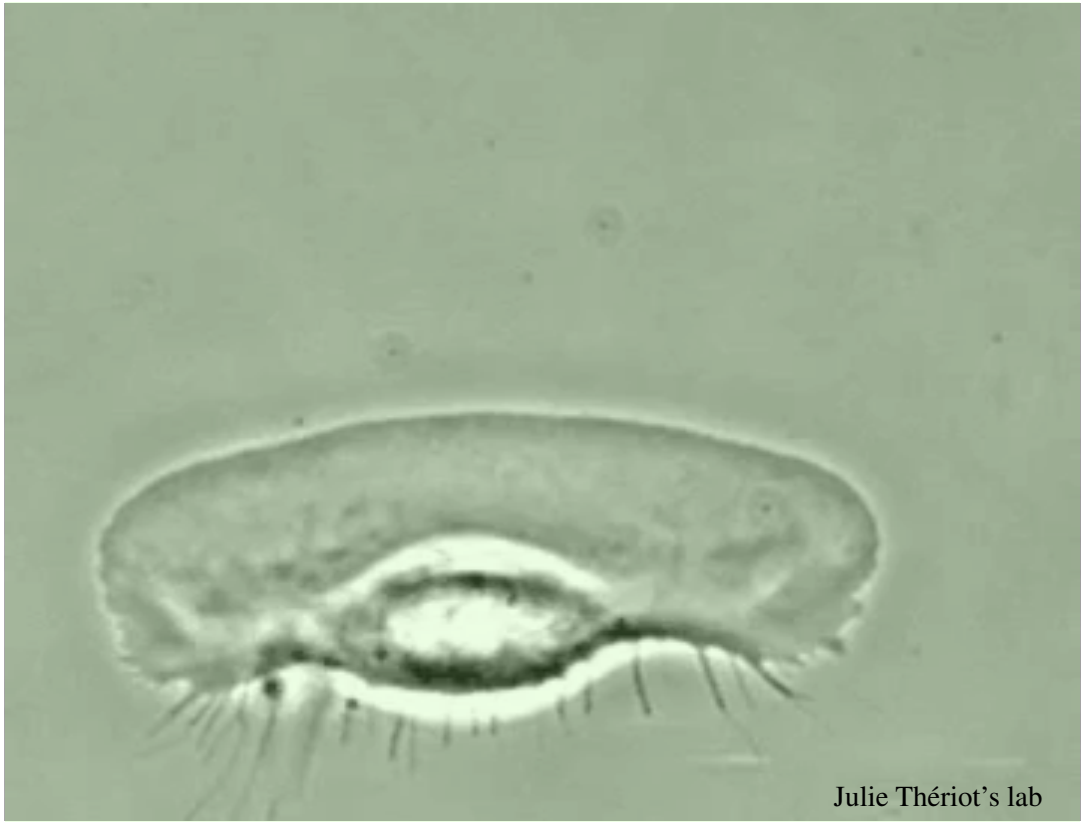
Cellular Motility



Course 2: Mechanics of cell crawling on substrate

Thomas Lecuit

chaire: Dynamiques du vivant

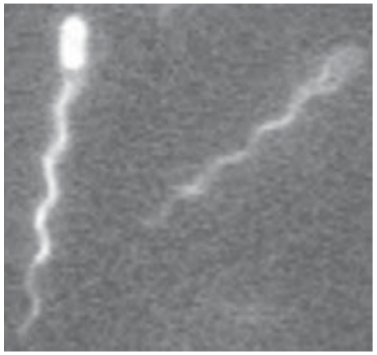
COLLÈGE DE FRANCE 1530

Summary - course 1

- Physical constraints on cell motility: low Reynolds number
  Inertia is negligeable. World dominated by viscosity

$$\mathrm{Re} = \frac{UL\rho}{\eta}$$

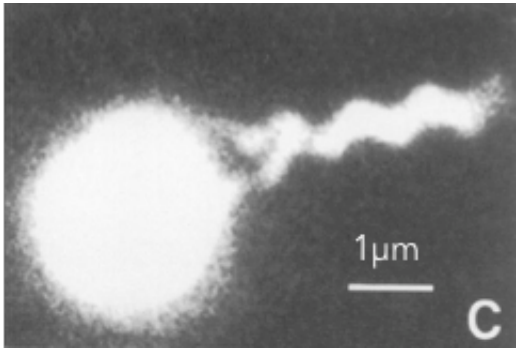
- Net forward movement requires non-reciproqual movement
  eg. Rotation of helical structures, beating of flexible filaments
- Convergent evolution of means of propulsion in viscous media (water, extracellular matrix in organisms).



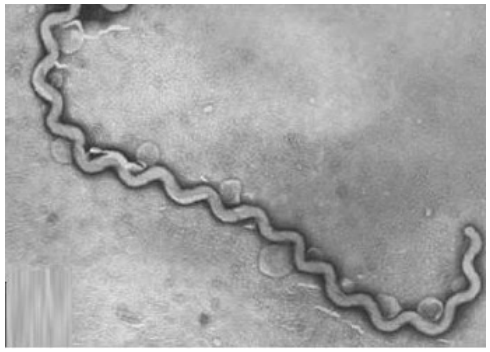
E. coli

20µm/s

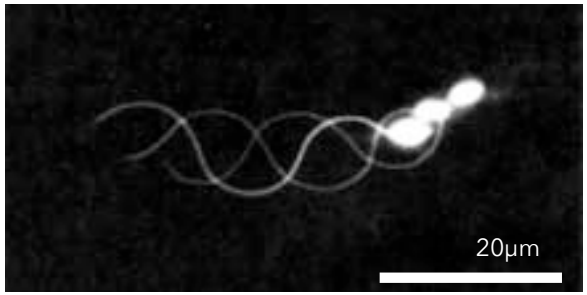
2 µm



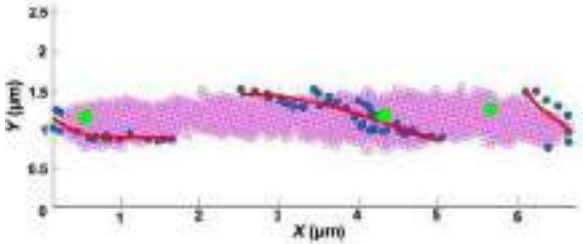
Archaea – Halobacteriaceae



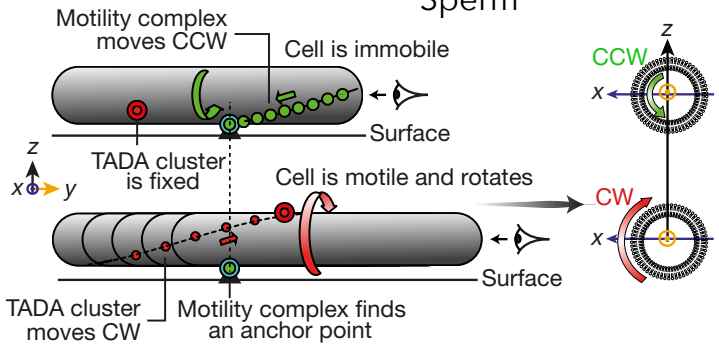
Spirochete



Sperm



Flavobacterium johnsoniae



Myxococcus xanthus 2µm/min

Summary - course 1

3 general problems

1. Decoding the environment: What is the nature of cues?
   - Cells don't move randomly but sense an external cue
   - What is the nature of external cues? Diversity of cues (chemical, mechanical, electric, light)
   - Temporal vs spatial decoding

2. Processing the cue: Cell polarisation
   - Symmetry breaking: converting external gradient into vectorial cell organisation
   - Deterministic vs Stochastic processing
   - Polarisation of a cell or a trajectory

3. Mechanical response: Principles of movement
   - Depends on environment
   - **Force generation:** Active processes: actin pushing forces, actin flow, actomyosin contractility
   - **Force transmission:** Passive resistance: friction/adhesion, viscous resistance of medium.

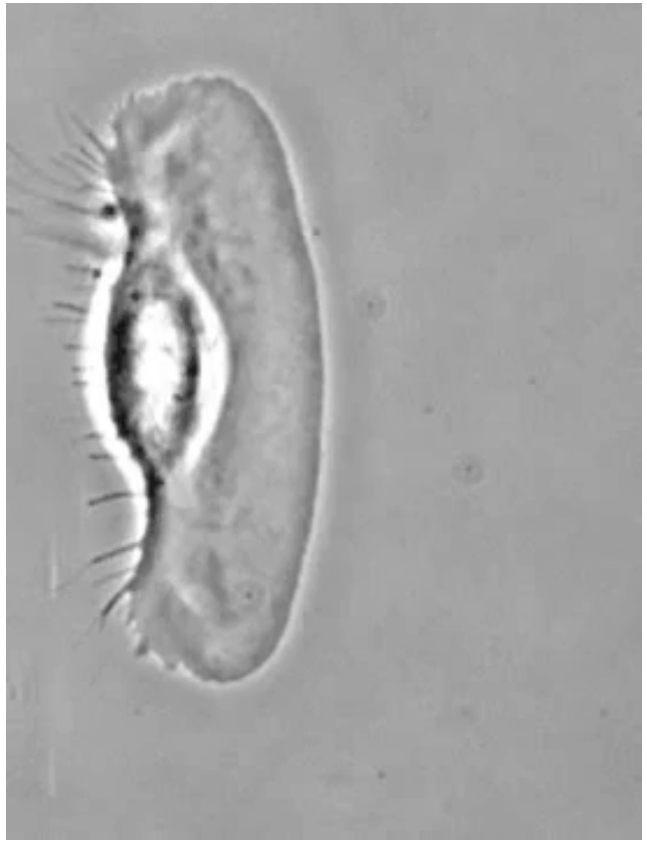
Mechanics of cell crawling on substrate in 2D

1. **Force generation:** Active processes: actin pushing forces, actin flow, actomyosin contractility

2. **Force transmission:** Passive resistance: friction/ adhesion, viscous resistance of medium.

2D cell motility on a substratum

- Cell adhesion to substrate
- The engine of motility:
  - — actin polymerisation
  - — membrane tension
  - — cell adhesion
  - — cell contraction

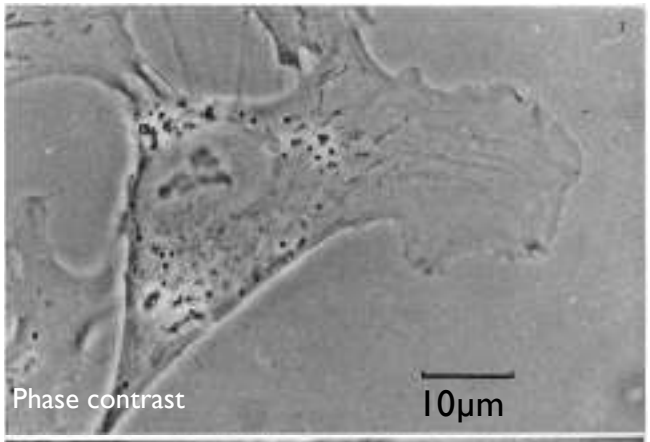


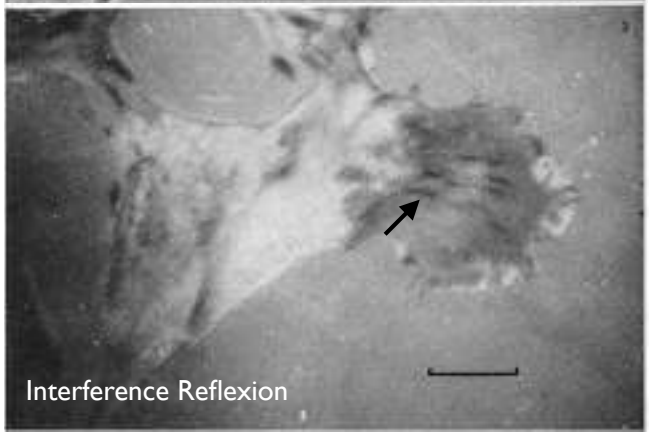
Julie Thériot's lab

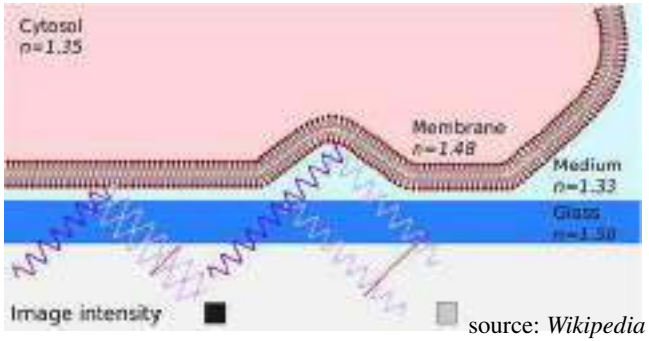
Cell contacts with a substrate

- Cells were first recognised to be pinned on the surface, at **adhesion sites**:
  - Use of reflexion interference microscopy
  - Focal contacts within 10-20 nm of glass surface
  - Coined by Izzard and Lochner (1976)
- Focal contacts are adhesion sites: stationary as cells move
- Cells are motile so motility entails dynamics of adhesion sites

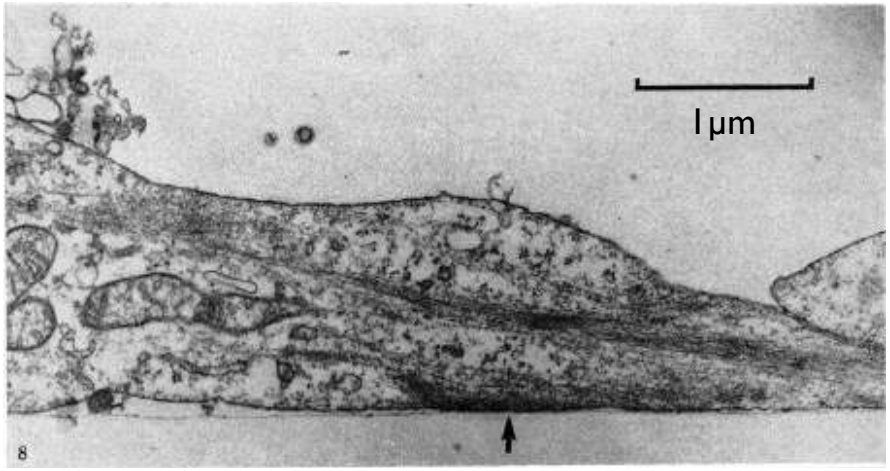
primary chick embryo heart fibroblast







source: *Wikipedia*

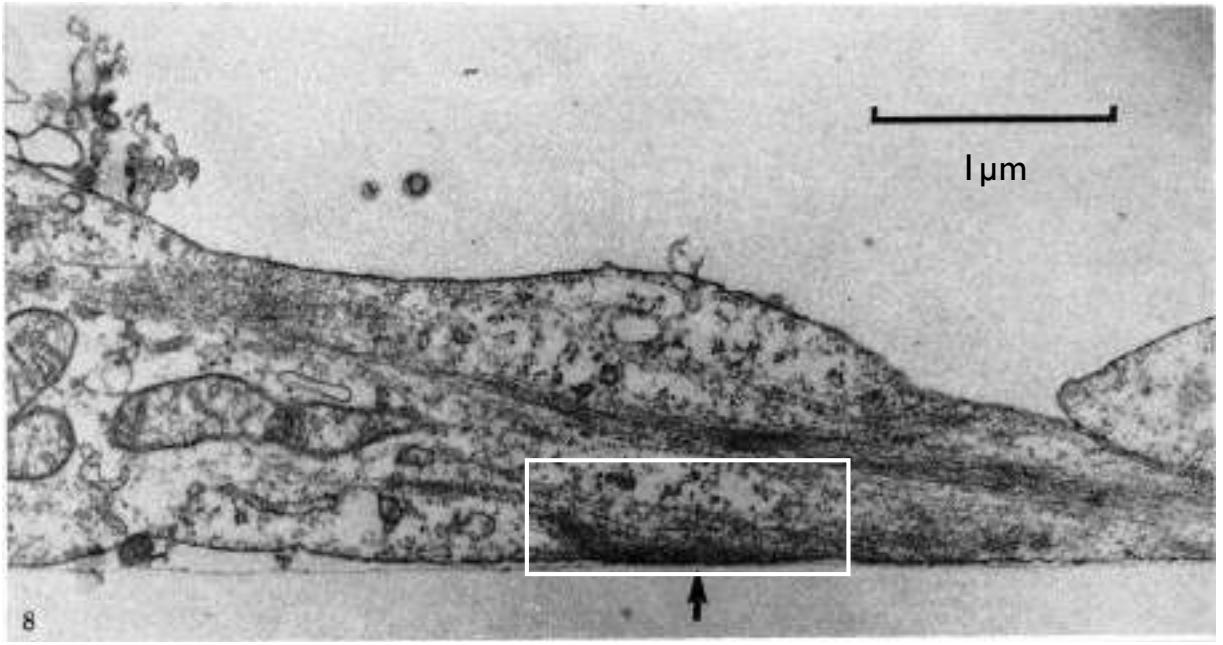


M. Abercrombie The Croonian Lecture: The Crawling Movement of Metazoan Cells *Proceedings of the Royal Society of London.* 207, 129-147 (1978)

Curtis, A. S. G. *J. Cell Biol.* 20:199-215. (1964)

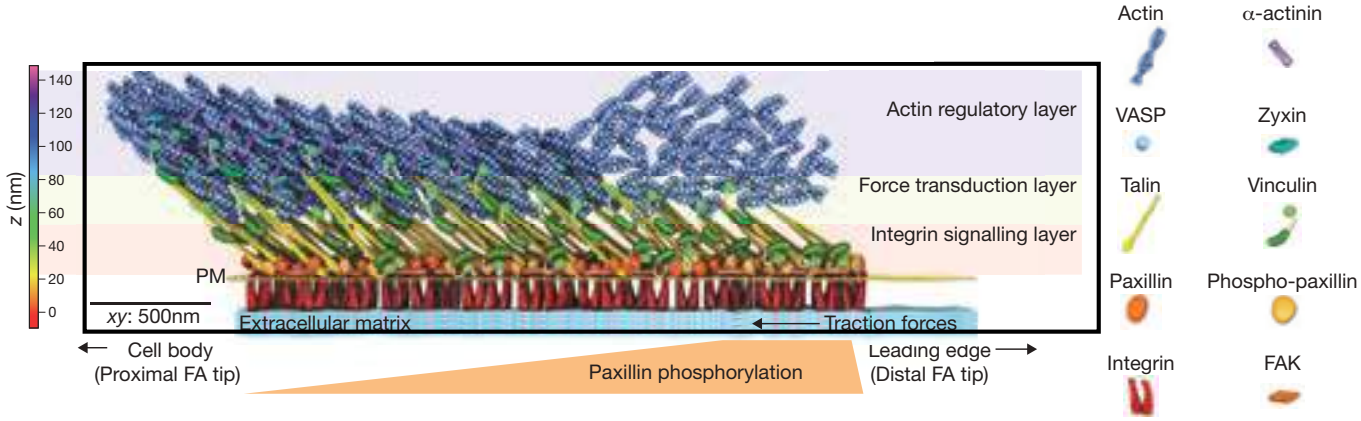
Izzard and Lochner *J. Cell Sci.* 21:129-160. (1976)

Adhesion to the substrate



M. Abercrombie *Proc. Royal Society.* 207:129-147 (1978)

PALM/STORM microscopy



Lindsay B Case and Clare Waterman *Nat Cell Biol*. 17(4):955-963 (2015)

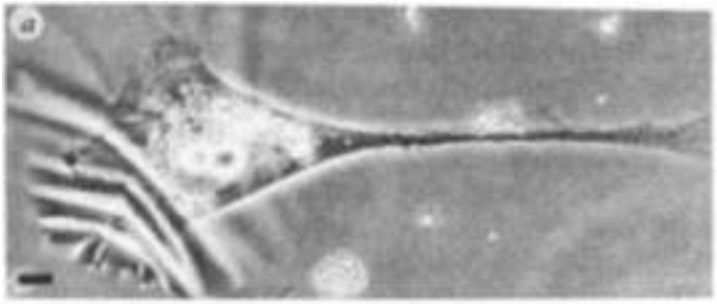
Kanchanawong, P. *et al.*. *Nature* **468,** 580–584 (2010).

