2015.

Origin of actomyosin contraction

Disordered contraction

Self-organisation of minifilament in stacks





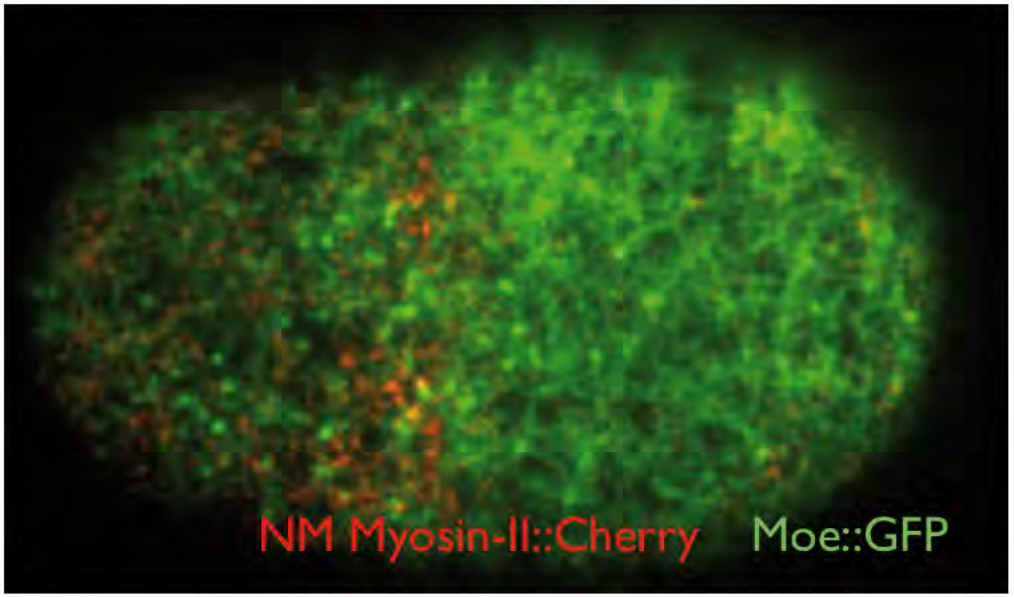
Hu S. et al. and Bershadsky A. *Nat Cell Biol*. 19(2):133-141. 2017.

Origin of actomyosin contraction

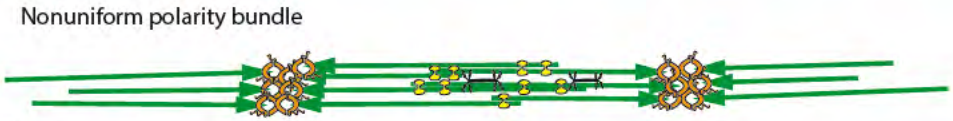
Disordered contraction in isotropic or anisotropic networks

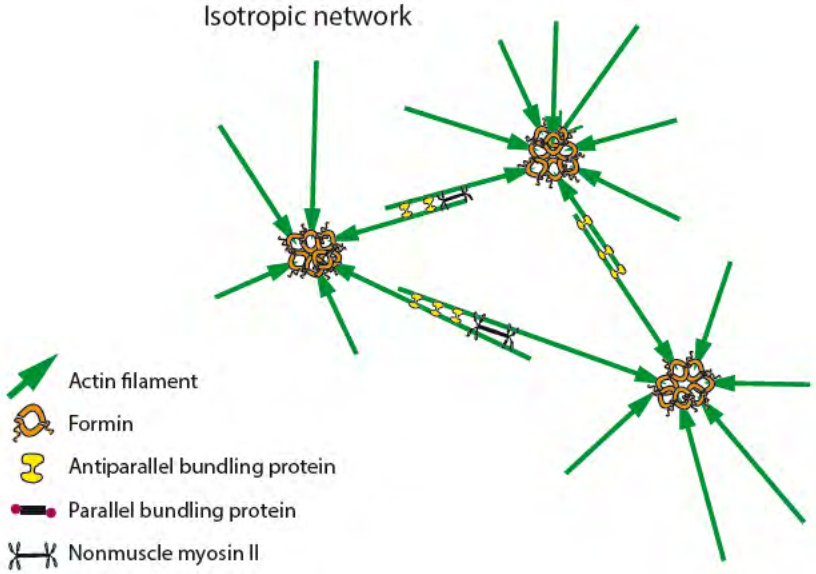
Importance of parallel/antiparallel organisation of actin filaments

Cytokinesis



Edwin Munro. Univ. of Chicago



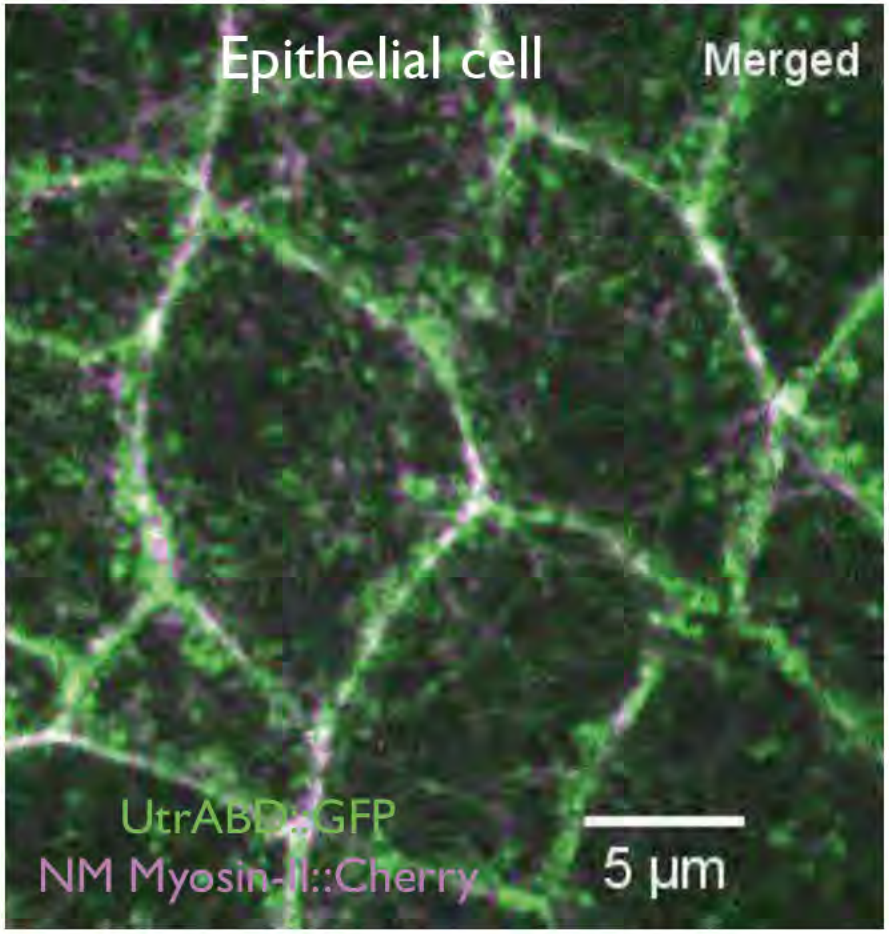


Skau CT. and Waterman CM. *Annu. Rev. Biophys.* 44:285–310. 2015.

