Spontaneous Waves and Patches of F-Actin in Cells

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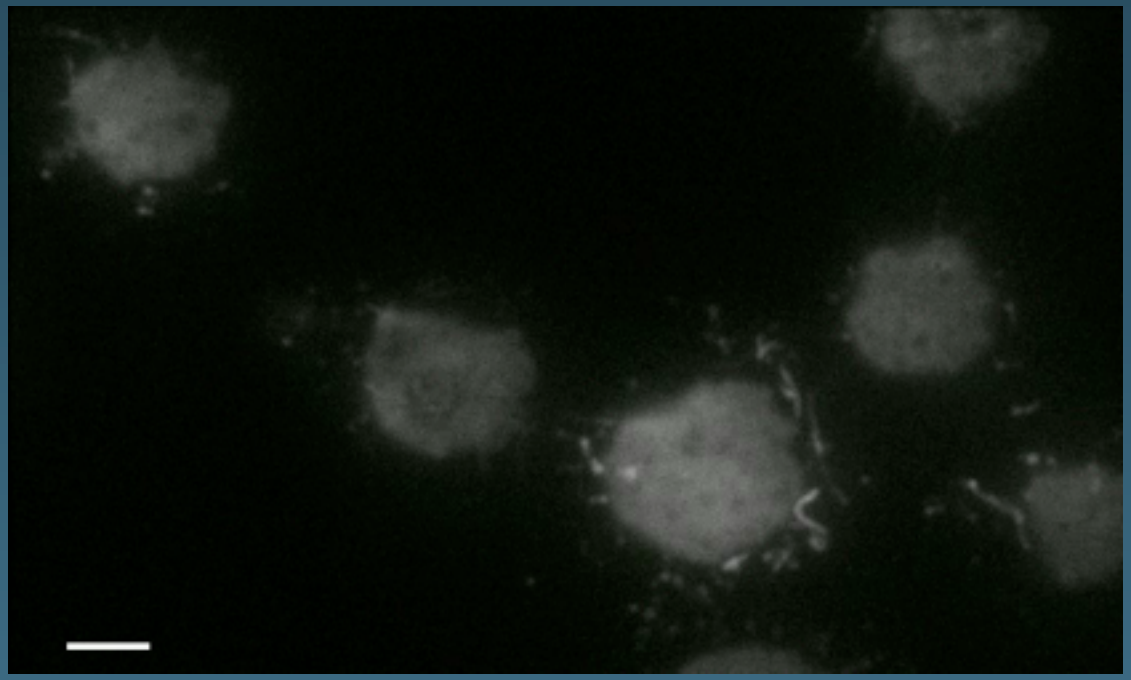
in St Louis

Polymerized actin often exhibits spontaneous dynamic behaviors, such as waves, patches, and uniform oscillations

These waves may help cells explore their environment, exert force, and sense tension

- Does known actin biochemistry lead to spontaneous wave formation?
- What types of feedback mechanisms lead to spontaneous actin dynamics?

Actin Waves Appear After Recovery from Latrunculin Treatment



5 µm

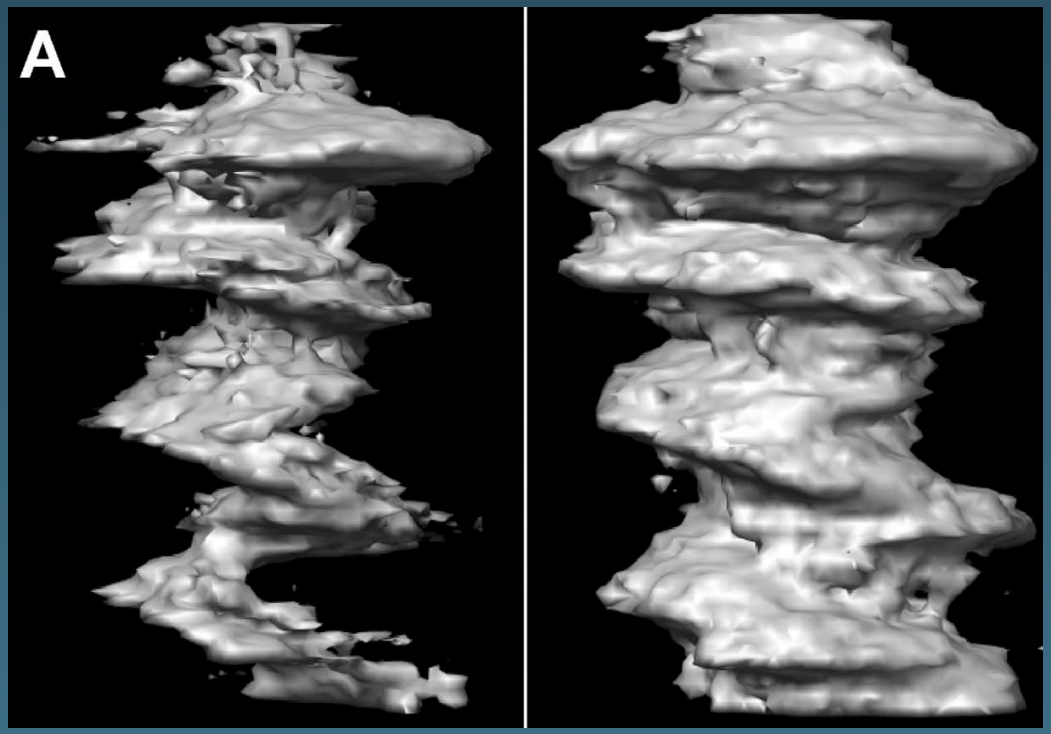
(Bretschneider et al 2004)

- Spontaneous waves and patches of actin assembly
- Over time, get patches first, then waves
- Wave speed is 0.1-0.2 µm/s