Cell induced substrate deformation

Cells exert traction forces on substrates

Fibroblast traction as a mechanism for collagen morphogenesis

Albert K. Harris, David Stopak & Patricia Wild
Department of Zoology, University of North Carolina, Wilson Hall 045-A, Chapel Hill, North Carolina 27514, USA

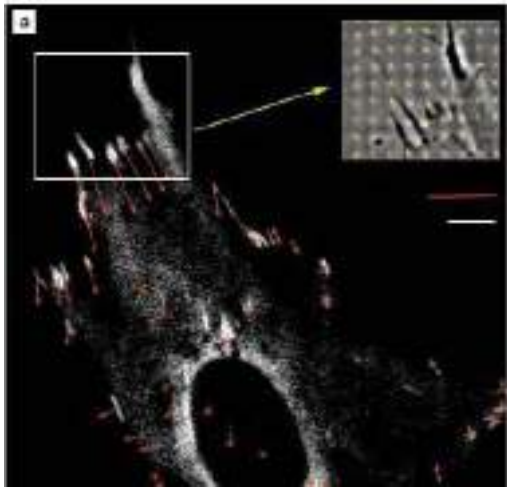


A. Harris, D. Stopak and P. Wild. *Nature*. 290:249-251 (1981)

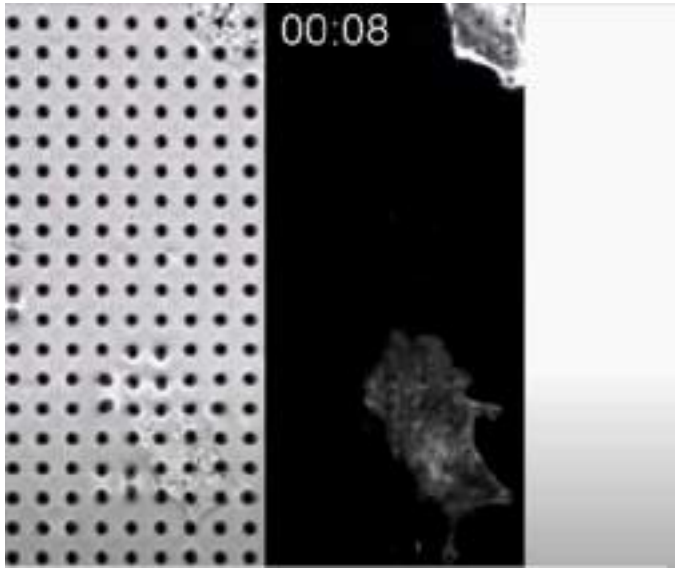
traction force microscopy

Human skin fibroblasts

GFP–vinculin



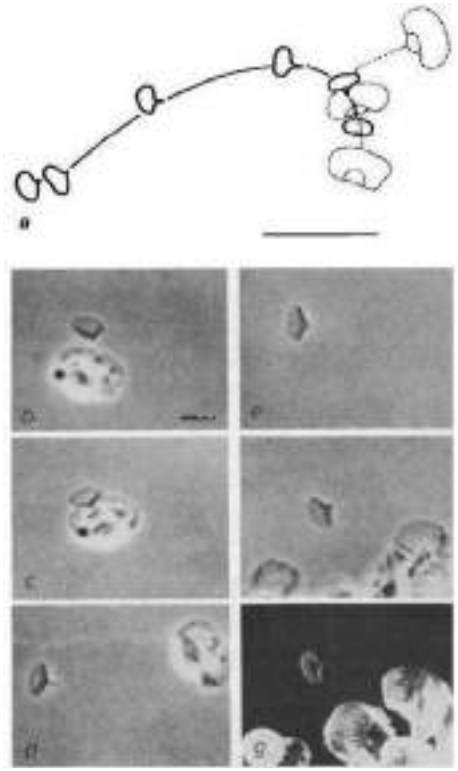
N. Balaban et al. and B. Geiger. *Nature Cell Biology*. 3: 466-472 (2001)



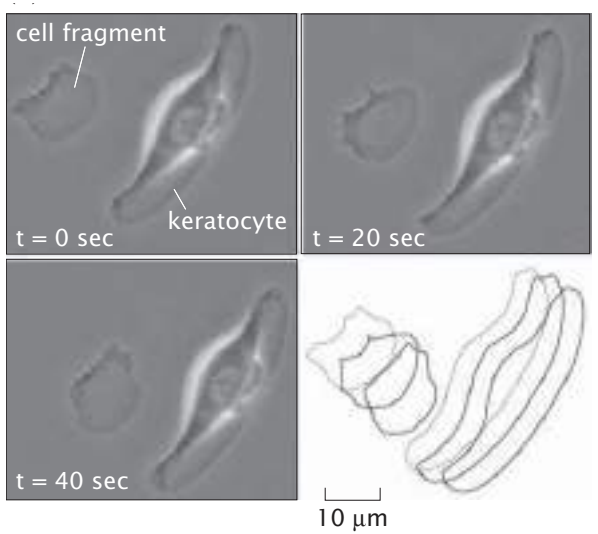
A GFP-integrin beta 1 expressing MDCK cell crawling on a miniature pillar array

The Lamellipodium: the « engine » of substrate motility

Cell fragments devoid of nucleus and microtubules are motile

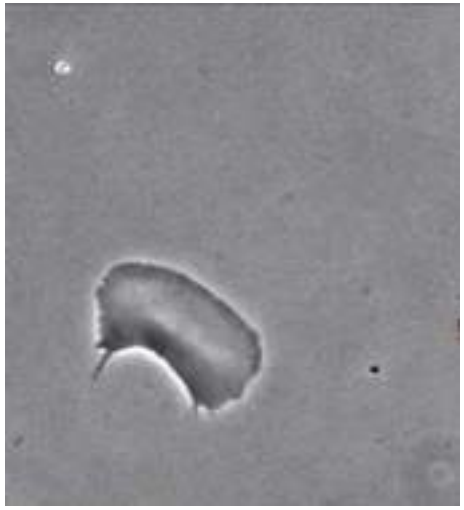


Euteneuer U, Schliwa M. *Nature*. 310(5972):58-61 (1984)



R. Phillips, J. Thériot, J. Kondev, H. Garcia *PBOC*

- cell fragment motility is persistent
- Its determinants (the « engine » of motility) must be present at the lamellipodium itself

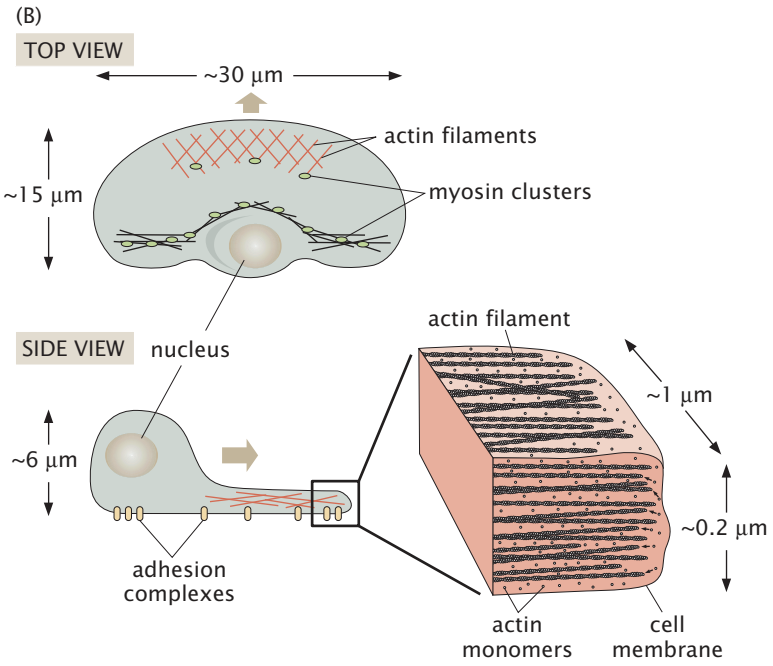


Erin Barnhart (Thériot lab), Stanford

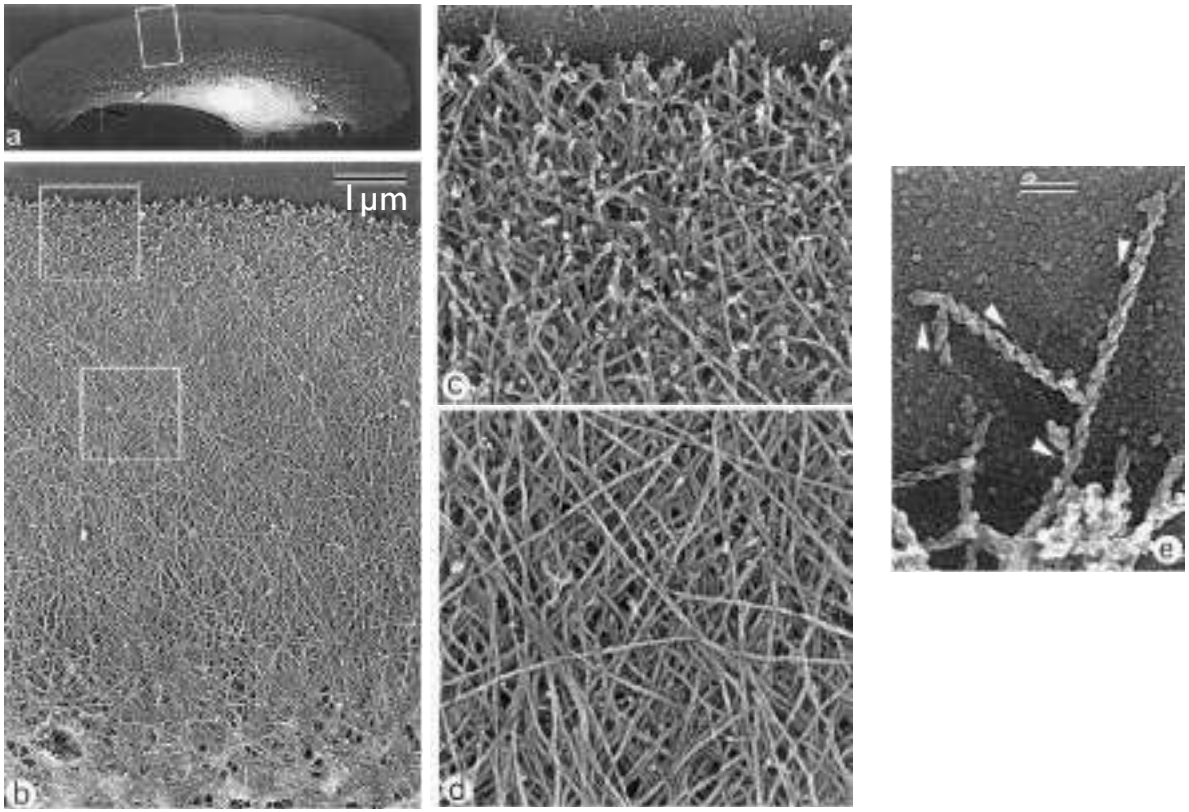
Composition of lamellipodium

Actin architecture

- A branched actin network in the lamellipodium
- Barbed end of actin filaments towards the leading edge



R. Phillips, J. Thériot, J. Kondev, H. Garcia *PBOC*



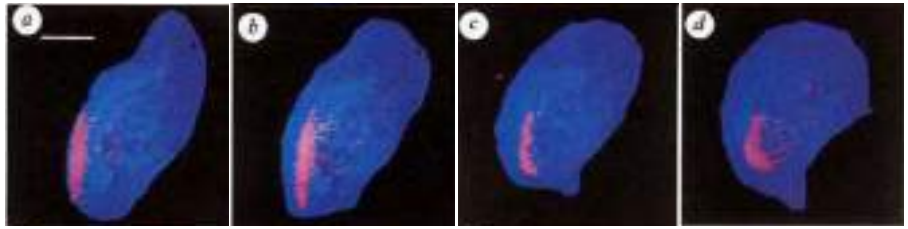
T. Svitikina and G. Borisy. *Journal of Cell Biology*, 139, 397–415 (1997)

Dynamics of lamellipodium

Actin turnover and treadmilling

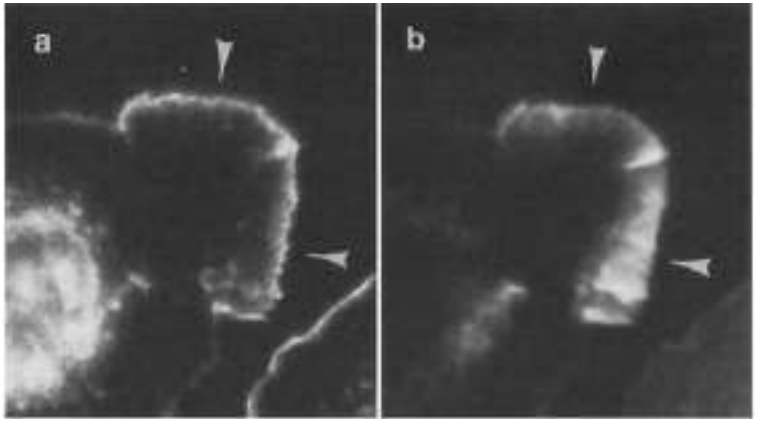
- actin filaments grow from the margin of the lamellipodium
- filaments are « transported » inward in cells but remain stationary in the substrate referential
- The speed of retrograde actin flow correlates with cell movement
- **Model: Treadmilling of actin from the margin**

Caged fluorescent G-actin



4s 48s 81s 136s

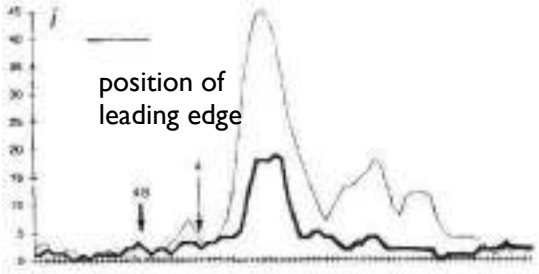
(time after photoactivation/un-caging)



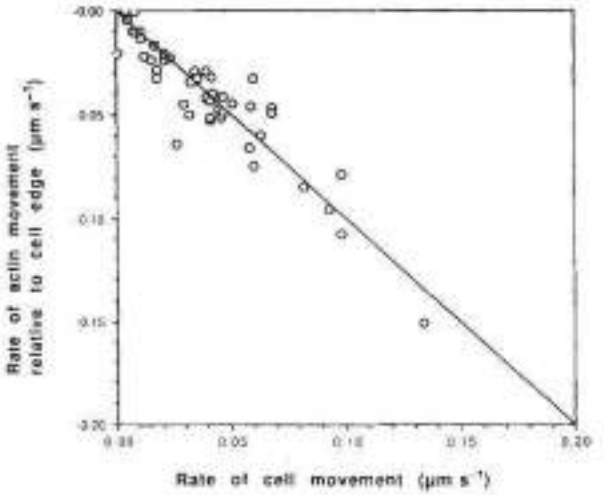
cells were permeabilized in the presence of fluorescein-phalloidin for 2 min, rinsed over the course of 2 min, and subsequently incubated for 4min with Rhodamine-actin. (a) Rhodamine , and (b) fluorescein-phalloidin stain

Symons, M.H., and Mitchison, T.J. *J. Cell Biol. 114*, 503–513. (1991)

fluorescence intensity



position in lab/substrate referential



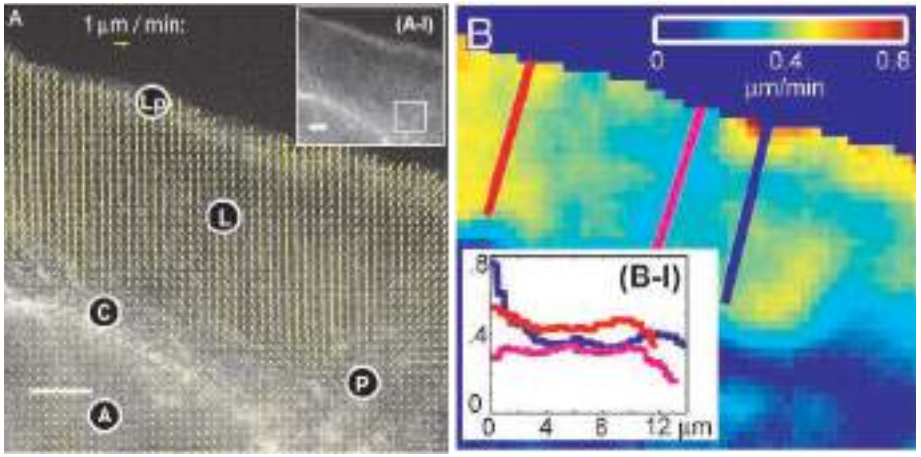
Theriot, J.A., and Mitchison, T.J. *Nature* 352, 126–131. (1991)

Dynamics of lamelipodium

Actin turnover and treadmilling

- **Fluorescent speckle microscopy:**

—Based on low incorporation rate of fluorescenct G-actin
—speckle tracking (particle imaging velocimetry)

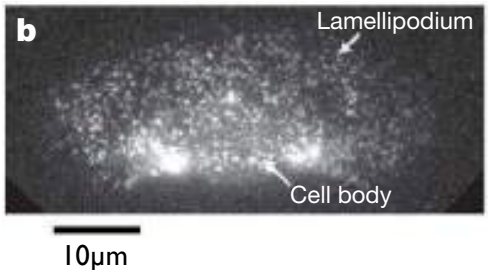


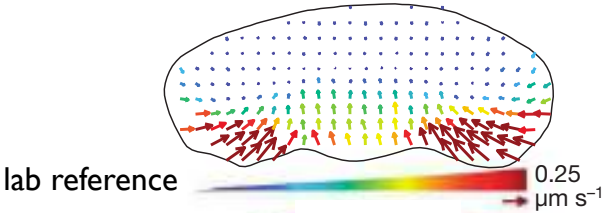
—Calculate assembly and disassembly maps from actin intensity $I(t)$ and divergence of FSM flow field $\nabla \cdot \mathbf{v}(\mathbf{x}, t)$

Net turnover rate of actin:

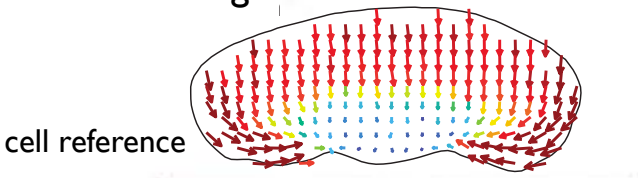
$$\sigma(\mathbf{x}, t) = \partial I(\mathbf{x}, t)/dt + I(\mathbf{x}, t)\mathrm{div}(\mathbf{v}(\mathbf{x}, t)) + \nabla I(\mathbf{x}, t)\mathbf{v}(\mathbf{x}, t).$$

Vallotton, P., Gupton, S. L., Waterman-Storer, C. M. & Danuser, G. *PNAS.* 101, 9660–9665 (2004).





- F-actin retrograde flow



- Polarization of assembly/disassembly of F-actin

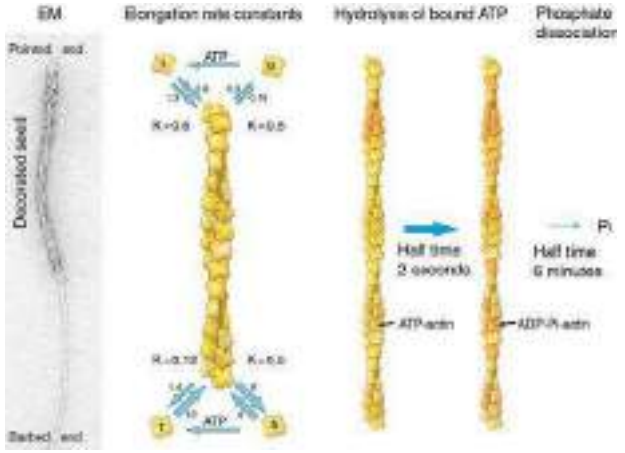
C. Wilson et al. and G. Danuser and Julie Thériot. *Nature* 465, 373–379. (2010)

Dynamics of lamelipodium

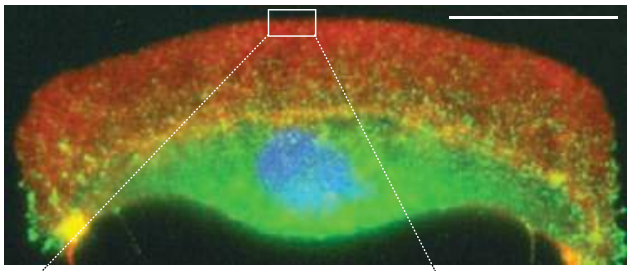
Polarized actin nucleation at the leading edge
Actin turnover

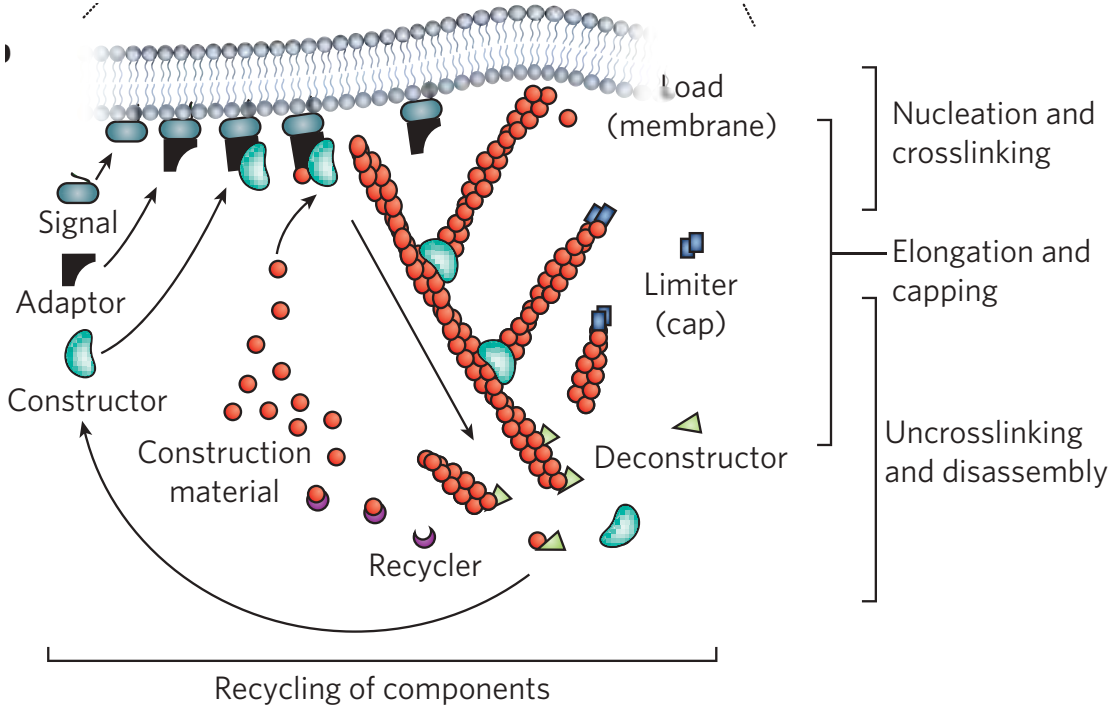
- Actin treadmils very slowly on its own
- Actin nucleators promote faster treadmilling at the leading edge

- Branched actin nucleation by the Arp2/3 complex
- Individual filaments do not treadmil, rather, the actin array treadmils as a whole by nucleation at the cell front and disassembly at the back



dissociation constant *K* (μM) of ATP-actin at barbed end lower than at pointed end





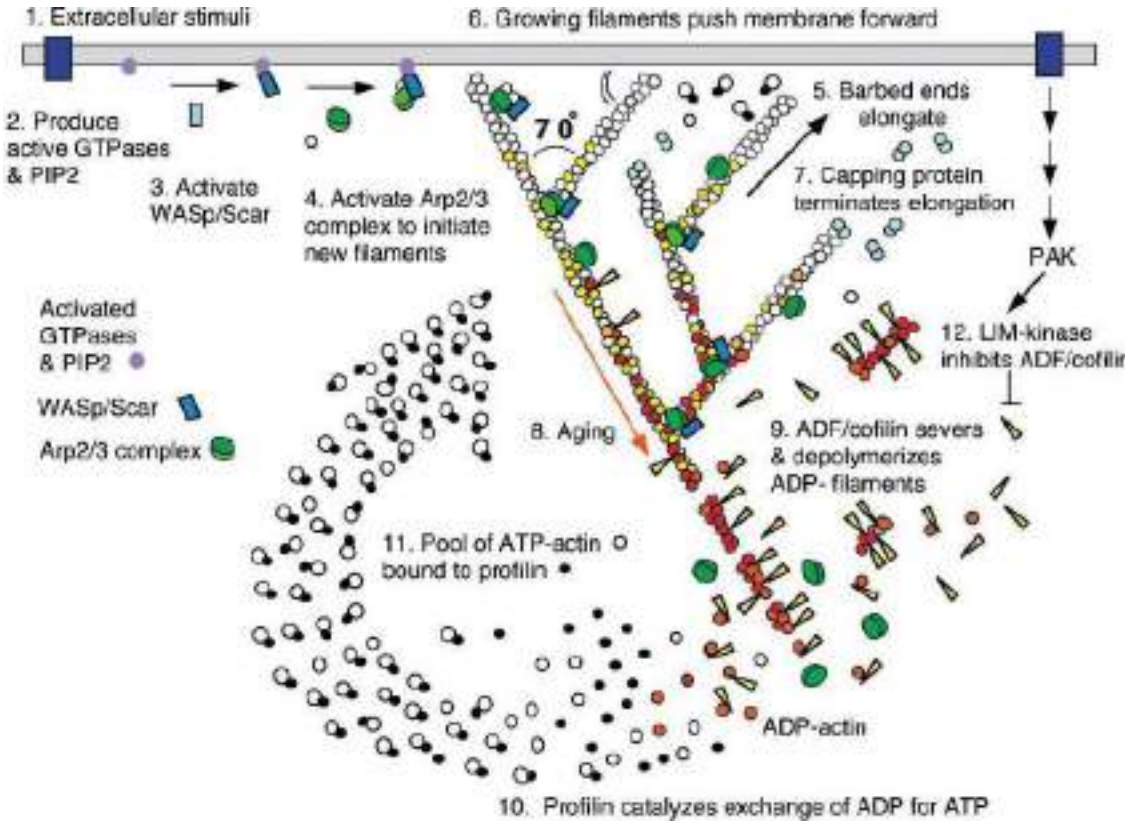
DA Fletcher, RD Mullins *Nature* 463 (7280), 485-492

Dynamics of lamelipodium

Polarized actin nucleation at the leading edge
Actin turnover

- **Mechanisms of fast actin polymerization**
  — polymerization competent subunits at a 100x the critical concentration for pure actin
  —Profilin associates with ATP-actin and blocks incorporation at pointed ends
  —Mechanisms that reduce polymerization to maintain a sufficient pool of competent subunits far from equilibrium: Capping at barbed ends and ADF/Cofilin which breaks filaments.

