topics:

fluorescence speckling microscopy

evidence that actin assembly drives cell protrusion

how does actin assembly generate force

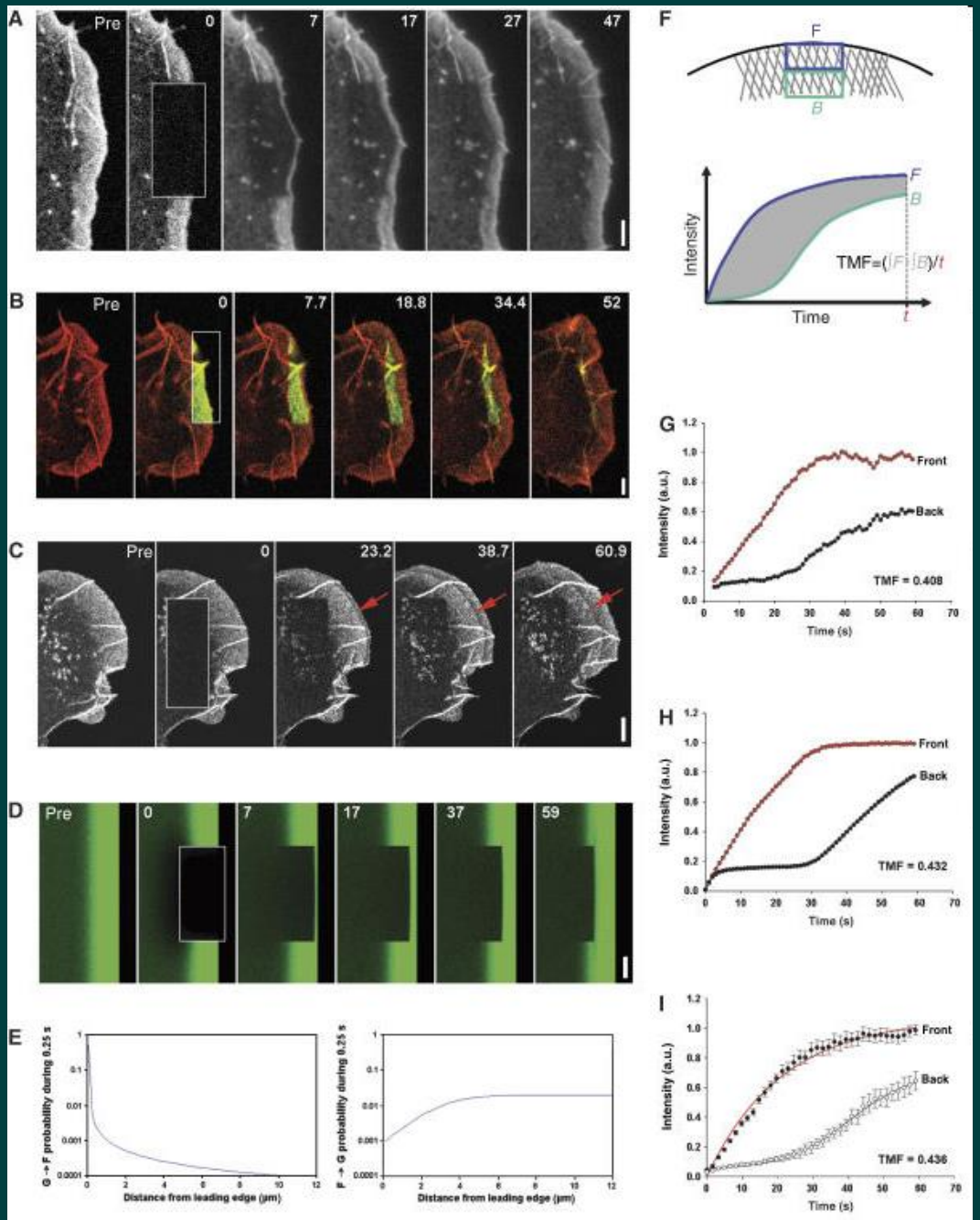
mechanism of actin assembly at the cell edge: how does it go so fast?

labeling part of the filament for fluorescence microscopy:

photobleacing (FRAP: fluorescenece recovery after photobleaching), photoactivation and their modifications (e.g. FLAP)

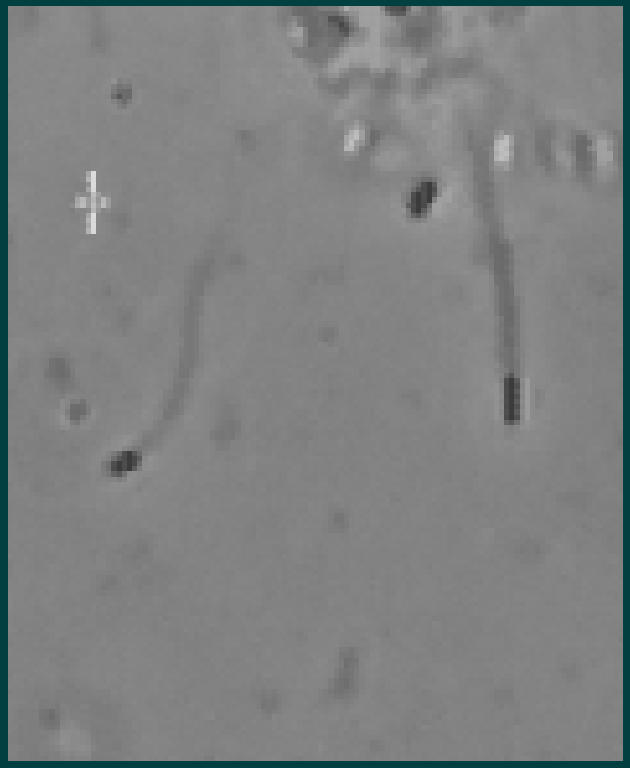
fluorescent speckle microscopy (FSM) including single molecule speckling

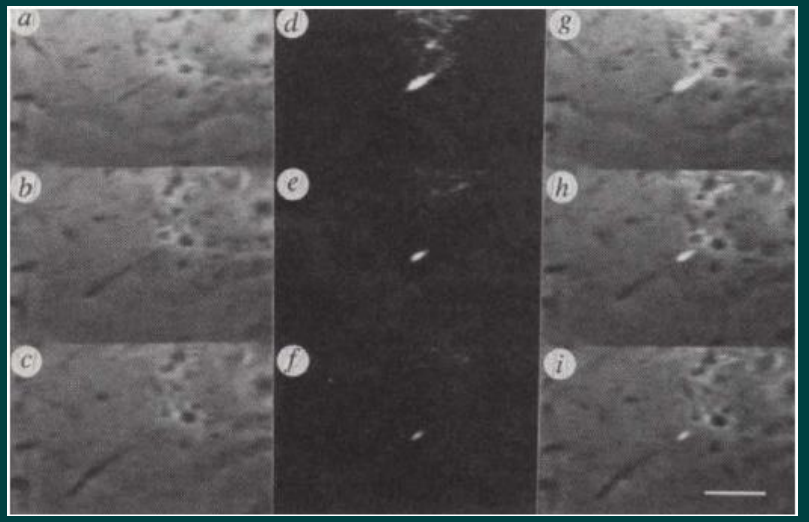
Photobleaching shows predominant actin assembly at the cell edge



Lai et al., 2008

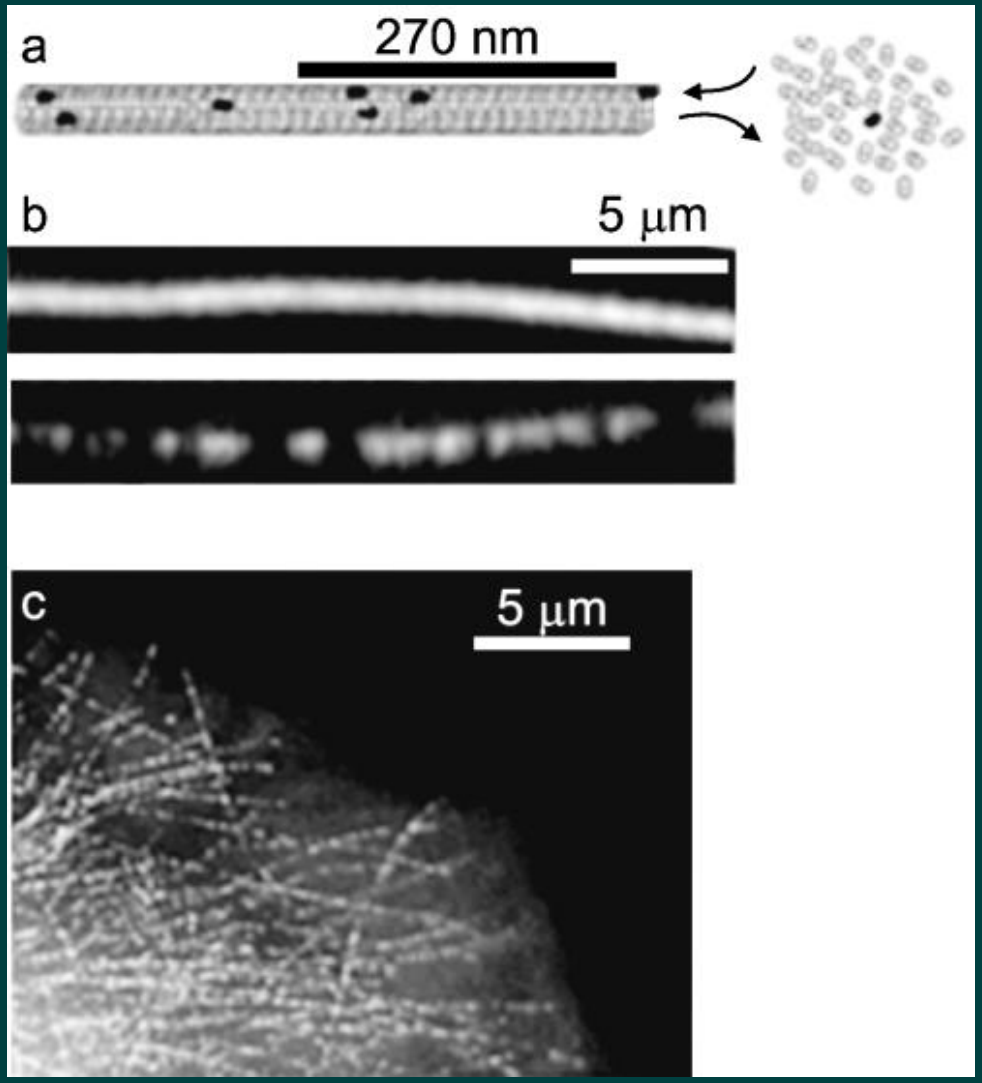
Actin tails of intracellular pathogen Listeria: photoactivation shows that actin is stationary as the tail grows (similar to the leading edge)



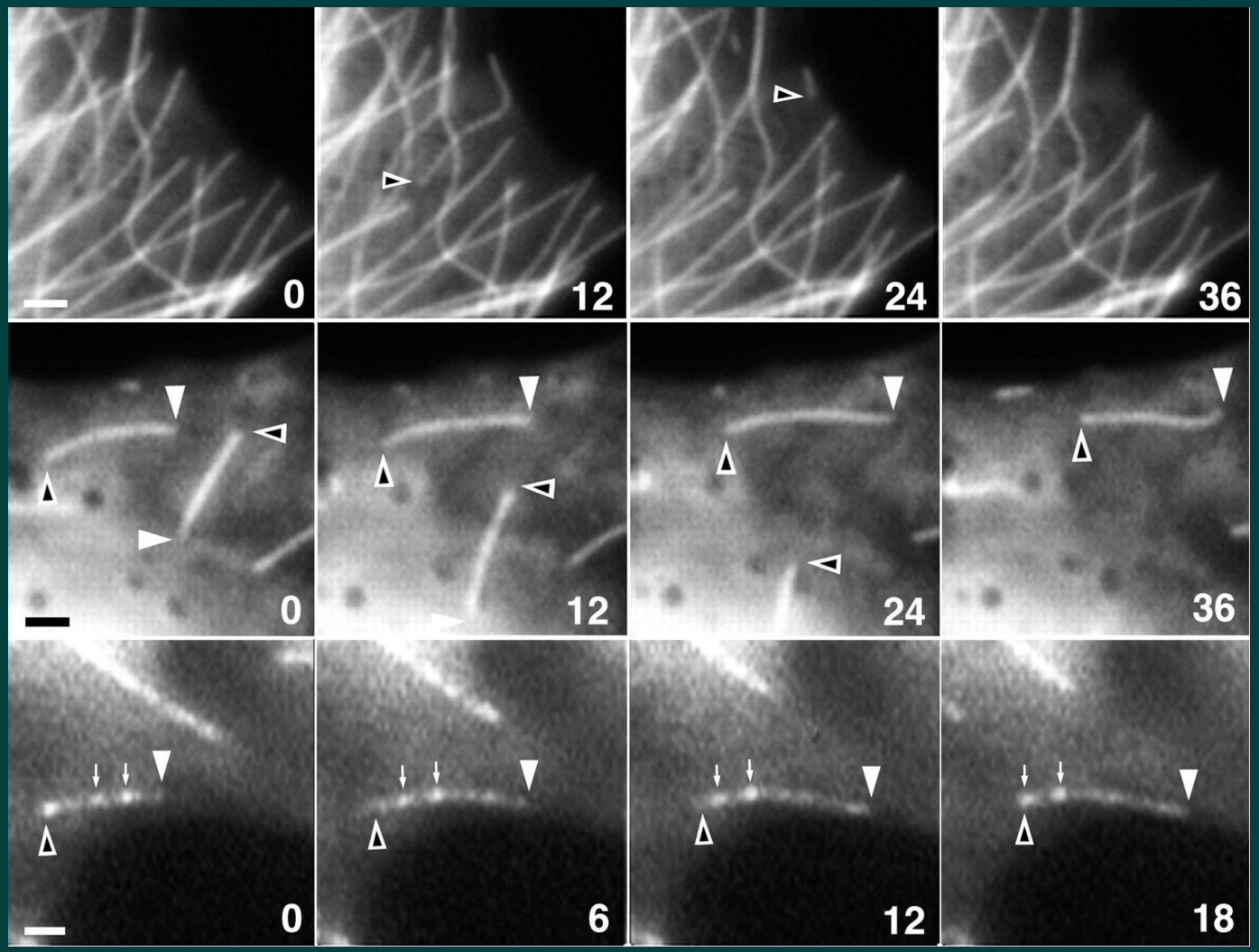


Photoactivation, Theriot et al., 1992

Fluorescent speckling microscopy (FSM)



