Actin cytoskeleton and cell motility

Julie Plastino, UMR 168

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Course outline

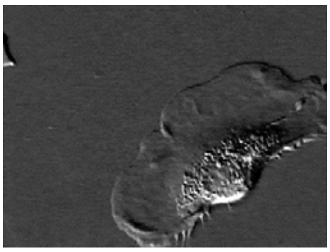
I. Introduction to actin biochemistry and biomimetic systems (Listeria, beads)
…general overview of the field and review of work on the subject (including our work)

II. The moving cell: force production, retrograde flow

III. The blebbing cell and in vivo cell migration including invasive cancer cell motility

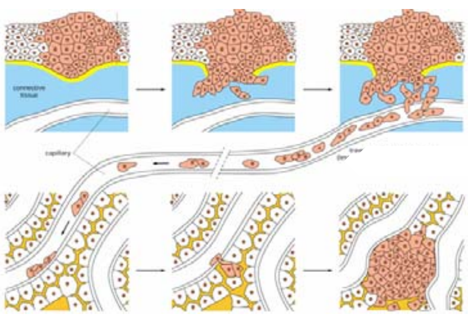
Cell movement

Keratocyte



Verkhovsky et al., 1999

Cancer Metastasis



Alberts et al., 1994

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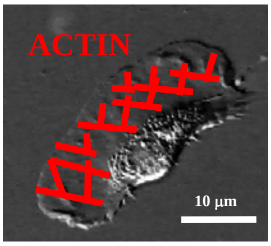
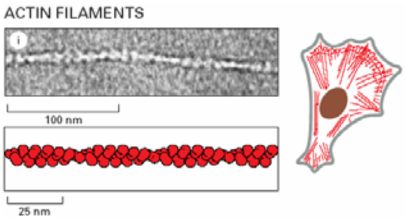
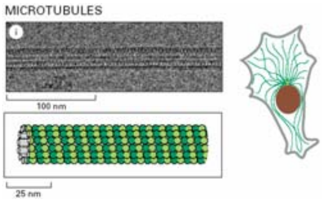
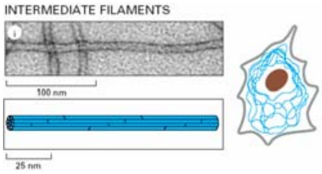
Components of the cytoskeleton: protein polymers









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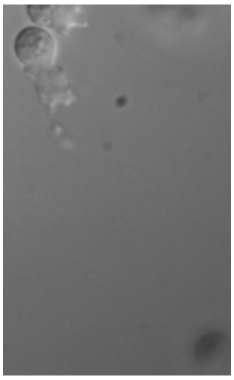
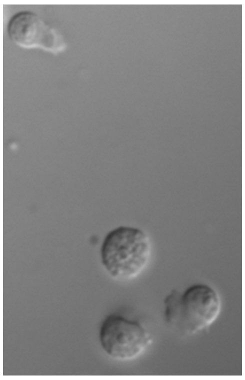
Alberts et al., 2002

The exception…nematode sperm cells

No actin, no myosin, no microtubules

More about this on Monday…





Polymerization and depolymerization of (MSP) Major Sperm Protein

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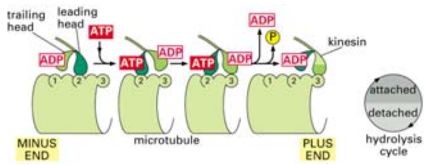
Plastino, unpublished

Movements in the cell…

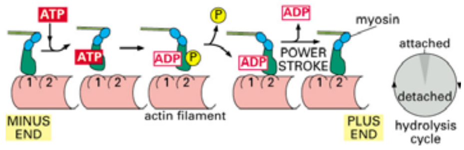
4 ancient mechanisms account for (almost) all intracellular displacements in eucaryotes:
Molecular motor-based

1) **Microtubules + kinesins and dynein**



Kinesins walk along microtubules

2) **Actin + myosins**

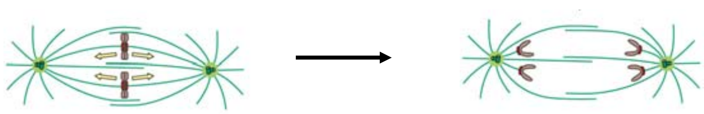


Myosins walk along actin filaments

Alberts et al, 2002

Movements in the cell…

Polymerization/depolymerization-based
3) Polymerization and depolymerization of MTs



Depolymerization of microtubules pull chromosomes apart during anaphase

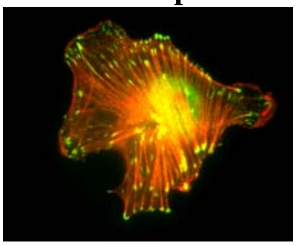
4) Polymerization and depolymerizaton of actin

…Polymerization and depolymerization of Major Sperm Protein (MSP) in nematode sperm cells

Introduction to actin

Muscle actin
20% of total protein

Non-muscle (cytoplasmic) actin
5% of total protein



Fradelizi unpublished results (1999)

Evolutionarily conserved: muscle, cytoplasmic, protozoan actin <5% different

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Structural polarity of actin

Binds ATP and Mg^{2+} or Ca^{2+}

Kabsch et al., 1990

42 kD Monomer:
globular or "G-actin"

EM

Pointed end

Pollard and Borisy, 2003

Barbed end

Polymer:
filamentous or "F-actin"

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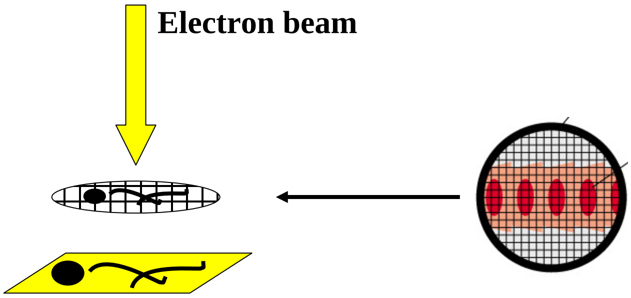
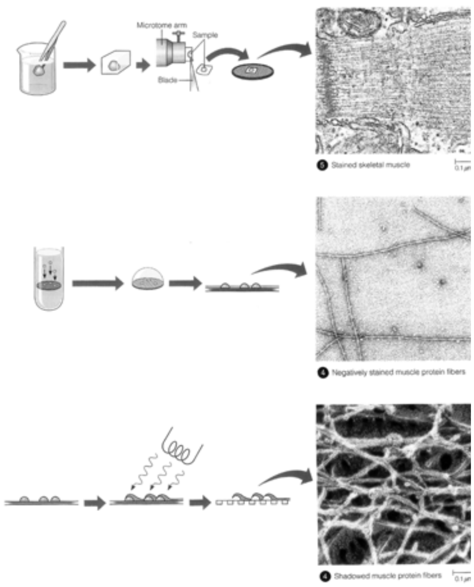
Electron microscopy



Electrons blocked by objects—resulting « shadows » observed on a phosphorescent screen or captured on photographic film



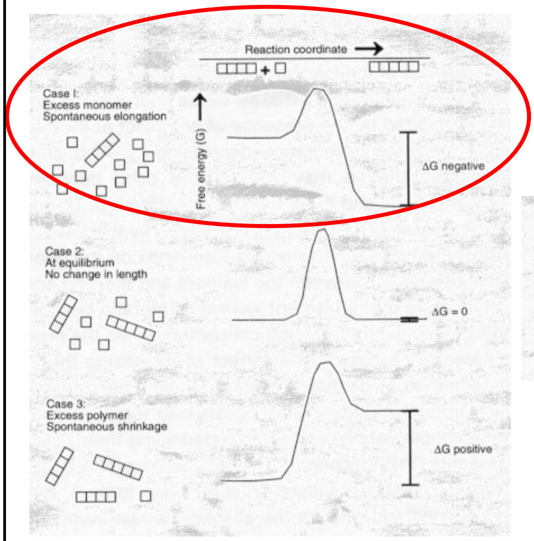
1. Plastic embedding and sectioning

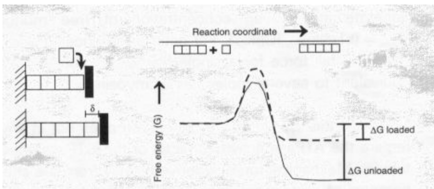
2. Negative staining

3. Platinum shadowing (critical point drying)

Modified from Mathews et al. Biochemistry 3rd ed.

Mechanical force from the chemical potential of protein polymerization





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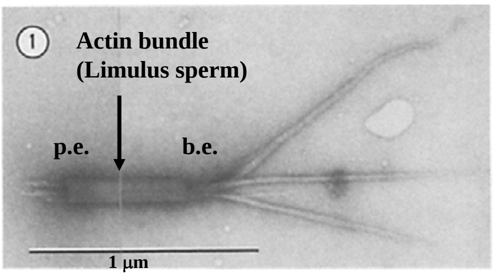
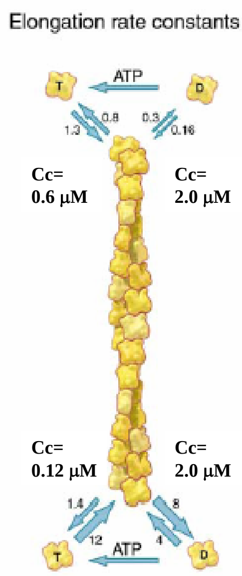
Biochemical polarity of actin



Pollard, 1986



Pollard and Earnshaw, 2002

Cc: (k_{off}/k_{on})
monomer concentration giving equal rates of association and dissociation

TIRF microscopy: confirm rate constants

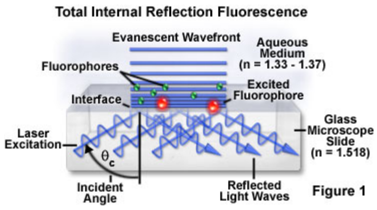
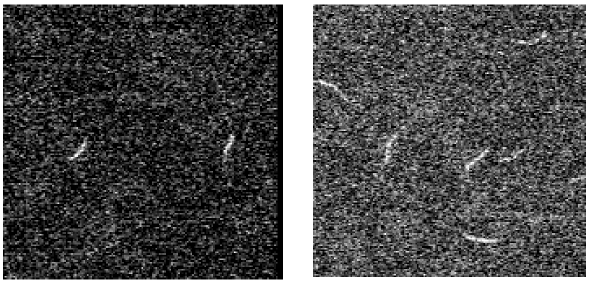


Figure 1

Total internal reflection at the glass/aqueous interface generates an evanescent wave… decays exponentially = illuminates to depth of ~ 100 nm



Fujiwara et al., 2007

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Aside about diffusion limitation

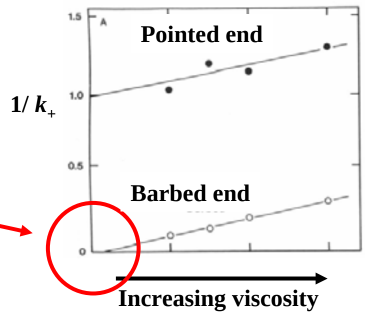
$$k_+ = 4\pi\, k\, f_{elec}\, b(D_M + D_F)\, N_o\, 10^{-3}$$

Debye-Smoluchoski

k steric factor
f_{elec} electrostatic factor
b interaction radius
D_M diffusion coeff monomer
D_F diffusion coeff filament
N_o Avogadro's number

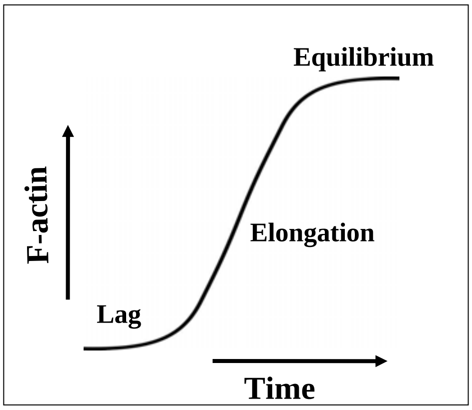
Can estimate k_+ at about 0.1-75 $\mu M^{-1}s^{-1}$
So both barbed and pointed end COULD be diffusion-limited…