Visualization via total internal reflection fluorescence microscopy

F-actin Waves Are Correlated with Edge Protrusion

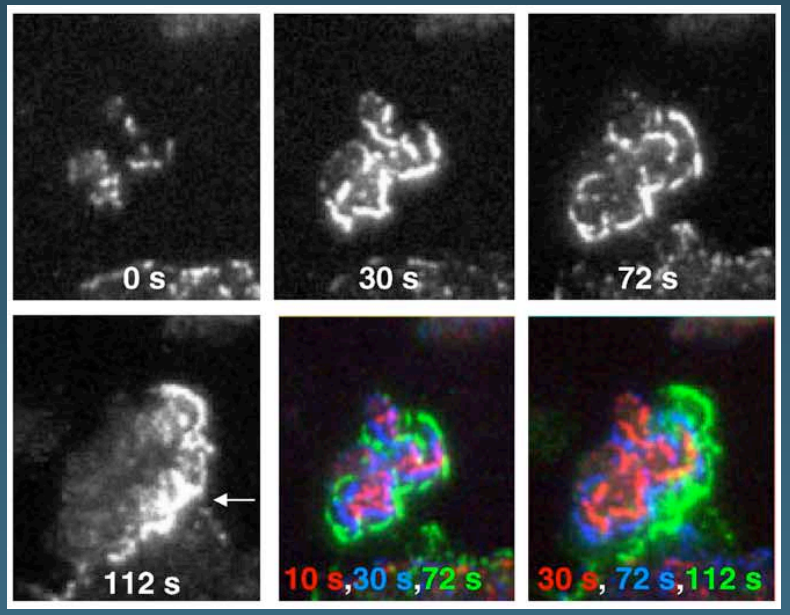
F-Actin Intensity Cell Edge



- Actin waves appear to push membrane out

(Gerisch lab, 2009)

Hem-1 Waves in Chemoattractant-Stimulated Neutrophils



Red, blue, and green are successive times

Waves correlate with edge protrusion

(Weiner et al, 2007)

Actin waves also seen in T cells (Upadhyaya lab)

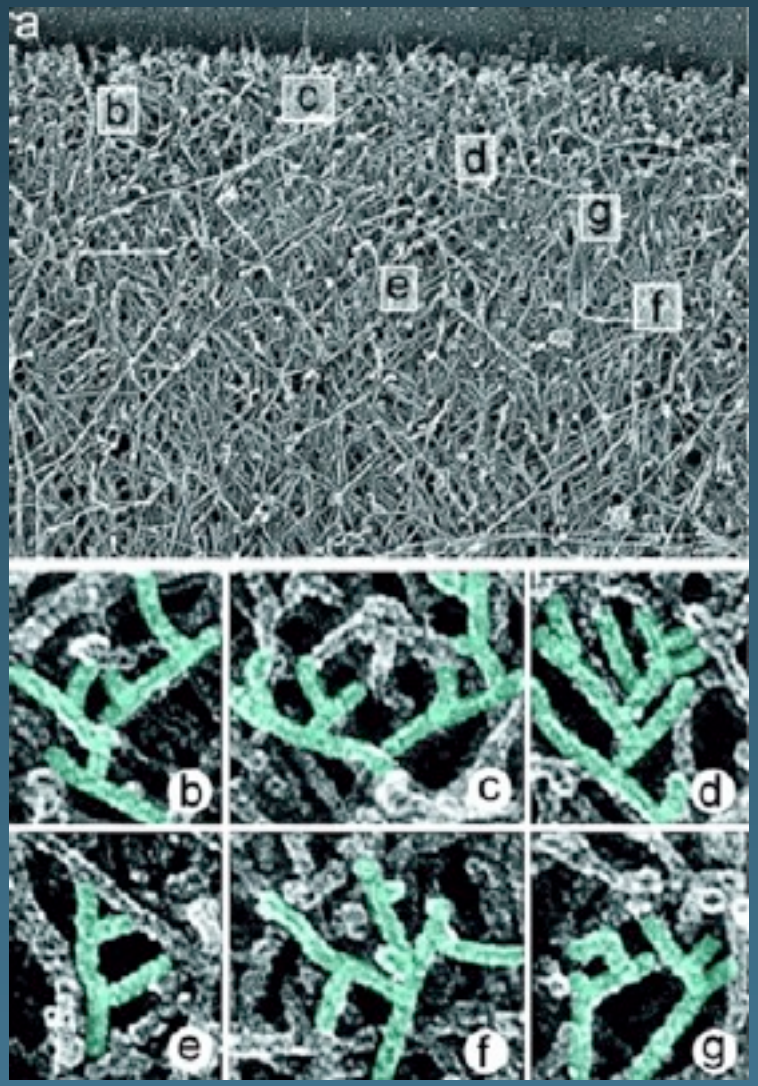
Coarse-Grained Models of Actin Waves

- (Weiner et al 2007, Doubrovinski and Kruse 2008, 2010). Actin filaments plus F-actin nucleators which act cooperatively and are active when at the membrane.
- (Whitelam et al 2009) Nonlinear F-actin field with built-in positive feedback and spontaneously arising orientation anisotropy

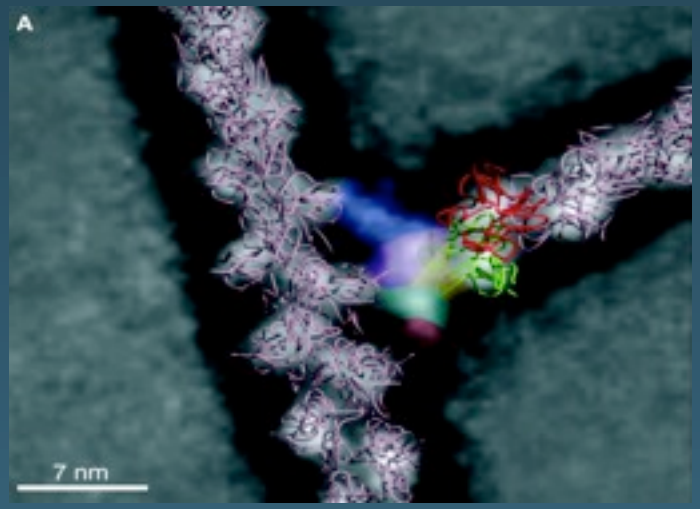
Related work: Falcke, Allard and Mogilner, Vavylonis, Levine and collaborators

