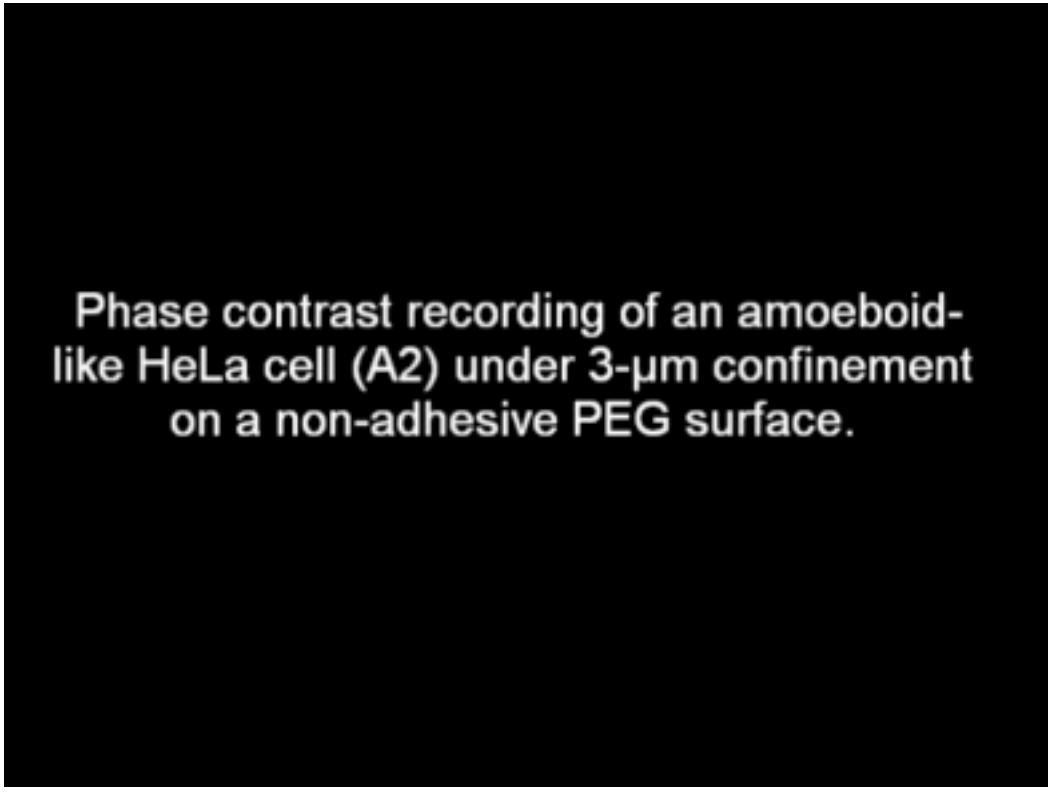
Cellular Motility



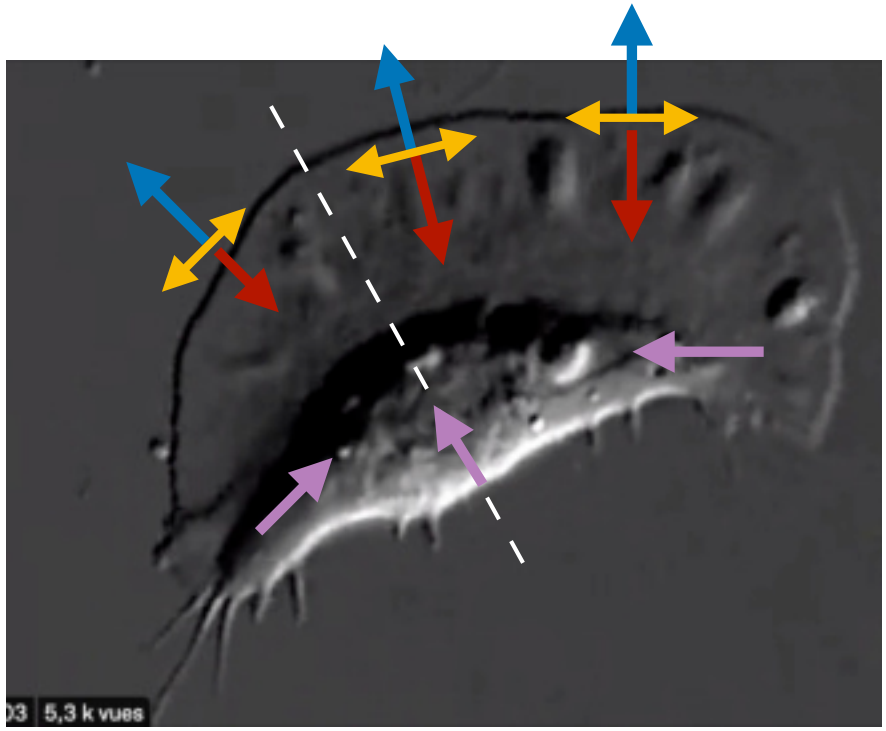
Course 3: Mechanics II - crawling under confinement

Thomas Lecuit
chaire: Dynamiques du vivant

COLLÈGE DE FRANCE 1530

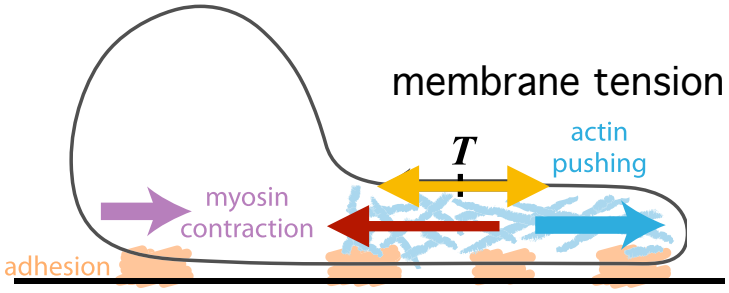
Summary - Crawling on a 2D substrate

In Eukaryotes: cell crawling is associated with cell deformation



Dylan Burnette @MAG2ART

- **Force production:** actin polymerization
  — Brownian ratchet model
- **Force transmission:** actin retrograde flow and substrate adhesion
  — Clutch model (force dependent reinforcement)
- Membrane tension:
  — limits force production at front
  — couples front and back of cell
  — can be tuned

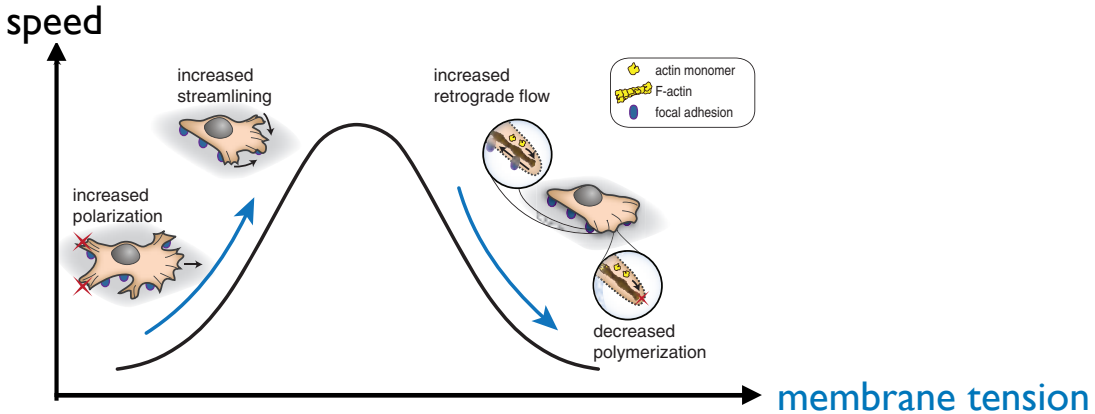


actin retrograde flow

Summary - Crawling on a 2D substrate

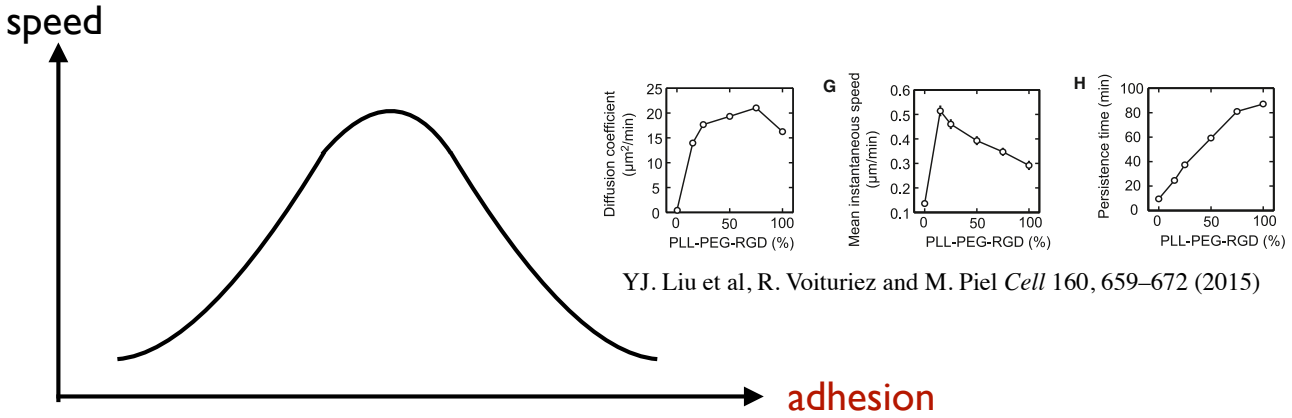
- Feedback regulation:
- Excess membrane tension and adhesion inhibit motility (negative feedback)
- Mechanical adaptation via feedbacks - impact on environment sensing (see course #6)

Membrane tension



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

Adhesion



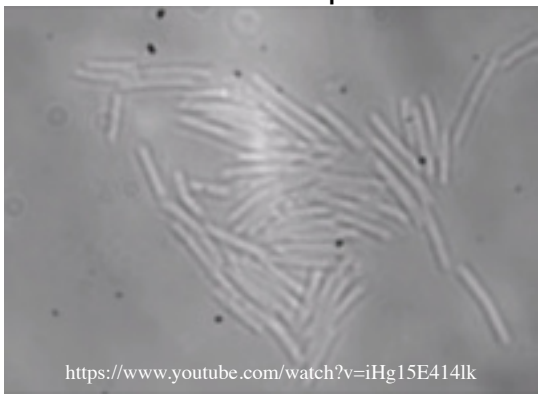
YJ. Liu et al, R. Voituriez and M. Piel *Cell* 160, 659–672 (2015)

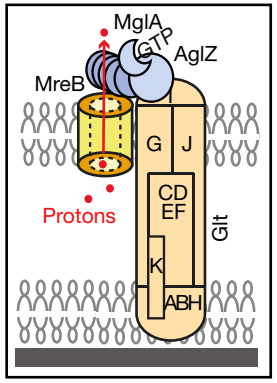
P. DiMilla et al. and D. Lauffenburger *JCB* 122: 729-737 (1993)

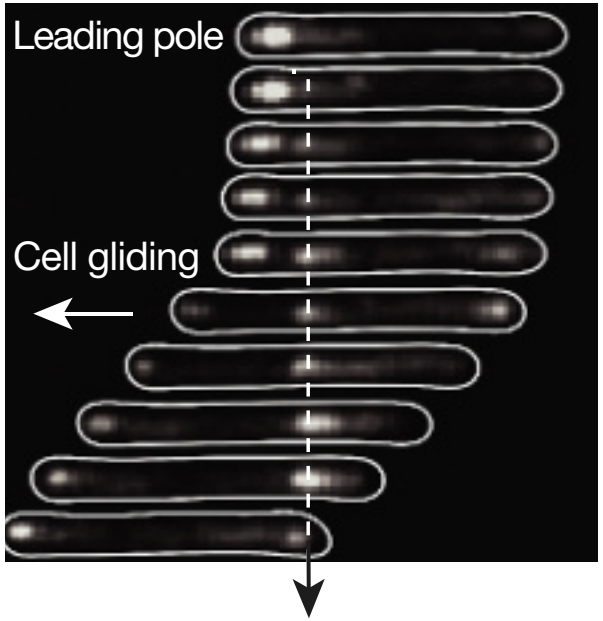
Crawling of rigid cells on a substrate: gliding

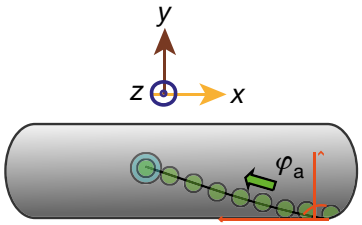
Myxococcus xanthus (ProteoB),

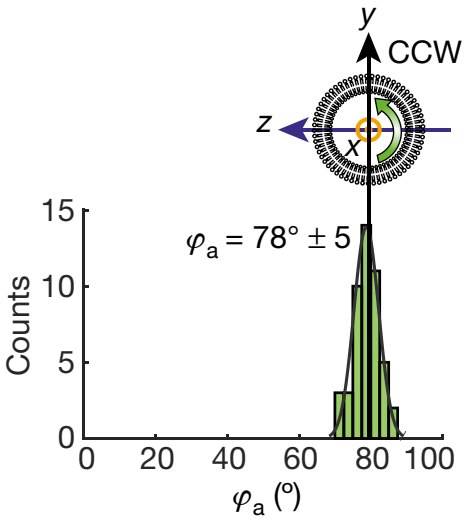
Moves at 2-4 µm/min









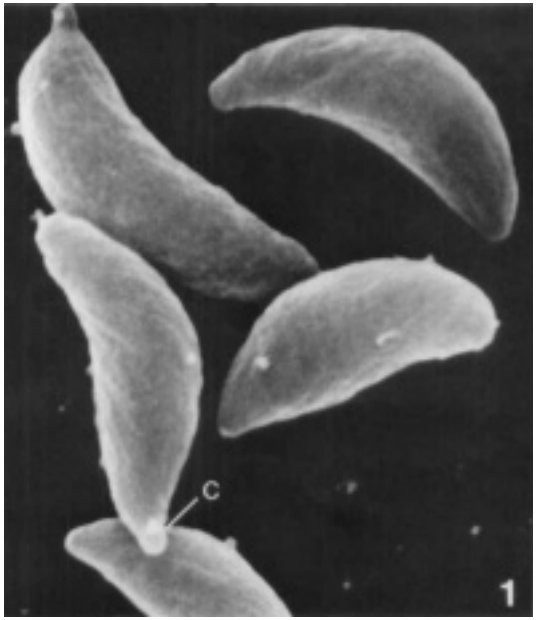


L. Faure et al, and T. Mignot *Nature* 2016, 539:530-535

Crawling of rigid cells on a substrate: gliding

Apicomplexa (Sporozoa): e.g. parasitic agent of malaria, toxoplasmosis

P. Keeling et al. *Plos Biol*. (2014)
https://doi-org.insb.bib.cnrs.fr/10.1371/journal.pbio.1001889

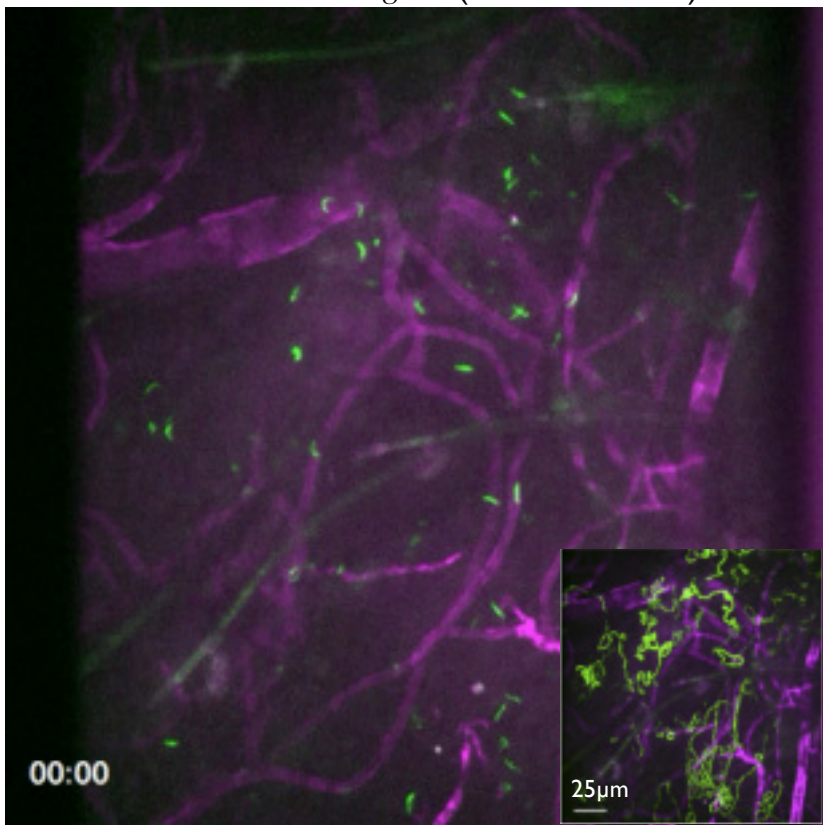


Chiappino et al *J. Protozool* 31:288-292 (1984)

Crawling of rigid cells on a substrate: gliding

A rich repertoire of gliding behaviors on a substrate or in situ

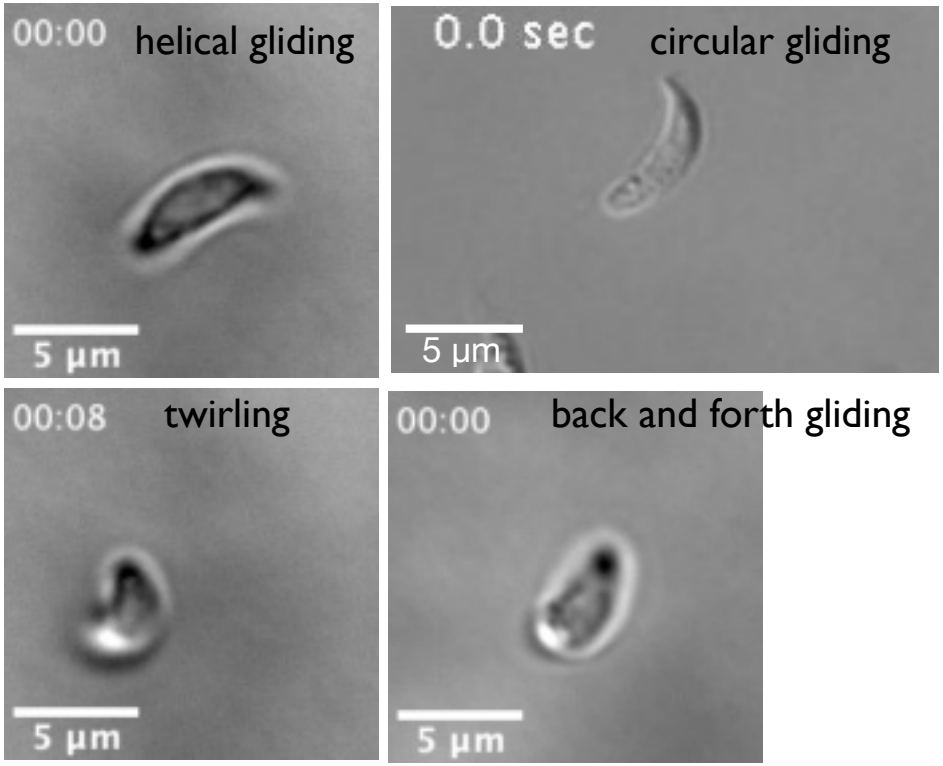
Plasmodium berghei (rodent malaria)