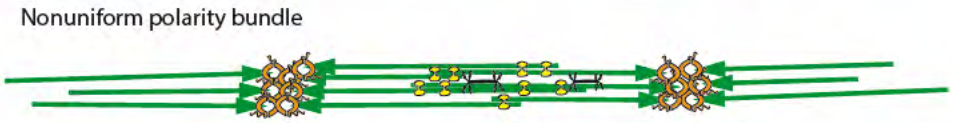
Origin of actomyosin contraction

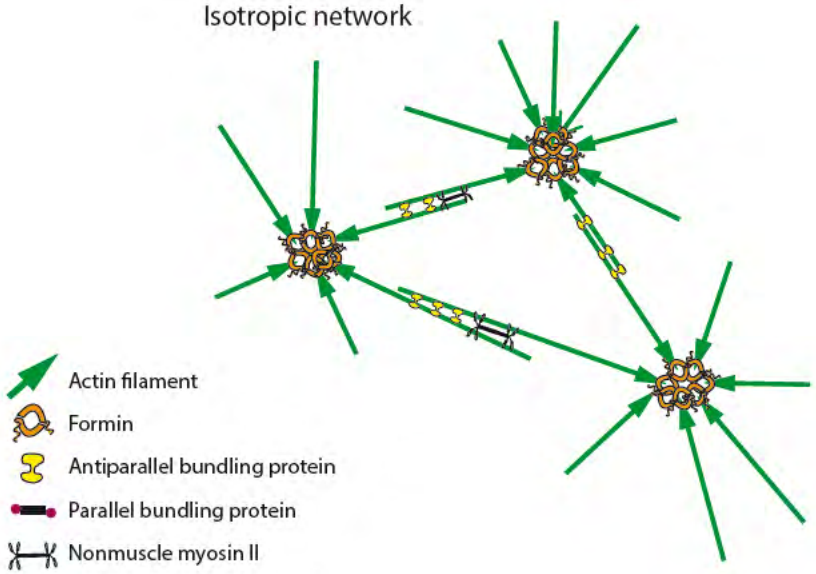
Disordered contraction in isotropic or anisotropic networks

Importance of parallel/antiparallel organisation of actin filaments



Munjal A. et al. Lecuit T. *Nature*. 524:351. 2015
Rauzi M. et al. Lenne PF. and Lecuit T. *Nature*. 468:1110. 2010

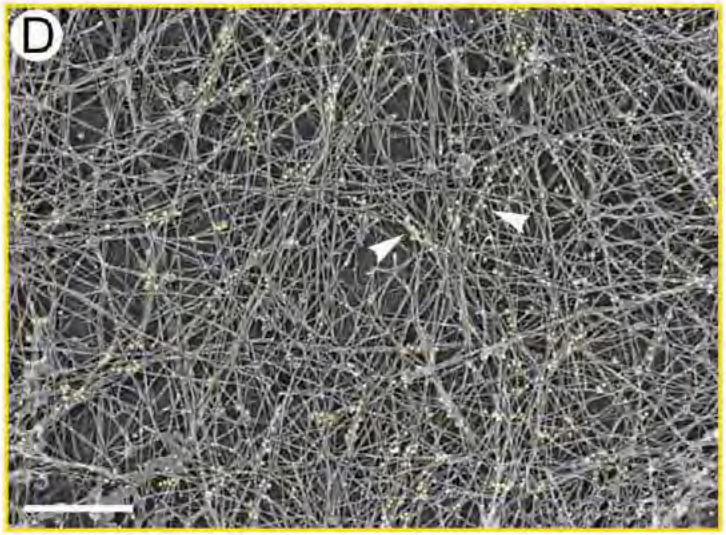


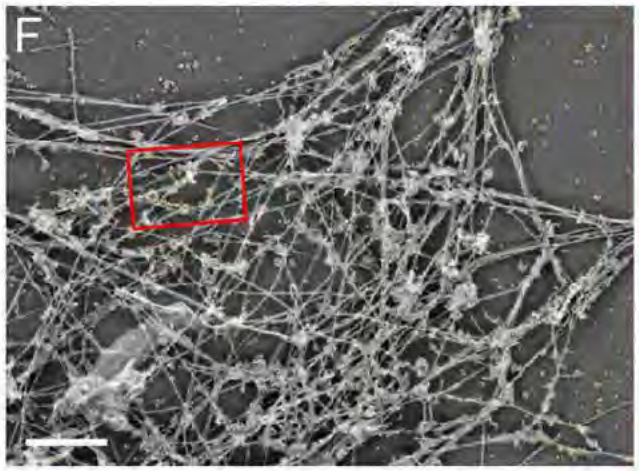


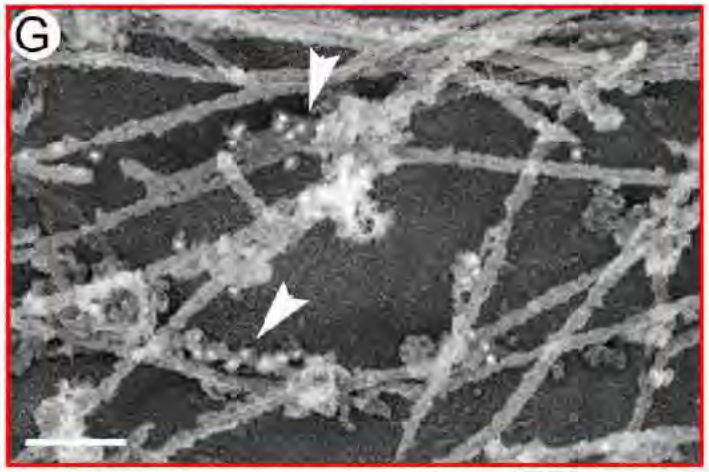
Skau CT. and Waterman CM. *Annu. Rev. Biophys*. 44:285–310. 2015.