At zero viscosity,
k_+ is infinite =
diffusion limited…
only for barbed end



Drenckhahn and Pollard, 1986

Actin polymerization



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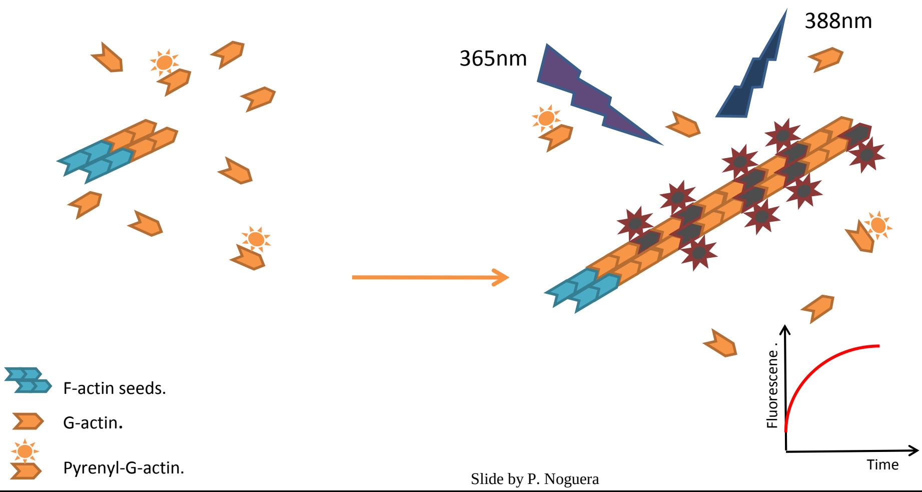
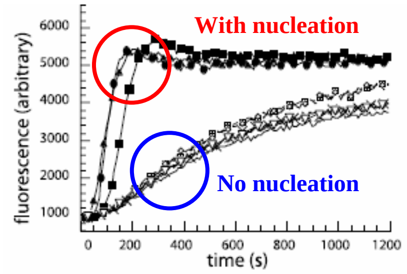
The Pyrene Assay

•Follow amount of filamentous actin formed over time

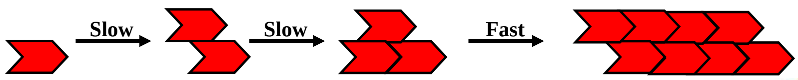


Slide by P. Noguera

Nucleation

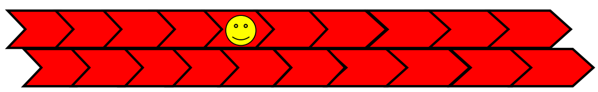


Yarar et al., 2002



Polar growth and treadmilling

ATP-actin **ADP-actin**

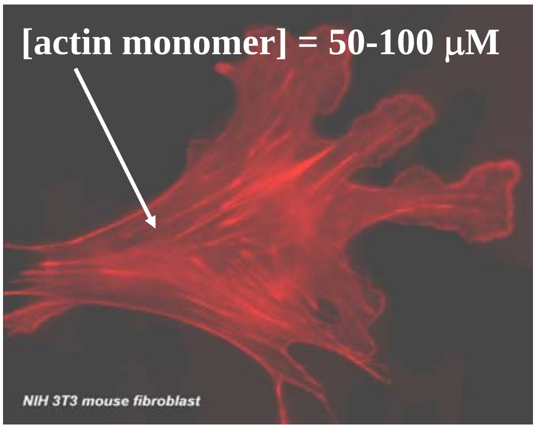


Polymerization of ATP monomers

Depolymerization of ADP monomers

Treadmilling = no net consumption of monomers

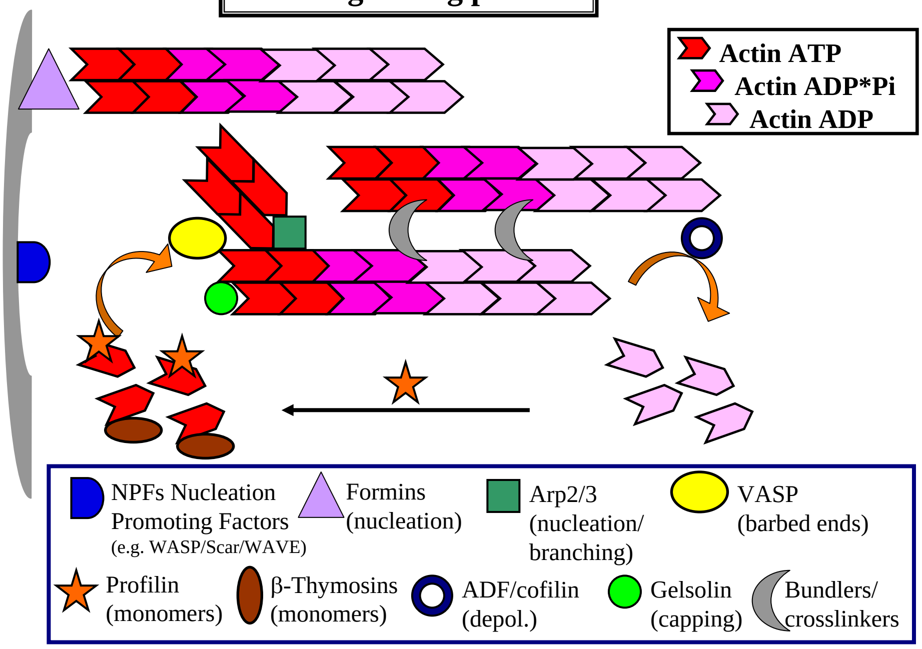
Actin in the cell



500-1000x higher than the critical concentration of barbed ends!!!!

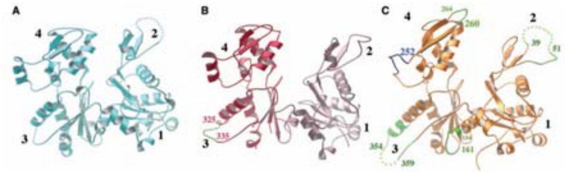
Tight control of nucleation and monomer sequestering

Actin organizing proteins

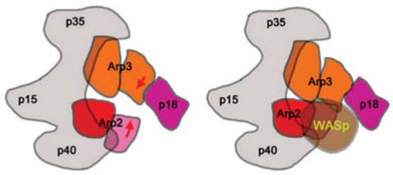


How the Arp2/3 complex nucleates

Actin **Arp2** **Arp3**



Robinson et al., 2001



Rodal et al., 2005

NPFs bind monomer
Cause conformational change of Arp2/3 complex

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…and branches

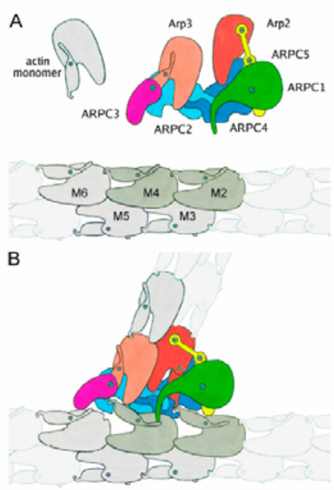

Figure 5. **Schematic representations of the precursors and assembled components of the branch junction.** (A) Drawing of the inactive Arp2/3 complex and a standard actin filament. (B) Model of the branch junction. The following conformational changes are proposed to occur: (1) opening the nucleotide-binding clefts of mother filament subunits M2 and M4; (2) converting subunit M4 from a filament to a monomeric conformation; (3) converting Arp3 into filament conformation; (4) moving Arp2 tethered by ARPC5 next to Arp3 to form the first two subunits of the daughter filament; and (5) converting Arp2 into filament conformation.

Rouiller et al., 2008

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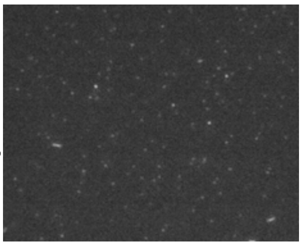
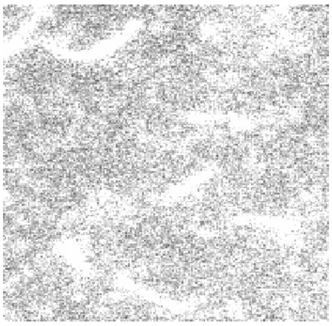
TIRF microscopy of Arp2/3 branching

No Arp2/3



With Arp2/3



Amann and Pollard, 2001

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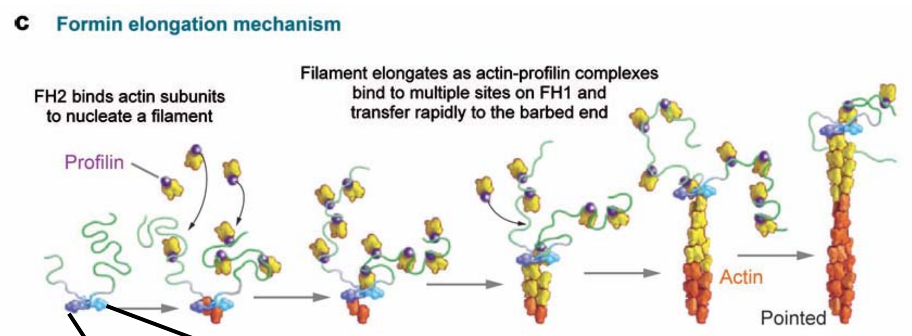
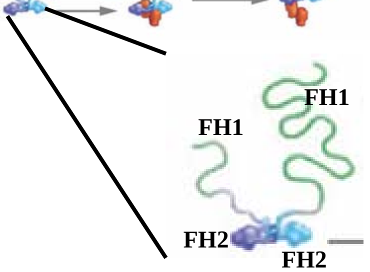
Formins: nucleation without branching



Pollard, 2007



Unlike other nucleators (Arp2/3, Spire, Cobl), formin remains associated to barbed end!!!

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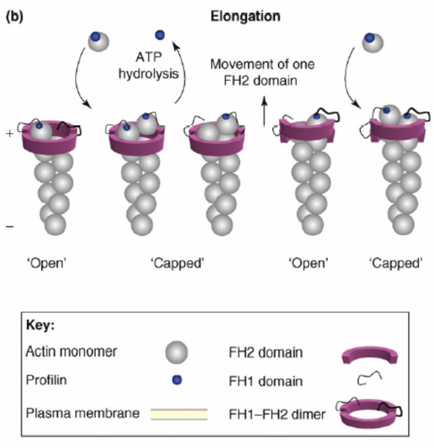
Zoom in on the barbed end/formin interaction



Processive capping

k_+ 110 $\mu M^{-1}s^{-1}$:
10 x acceleration of barbed end polymerization (mystery!! since b.e. pol. already diffusion-limited)

Renault et al., 2008

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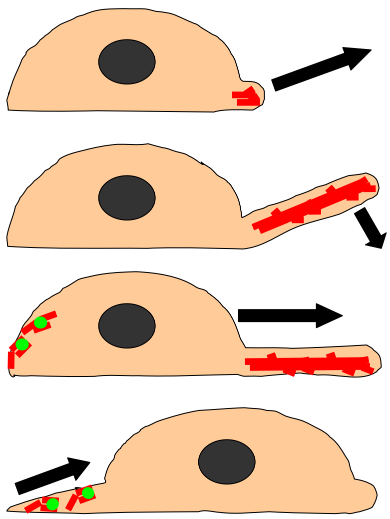
Steps in cell motility



Protrusion

Adhesion

Traction

Retraction

Actin

Myosin

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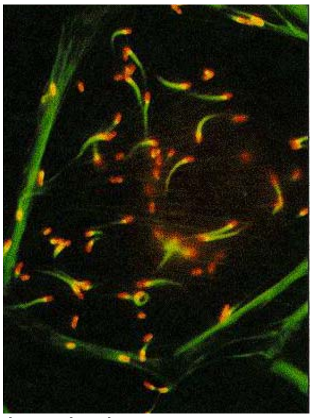
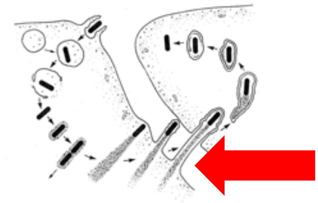
Modified from Mitchison and Cramer, 1996