- Importance of capping: restricts filament growth at the leading edge. Induce stronger pushing forces (see later).



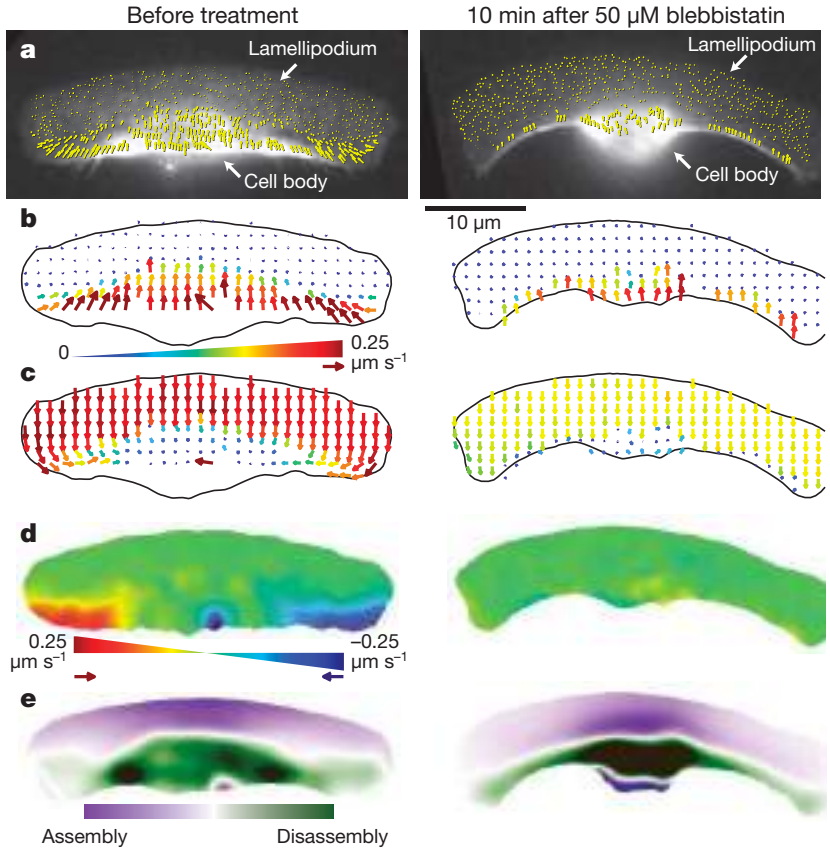
T. Pollard and G. Borisy. *Cell* 112:453-465 (2003)

Actin turnover requires cell contraction

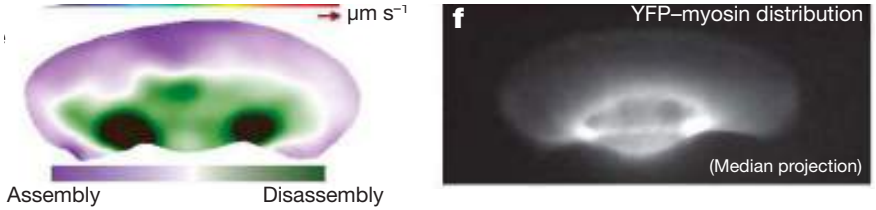
Keratocytes

- ADF/Cofilin breaks filaments and contributes to maintaining a high pool of monomers to sustain polymerization
- Actomyosin contraction also contributes to actin filaments disassembly.

Inhibition of myosin II with blebbistatin blocks inward flow and alters the pattern of disassembly of the actin network



Correlation between actin disassembly and MyosinII localisation



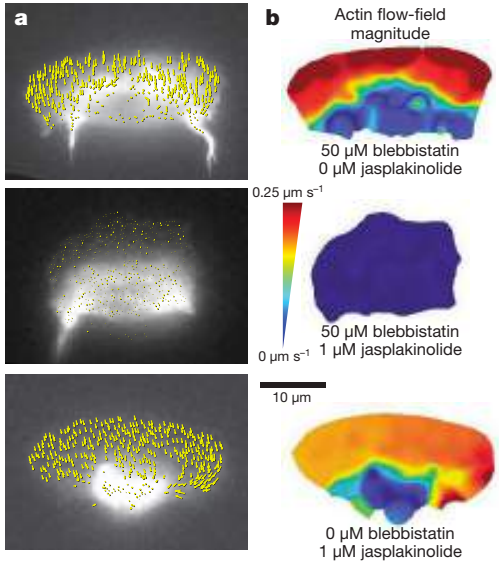
C. Wilson et al. and G. Danuser and Julie Thériot. *Nature* 465, 373–379. (2010)

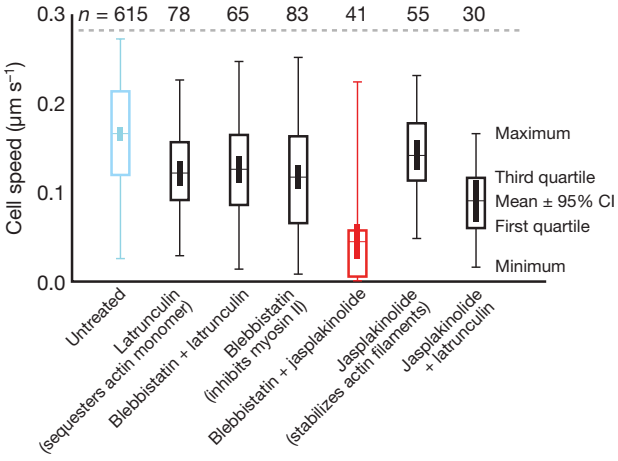
Actin turnover requires actomyosin contraction

Keratocytes

- Two parallel pathways for actin disassembly in a keratocyte:
- MyosinII induced disassembly and actin turnover (induced by cofilin and other regulators)

Jasplakinolide reduces actin turnover by blocking depolymerization.
Belbbistatin inhibits MyosinII
—Synergistic effect of jasp and blebbistatin
Cell movement and actin retrograde flow are completely blocked





C. Wilson et al. and G. Danuser and Julie Thériot. *Nature* 465, 373–379. (2010)

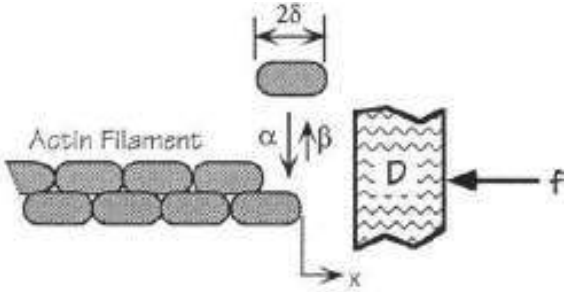
Mechanism of force production by actin polymerization

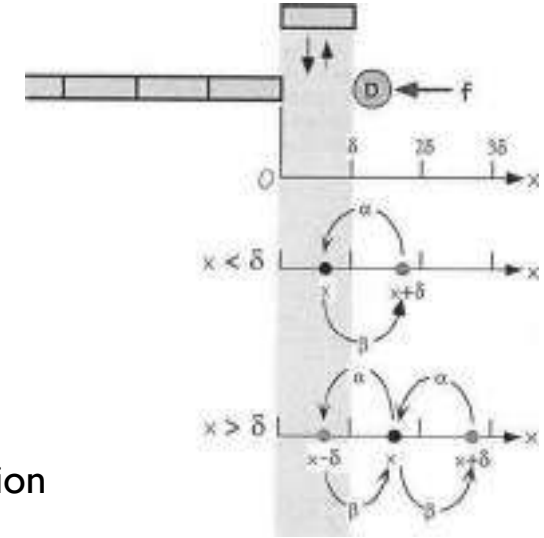
The model of « Brownian ratchet »

- **The problem:**

  Extension of lamelipodia is driven by actin polymerization, but no motor is involved.
  The free energy drop associated with polymerization is sufficient to deform the membrane
  However, what is the mechanism for energy transduction that produces a mechanical force?

- **The model:**





Polymerization rate: $R = k_{on}(x) \times M - \beta$ and $\begin{cases} k_{on}(x) \times M = \alpha & \text{if} \quad x \geq \delta \\ k_{on}(x) \times M = 0 & \text{if} \quad x < \delta \end{cases}$

Velocity without load: $v = \delta \times R \sim 0.75\,\mu m/s$ at $25\,\mu M$ actin monomer concentration

Velocity of polymerization ratchet $v = \delta[\alpha e^{-\omega} - \beta]$

where $\omega = f \times \delta / k_B T$

is the dimensionless load force
or, equivalently, the work done against load (ie. membrane) by adding one monomer

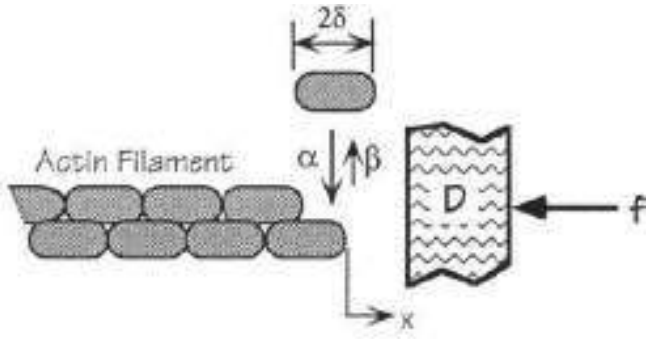
The polymerization rate α is weighted by the probability of the load allowing a gap the size of a monomer

C. Peskins, G. Odell and G. Oster. *Biophysical Journal* 65:316-324 (1993)

Mechanism of force production by actin polymerization

The model of « Brownian ratchet »

Not a biased random walk in which the jump probabilities are asymmetric and diffusion is biased
Here diffusion is unbiased



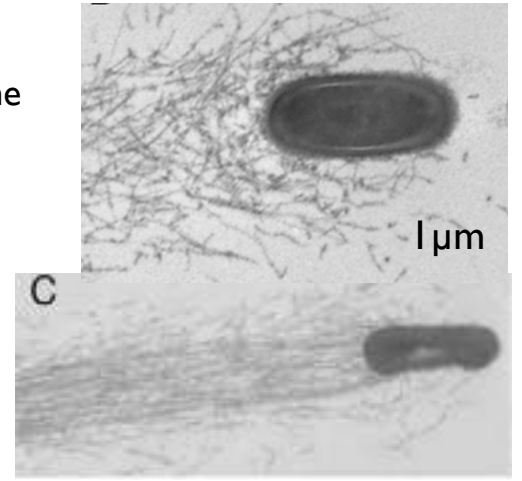
Actin polymerization rectifies the thermal fluctuations of the load occurring at the membrane: renders unidirectional the random fluctuations (diffusion) of load.

The origin of the force for movement arises from thermal fluctuation of the load.
But the free energy of binding of actin monomer to filament is large enough with respect to thermal fluctuations to drive the ratchet forward:

If $\Delta G_b \sim k_B T$ then monomer would dissociate before back diffusion of membrane or due to load associated with it

stall force: $f_0 = -\frac{k_B T}{\delta} \ln\left(\frac{\beta}{\alpha}\right) \sim 7.8$ pN

load force given membrane tension: 25 pN
So a few filaments can easily push the membrane



Brieher et al. *JCB.* 2004 http://www.jcb.org/cgi/doi/10.1083/jcb.200311040

This model also accounts for the motility of the bacteria *Listeria monocytogenes* in cells, driven by actin comet tails

C. Peskins, G. Odell and G. Oster. *Biophysical Journal* 65:316-324 (1993)

Mechanism of force production by actin polymerization

Elastic Brownian ratchet

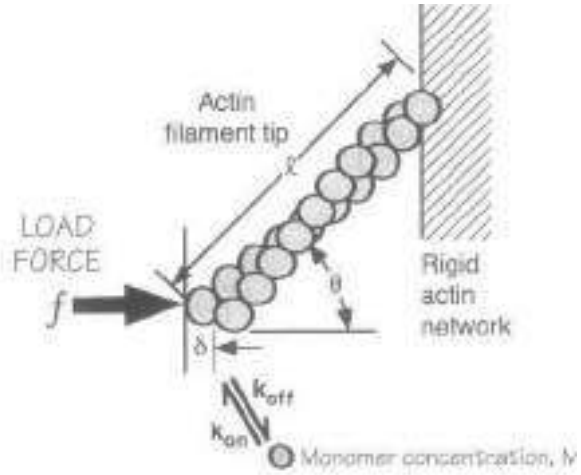
The thermal fluctuations of the load (membrane) are not quite sufficient to produce deformations
Brownian ratchet model: velocity depends on diffusion coefficient of load $v = \frac{2D}{\delta}\left[\frac{(\mu - \omega)(\omega^2/2)}{\omega^2 + (e^{\omega} - \omega - 1)\mu}\right]$

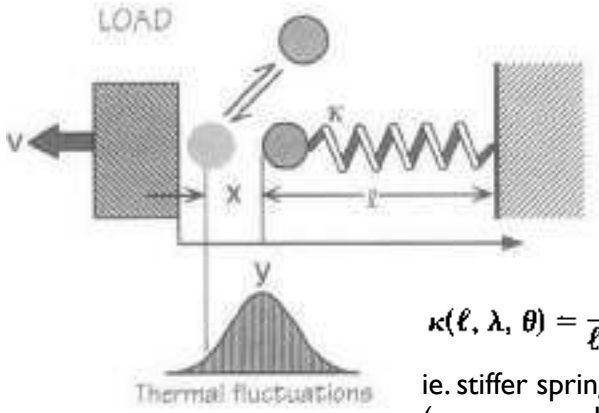
- **The model: consider now the thermal fluctuations of the elastic polymerizing filament**

  thermal fluctuations induce filament bending
  the bending modulus B of actin filaments determines the extent of fluctuations
  the persistence length λ of filament reflects this: $B = \lambda k_B T$

Andrew Ward et al, *Nature Materials* (2015)

an actin filament at angle θ with respect to a load is modelled as a 1D spring:





$$\kappa(\ell, \lambda, \theta) = \frac{4\lambda k_B T}{\ell^3 \sin^2(\theta)}$$

ie. stiffer spring for low angles (near perpendicular to membrane)

A. Mogilner and G. Oster. *Biophysical Journal* 71:3030-3045 (1996)

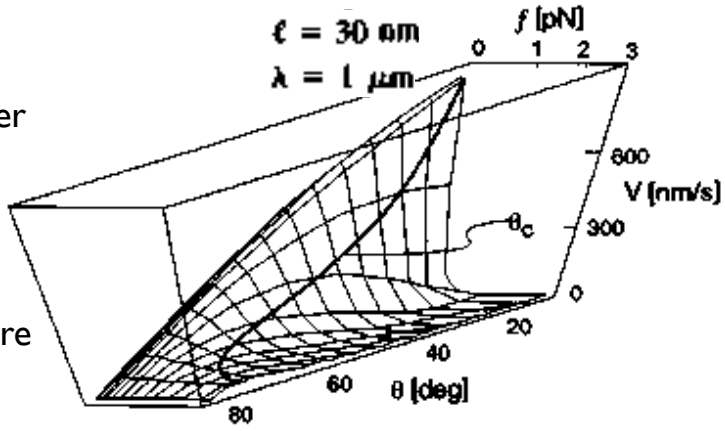
Mechanism of force production by actin polymerization

Elastic Brownian ratchet

- **Result:**

$$V \approx \delta \cos(\theta)[k_{on} M p(\theta, f) - k_{off}]$$

load-velocity relation: depends on angle



$p(\theta, f)$: probability of a gap of sufficient size and duration to allow addition of 1 monomer
It depends on spring stiffness, which depends on persistent length of filament and filament length. $\kappa(\ell, \lambda, \theta) = \frac{4\lambda k_B T}{\ell^3 \sin^2(\theta)}$

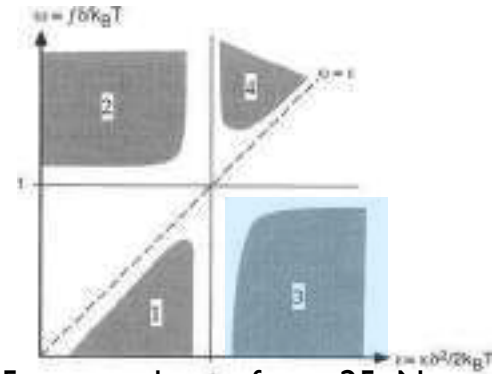
At low angles thermal fluctuations cannot sufficiently bend filament for filament growth. At large angles, the thrust associated with polymerization is lower. So there must be an optimum in between.

3 dimensionless parameters define the different regimes:

$\omega = f\delta/k_B T$, dimensionless work of the load force to bend a filament by δ

$\varepsilon = \kappa_0 \delta^2/2k_B T$, mean elastic energy stored in filament sufficiently bent to intercalate one monomer.

$\hat{f} = \omega/2\varepsilon = f/\kappa_0 \delta$ load force relative to the force required to bend a filament by one intercalation distance, δ



Lamelipodium

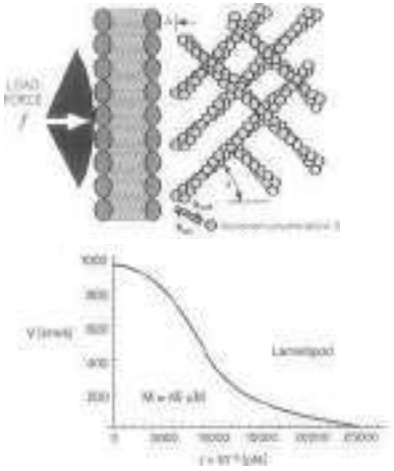
load force/filament

$f \sim 0.035$ pN

$\omega \sim 0.02 \ll 1$

$\kappa_0 \sim 0.6$ pN/nm, $\varepsilon \sim 0.6$

$\theta_c \sim 48°$



predicted stall force for strip of 5µm membrane front: 25nN
(5000 filaments and 5pN stall force/filament)
measurement: 45nN to stop advancing membrane in keratocyte

The model predicts that filaments grow more and more parallel to membrane as the resistance force increases

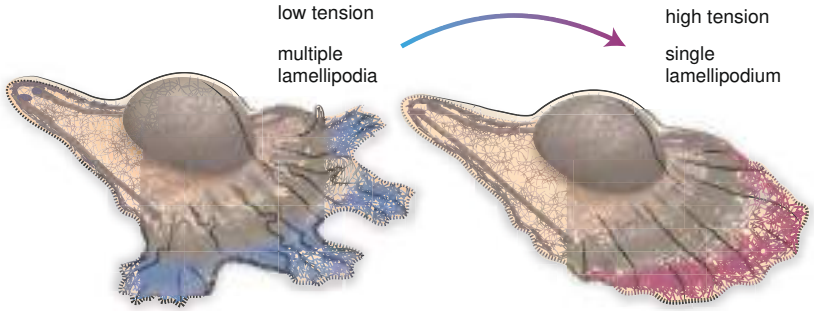
A. Mogilner and G. Oster. *Biophysical Journal* 71:3030-3045 (1996)

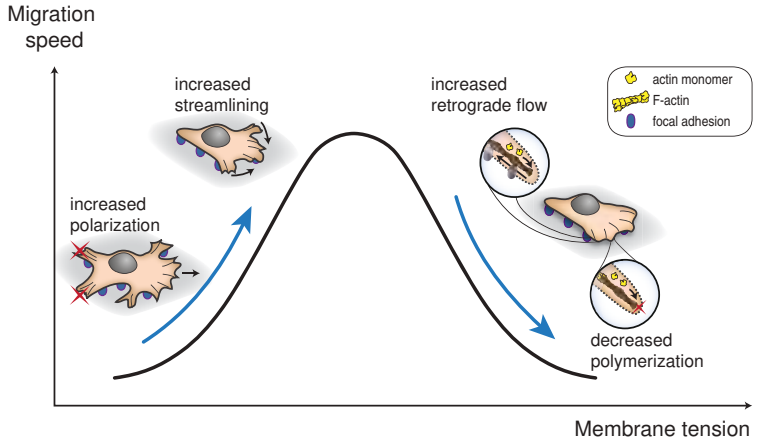
Importance of membrane tension

Feedback between membrane tension and actin polymerization during cell motility

- Membrane tension depends on available membrane area, cytoskeletal activity (actin polymerization) and cell-substrate adhesion (wetting forces)
- Membrane tension in turn affects actin polymerisation and cell migration