Origin of actomyosin contraction

Disordered contraction
Isotropic Networks



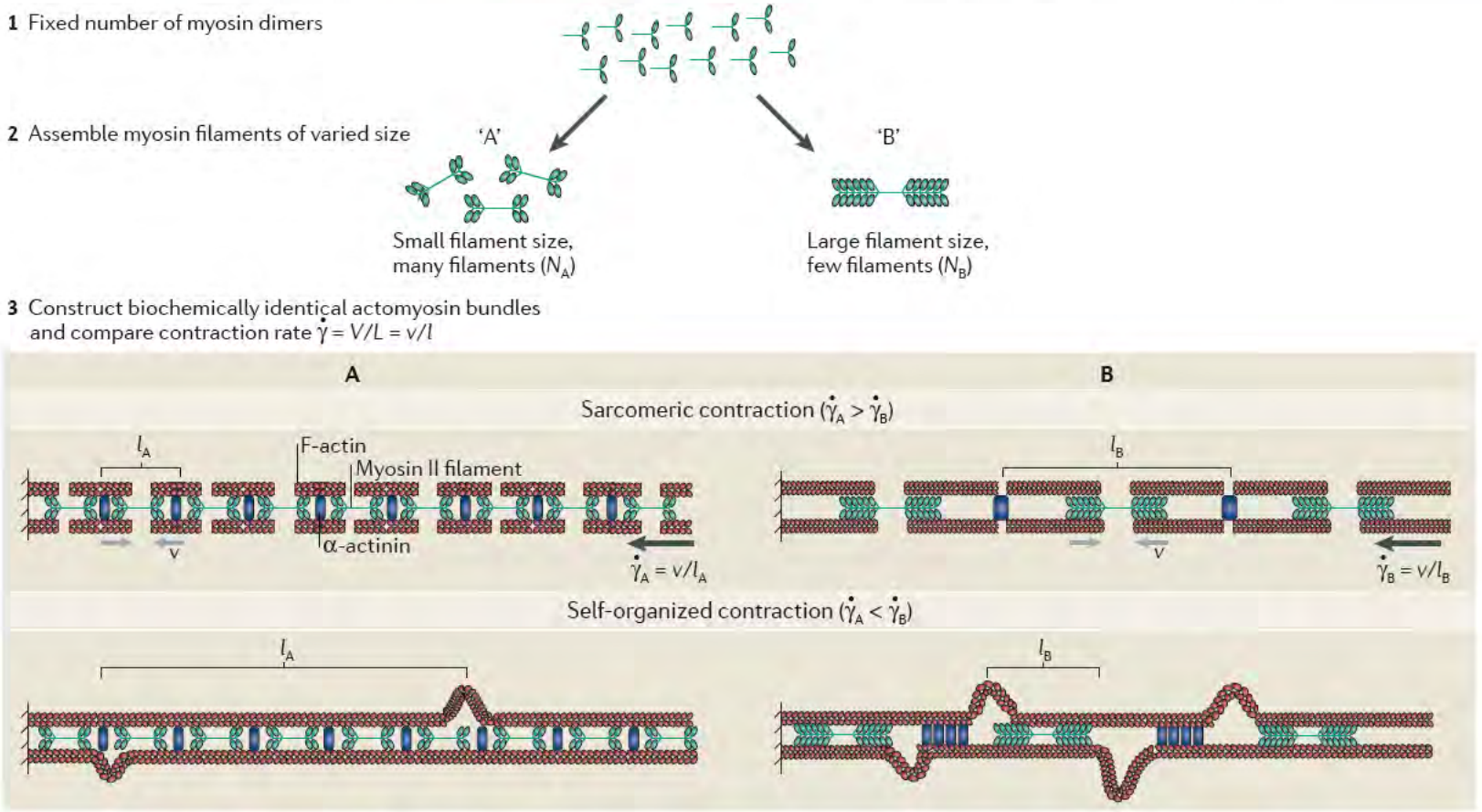




Shutova M1, Yang C, Vasiliev JM, Svitkina T. *PLoS One*. 7(7):e40814. 2012.

Origin of actomyosin contraction

Impact of filament size* on contraction rate



* Note on *Filament size:* Affected by any process that:

1) changes the number of motors/filament
2) increases the duty ratio of motors/filament: e.g. isoforms composition, phosphorylation, load etc
3) increases the fraction of active motors/minifilament: phosphorylation.

Murrell M, Oakes PW, Lenz M, Gardel ML. *Nat Rev Mol Cell Biol*. 16(8):486-98. 2015.

Origin of actomyosin contraction

Biophysical regulators of actomyosin force generation

- Passive cross linkers, Active cross linkers (MyoII)
- Actin filaments length
- Membrane attachement

Process (and factors involved)	Cellular location	Effect on actomyosin architecture	Effect on contractility	k_{off} (dissociation rate constant in s^{-1})	Refs
F-actin crosslinking					
Fascin	Filopodia	Unipolar bundling	Promotes dynamic non-contractile steady states, increased length scale*	$9\ s^{-1}$	69,72, 112–116
Filamin	Smooth muscle, stress fibres and cortex	Isotropic networks and apolar bundles	Increased length scale*	• $0.6\ s^{-1}$ ($F = 0^{\ddagger}$) • $0.087\ s^{-1}$ ($F > 0^{\S}$)	39,66,75, 117–122
α-actinin	Myofibrils, stress fibres, contractile ring and cortex	Isotropic networks and apolar bundles	Increased length scale*	• $0.4\ s^{-1}$ ($F = 0$) • $0.066\ s^{-1}$ ($F > 0$)	39,67,118, 122–126
Anillin	Cleavage furrow	Apolar bundles	Increased length scale*	Unknown	121, 127–129
Cortexillin	Cleavage furrow	Apolar bundles	Increased length scale*	Unknown	113, 130,131
Solution pH	Cytosol	Higher pH enhances F-actin crosslinking	Increased length scale	Unknown	121
F-actin length					
Gelsolin capping protein	Cell cortex	Reduced F-actin length	Increased speed and reduced length scale	Unknown	66,132
Membrane attachment					
Ezrin, moesin and filamin	Cell cortex	Adds viscous drag to F-actin, resisting its mobility	Reduced length scale	Unknown	53, 133–135

*Non-monotonic effect on contractility. Excessive crosslinker or capping protein inhibits contraction. $^{\ddagger}F = 0$ corresponds to unloaded (zero force) conditions. $^{\S}F > 0$ corresponds to loaded (non-zero force) conditions.

Murrell M, Oakes PW, Lenz M, Gardel ML. *Nat Rev Mol Cell Biol*. 16(8):486-98. 2015.

Cortical tension - Measurements

Measurements

Table 1. Experimental measurements of turnover of cortex components

Cell type	Protein	Turnover half-time	Refs
LLCPK1 cells during cytokinesis	Actin	45 s (polar cortex) 26 s (contractile ring)	[37]
NRK cells, anaphase and telophase	Actin	15 s	[36]
Dictyostelium, contractile ring	Myosin II	7s	[104]
Drosophila S2 cells	Myosin II	~6 s (metaphase) ~14 s (anaphase)	[105]
NRK cells	Alpha-actinin (crosslinker)	~8 s (equator in cytokinesis) ~19 s (poles in cytokinesis)	[40]
Dictyostelium	Dynacortin (crosslinker)	0.45 s (interphase) 0.98 s (equator in cytokinesis) 0.51 s (poles in cytokinesis)	[44]
Dictyostelium	Fimbrin (crosslinker)	0.26 s (interphase) 0.58 s (equator in cytokinesis) 0.31 s (poles in cytokinesis)	[44]
Dictyostelium	Cortexillin-I (crosslinker)	3.3 s (interphase) 5.4 s (equator in cytokinesis) 4.5 s (poles in cytokinesis)	[44]

Turnover times of cortical actin, myosin II, and crosslinkers measured by FRAP. Myosin and crosslinkers typically turn over faster than actin filaments. Notably, non-muscle myosin II aggregates in mini-filaments in the cortex and it is unclear whether FRAP experiments measure the timescale of turnover of individual myosins or of entire mini-filaments.

Table 2. Experimental measurements of cortical tension

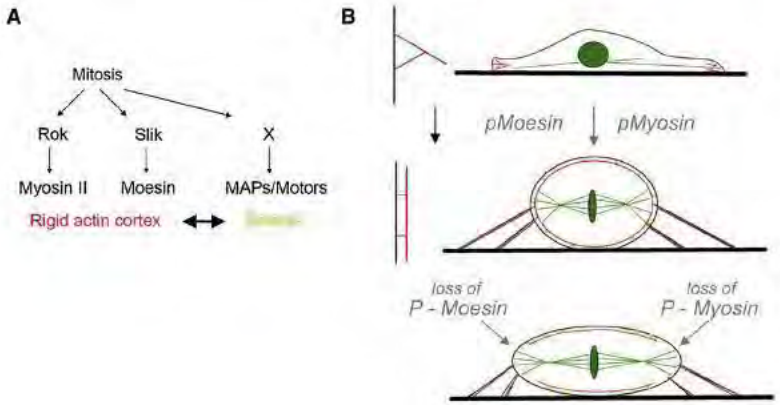
Cell type	Technique	Value (pN/μm)	Refs
Blood granulocyte	Micropipette	30–35	[48,106]
Dictyostelium	Micropipette	~1500	[30]
Dictyostelium	Micropipette	4330	[52]
Fibroblasts	Micropipette	400	[34]
Fibroblasts	Micropipette	~300	[107]
Zebrafish progenitor cells	AFM indentation	~50	[50]