Goal here: establish minimal model including known actin biochemistry

Basic Idea: Simulate Dendritic-Nucleation Model of Actin Filament Generation



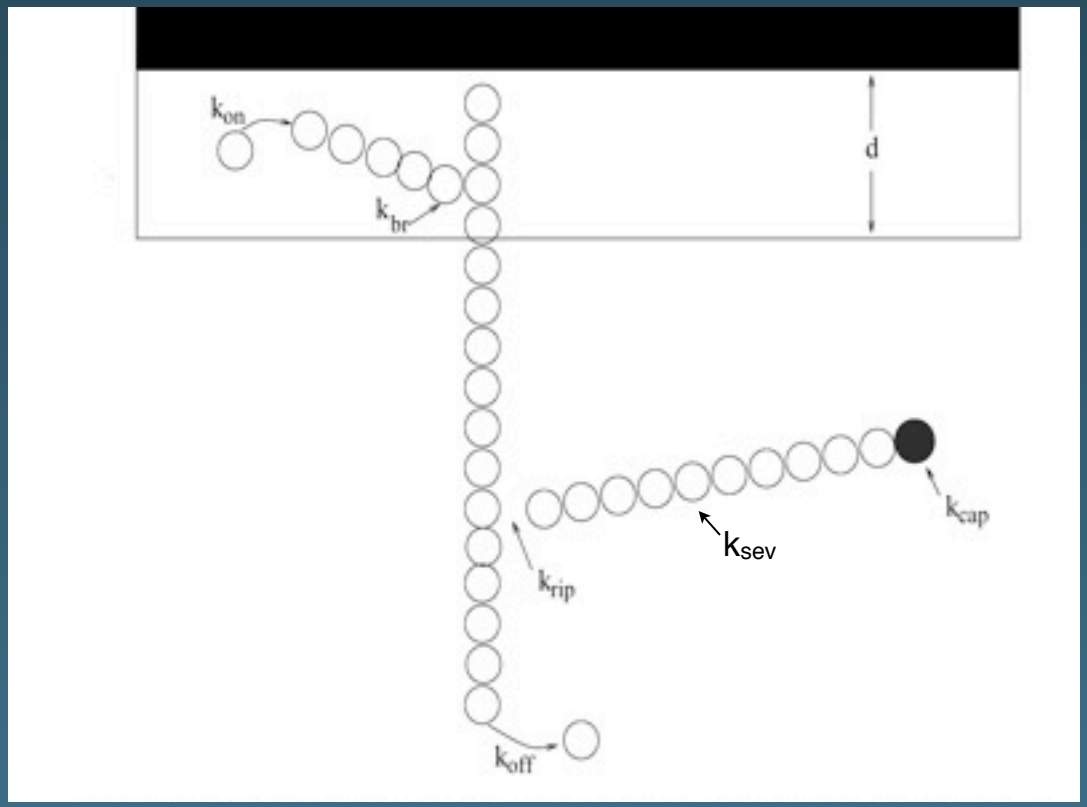
(Svitkina et al 1999)



(Volkmann et al, 2004)

- "Arp2/3 complex" is activated by nucleation-promoting factors (NPF) in membrane: "dendritic nucleation"
- In a "Lego-Block" fashion, it starts new filaments on the sides of existing ones

Simulation Approach: Stochastic "Topological" Processes Combined with Brownian Dynamics of Filament Motion

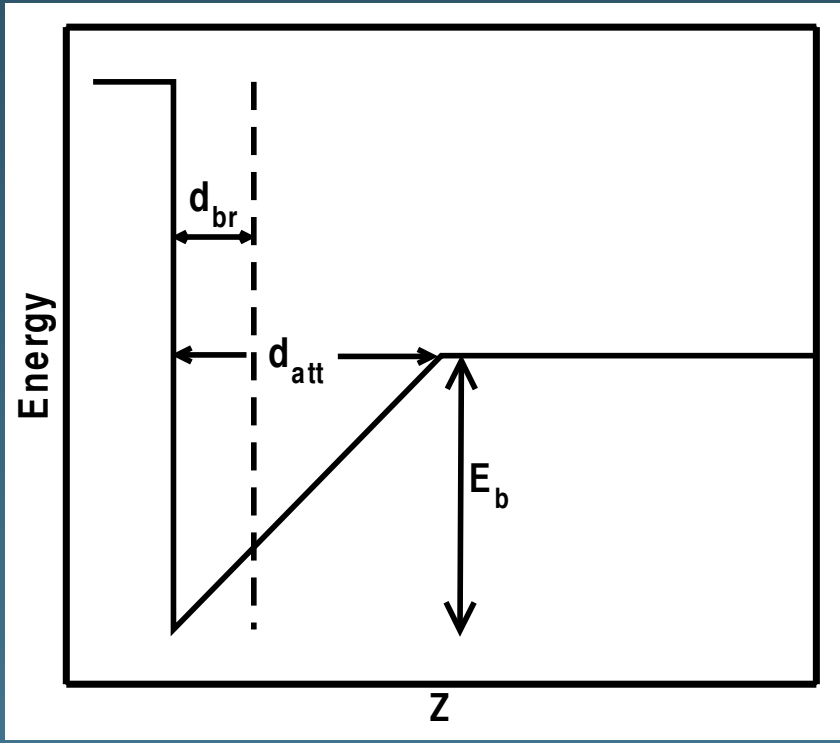


k_{on}: polymerization rate
k_{off}: depolymerization rate
k_{br}: branching rate
k_{cap}: capping rate
k_{det}: branch detachment rate
k_{sev}: severing rate