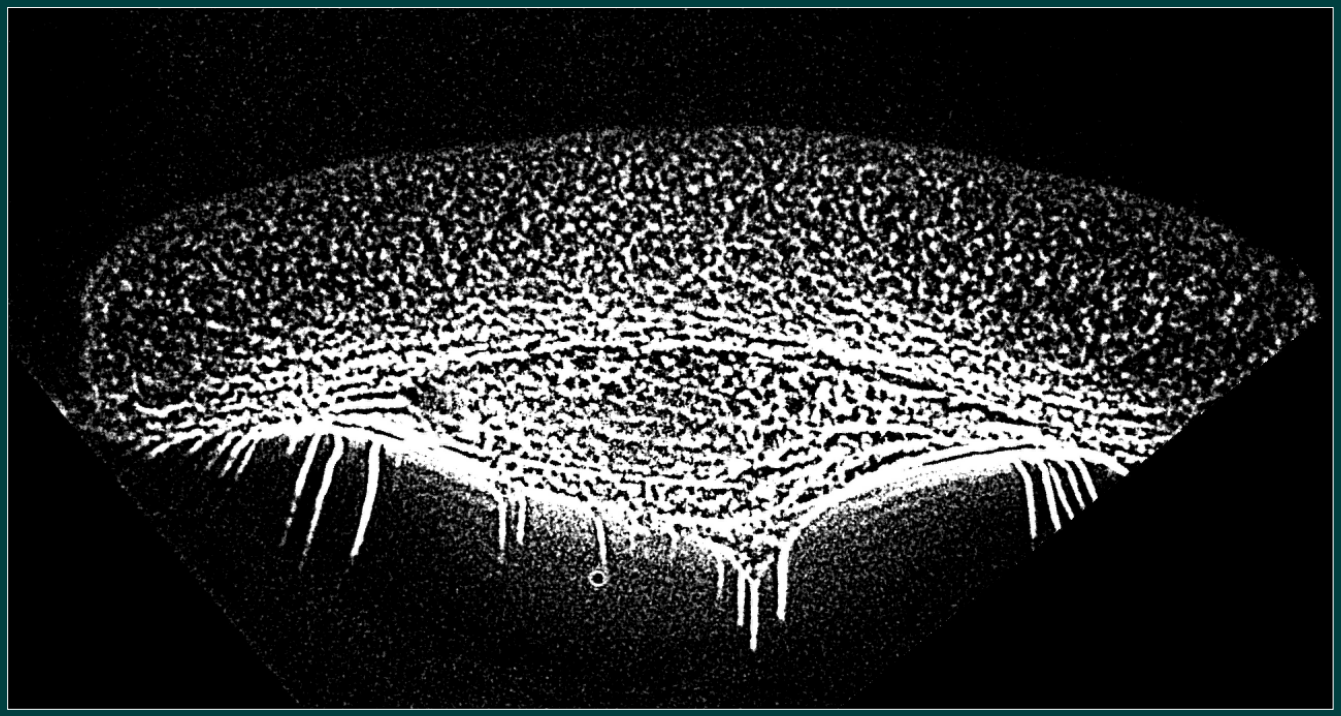
Danuser and Waterman-Storer, 2003



microtubule treadmilling with FSM

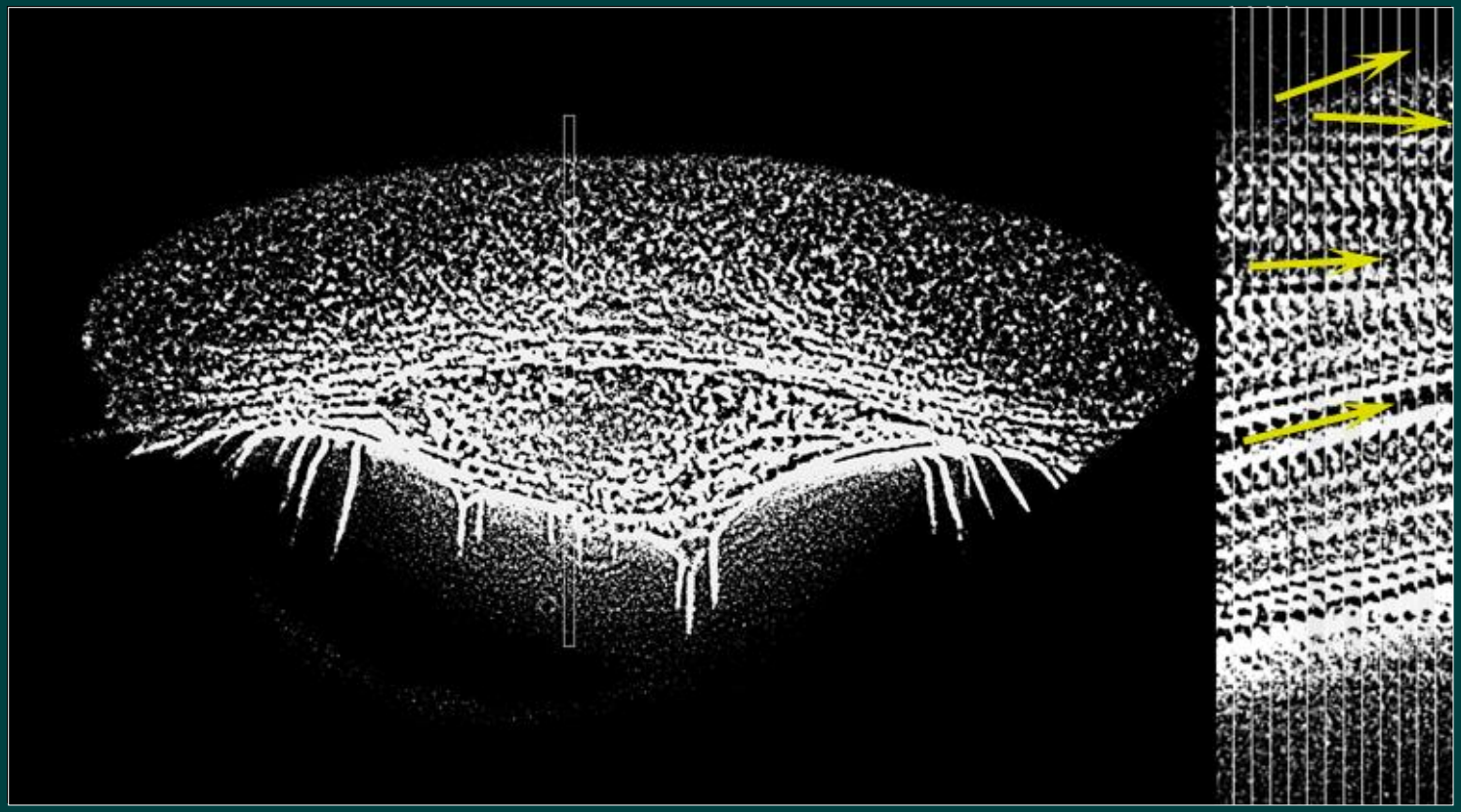
Rodionov et al., 1999

retrograde actin flow in rapidly moving keratocytes by FSM

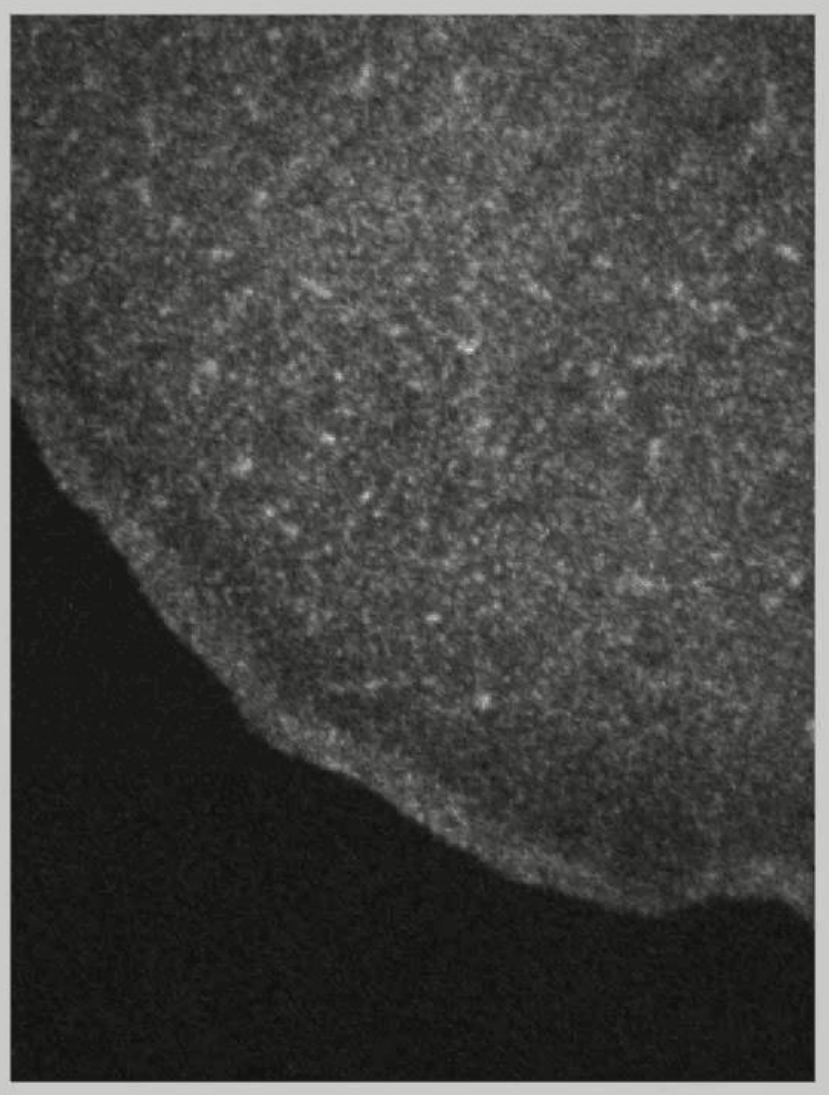


FSM
Schaub et al., MBC, 2007

Kymograph:
actin actually moves backward at the leading edge

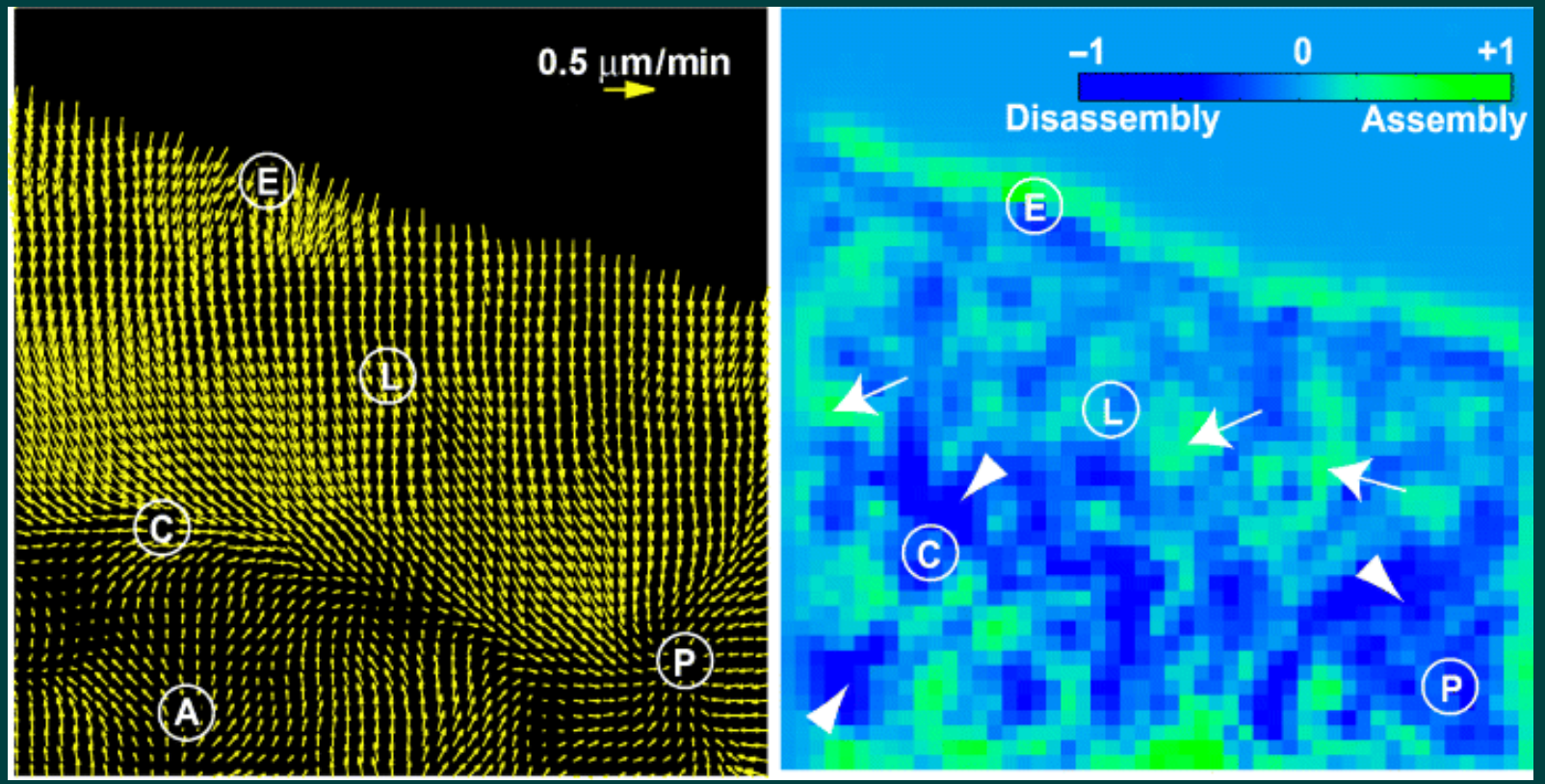


Retrograde actin flow with FSM in epithelial cells: two zones

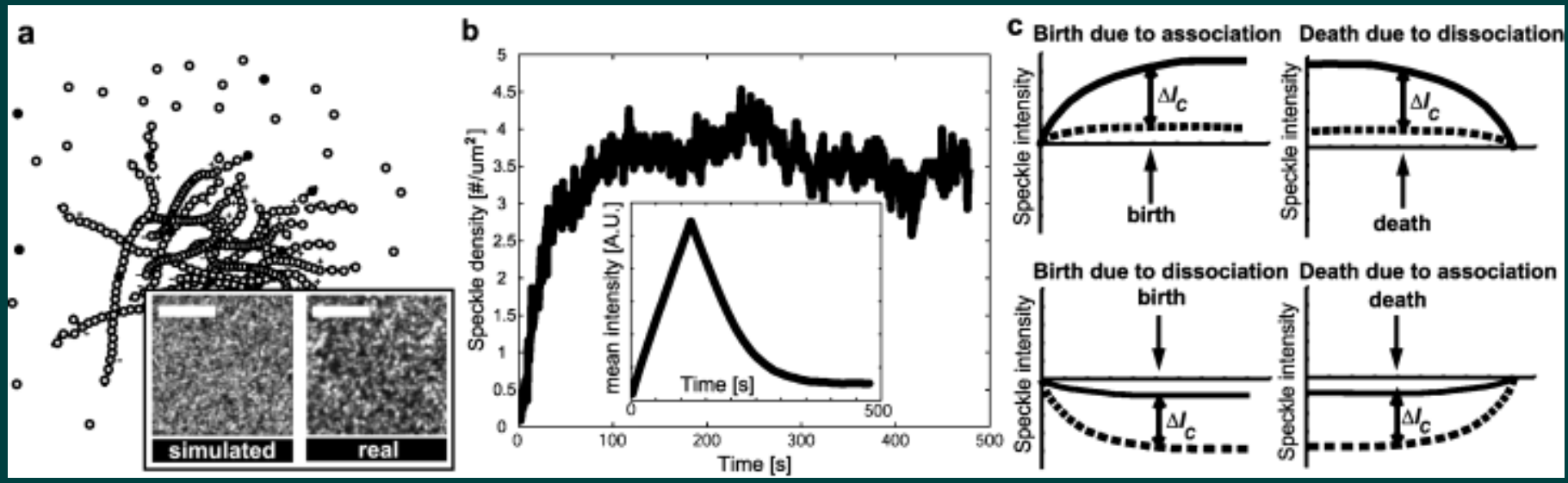


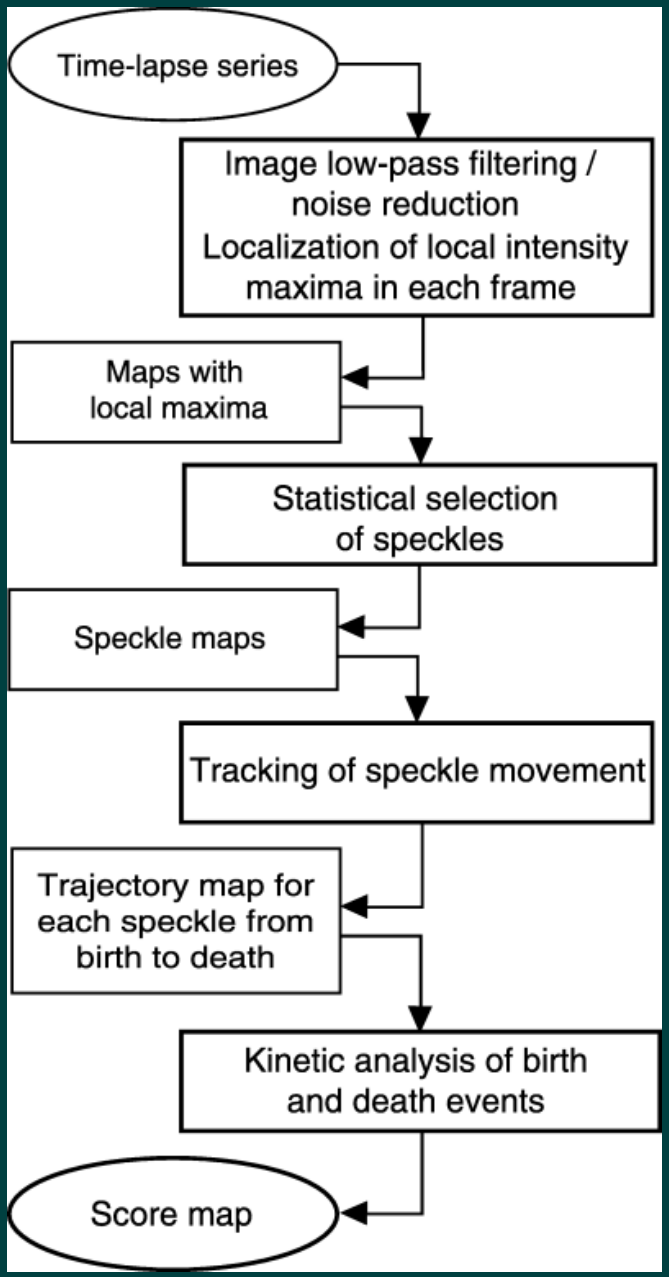
Ponti et al., 2004

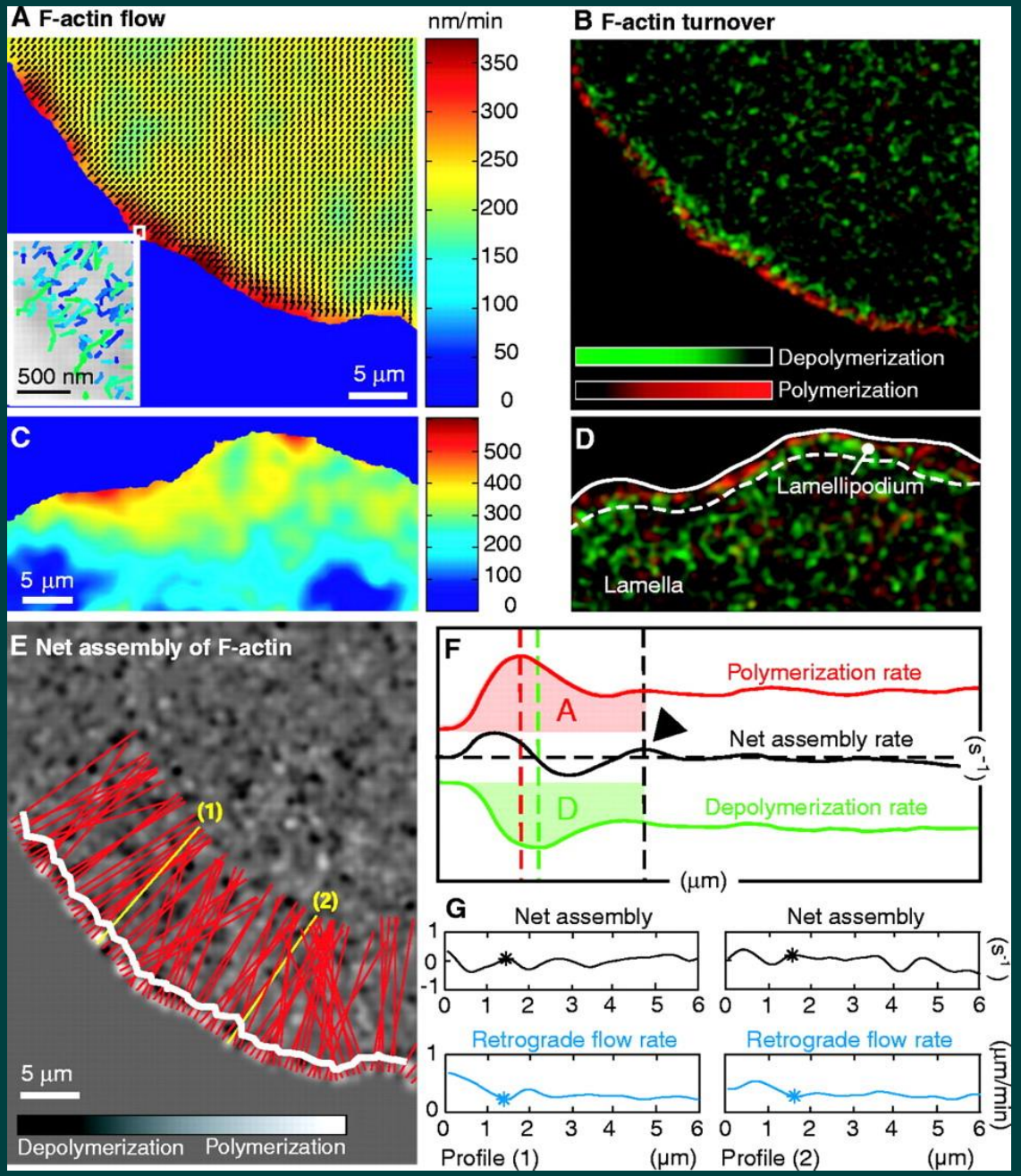
Tracking of speckle movement and computation of net polymer turnover



Actin turnover based on speckle birth and death

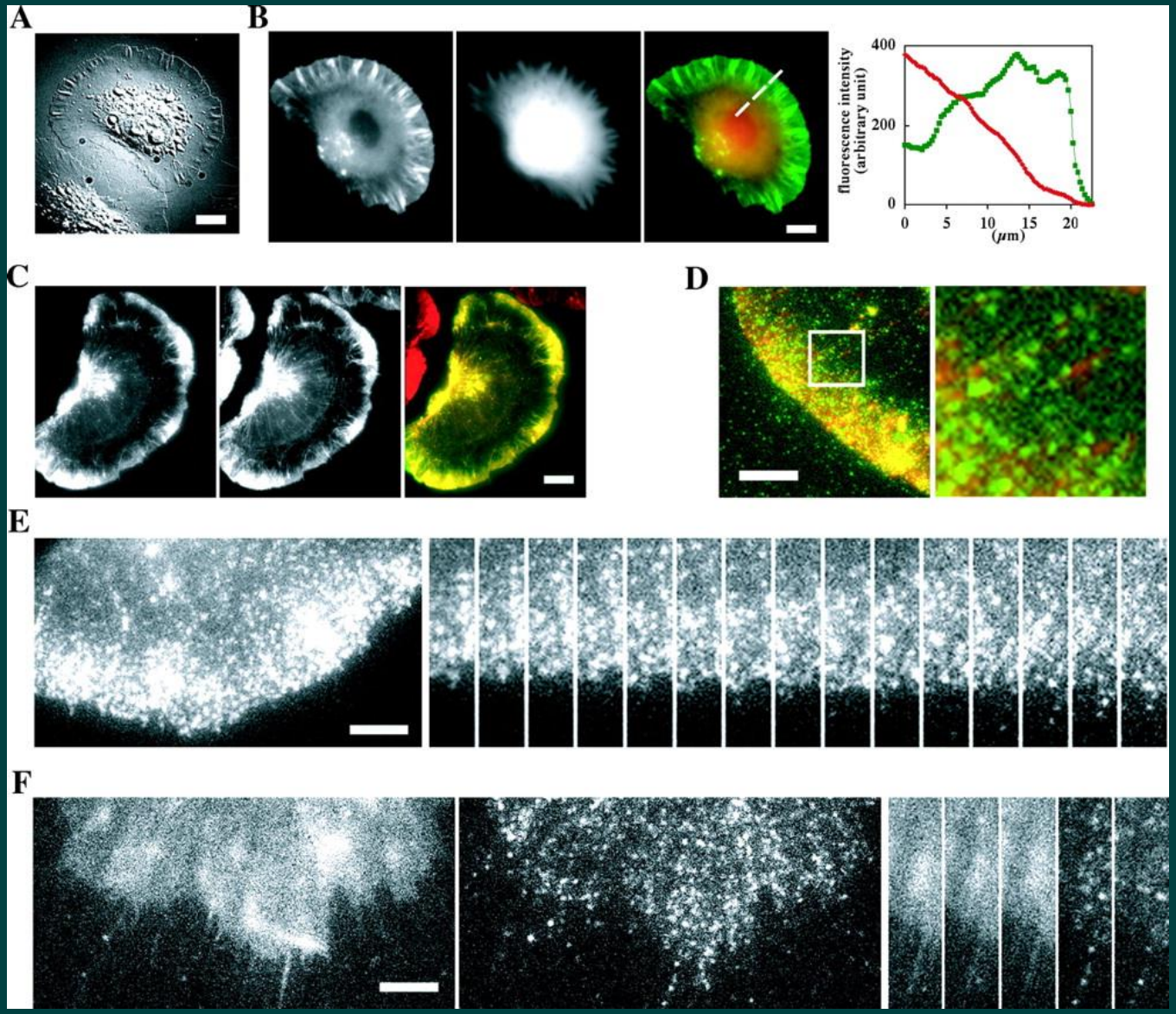






Ponti et al., 2004

Single molecular speckles

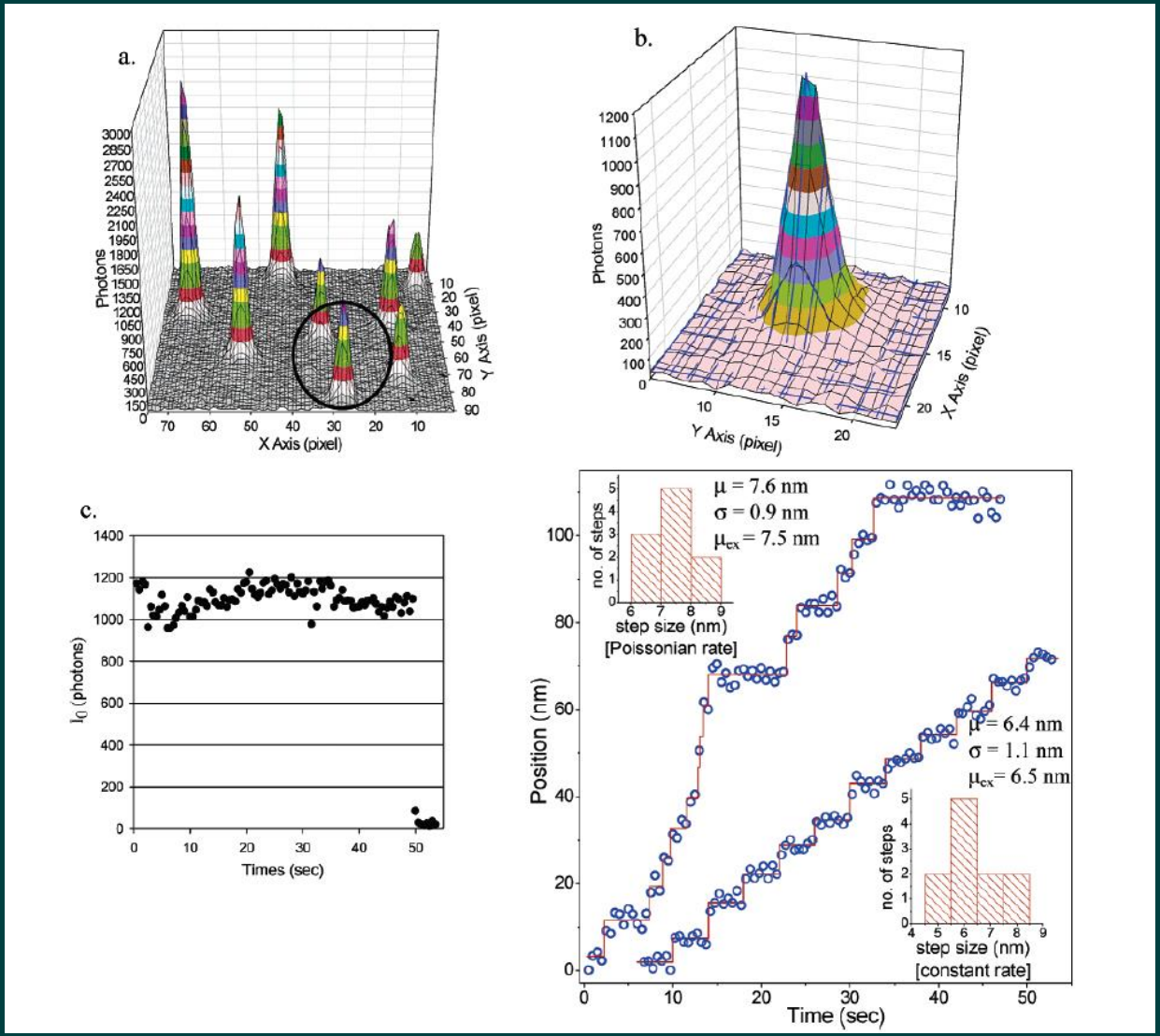


Watanabe and Mitchison, 2002

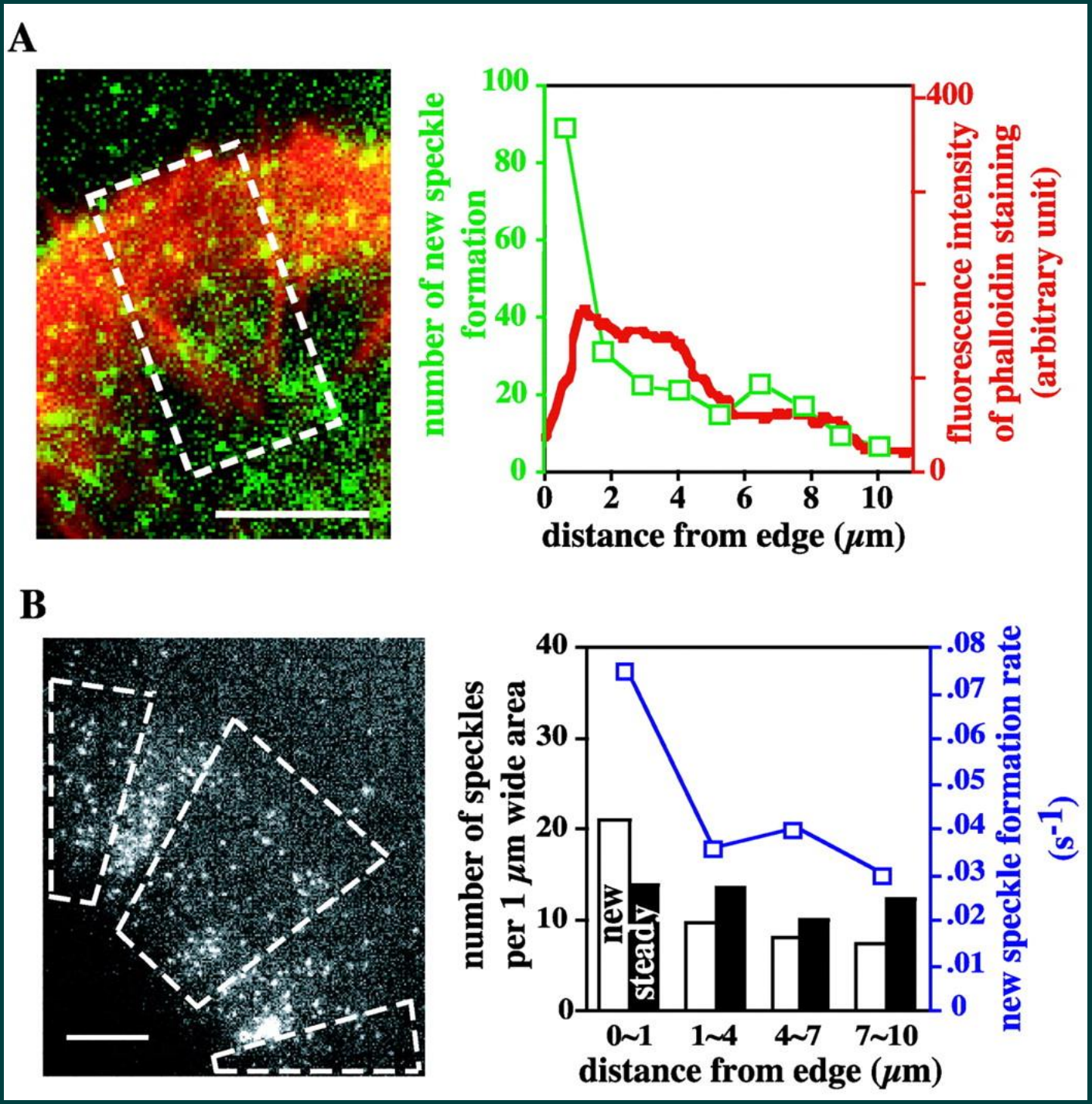
Fluorescense imaging with one nanometer accuracy (FIONA)