—load force in Brownian ratchet models



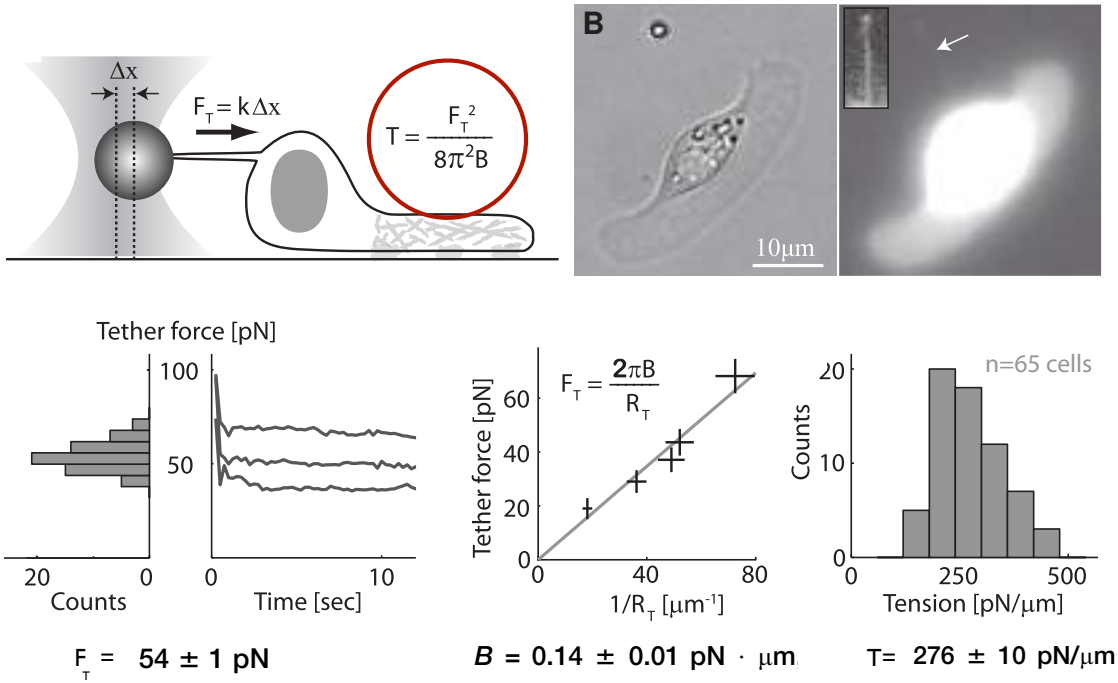


P. Sens and J. Plastino. *J. Phys.: Condens. Matter* 27 (2015) 273103 (13pp)

Membrane tension and cytoskeletal forces

Feedback between membrane tension and actin polymerization during cell motility

- **Measurement of cell membrane tension using an optical tweezer**



F_T = 54 ± 1 pN

Tether forces

B = 0.14 ± 0.01 pN · μm

Bending modulus (links tether force F_T and tether radius R_T)

T= 276 ± 10 pN/μm

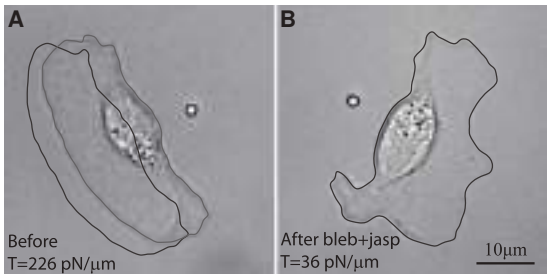
Membrane tension

Lieber AD. et al, Theriot J, and Keren K. *Current Biol.* 23:1409. (2013)
A. Houk et al. O. Weiner. *Cell* 148, 175–188 (2012)

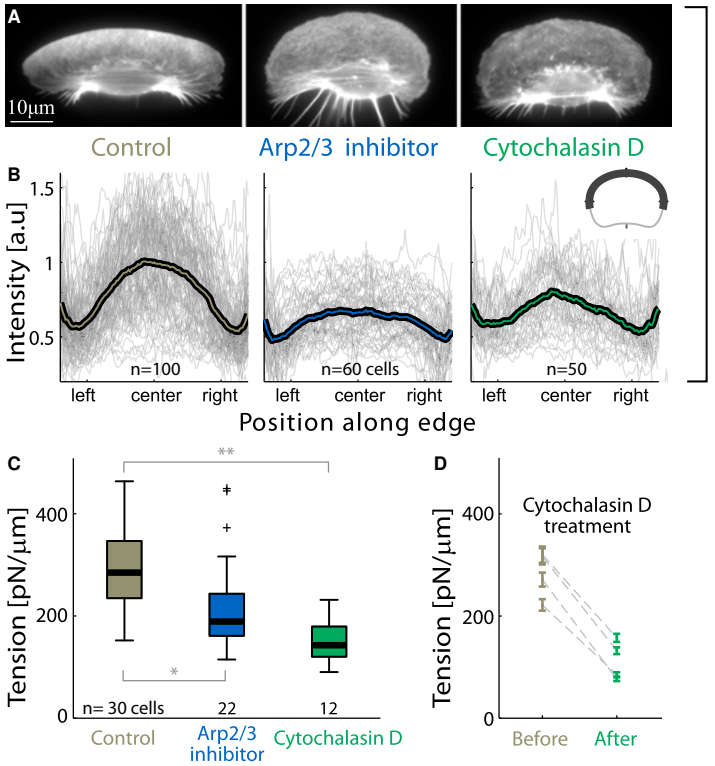
Membrane tension and cytoskeletal forces

Feedback between membrane tension and actin polymerization during cell motility

- Membrane Tension requires an « active » cytoskeleton (turnover and contraction)



- Tension is enhanced by actin based protrusive forces at cell front



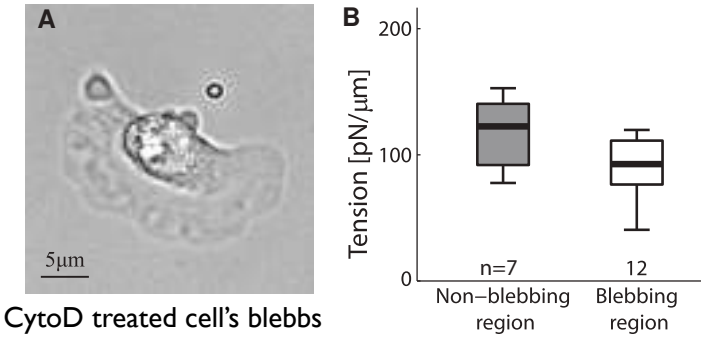
F-actin density (phalloidin)

Lieber AD. et al, Theriot J, and Keren K. *Current Biol.* 23:1409. (2013)
A. Houk et al. O. Weiner. *Cell* 148, 175–188 (2012)

Membrane tension and cytoskeletal forces

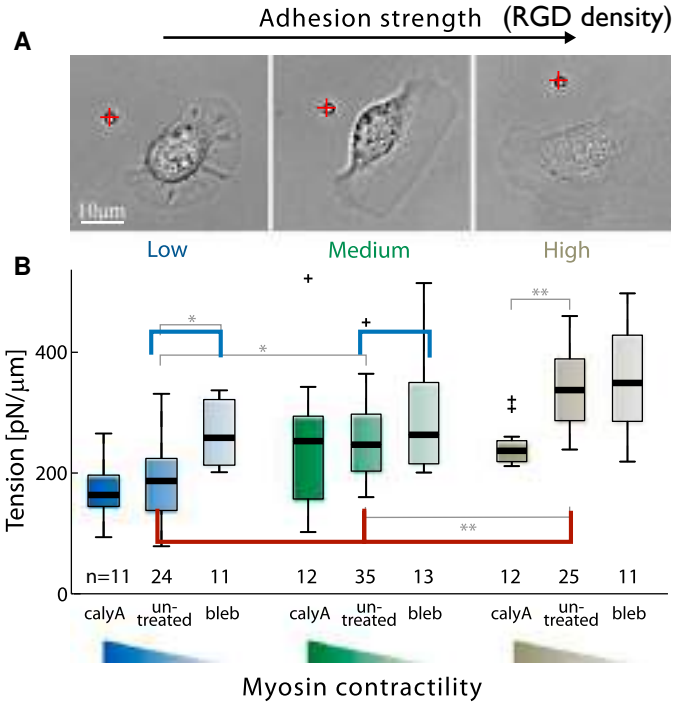
Feedback between membrane tension and actin polymerization during cell motility

- In-plane membrane tension is the main contributor of tension in keratocytes (tension in blebs similar to non-bleb regions)



CytoD treated cell's blebbs

- Tension is enhanced by cell-substrate adhesion and low contractility



Lieber AD. et al, Theriot J, and Keren K. *Current Biol.* 23:1409. (2013)
A. Houk et al. O. Weiner. *Cell* 148, 175–188 (2012)

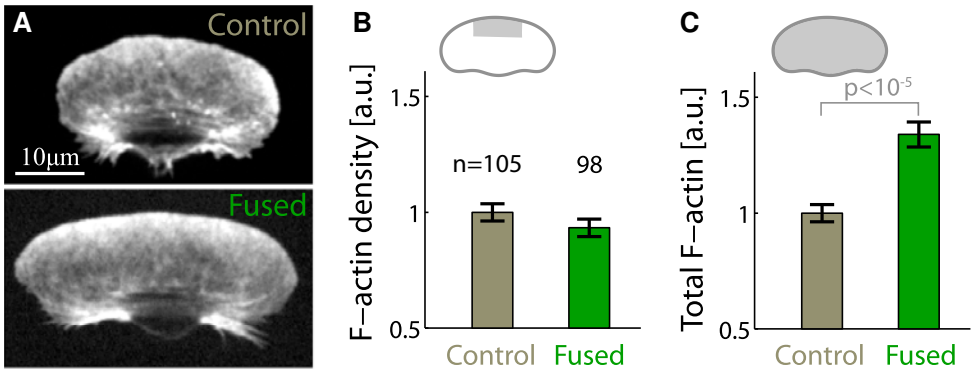
Membrane tension and cytoskeletal forces

Feedback between membrane tension and actin polymerization during cell motility

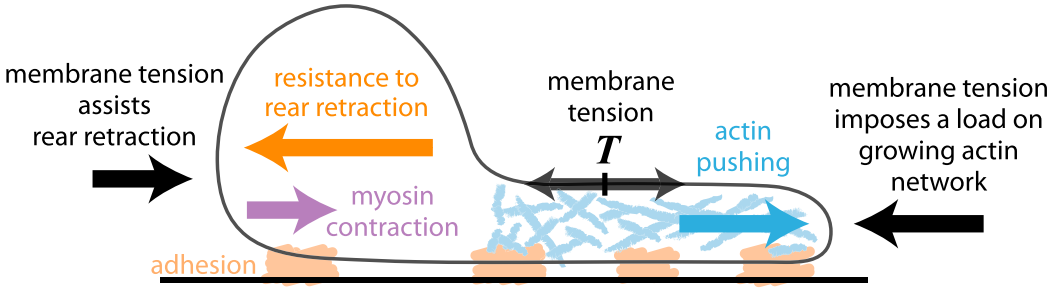
- Cells adjust actin polymerisation to membrane surface area so as to maintain membrane tension:

>Feedback of tension on actin polymerization (consistent with Brownian ratchet model)**?**

>Homeostasis

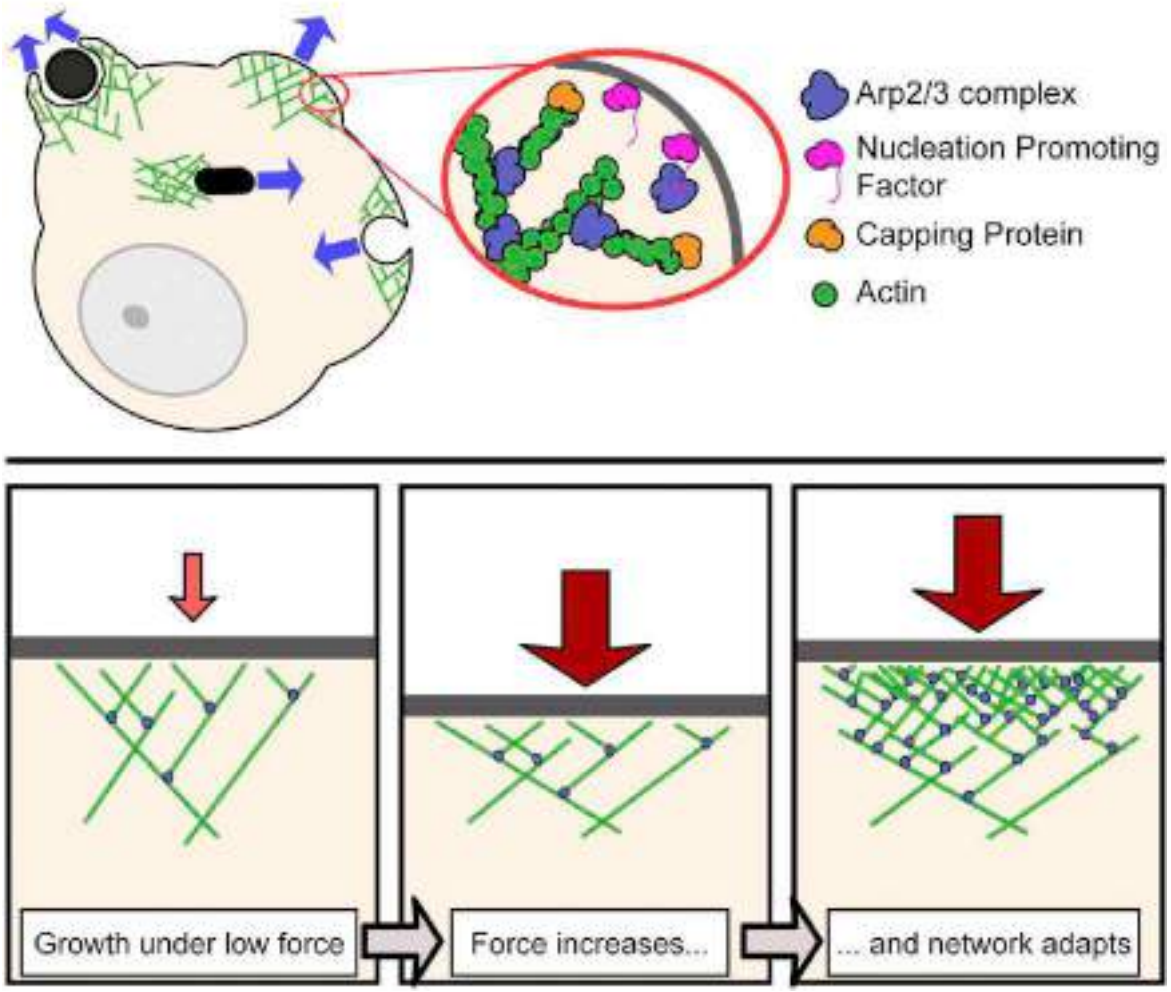


- Membrane tension is determined by mechanical force balance between actin pushing forces, load exerted by membrane tension, myosin contraction and adhesion to substrate.



Lieber AD. et al, Theriot J, and Keren K. *Current Biol.* 23:1409. 2013

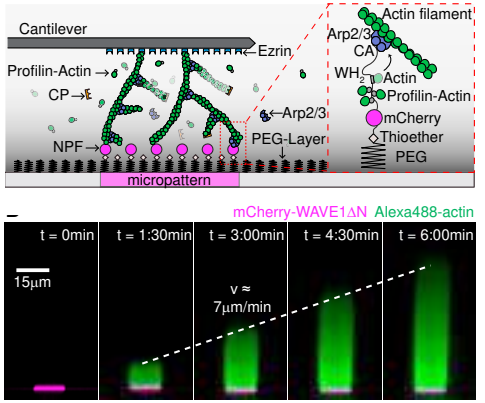
Force feedback on branched actin network architecture and mechanics



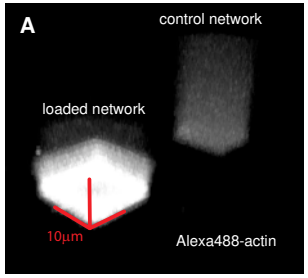
Bieling et al., D. Fletcher and D. Mullins *Cell* 164, 115–127 (2016)

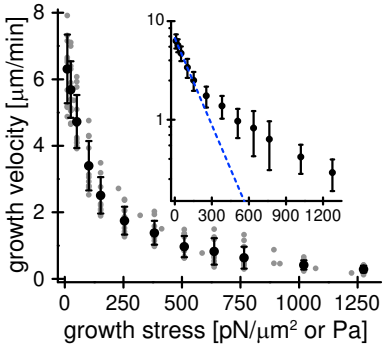
Force feedback on branched actin network architecture and mechanics

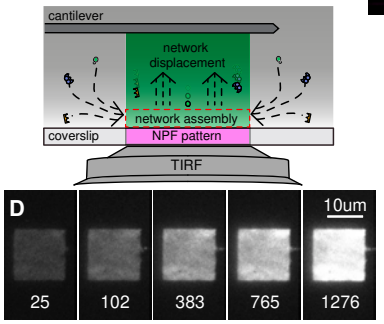
- An in vitro assay to assess actin assembly in response to a load



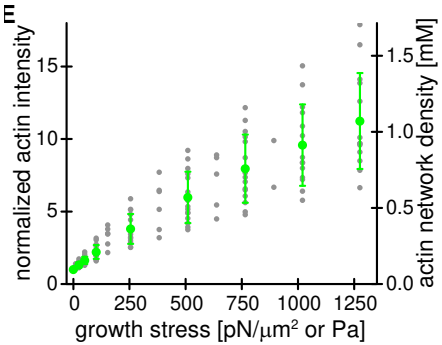
- Force feedback increases actin density

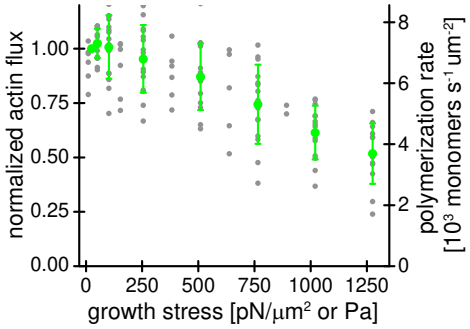


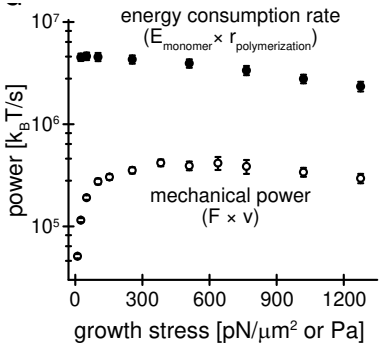


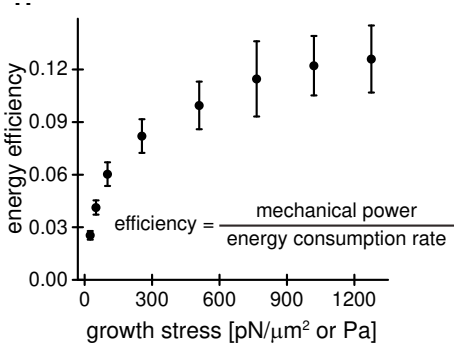


- Force feedback increase mechanical efficiency of actin network







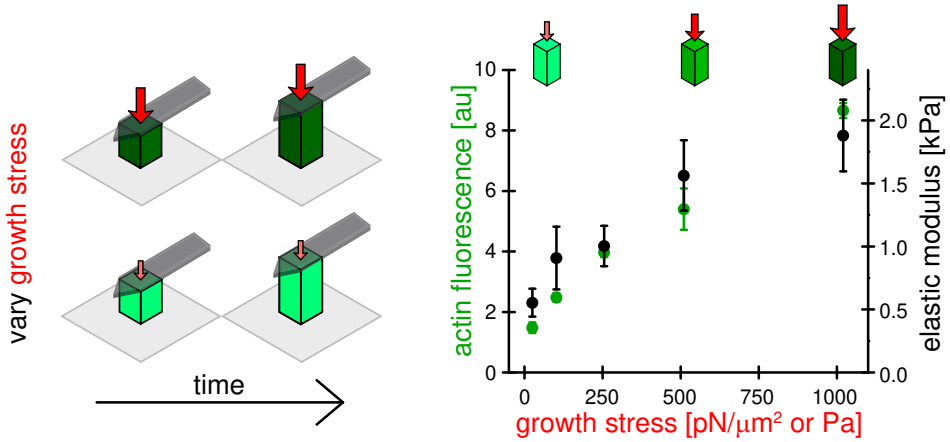


Bieling et al., D. Fletcher and D. Mullins *Cell* 164, 115–127 (2016)

Force feedback on branched actin network architecture and mechanics

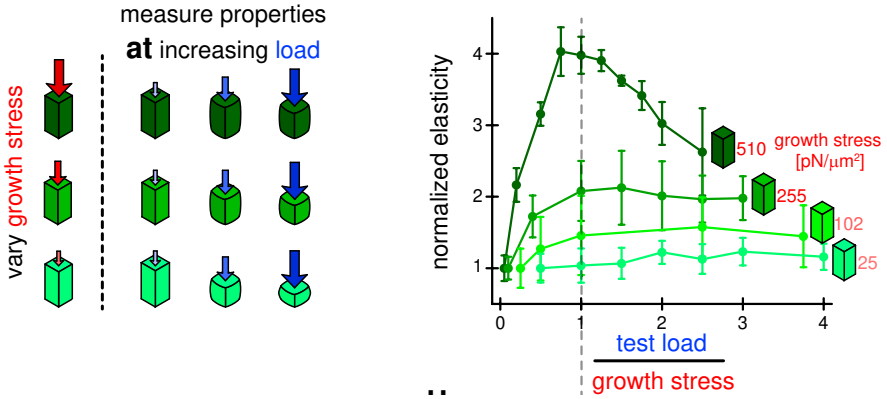
- How do load-induced changes in network architecture affect the ability of branched networks to transmit and resist forces?

Stiffness of network increases with filament density



- Growing branched actin networks adapt to a specific growth force to become maximally stiff and minimally viscous at that load.

branched actin networks are stiffest when the test load matches the original growth force experienced during its assembly.