Listeria monocytogenes

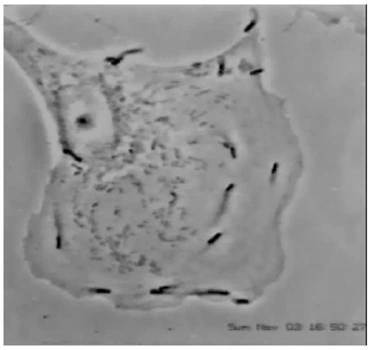


Theriot and Mitchison

Actin in green

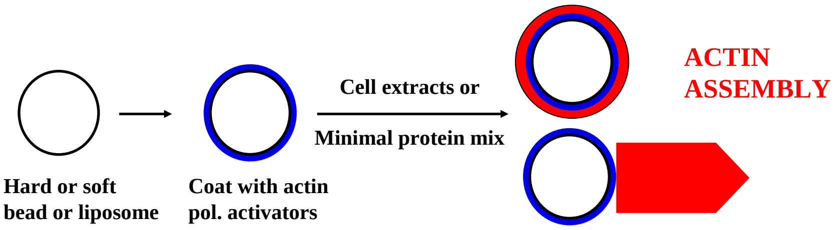


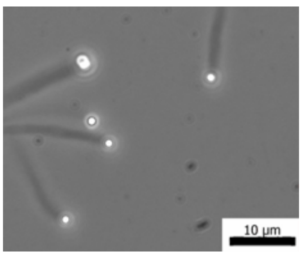
Tilney and Portnoy, 1989



Theriot et al. http://cmgm.stanford.edu/theriot

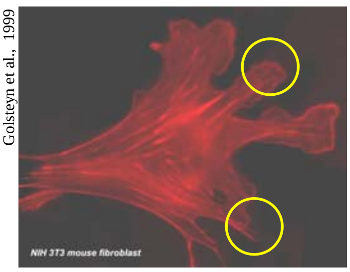
Motility reproduced *in vitro*





Plastino, unpublished

Golsteyn et al., 1999



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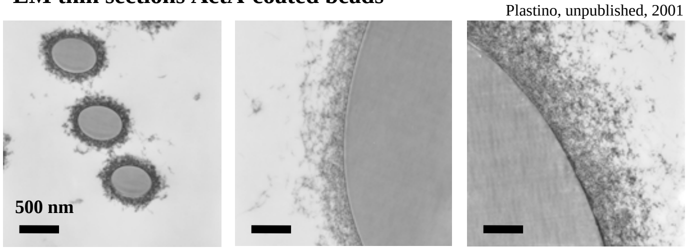
Actin networks on spherical surfaces

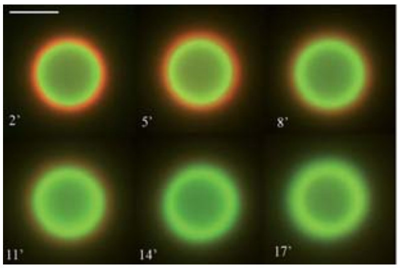
EM thin sections ActA-coated beads

Plastino, unpublished, 2001



r = 0.3 µm   r = 3.2 µm   r = 4.6 µm

Bigger beads support a thicker steady-state actin layer



A "living" actin layer
→2 color experiment
first red, then green

van der Gucht et al., *PNAS* 2005

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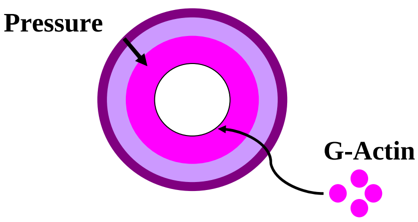
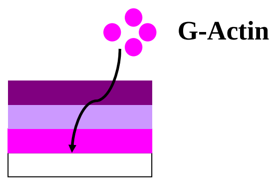
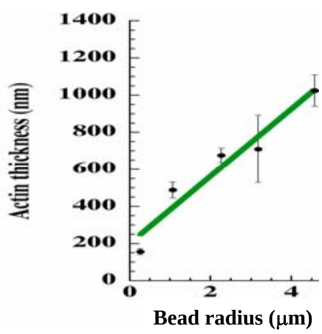
Stress build-up on spherical surfaces







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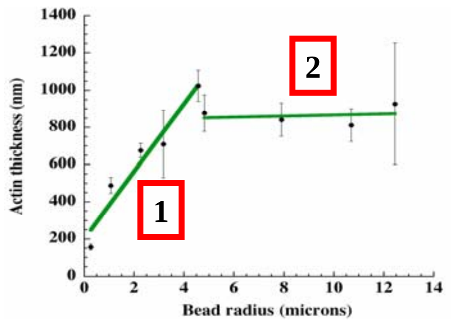
Two regimes of actin gel growth

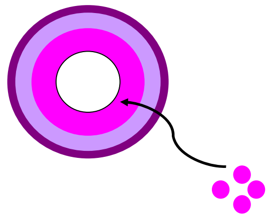


1 Stress-limited regime

2 Diffusion-limited regime

$$c_e k_+^b(\sigma_{\text{ortho}})\left[1 - \frac{e/e^*(\sigma_{\text{tang}})}{1+e/r}\right] = k_-^b(\sigma_{\text{ortho}}) + k_-^p(\sigma_{\text{tang}})$$

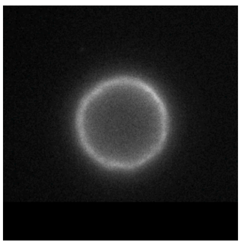
$$e^*(\sigma_{\text{tang}}) = \frac{D\xi^2 c_e}{k_-^p(\sigma_{\text{tang}})}$$

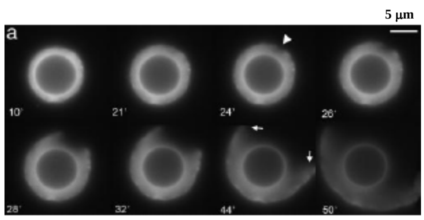


$D = 10^{-8}$ cm^{2}s^{-1}

Plastino et al., 2004

Stress release drives symmetry breaking



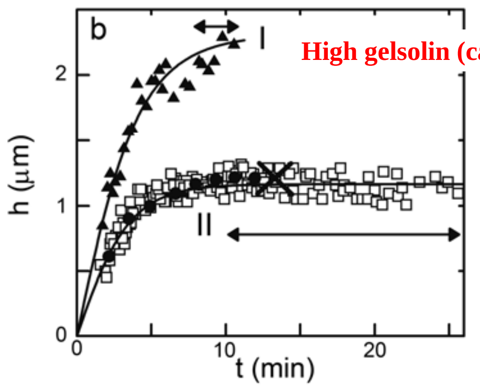


Spontaneous symmetry breaking

Polarization triggered by photodamage

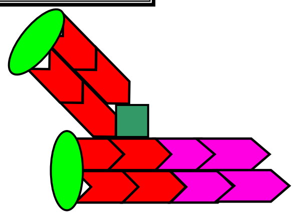
van der Gucht et al., *PNAS* 2005

Stress controlled by biochemical parameters



High gelsolin (capping)

Low gelsolin



What happens in high capping?
1—all b.e. capped, so monomer concentration = Cc p.e. (0.6 uM instead of 0.1 uM)
2—filaments are short = mesh size increases

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van der Gucht et al., *PNAS* 2005

Elasticity and fracture…two scenarios

Noireaux et al., *Biophys. J.* 2000

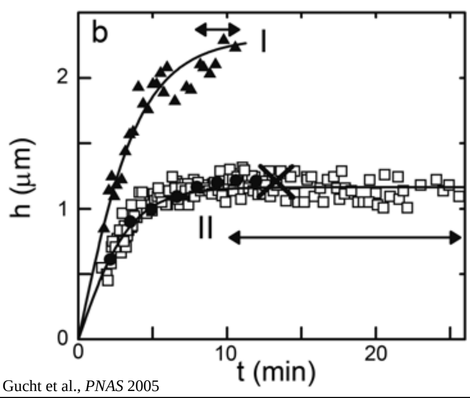
Steady-state thickness:

$$h^s \cong R\left(\frac{\Delta\mu}{E\xi^2 a}\right)^{1/2}$$

Critical thickness:

$$h^f \cong R\left(\frac{\Gamma}{Ed}\right)^{1/2}$$

Δμ, chemical energy of polymerization, ξ, mesh size of actin gel, *Γ*, fracture energy per unit surface, *d*, pre-existing crack size



Case I : $h^f < h^s$

→ no plateau

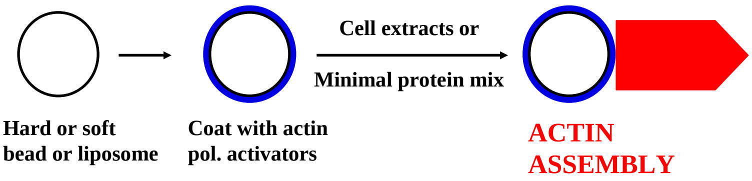
Case II : $h^f > h^s$

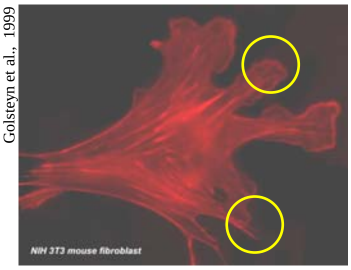
→ plateau

→ delayed fracture (energy barrier)

van der Gucht et al., *PNAS* 2005

Use the *in vitro* system to answer biological questions





Golsteyn et al., 1999

Use this system to study two cytoskeletal elements important for leading edge morphology and dynamics: the Arp2/3 complex and Ena/VASP proteins

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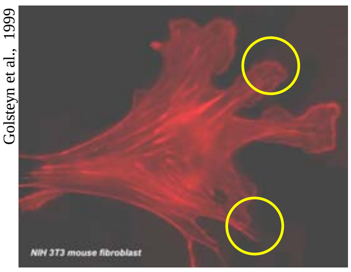
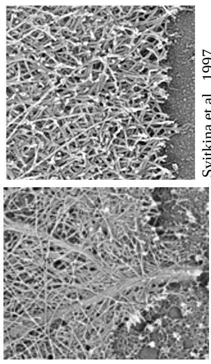
Actin structures in moving and adhering cells

Arp2/3

VASP

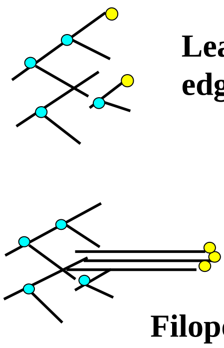


Golsteyn et al., 1999



Svitkina et al., 1997

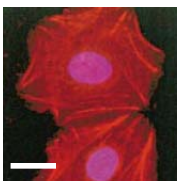
Svitkina et al., 2003



Leading edge

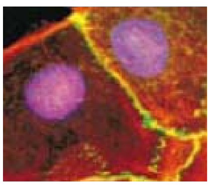
Filopodia

15 µm



Arp2/3

VASP



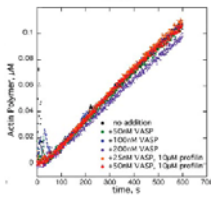
Vasioukhin et al., 2000

red = actin;
green = cadherin;
yellow = colocalization

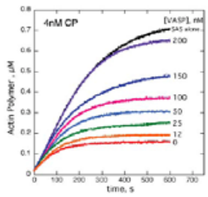
What does VASP do?

→VASP does not interact directly with the Arp2/3 complex.

→VASP is not an actin nucleating protein.

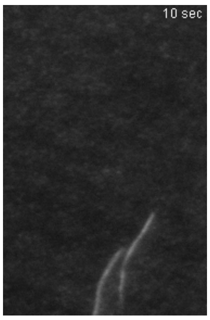


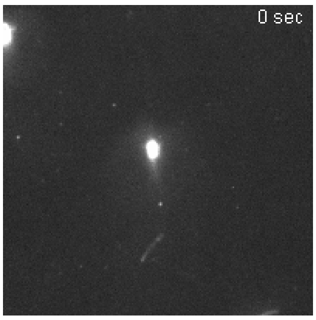
→VASP is an anticapping protein.