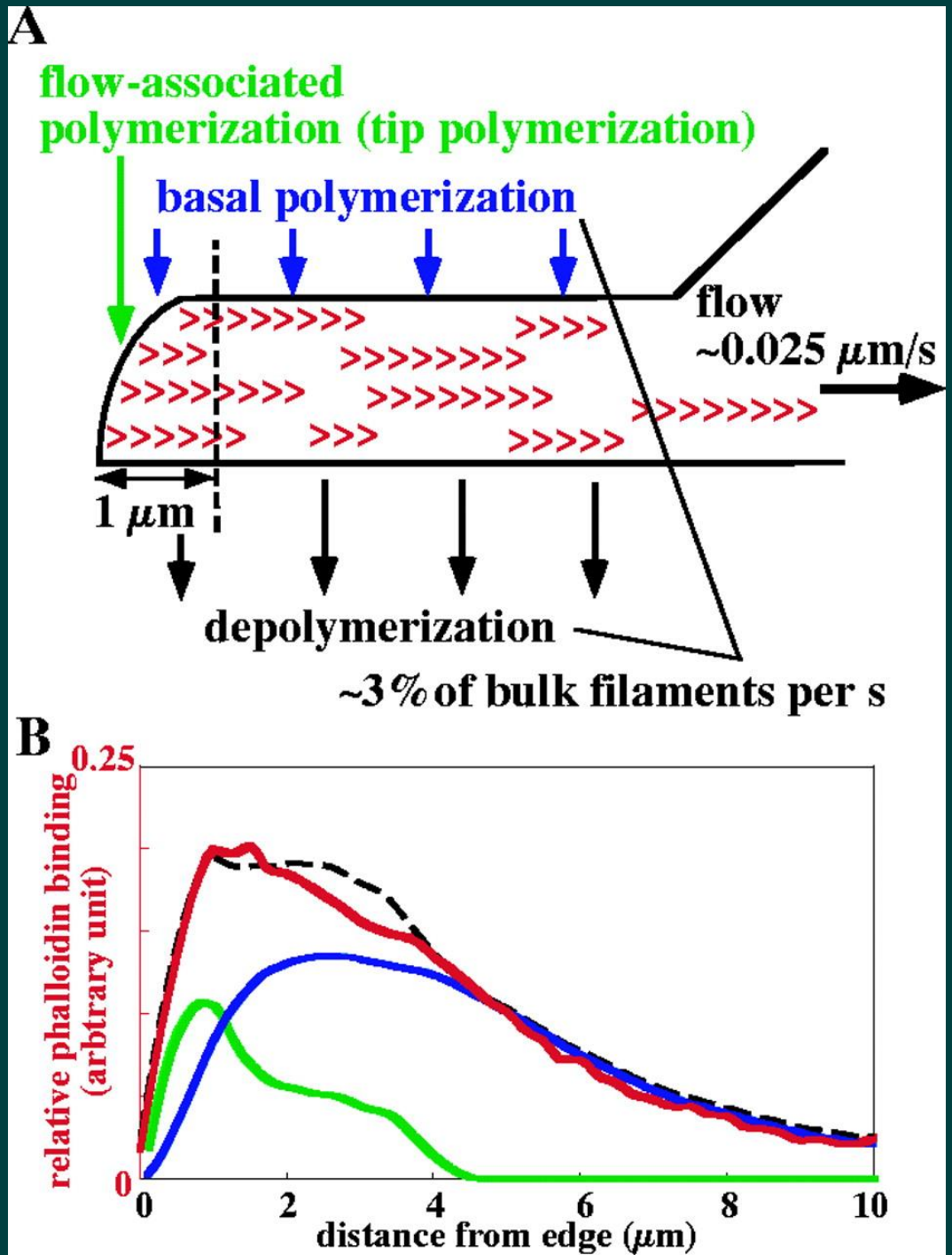
$$\sigma_{\mu_i} = \sqrt{\left(\frac{s_i^2}{N} + \frac{a^2/12}{N} + \frac{8\pi s_i^4 b^2}{a^2 N^2}\right)}$$

Thompson et al., Biophys. J., 2002



Yildiz and Selvin, Acc. Chem. Res., 2005


A
number of new speckle formation
fluorescence intensity of phalloidin staining (arbitrary unit)
distance from edge (μm)
B
number of speckles per 1 μm wide area
new speckle formation rate (s-1)
new
steady
0~1
1~4
4~7
7~10
distance from edge (μm)


A
flow-associated
polymerization (tip polymerization)
basal polymerization
flow
~0.025 μm/s
1 μm
depolymerization
~3% of bulk filaments per s
B
relative phalloidin binding
(arbtrary unit)
0.25
0
0
2
4
6
8
10
distance from edge (μm)

Evidence that protrusion at the leading edge of the cell is driven by actin assembly

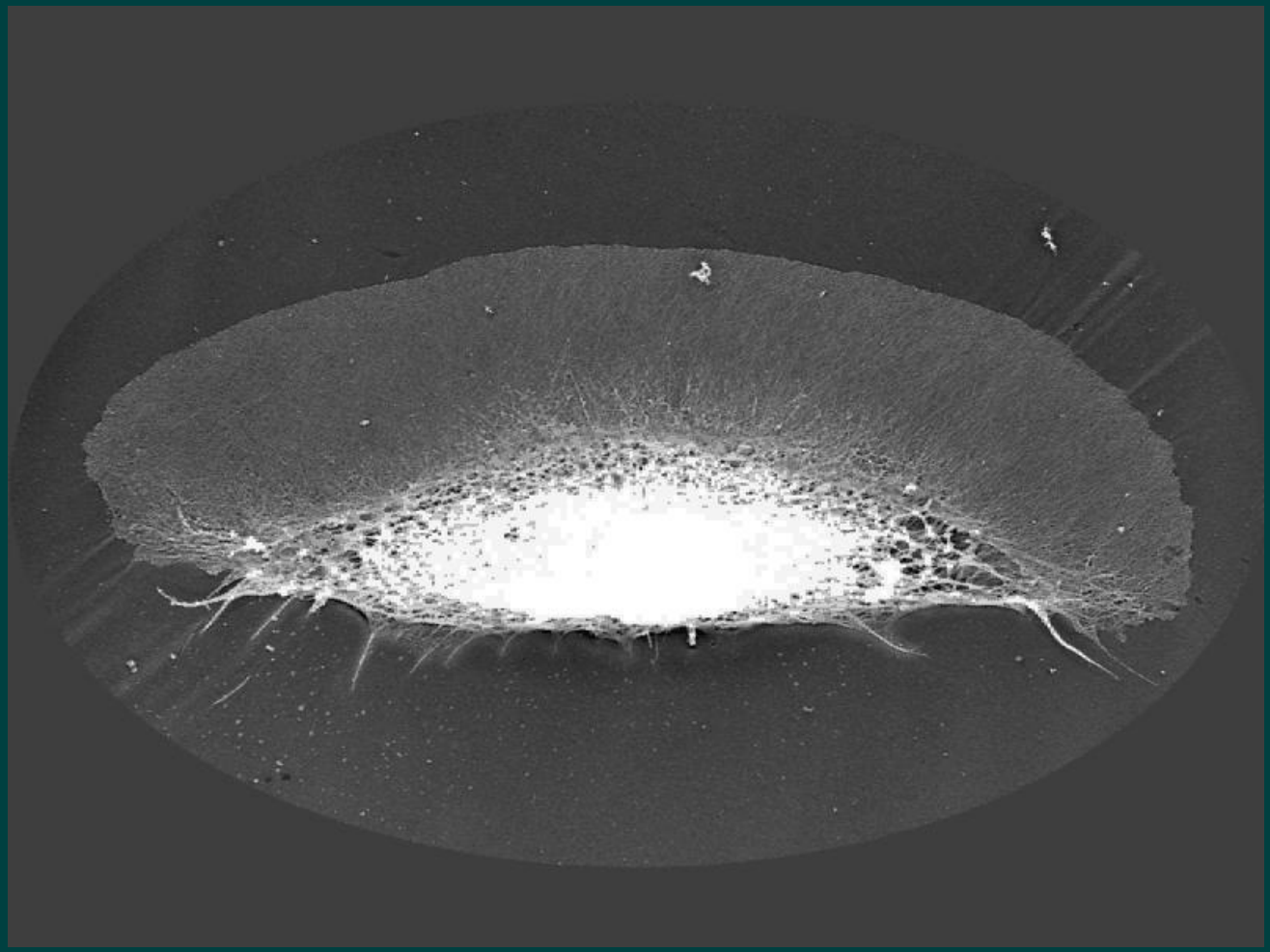
1. Actin assembles mostly at the front and assembled actin does not move forward

2. Actin organization: dense network, proper polarity

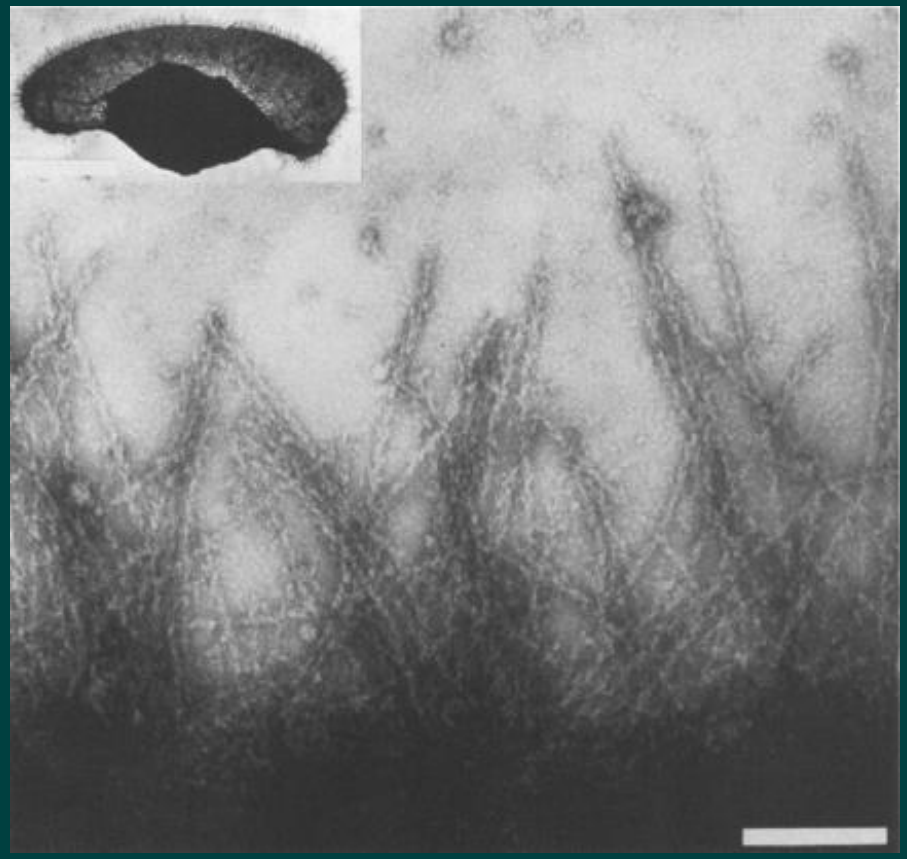
3. Cytochalasins (actin assembly inhibitor) block protrusion

4. Protrusion can be reconstituted in a system containing actin and a few other proteins

organization of actin in moving cells: dense filament network all the way to the front

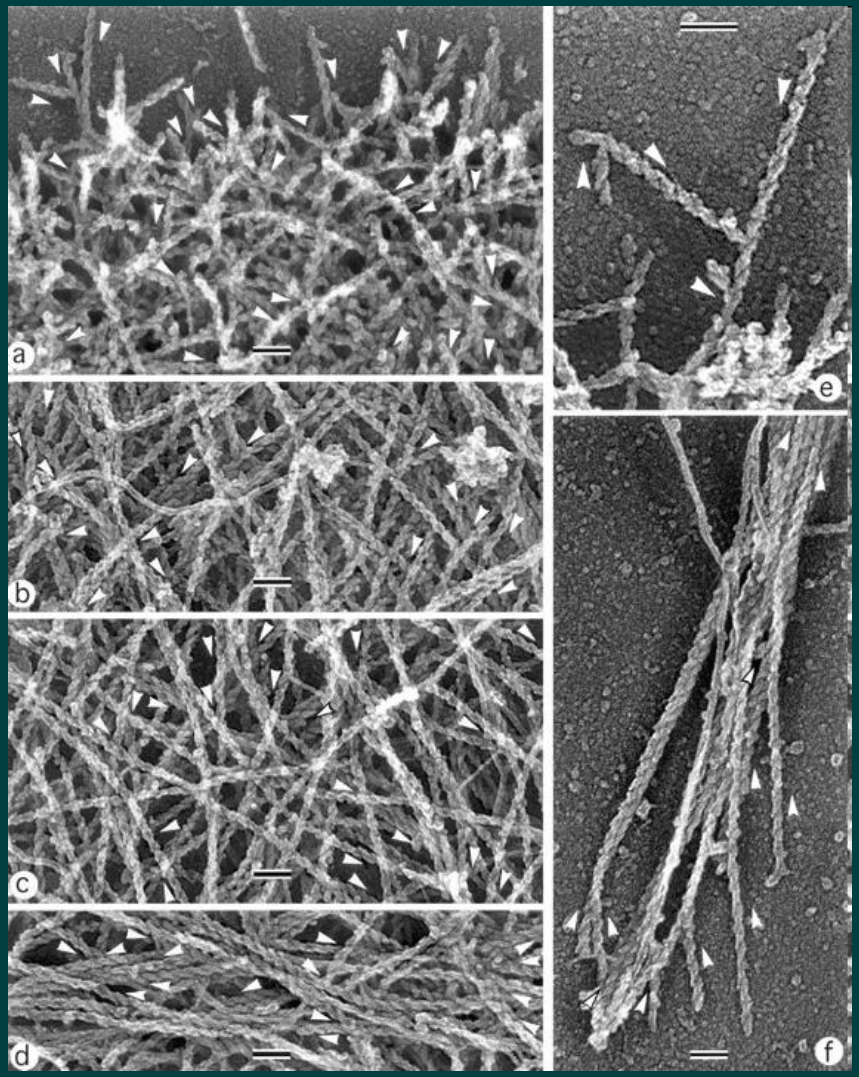


Fast assembling (barbed) filament ends are towards the edge in migrating cells



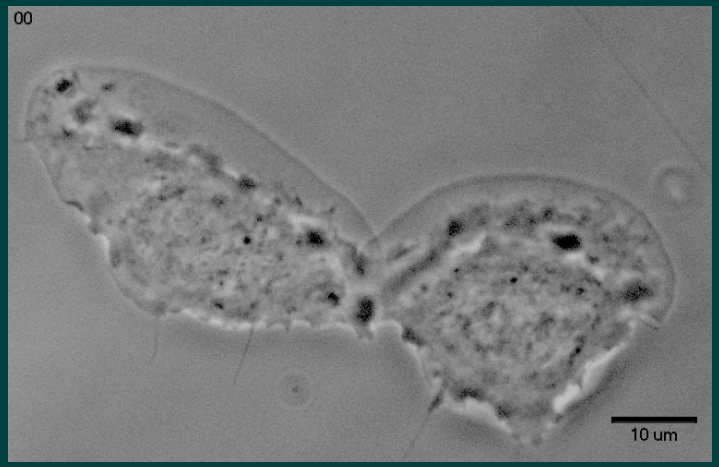
Small et al., 1995

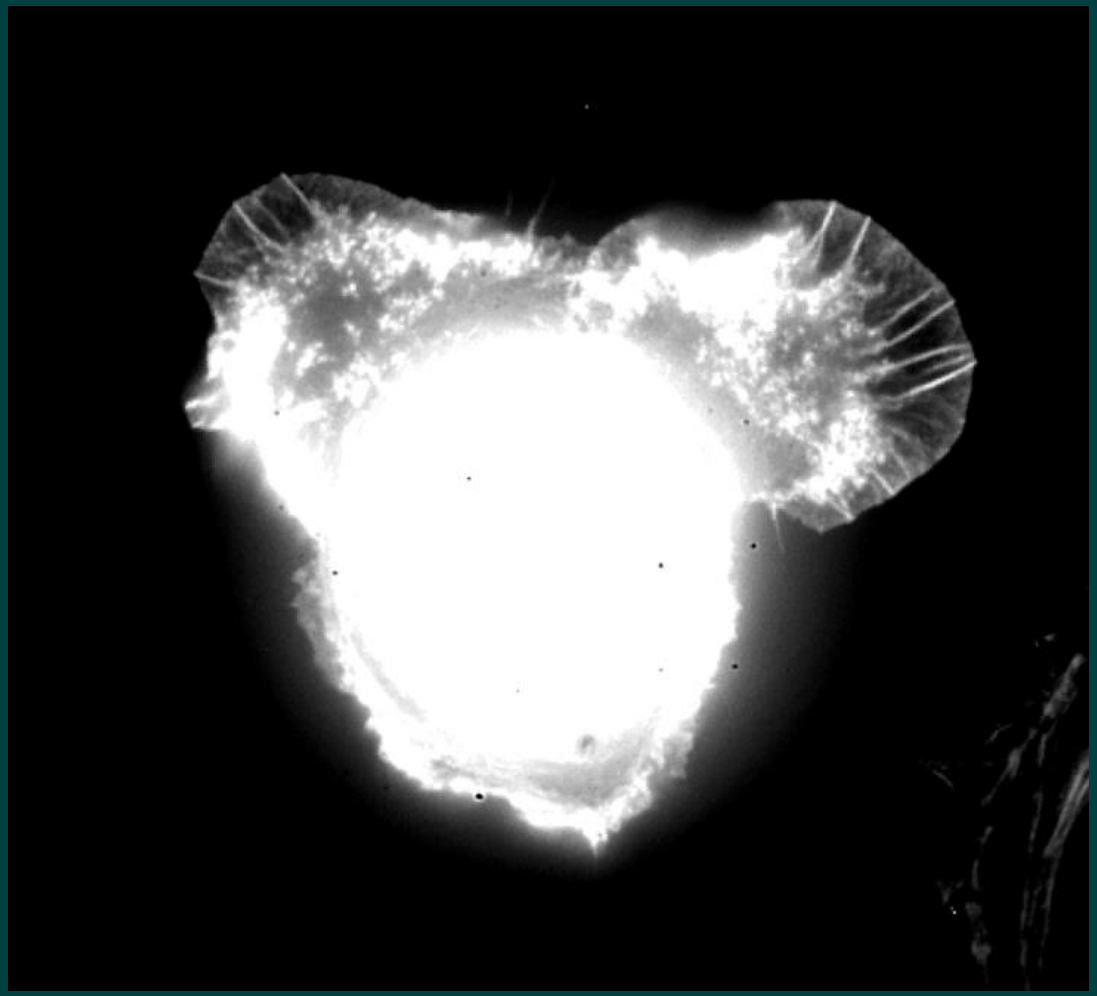
Actin filaments: barbed (+)-end towards the cell edge



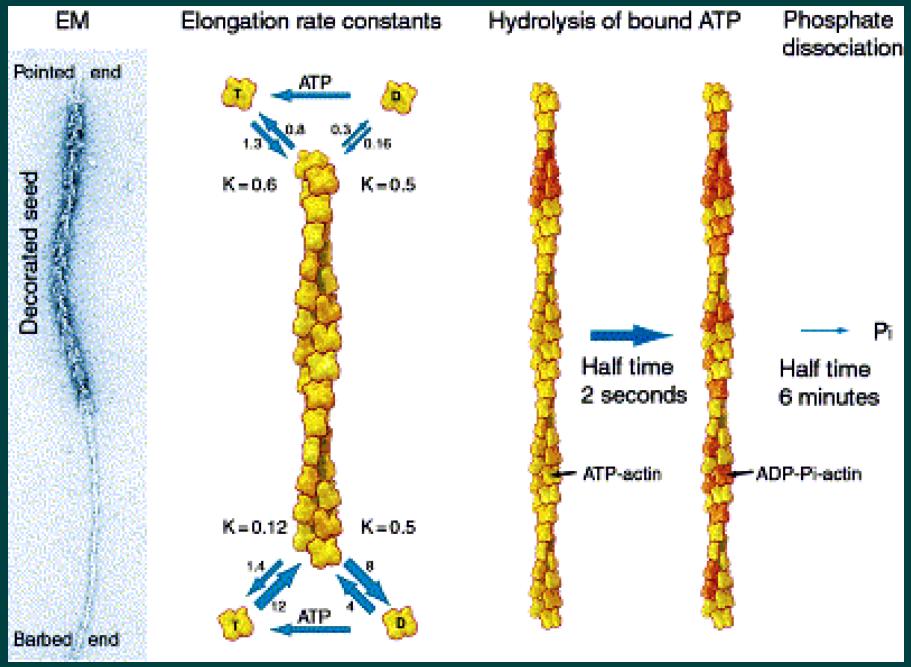
Where is the assembly happening?