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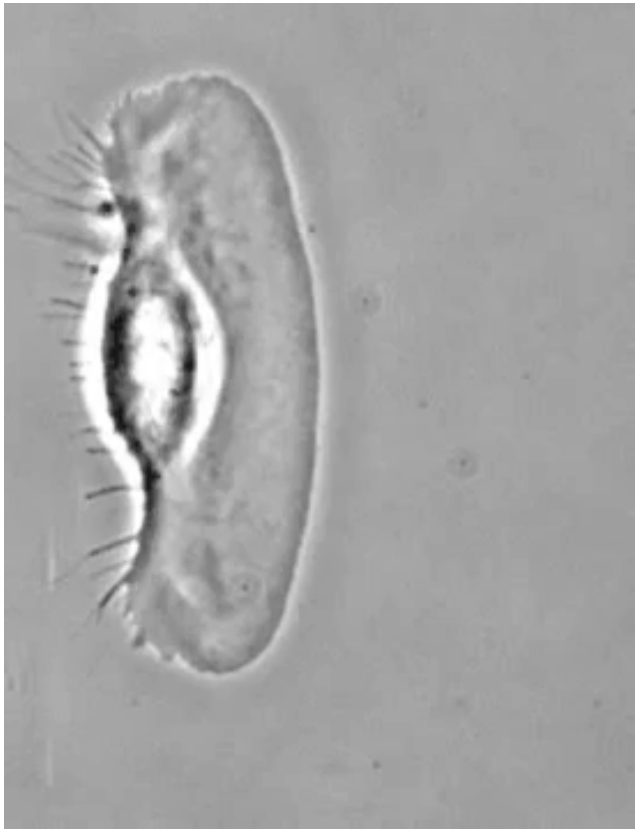
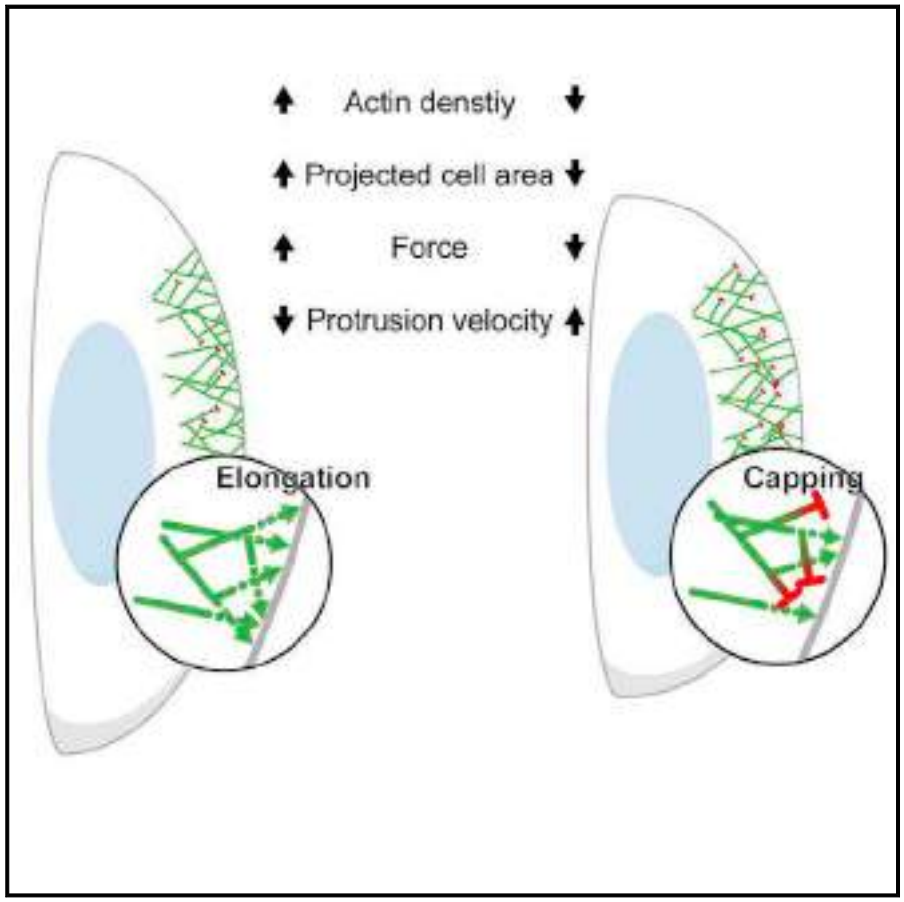
Bieling et al., D. Fletcher and D. Mullins *Cell* 164, 115–127 (2016)

Mechanism: https://www.biorxiv.org/content/10.1101/2021.05.24.445507v1

Load adaptation of lamellipodial actin networks

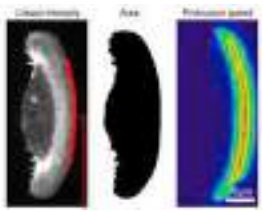


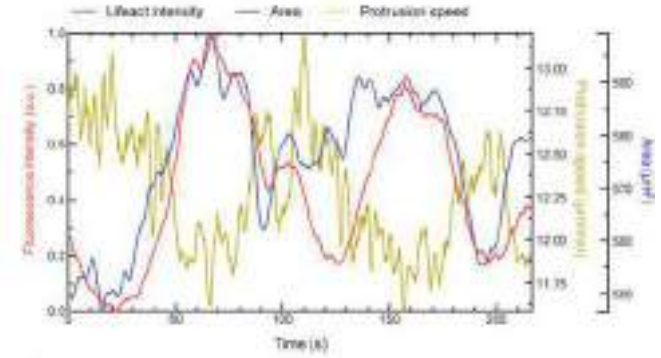


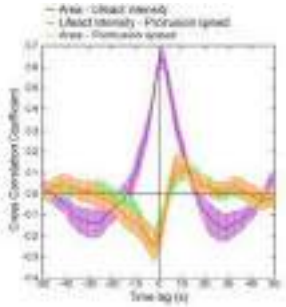
J. Mueller et al., and M. Sixt *Cell* 171, 188–200 (2017)

Load adaptation of lamellipodial actin networks

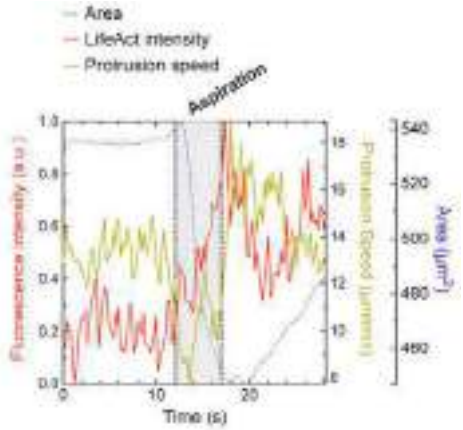
- As cell projected area increases, actin density increases and protrusion speed decreases
  —cell area increases *correlates* with increased membrane tension

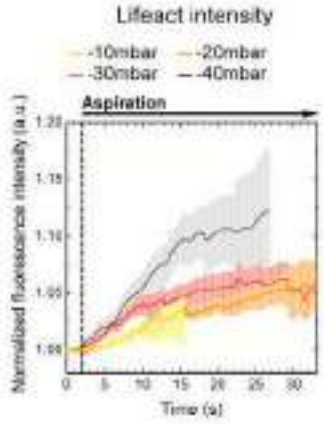


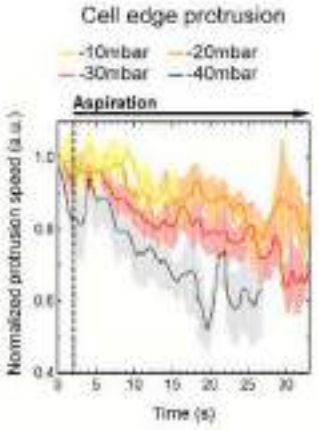


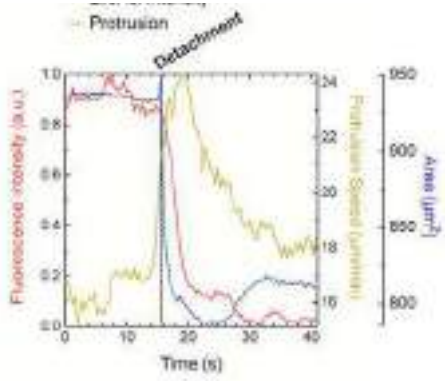


- Increase in membrane tension increases actin density and reduces velocity (and vice versa)
  —Membrane aspiration is used to increase membrane tension
  Detachement reduces membrane tension
  — Test of *causality*







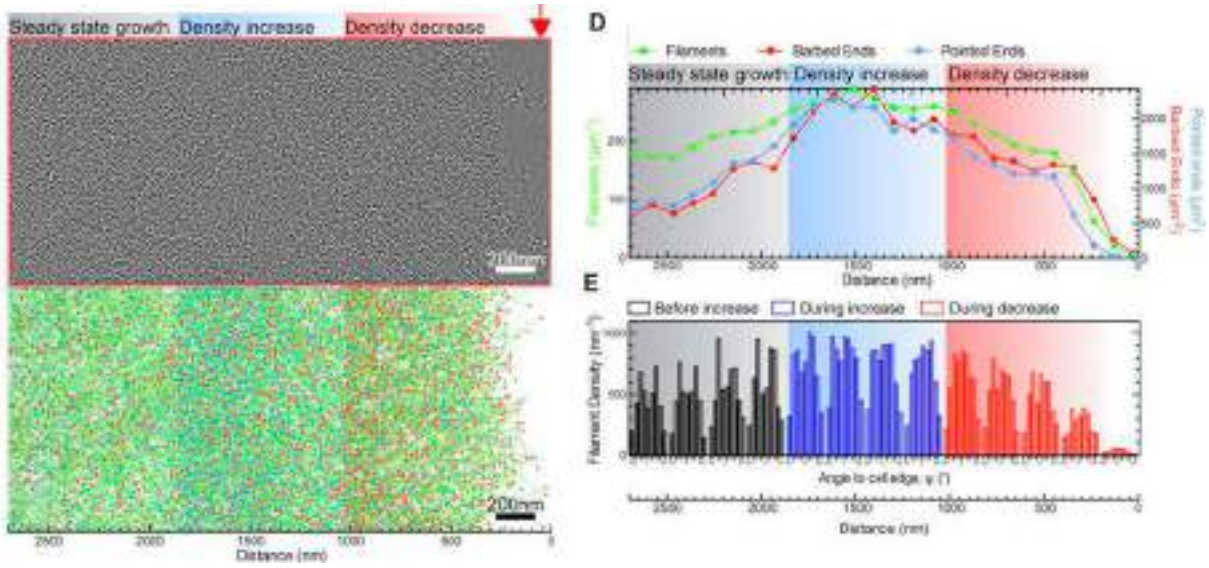


J. Mueller et al., and M. Sixt *Cell* 171, 188–200 (2017)

Load adaptation of lamelipodial actin networks

—EM tomography reveals changes in branched actin network architecture following changes in membrane tension (ie. mechanical load on actin)

- Geometry
  - angles of actin filaments are normally predominantly at around +/- 35°
  - Following transient increase in membrane tension, angles at 0-20° and 50-70° are more frequent
  - When tension is decreased, angles at 0° become predominent



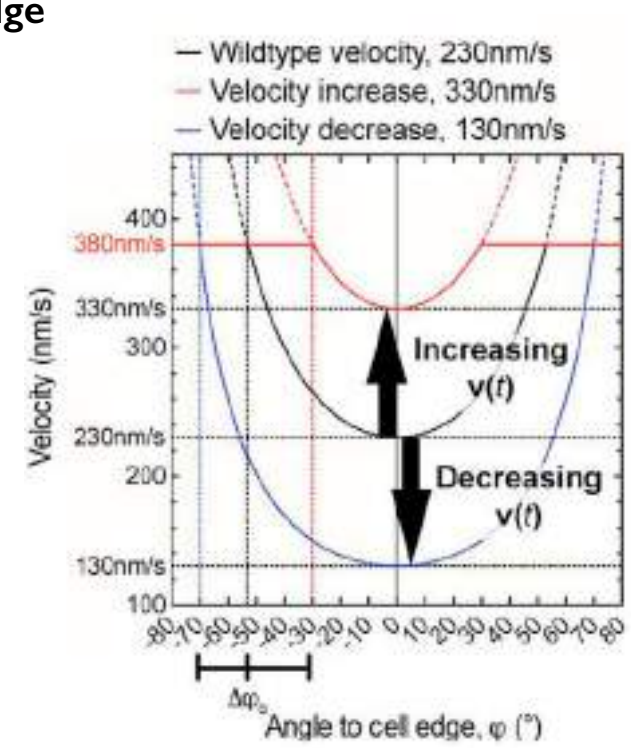
J. Mueller et al., and M. Sixt *Cell* 171, 188–200 (2017)

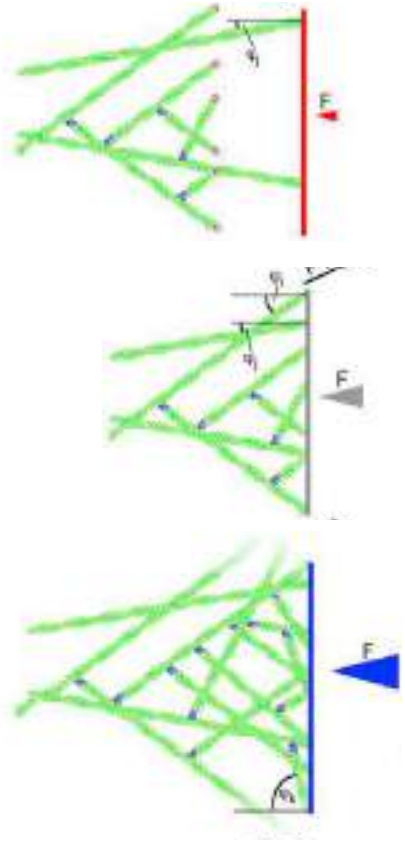
Load adaptation of lamelipodial actin networks

—Stochastic computational model based force velocity curves from Mogilner & Oster 1996

- **Filaments away from the leading edge membrane are not protected from capping and stop elongating**

- Given a certain value of protrusion speed, filaments that grow at angle φ need to grow at velocity $1/cos\varphi$ faster to keep up with the membrane
- If load is reduced, speed increases, and filaments that grow at lower angles reach the plasma membrane faster than other filaments, which thus are capped.
- Conversely, if load is increased filaments are larger angles are selected as well.





J. Mueller et al., and M. Sixt *Cell* 171, 188–200 (2017)

Mechanics of cell crawling on substrate in 2D

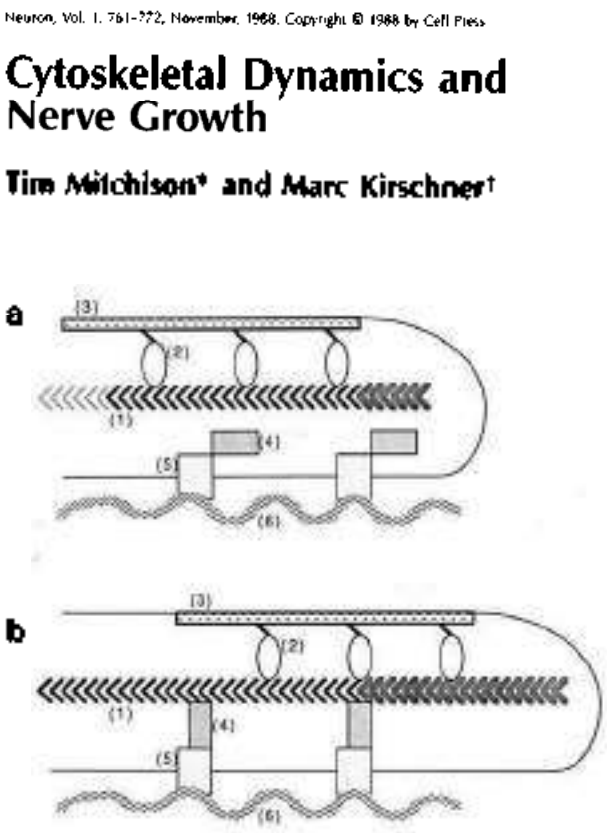
1. **Force generation:** Active processes: actin pushing forces, actin flow, actomyosin contractility

2. **Force transmission:** Passive resistance: friction/ adhesion, viscous resistance of medium.

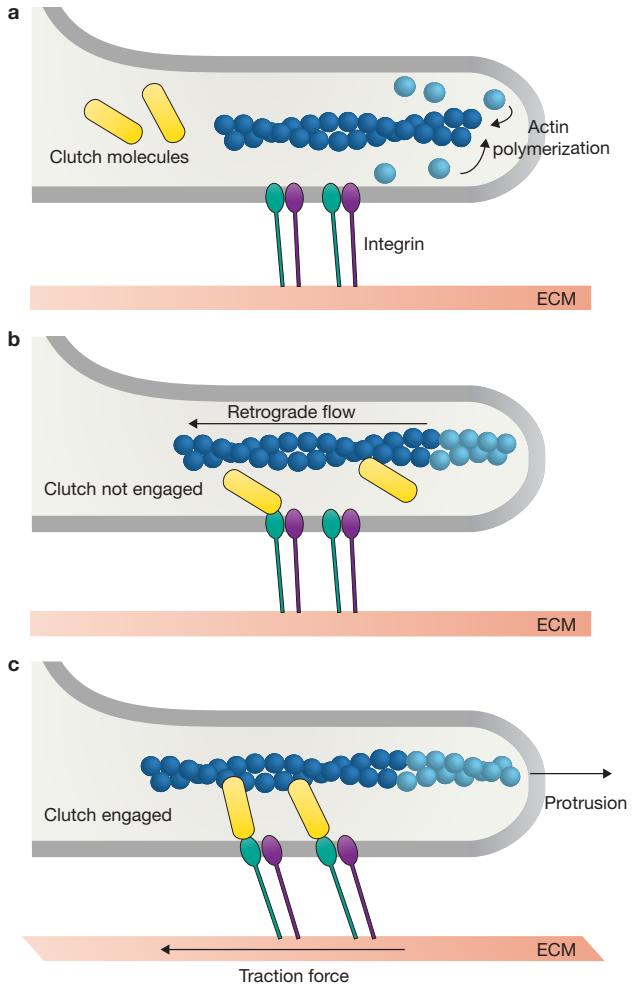
Cell adhesion and force transmission

The molecular clutch model

- free retrograde flow: no movement
- Molecular coupling to substratum (ECM): protrusion



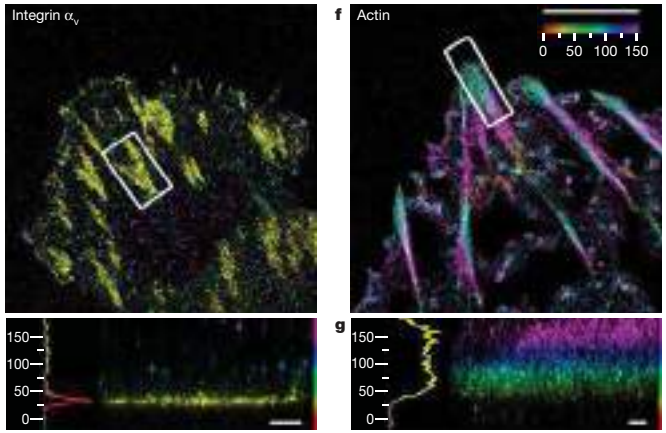
T. Mitchison and M. Kirschner *Neuron* 1:761-772 (1988)

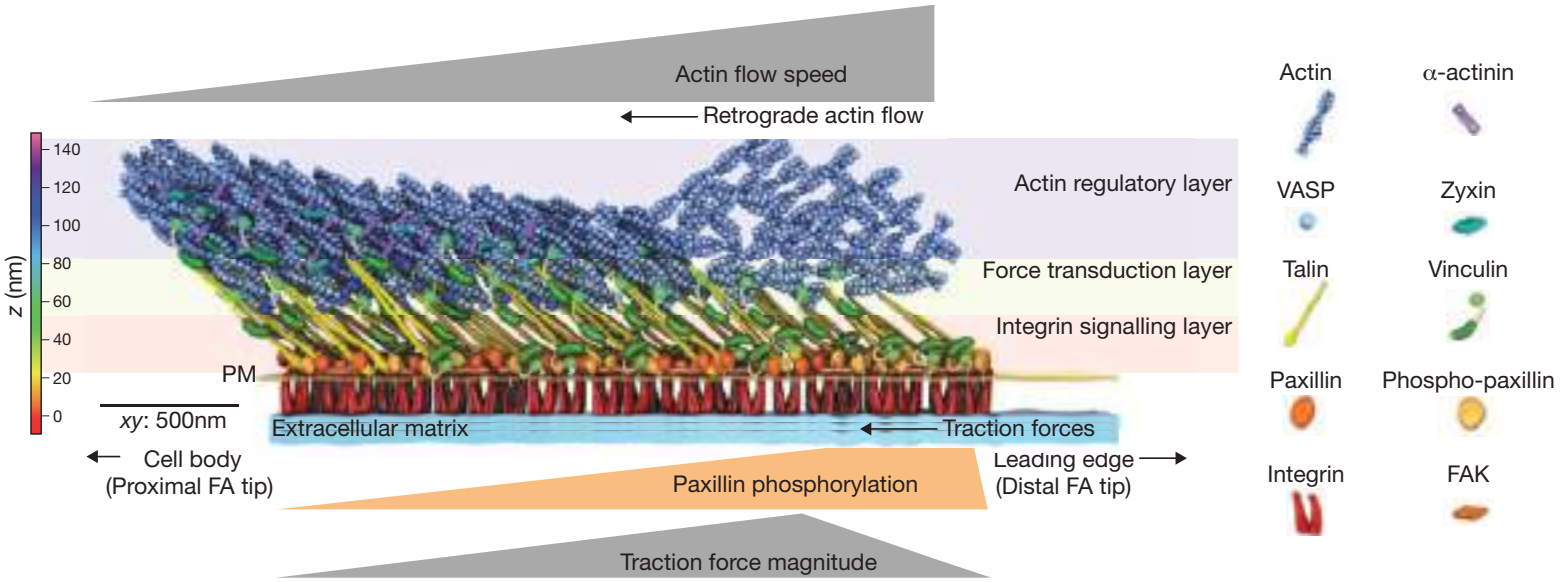


L. Case and C. Waterman *Nature Cell Biology* 17:955-963 (2015)

Cell adhesion and force transmission

Mechanosensitivity



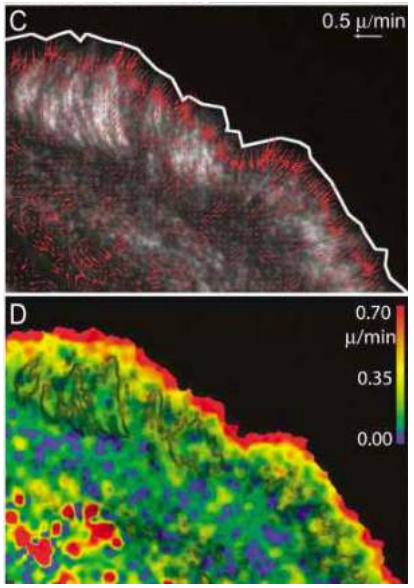


Kanchanawong, P. *et al.*. *Nature* **468,** 580–584 (2010).