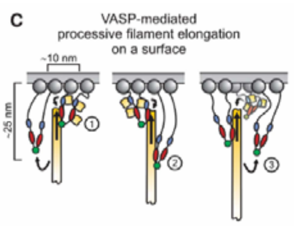


Barzic et al., 2004

VASP drives processive elongation like formin







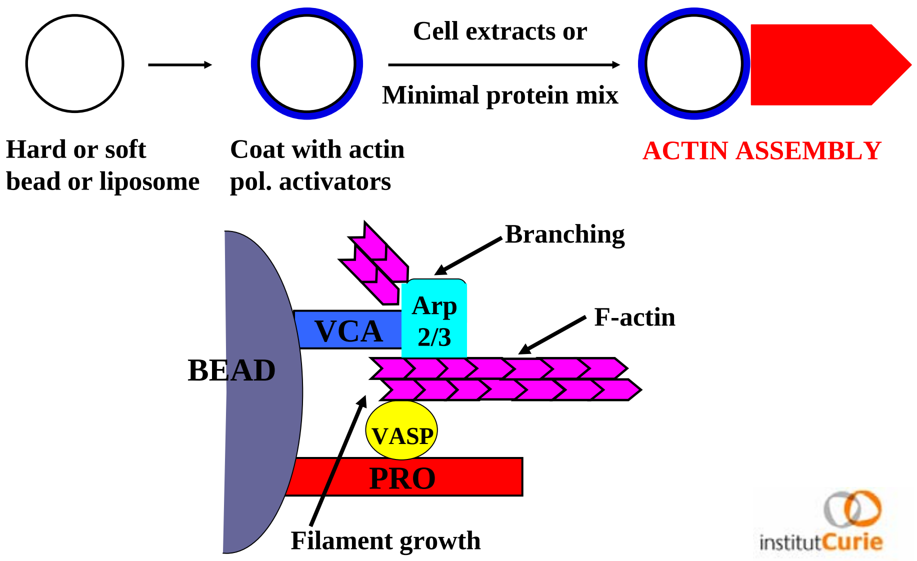
-VASP 10.5 subunits/sec
+VASP 74 subunits/sec

Clustering on bead, VASP becomes processive
65 subunits/sec

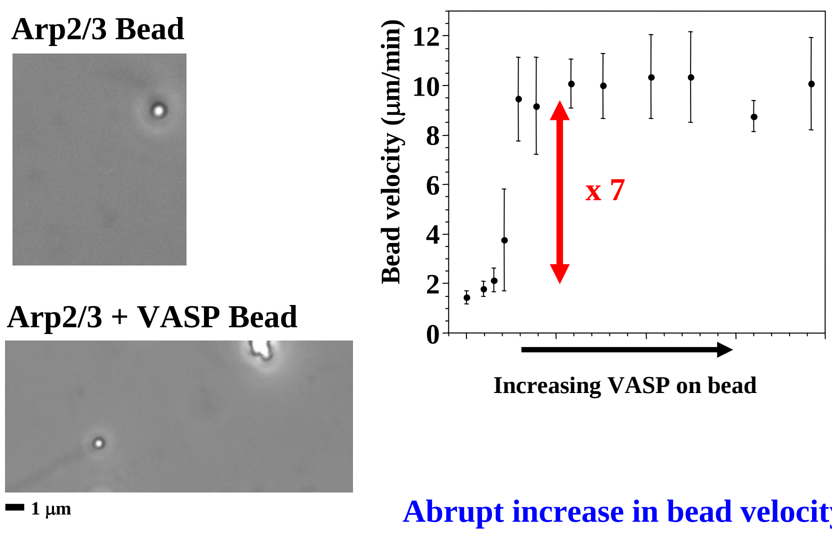
N.B. Dictyostelium VASP
Low salt conditions (50 mM)

Breitsprecher et al., 2008

Bead system applied to Arp2/3-VASP interplay



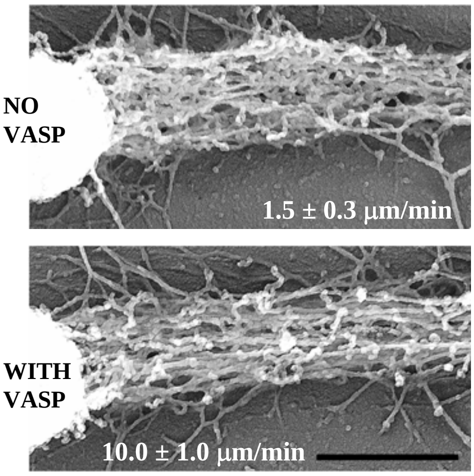
Effect of increasing VASP on bead velocity



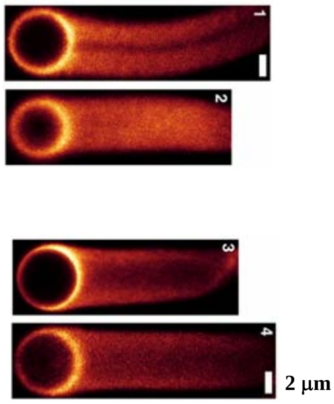
Plastino et al., 2004

Abrupt increase in bead velocity with increasing amounts of VASP

+VASP = Increased velocity, filament alignment, hollow comets



Bar 500 nm



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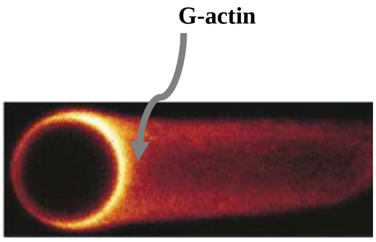
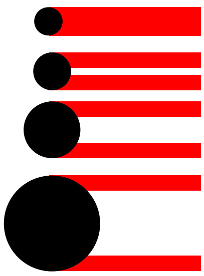
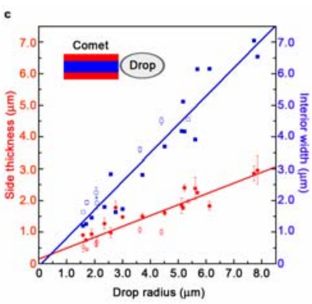
Plastino et al., 2004

Hollow comets NOT due to diffusion limitation



If diffusion-limited, would expect:





What we observe: wall thickness increases with bead size

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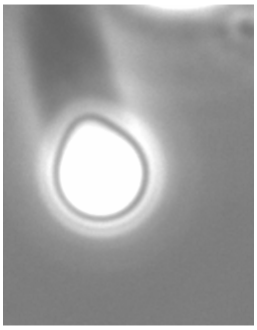
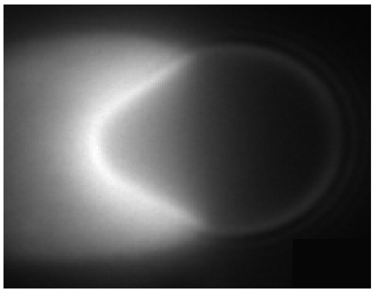
Force distribution on deformable beads

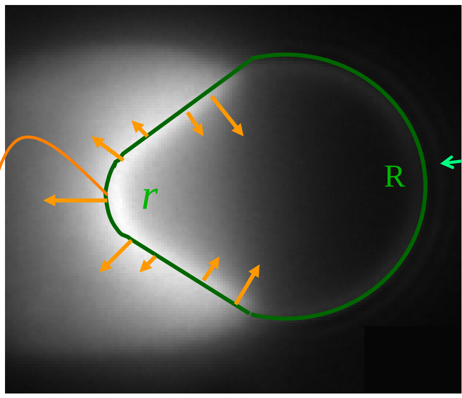


1.5 µm

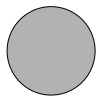


Boukellal et al., 2004

Apply Laplace…



NB: A sphere has two curvatures
$C_1=C_2=1/R$



Laplace equation

$$\Delta P = \gamma(\text{surface tension})\left(\frac{1}{R_1}+\frac{1}{R_2}\right)$$

$$\Delta P = 2\gamma\left(\frac{1}{R}\right)$$

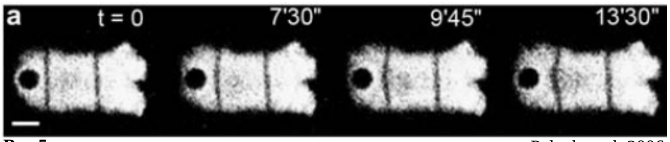
$$\Delta P = 2\gamma\frac{1}{r} + \sigma$$

σ: normal stress exerted on the droplet by the actin gel
32nN/µm² at the rear

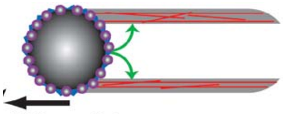
Boukellal et al., 2004

Hollow comets due to stresses in the gel?

Gel moving faster on the sides, pulling on the center.



Bar 5 µm Paluch et al. 2006



Why rupture only with VASP?
VASP weakens network/surface link?
1) **"Detaching activity" Samarin et al., 2004?**
2) **Result of decrease in branching?**

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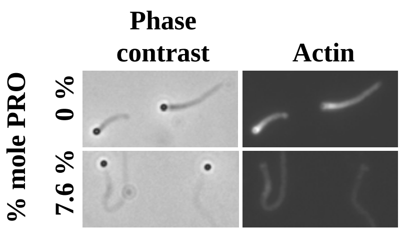
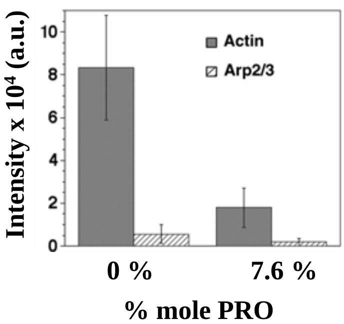
Enhanced speed ⟷ Decreased actin density





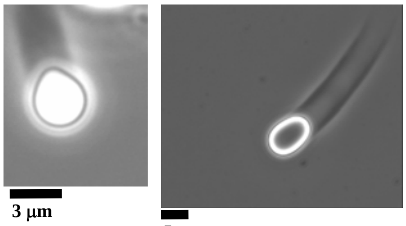
With VASP = comet 4x less dense, but bead moves 7x more quickly. So per unit time, ~2x more actin incorporated in the presence of VASP. What then accounts for the 7x increase in bead speed??

Global change in how actin comet acts on surface.

Jumping behavior with VASP

Arp2/3 only
0.15 µm/min

Arp2/3 + VASP
0.7 µm/min

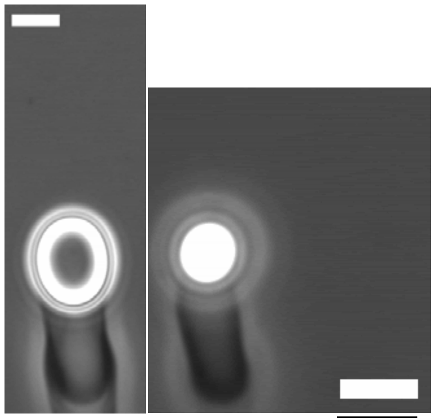


3 µm

5 µm

Jumping behavior only with VASP

4 µm



4 µm

Drops with VASP:
--5-fold velocity increase
--kiwi shape
--hollow comets

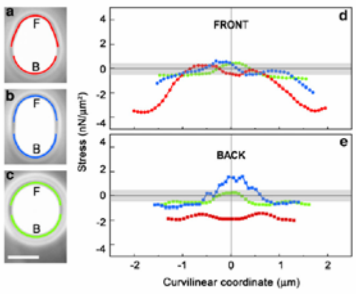
Trichet et al., 2007

Calculate stresses during jumping

Just after a jump

Just before a jump

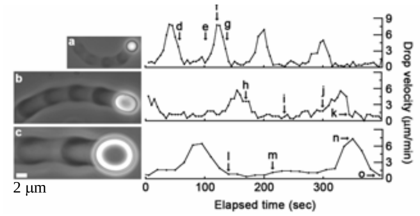
During the jump



Trichet et al., 2007

Jumping behavior of VASP-recruiting drops

--never observed without VASP
--not observed on hard beads under same conditions
--occurrence dependant on drop size



Droplet radius (µm)	% Hopping	Comet count
0-1	**43.3**	**30**
1-2	**35.2**	**88**
2-3	**18.2**	**55**
3-4	**14.9**	**47**
4-5	**12.2**	**41**
5-6	**15.4**	**26**
6-7	**7.1**	**14**
7-8	**0**	**8**