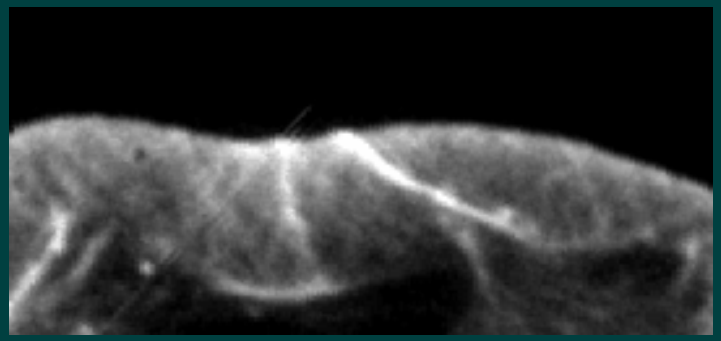
When actin assembly is blocked by cytochalasin D,
front protrusion stops





Natural explanation for steady state protrusion - actin treadmilling

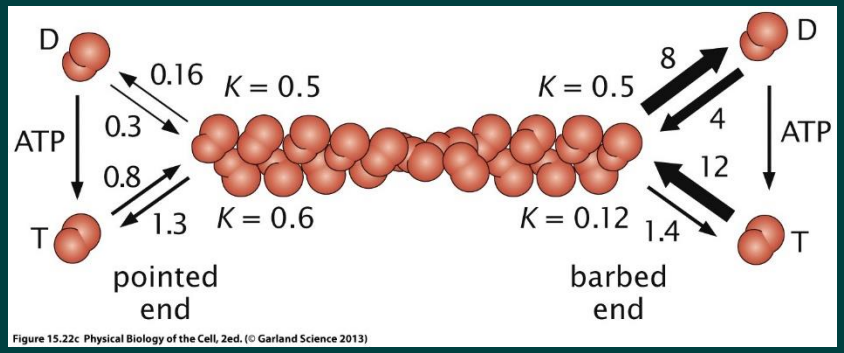


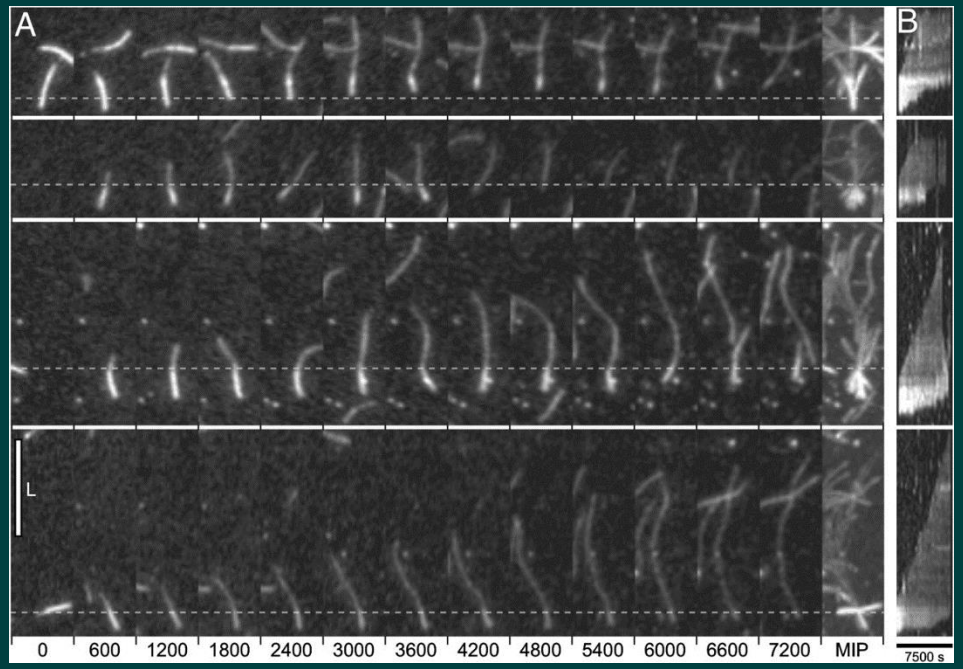


Protrusion of the cell's leading edge:
up to 20 μm/min
can last for hours without interruption (steady state)

Problem: treadmilling of pure actin is extremely slow (micrometers in hours instead of minutes)

TIRF
measurement of
actin assembly





:

Kuhn JR, Pollard TD. Biophys J. 2005 Feb;88(2):1387-402.

Questions:

What is the force that actin assembly can generate?

Apparent paradox: in order to push actin filament tip should be in contact with the membrane, but in order to grow there should be a space between the tip and the membrane for monomer insertion

How can actin treadmilling be fast enough to account for cell speed?

maximal force at equilibrium polymer assembly

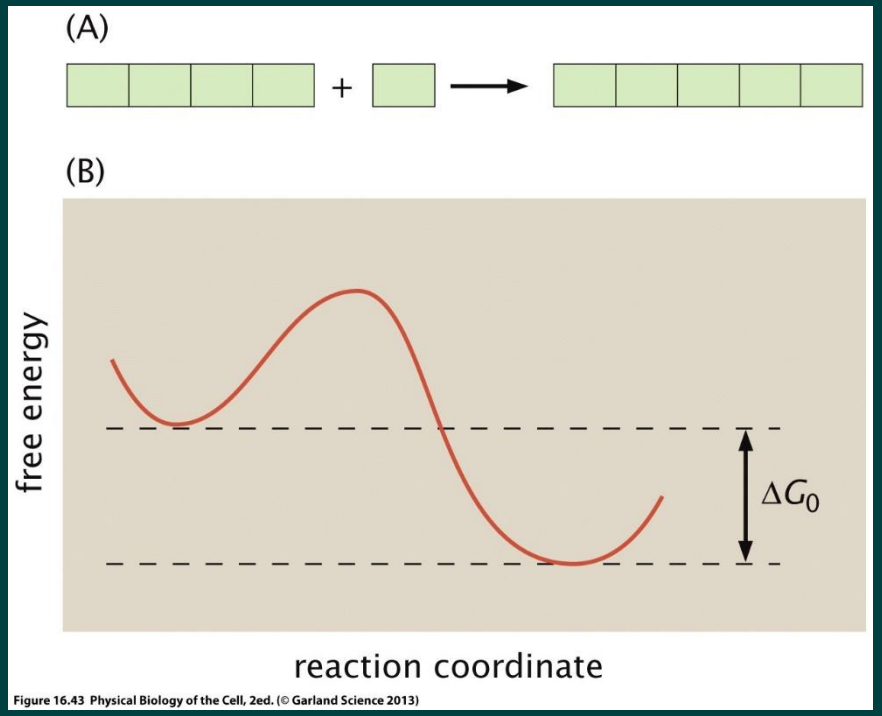


Figure 16.43 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

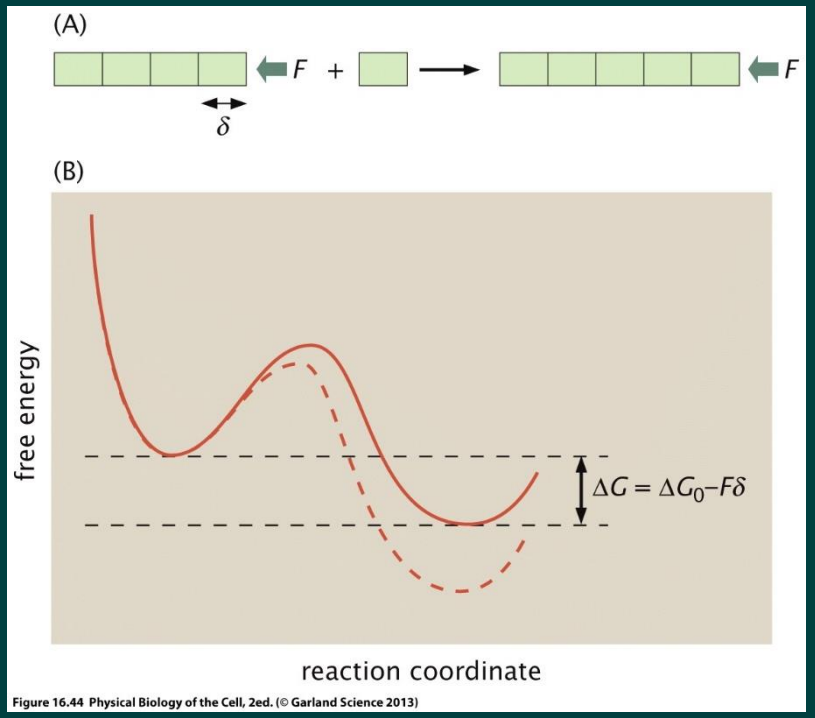


Figure 16.44 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

using chemical potential:

$$\mu_i = \mu_{i0} + k_B T \ln \frac{c_i}{c_{i0}}$$

$$F_{max} = \frac{k_B T}{\delta} \ln \frac{m}{m^*}$$

$$m^* = K_d(0)$$

$$F_{max} = \frac{4 \text{ pN nm}}{2.7 \text{ nm}} \times \ln 100 \approx 7 \text{ pN}$$

Polymerization Brownian ratchet

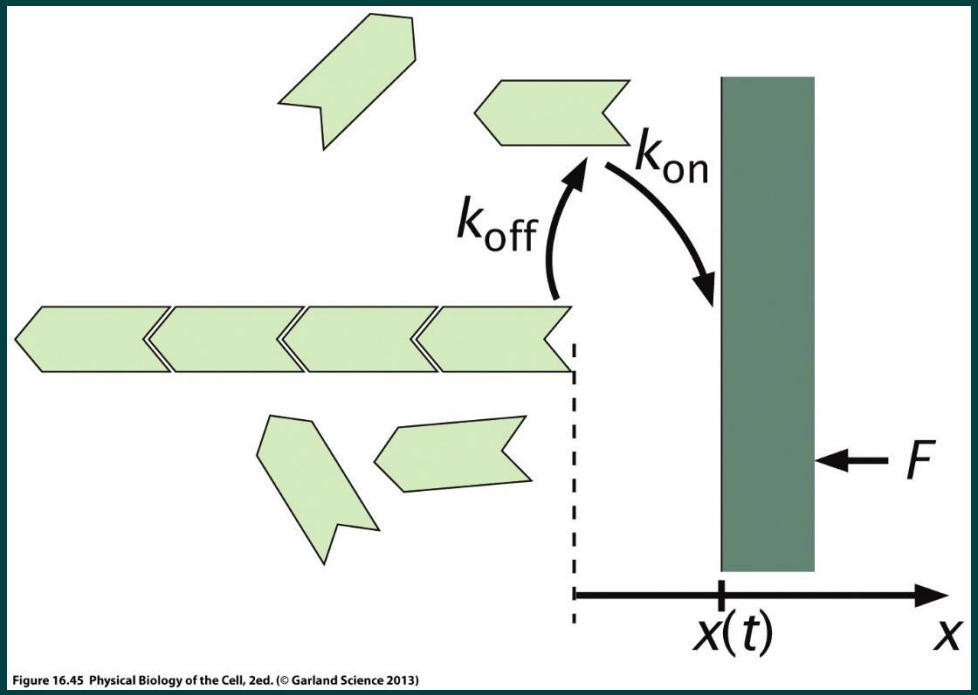


Figure 16.45 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

$$v = \delta(k_{on} m p_{eq}(x > \delta) - k_{off})$$

$$p_{eq}(x > \delta) = \int_{\delta}^{\infty} p_{eq}(x)\mathrm{d}x$$

Boltzmann distribution

$$p(E_i) = \frac{1}{Z} e^{-E_i/k_B T}$$

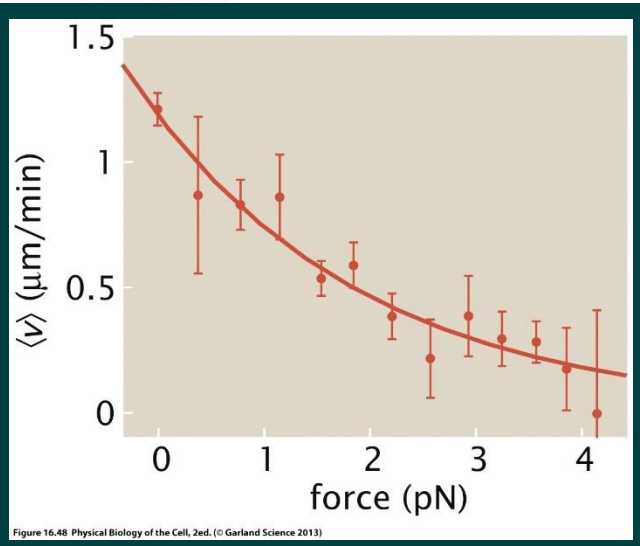
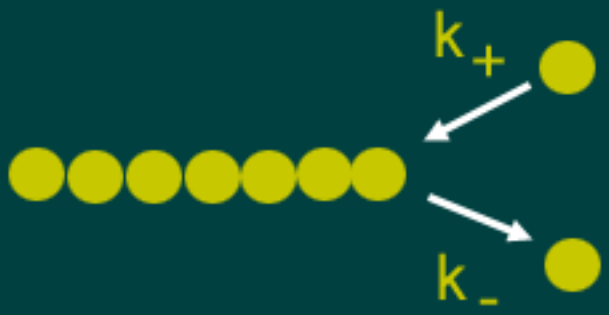


Figure 16.48 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

$$v = \delta[k_{on} m e^{-F\delta/k_B T} - k_{off}]$$

how comes actin assembly is so fast in the cell?



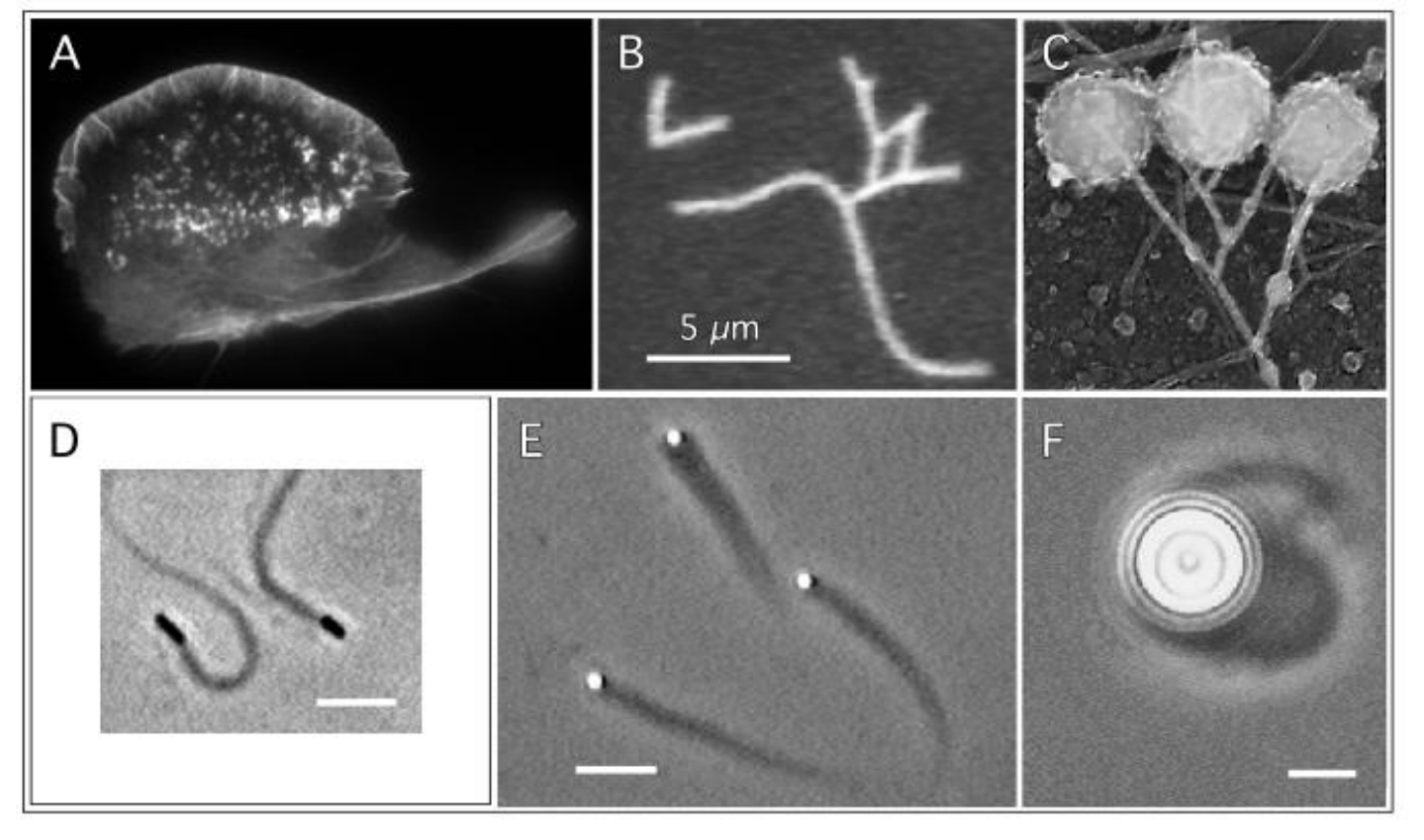
$$dP/dt = k_+ C\ N - k_-\ N$$

$dP/dt = 0$ $\qquad$ $C_c = k_-/k_+$

	ATP–actin		ADP–actin	
	Barbed	Pointed	Barbed	Pointed
k_+ ($\mu M^{-1}s^{-1}$)	11.6	1.3	3.8	0.16
k_- (s^{-1})	1.4	0.8	7.2	0.27

P, polymer concentration
C, monomer concentration
N, number of filament ends

Novel Dimensions of Cell Motility



Dominique Pantaloni, Christophe Le Clainche and Marie-France Carlier

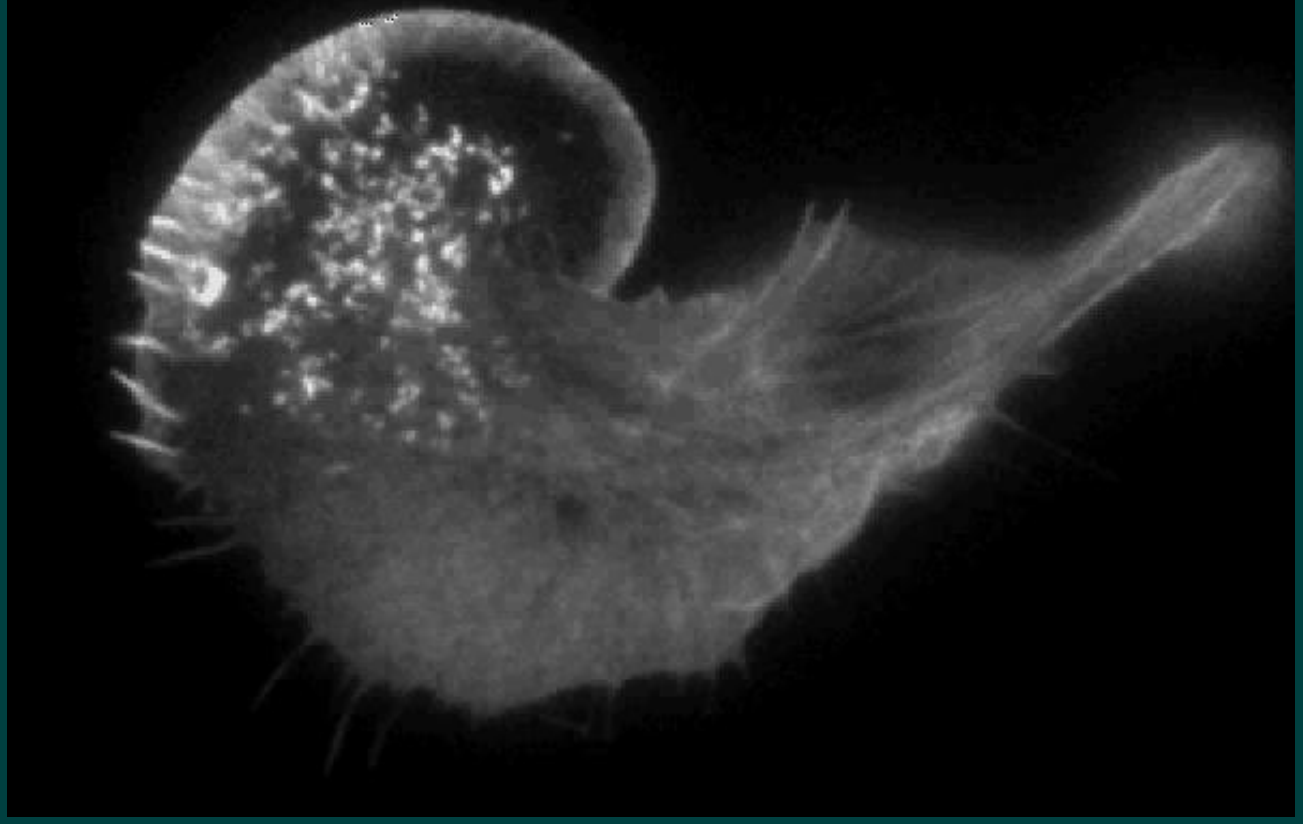
Dynamique du Cytosquelette, Laboratoire d'Enzymologie et Biochimie structurales, C.N.R.S., Avenue de la Terrasse, 91198 Gif-sur-Yvette, France

http://www.sciencemag.org/cgi/content/full/292/5521/1502/DC1

Actin-based Motility

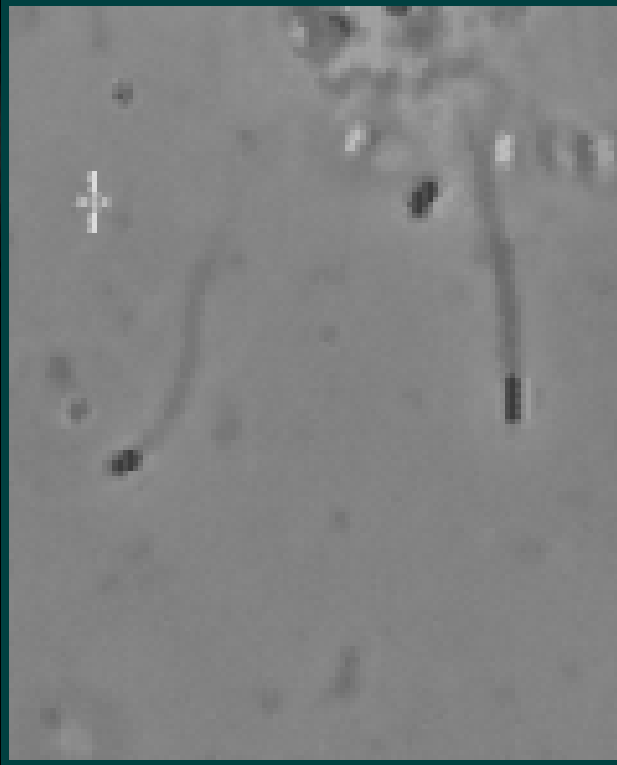
Actin-GFP

Lamellipodium and filopodium extension



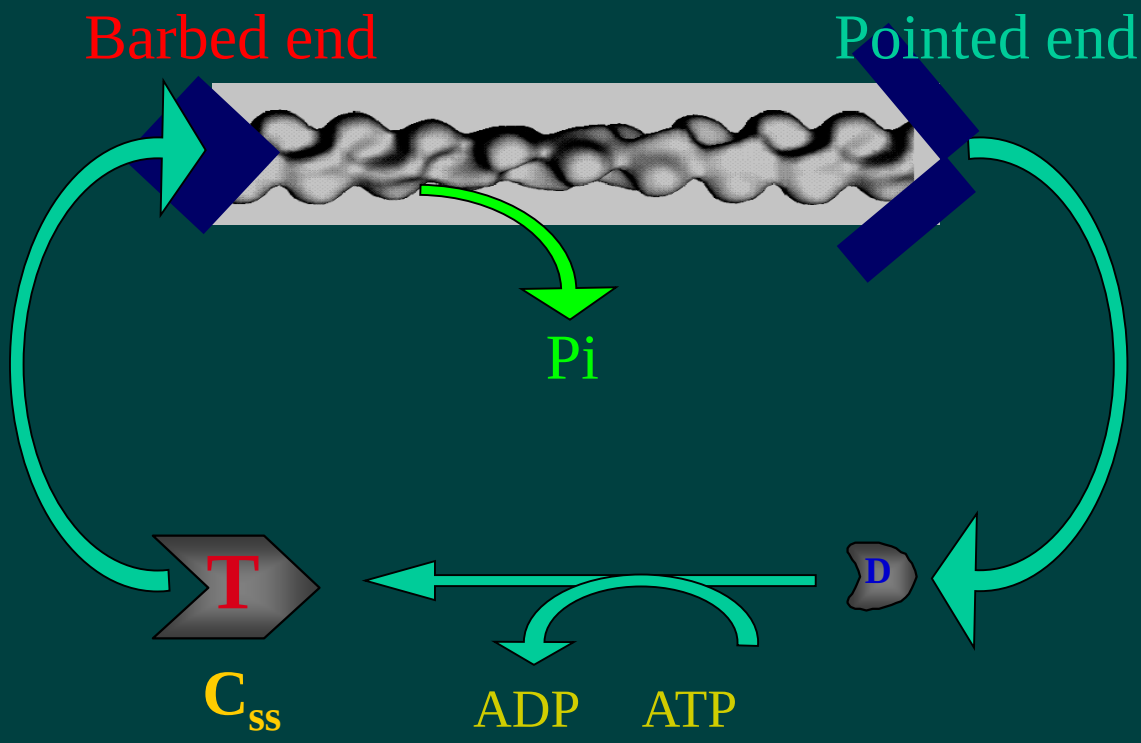
(Vic SMALL)