New branches appear within distance d of membrane

Filaments Move via Brownian Dynamics in Membrane's Force Field

Membrane has
1) repulsive interactions with all filament tips
2) attractive interactions with uncapped barbed ends

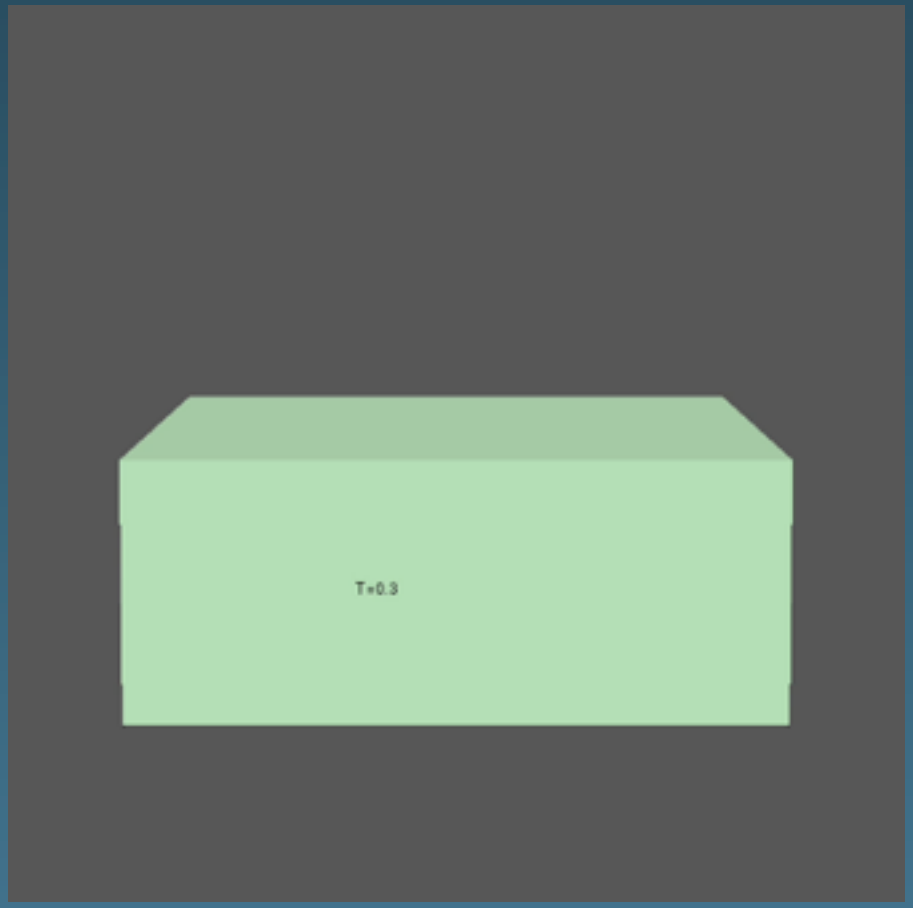


$$\Delta R_i = (D/k_B T) F_i \Delta t + \eta\sqrt{2D\Delta t}$$

d_{br} = 10 nm
d_{att} = 10 nm
E_b = 4.5kT

Requires time step of about 10^{-7} s
Treat dendritic clusters as rigidly moving units and ignore cluster-cluster interactions

Dynamics of Actin at Membrane: Initiation of Polymerization



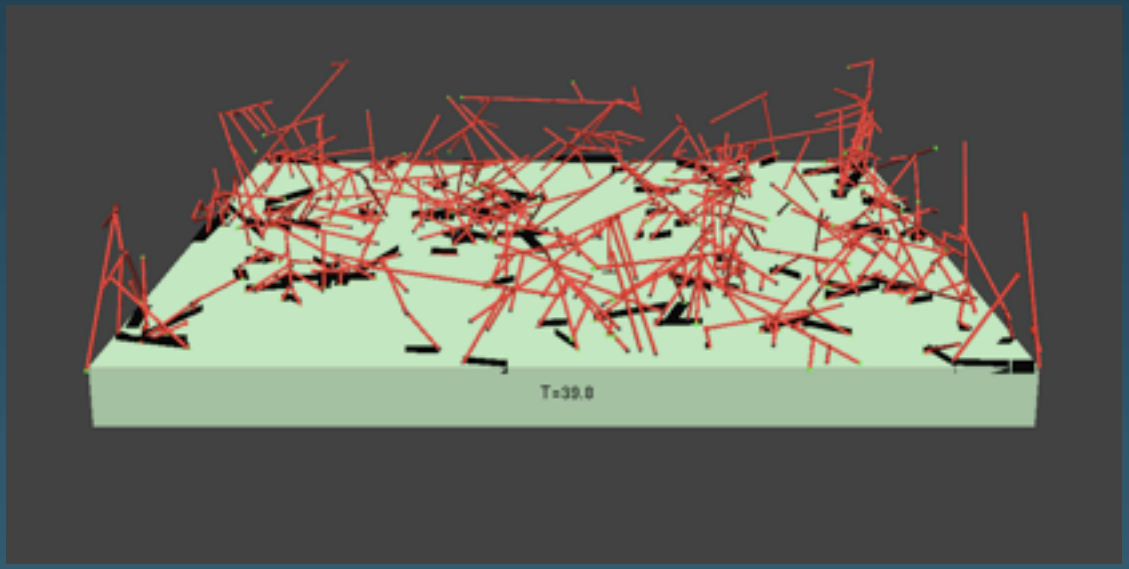
Bright green: uncapped barbed ends

Filaments nucleate at membrane and branch if they stay attached long enough

Most filaments leave the membrane before they branch, giving a large critical cluster size

This is similar to the effect of a threshold for positive feedback in continuum models