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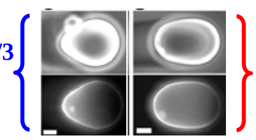
Trichet et al., 2007

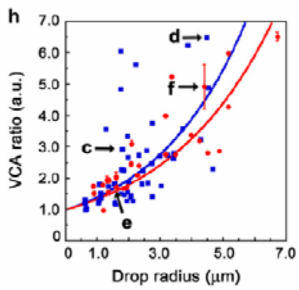
What defines soft bead jumps?
…Clues from protein clustering on fluid surfaces

Arp2/3 Drop



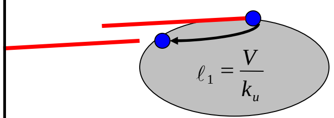
Arp2/3 + VASP Drop

Bars 2 µm

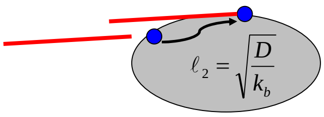


Exponential increase in NPF clustering with increasing drop size

convection/unbinding



diffusion/binding



V **drop velocity**

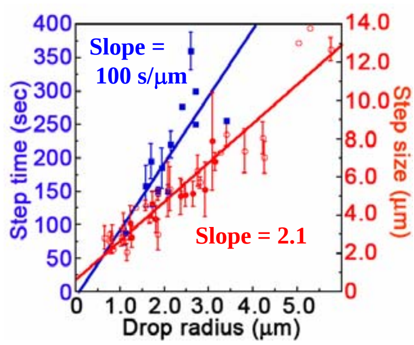
D **diffusion coefficient**

k_u unbinding constant

k_b binding constant

Trichet et al., 2007

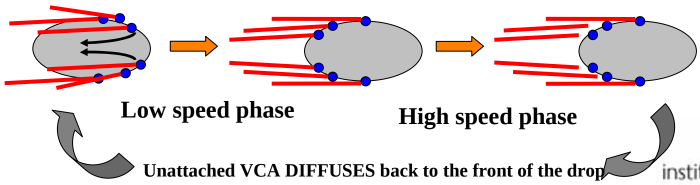
Convection and diffusion control steps



CONCLUDE:

1) Travels one radius length in low speed phase
From step time = 100 s/µm x R, low speed velocity 0.01 µm/s

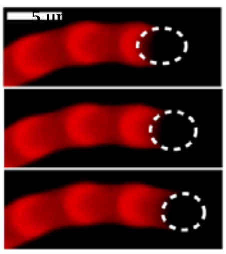
2) Travels one radius length in high speed phase
Total step 2R

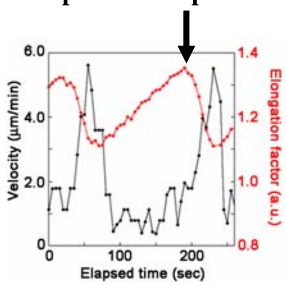


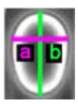
Why jumping only with VASP?

VASP weakens clustering i.e., enhances diffusive effect or reduces convective effect…due to decreased surface attachment of network?

Attachment catastrophes in the presence of VASP



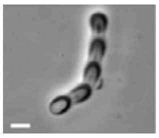


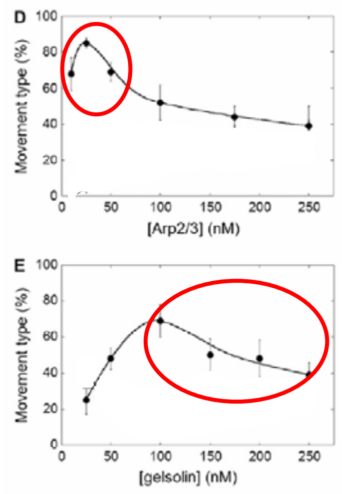


Elongation factor = b/a

Trichet et al., 2008

Jumping also with low branching or high capping





Low Arp2/3 complex or high gelsolin = jumping

Delatour et al., 2008

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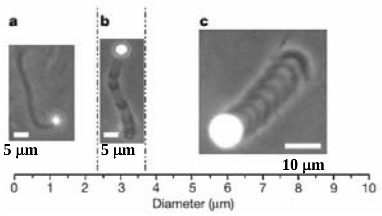
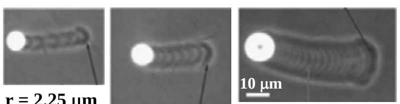
Jumping hard beads show a completely different behavior



Bernheim-Grosswasser et al., 2002

↑ Bead Size = ↑ Jumping behavior



r = 2.25 µm

r = 3.15 µm

r = 4.55 µm

Bernheim-Grosswasser et al., 2005

↑ Bead Size = ↓ Step Size Step Time

For hard spheres, friction and gel elasticity control steps

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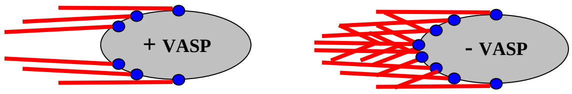
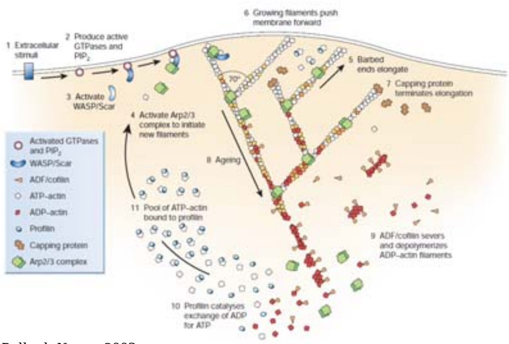
Overall conclusion: global effect of VASP is to weaken actin network-surface attachment

→on beads/drops, results in faster movement, hollow comets or jumping movement
→in cells, allows cell membrane to move/evolve and actin cytoskeleton to remodel

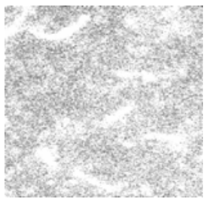
→mechanism…related to reduced branching in the presence of VASP??



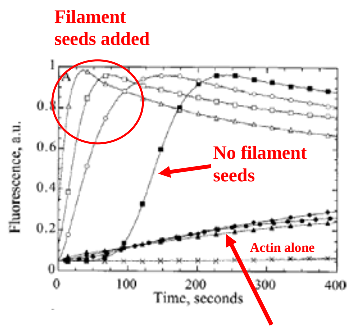
New insights: How can side-branching produce motility?



Pollard, *Nature* 2003



Amann and Pollard, 2001

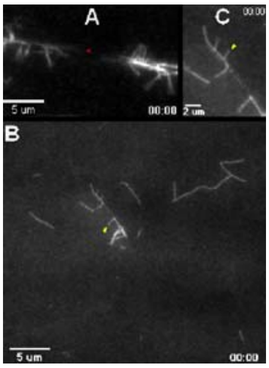


Machesky et al., *PNAS* 1999

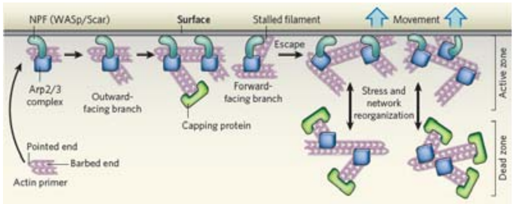
Barbed ends not preferentially oriented toward the surface

TIRF microscopy on rods

Fluo actin that bleaches so only see new polymer



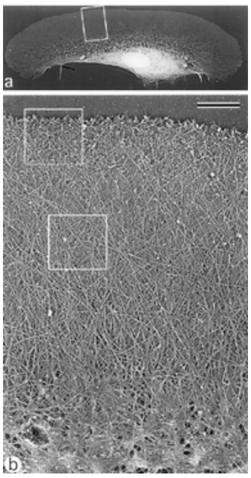
Achard et al., *Curr. Biol.* 2010



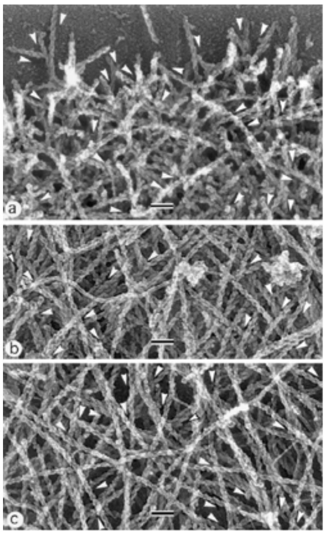
Sykes and Plastino, *Nature* 2010

In absence of capping, Arp2/3 nucleated filaments « escape » from the surface

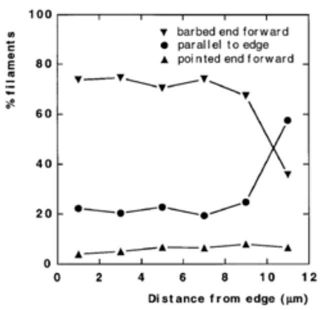
But in cells, most barbed ends are pointing forward…



EM of actin filaments in cells

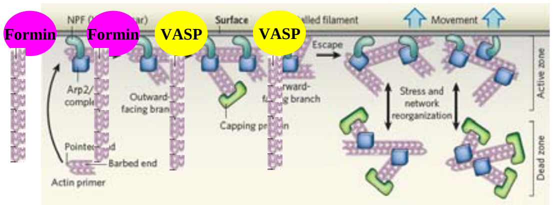


Determine polarity with myosin labeling

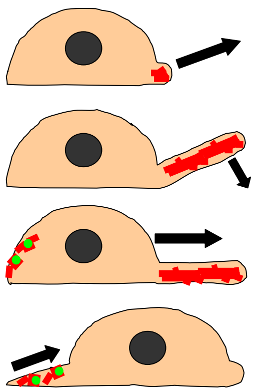


Svitkina et al., *J. Cell Biol.* 1997

Barbed end anchoring proteins in cells: formins, Ena/VASP?



Part II. Force production in the moving cell



Protrusion — Part I. How protrusion is produced by actin polymerization

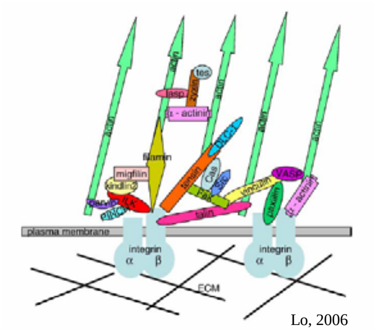
Adhesion

Traction — **Actin** / **Myosin** — Part II. How protrusion is converted into motility

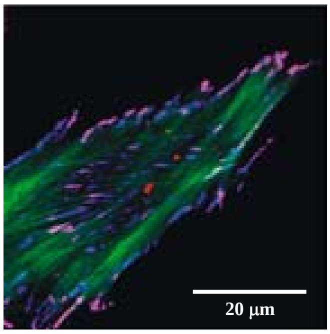
Retraction

Cell-substrate adhesion

3 parts of a cell-substrate adhesion:
<u>Actin</u> filaments
<u>Integrins</u>—transmembrane proteins that bind the substrate
<u>All the rest</u> (vinculin, paxillin, zyxin etc.) —proteins that mediate the link actin-integrin



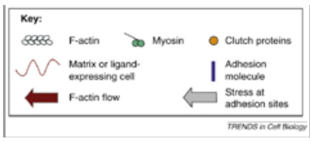
Lo, 2006

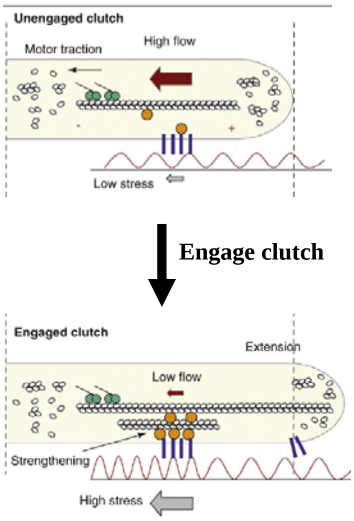


Cavalcanti-Adam et al., 2007

green = actin;
purple = coloc. vinculin and β3 integrin

« The molecular clutch »