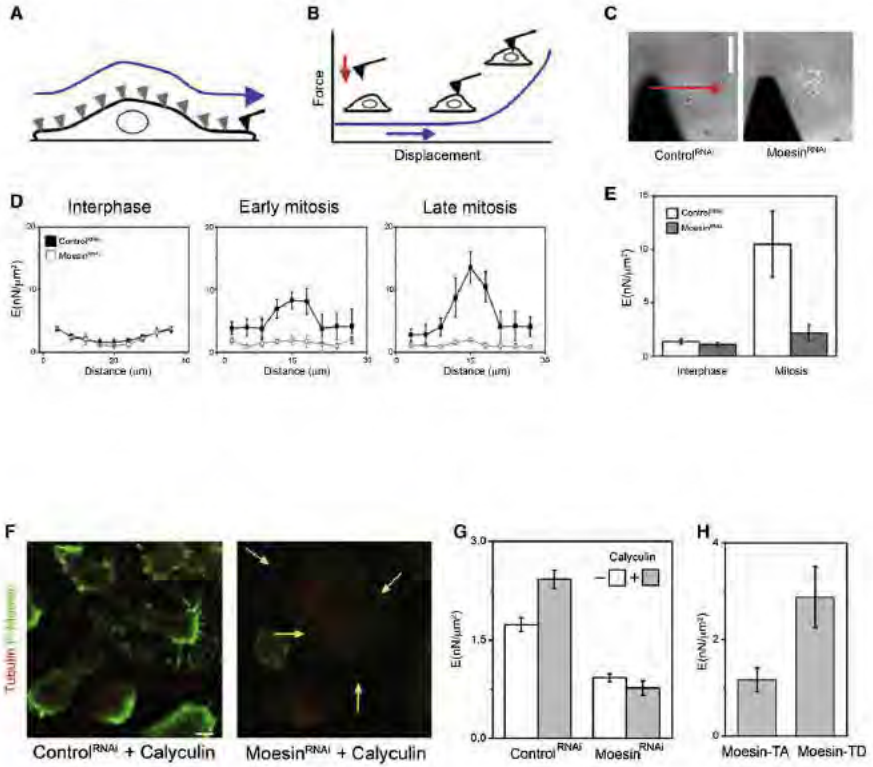
Review: Salbreux G, Charras G. and Paluch E. *Trends Cell Biol*. 22:536. 2010

Regulation of Cortical tension

- Mitotic cell rounding
- Increase in cortex stiffness
- There is also a contribution of osmotic pressure

Stewart M. et al, Hyman AA. *Nature*. 469:226:. 2011



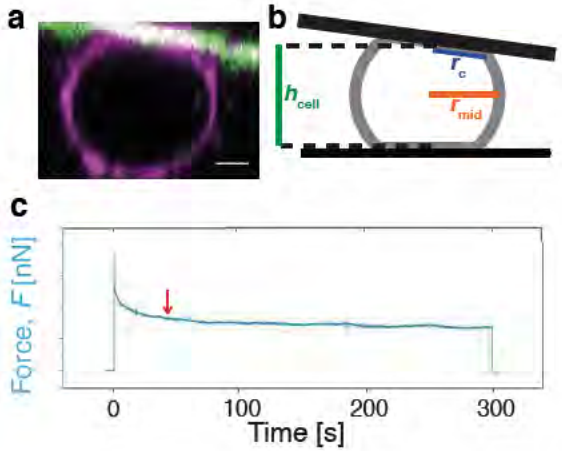


Kunda P. et al, Baum B. *Current Biol*. 18:91-101 2008
Matthews HK. et al., Baum B. *Dev Cell*. 23:371-383 2012

Regulation of Cortical tension

Importance of network architecture

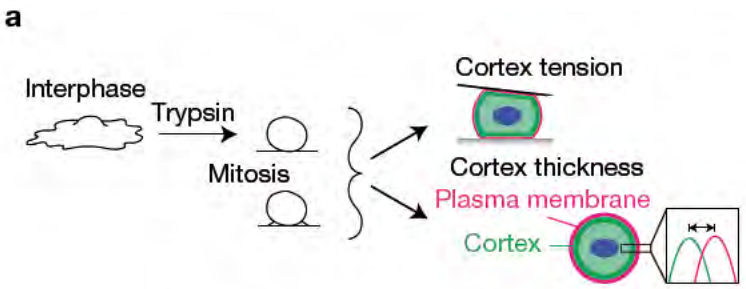
- Cortex tension increases during mitosis
- Associated with a reduction of cortex thickness

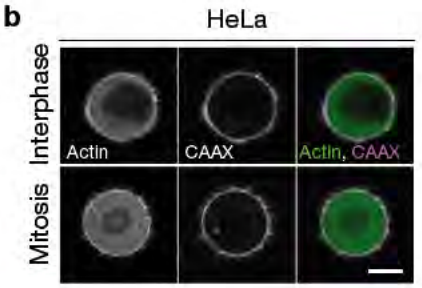


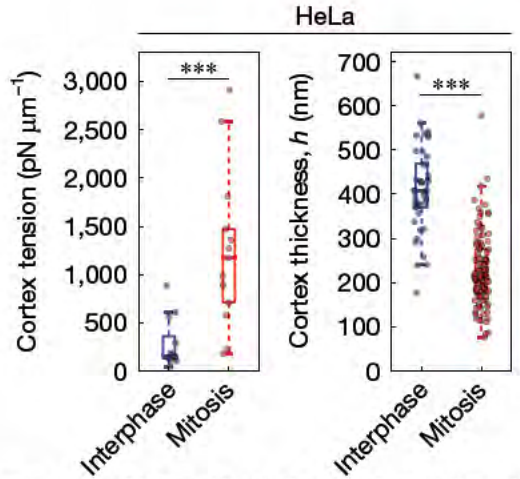
Force exerted on AFM cantilever

$$T = \frac{F\left(\frac{r_{mid}^2}{r_c^2} - 1\right)}{2\pi r_{mid}}$$

Tension





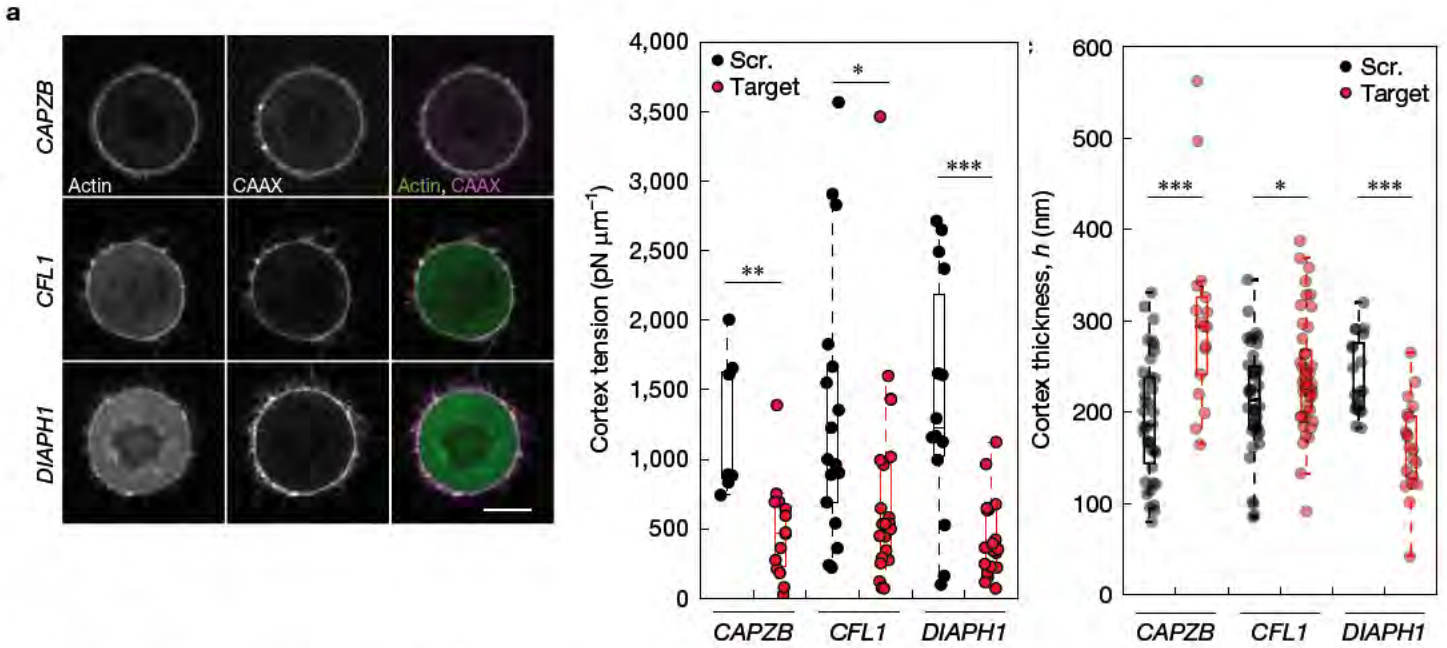


Chugh et al., Charras G. Salbreux G. and Paluch E. *Nature Cell Biology*. 2017

Regulation of Cortical tension

Importance of network architecture

- Perturbation of actin filament length regulators affects cortical tension



Effect on actin filament length

- negative { CAPZB: barbed end capping protein (limits barbed end polymerisation)
CFL1: actin severing protein cofilin (severs actin filaments)