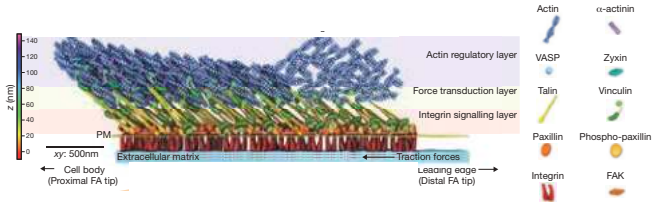
Cell adhesion and force transmission

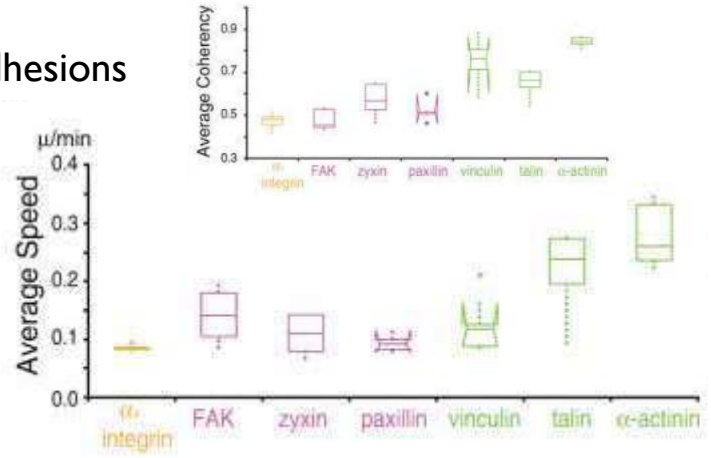
Differential transmission of actin motion within focal adhesions

- Retrograde actin flow velocity is reduced at focal adhesions consistent with frictional molecular coupling with Integrins limiting flow
- Flow is not blocked
- Method used Fluorescence speckle microscopy (FSM) and TIRF (submicron resolution)



- Shear at focal adhesion: gradient of velocity within Focal adhesions
- Slippage interface



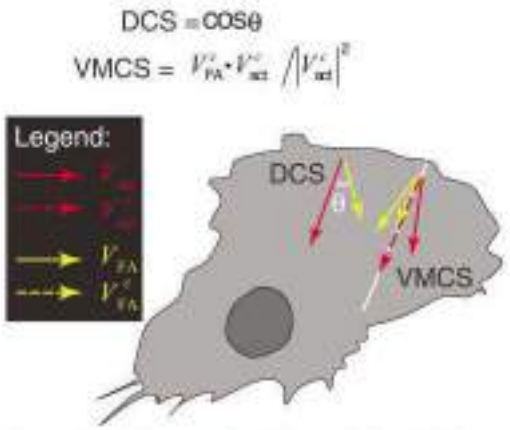


Ke Hu *et al.* G. Danuser and C. Waterman *Science* **315**, 111 (2007)

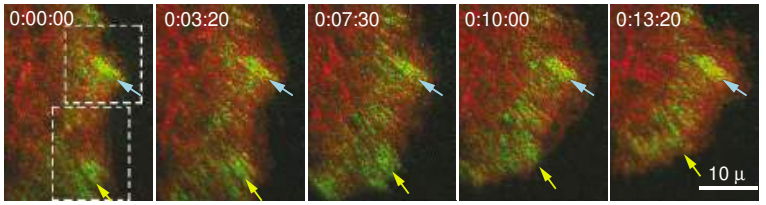
Cell adhesion and force transmission

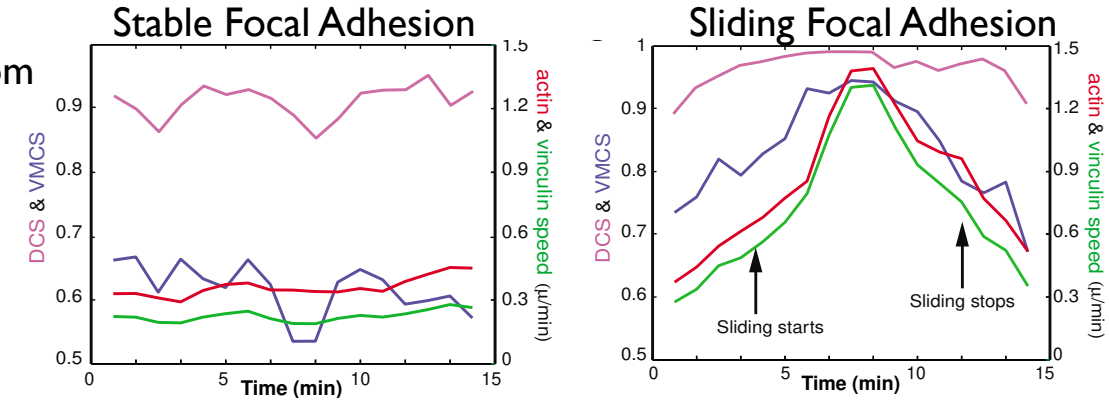
Differential transmission of actin motion within focal adhesions

- Correlation between actin flow and vinculin dynamics



- In protrusions, focal adhesions are stationary
- At stationary FAs: stable correlation of velocities
- In retractions, FAs disassemble
- This is associated with increased velocities and correlation during slippage up to a maximum
- Suggests that dissociation of Vinculin from more stationary FA components causes slippage.





Ke Hu *et al.* G. Danuser and C. Waterman
Science **315**, 111 (2007)

Cell adhesion and force transmission

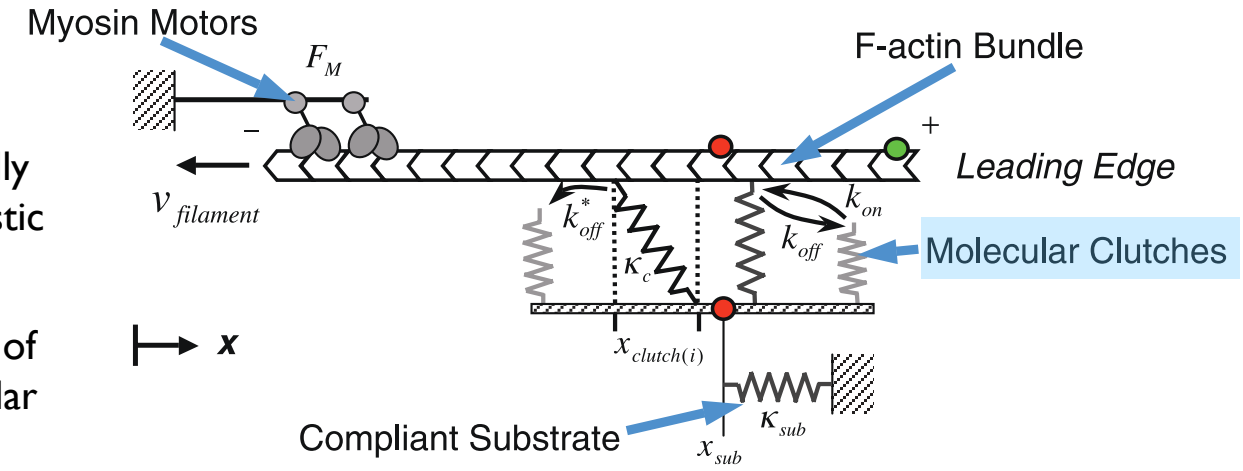
mechanics of force transmission at the molecular clutches: impact of substrate stiffness

- Model

  Elastic coupling at molecular clutches

  Tension along engaged clutches increases their off- rate constant, k_{off}^*, exponentially according to Bell's Law with a characteristic breaking force Fb

  Force balance between elastic resistance of substrate and tension engaged in molecular clutches



C. Chan and D.J. Odde
Science **322**, 1687-1691 (2008)

Cell adhesion and force transmission

- Predictions:

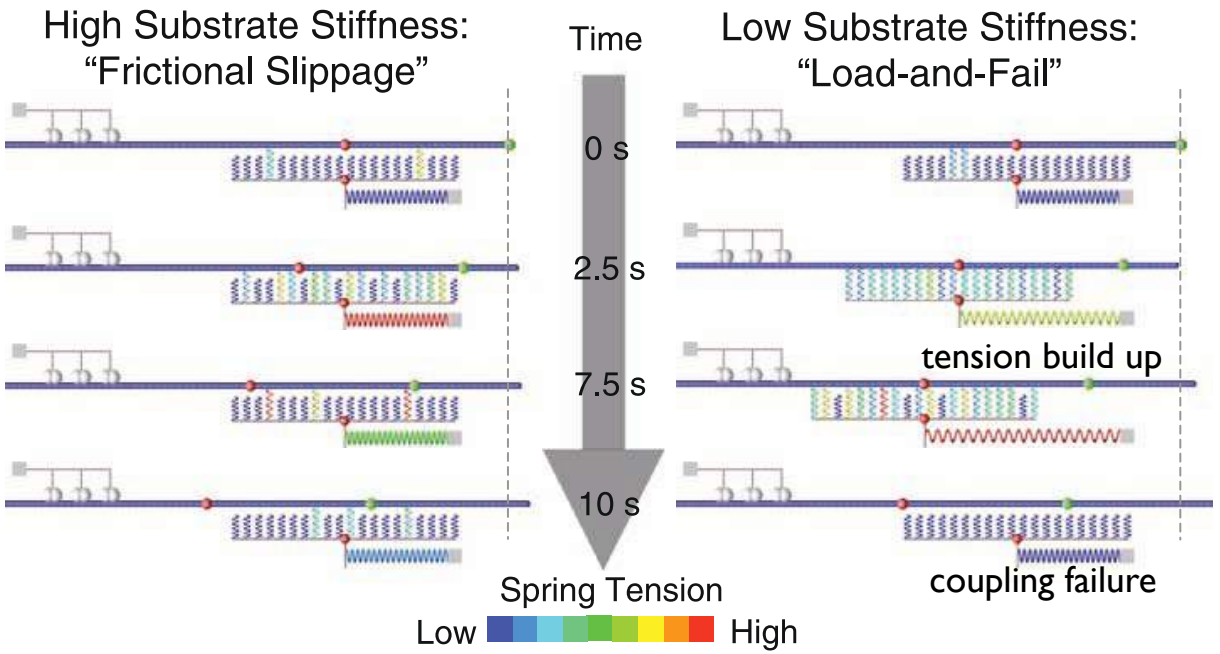
—High substrate stiffness: Frictional slippage
as elastic bonds in clutches soon reach breaking force, so life time of elastic bonds is very short

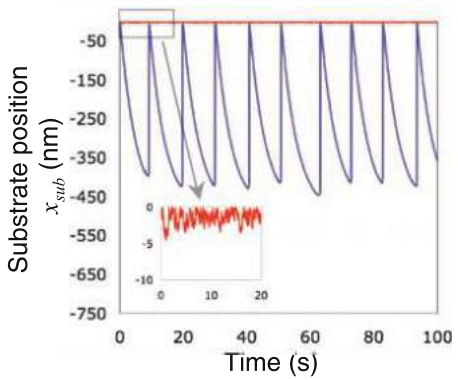
Slippage at constant velocity

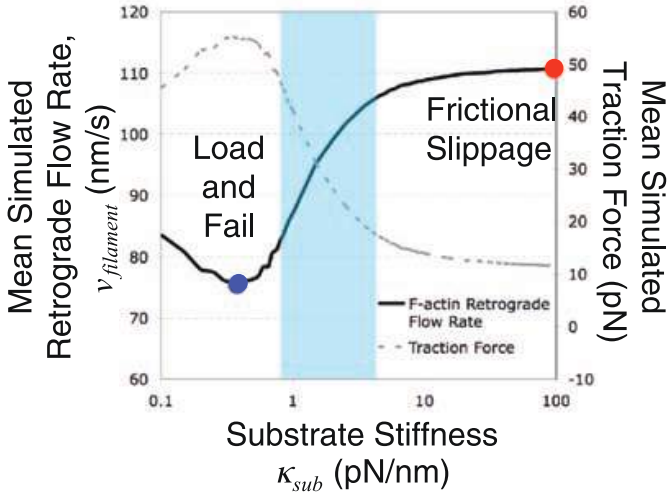
—Low substrate stiffness: Load and Fail
Substrate compliance slows down tension build up in clutches and increases the life time of actin/clutches interaction

Low resistance causes high rates of actin flow. As the substrate strains and **tension builds up,** more and more clutches are engaged as they share the load. This in turn increases mechanical resistance and reduces actin flow
Stochastic loss of a clutch induces catastrophic collapse of all clutches, **coupling failure** and rapid rise in actin flow rate

Emergent oscillatory dynamics







C. Chan and D.J. Odde
Science **322**, 1687-1691 (2008)

Cell adhesion and force transmission

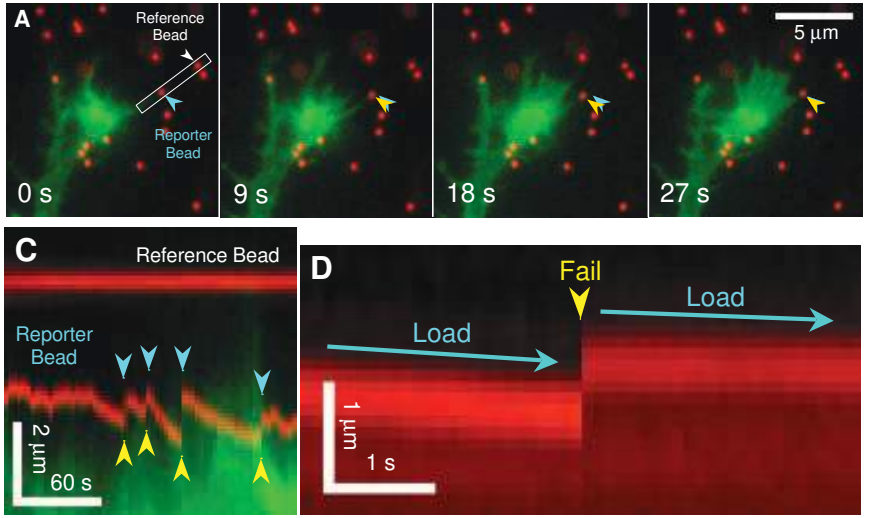
mechanics of force transmission at the molecular clutches: impact of substrate stiffness

- Experimental tests:

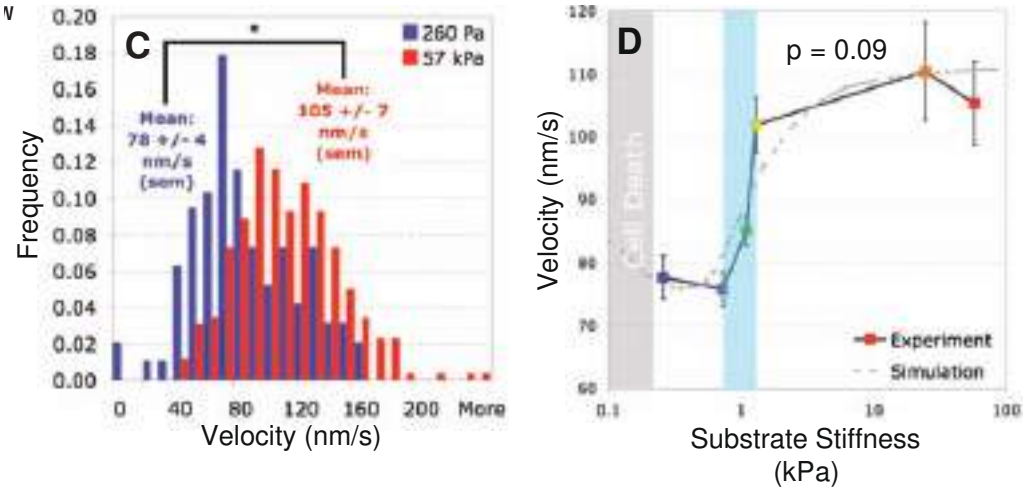
Observation of substrate deformation induced by filopodia on growth cones

Reveals Load and Fail dynamics on soft substrates

730-Pa PAG with embedded fiduciary marker beads



Force Velocity curves reveal a sharp transition at 1kPa substrate stiffness similar to model predictions



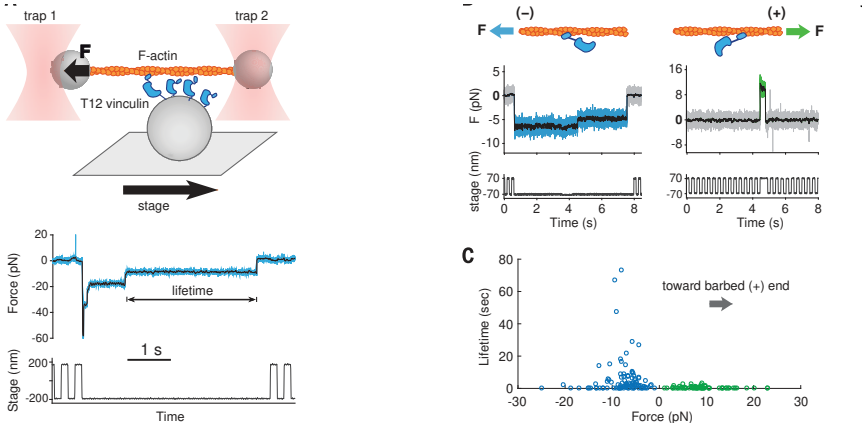
C. Chan and D.J. Odde
Science **322**, 1687-1691 (2008)

Cell adhesion and force transmission

Directionally asymmetric catch bond between the clutch and Actin

Measurement of Vinculin Actin association under load using optital tweezers

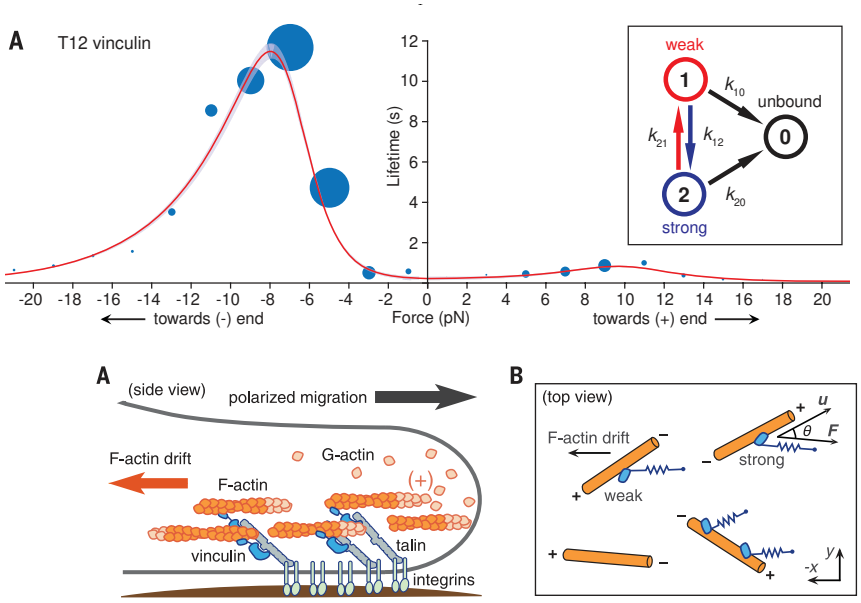
optical trap (OT)–based assay to define the load dependence of the binding interaction between vinculin and F-actin



A 2-state catch bond model best fits the data

(similar to E-cadherin based adhesion cf Cours 2017-2018)

Could impact actin filaments orientation

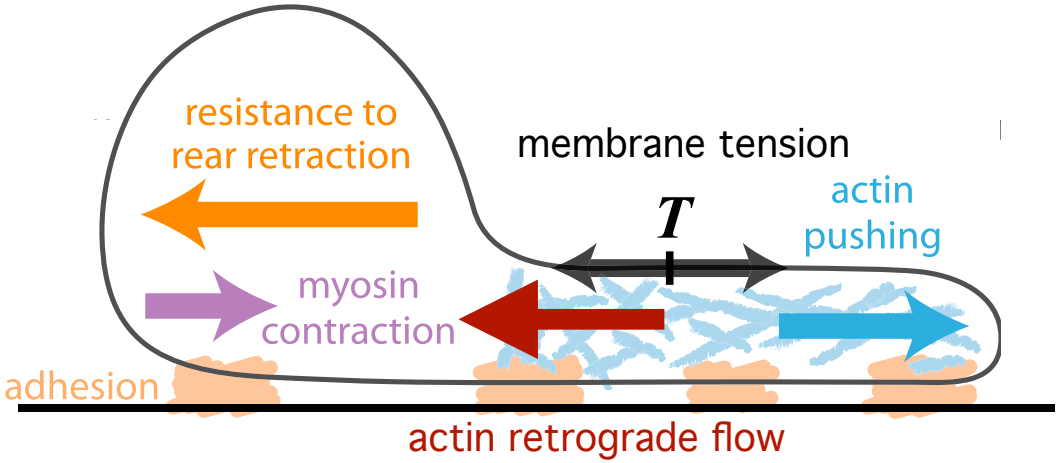


D. Huang et al., A. Dunn. *Science* 357, 703–706 (2017)

Cell propulsion and contractility

Cell motility requires coordination of anterior protrusion and posterior retraction, as well as translocation of the cell body

But not necessarily as in neurons where axon extension requires motility of growth cone.



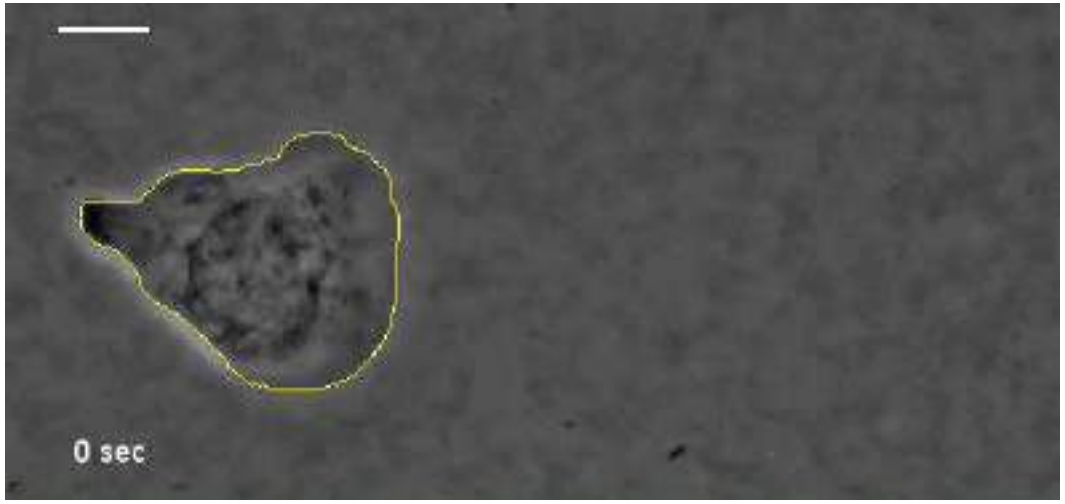
Mechanisms of Front-Rear Coupling :

- Temporal coupling ensures overall cell translocation, instead of cell elongation (eg. neurons)
- Spatial coupling is associated with cell polarization and persistence of motility

Mechanisms of Front-Rear Coupling: Spatial

Membrane Tension and cell polarization

- Cells exhibit front rear polarity:
- Questions:
  —Mechanisms of unipolar polarity (in spite of dynamic repolarization)
  —Persistence of cell polarization?