Listeria propulsion



(V.Laurent. M-F. Carlier)

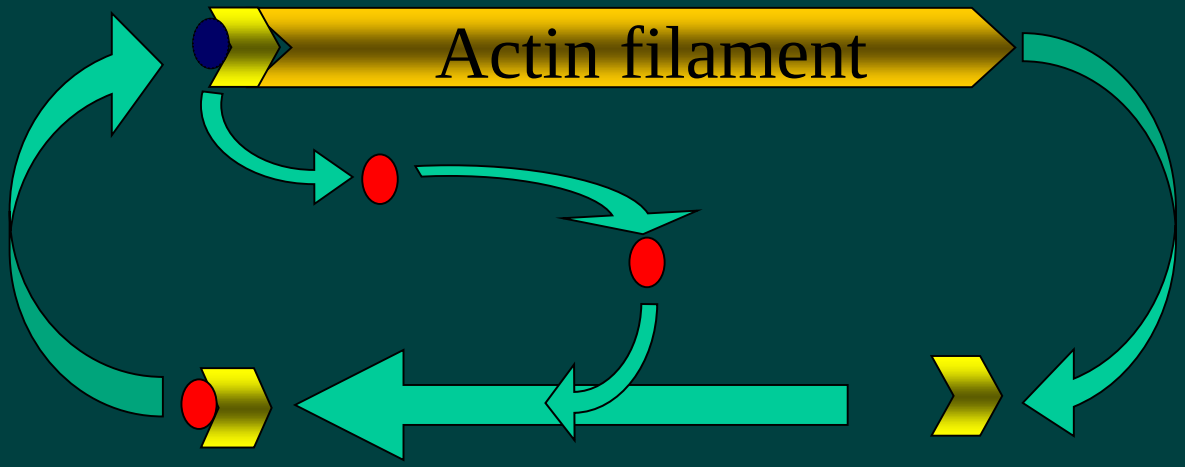
Treadmilling (Wegner, 1976)



	C_{ss}	Filament turnover
Pure actin:	0.1 µM	3µm / 90 min
Lamellipodium:	2 µM	3 µm / 1 min

Profilin and proteins of the actobindin family (Ciboulot : 3-ß-thymosin repeats) increase the rate of actin-based motility

- Profilin or Ciboulot



complex could be added to barbed end only

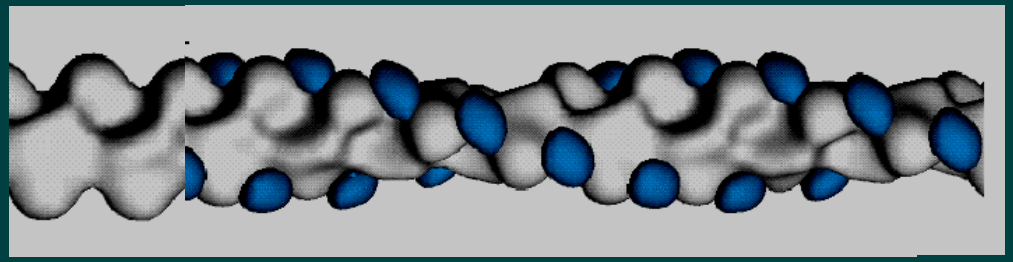
(Boquet *et al.*,Cell. Oct. 2000)

Actin Depolymerizing Factor (ADF) / Cofilin:

an Actin Dynamizing Factor in cell motility

- Ubiquitous, conserved, stimulus-responsive regulators of actin dynamics in motile processes:
  - Embryonic development (Bamburg, Obinata, Abe, Ono, 1993)
  - Yeast endocytosis (Lappalainen and Drubin, 1998)
  - Cytokinesis (Gunsalus, 1995)
  - *Listeria* propulsion (Carlier et al.; Rosenblatt and Mitchison, 1997)
- Localized in motile regions of cells (Svitkina and Borisy, Bailly and Condeelis, 1997)

ADF increases the treadmilling of F-actin (Carlier et al., 1997)



Increase in **Css**

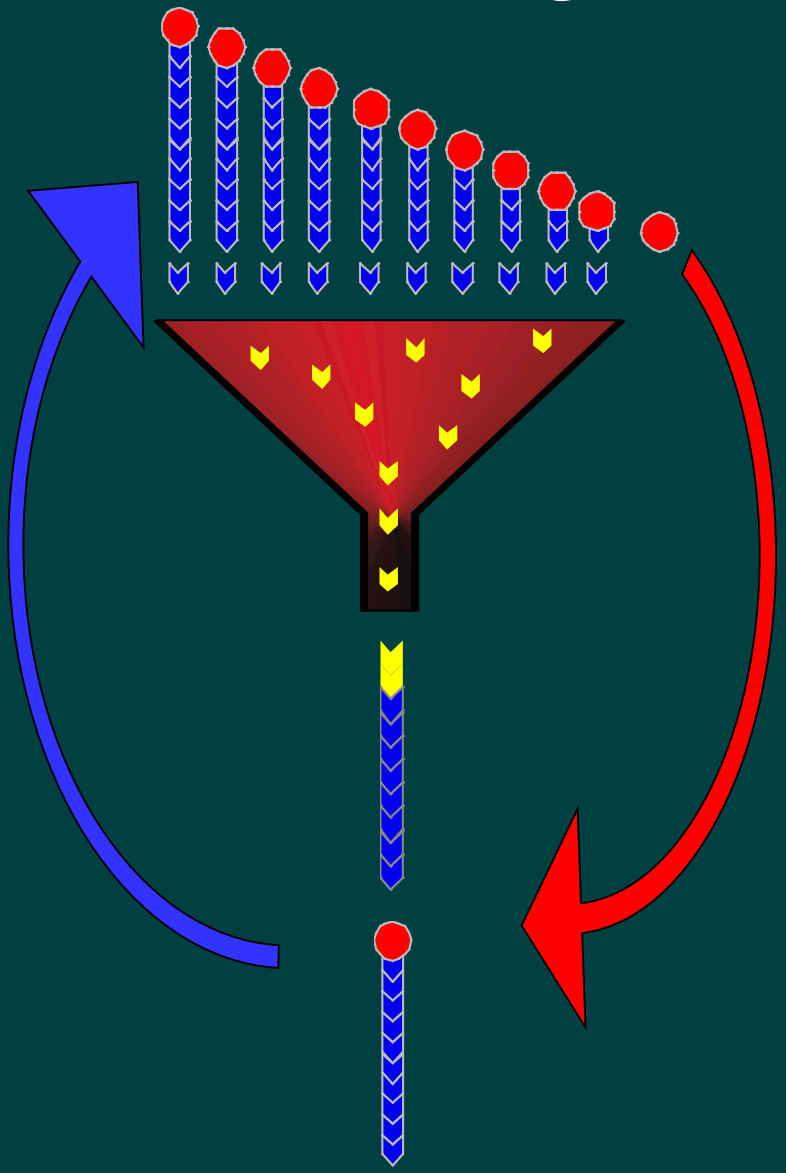
=> faster barbed end growth

Role of capping proteins in motility: funnelling the treadmilling

✶ Capping proteins are known to be required for efficient motility *in vivo* (J. Cooper; H. Yin; T. Stossel).

✸ Capping proteins block most of the barbed ends, hence they increase the concentration of monomeric ATP-actin.

Role of capping proteins in motility: funnelling the treadmilling



Slow pointed end disassembly from many capped filaments

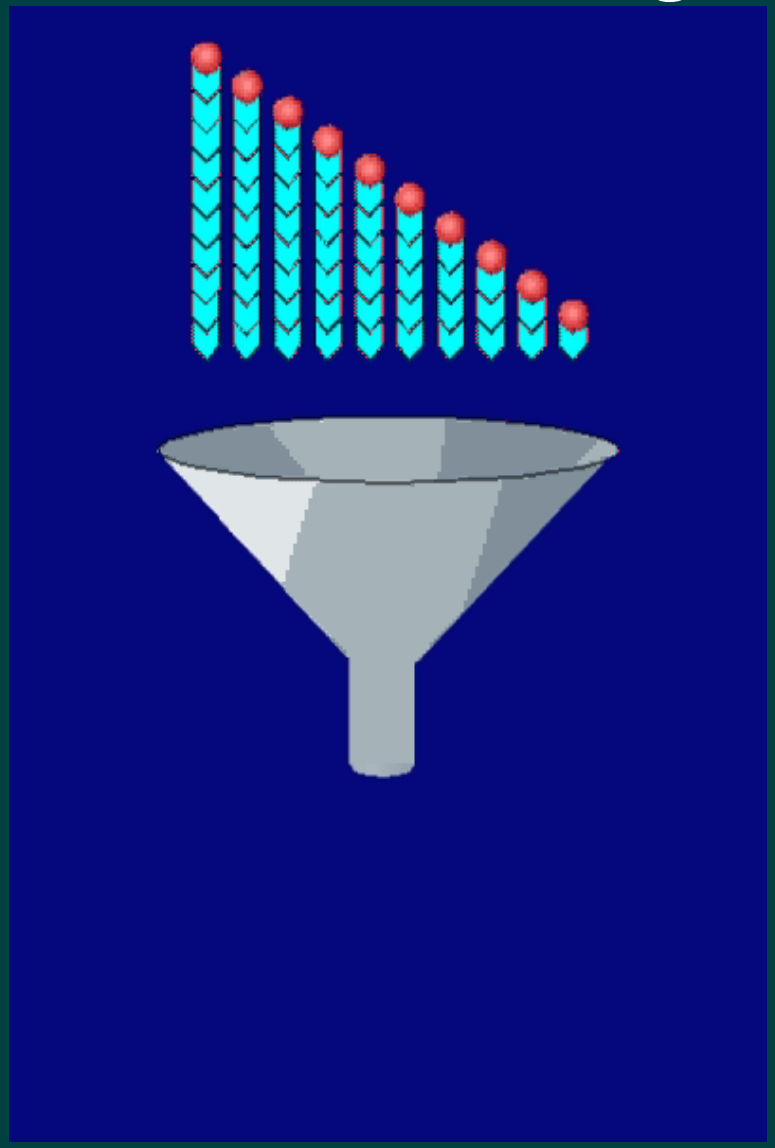
Nucleotide exchange

Fast barbed end assembly of few uncapped filaments

and

Capping

Role of capping proteins in motility: funnelling the treadmilling



Slow pointed end disassembly from many capped filament

Nucleotide exchange

Fast barbed end assembly of few uncapped filaments

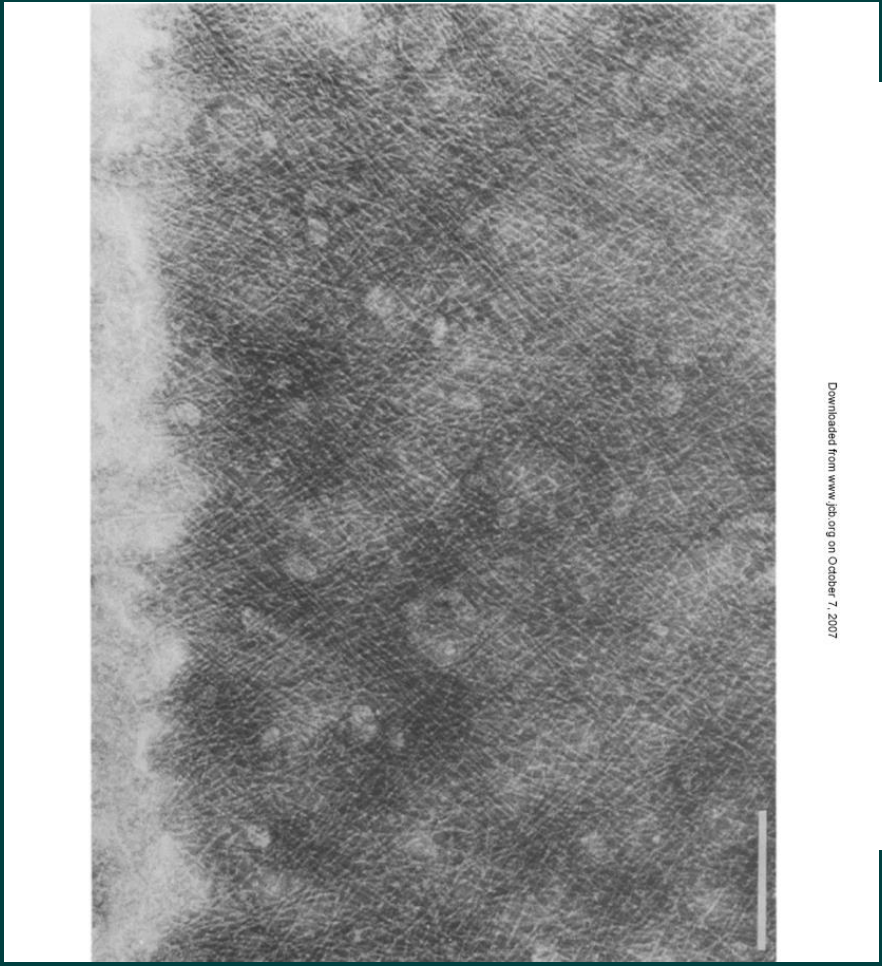
and

Capping

Clues from the structure:

are these the same filaments that grow at +end and shrink at -end?

What is filament organization in the lamellipodium?



Small, Herzog and Anderson, 95

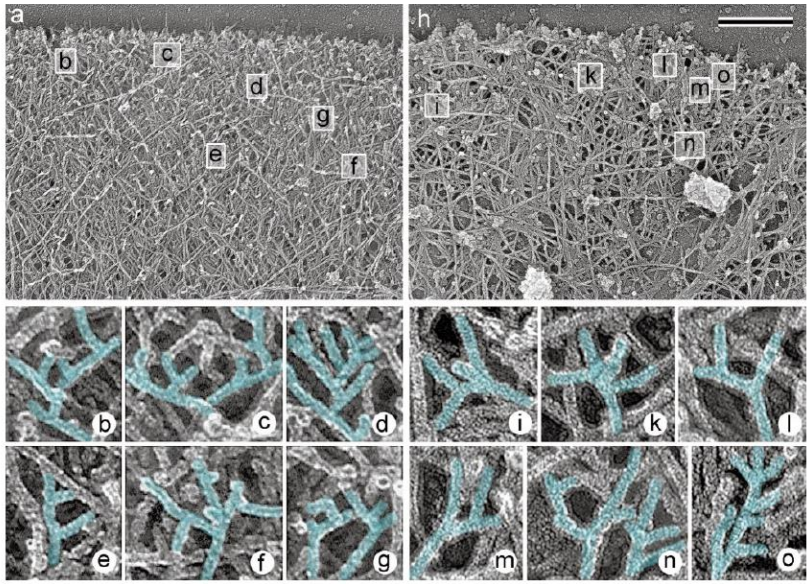
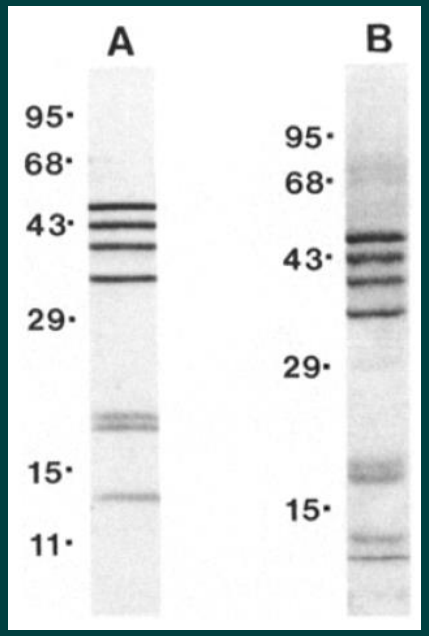
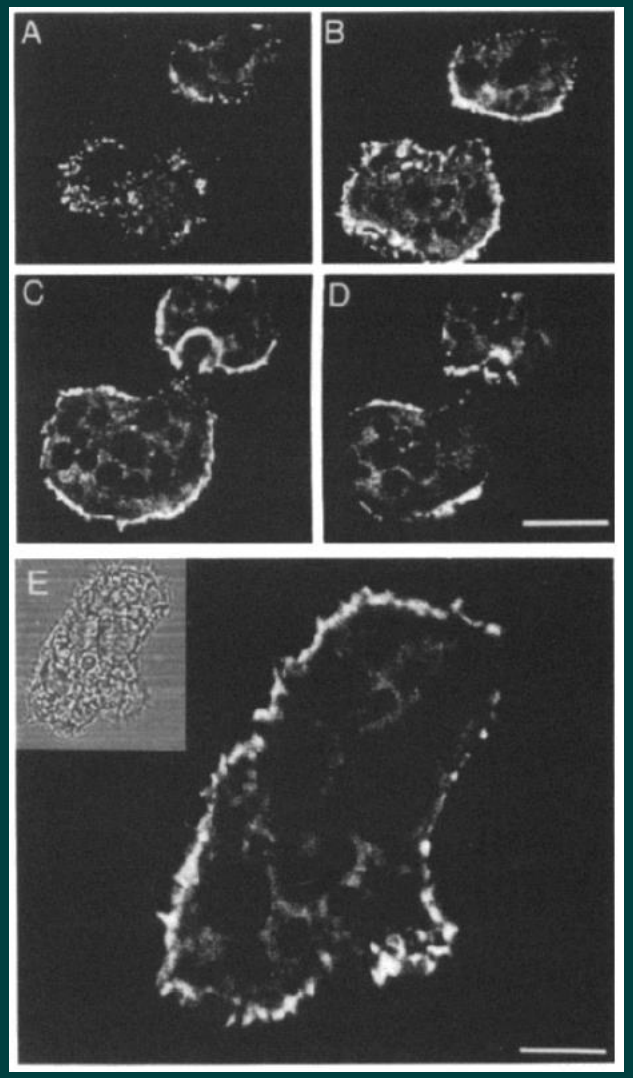


Figure 1. Multiple branching of actin filaments in lamellipodia. EM of lamellipodia of *Xenopus* keratocytes (a–g) and fibroblasts (h–o) showing overviews of the leading edge (a and h) and enlargements of the boxed regions (b–g and i–o). Many examples of filaments with tightly spaced multiple branches (cyan) can be visualized in lamellipodia despite the high overall density of the actin network. Bar, 0.5 μm.

Svitkina and Borisy, 99

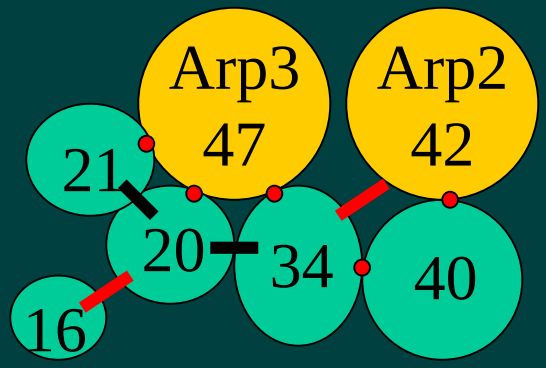
Purification and localization of Arp2,3 complex in Acanthamoeba





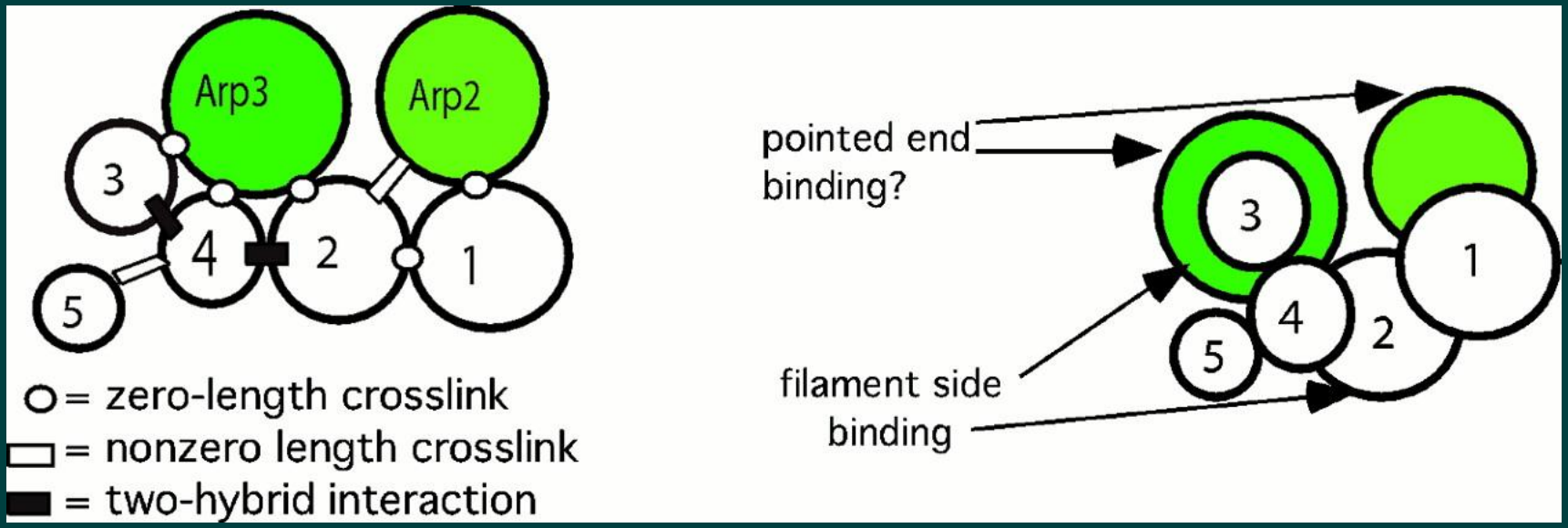
Machesky et al., JCB, 1994

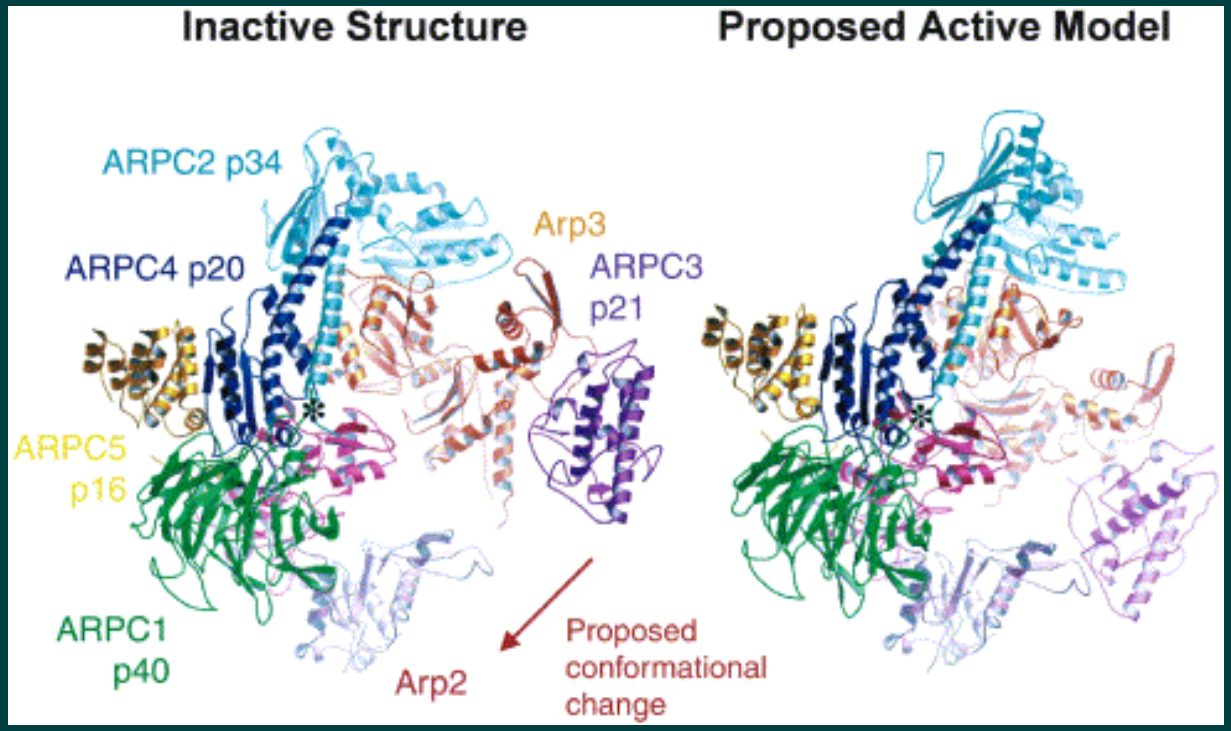
The Arp2/3 complex: downstream target of multiple signaling pathways leading to actin assembly