Giannone et al., *Trends Cell Biol.* 2009

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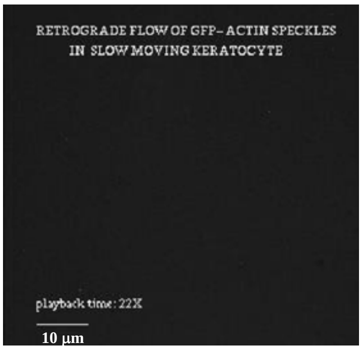
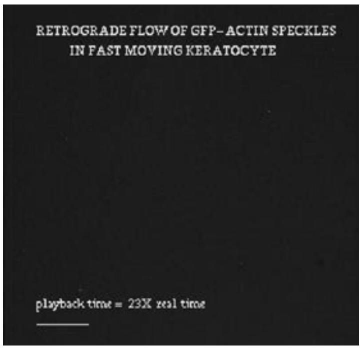
Actin flow in cells



High flow in immobile cell
--motor turning over, but clutch not engaged = cell not going anywhere



Low flow in mobile cell
--clutch engaged = polymerization converted into movement
…but still some residual flow

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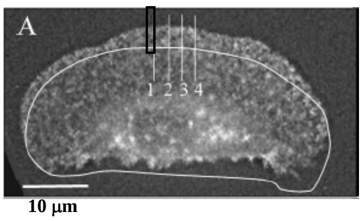
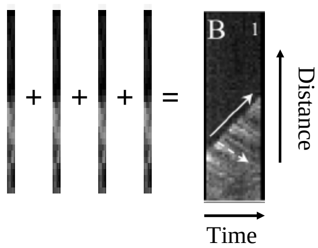
Jurado et al., *MBC* 2008

Measuring actin flows in cells: kymograph analysis

Speckle labeling (low amounts of fluo species)





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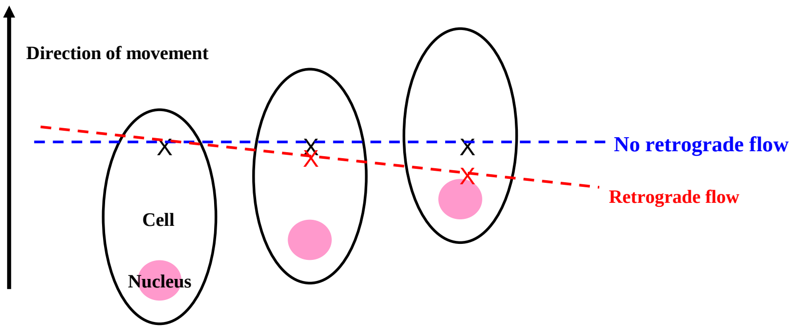
Jurado et al., *MBC* 2008

ASIDE: All motile cells characterized
to date display some degree of retrograde flow…
???keep the clutch lightly engaged for reactivity???



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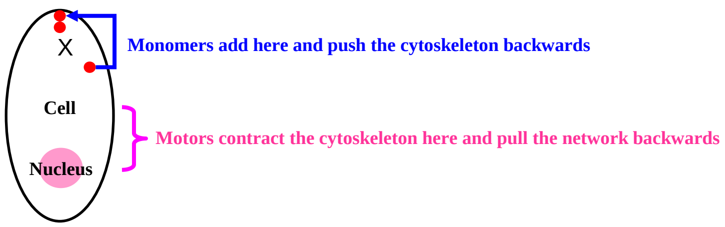
What causes retrograde flow??



So which is it?
--Conflicting results in the literature--
Bottom line: probably cell-type and cell region dependent

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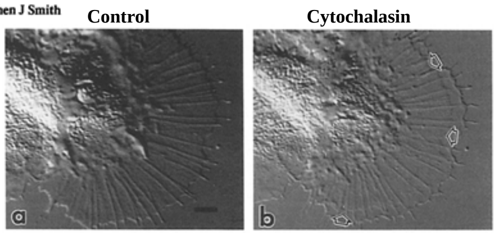
JCB (1988), 107: 1505-1516

Myosin creates retrograde flow

Actions of Cytochalasins on the Organization of Actin Filaments and Microtubules in a Neuronal Growth Cone

Paul Forscher and Stephen J Smith

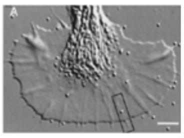
Control   Cytochalasin

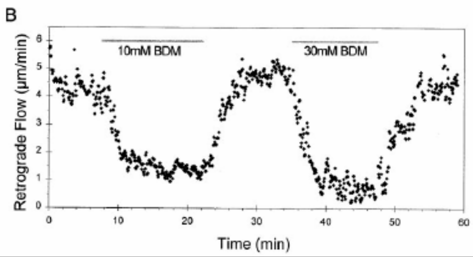


Neuron, Vol. 16, 769–782, April, 1996, Copyright ©1996 by Cell Press

Myosin Drives Retrograde F-Actin Flow in Neuronal Growth Cones

C. H. Lin,* E. M. Espreafico,† M. S. Mooseker,‡ and P. Forscher‡





BDM =
2,3-butanedione-2-monoxime
(inhibits myosin ATP-ase activity)

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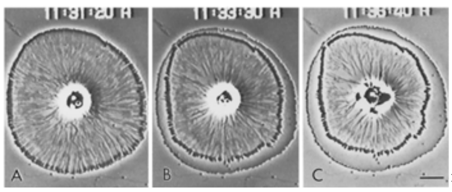
Molecular Biology of the Cell
Vol. 10, 4075–4090, December 1999

Maybe it's more than myosin…

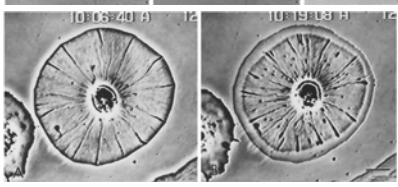
Two Components of Actin-based Retrograde Flow in Sea Urchin Coelomocytes

John H. Henson,*†‡ Tatyana M. Svitkina,§ Andrew R. Burns,* Heather E. Hughes,* Kenneth J. MacPartland,* Ronniel Nazarian,*† and Gary G. Borisy§

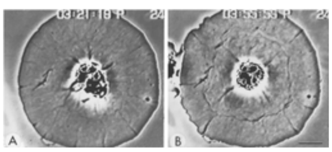


Cytochalasin D treatment
(blocks polymerization)

10 µm



BDM (inhibit myosin)



Kinase inhibitors (inhibit myosin)

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It’s both myosin AND polymerization in two different regions of the leading edge

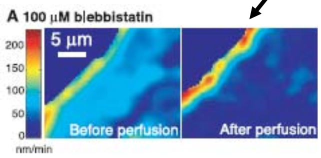
Two Distinct Actin Networks Drive the Protrusion of Migrating Cells

A. Ponti, M. Machacek, S. L. Gupton, C. M. Waterman-Storer, G. Danuser

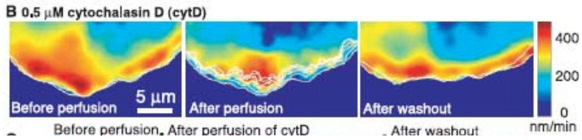
17 SEPTEMBER 2004 VOL 305 SCIENCE

Retrograde flow velocity maps in migrating epithelial cells

Front of the moving cell



Inhibit myosin = no effect cell edge, slows down in interior



Inhibit polymerization = little effect cell interior, slows down edge (and engenders retraction…the white lines)

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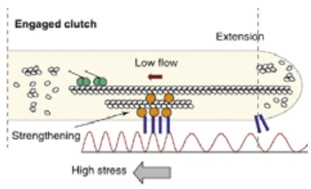
Molecular clutch predicts:
Inverse linear relation between flow and movement



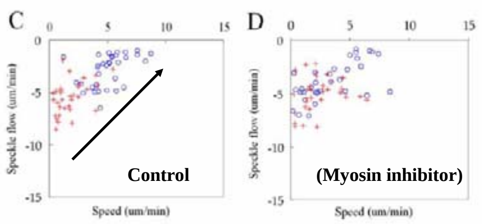
Generally true…one example:

Molecular Biology of the Cell
Vol. 16, 507–518, February 2005

Slipping or Gripping? Fluorescent Speckle Microscopy in Fish Keratocytes Reveals Two Different Mechanisms for Generating a Retrograde Flow of Actin

Carlos Jurado, John R. Haserick, and Juliet Lee*

Speed increases,
Flow decreases
Sum remains constant



Natural variability:
Blue cells—fast
Red cells—slow

Fast moving cells slow down and retrograde flow increases

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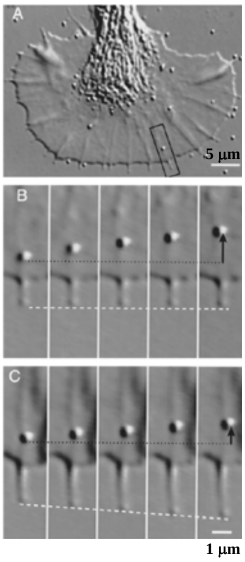
Another example:



Neuronal growth cone

**Control: retrograde flow of a bead,
no filopodia extension**

**Inhibit myosin: retrograde flow of a bead slows,
filopodia extension speeds up**

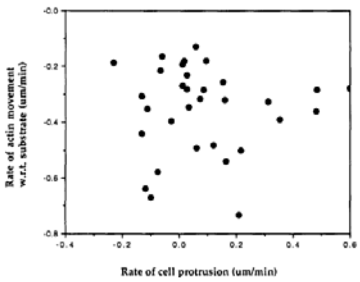
Lin et al, *Neuron* 1996

Some exceptions…

Comparison of Actin and Cell Surface Dynamics in Motile Fibroblasts

Julie A. Theriot and Timothy J. Mitchison*

© The Rockefeller University Press, 0021-9525/92/10/367/11 $2.00
The Journal of Cell Biology, Volume 118, Number 2, October 1992 367–377



No correlation between protrusion speed and retrograde flow

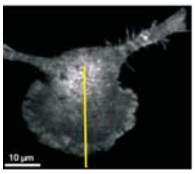
Adaptive force transmission in amoeboid cell migration

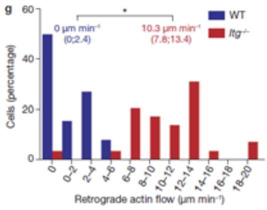
Jörg Renkawitz[1], Kathrin Schumann[1], Michele Weber[1], Tim Lämmermann[1], Holger Pflicke[1], Matthieu Piel[2], Julien Polleux[3,4], Joachim P. Spatz[4] and Michael Sixt[1,5]

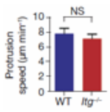
NATURE CELL BIOLOGY VOLUME 11 | NUMBER 12 | DECEMBER 2009

As expected, retro increases…but movement stays the same!!