+ positive DIAPH1: formin, unbranched actin nucleator

Chugh et al., Charras G. Salbreux G. and Paluch E. *Nature Cell Biology*. 2017

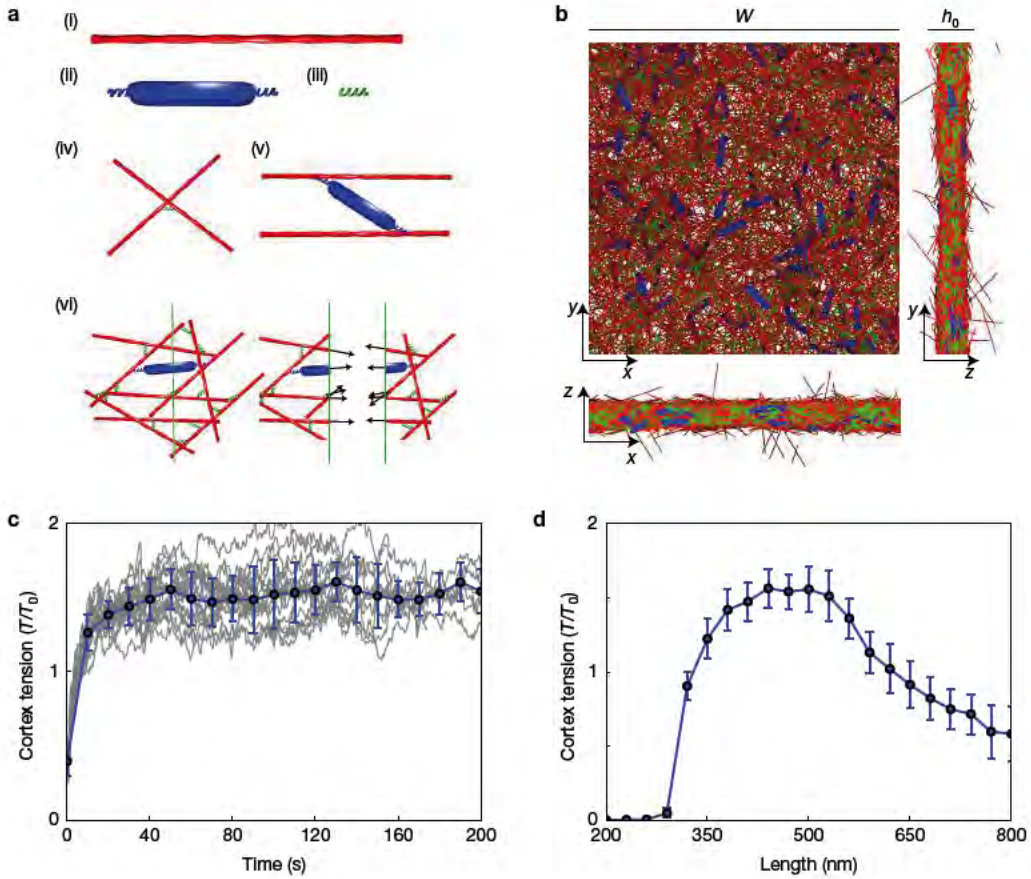
Regulation of Cortical tension

Importance of network architecture

Computational model: Maximum tension predicted at intermediate filaments length

- **Ingredients of model:**
Actin filaments, rigid motors, passive crosslinkers with elastic links.
Motors form bipolar mini filaments that wall towards + end.
Frictional resistance to motor movement

- **Result:** Effect of filament length but not of cortex thickness *per se*



Chugh et al., Charras G. Salbreux G. and Paluch E. *Nature Cell Biology*. 2017

Regulation of Cortical tension

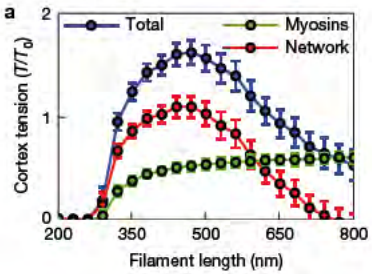
Importance of network architecture

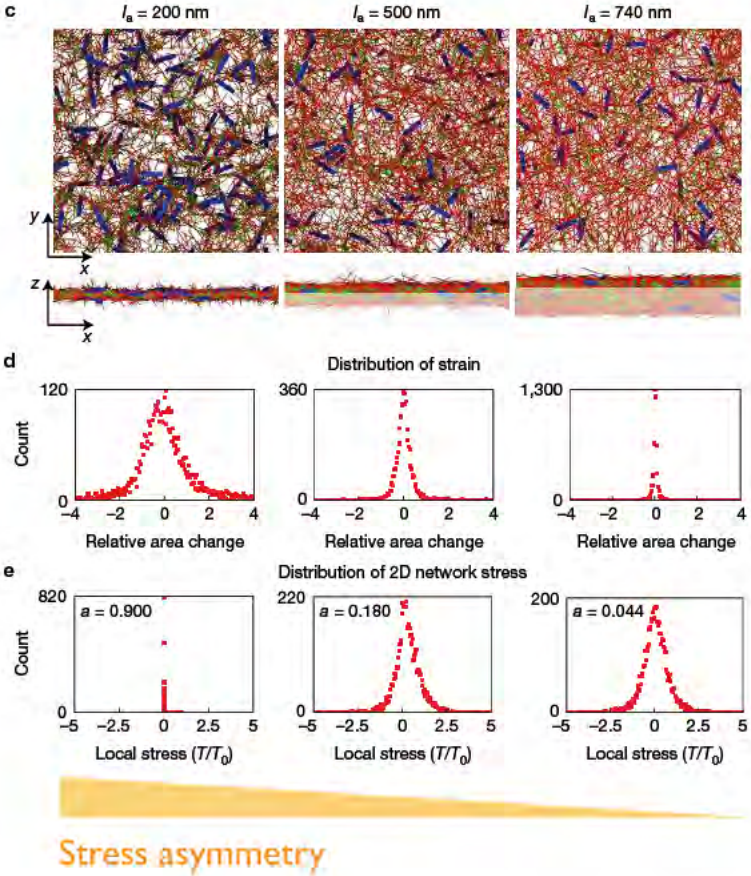
- Impact of length of filaments on stress asymmetry

- Stress in motors increases and saturates as filament length increases
- Stress in network has a maximum
- So effect of filament length due to response of network to motor driven stresses.

- Strain is higher for shorter filaments (more compliants)
- Strain symmetry for any length of filaments

- Stress asymmetry for shorter filaments.