P. berghei sporozoites 10 min after intradermal inoculation with CD31-labeled vascular endothelial cells.

Hopp et al. *eLife* 2015;4:e07789. DOI: 10.7554/eLife.07789

Toxoplasma gondii



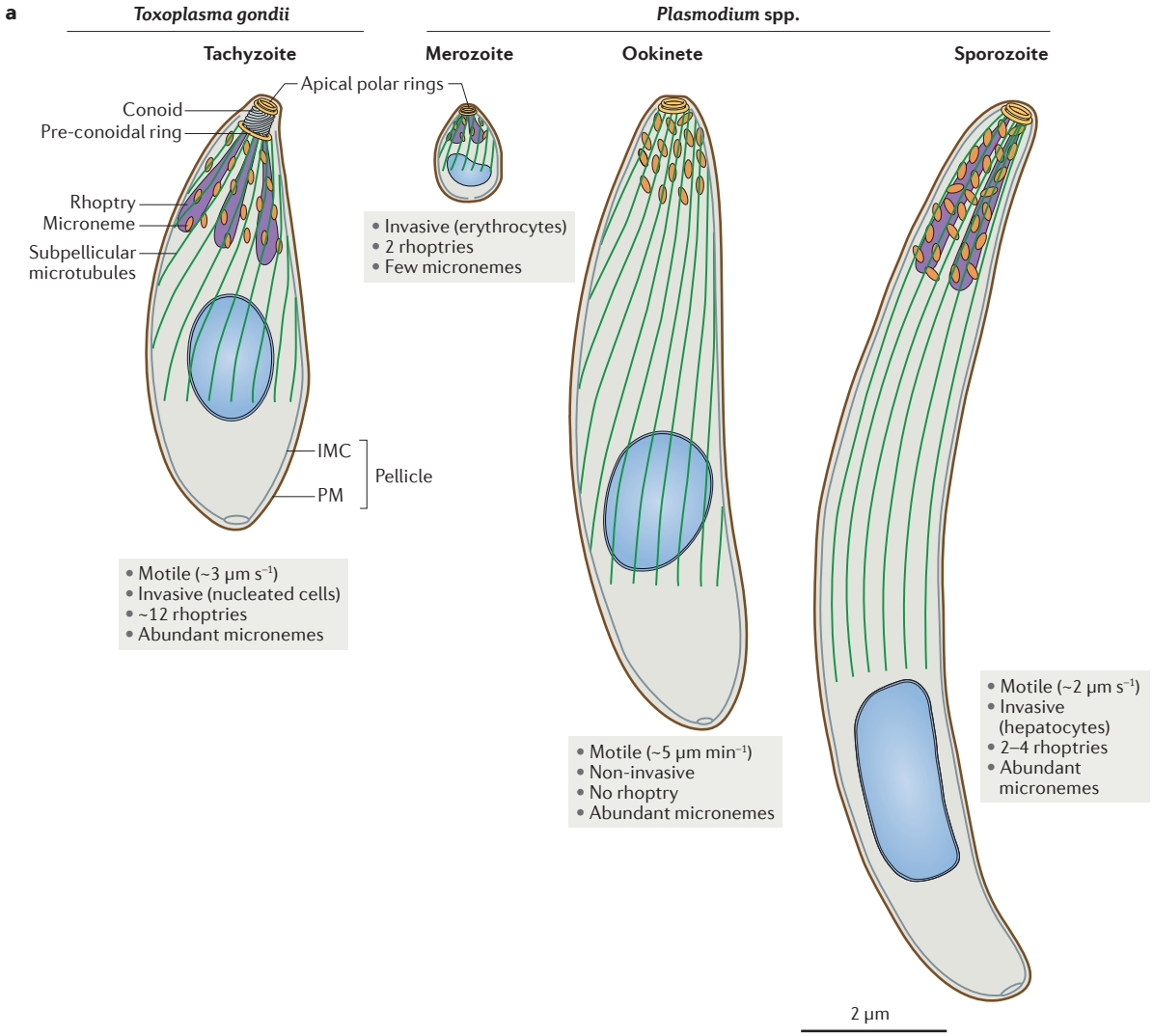
K. Frénal et al. and D. Soldati-Favre. *Nature Rev. Micro*. 15:645-660 (2017)

Crawling of rigid cells on a substrate: gliding

Structural organisation

Cells have a rigid, curved architecture

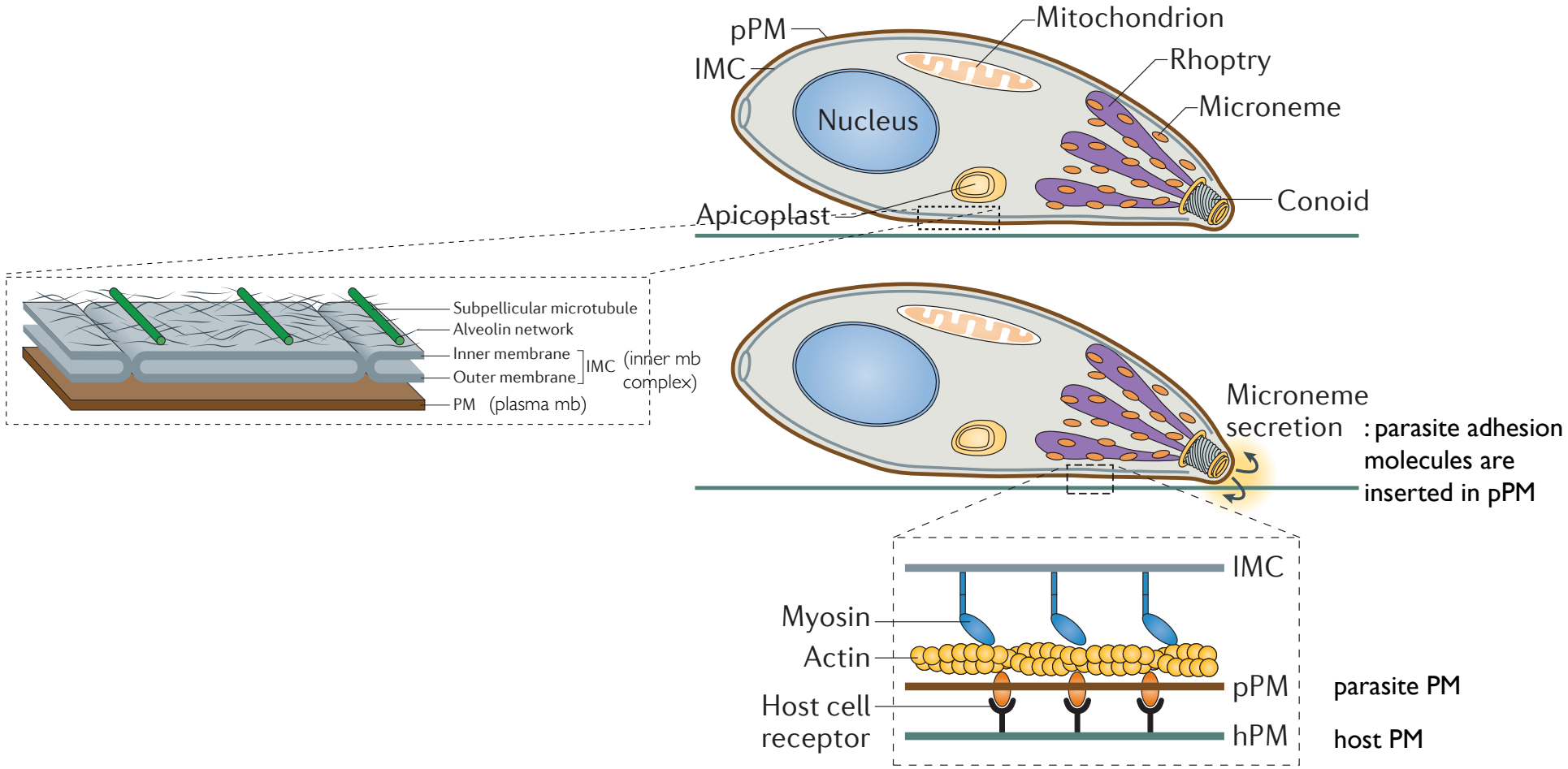
Microtubules dictate twisted cell shape



K. Frénal et al. and D. Soldati-Favre. *Nature Rev. Micro*. 15:645-660 (2017)

Crawling of rigid cells on a substrate: gliding

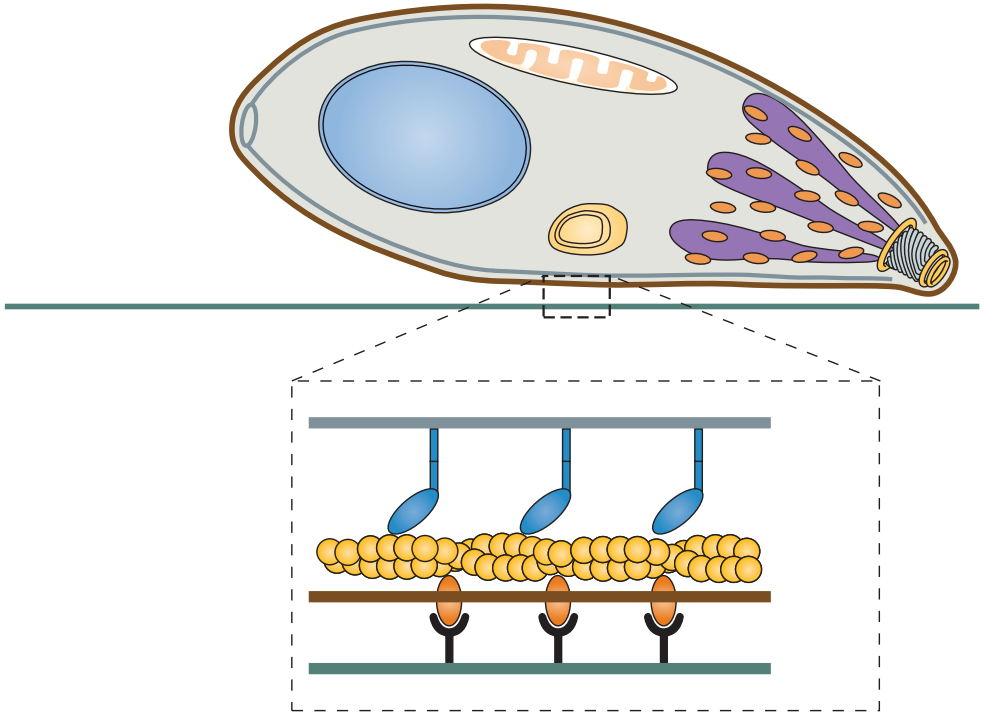
Anatomy of the motor system



K. Frénal et al. and D. Soldati-Favre. *Nature Rev. Micro*. 15:645-660 (2017)

Crawling of rigid cells on a substrate: gliding

Polar actin flow against adhesin drive motility



Backward movement of the adhesins

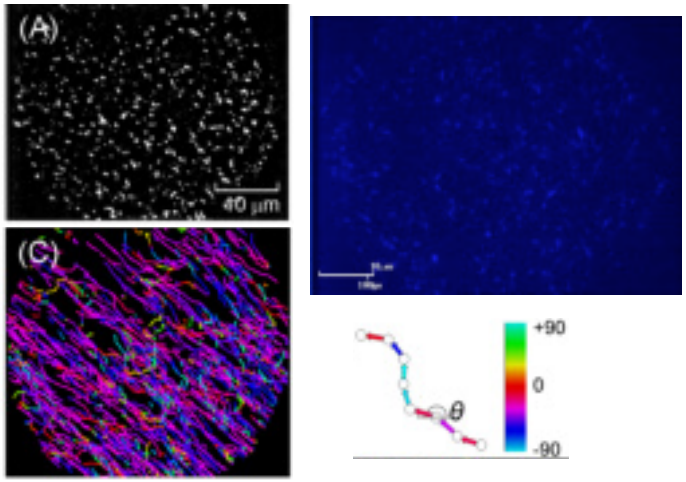
Forward movement of the parasite

K. Frénal et al. and D. Soldati-Favre. *Nature Rev. Micro*. 15:645-660 (2017)

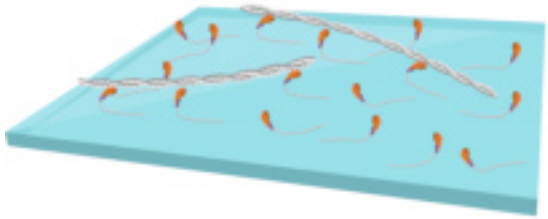
- in vitro actin motility assay on substrate conjugated with Myosin motors
- Alignement and flow of short actin filaments

instantaneous image

temporal projection

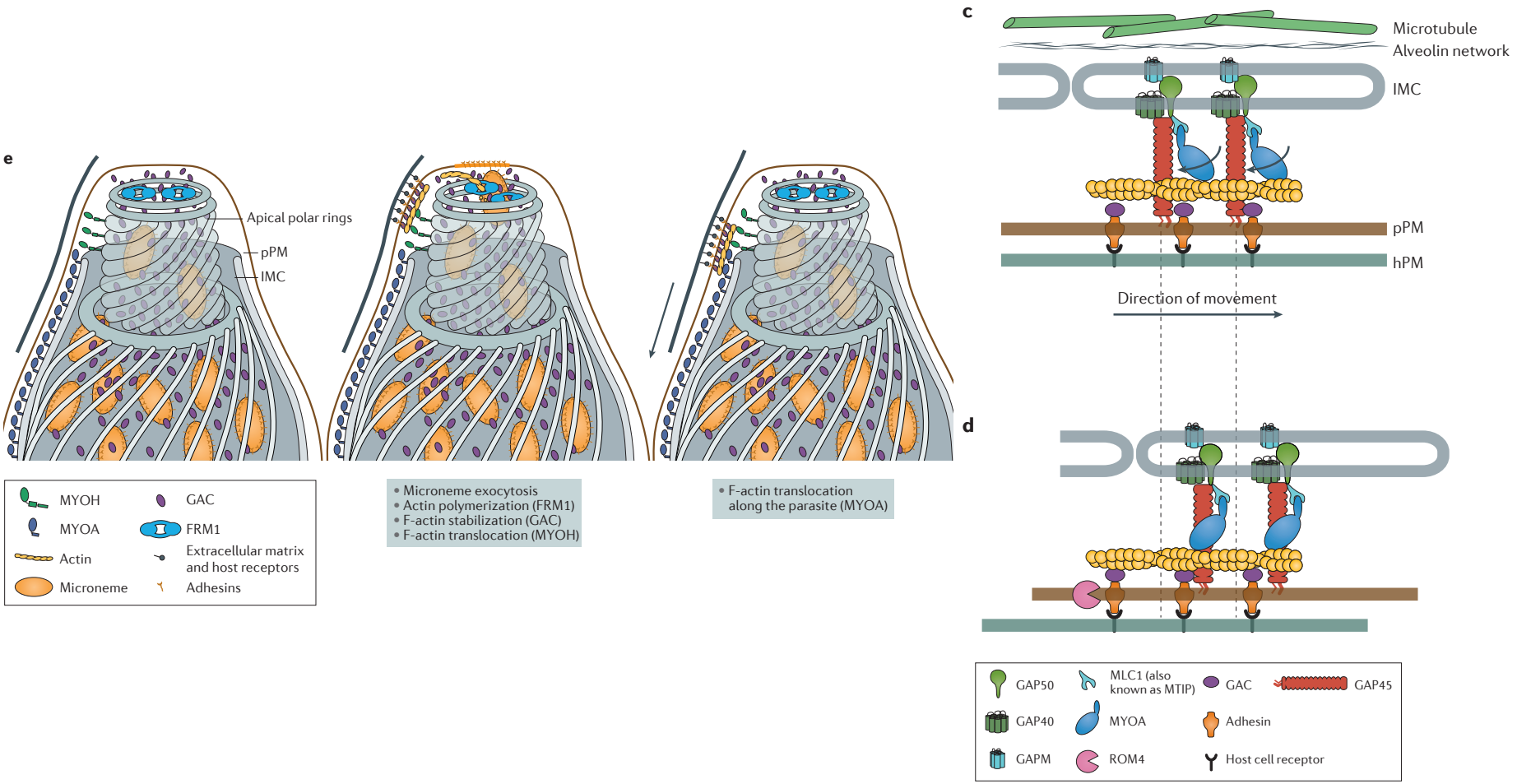


T. Butt et al. *J. Biol.Chemistry*. 285:4964–4974 (2010)



Crawling of rigid cells on a substrate: gliding

Initiation of motility at apical pole and propagation by glideosome complex

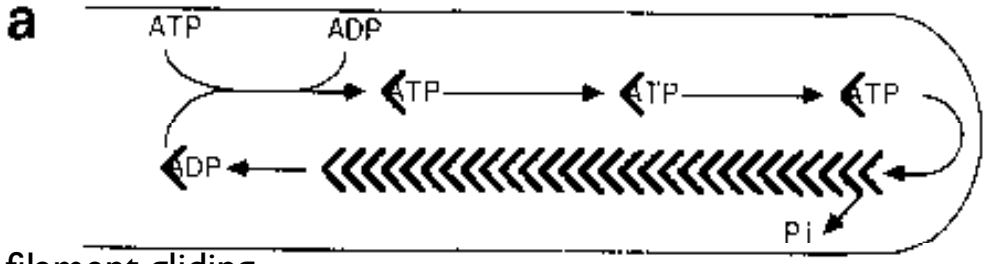


K. Frénal et al. and D. Soldati-Favre. *Nature Rev. Micro*. 15:645-660 (2017)

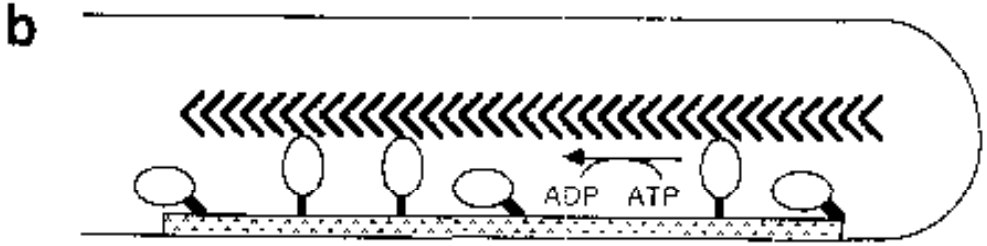
Actin flow and motility

Two ways to power actin flow

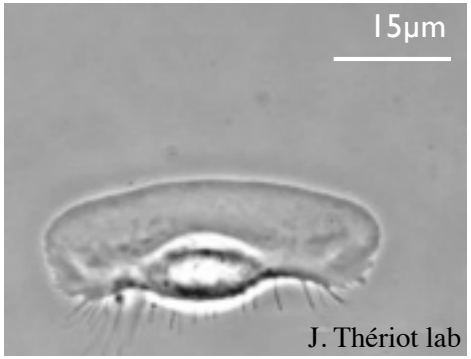
- actin treadmilling and polarized nucleation



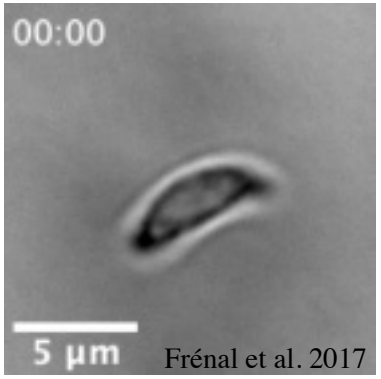
- motor drivin filament gliding



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



Keratocyte

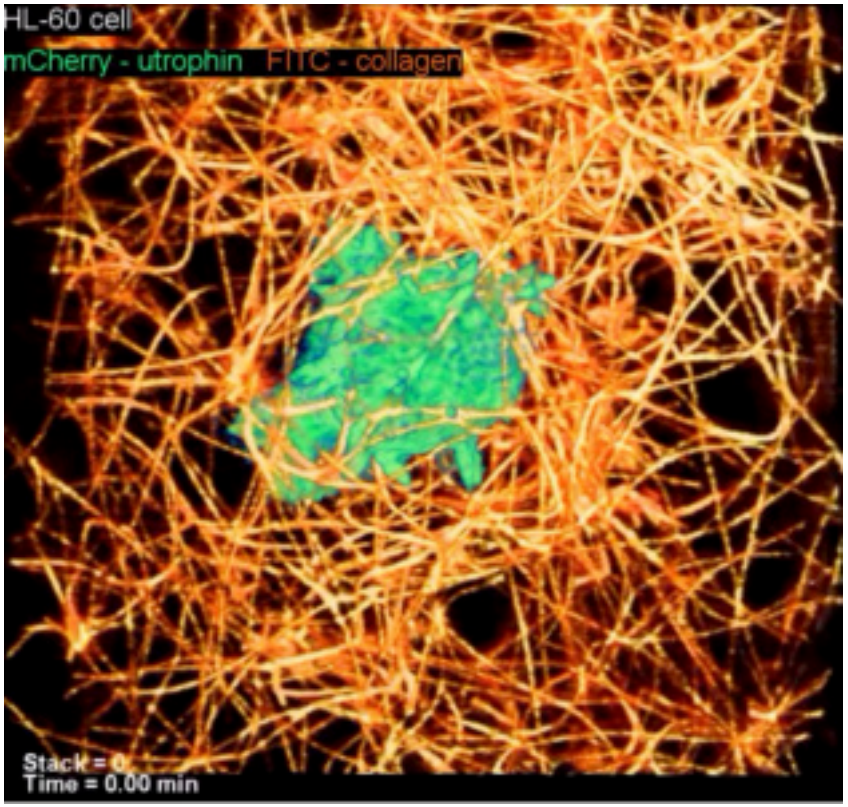


Toxoplasma gondii

Crawling in confinement - Plan

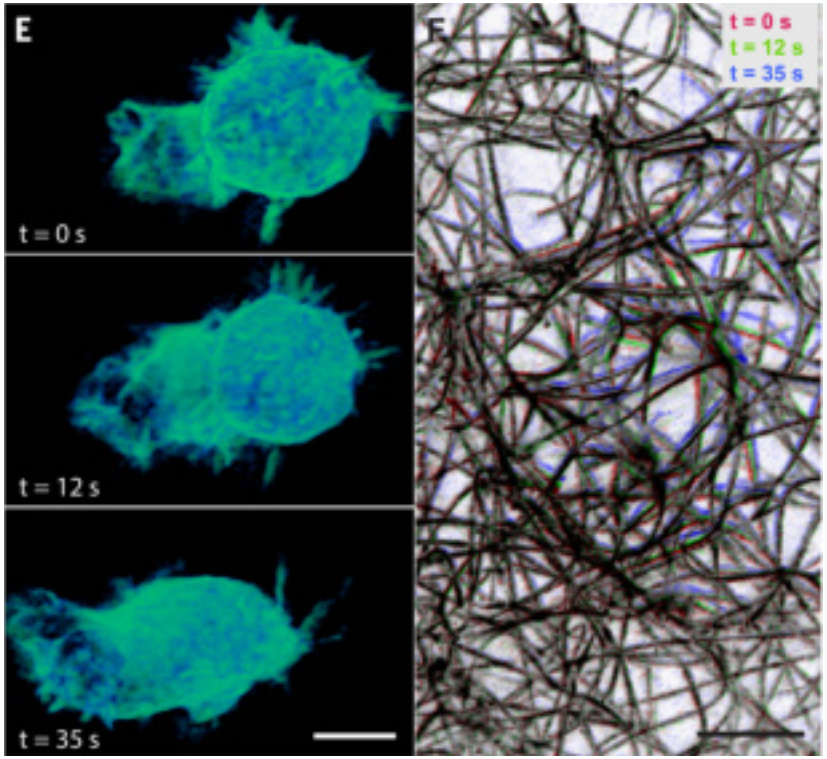
- Manifestations of cell motility under confinement
  —Adhesion independent motility
- Mechanisms of propulsion:
  —actin retrograde flow and friction
  —environment topography
  —confinement sensing
- Mechanisms of protrusion -
  —Blebbs
- Evolutionary origin of ameboid motility

How do cells move in the complex 3D in vivo environment?