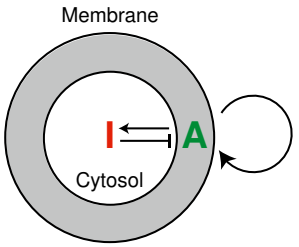
T. .Tsai et al. and J. Ferrell and J. Theriot, *Developmental Cell* 49, 189–205 (2019)

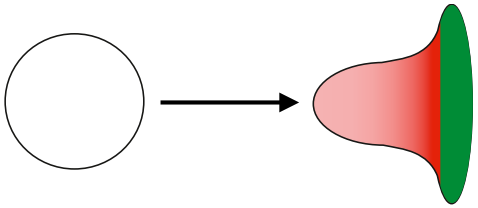
HL60 cell: human leukocyte

Spatial Front-Rear Coupling: Polarization

Conceptual mechanisms of Unipolar polarization
Local excitation (positive feedback loop) + Global inhibition

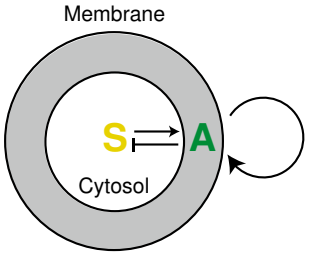
Diffusible inhibitor
(Turing instability)

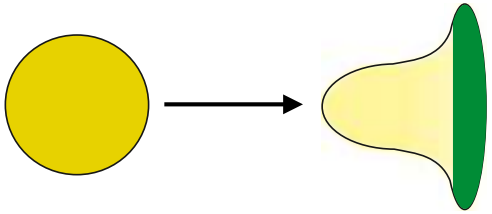




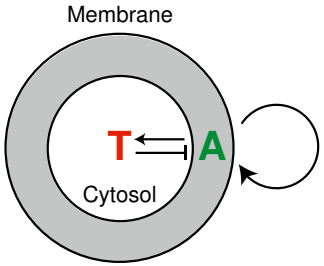
see course 27 Nov 2018
https://www.college-de-france.fr/site/thomas-lecuit/course-2018-11-27-10h00.htm

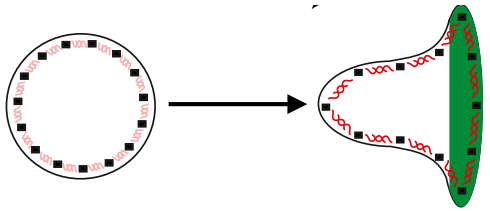
Limiting substrate
(Meinhardt)





Membrane tension





A. Houk et al. O. Weiner. *Cell* 148, 175–188 (2012)

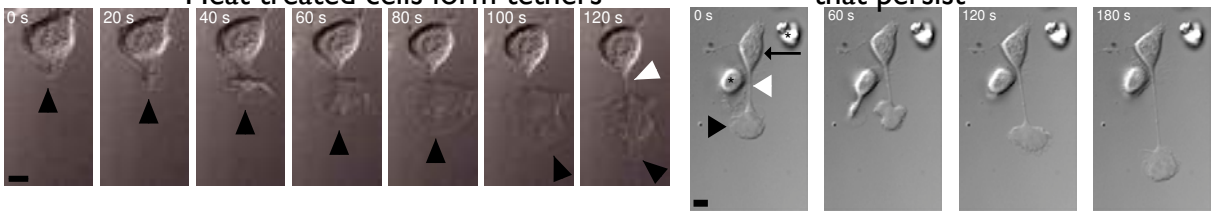
Spatial Front-Rear Coupling: Polarization

Membrane tension

Cell polarity persists in tethered cells: **long range inhibitor propagates through tether** (a priori incompatible with diffusion)

HL60 cell: human leukocyte

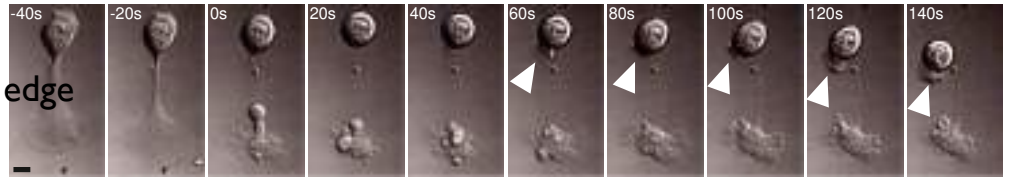
Heat treated cells form tethers that persist



Pseudopod formation after cell severing

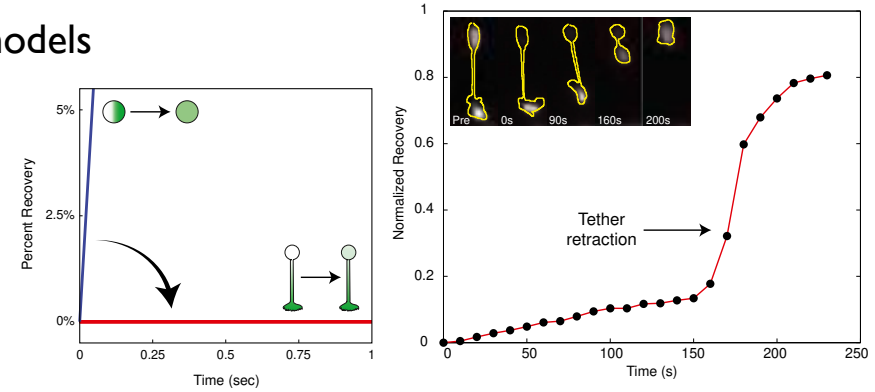
Argues for short-lived inhibitor generated at the leading edge

This short-lived inhibitor could be due to mechanical tension, a rapidly synthesized limiting component, or a diffusible inhibitor with a short half-life.



Low diffusivity through tether incompatible with biochemical models

- Limiting substrate model: the rate of pseudopod recovery requires a high rate of protein synthesis of substrate (6 p/s), which has to be balanced by exceedlingly high diffusion coefficient (>300µm2/s) to maintain polarity in tethered cells
- Inhibitor: short-lived inhibitor to explain recovery but given time of diffusion through tether, would not repress at long range in tethered cell.

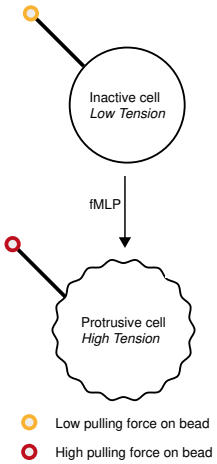


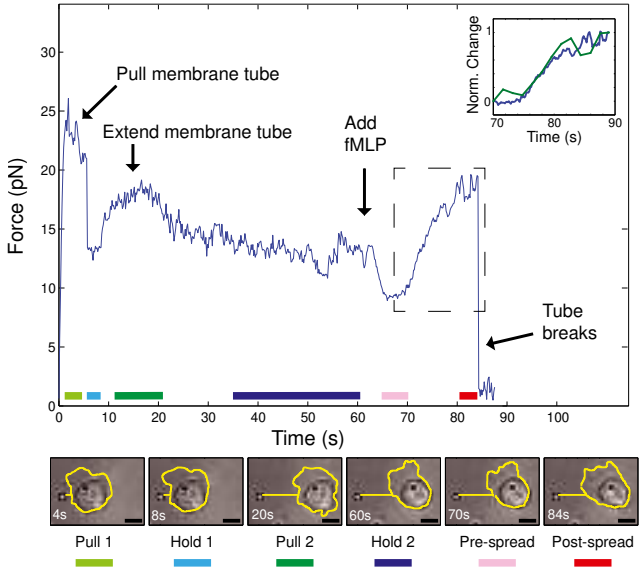
A. Houk et al. O. Weiner. *Cell* 148, 175–188 (2012)

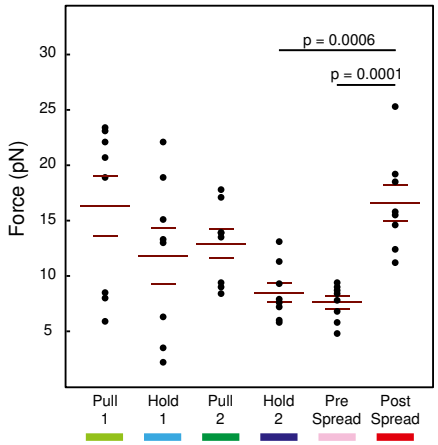
Spatial Front-Rear Coupling: Polarization

Membrane tension

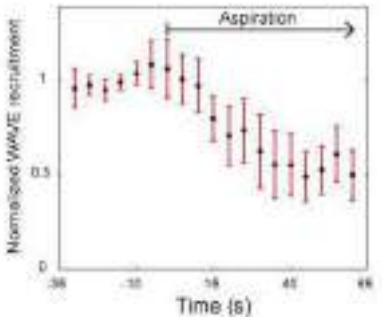
- **Membrane tension probed with tether increases during cell protrusion**

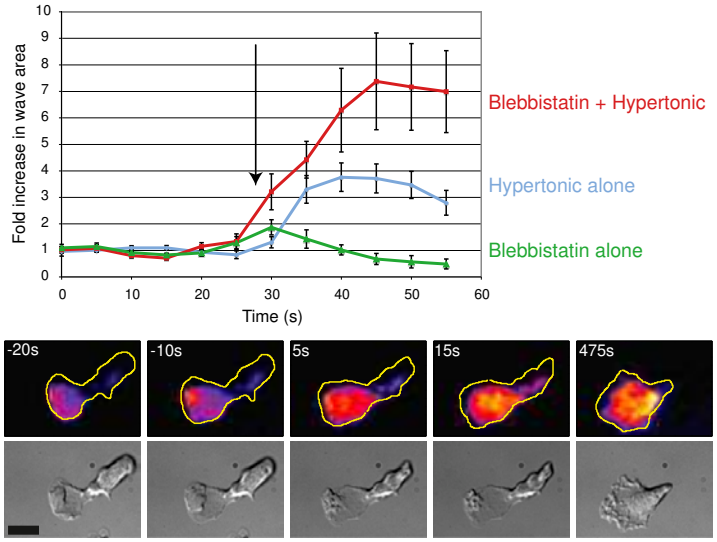


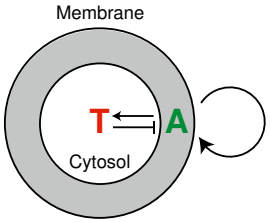




- Cell aspiration increases membrane tension and inhibits Scar/WAVE at leading edge
- Reduced membrane tension (ie. hypertonic medium and MyoII inhibition) increases WAVE at the cell front
- **Membrane tension works as a long range inhibitor of actin nucleation.**



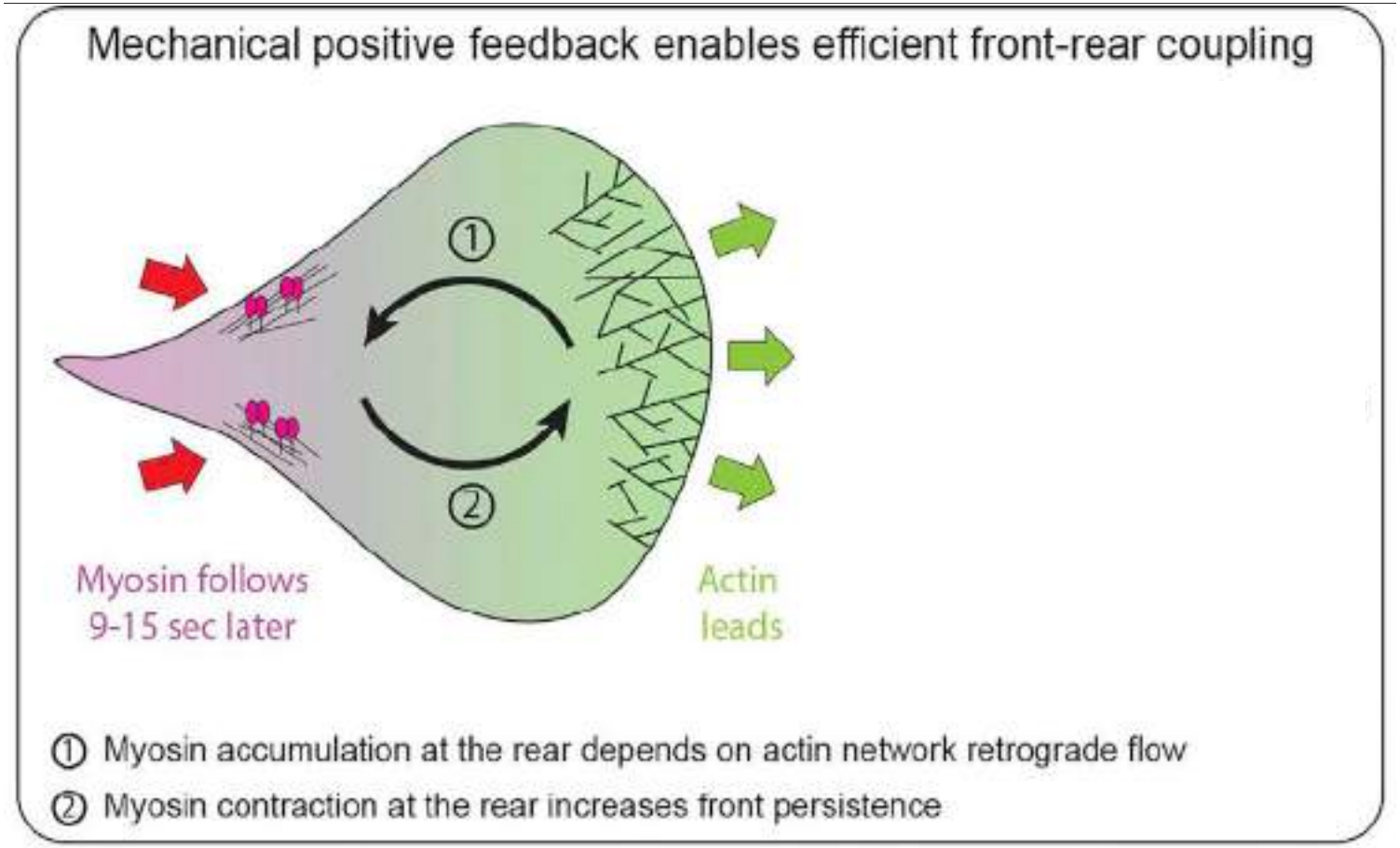




A. Houk et al. O. Weiner. *Cell* 148, 175–188 (2012)

Mechanisms of Front-Rear Coupling

Actin retrograde flow and Myosin advection

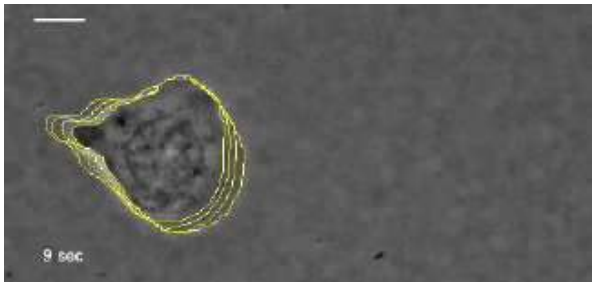


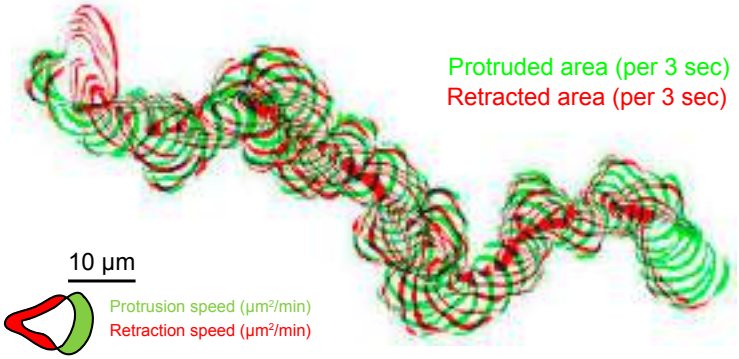
T. .Tsai et al. and J. Ferrell and J. Theriot, *Developmental Cell* 49, 189–205 (2019)

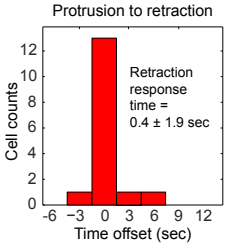
Mechanisms of Front-Rear Coupling

Actin retrograde flow and Myosin advection

- Cell protrusion at the front and cell retraction at the back are synchronous (cell area is constant)
- Coupling is **instantaneous**, calling for mechanical coupling (ie. membrane tension)
- Incompatible with diffusion of molecule (diffusion over 30 µm would take around 30s).







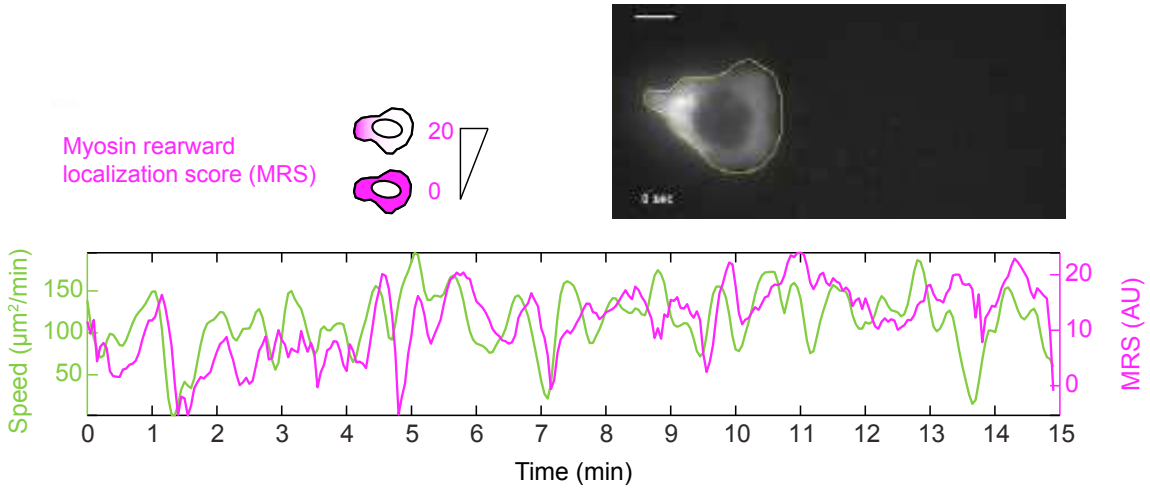
T. .Tsai et al. and J. Ferrell and J. Theriot, *Developmental Cell* 49, 189–205 (2019)

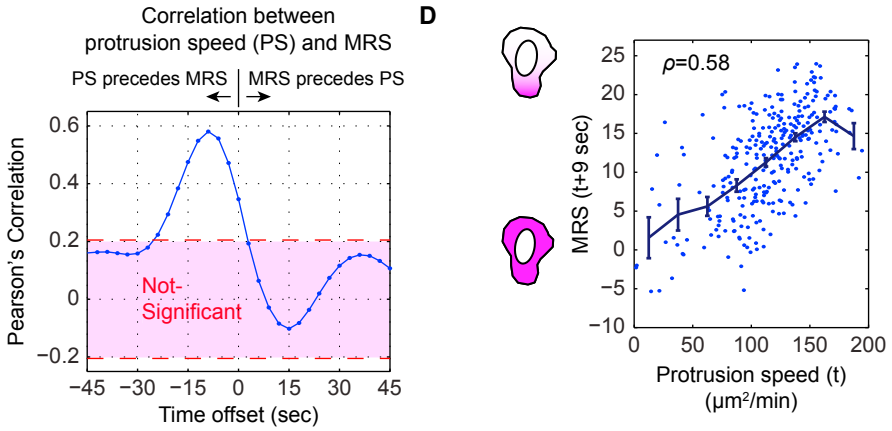
Mechanisms of Front-Rear Coupling

Actin retrograde flow and Myosin advection

- MyosinII is polarized at the back of cells
- MyosinII polarity fluctuates

- High correlation with cell protrusion with a consistent 9s delay
- This delay is incompatible with MyosinII contractility causing cell retraction
- MyoII polarity correlates with protrusion speed



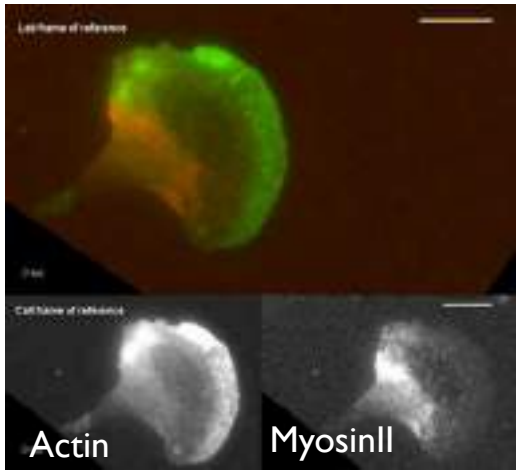


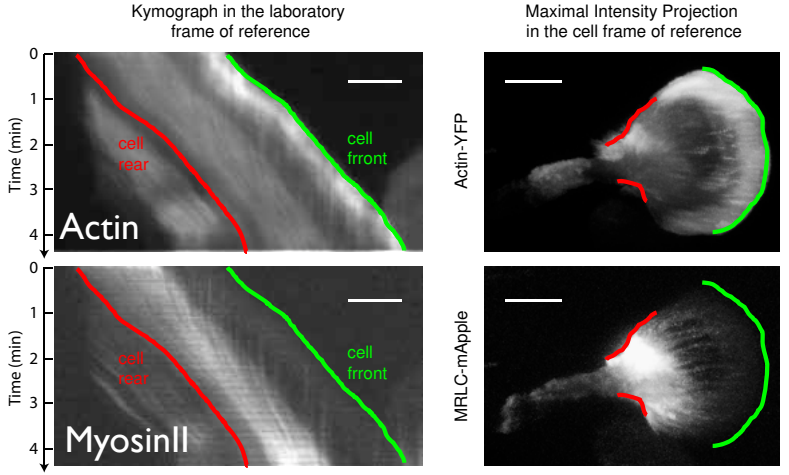
T. .Tsai et al. and J. Ferrell and J. Theriot, *Developmental Cell* 49, 189–205 (2019)

Mechanisms of Front-Rear Coupling

Actin retrograde flow and Myosin advection

- MyosinII is transported from the front to the back of the cells during motility



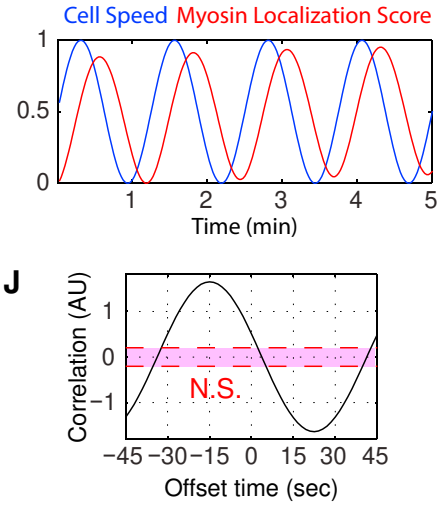


- Modeling of diffusion, advection and association/ dissociation explains dynamic relocalisation of MyoII and correlation with cell protrusion (with 10s delay)

advection

$$\frac{\partial M_b}{\partial t} = \underbrace{-V\frac{\partial M_b}{\partial x}}_{\text{drift of bound myosin}} + \underbrace{k_{on}M_f}_{\text{attachment}} - \underbrace{k_{off}M_b}_{\text{detachment}}$$

$$\frac{\partial M_f}{\partial t} = \underbrace{D\frac{\partial^2 M_f}{\partial x^2}}_{\text{diffusion of free myosin}} - \underbrace{k_{on}M_f}_{\text{attachment}} + \underbrace{k_{off}M_b}_{\text{detachment}}$$



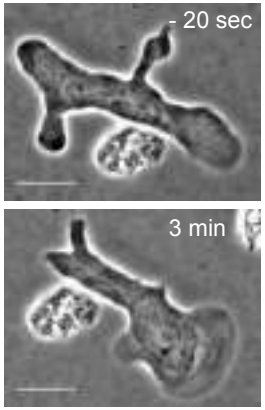
T. .Tsai et al. and J. Ferrell and J. Theriot, *Developmental Cell* 49, 189–205 (2019)

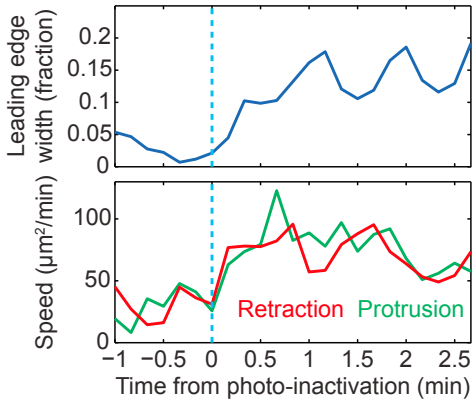
Mechanisms of Front-Rear Coupling

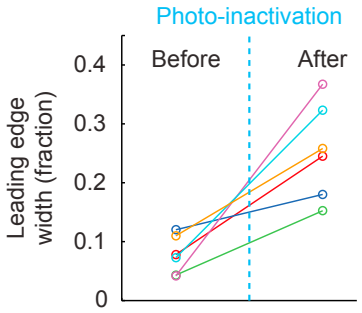
Actin retrograde flow and Myosin advection:

- Front and back of leukocytes are mutually reinforcing.