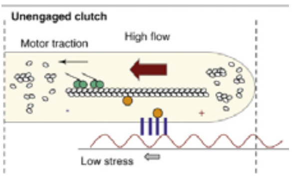
Dendritic cells (immune system) k.o. all integrins, but confine = motile



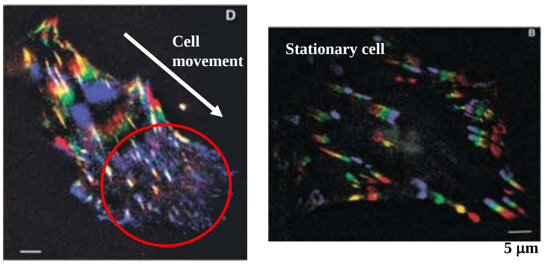




Molecular clutch generally portrayed as actin-linker or linker-integrin slippage NOT integrin-ECM slippage



Only a few reports of possible integrin-ECM slippage



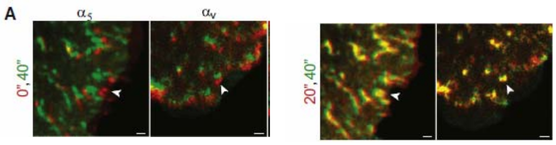
Smilenov et al., *Science* 1999

Imaging fluorescent integrins
Red…yellow…green…purple
Time 0………………...time t

Rainbow = integrins moving

No rainbow at front of moving cell

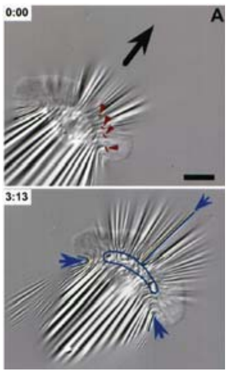
After release of myosin inhibition = transient slippage then stops



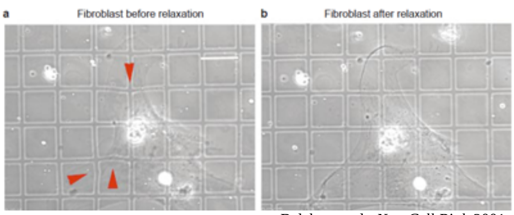
Aratyn-Schaus et al., *Curr. Biol.* 2010

Force transmission to the substrate: traction forces

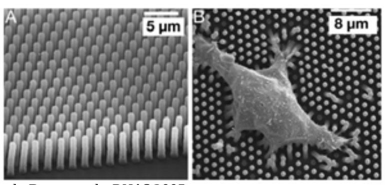
Visualize forces exerted on the substrate using deformable elastomer substrates (track wrinkles, embedded beads, pattern deformation, microstructures…)



Burton et al., *Mol. Biol. Cell* 1999



Balaban et al., *Nat. Cell Biol.* 2001



du Roure et al., *PNAS* 2005

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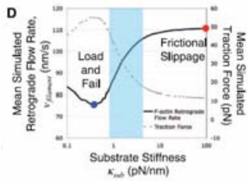
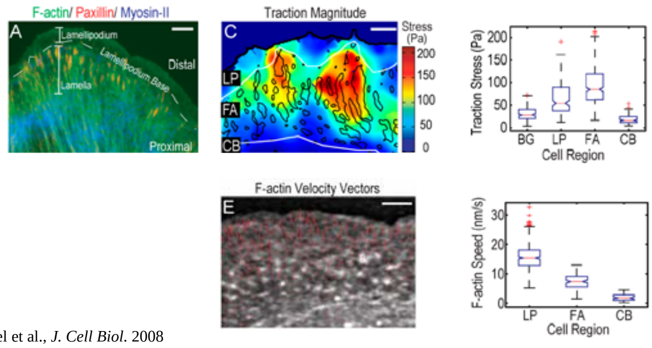
Molecular clutch predicts:
Low retro flow should be associated with increased traction force



Filopodia traction forces in growth cones

Chan and Odde, *Science* 2008

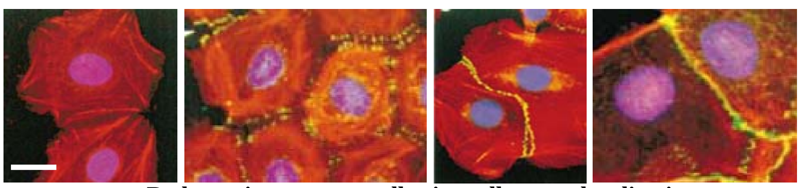
But maybe more complex in other cell types…depends on age of adhesion



Gardel et al., *J. Cell Biol.* 2008

Retrograde flow and force production in 3D…
Cell-cell adhesion

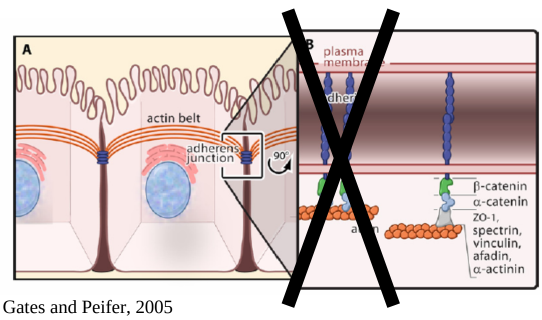
Time ⟶

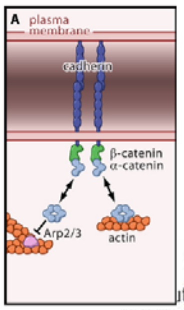


15 μm

Red = actin; green = cadherin; yellow = colocalization

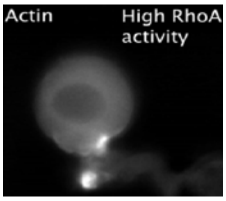
Vasioukhin et al., 2000

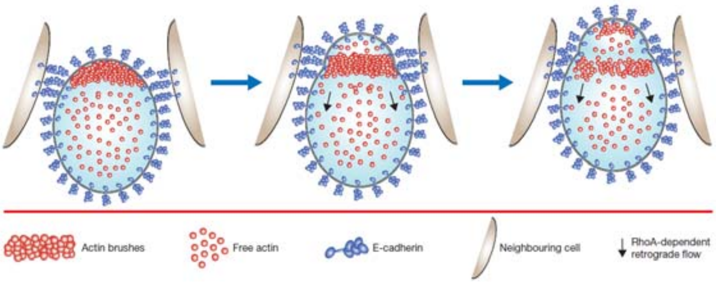




Gates and Peifer, 2005

Zebrafish germ cells—migrate to gonad during embryonic development





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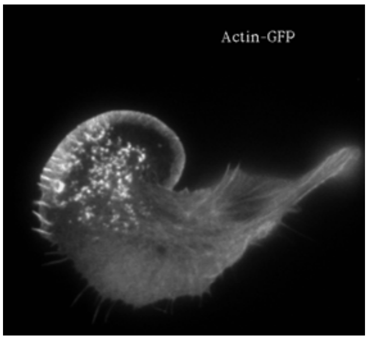
Kardash et al., 2010

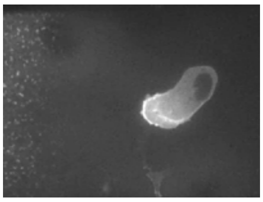
Part III. Blebbing phenomenon and *in vivo* cell migration

B16 mouse melanoma

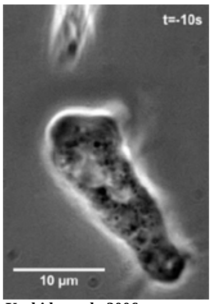


Vic Small, Klemens Rottner
Cell 30-50 µm wide

Deep cells killifish embryo



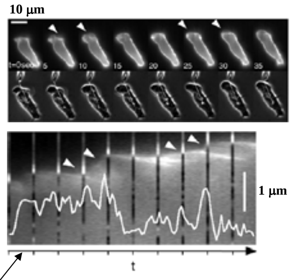
Rachel Fink webpage, Mt Holyoke College
Cell 20-40 µm long



***Dictyostelium* amoeba**

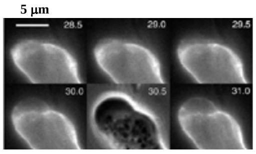
Yoshida et al., 2006

Dictyostelium moves by bleb formation

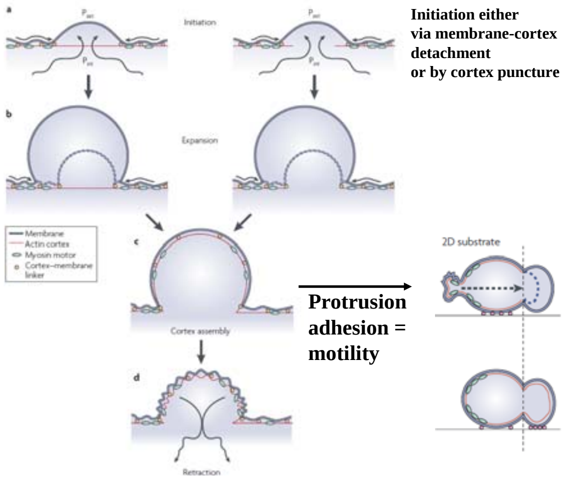


5 sec

White trace =
Centroid velocity
Highest velocity corresponds
to bleb events



Yoshida et al., *J. Cell Sci.* 2006



Initiation either
via membrane-cortex
detachment
or by cortex puncture

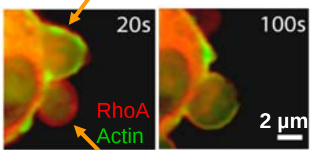
Protrusion
adhesion =
motility

Charras and Paluch, *Nat. Rev. Mol. Cell Biol.* 2008

A few words about the cortex

What we have seen up until now hasn't been cortical…

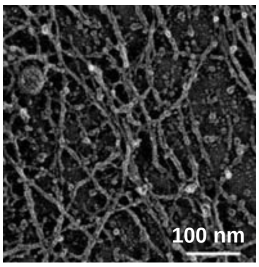
Cortex fully reformed in an old bleb



Charras et al.,
J. Cell Biol. 2006

No cortex in a new bleb

- Regulation: Cdc42, Rac, RhoA (regulatory proteins)
- Nucleation: unclear
- Tethering: ERM proteins
  (Ezrin, Radixin, Moesin)
- Myosin II



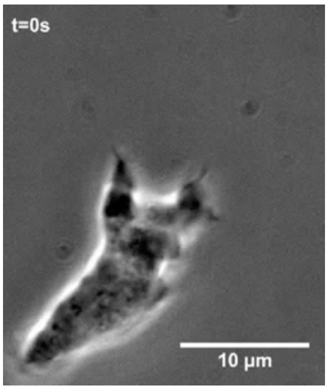
Morone et al., *J. Cell Biol.* 2006

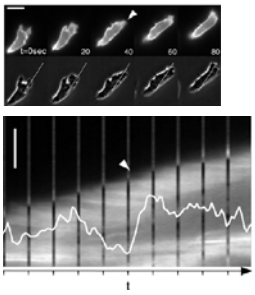
EM of a cortex of a fibroblast

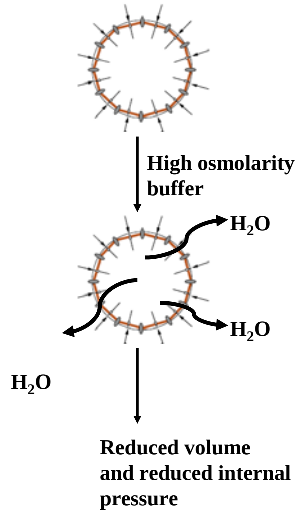
- Filaments next to the membrane
- No specific orientation
- Meshsize ~100nm
- 50nm < thickness < 2µm

Internal pressure drives bleb formation

Decrease internal pressure with osmotic shock = suppress blebbing





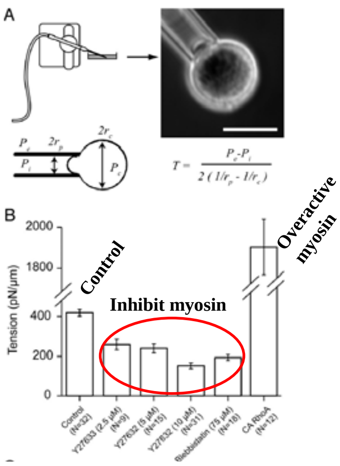


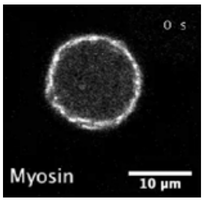
Yoshida et al., *J. Cell Sci.* 2006

Cortical tension is important for blebbing

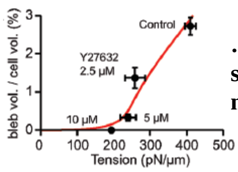
Decrease myosin activity = suppress blebbing

Myosin (+ actin) create cortical tension





Trigger blebs…

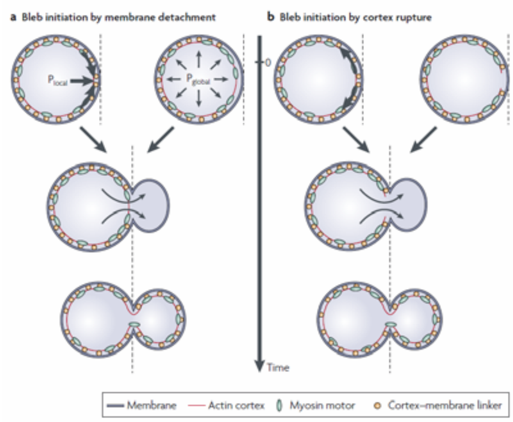


…measure bleb size under myosin inhibition

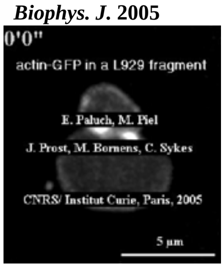
Tinevez et al. *PNAS* 2009

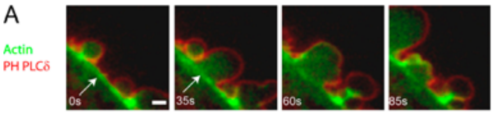
Cortex rupture versus membrane detachment