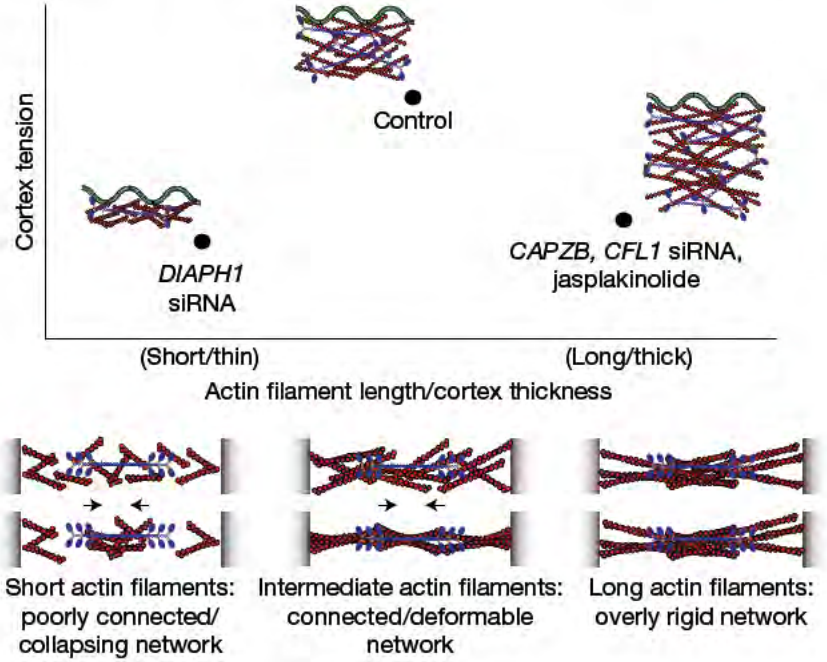


Chugh et al., Charras G. Salbreux G. and Paluch E. *Nature Cell Biology*. 2017

Regulation of Cortical tension

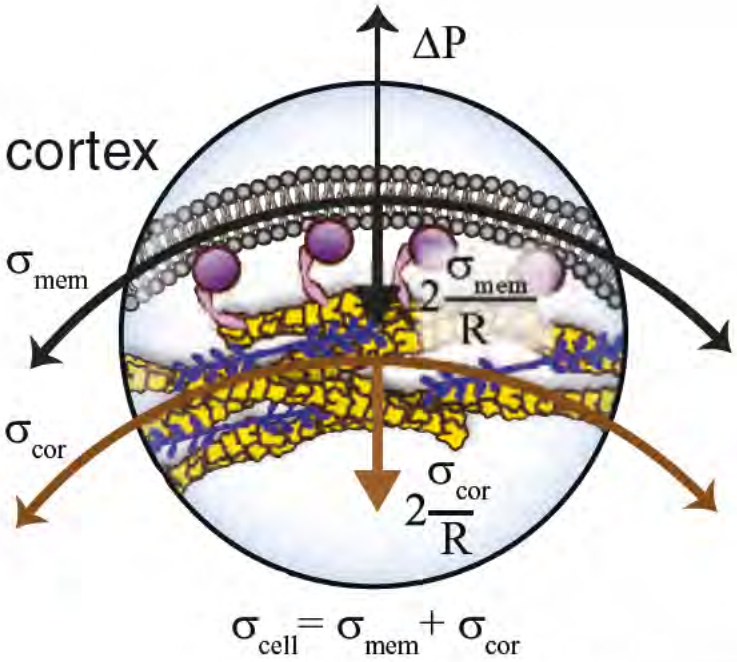
Importance of network architecture: actin filaments length

- Trade-off between network connectedness and stress asymmetry underlies tension generation in actomyosin networks



Chugh et al., Charras G. Salbreux G. and Paluch E. *Nature Cell Biology*. 2017

Membrane vs Cortical Tension



- In migrating cells membrane tension is often a control parameter.
- What about epithelial cells: at cell interfaces, adhesive processes, with strong coupling to contractile actomyosin network.

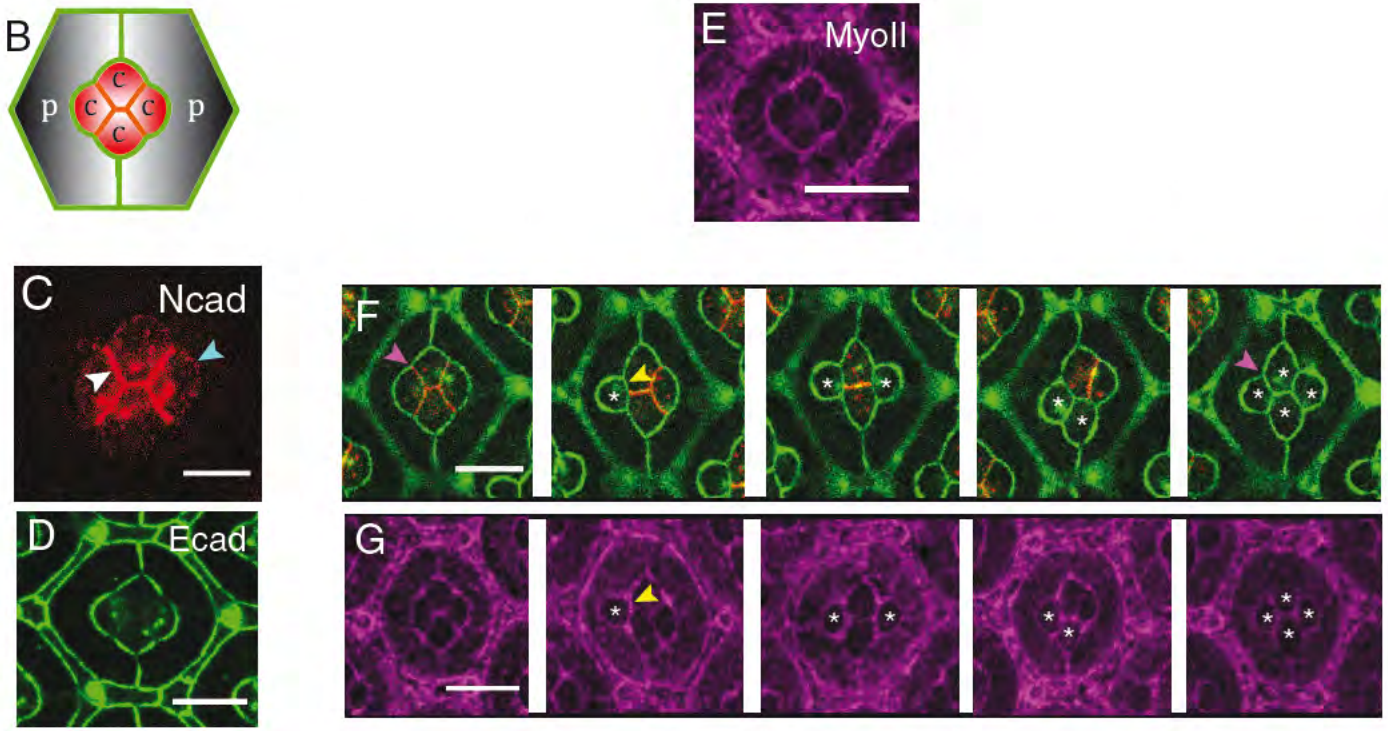
- Cell tension = Membrane tension + Cortical tension

Sens P and Plastino J. *J. Phys.: Condens. Matter.* 27:273103. 2015

Contribution of cortical tension to interfacial tension

Configuration of cell aggregates: adhesion versus cortical tension

- Cell arrangements dependent on differential expression of N-cad and E-cad.
- But Myosin-II is differentially localised at cell interfaces
- N-cadherin affects Myosin-II distribution (adhesion impacts on cortical tension?)