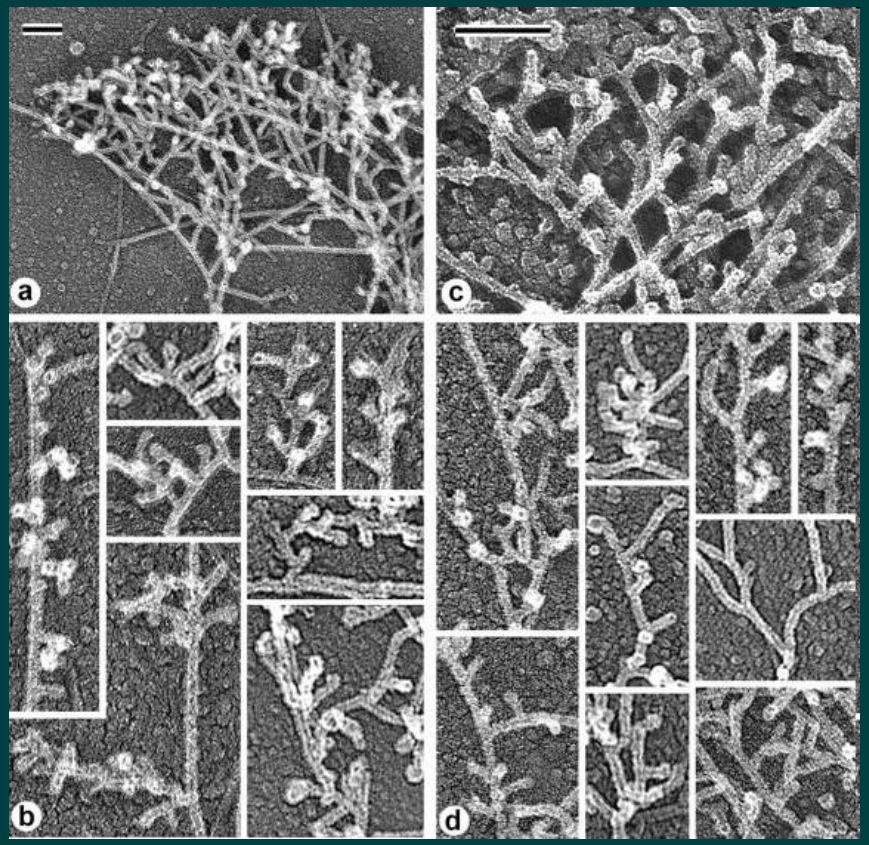
(Higgs and Pollard, 1999)

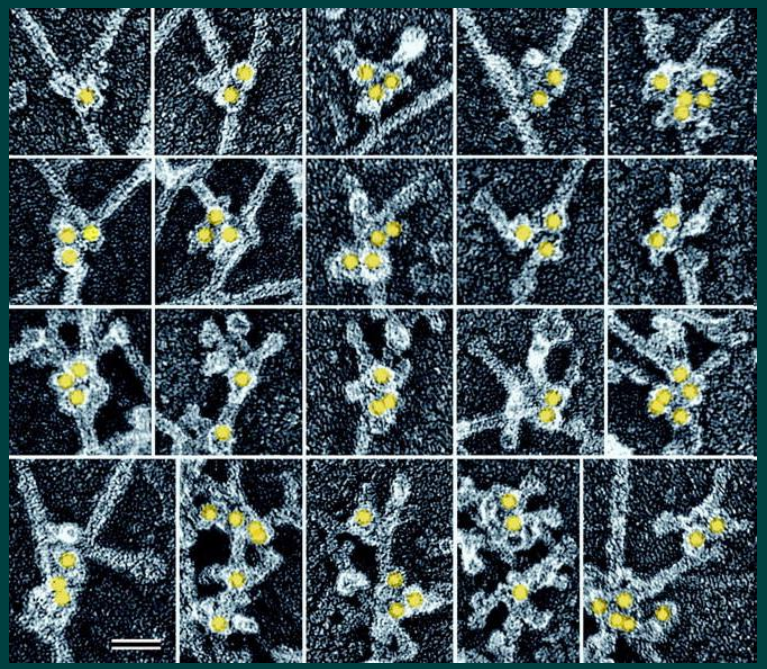
- Seven conserved subunits including Arp2 and Arp3 (Machesky et al., 1994).
- Is activated at the surface of *Listeria* by the ActA protein to stimulate actin polymerization and bacterial propulsion (Welch et al., 1998).
- Localizes at motile regions of cells (Svitkina and Borisy, 1999).
- Generates new actin filaments in a stimulus-responsive and spatially controlled fashion.


Arp3
Arp2
3
4
2
1
5
= zero-length crosslink
= nonzero length crosslink
= two-hybrid interaction
pointed end
binding?
filament side
binding


Inactive Structure
Proposed Active Model
ARPC2 p34
Arp3
ARPC4 p20
ARPC3
p21
ARPC5
p16
ARPC1
p40
Arp2
Proposed
conformational
change

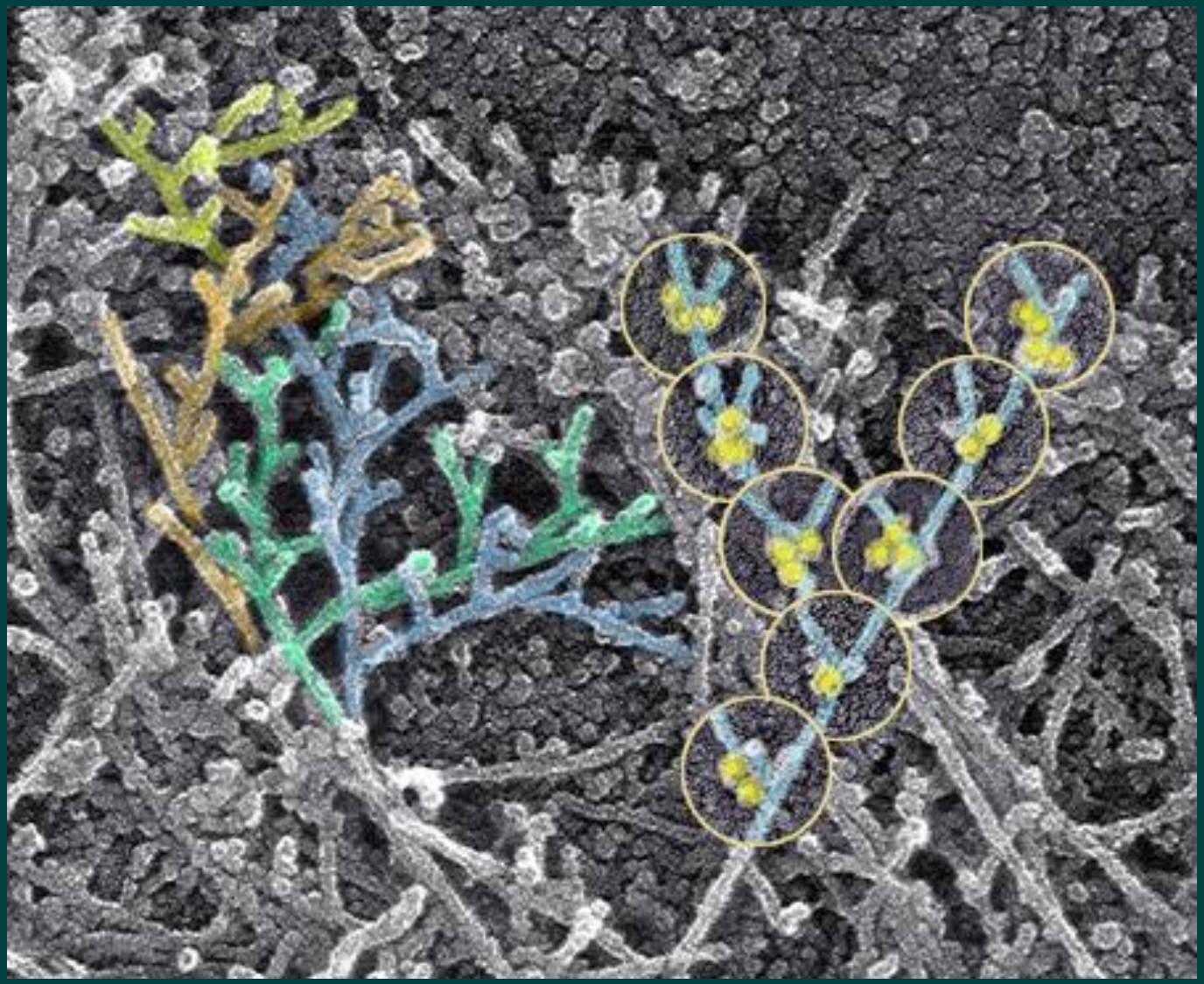
Arp2,3 complex is at the branching sites in actin network





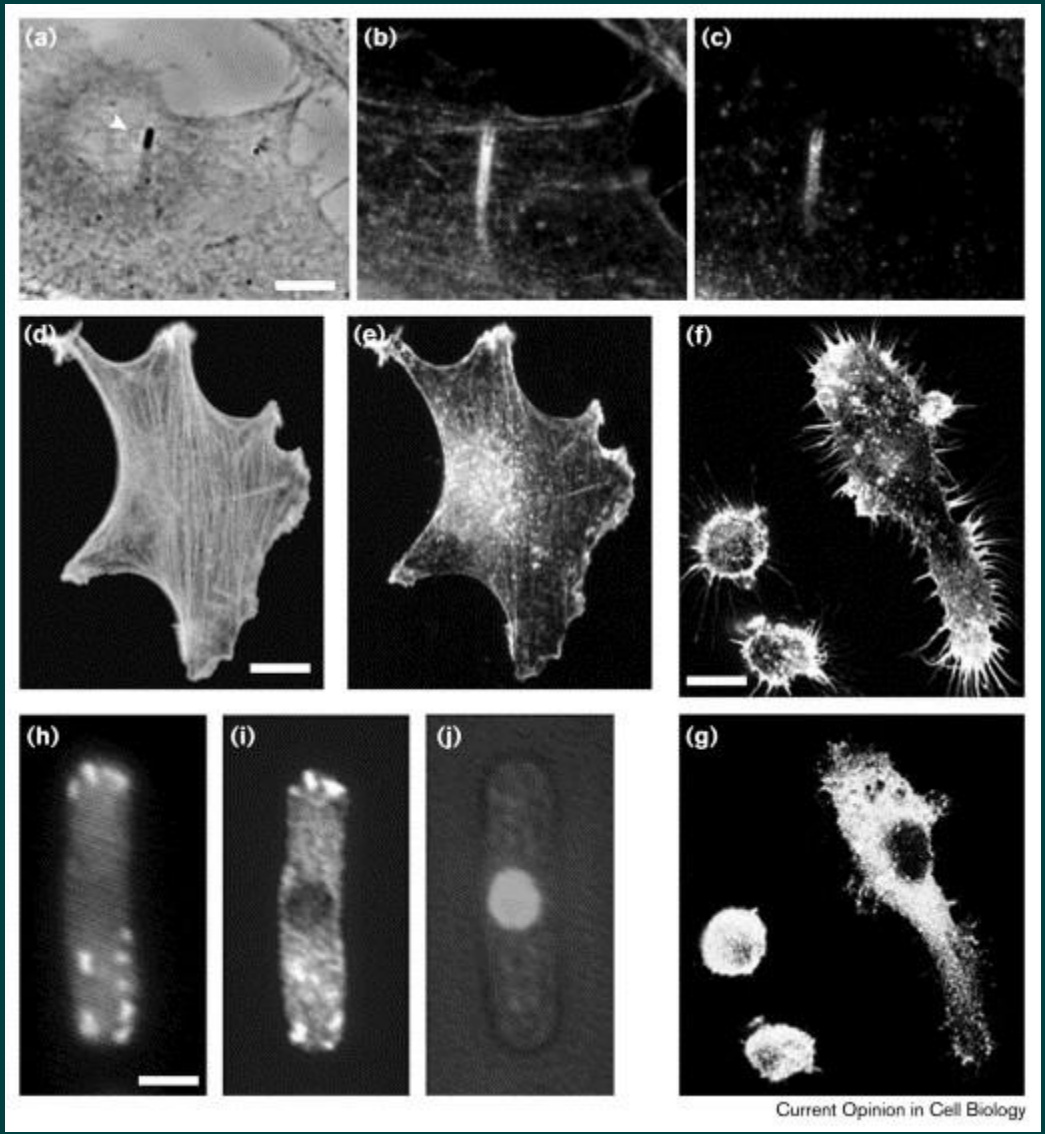
Svitkina and Borisy, JCB, 1999

branching actin network



Svitkina and Borisy, 1999

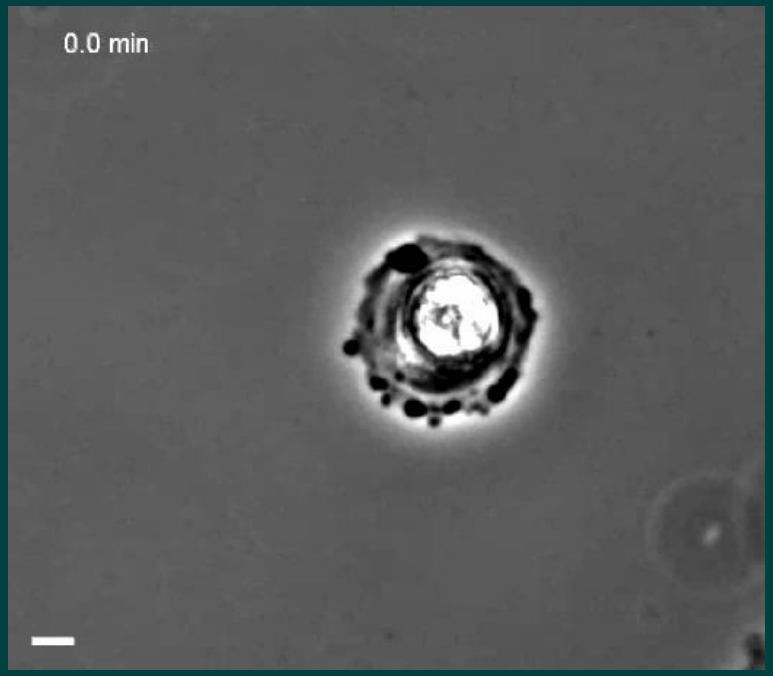
Arp2,3 in various actin structures



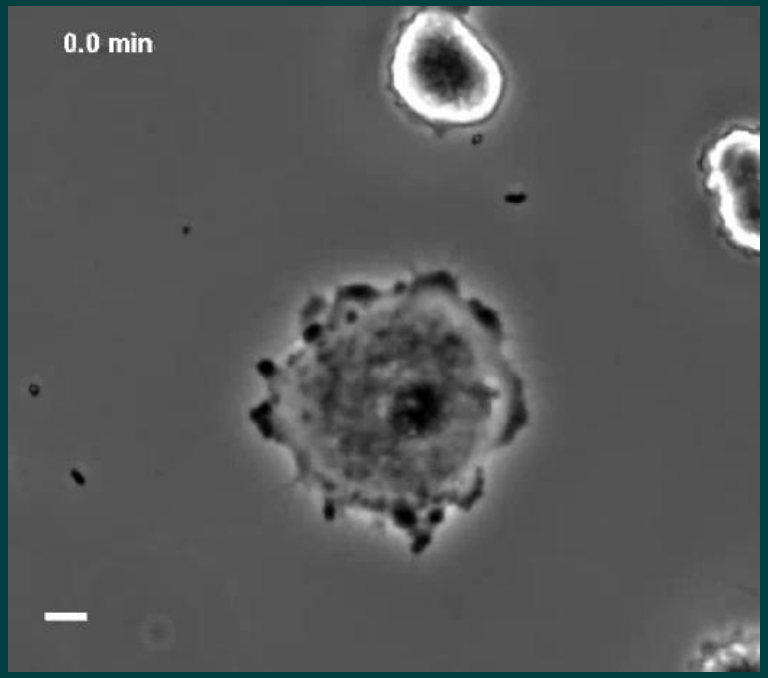
Machesky and Gould, 1999

Arp2,3 is essential for lamellipodia

ARPC3+/+



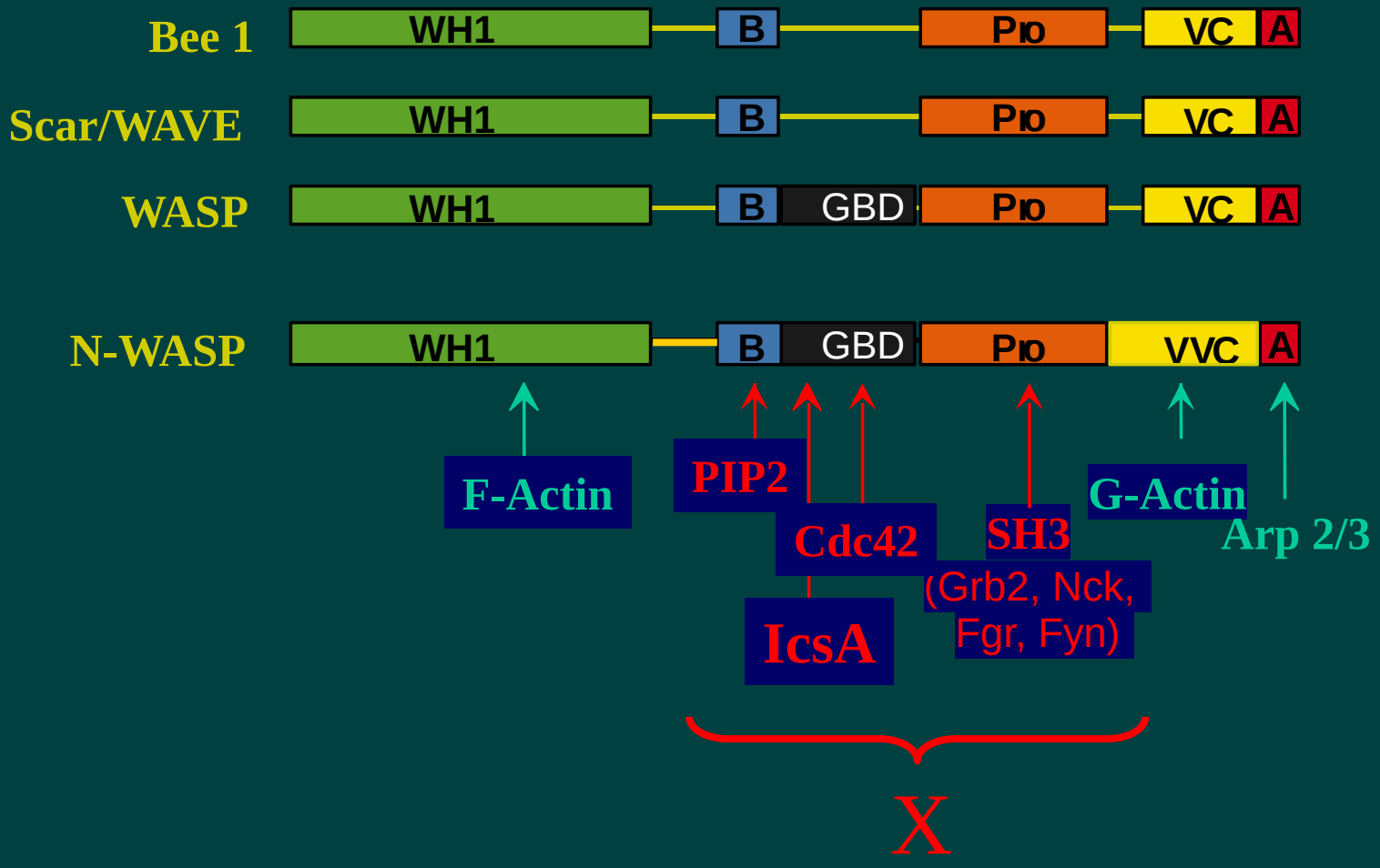
ARPC3-/-



Suraneni et al., JCB, 2012

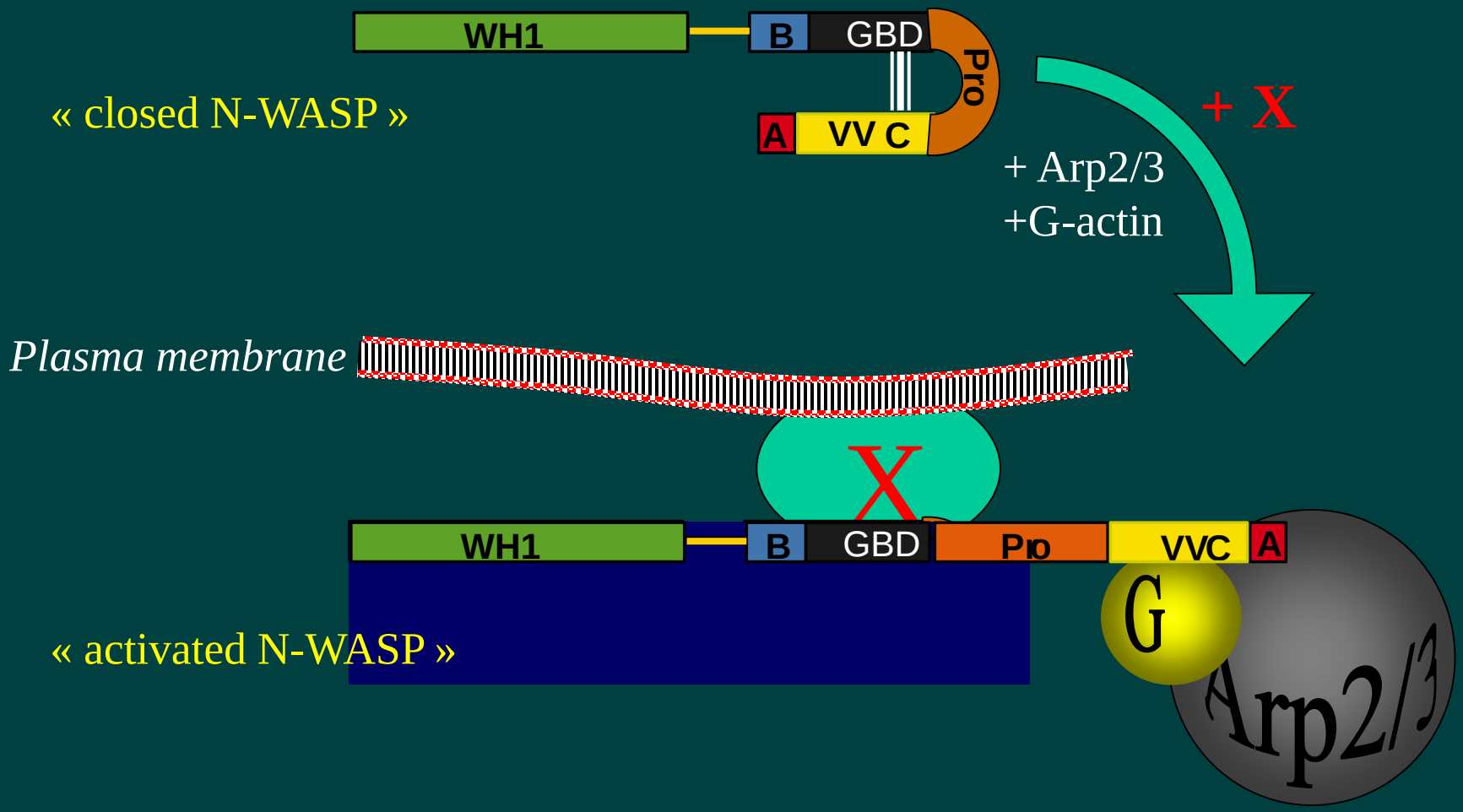
WASP family proteins :

activators of Arp2/3 complex



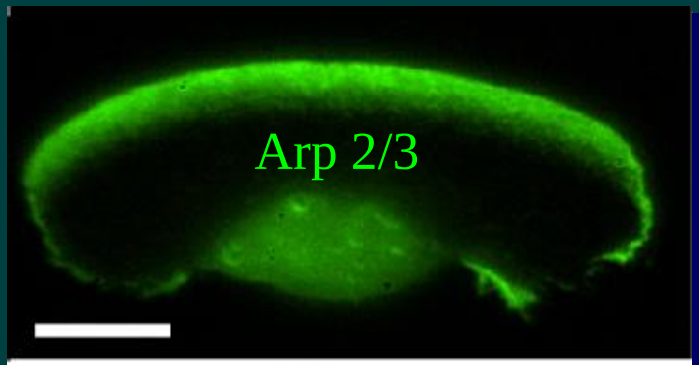
(Miki et al., 1998; Machesky and Insall, 1998; Rohatgi et al., 1999; Egile et al., 1999; Kim, Rosen et al., 2000; Prehoda et al;, 2000)