- MyosinII inhibition does not block instantaneous coupling between protrusion and retraction (consistent with some other mechanism (ie. membrane tension) required for this)
- But MyosinII activation locally (blebbistatin and photoinactivation) causes expansion of leading edge and increased velocity, as well as increased persistence.





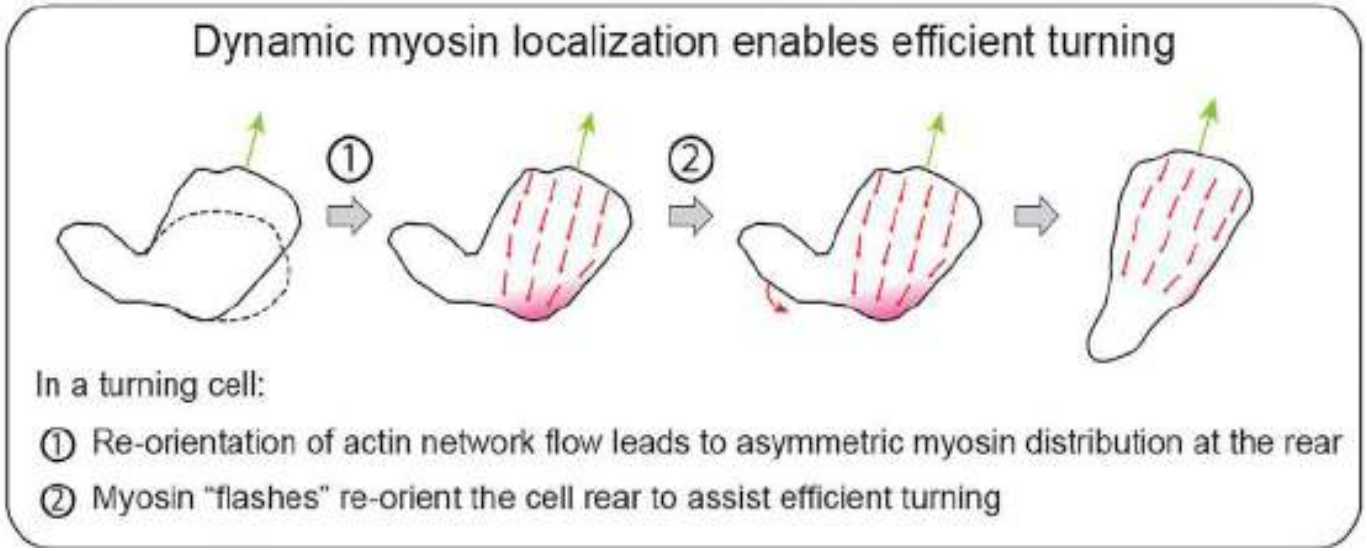


T. .Tsai et al. and J. Ferrell and J. Theriot, *Developmental Cell* 49, 189–205 (2019)

Mechanisms of Front-Rear Coupling

— causes adaptation of cell to changed environment

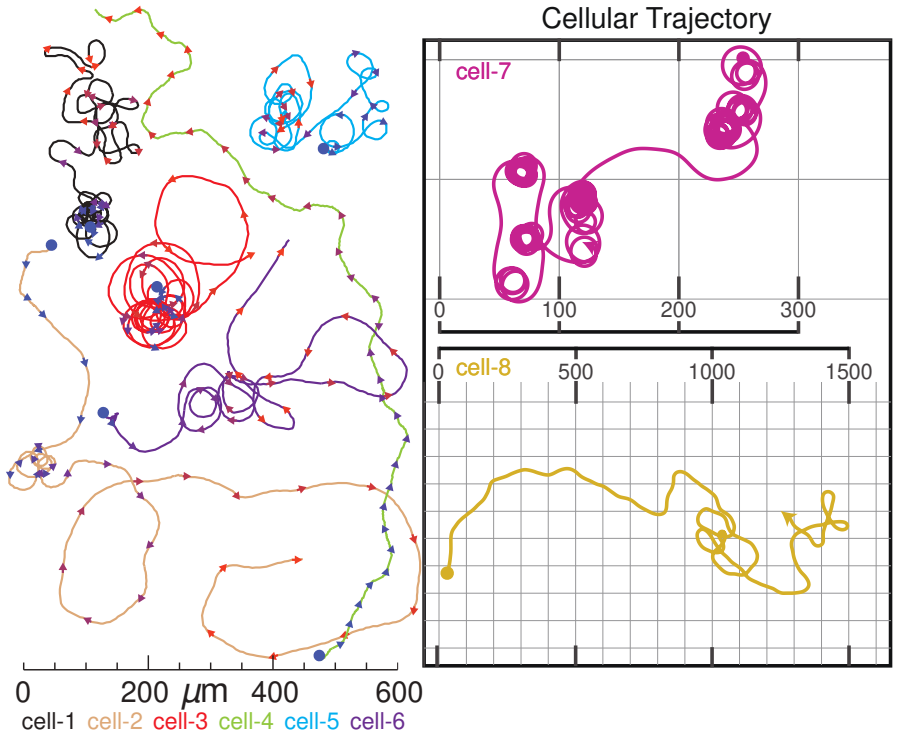
Actin retrograde flow and Myosin advection:

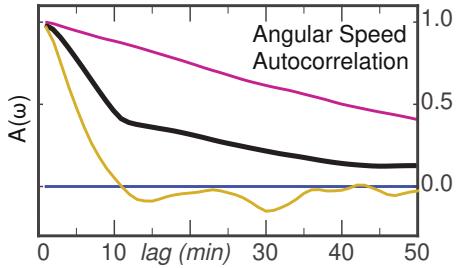


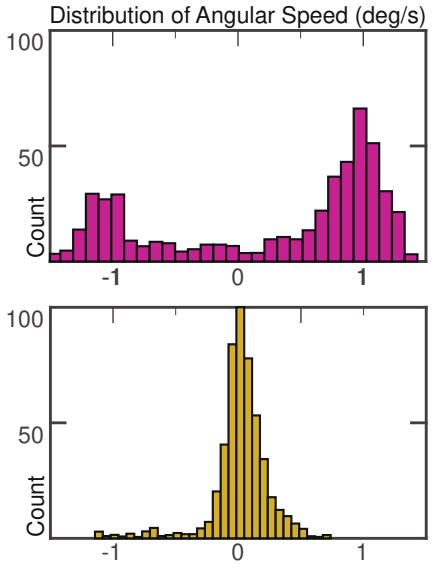
T. .Tsai et al. and J. Ferrell and J. Theriot, *Developmental Cell* 49, 189–205 (2019)

Feedback mechanisms underlie cell turning

Long-term trajectories of cells exhibit Persistent Turning States





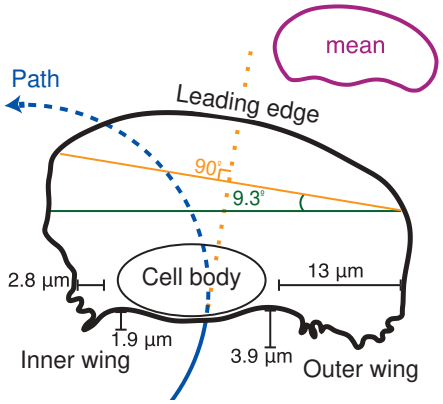


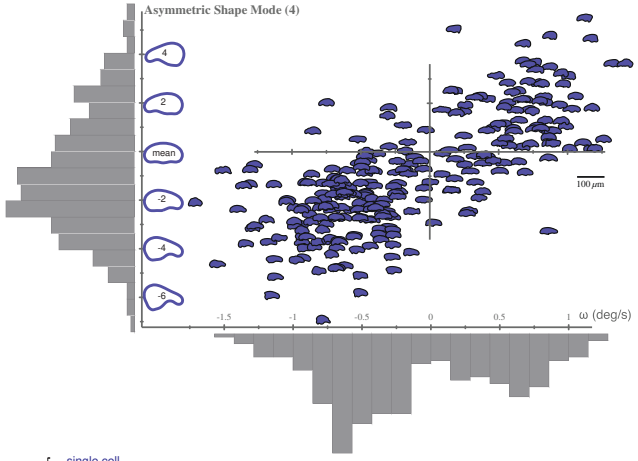
Allen et al., Julie Thériot and A/ Mogilner, *Cell Systems* 11, 286–299 (2020)

Feedback mechanisms underlie cell turning

Cell asymmetry during cell rotation

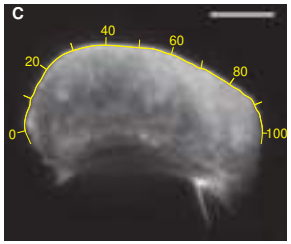
- **Asymmetric shape** is associated with cell turning

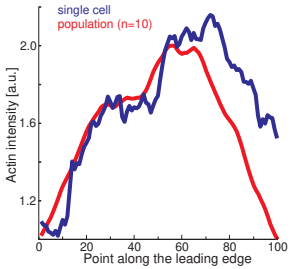




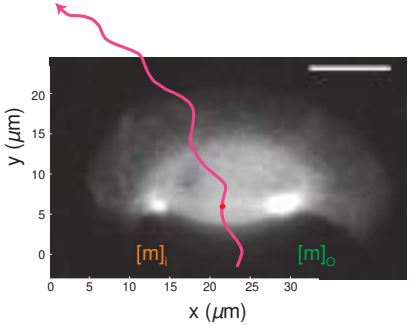
- **Asymmetric actin density** underlies cell turning: higher rate in the outer side of the turn
  cells pivot around turns with faster lamellipodial protrusion on the outer side of the turn

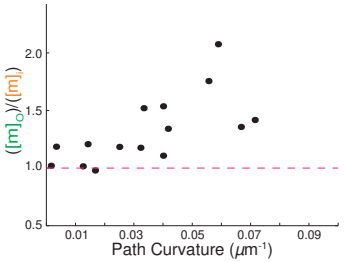
  This is not sufficient to account for turning

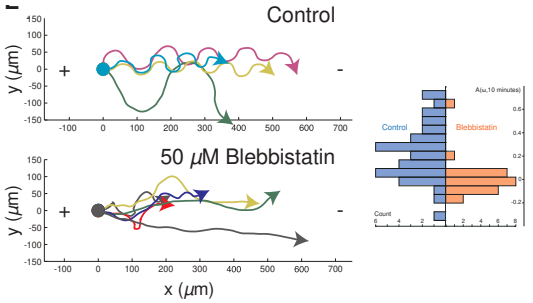




- **Asymmetric MyosinII**: Higher concentration in outer side at the cell rear. This is required for persistent turning.





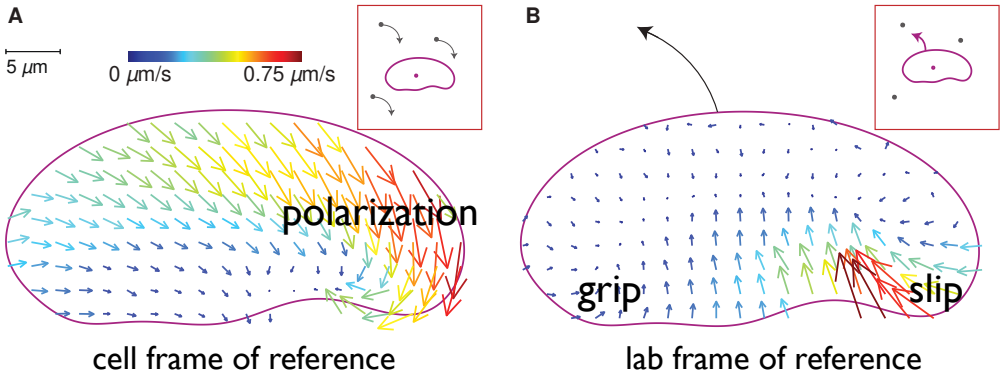


Allen et al., Julie Thériot and A/ Mogilner, *Cell Systems* 11, 286–299 (2020)

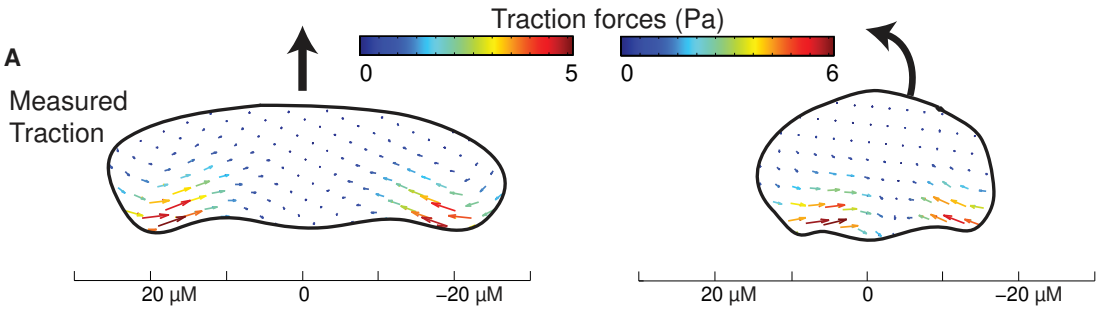
Feedback mechanisms underlie cell turning

Cell mostly steered from the rear

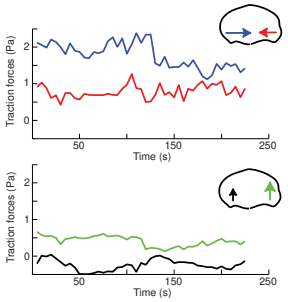
- Asymmetric **Actin flow patterns**
- In cell frame of reference, flow explains MyosinII enrichment by advection
- In lab frame of reference, minimal symmetric flow at cell front reflects high coupling to substrate (adhesion)
- asymmetric centripetal flow at the rear reflects grip (left) and slip (right)



- Asymmetric **Traction forces**
- Symmetric propulsive forces at the front
- **Asymmetric** resistive forces at the rear



weaker inward traction at rear right (outer side of turn)
stronger forward traction at rear right

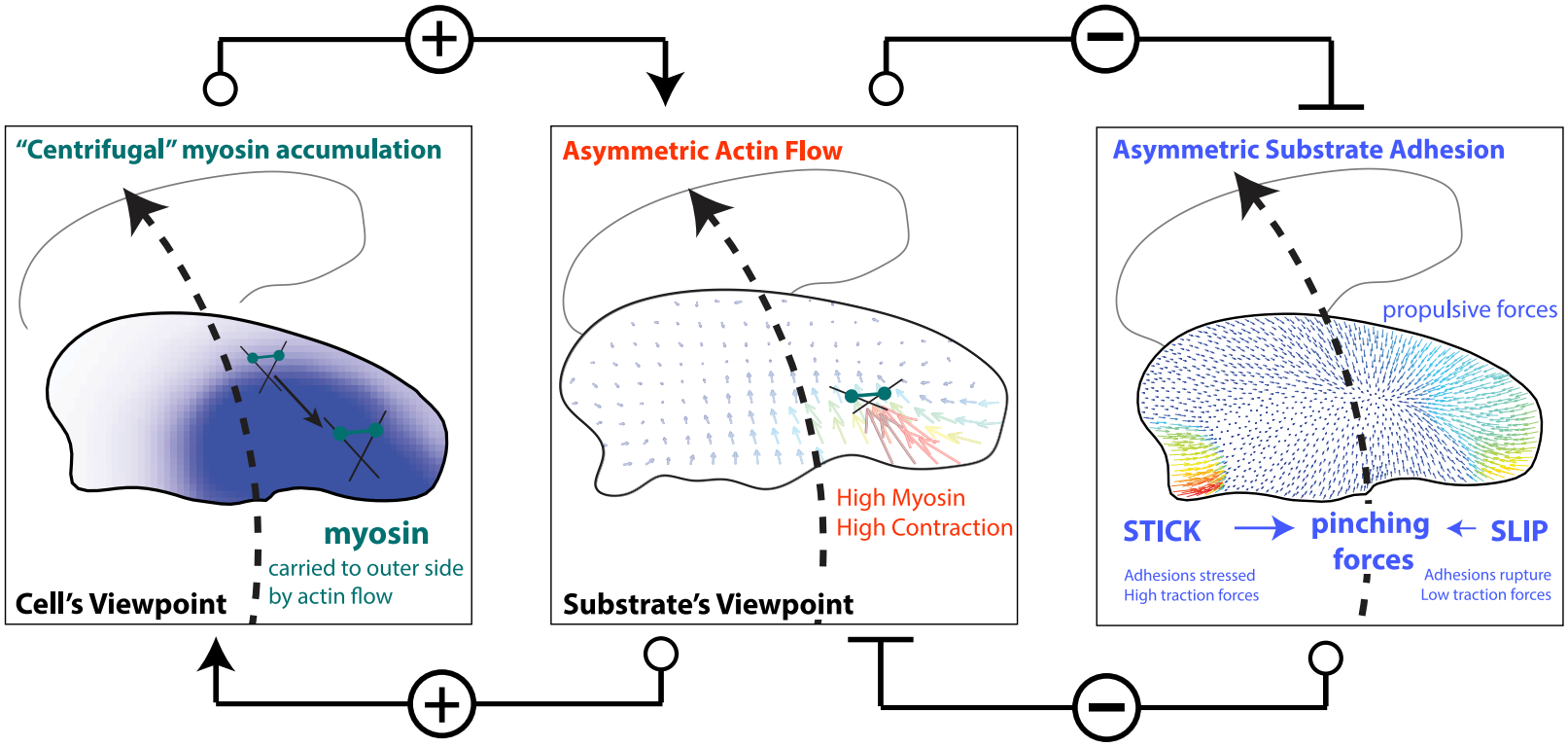


Allen et al., Julie Thériot and A/ Mogilner, *Cell Systems* 11, 286–299 (2020)

Feedback mechanisms underlie cell turning

Feedback loops cause persistent turning

- High flow speed associated with low adhesion
- Low flow speed associated with high adhesion



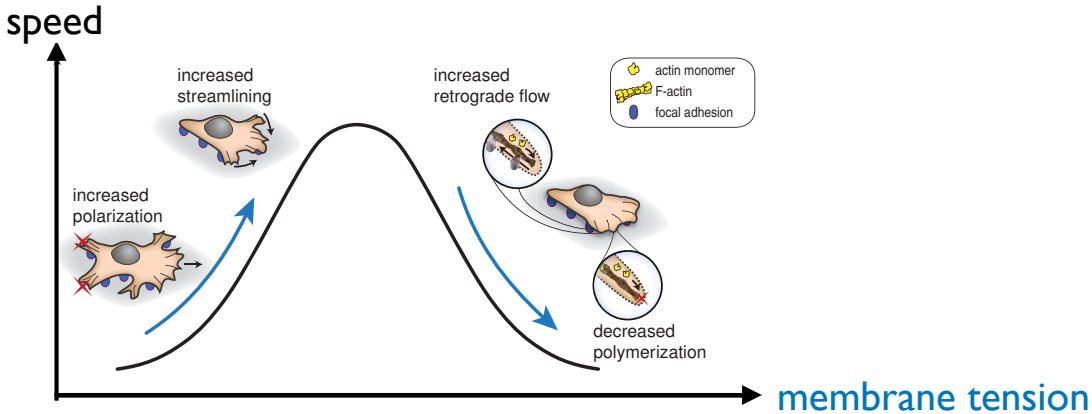
Increased myosin contractility and actin flow on the outer side of the turning cell breaks adhesions on the outer edge of the cell (SLIP), weakening traction forces (colored vectors)

Allen et al., Julie Thériot and A/ Mogilner, *Cell Systems* 11, 286–299 (2020)

Conclusions

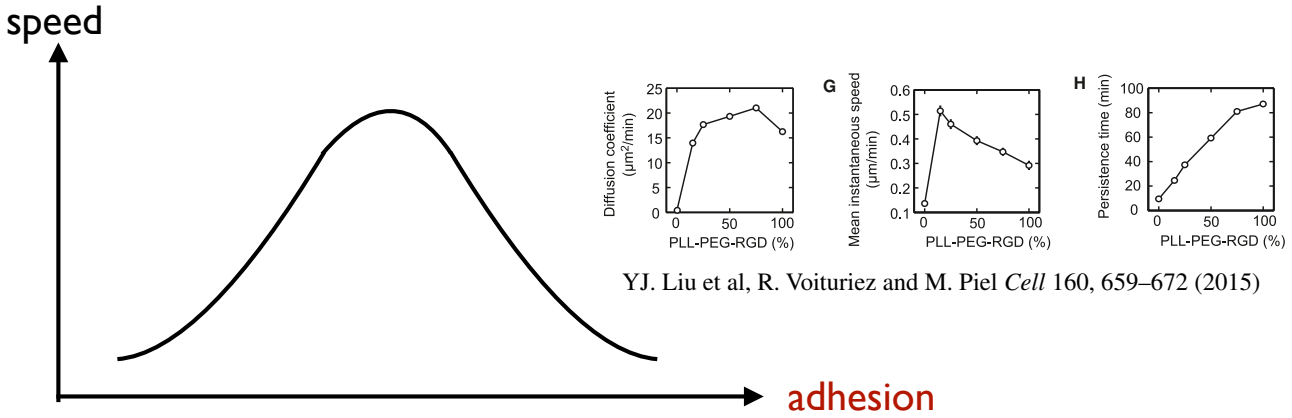
- Force production: actin polymerization
- Force transmission: substrate adhesion
- But excess mb tension and adhesion inhibit motility (negative feedback)
- Mechanical adaptation via feedbacks - impact on environment sensing

Membrane tension



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

Adhesion



YJ. Liu et al, R. Voituriez and M. Piel *Cell* 160, 659–672 (2015)

P. DiMilla et al. and D. Lauffenburger *JCB* 122: 729-737 (1993)