HL-60 cells
mCherry-Utrophin (F-actin)

collagen labeled with FITC



Lattice light sheet microscopy of a neutrophilic HL-60 cell expressing mCherry-utrophin (marks F-actin) in a 3D collagen matrix labeled with FITC

Bi-Chang Chen et al. and E. Betzig. *Science* 346, (2014)

DOI: 10.1126/science.1257998

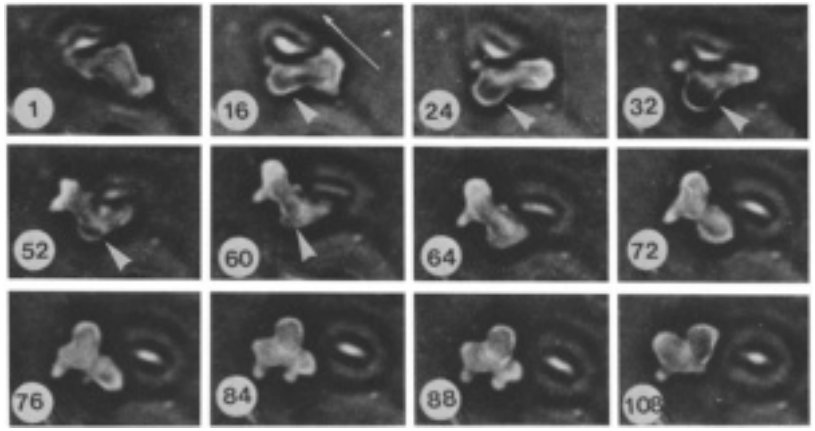
Evidence of Adhesion-free motility

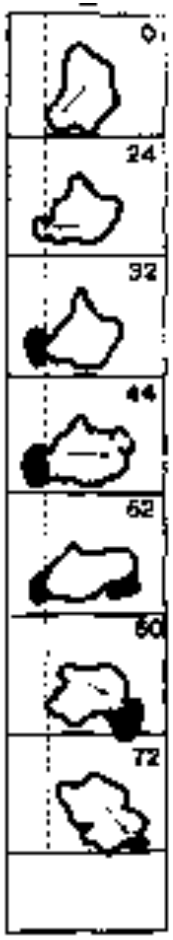
Lymphocyte Locomotion and Attachment on Two-dimensional Surfaces and in Three-dimensional Matrices

WENDY S. HASTON, JAMES M. SHIELDS, and PETER C. WILKINSON
Department of Bacteriology and Immunology, University of Glasgow, Glasgow G11 6NU, Scotland

A sequence from a time-lapse film of a lymphocyte moving through a collagen gel enlarged to show the "anchoring" pseudopodia.

Lymphocytes poorly adhere to 2D substrate, yet are highly motile on 3D gel of collagen, indicating adhesion independent motility





Haston WS, Shields JM, Wilkinson PC. *J. Cell Biol*. 92:747–52 (1982)

Evidence of Adhesion-free motility

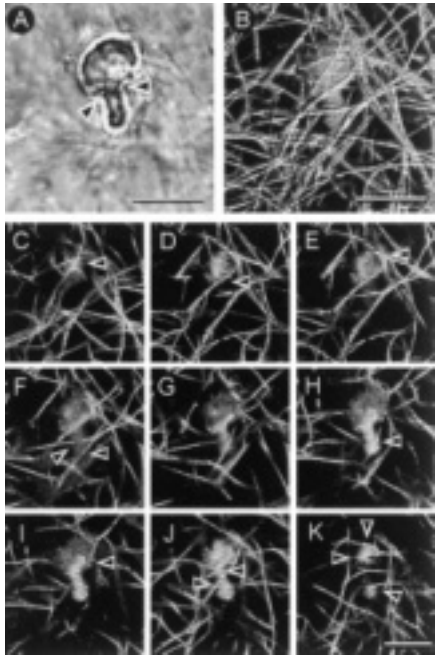
- Cell motility (random motility and chemotaxis) is unaffected by EDTA or antibodies against Integrins
- Motility is enhanced when cells are sandwitched between slide and coverslip (chimneying model)

Random locomotion and chemotaxis of human blood polymorphonuclear leukocytes (PMN) in the presence of EDTA: PMN in close quarters require neither leukocyte integrins nor external divalent cations

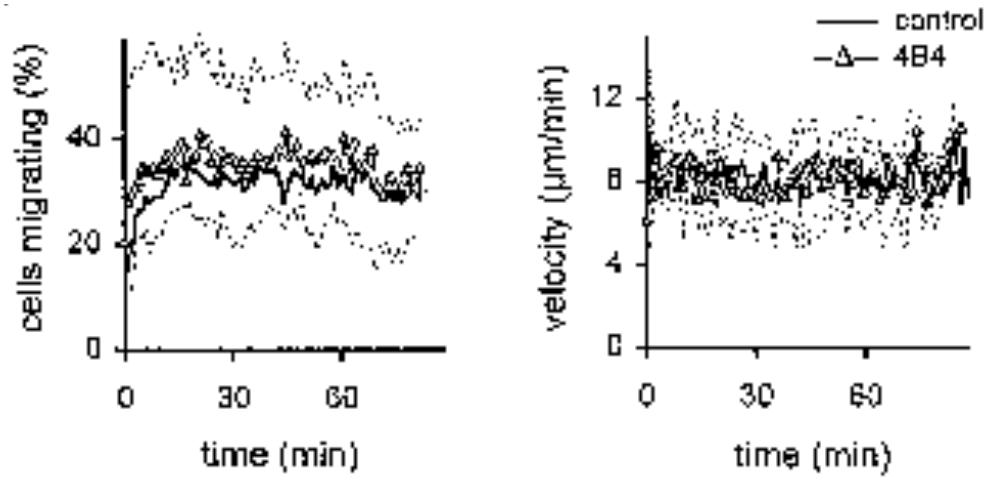
STEPHEN E. MALAWISTA*† AND ANNE DE BOISFLEURY CHEVANCE‡

S. Malawista and A. Boisfleurry Chevance *PNAS* 94, 11577–11582, (1997)

- Amoeboid motion of a T lymphocyte within a 3-D collagen matrix.



Anti ß-integrin blocking antibody does not affect cell motility in collagen matrix



P. Friedl et al *Eur. J. Immunol*. 28: 2331–2343 (1998)

reviewed in P. Friedl et al *J. Leukoc. Biol*. 70: 491–509 (2001).

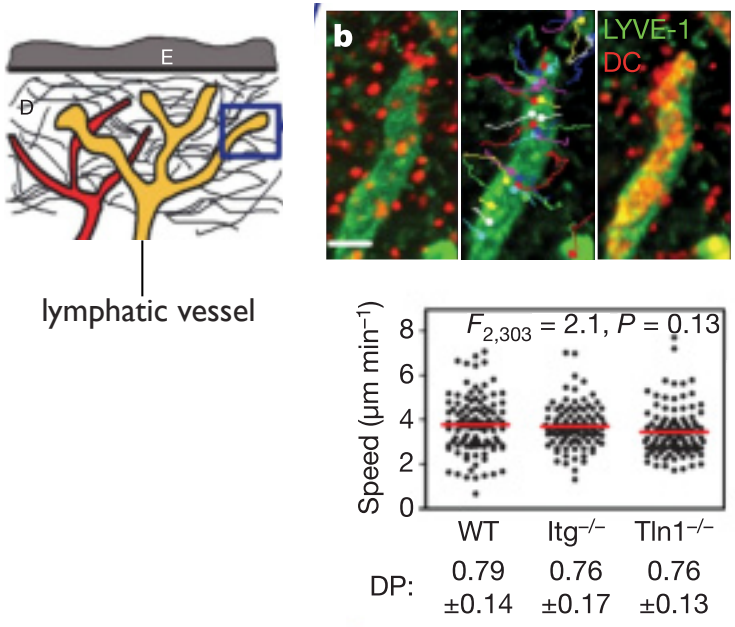
Demonstration of Integrin/adhesion independent motility

Rapid leukocyte migration by integrin-independent flowing and squeezing

Tim Lämmermann[1], Bernhard L. Bader[3], Susan J. Monkley[4], Tim Worbs[5], Roland Wedlich-Söldner[2], Karin Hirsch[1], Markus Keller[3], Reinhold Förster[5], David R. Critchley[4], Reinhard Fässler[1] & Michael Sixt[1]

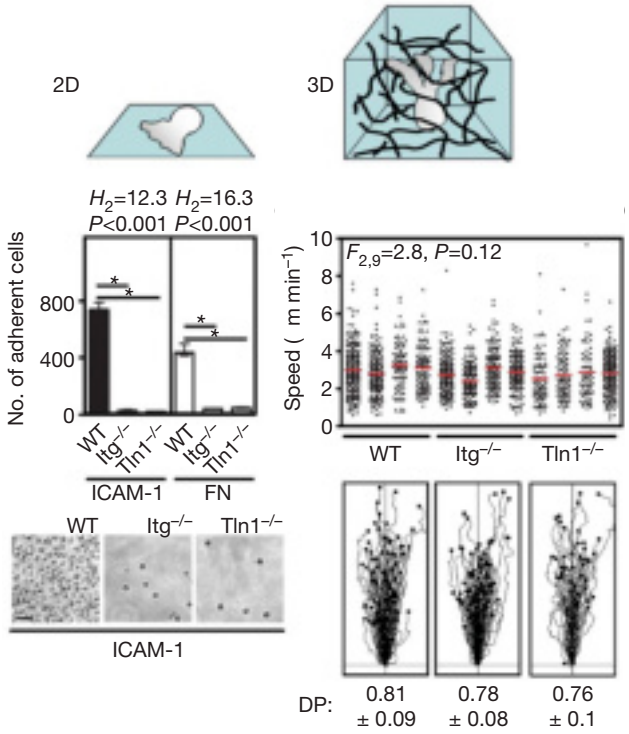
Interstitial migration of dendritic cells (DC) within skin and entry into lymphatic vessels

Integrin and Talin mutants have normal migration speed



wild-type (WT), integrin$^{-/-}$ (Itg$^{-/-}$) and talin1$^{-/-}$ (Tln1$^{-/-}$) DCs

Velocities of chemotaxing leukocytes in 3D collagen matrices is unaffected in integrin and Talin mutants



Adhesion: affected Migration: unaffected

Lämmermann, T. *et al*. and M. Sixt. *Nature* 453, 51–55 (2008).

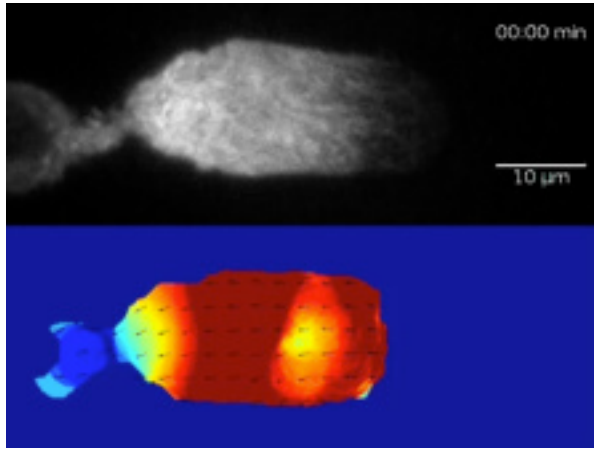
Integrin/adhesion independent motility

- What is the nature of propulsive forces?

—contractility at the cell rear
—cell expansion at the front by fluid flow in actin free regions (blebbs)
—actin retrograde flow

- What allows force transmission?

—Confinement and Friction with surrounding in 3D



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

Crawling in confinement - Plan

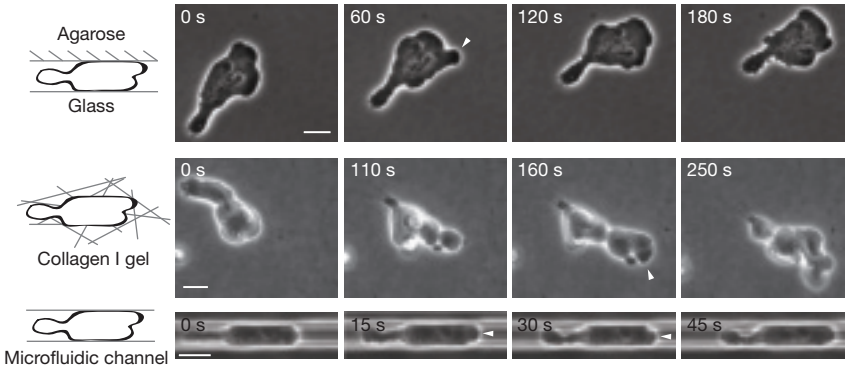
- Manifestations of cell motility under confinement
  —Adhesion independent motility
- Mechanisms of propulsion:
  —actin retrograde flow and friction
  —environment topography
  —confinement sensing
- Mechanisms of protrusion - Blebbs
- Evolutionary origin of ameboid motility

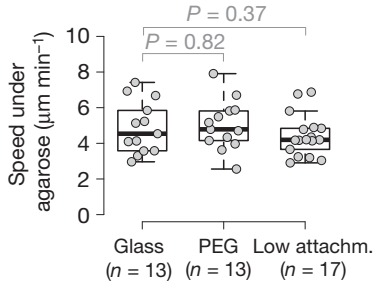
Cell confinement in 3D induces motility

Manifestation of 3D confined cell motility

- Cell movement in different 3D environments

Time lapses of migrating blebbing Walker cells under agarose, within a 3D collagen-I gel or in a BSA-coated microfluidic channel

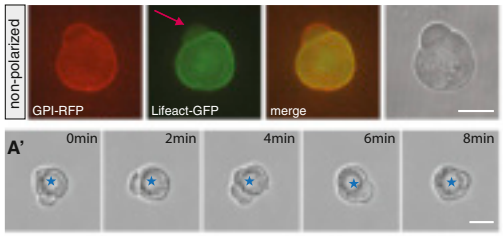




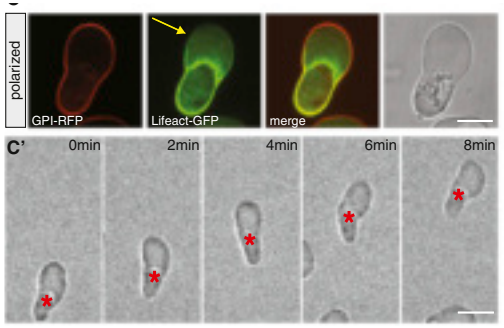
Non adhesive substrates

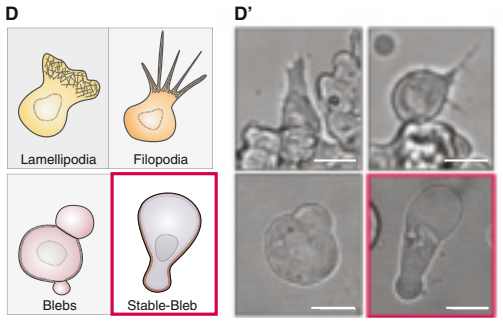
Bergert M, et al and G. Salbreux and E. Paluch. *Nat. Cell Biol.* 17:524–29 (2015)

- Zebrafish germ layer progenitor cells adopt a polarized motile state in presence of serum in confinement in vitro



+serum





immobile

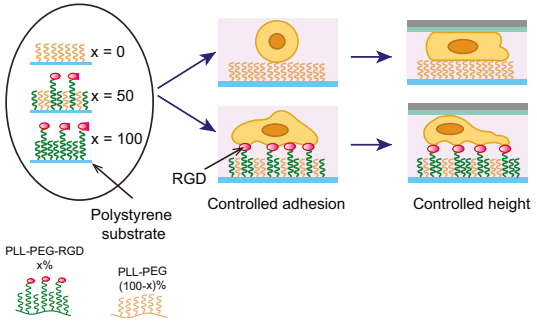
motility is associated with cell polarisation

V. Ruprecht et al. and R Voituriez and M. Piel. *Cell* 160, 673–685 (2015)

Cell confinement in 3D induces motility

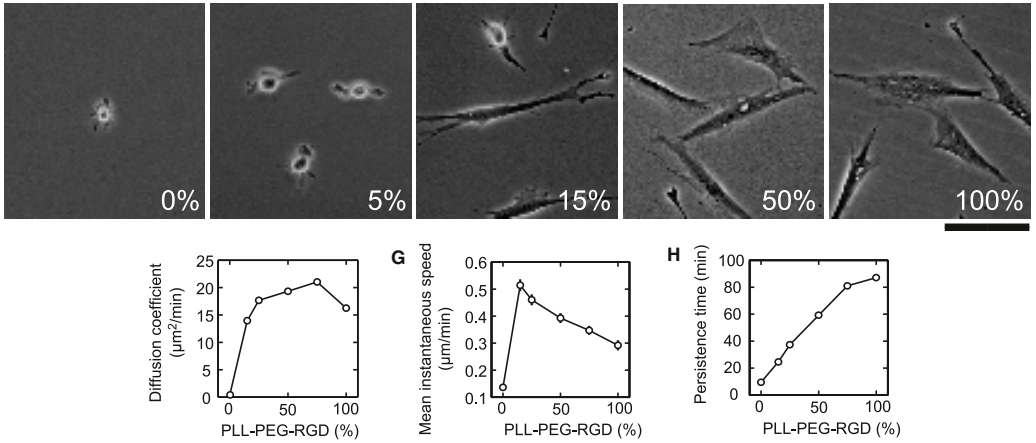
Change in cell morphology due to confinement

- strength of adhesion is tuned by % of RGD peptide in vitro
- height of confinement is tuned



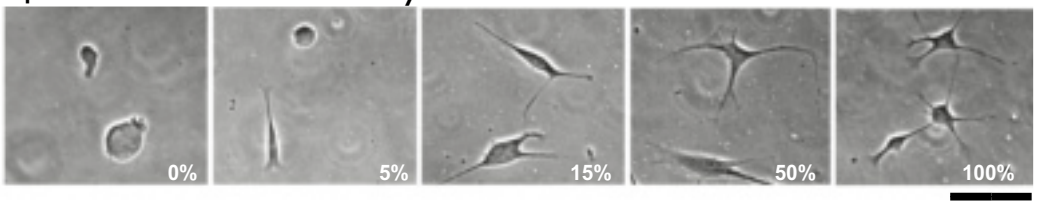
- Adhesion to 2D substrate

Normal human dermal fibroblast cells (NHDF) migrate in a mesenchymal fashion: lamelipodia, filipodia



- 3D confinement: cells adopt different morphologies

5μm confinement: cell body alone is deformed

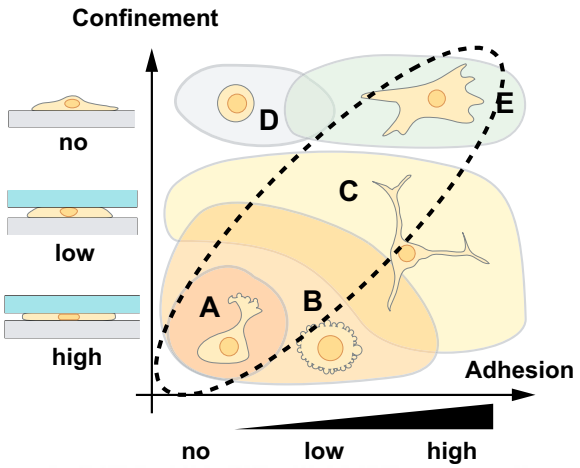


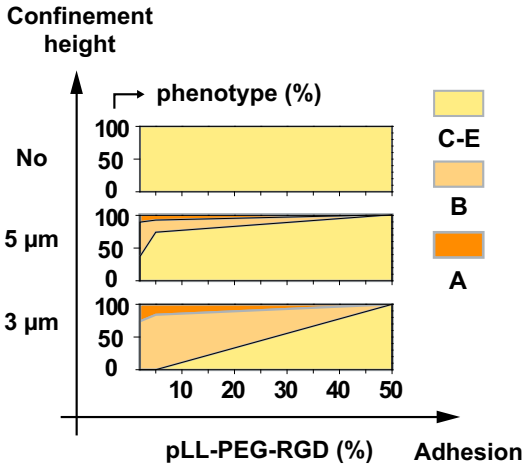
YJ. Liu et al, R. Voituriez and M. Piel *Cell* 160, 659–672 (2015)