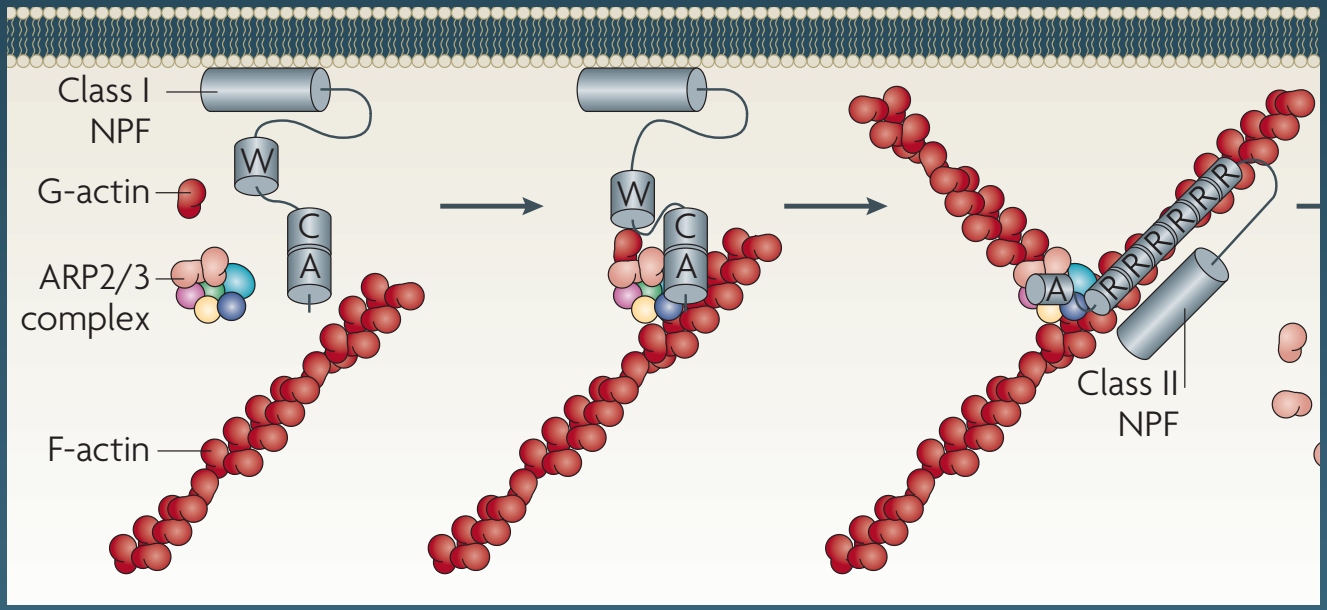
"Bare" Dendritic Nucleation Model Gives no Identifiable Waves or Patches



3μm

80 μM actin

Origin of Waves May Lie Upstream of Actin

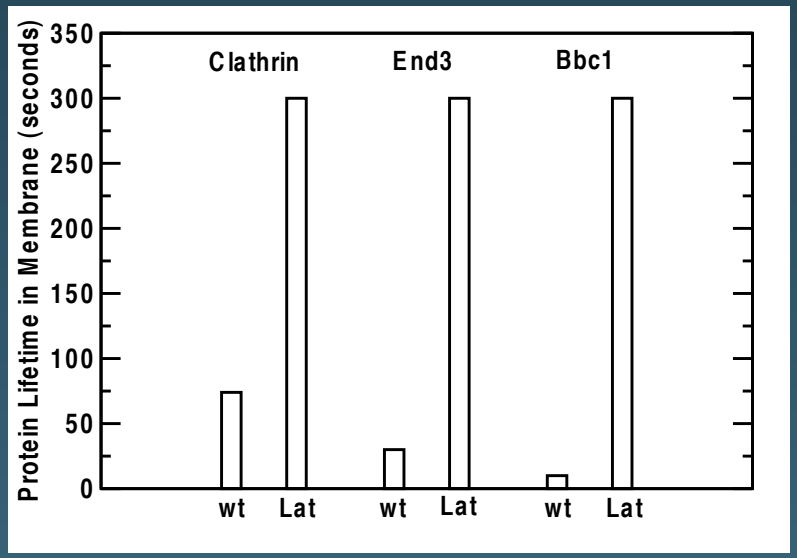


(Welch, 2010)

Nucleation-promoting factors (NPFs) act upstream of actin polymerization and require membrane localization to efficiently activate Arp2/3 complex

Assumption: F-Actin Detaches/ Inactivates Upstream NPFs

Weiner et al (2007) showed that removal of the NPF Hem-1 from the membrane is greatly slowed by latrunculin treatment



Data for upstream actin patch proteins in yeast (Drubin lab, 2005)

Upstream protein lifetimes are much longer with latrunculin treatment

Include F-Actin-Induced NPF Detachment in Model

$$\frac{\partial n_a}{\partial t} = -k_{\text{det}} F(x,y) n_a + k_{\text{att}} \bar{n}_d$$

$$\frac{d\bar{n}_d}{dt} = \frac{-1}{A} \int \frac{\partial n_a}{\partial t} dxdy$$

n_a: 2D density of attached (active) NPFs
n_d: 2D density of detached NPFs (constant)
A: membrane area
F: density of F-actin near membrane
k_{det}, k_{att}: detachment and attachment constants