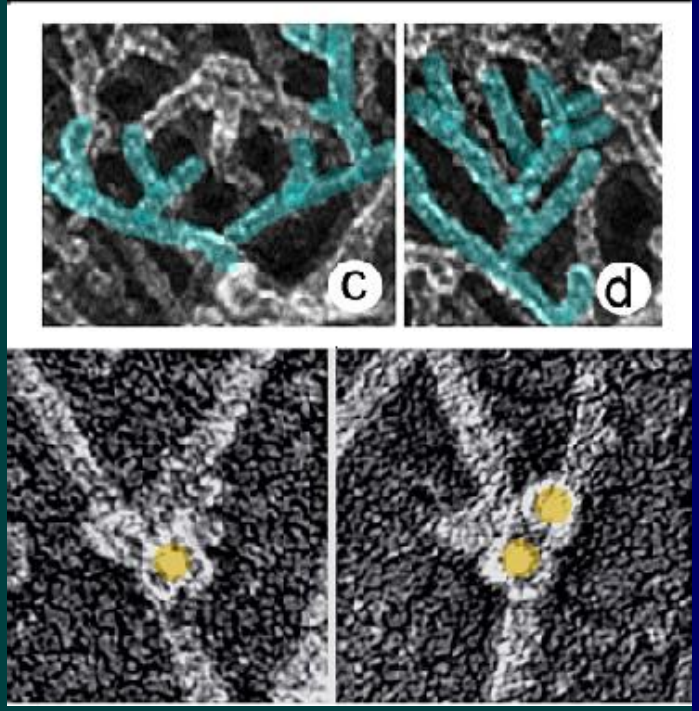
WASP family proteins :
activators of Arp2/3 complex

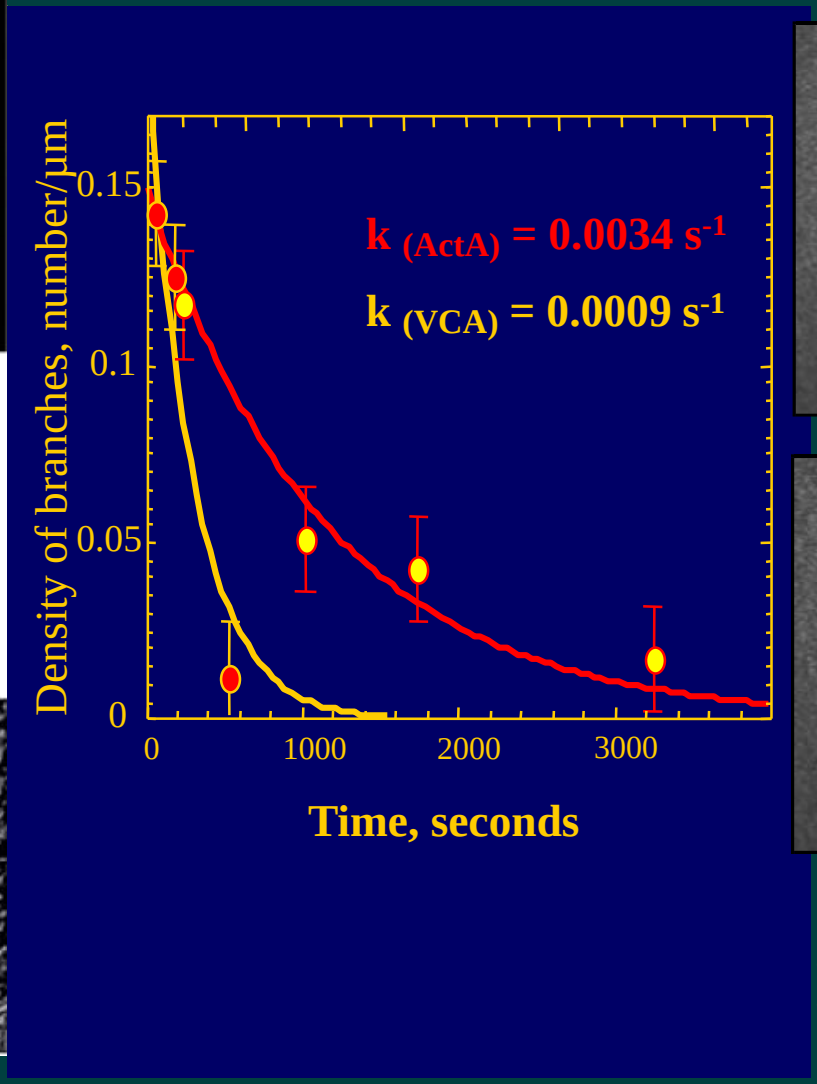


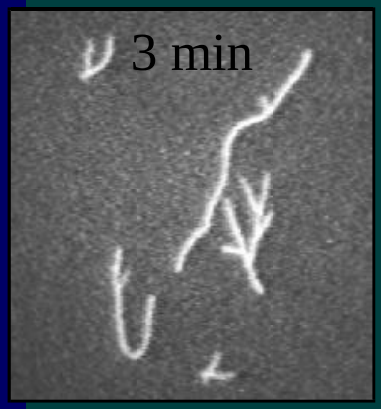
(Miki et al., 1998; Machesky and Insall, 1998; Rohatgi et al., 1999; Egile et al., 1999; Kim, Rosen et al., 2000; Prehoda et al;, 2000)

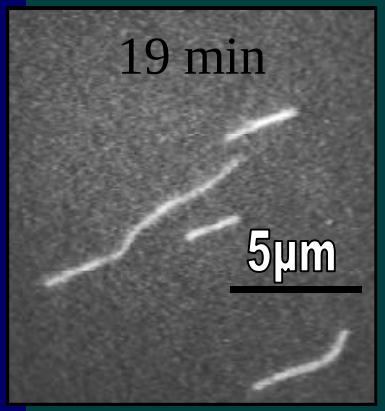
Filament branching array in lamellipodia







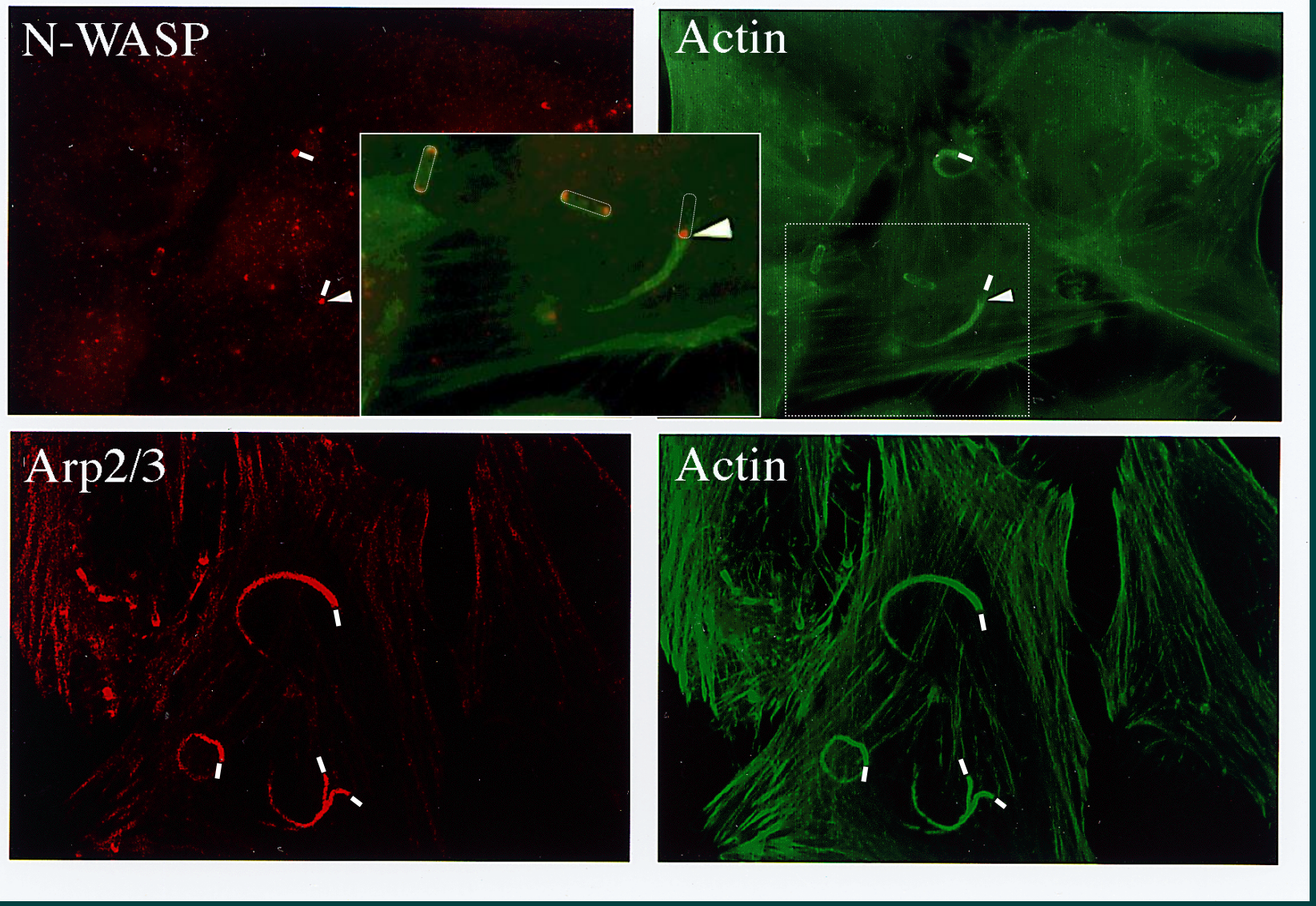




Debranching

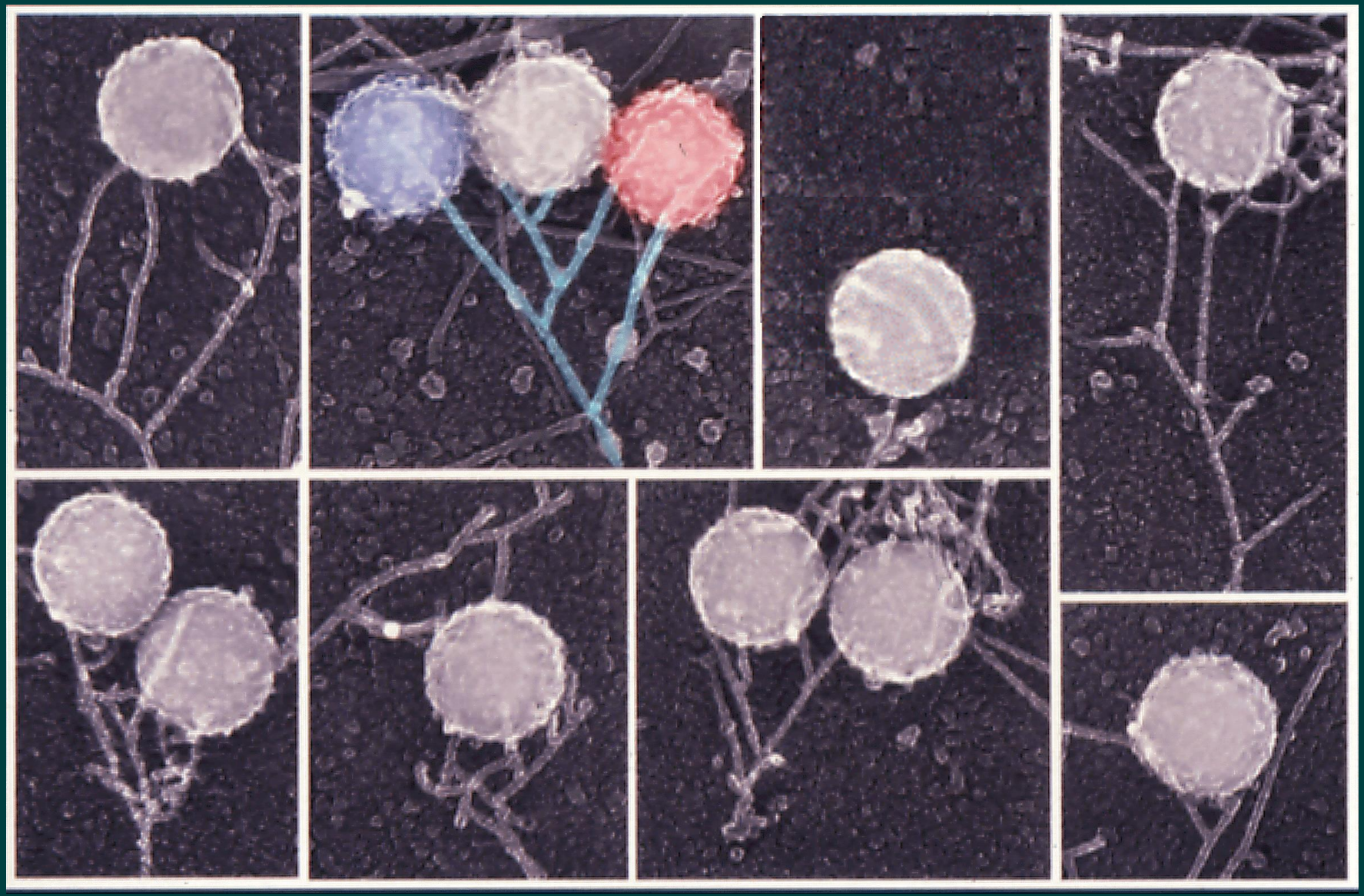
(T. Svitkina and G.G. Borisy, 1999; Blanchoin et al.; Pantaloni et al., 2000)

N-WASP and Arp2/3 localisation in *Shigella*-infected cells (Egile et al., 1999)

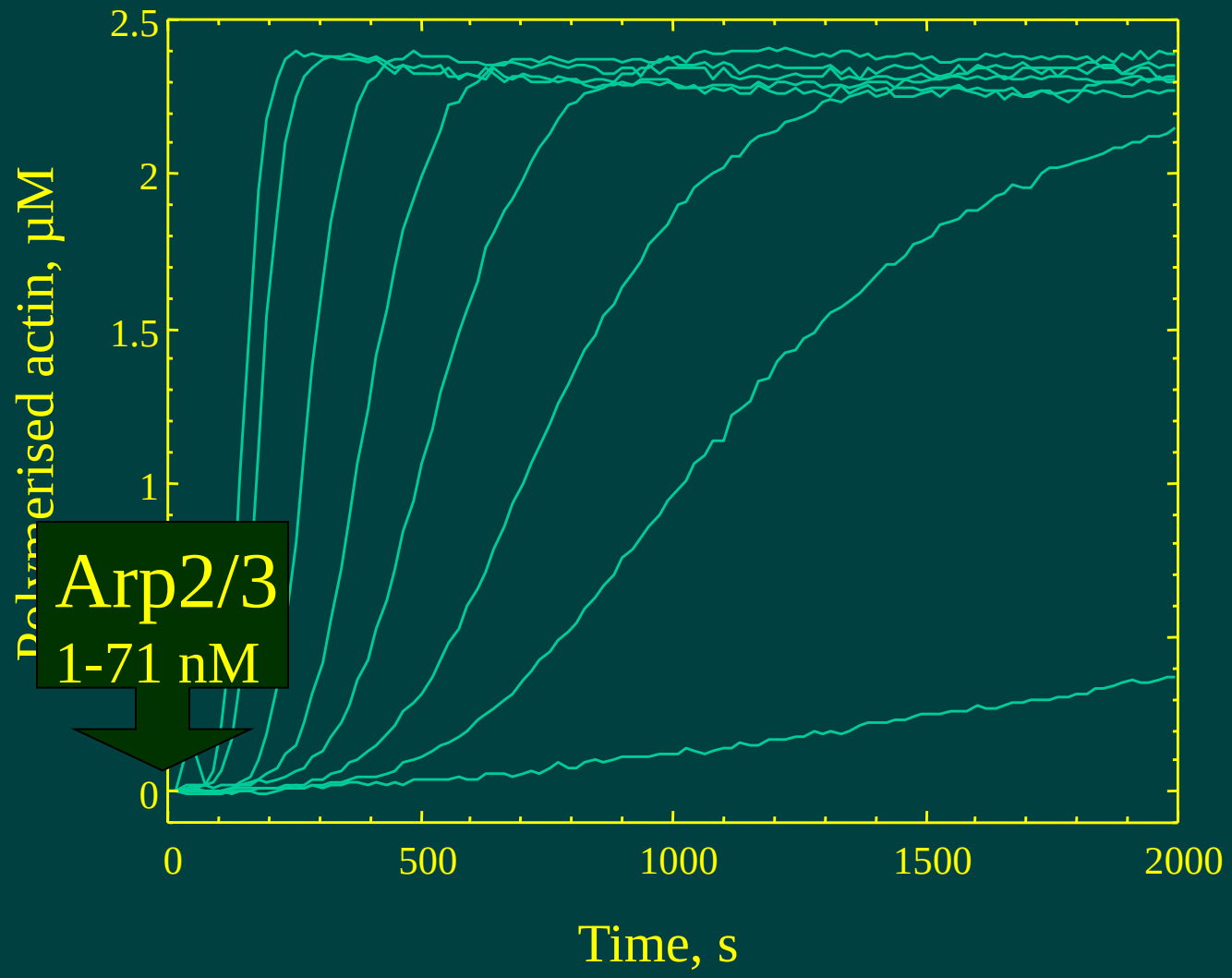


Dendritic nucleation on ActA beads

(Svitkina and Borisy) .