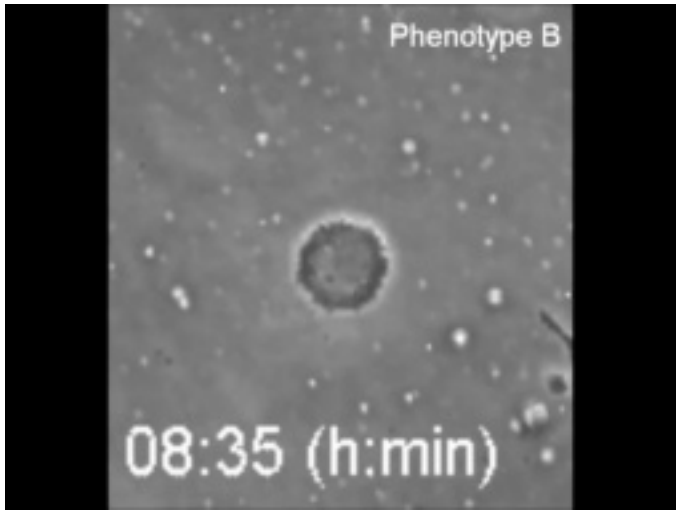
Cell confinement in 3D induces motility

Competing effects of adhesion and confinement on motility

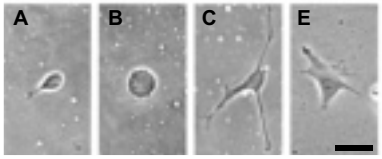
- Without confinement: adhesion promotes motility (E vs D)
- With high confinement:
  - —no motility at intermediate adhesion (B)
  - —rapid motility without adhesion (A)

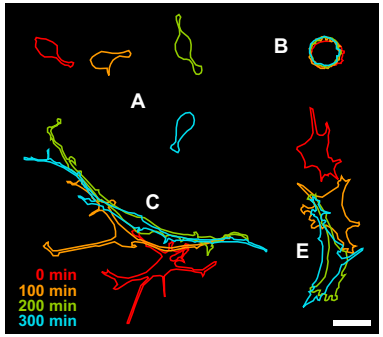


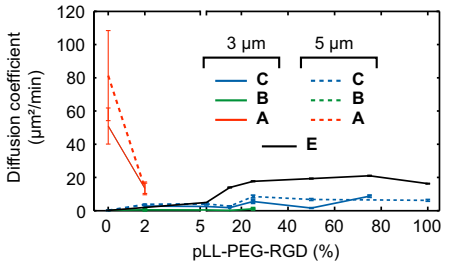


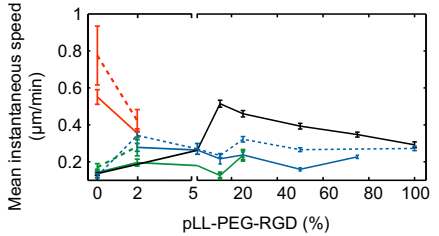


3µm confinement: cell body and nucleus deformed









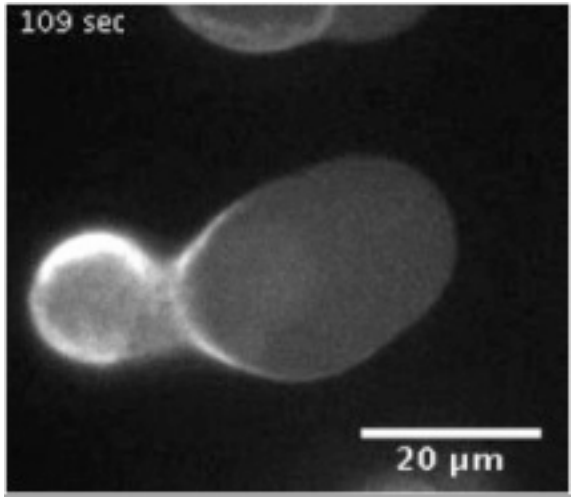
YJ. Liu et al, R. Voituriez and M. Piel *Cell* 160, 659–672 (2015)

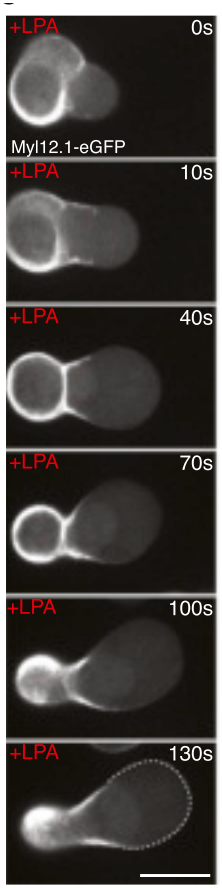
Cell confinement in 3D induces motility

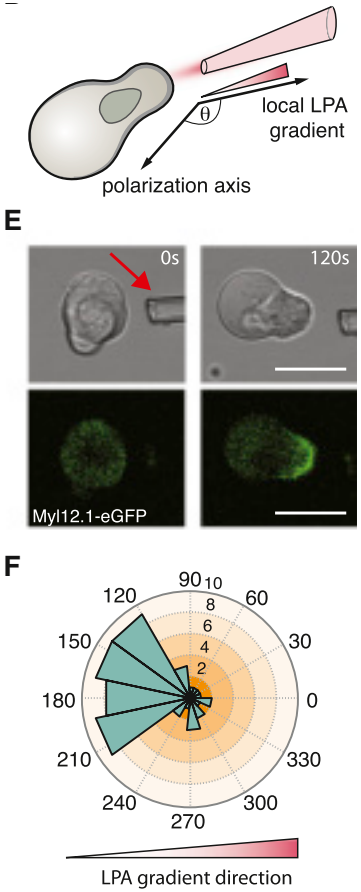
Induced polarization in adhesion free motility by chemical gradient

- LPA (lysophosphatidic acid) induces a switch from mesenchymal to amoeboid motility
- LPA induces Myosin-II contractility (inhibiting MyoII blocks the amoeboid state)
- LPA induces actomyosin and cell shape polarization

+ Blebbistatin (Myosin II inhibitor)





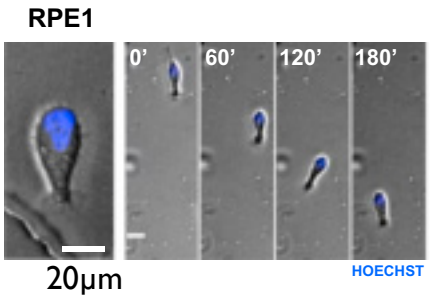


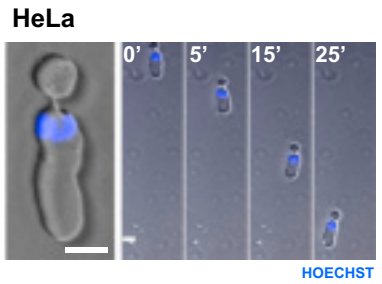
V. Ruprecht et al. and R Voituriez and M. Piel. *Cell* 160, 673–685 (2015)

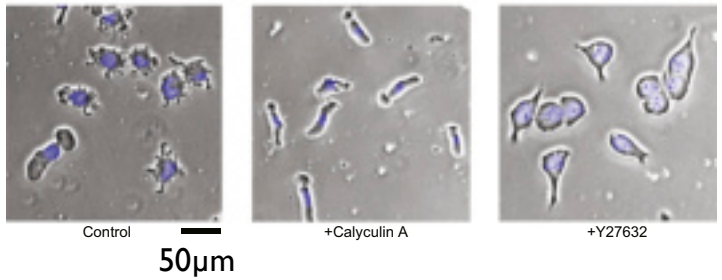
Cell contractility is required for confined motility

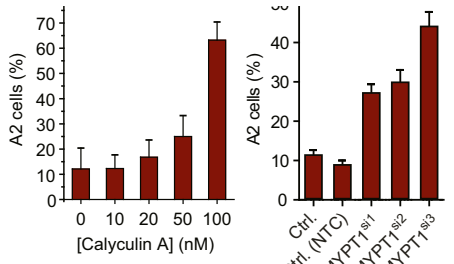
Spontaneous polarization in adhesion free motility is enhanced by cell contractility

Phase contrast recording of an amoeboid-like HeLa cell (A2) under 3-µm confinement on a non-adhesive PEG surface.

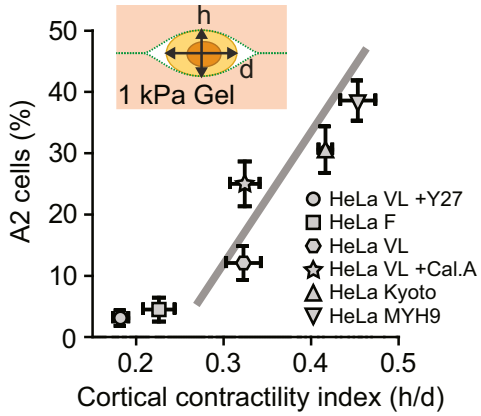








- The proportion of rapid amoeboid cells scales with cell cortex contractility



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

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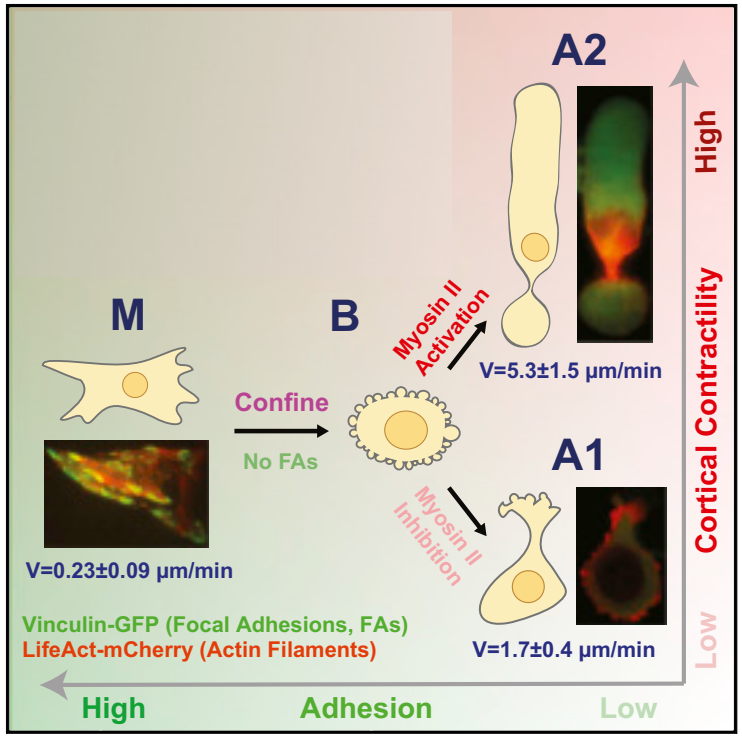
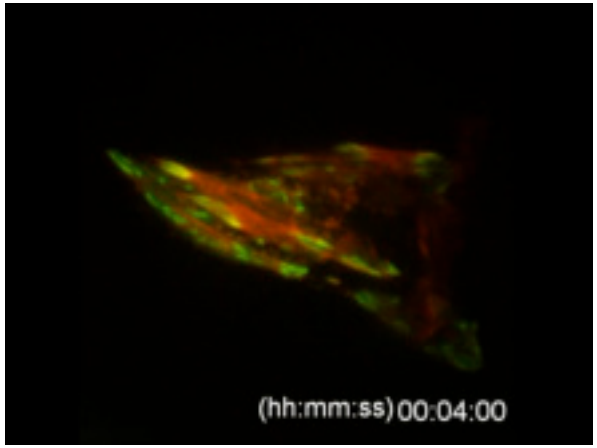
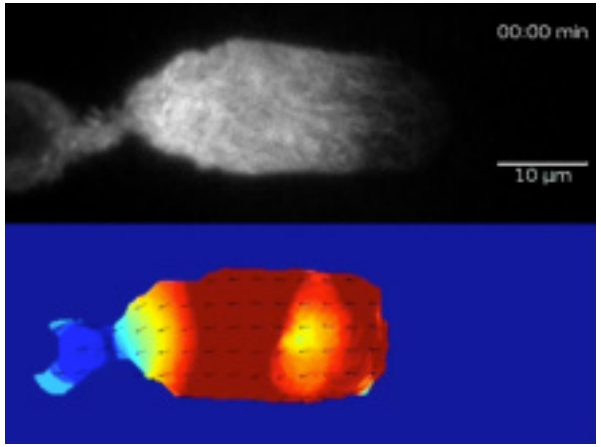




LifeAct-mCh (F-actin)
Vinculin-GFP (adhesion)



LifeAct-mCh (F-actin)

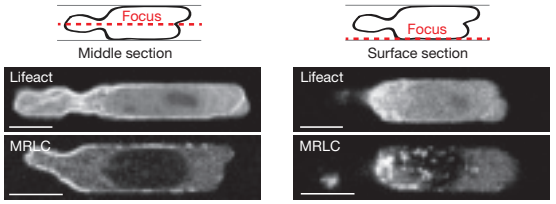
Retrograde flow

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

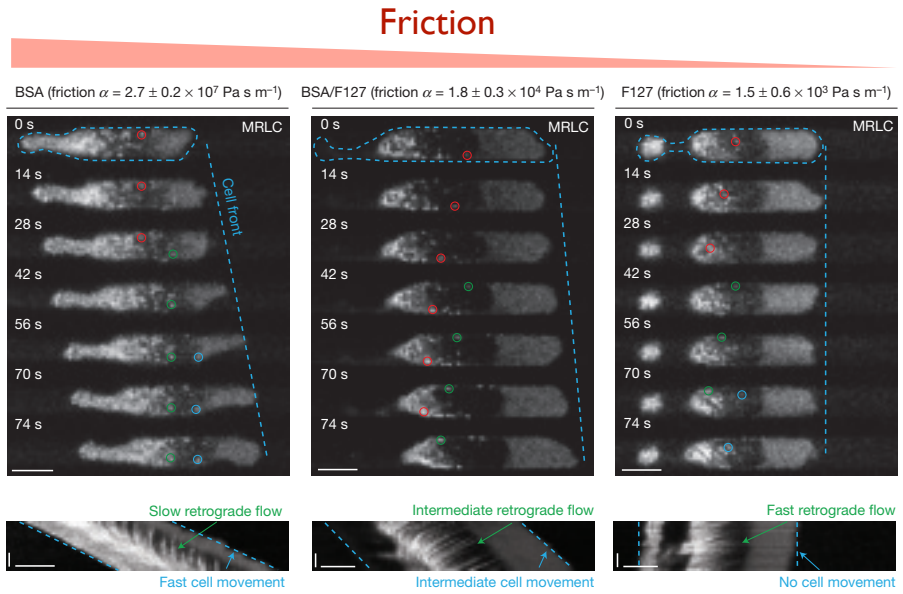
Actin retrograde flow and Force transmission by friction

Impact of friction

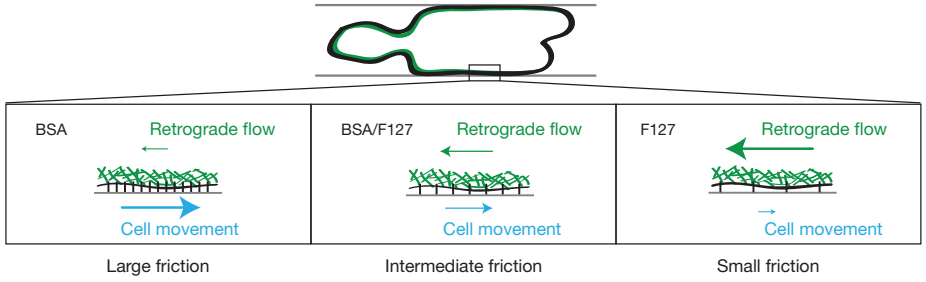
- Cells in confinement have a clear front-rear polarity:
  —MyosinII and F-actin are enriched at the posterior



- Retrograde actin flow in referential of the cell
- The actin flow in lab referential depends on friction coefficient with walls of capillary:
  —If very low friction, rapid flow in lab referential and no cell movement
  —if high friction, no flow in lab referential and rapid cell movement



- This supports the view that **frictional forces are essential for forward movement**



Bergert M, et al and G. Salbreux and E. Paluch. *Nat. Cell Biol.* 17:524–29 (2015)

Mechanical model of confined motility

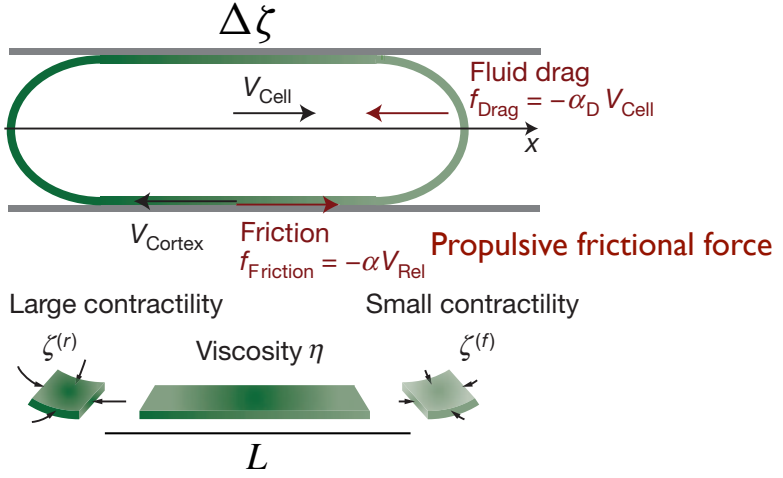
Force transmission by frictional forces

- **Mechanical model**
- Propulsive frictional forces result from retrograde actin flow velocity and allows forward movement

- Gradient of cortical tension $\Delta\zeta$ drives posterior actomyosin flow (see course 4th Dec 2018, https://www.college-de-france.fr/media/thomas-lecuit/UPL9034741296314664690_LECUIT_2018_Cours_3.pdf)

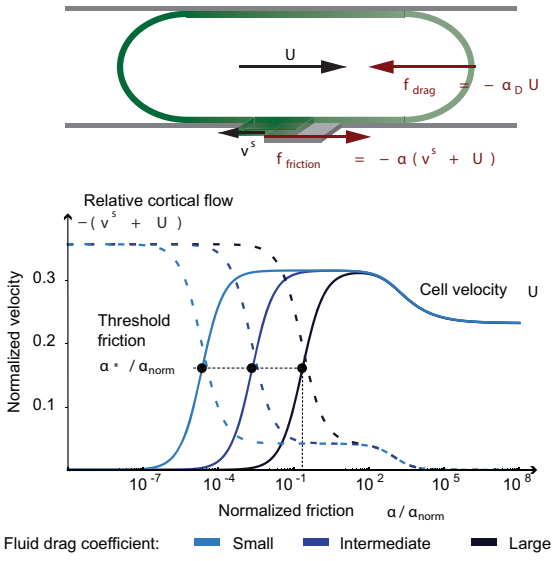
$$v_{norm} = (\zeta(r) - \zeta(f))L/\eta$$

- Fluid drag force opposes forward movement

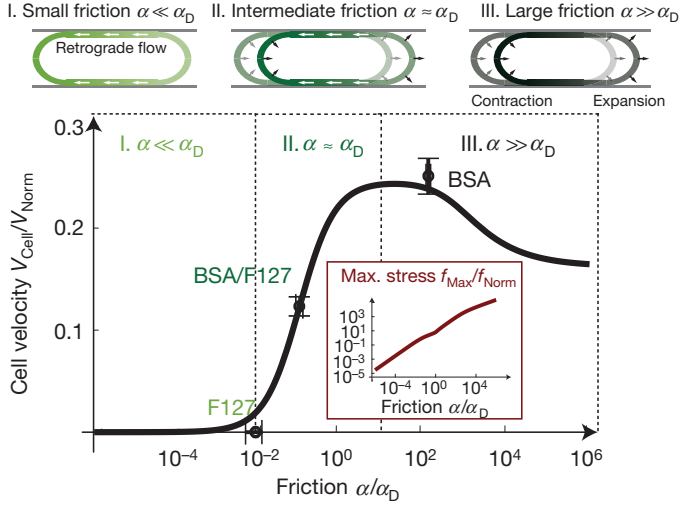


- Cortical flow in cell referential and cell velocity depend on friction.
- Above a friction threshold α^* cell movement occurs
- α^* depends on drag coefficient α_D

$$\alpha^* \approx \frac{R\alpha_D}{2L}.$$



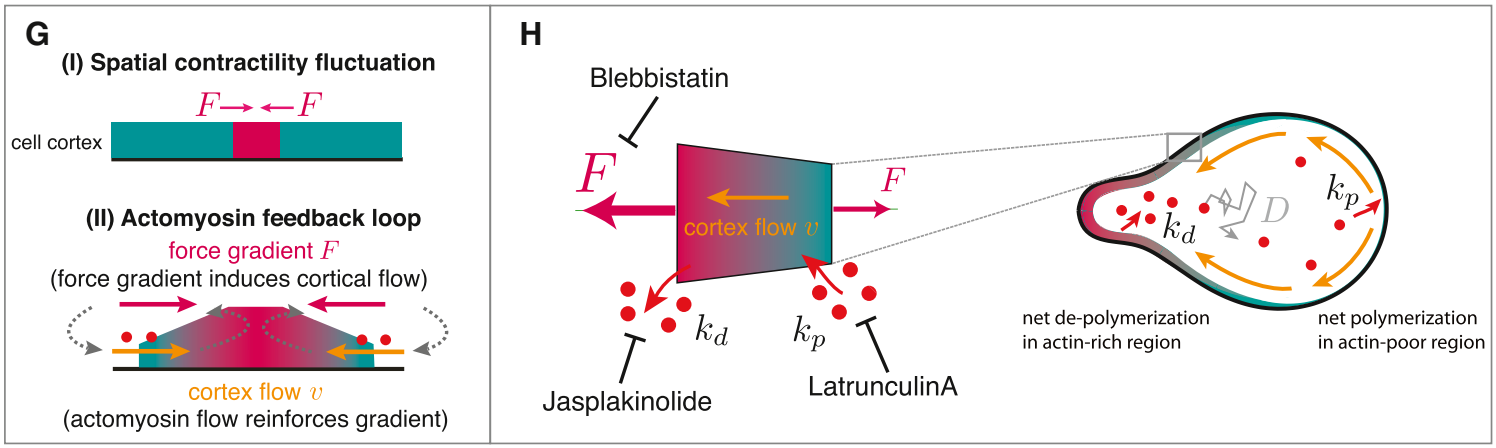
- Cell motility if frictional forces become equal to or larger than fluid drag force