NPFs are detached by F-actin, then reattach spontaneously

Detachment inactivates the NPFs

Arp2/3 activation $\propto n_a^2$

k_{on}^{B}	8.7 $\mu M^{-1}s^{-1}$	[3]
k_{on}^{P}	1.3 $\mu M^{-1}s^{-1}$	[4]
k_{cap}	8.0 $\mu M^{-1}s^{-1}$	[5]
k_{uncap}	0.0004 s^{-1}	[5]
k_{nuc}	0.001 - 0.009 $\mu M^{-1}s^{-1}$	
k_{br}	0.018 $\mu M^{-3}s^{-1}$	
k_{dis}	$0.04s^{-1}$	[6]
k_{sev}	$0.005s^{-1}$	[7]
k_{att}	0.025-0.075 s^{-1}	
k_{det}	0.005-0.015$\mu M^{-1}s^{-1}$	
A_c^B	0.07 μM	[5]
A_c^P	0.69 μM	[5]
$[A]$	10-40 μM	
$[CP]$	0.15 - 0.5 μM	
$[Arp2/3]$	1.0 μM	[8]
E_b	$2 - 5k_{\mathrm{B}}T$	
D_{memb}	0.04 $\mu m^2 s^{-1}$	[9]
D_{mon}	4 $\mu m^2 s^{-1}$	[10]

Parameter Values

On-and-off rates taken from in vitro measurements, other parameters used to obtain reasonable network structures or varied within reasonable ranges

Simulations Show that Branched Actin Networks Form Waves Under Some Conditions

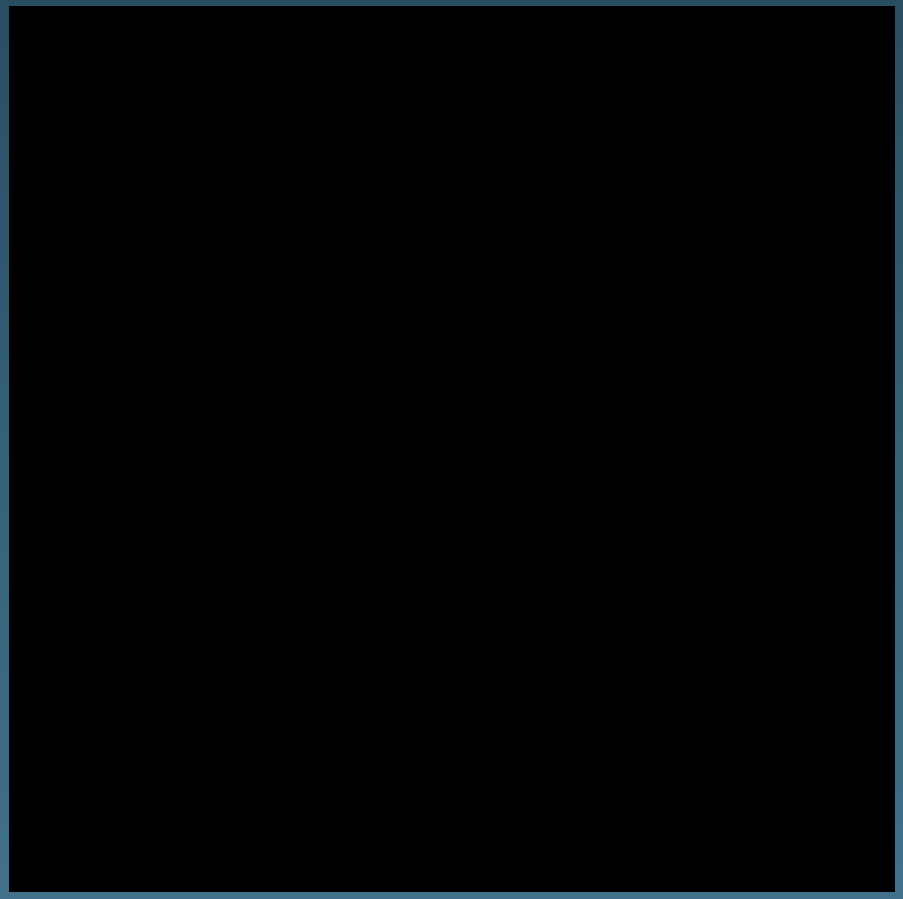
3μm

22.5 μM actin

(PRL, 2010)

Burst of actin polymerization is followed by loss of NPF, which forces actin growth forward

Simulated Fluorescence of Waves



Patchy state can appear as interloper

Why Does Dendritic Actin Nucleation Lead to Wave/Patch Formation?

Generic mechanisms leading to wave/patch formation:

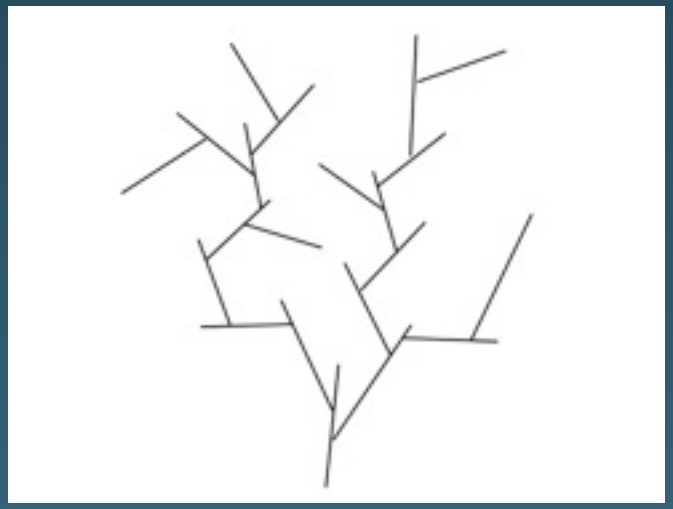
- Positive feedback
- Diffusive spreading
- Delayed negative feedback

$$\dot{u} = -u + H(u - a) - v + \nabla^2 u$$
$$\tau \dot{v} = \mu u - v + L^2 \nabla^2 v ,$$