Charras and Paluch, *Nat. Rev. Mol. Cell Biol.* 2008



Previous movie
See also Paluch et al., *Biophys. J.* 2005





Arrows: cortex intact during bleb expansion

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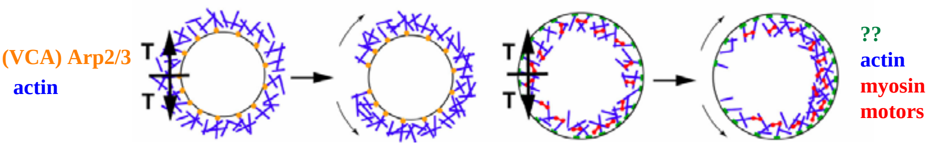
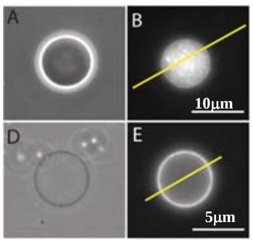
Tie-in with bead symmetry breaking



Paluch et al., *J.Cell Biol.* 2006

On beads, stresses generated by spherical geometry
In cells, stresses generated biochemically

Next step, acto-myosin cortexes in liposomes…towards an artificial cell
Cécile Sykes's group
Institut Curie



Controlled production of an actin cortex in a liposome

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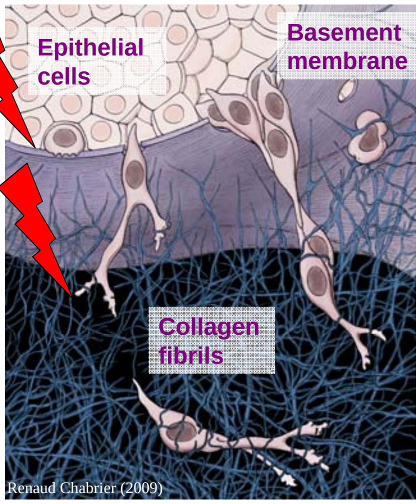
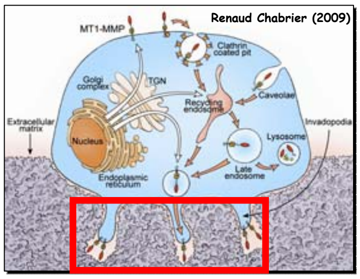
Pontani et al., *Biophys. J.* 2009

Dictyostelium aside, *in vivo* significance of blebbing… tumor cell invasion and motility

Invasion and migration require protease degradation of basement membrane and ECM

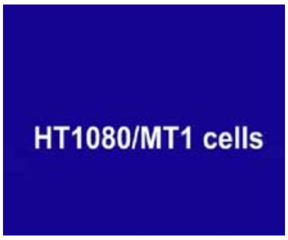


http://animals.howstuffworks.com/insects/housefly

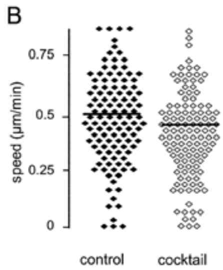


Membrane-tethered matrix metalloproteases (MT-MMP)

Inhibit proteases = tumor cells switch to blebbing mode
(in *in vitro* reconstituted ECM)



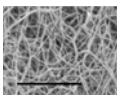
Migration in 3D collagen matrix (reconstituted)



Wolf et al., *J. Cell Biol.* 2003

Probably an artifact of artifical matrix…cell balls (same cells as previous study)

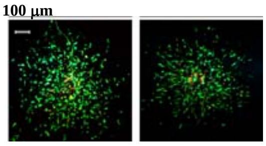
Pepsin extracted



5 µm

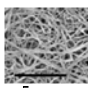
(like in Wolf et al.)

100 µm



Control | **No protease (MT1 RNAi)**

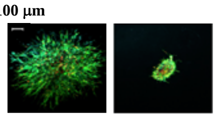
Acid extracted



5 µm

More *in vivo*-like

100 µm

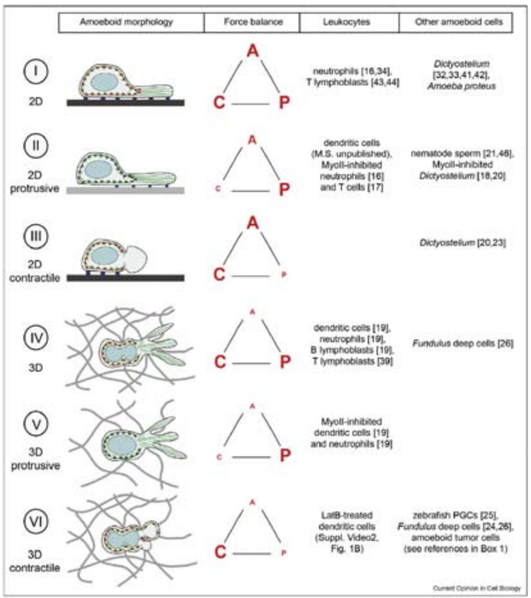


Control | **No protease (MT1 RNAi)**

Sabeh et al., *J. Cell Biol.* 2009

Nevertheless, shows that cells can employ mutliple strategies

A adhesion
C contraction
P polymerization



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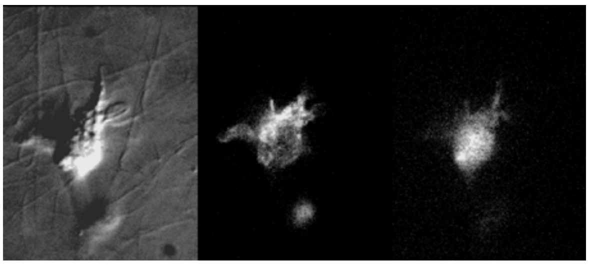
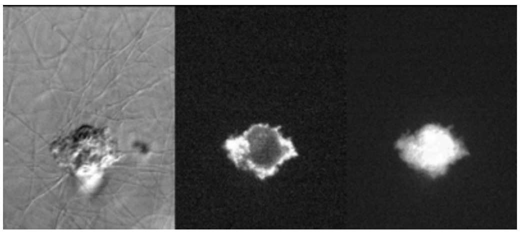
Lammermann and Sixt *Curr. Opin. Cell Biol.* 2009

Whatever works…



Dendritic cell chemotaxis in a 3D collagen matrix



Same cells.
Drub (LatB) which prevents polymerization (but leaves cortical actin intact)

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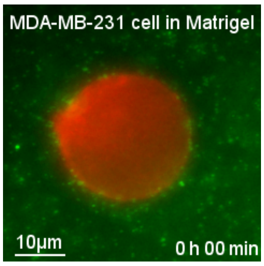
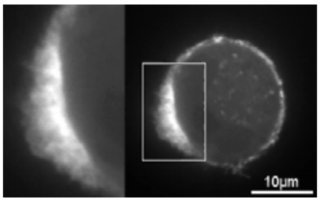
Lammermann and Sixt *Curr. Opin. Cell Biol.* 2009

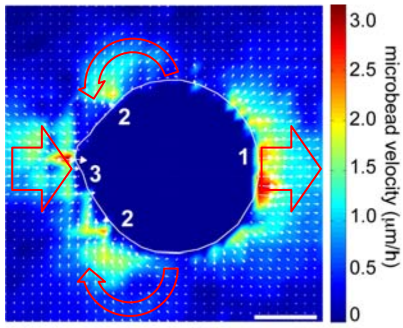
Rounded polarized morphology

MDA-MB-231 cells migrate in Matrigel





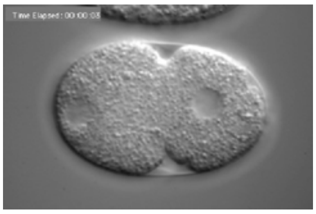
Particle Image Velocimetry (PIV)



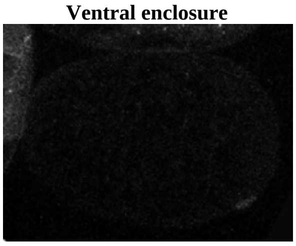
Poincloux et al., *PNAS* 2011

Putting cell motility, cell-substrate and cell-cell adhesions all together in tissues:
morphogenesis in the *C. elegans* embryo

How a fertilized egg becomes a worm

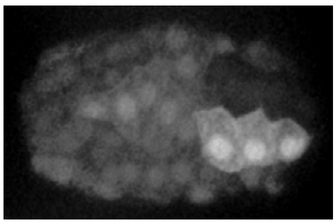


Ventral enclosure



***DLG-1::GFP* translational reporter**

Dorsal intercalation



***lbp-1::gfp* transcriptional reporter**

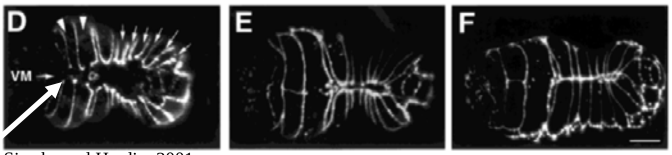
Wormbook

Ventral enclosure

1) Leading cell migration

2) Leading cell junction formation and fusion

3) Ventral pocket closure



Simske and Hardin, 2001

JAM-1-GFP
Total time 5-10'
Bar 10 µm

Actin and α-catenin-rich filopodia

Actin and (probably) myosin dependent

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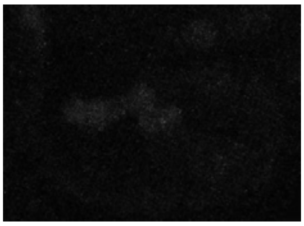
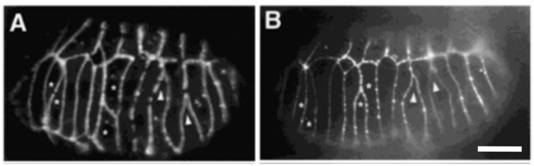
Dorsal intercalation



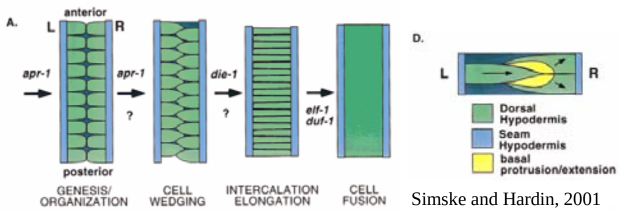
Wormbook

***DLG-1::GFP* translational reporter**



Simske and Hardin, 2001

JAM-1-GFP
Bar 10 µm
Time between frames 10'



Simske and Hardin, 2001

Actin and microtubule dependent

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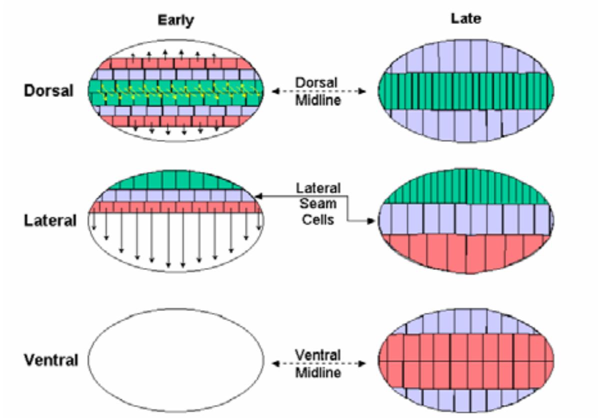
Summary of the cell shape changes associated with ventral enclosure and dorsal intercalation



Piekny and Mains, 2003

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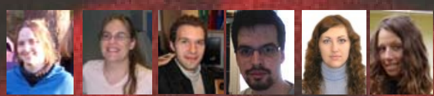

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