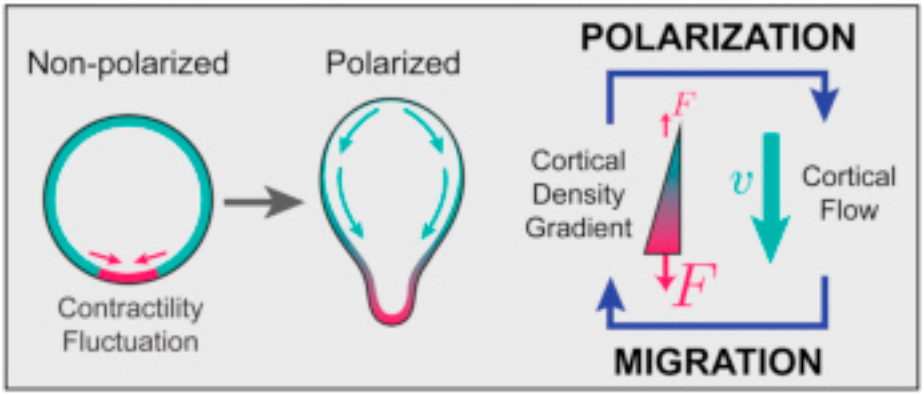


Bergert M, et al and G. Salbreux and E. Paluch. *Nat. Cell Biol.* 17:524–29 (2015)

A positive feedback loop underlies polarization and motility

Contractile gradient drives flow which reinforces the gradient
Positive feedback (similar to 2D crawling)



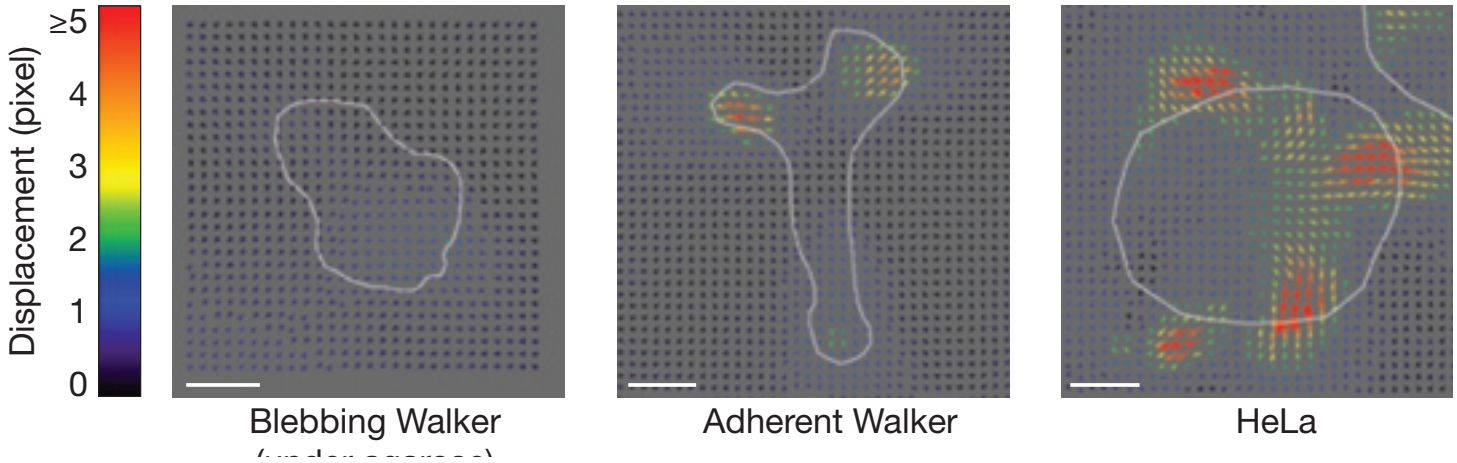


V. Ruprecht et al. and R Voituriez and M. Piel. *Cell* 160, 673–685 (2015)

Traction forces in 2D substrate vs 3D confinement

- traction force in 3D confinement are much smaller than in cells adhering to a substratum

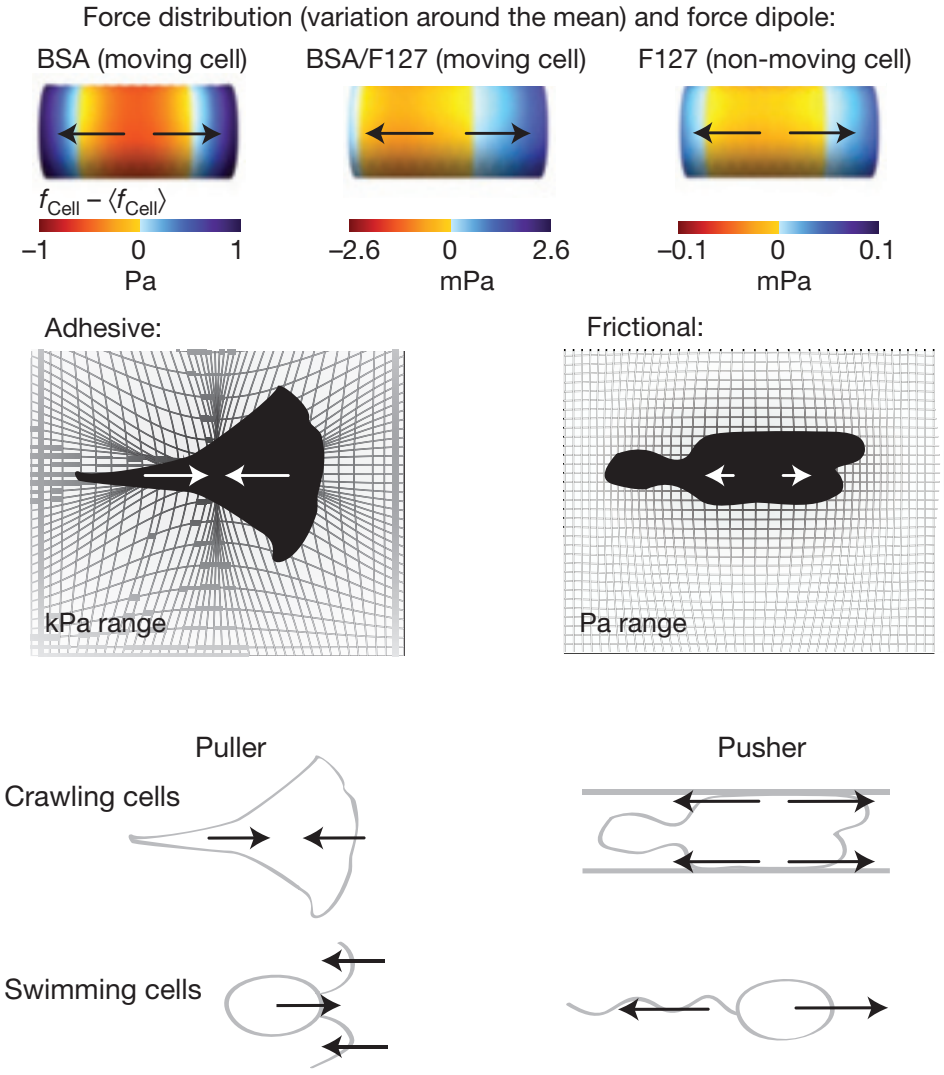
—Adhesion: nN range per μm² : 1kPa range
—Adhesion free: pN range per μm²: 1 Pa range



Bergert M, et al and G. Salbreux and E. Paluch. *Nat. Cell Biol.* 17:524–29 (2015)

In confinement, cells are pushers, and not pullers

- Opposite force dipole in adhesion and adhesion independent motility
- **Adhesion dependent:** Negative force dipole of traction forces reflects combined effect of retrograde actin flow and cell contraction
  Contraction is used to de-adhere
- **Adhesion free:**
  Positive force dipole reflects expansion due to contraction at the back and frictional resistance



Bergert M, et al and G. Salbreux and E. Paluch. *Nat. Cell Biol.* 17:524–29 (2015)

Comparison of adhesion and adhesion-free motility

Migration Mode	Adhesive	Non-adhesive
Protrusion type	Usually lamellipodia	Usually blebs
Propelling force generation	Filament extension/actin flow	Cortex flow
Force transmission	Focal adhesion	Friction, protrusion intercalations, etc.
Substrate interaction	Specific	Non-specific
Duration of cell-substrate interactions	Longer than dwell time	Shorter than dwell time
Speed-substrate interaction strength relationship	Bell curve	Plateau
Environment	2D surfaces and 3D environments	3D confinement
Migration speed[a]	~0.1–1 μm/min	~1–10 μm/min
Stresses exerted on substrate[b]	~10^2 – 10^5 Pa	<1Pa
Actin flow profile	Mainly in lamellipodium	At the cortex all along the cell body, max velocity in cell center
Force dipole	Contractile	Expansile

Bodor et al. and E. Paluch. *Developmental Cell*. 52: 550-562 (2020)

- Friction-based migration is only possible in 3D confinement (unlike adhesion based motility)
- For 2D substrate motility, the strength and duration of molecular bonds must be strong enough to counteract Brownian motion (see catch bond and mechanical amplification mechanisms at Integrin foci)
- **In 3D, confinement prolongs the contacts of weak molecular interactions and multiply them over the entire cell surface**
- Cell substrate interactions shorter than cell dwell time in non-adhesive motility, but longer than cell dwell time in adhesive motility (thus requiring de-adhesion mechanisms). **In non-adhesive motion, friction does not interfere with cell retraction.**
- Therefore, **increasing friction does not lead to a plateau of migration speed, and no slowing down is expected even at very high friction.**

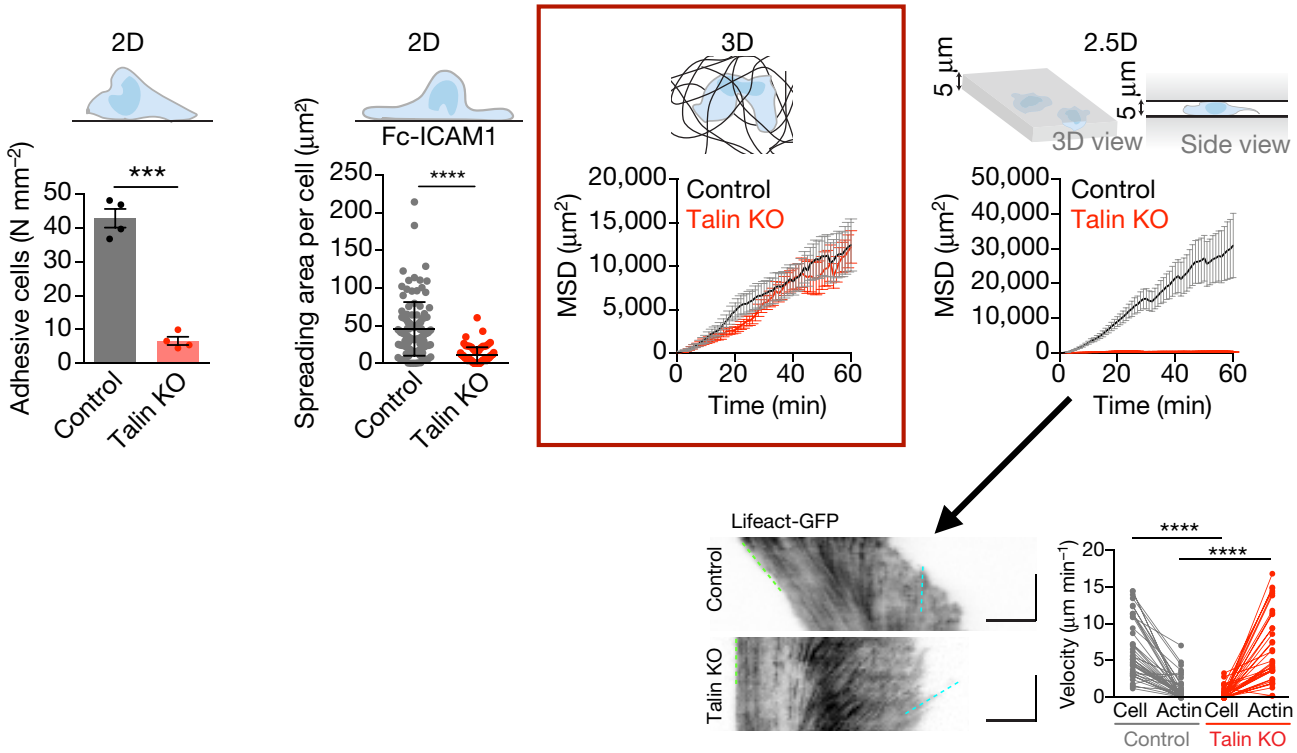
Crawling in confinement - Plan

- Manifestations of cell motility under confinement
  —Adhesion independent motility
- Mechanisms of propulsion:
  —actin retrograde flow and friction
  —environment topography
  —confinement sensing
- Mechanisms of protrusion - Blebbs
- Evolutionary origin of ameboid motility

Role of substrate topography in confined motility

T lymphocyte motility is dependent on Integrin on a 2D substrate, or in 3D confinement between glass and coverslip

However in 3D matrigel, motility is integrin independent



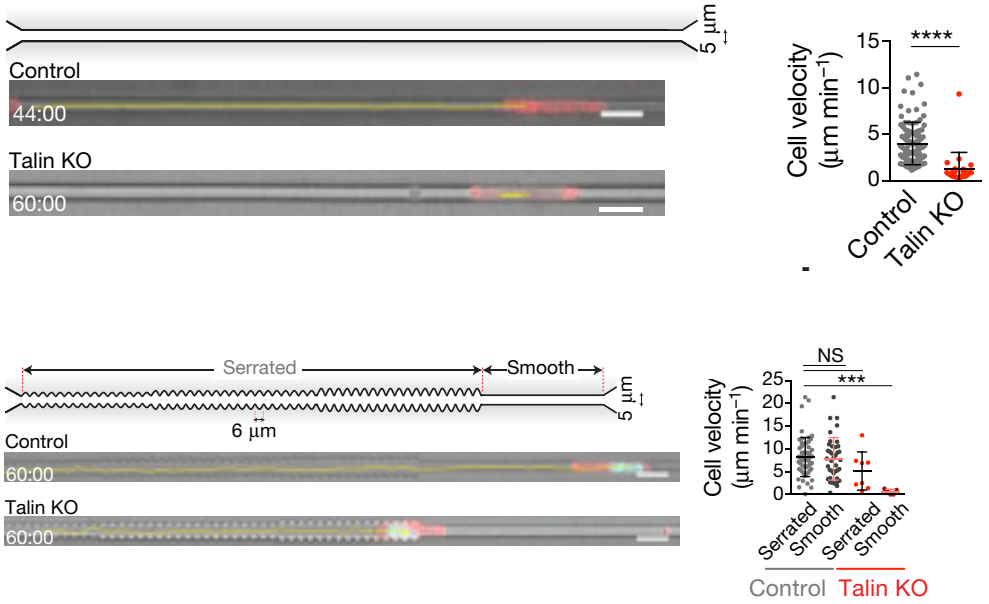
Question: what feature of 3D matrix triggers integrin dependent motility?

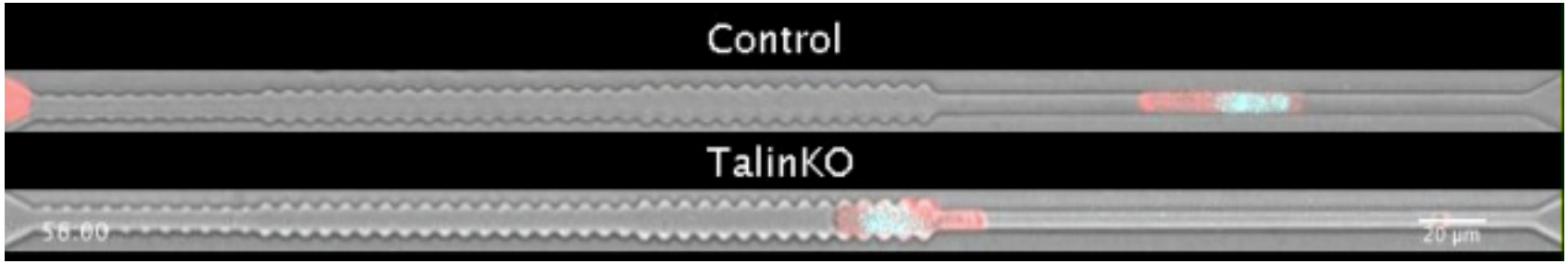
Hypothesis: Geometry/Topography of environement

A. Reversat et al. and R. Voituriez and M. Sixt. *Nature*. 582(7813):582-585. (2020) doi: 10.1038/s41586-020-2283-z.

Role of substrate topography in confined motility

Testing the importance of environment topography using smooth and serrated channels





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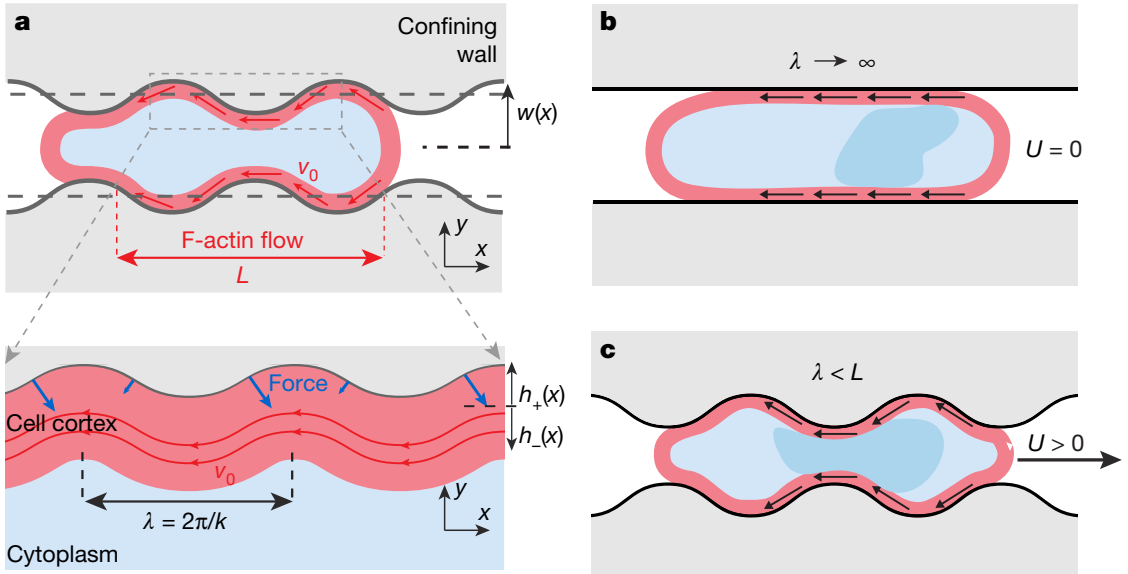
Role of substrate topography in confined motility

mechanical model of adhesion/friction free cell motility

- For a static fluid **normal forces** are homogeneous and sum up to zero
- In the **presence of a retrograde actin flow, they are inhomogeneous and sum up globally to a propulsion force**, which enables migration.
- An **irregular geometry** bends the actin flow lines and induces shear forces in the actin cytoskeleton. To maintain the actin flow and balance shear forces, a **pressure gradient** along the cell polarity axis is therefore required in irregular geometries, and locally **induces non homogeneous normal forces**: forward facing boundaries are subject to larger forces.



$$F_x = -\frac{8\pi^6}{3}\frac{z_0 L v_0 \eta}{\delta}\left(\frac{\delta}{\lambda}\right)^6 \epsilon^2,$$