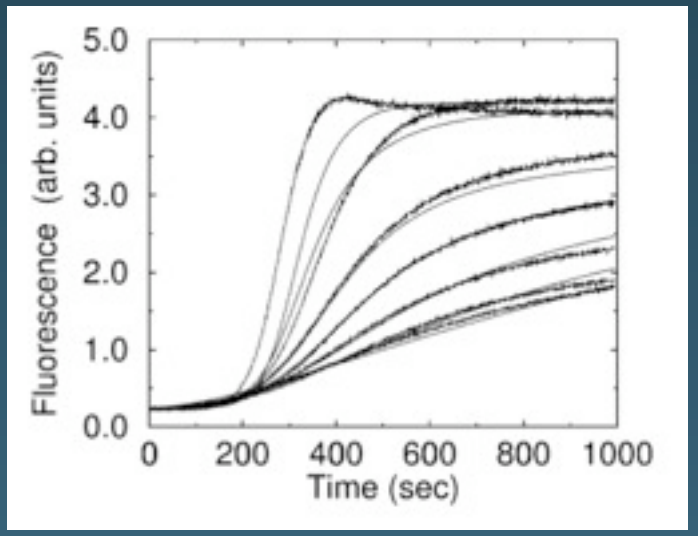
(Krischer and Mikhailov 1994)

u=activator (F-actin)
H= positive feedback (step fcn)
v = inhibitor (absence of NPF)

Branching Causes Positive Feedback



Bulk polymerization with Arp2/3 complex



Martin Wear (Cooper Lab), 2004

(Fluorescence measures polymerized-actin content)

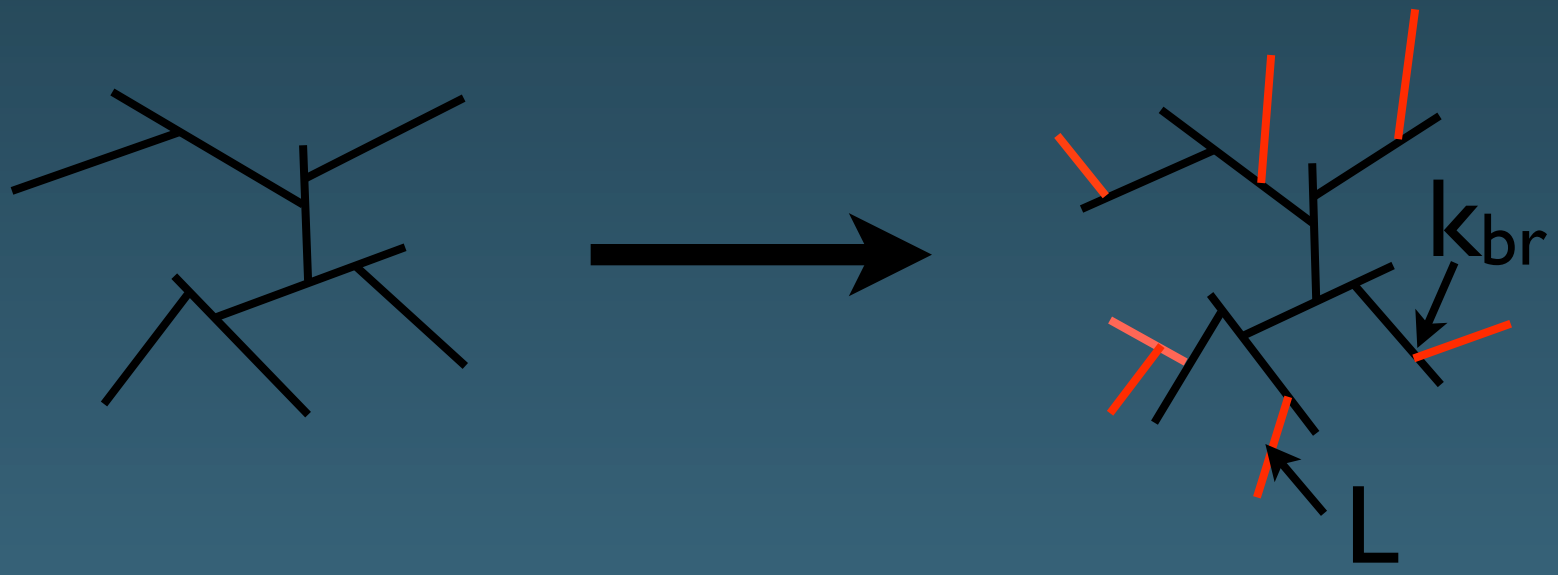
Number of filaments

$$dN/dt = k_{br} N$$

Branching rate

Polymerization has lag phase followed by exponential growth, characteristic of positive feedback

Diffusionlike Spreading of F-Actin Clusters Results from Branching



$$D_{eff} \approx k_{br}\, L^2/12 \approx 0.01\,\mu m^2/s$$

D_{eff} is probably greater than the physical diffusion coefficient D because of attachment of dendritic clusters to membrane

Wave/Patch Formation is Favored by:

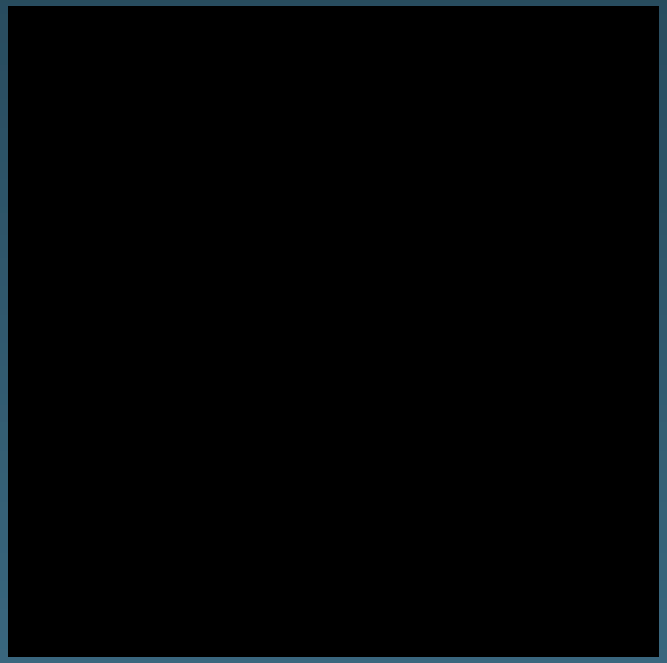
- Slow NPF reattachment
- Slow spontaneous nucleation of actin filaments
- Optimal values of actin polymerization rate and binding strength to membrane