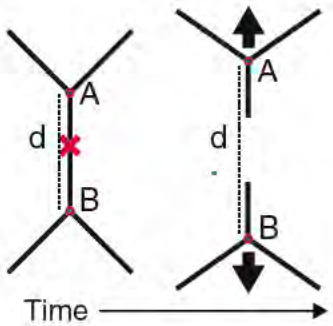


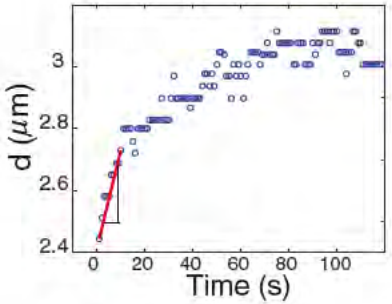
Chan E. HY. et al. Lenne PF. *eLife* 2017;6:e22796

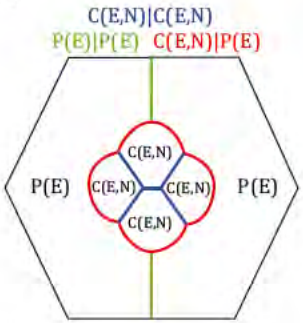
Contribution of cortical tension to interfacial tension

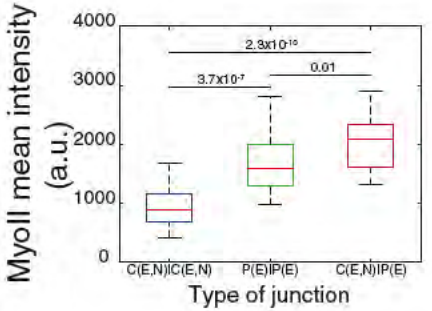
Configuration of cell aggregates: adhesion versus cortical tension

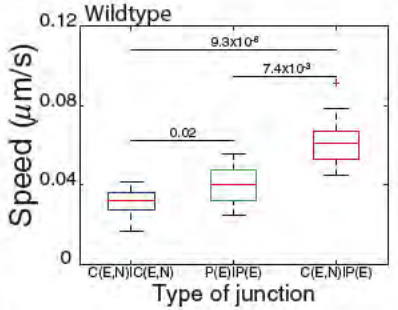
- Myosin-II distribution correlates with interfacial tension
- Free N-cadherin (not homophilically engaged) affects interfacial tension











Chan E. HY. et al. Lenne PF. *eLife* 2017;6:e22796

Contribution of cortical tension to interfacial tension

Configuration of cell aggregates: adhesion versus cortical tension

- Thermodynamic model

$$E = \sum_{\text{contact } ij} \gamma_{locij} l_{ij} + \sum_{\text{cells } i} \frac{K}{2} \frac{(p_i - p_{0i})^2}{p_{0i}}$$

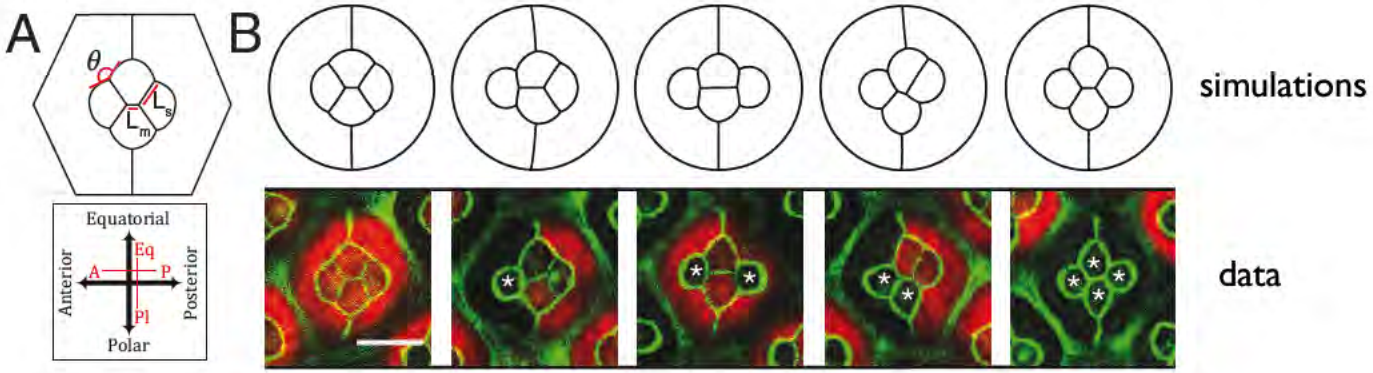
$$\gamma_{loc} \approx \gamma - 2K \frac{\Delta p}{p_o} \qquad \frac{\Delta p}{p_o} \approx 8\% \quad \text{(laser cuts)}$$

γ: global interfacial tension (from ablation)

elastic term

K used as fit parameter between simulations and data

$K \approx 4.2$ (normalised to $\gamma_{C(E,N)|C(E,N)}=1$)



* N-cad mutant cells

Chan E. HY. et al. Lenne PF. *eLife* 2017;6:e22796

Contribution of cortical tension to interfacial tension

Configuration of cell aggregates: adhesion versus cortical tension

- Thermodynamic model: cortical Myosin-II tension dominates adhesion contribution to interfacial tension

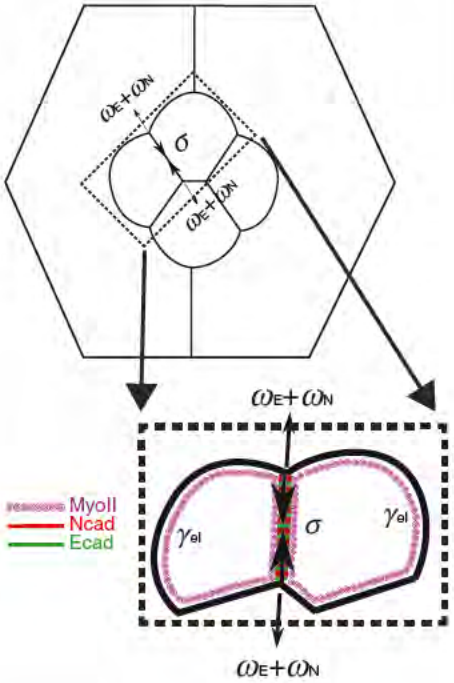
$\gamma_{loc} = \sigma - \omega$ and $\omega = \omega_E + \omega_N$

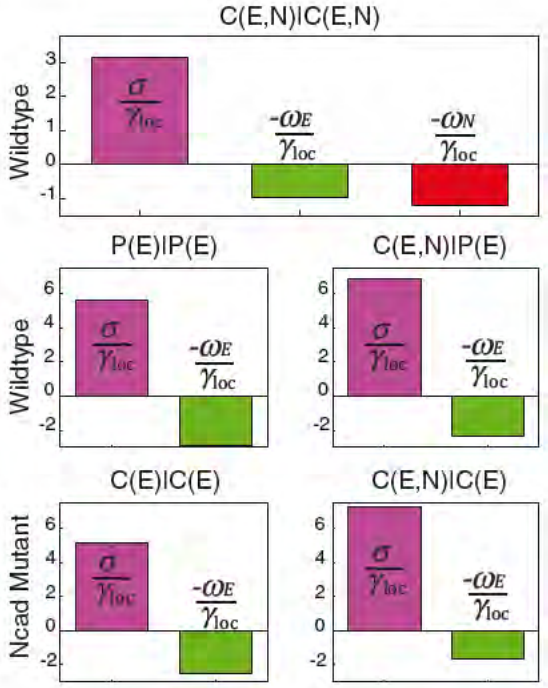
σ: cortical tension; ω: adhesion

γ_{loc} obtained from ablation data (γ) and modeling

σ, ω_E, ω_N set to be proportional to [MyoII] and [E-cad], [N-cad]

and extracted using least square fit method

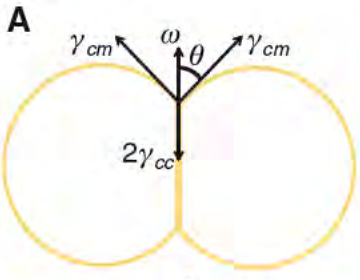




Chan E. HY. et al. Lenne PF. *eLife* 2017;6:e22796

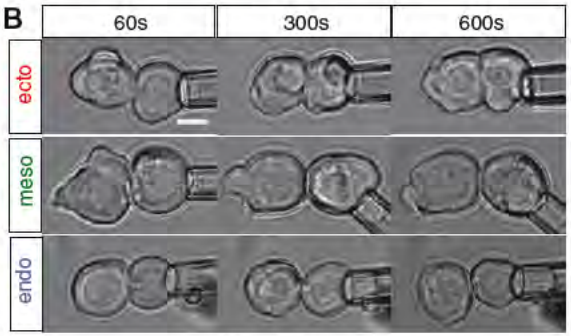
Contribution of cortical tension to interfacial tension