$w(x) = w_0 + w_1 \sin(kx)$

$\lambda = 2\pi/k$ $\quad w(x) - \delta < y < w(x)$

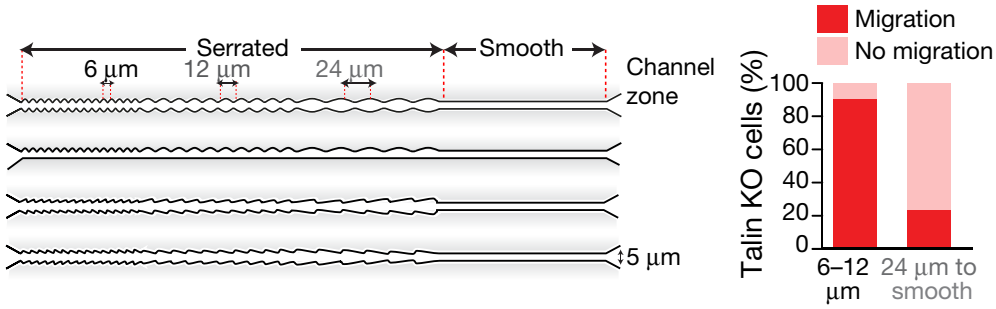
Predictions: dependency of force Fx on flow velocity and inverse of length scale of serrations

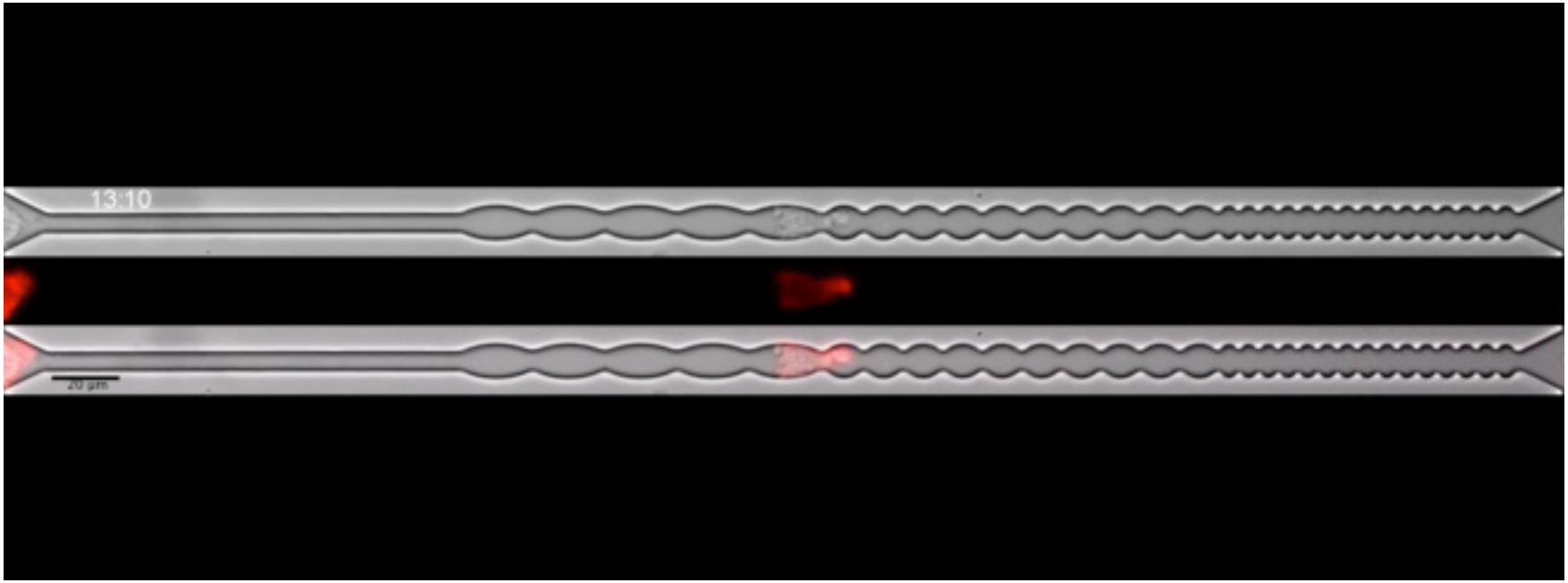
For classical values $\eta \sim 10^5$ Pa.s and $v_0 \sim 1 - 10$ μm/min, for realistic geometries used in experiments, for which λ ranges from 5 to 25 μm, the channel depth $z_0 \sim 10$μm, cell length $L \sim 20$ μm, and $\varepsilon = w_1/h_0 \sim 1$, we find forces in the range 0.1 – 100 nN, which is an expected order of magnitude for migrating cells

A. Reversat et al. and R. Voituriez and M. Sixt. *Nature*. 582(7813):582-585. (2020)

Role of substrate topography in confined motility

- Predictions: dependency of force Fx on inverse of length scale of serrations





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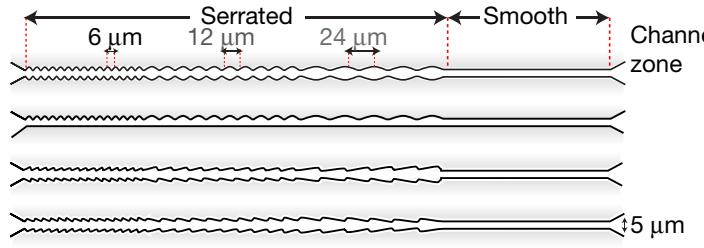

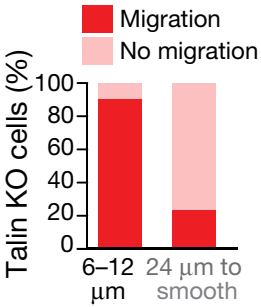

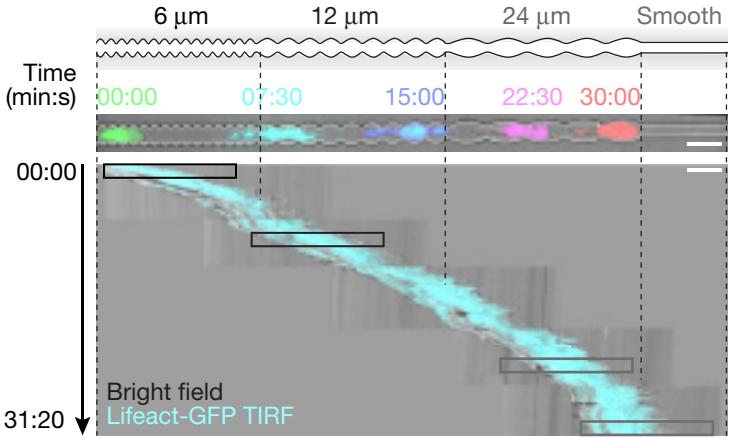

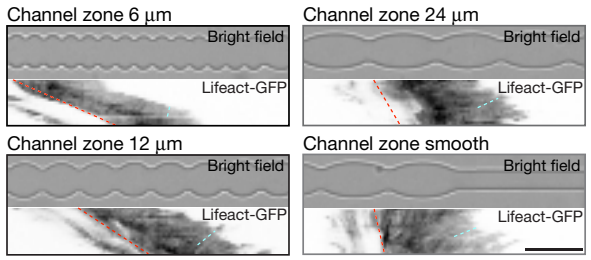

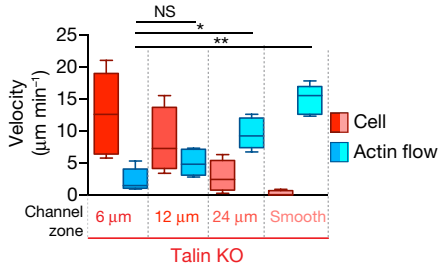

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How do cells sense confinement and induce contractility?

- Cells induce a retrograde actin flow which is powered by a gradient of cell contractility
- Blocking Myosin-II activation blocks cell motility
- How is cell contractility induced when cells are confined?

- Sensor of cell deformation under confinement
- The nucleus is a mechanical sensor

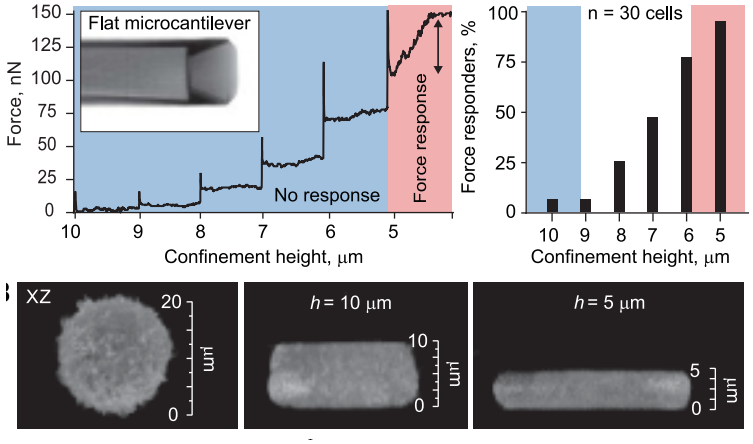
A. Lomakin et al. and D. Müller and M. Piel. *Science*. 370(6514):eaba2894. (2020) doi: 10.1126/science.aba2894
V. Venturini et al. and V. Ruprecht. *Science*. 370(6514):eaba2644. (2020) doi: 10.1126/science.aba2644

How do cells sense confinement and respond?

Cell confinement induces cortical contractility above a threshold

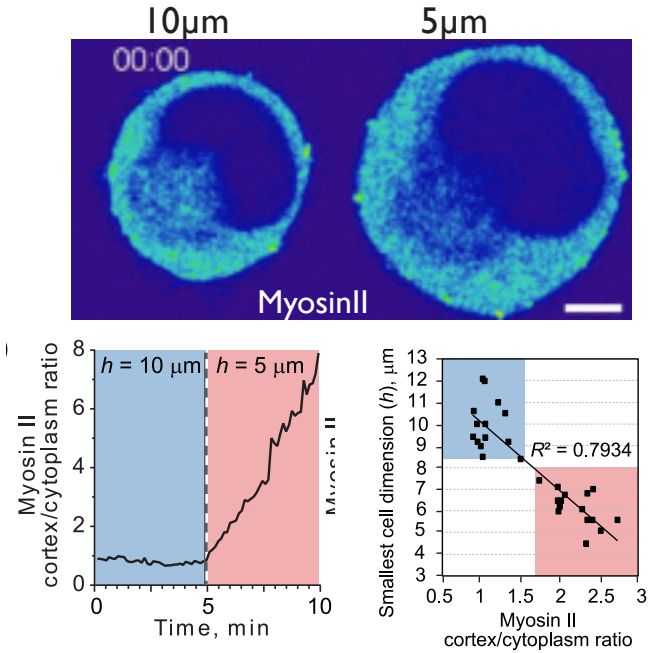
Experimental assay:

- Confinement of single nonadherent, initially rounded, interphase cells by using an ion beam–sculpted flat silicon microcantilever mounted on an atomic force microscopy (AFM) setup
- Monitor the acto-myosin cytoskeleton dynamics and contractile force generation by employing confocal video- microscopy and AFM-based force spectroscopy
- Stepwise compression from 10 to 5 µm (from initial diameter 20µm)



Results:

- most cells are insensitive to 10µm confinement. All cells responded at 5µm confinement
- Activation of cortical MyosinII and cell blebbing

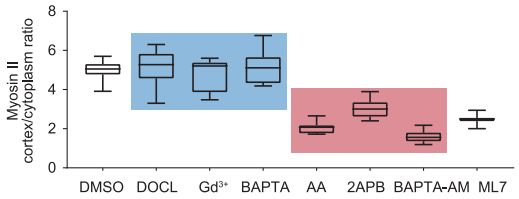


A. Lomakin et al. and D. Müller and M. Piel. *Science*. 370(6514):eaba2894. (2020)

How do cells sense confinement and respond?

Compression induced cell contractility depends on mechanisms associated with nuclear–ER membrane stretch

- Myosin-II cortical activation is sensitive to factors that affect tension in Endoplasmic reticulum/Nuclear Envelope (ER/NE) and intracellular calcium



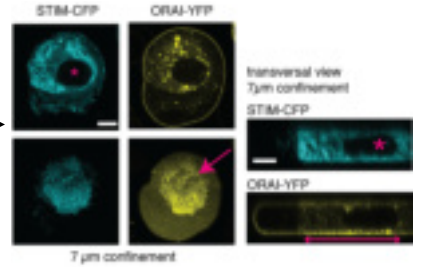
Perturbations:

PM tension/$[Ca^{2+}]_{out}$

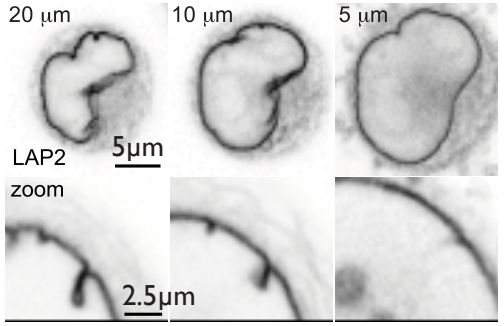
ER/NE tension/$[Ca^{2+}]_{in}$

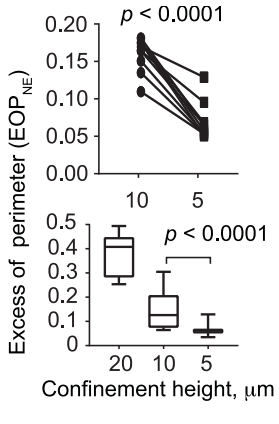
$GdCl_3$, gadolinium chloride inhibits stretch-sensitive ion channels at plasma membrane
DOCL, sodium deoxycholate, reduces plasma membrane tension
BAPTA chelates *extra*-cellular Ca^{2+}

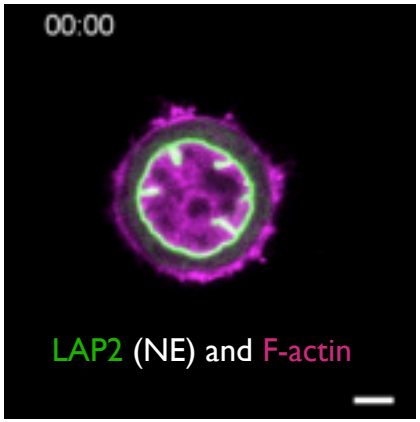
2APB: inhibits stretch sensitive calcium channels InsP3Rs and ORAI-STIM complex
BAPTA-AM chelates *intra*-cellular Ca^{2+}



- The nuclear envelope is convoluted (excess of perimeter - EOP)
- As cells are confined, the nuclear envelope unfolds and becomes smooth at 5µm







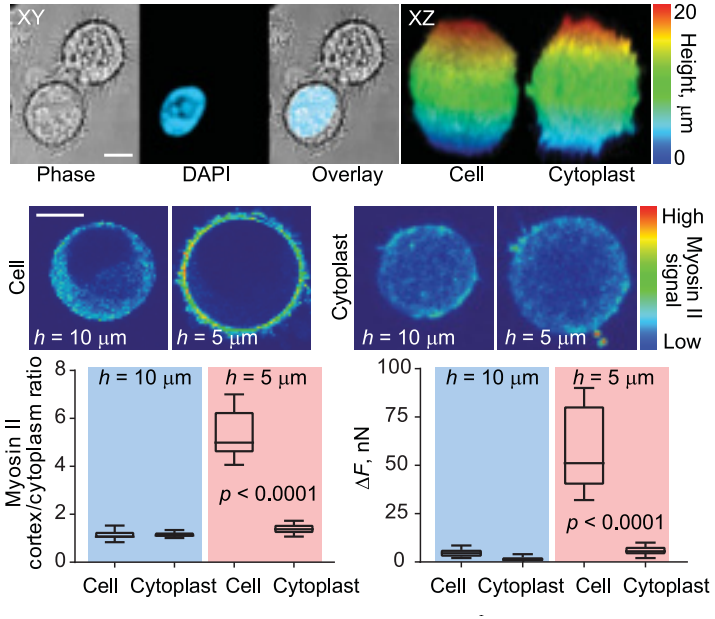
A. Lomakin et al. and D. Müller and M. Piel. *Science*. 370(6514):eaba2894. (2020)

V. Venturini et al. and V. Ruprecht. *Science*. 370(6514):eaba2644. (2020)

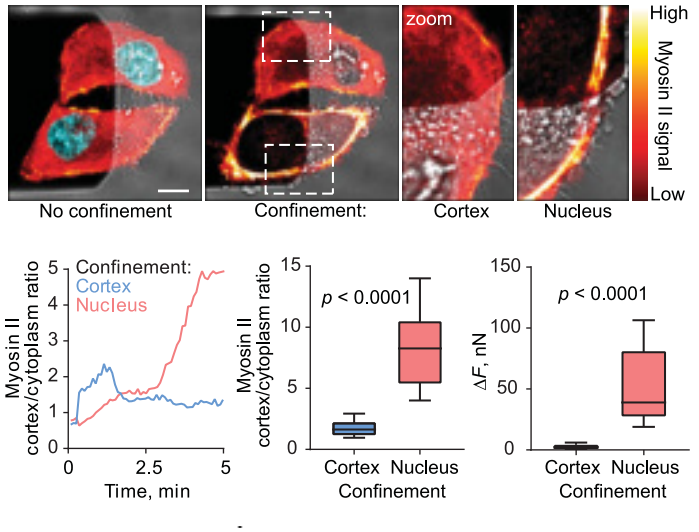
How do cells sense confinement and respond?

The nucleus is required for cortical Myosin-II activation in confined cells

- Cell enucleation (cytoplast) blocks MyosinII activation
- Yet inhibition of transcription (and translation) is not required for MyosinII activation



- Partial compression of cells: only cells where nuclei is compressed activate Myosin-II
- When half the cell is compressed by the nucleus is not, MyosinII is *not* activated at the cell cortex



A. Lomakin et al. and D. Müller and M. Piel. *Science*. 370(6514):eaba2894. (2020)

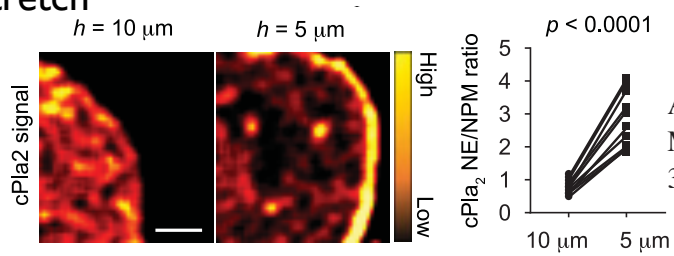
How do cells sense confinement and respond?

Activation of PLA2 at the inner Nuclear Envelope underlies nuclear mechanosensing

- Phospholipase A2 is sensitive to nuclear membrane stretch

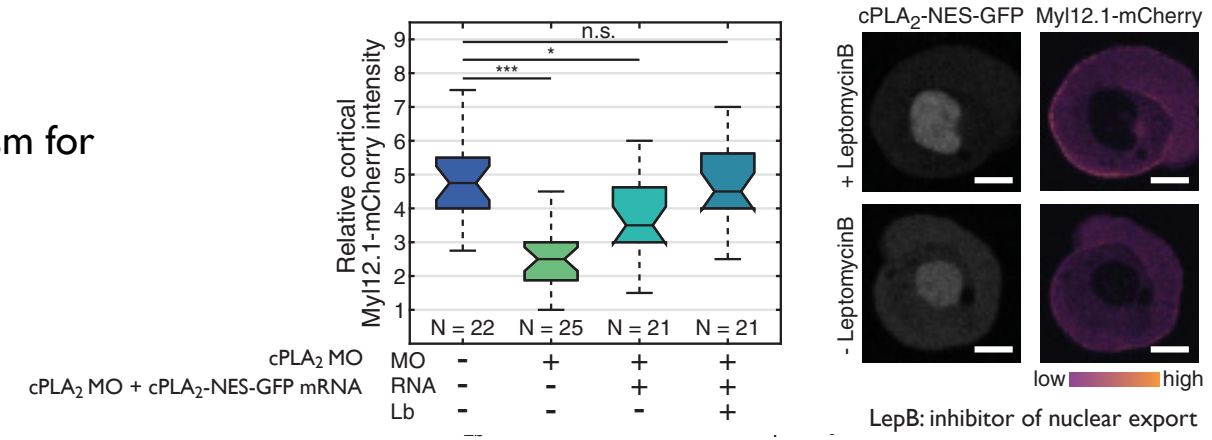
  Enyedi et al. *Cell* 165, 1160–1170 (2016)

- Pla2 is recruited at the nuclear membrane when cells are compressed (5µm)

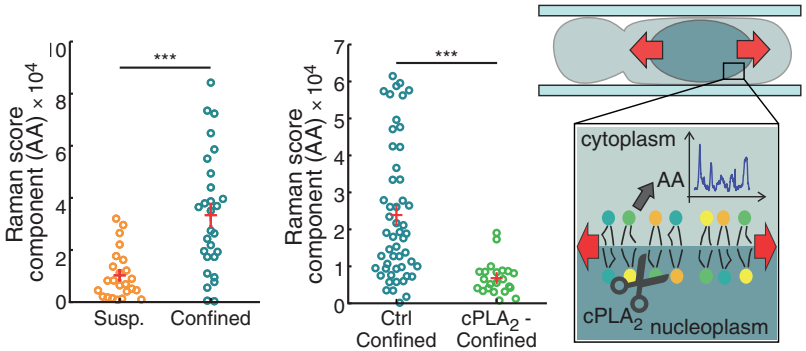


A. Lomakin et al. and D. Müller and M. Piel. *Science*. 370(6514):eaba2894. (2020)

- Pla2 is required in nucleoplasm for cortical Myosin-II activation



- Nuclear swelling induces eicosanoid synthesis (Arachidonic acid, AA) via activation of Pla2.