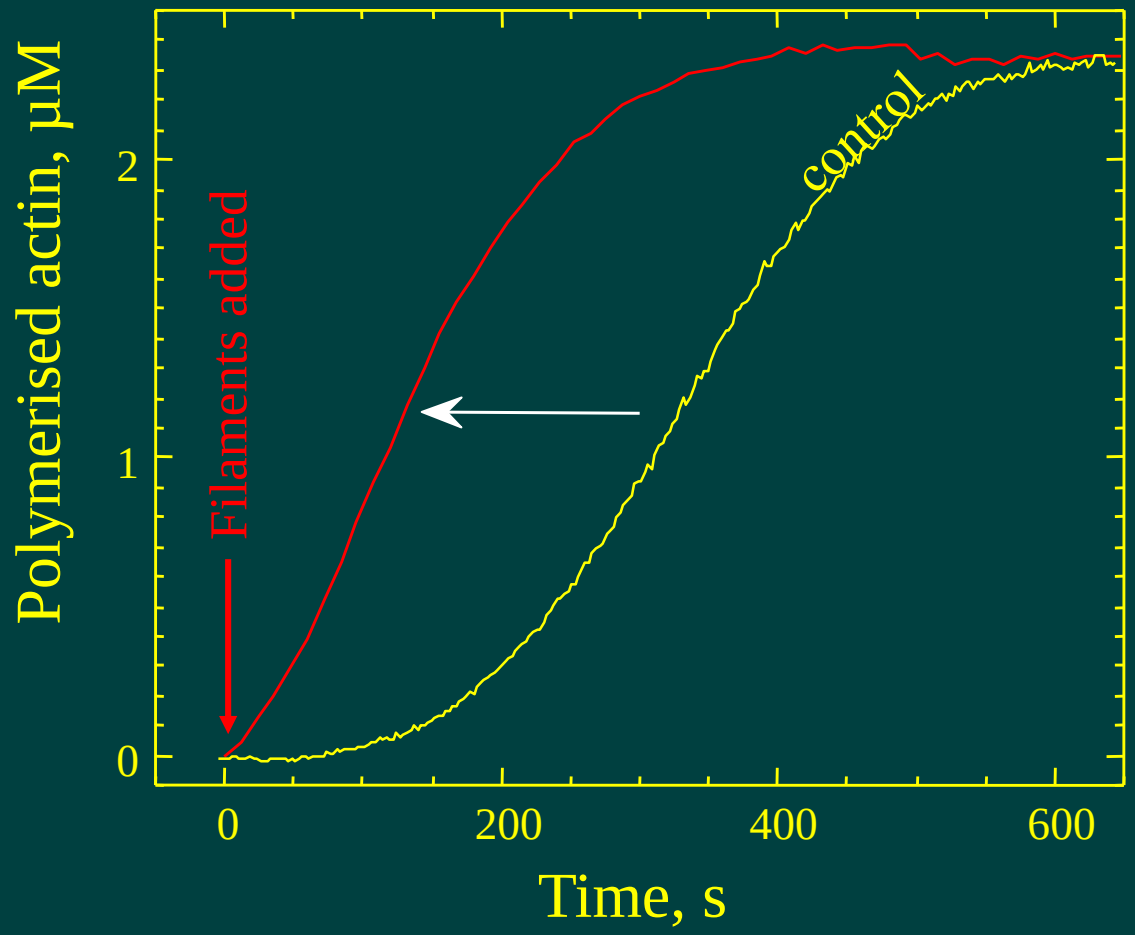


Arp2/3-stimulated actin polymerization is an autocatalytic process



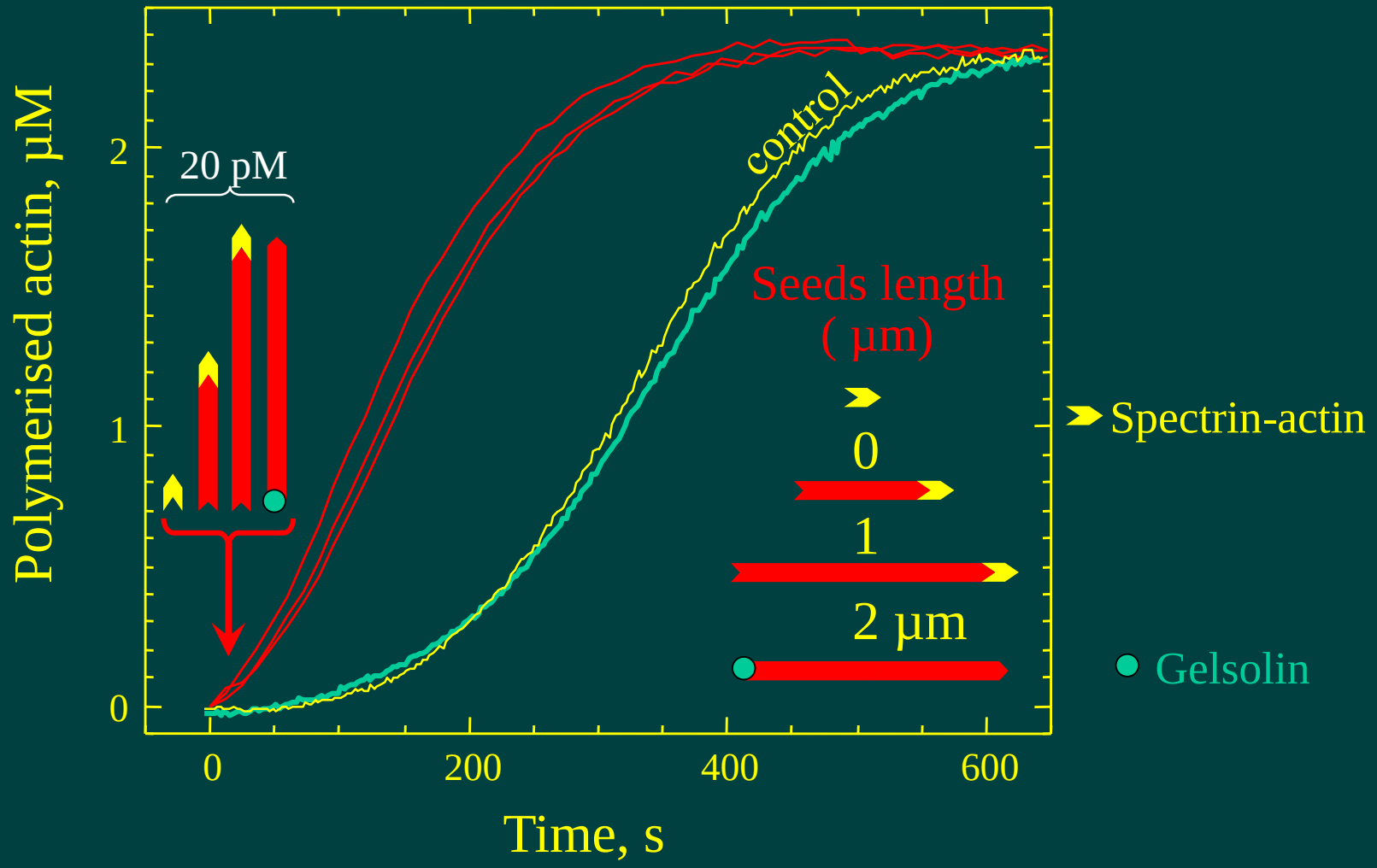
Arp2/3 interacts with filament to stimulate polymerization

(Higgs et al 1999)

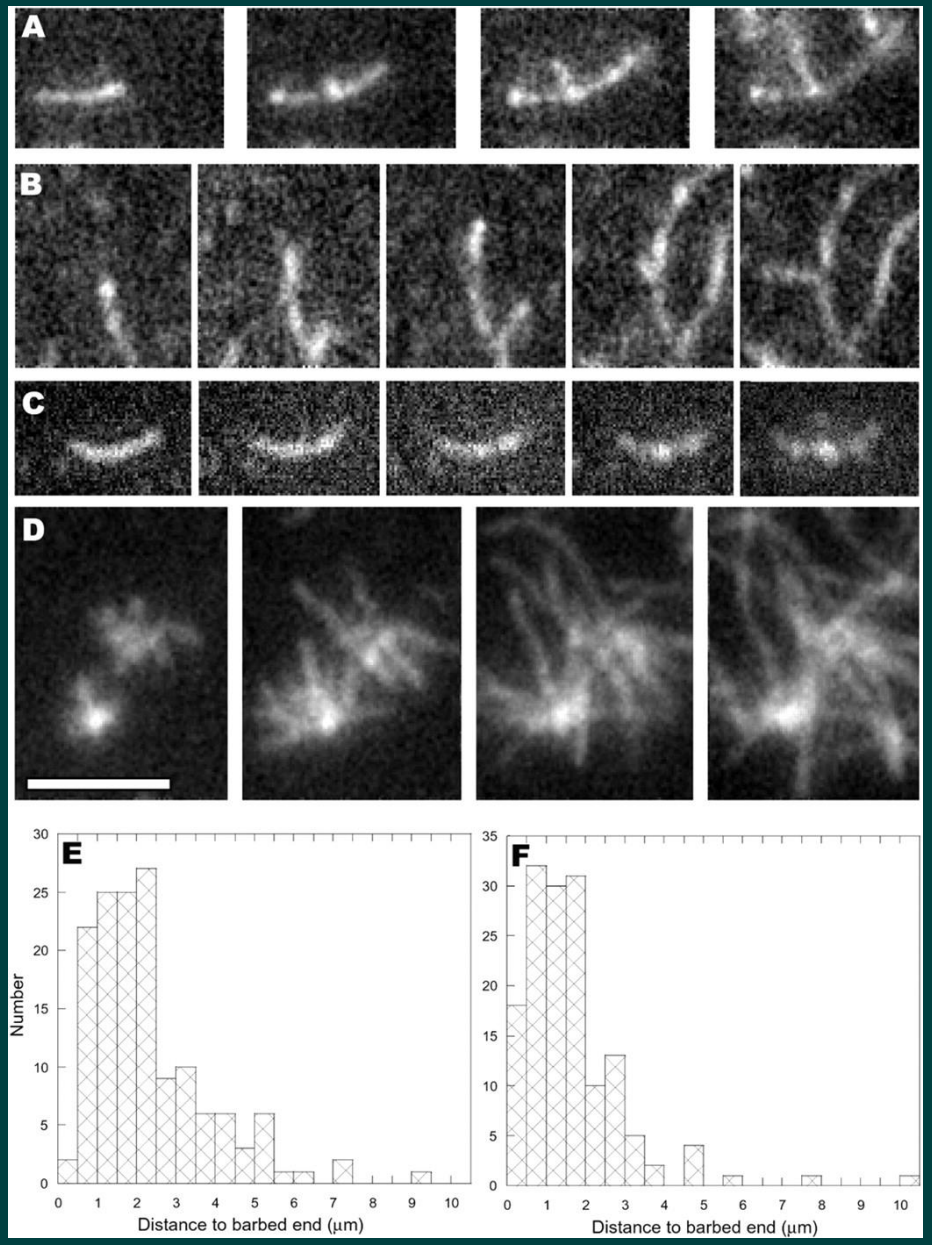


Arp2/3 interacts with barbed ends, independently of filament length

(D.Pantaloni *et al.* 2000)

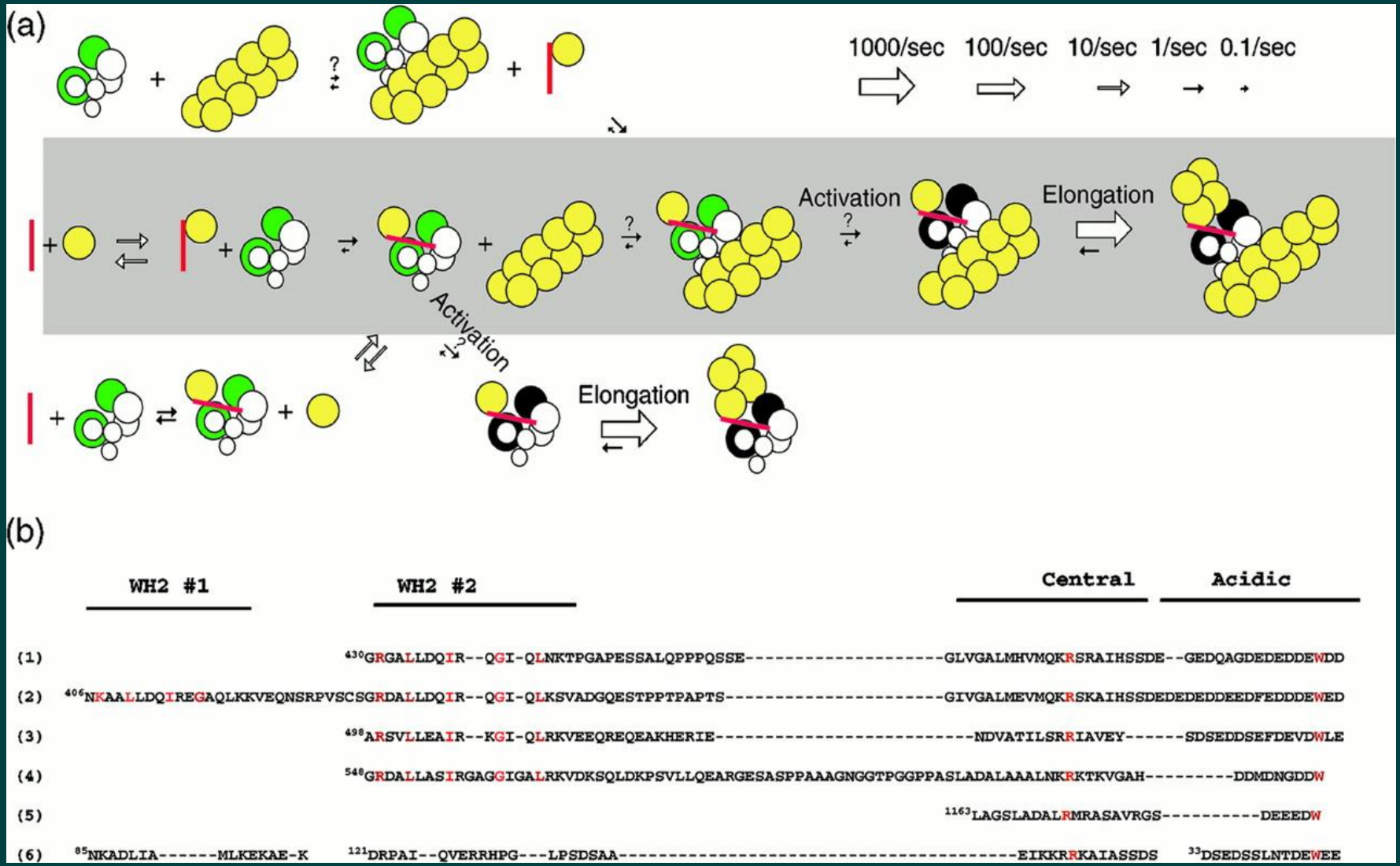


Side-branching by the Arp2/3 complex: TIRF observation



Amann and Pollard
PNAS, 2001

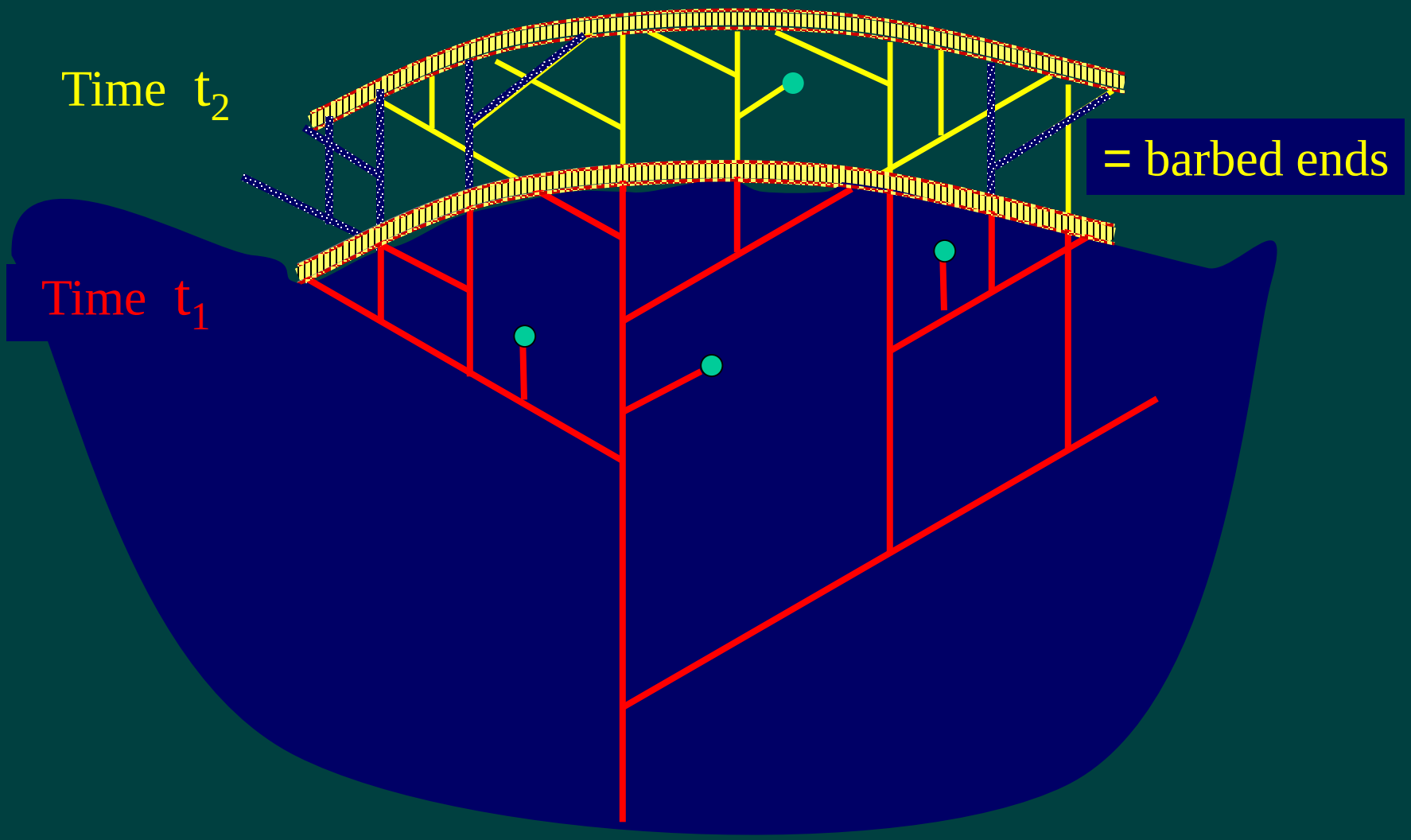
kinetic pathway of dendritic nucleation



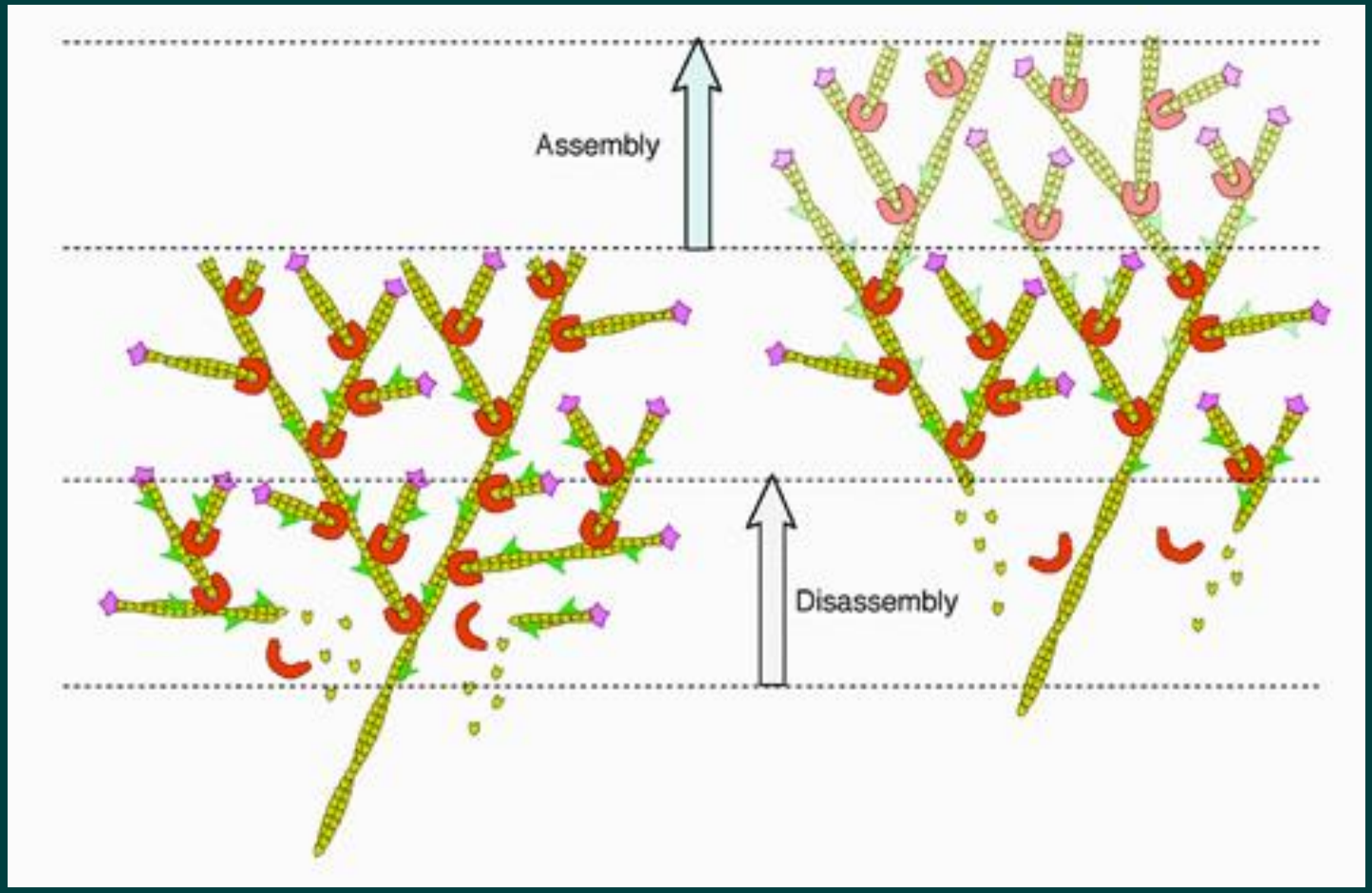
Higgs HN, Pollard TD..

Annu Rev Biochem. 2001;70:649-76.

Steady Branching Model

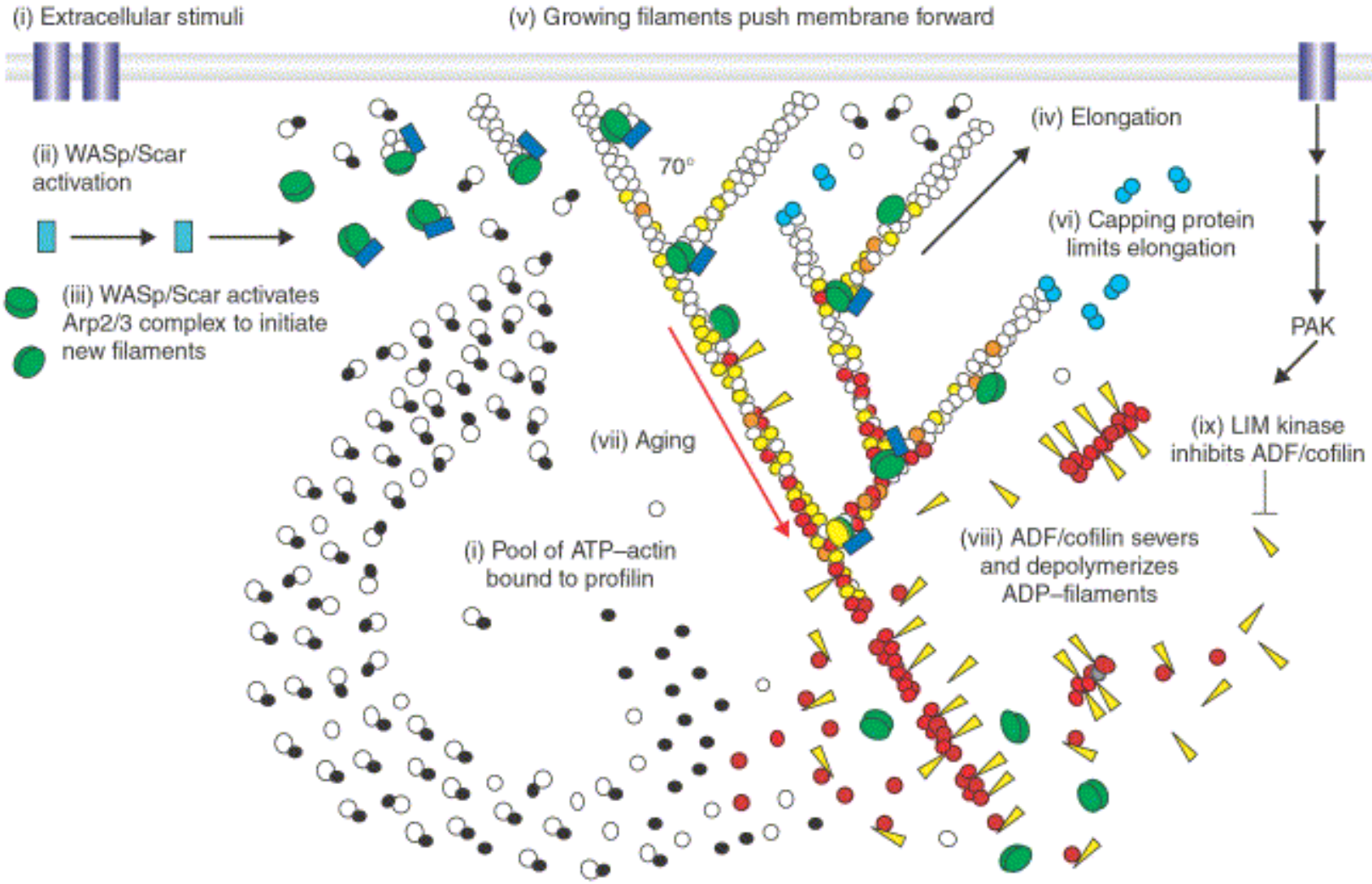


array treadmilling mechanism



Svitkina and Borisy, 2000

Dendritic nucleation model of actin assembly in the lamellipodia



next time:

reconstitution of force generation by branching actin array in vitro

other ways of fast actin assembly

various models of force generation by actin assembly

experimental force measurement

begin molecular motors