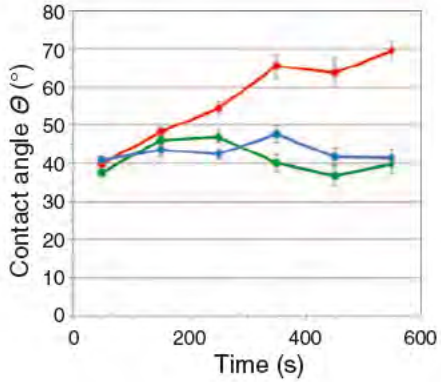
Embryonic germ layers: adhesion versus cortical tension
(Zebrafish)



$$\cos\theta = \frac{2\gamma_{cc} - \omega}{2\gamma_{cm}}$$

$$\gamma_i = 2\gamma_{cc} - \omega$$





$$\theta_{ecto} > \theta_{meso \text{ or } endo}$$

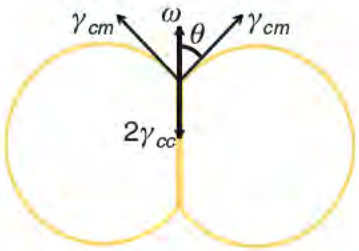
$$\left(\frac{\gamma_i}{\gamma_{cm}}\right)_{ecto} < \left(\frac{\gamma_i}{\gamma_{cm}}\right)_{meso \text{ or } endo}$$

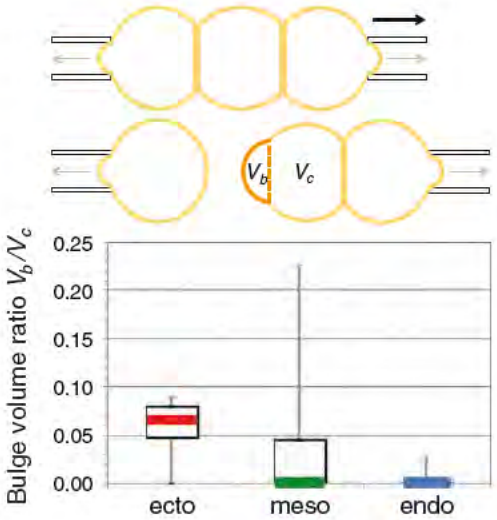
Maître JL. et al, Salbreux G. Jülicher F. Paluch E. and Heisenberg CP. *Science* 338:253-256 2017

Contribution of cortical tension to interfacial tension

Embryonic germ layers: adhesion versus cortical tension
(Zebrafish)

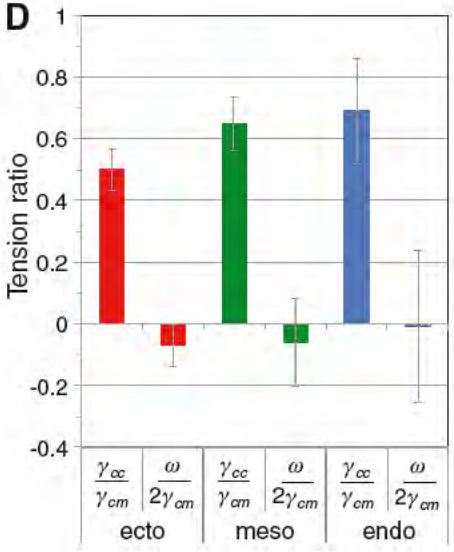
Determination of $\left(\frac{\gamma_{cc}}{\gamma_{cm}}\right)$

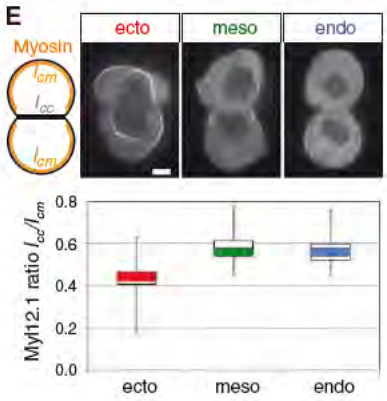




$Vb/Vc \propto 1/\left(\frac{\gamma_{cc}}{\gamma_{cm}}\right)$

$\left(\frac{\gamma_{cc}}{\gamma_{cm}}\right)_{ecto} < \left(\frac{\gamma_{cc}}{\gamma_{cm}}\right)_{meso}$





> Adhesion ω has little contribution to interfacial tension

Interfacial cortical tension correlates with Myosin-II density

Chief contribution of Myosin-II dependent cortical tension

Maître JL. et al, Salbreux G. Jülicher F. Paluch E. and Heisenberg CP. *Science* 338:253-256 2017

Back to cell sorting: a role for cortical tension?

Is Cell Sorting Caused by Differences in the Work of Intercellular Adhesion? A Critique of the Steinberg Hypothesis

ALBERT K. HARRIS

J. theor. Biol. (1976) 61, 267–285

- Differences between cell aggregates and liquids:

1. Cells are « active particles ».

2. Adhesion is much more than « close range attraction ». The forces that attract cells are not necessarily the same as those that hold cells together.

3. The work of adhesion need not be the same as the work of de-adhesion.

4. Adhesion molecules are not distributed uniformly and are mobile units.

- Alternative model of surface tension: differential cortical tension

(A) FIRST ALTERNATIVE: A DIFFERENTIAL SURFACE CONTRACTION HYPOTHESIS

The more strongly contractile a given cell type is over its exposed surface, the more internally it should sort out relative to other, less contractile, cell types. It should be made clear at this point that this hypothesis also presumes a degree of intercellular adhesiveness, and differs from Steinberg's hypothesis not in respect to whether intercellular adhesiveness occurs, but as to whether cell sorting and related phenomena are attributable to quantitative differences in adhesiveness.

A.K. Harris, *J. Theor. Biol.* (1976) 61:267

Conclusions

- Cortical tension is an important component of cell surface tension
- It emerges from Myosin motor contractility acting against cross linked actin filaments.
- Myosin are complex mechanoenzymes that follow an ATP-driven kinetic cycle converting the free energy of ATP binding, hydrolysis and ADP+Pi release into mechanical work.
- Myosins are kinetically tuned to adjust their duty ratio through the regulation of mini filaments assembly and collective dynamics.
- Actomyosin mechanical tension depends on motor activity, cross linkers dynamics and actin filament buckling that collectively allow the the buildup of internal stress.
- Cortical tension is tuned by actin cross linkers dynamics, actin filaments length and spatial organisation (parallel/antiparallel, isotropic/anisotropic, etc).
- Cortical tension exhibits spatial and temporal patterns in cells (*eg.* flows).
- It is the principal contributor of interfacial tension in adherent cells.

Perspectives

From cells to tissues…

- Adhesion and cortical tension are interdependent: mechanical feedbacks, boundary conditions, etc.
- Integration of adhesion and cortical tension: junction reinforcement or remodelling?
- Control of spatial patterns: impact on tissue patterns and dynamics.
- Self organisation: pulses, flows, trigger waves at cell and tissue levels.
- Mechanochemical coupling: material properties at cellular and tissue scales.

Colloque: le 10 Avril 2018

COLLÈGE DE FRANCE 1530

Mechanics and morphogenesis of cells, tissues and organs

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