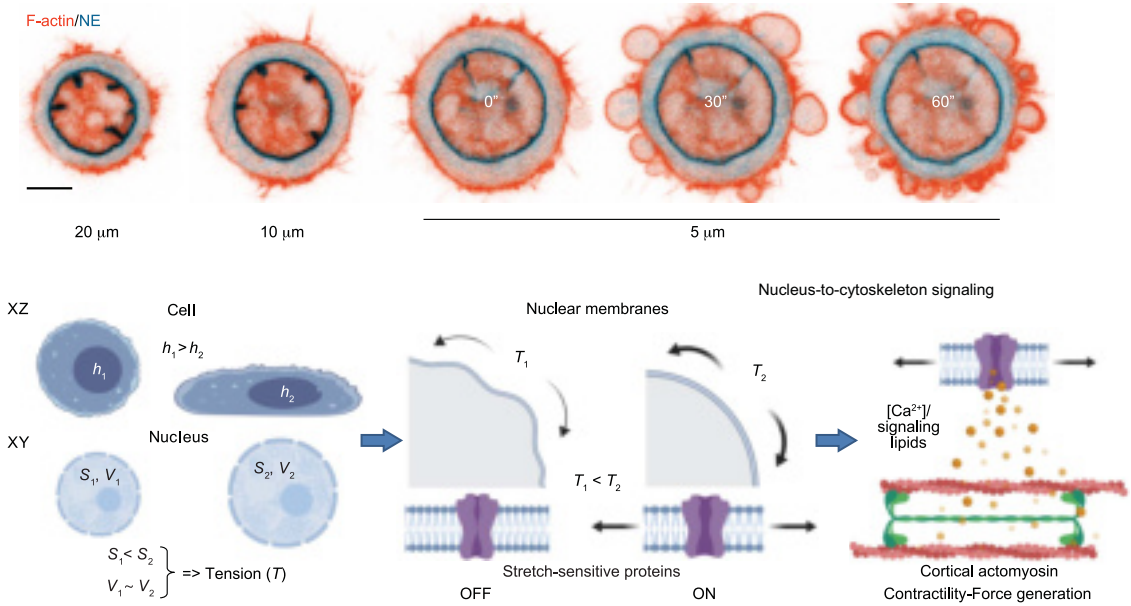
V. Venturini et al. and V. Ruprecht. *Science*. 370(6514):eaba2644. (2020) doi: 10.1126/science.aba2644

How do cells sense confinement and respond?

Working mechanical model

Cell compression (confinement) induces, above a certain threshold, inner nuclear membrane stretching.

This activates stretch sensitive calcium channels and cPLA2, which leads to synthesis of Arachidonic acid and together activate Myosin-II at the cell cortex



A. Lomakin et al. and D. Müller and M. Piel. *Science*. 370(6514):eaba2894. (2020)

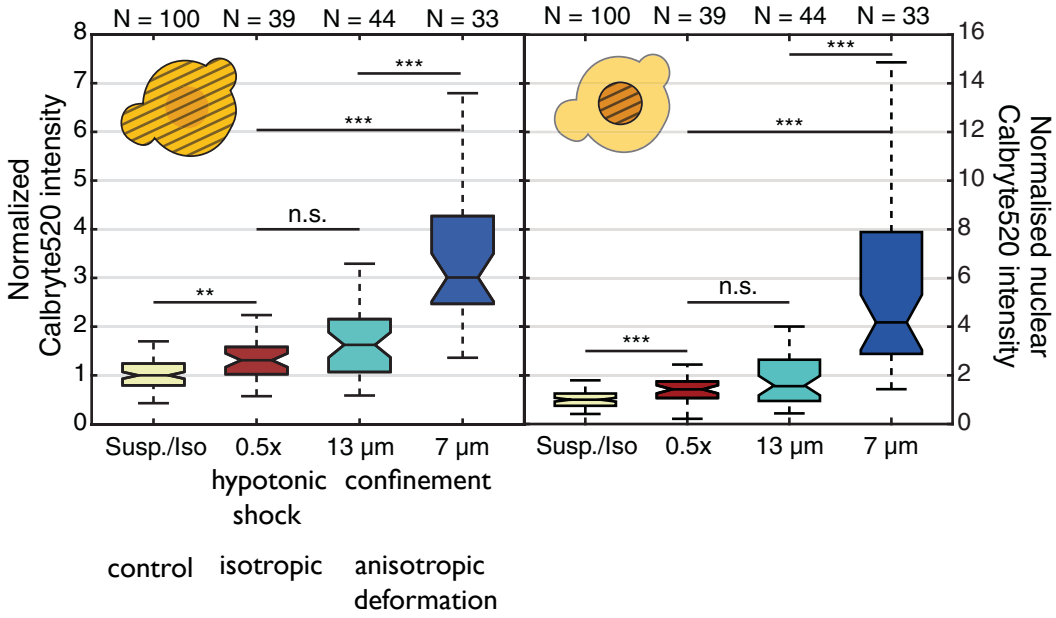
Q: Is nuclear envelope stretching sufficient to induce cell contractility?

How do cells sense confinement and respond?

Intracellular Ca^{2+} is associated with and required for cell compression induced cell contractility, and cell motility

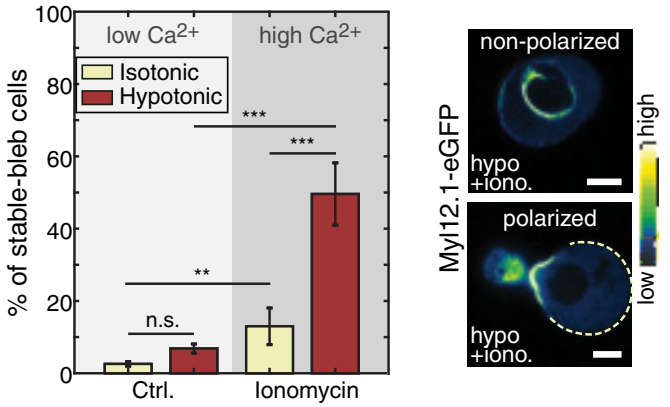
- Intranuclear and cellular Ca^{2+} increases when cells are compressed/confined
- Calcium accumulates mostly when cells are compressed and not in hypotonic condition
- Cells can distinguish between isotropic (hypotonic shock) and anisotropic deformations
- NE deformation is not sufficient for motility

Calbrytes measures Ca^{2+} levels



- Cell expansion by hypotonic medium (membrane tension increase) and increased calcium concentration (ionomycin) lead to increased cell contractility and induction of motile blebbing cell
- Cell motility requires both intracellular calcium and nuclear deformation

Ionomycin increases intracellular Ca^{2+}

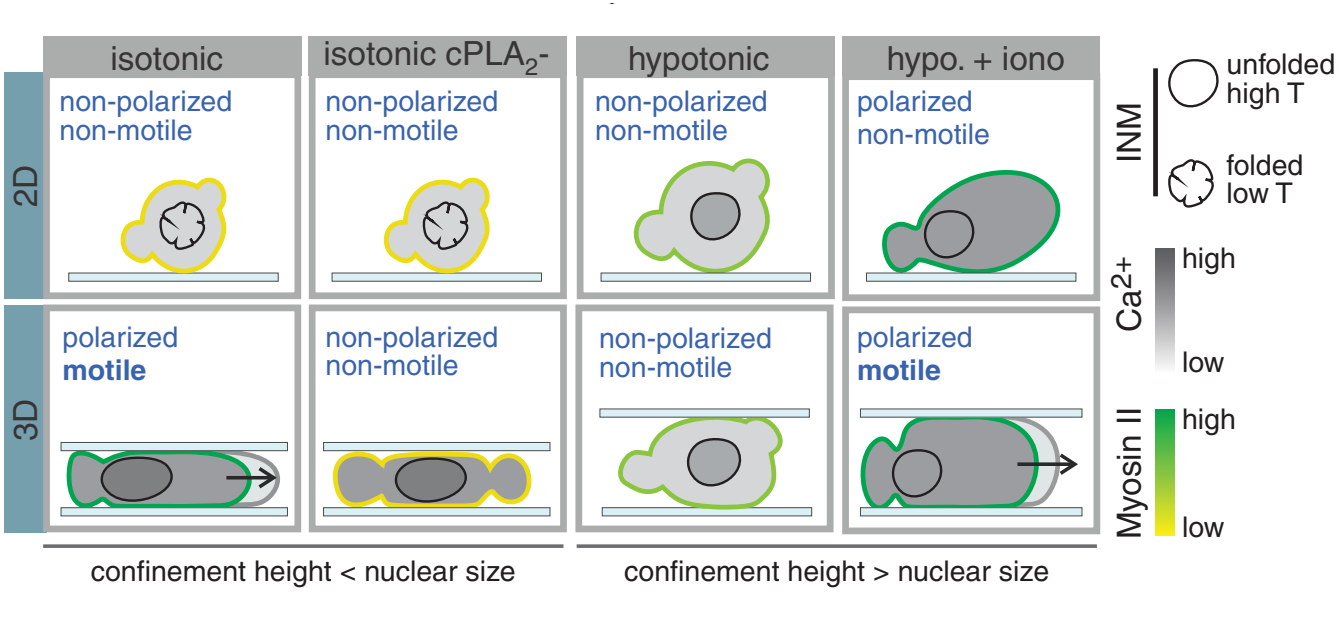


V. Venturini et al. and V. Ruprecht. *Science*. 370(6514):eaba2644. (2020) doi: 10.1126/science.aba2644

How do cells sense confinement and respond?

Cell motility induced by confinement requires both intracellular calcium (dependent on ER/PM contact) and nuclear envelope stretch

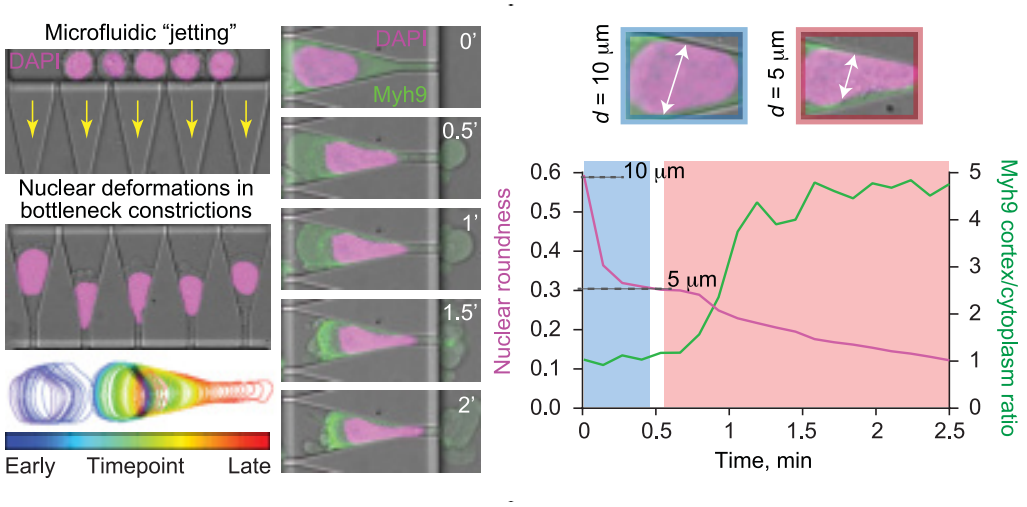
INM unfolding and intracellular calcium levels enable cells to decode isotropic stretch versus cell squeezing in confinement

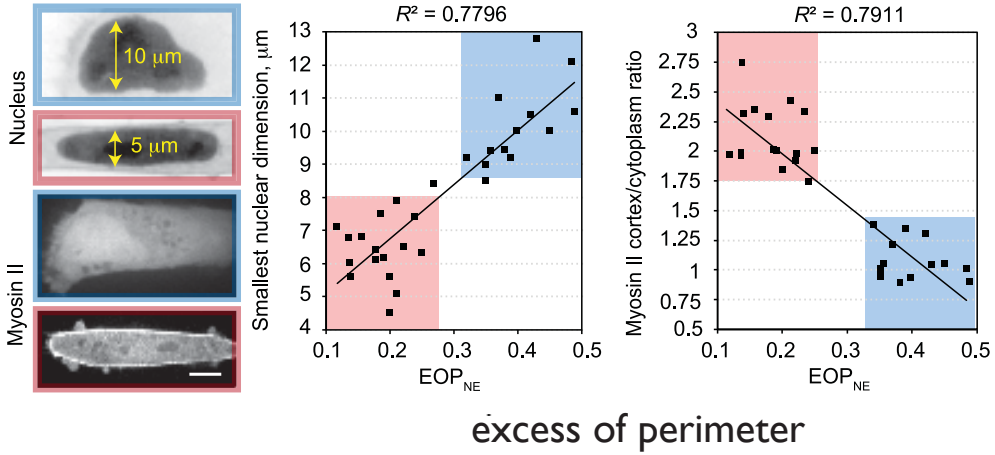


V. Venturini et al. and V. Ruprecht. *Science*. 370(6514):eaba2644. (2020) doi: 10.1126/science.aba2644

How do cells sense confinement and respond?

Correlation between nuclear stretching and cortical recruitment of myosin in confined and motile cells



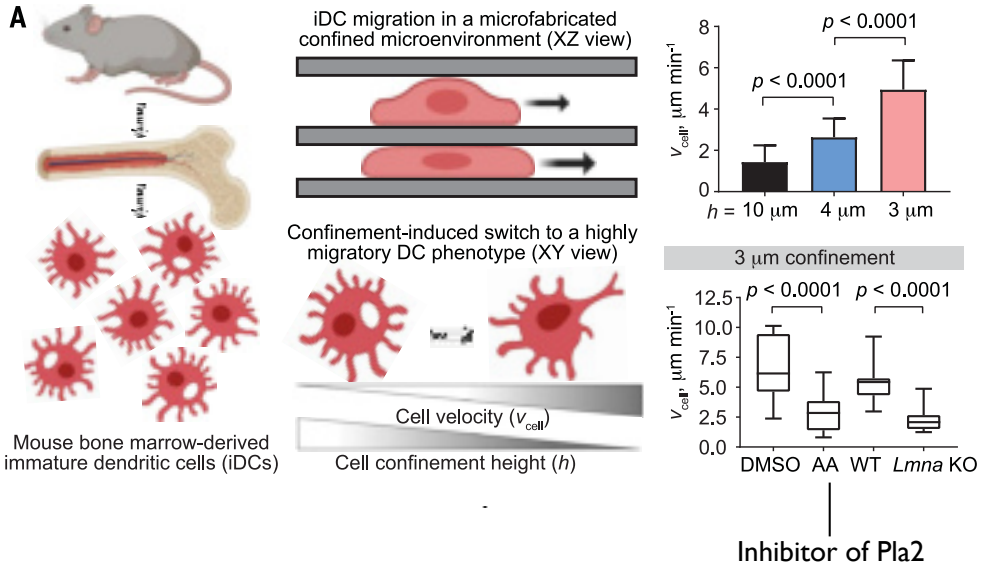


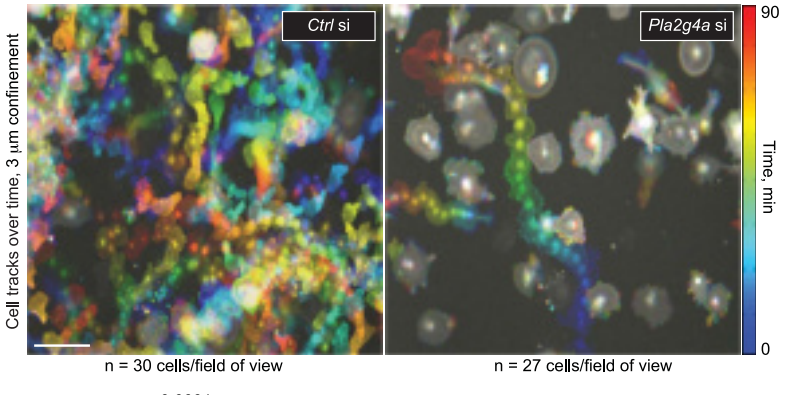
excess of perimeter

A. Lomakin et al. and D. Müller and M. Piel. *Science*. 370(6514):eaba2894. (2020)

How do cells sense confinement and respond?

Dendritic cell motility requires nuclear mechanosensing via Pla2





A. Lomakin et al. and D. Müller and M. Piel. *Science*. 370(6514):eaba2894. (2020)

Crawling in confinement - Plan

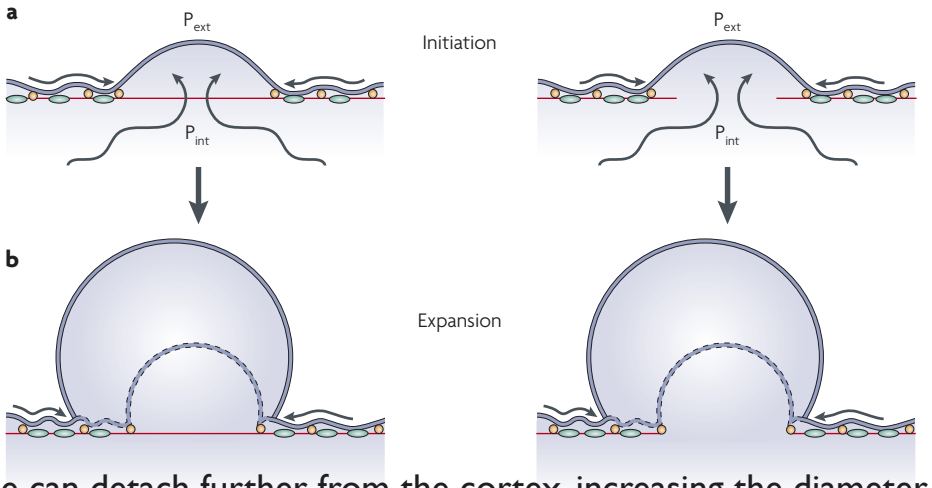
- Manifestations of cell motility under confinement
  —Adhesion independent motility
- Mechanisms of propulsion:
  —actin retrograde flow and friction
  —environment topography
  —confinement sensing
- Mechanisms of protrusion - Blebbs
- Evolutionary origin of ameboid motility

Cell expansion by blebbing

Bleb initiation can result from:

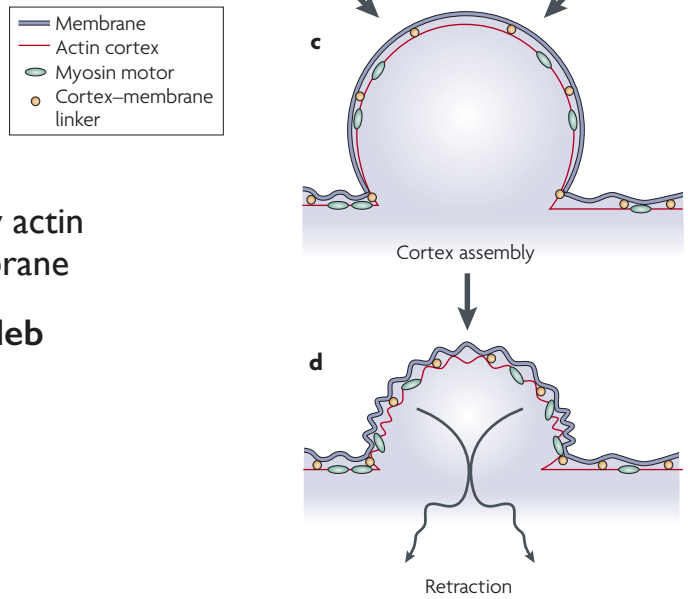
Local **detachment of the cortex from the membrane**

local **rupture of the cortex** or via hole in the cortex



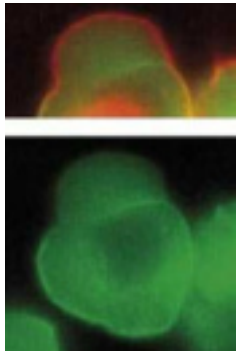
Hydrostatic pressure in the cytoplasm (P_{int}) then drives membrane expansion by propelling cytoplasmic fluid through the remaining cortex

the membrane can detach further from the cortex, increasing the diameter of the bleb base

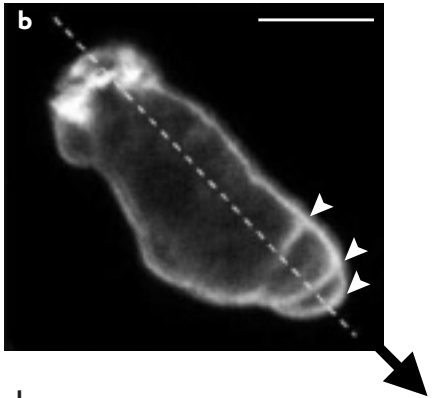


As bleb expansion slows down, a new actin cortex reforms under the bleb membrane

Recruitment of MyosinII leads to bleb retraction



zebrafish primordial germ cell

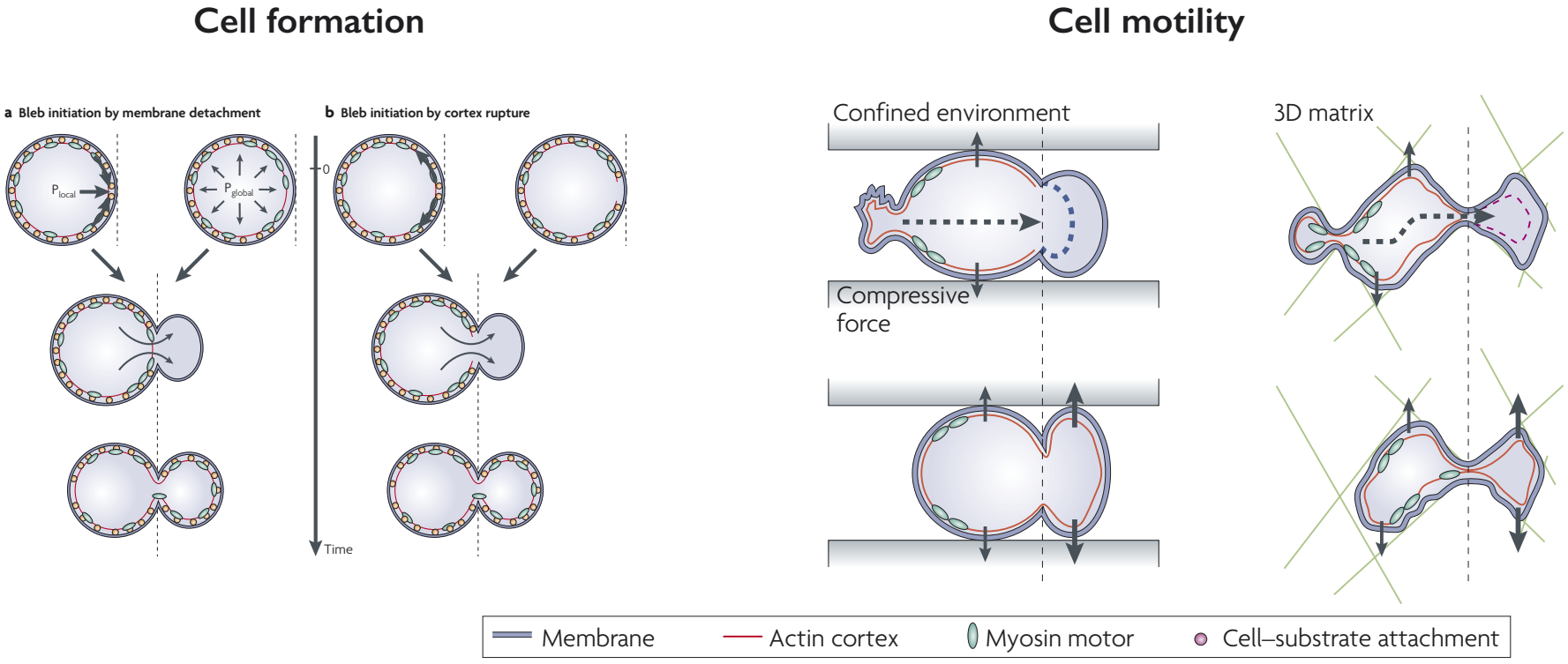


Dictyostelium

G. Charras and E. Paluch *Nature Rev. Molecular Cell Biology* 9:730-736 (2008)

Cell expansion by blebbing

- Contractility can have a local or non local effect on the probability of detachment



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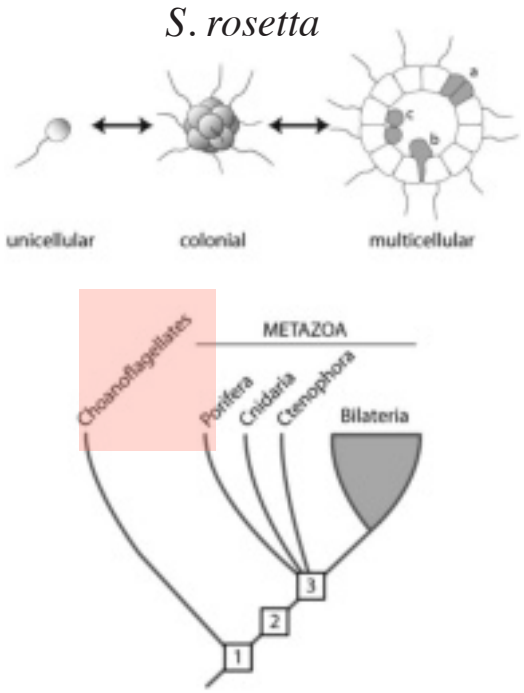
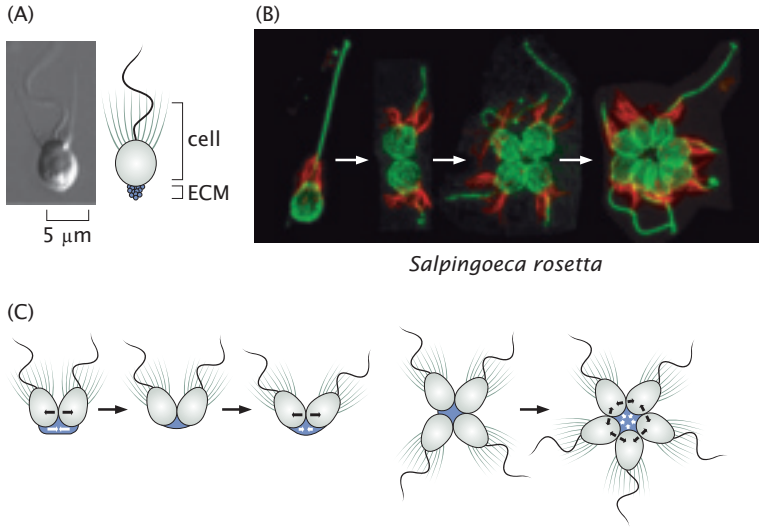
evolutionary origin of confined motility



King N. *Developmental Cell* 2004

Specialized crawling (amoeboid) cell types are present in multiple animal lineages, including sponges (archeocytes), ctenophores (stellate cells), cnidarians (amoebocytes), invertebrate bilaterians (amoebocytes), and vertebrates (white blood cells and mesenchymal cells)

What about amoeboid phenotypes in the closest relatives to animals, choanoflagelates?

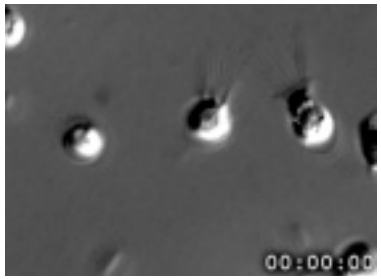


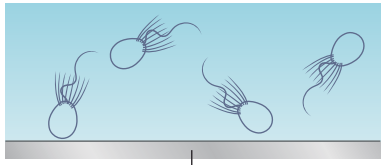
Brunet et al. and N. King. *eLife* 2021;10:e61037. DOI: https://doi.org/10.7554/eLife.61037

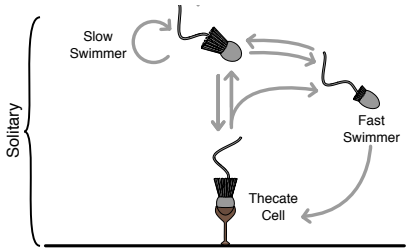
evolutionary origin of confined motility

A flagellate to amoeboid switch in the closest relatives of animals

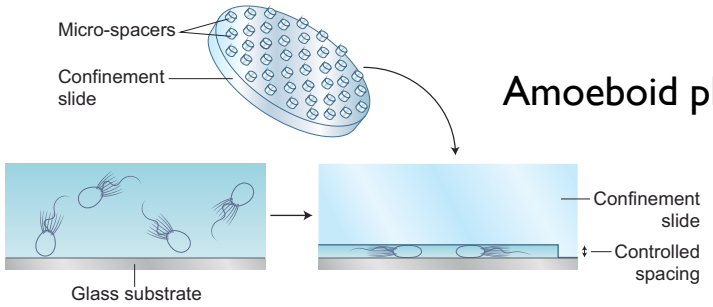
Swimming phenotype

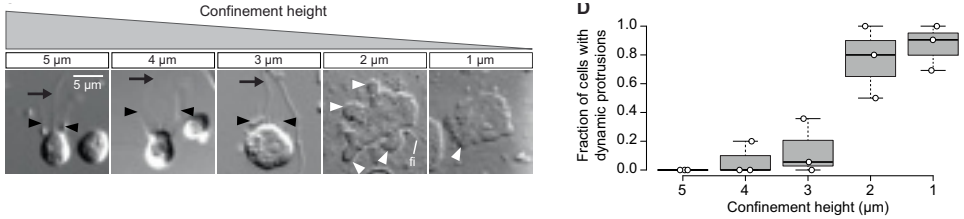


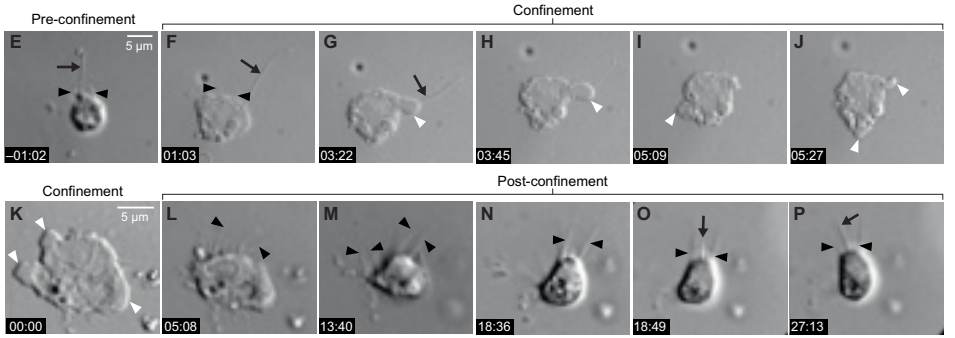




Amoeboid phenotype







M. Dayel et al. and N. King. *Developmental Biology* 357 (2011) 73–82 (2011)

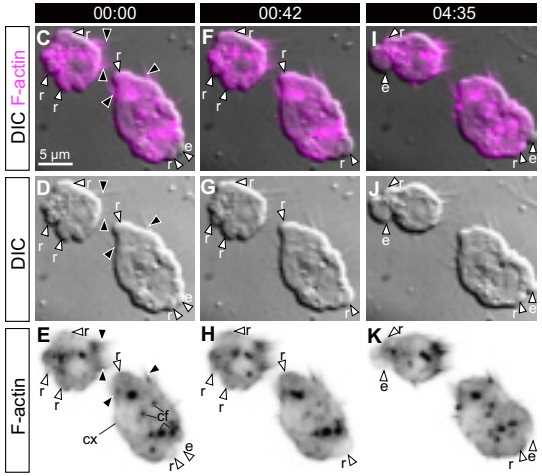
Brunet et al. and N. King. *eLife* 2021;10:e61037. DOI: https://doi.org/10.7554/eLife.61037

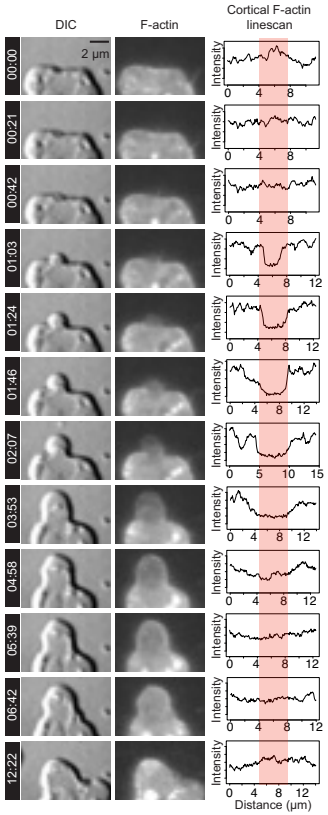
evolutionary origin of confined motility

A flagellate to amoeboid switch in the closest relatives of animals

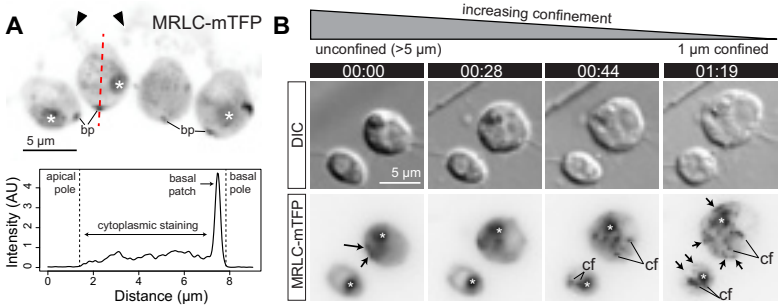
Cell blebbing of *S. rosetta* under confinement

Cortical actin dynamics in blebbs





Myosinll recruitment in blebbs

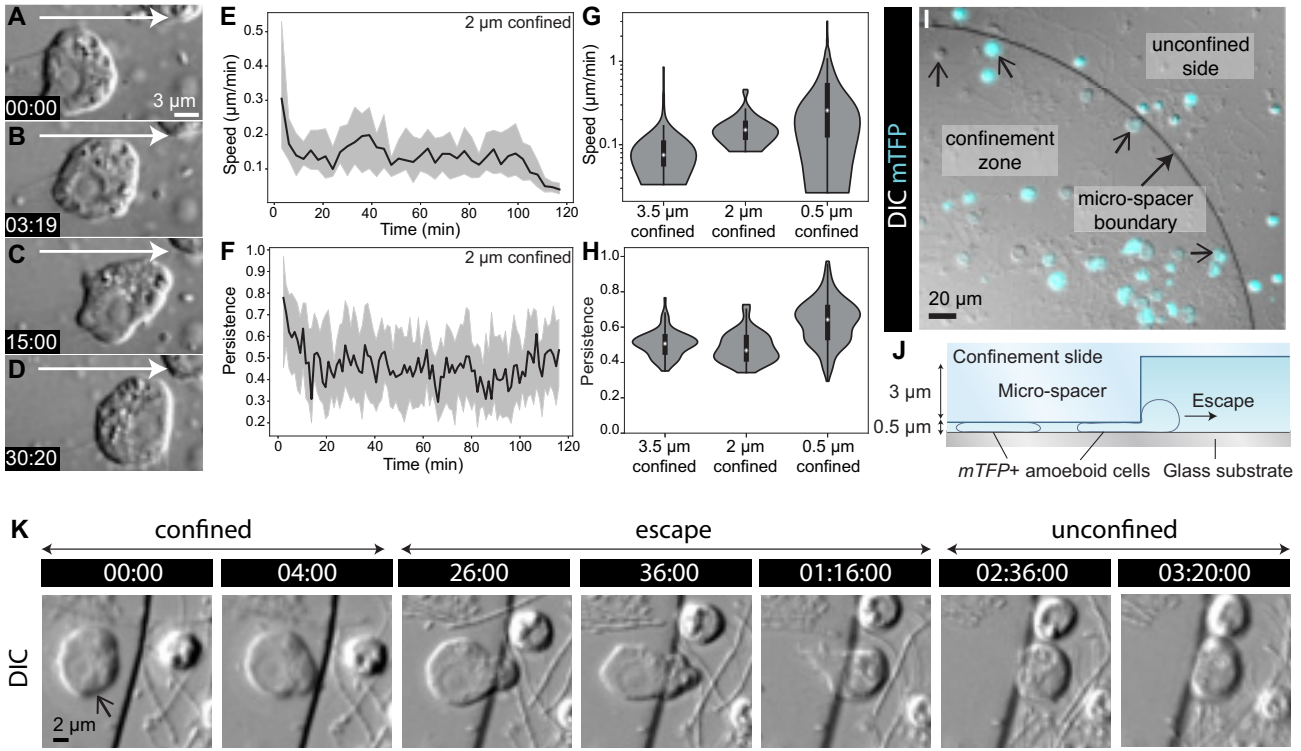


Brunet et al. and N. King. *eLife* 2021;10:e61037. DOI: https://doi.org/10.7554/eLife.61037

evolutionary origin of confined motility

Confinement is associated with motility and cell escape from confinement zone

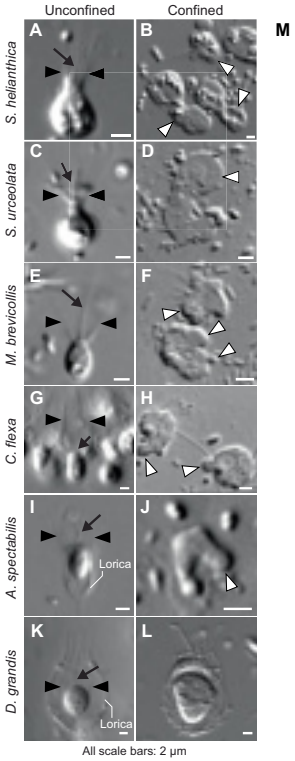
The switch from a flagellate to a amoeboid behavior due to confinement might have been part of an ancestral stress response in the last common choanozoan ancestor

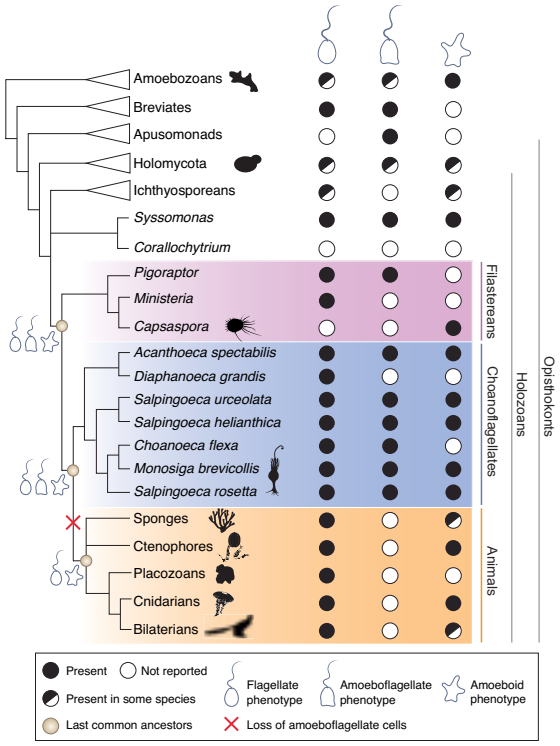


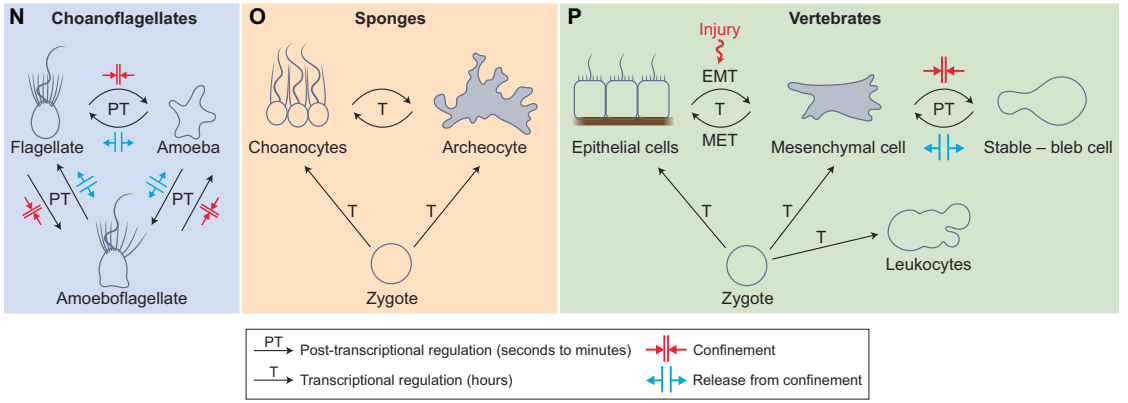
Brunet et al. and N. King. *eLife* 2021;10:e61037. DOI: https://doi.org/10.7554/eLife.61037

evolutionary origin of confined motility

- Widespread occurence of amoeboid switch among choanoflagellates
- Strengthens the pre-metazoan origin of amoeboid confined motility
- Might reflect ancestral stress response to confinement







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Comparison of adhesion and adhesion-free motility

Migration Mode	Adhesive	Non-adhesive
Protrusion type	Usually lamellipodia	Usually blebs
Propelling force generation	Filament extension/actin flow	Cortex flow
Force transmission	Focal adhesion	Friction, protrusion intercalations, etc.
Substrate interaction	Specific	Non-specific
Duration of cell-substrate interactions	Longer than dwell time	Shorter than dwell time
Speed-substrate interaction strength relationship	Bell curve	Plateau
Environment	2D surfaces and 3D environments	3D confinement
Migration speed[a]	~0.1–1 μm/min	~1–10 μm/min
Stresses exerted on substrate[b]	~10^2 – 10^5 Pa	<1Pa
Actin flow profile	Mainly in lamellipodium	At the cortex all along the cell body, max velocity in cell center
Force dipole	Contractile	Expansile

Bodor et al. and E. Paluch. *Developmental Cell*. 52: 550-562 (2020)

- Friction-based migration is only possible in 3D confinement (unlike adhesion based motility)
- For 2D substrate motility, the strength and duration of molecular bonds must be strong enough to counteract Brownian motion (see catch bond and mechanical amplification mechanisms at Integrin foci)
- **In 3D, confinement prolongs the contacts of weak molecular interactions and multiply them over the entire cell surface**
- Cell substrate interactions shorter than cell dwell time in non-adhesive motility, but longer than cell dwell time in adhesive motility (thus requiring de-adhesion mechanisms). **In non-adhesive motion, friction does not interfere with cell retraction.**
- Therefore, **increasing friction does not lead to a plateau of migration speed, and no slowing down is expected even at very high friction.**