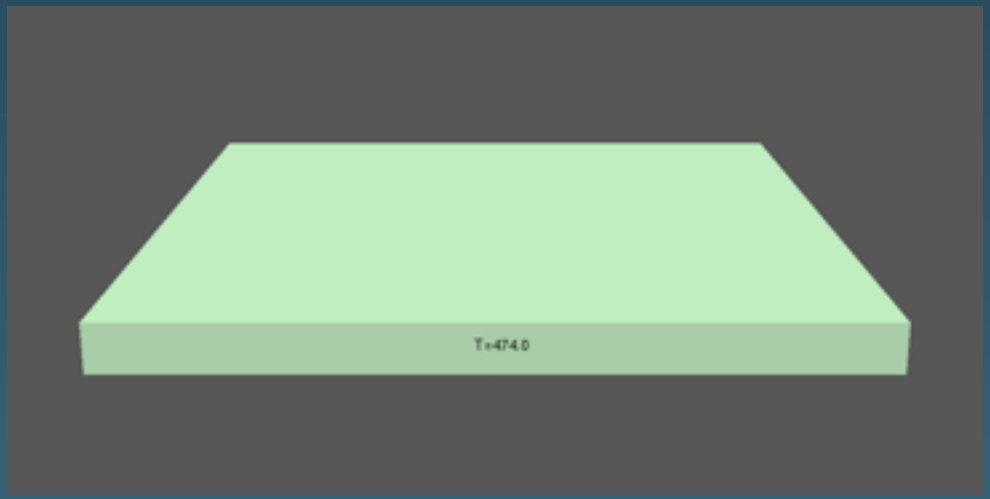
Differences from “Ordinary” Chemical Waves:

- Spreading is not via Brownian motion
- Positive feedback is strongly stochastic

Concrete Predictions:

Reducing actin concentration will cause wave-patch transition



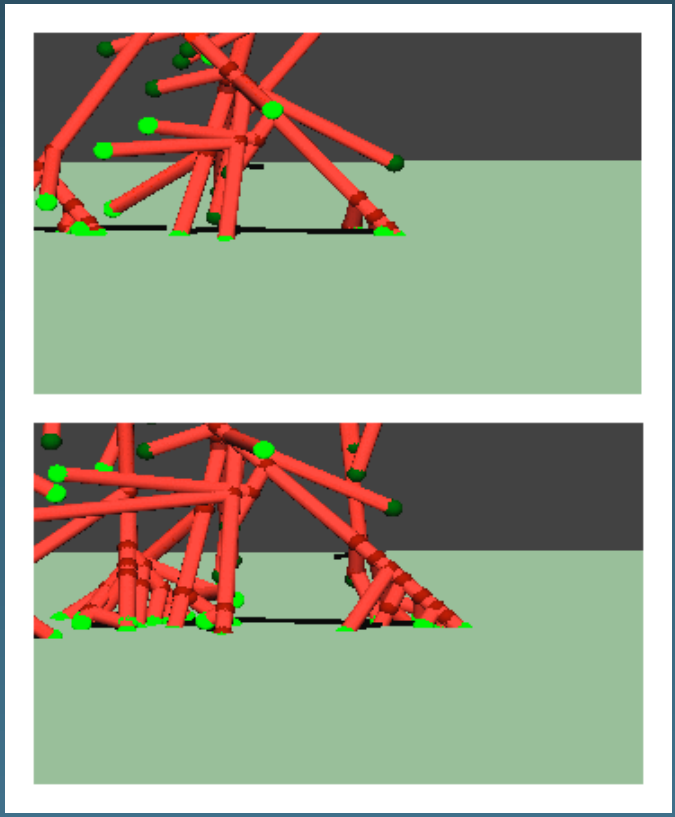


15 μM actin

Patches form because of depletion effects

Consistent with patch-wave transition seen during actin recovery after depletion (Bretschneider et al 2004)

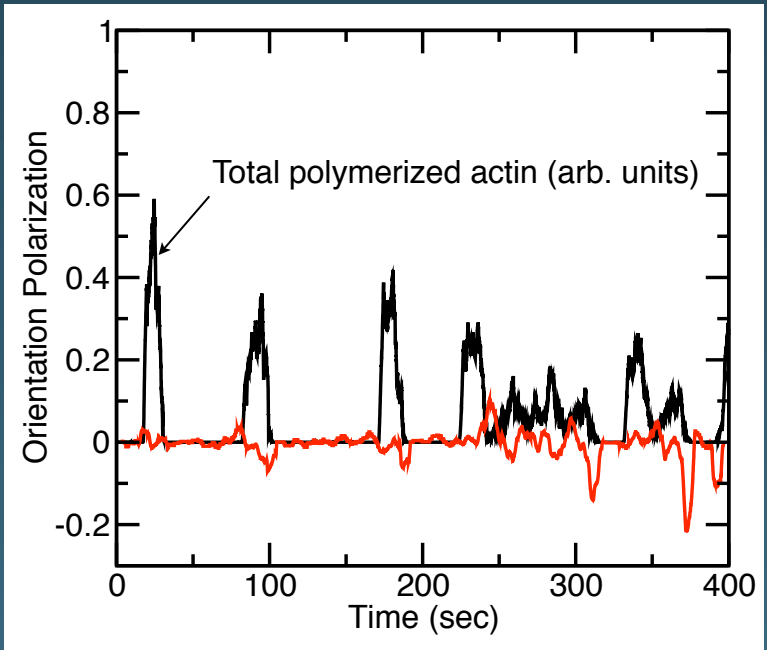
Waves and patches propagate by treadmilling based on branching at edges of waves/patches - not by physical motion of F-actin

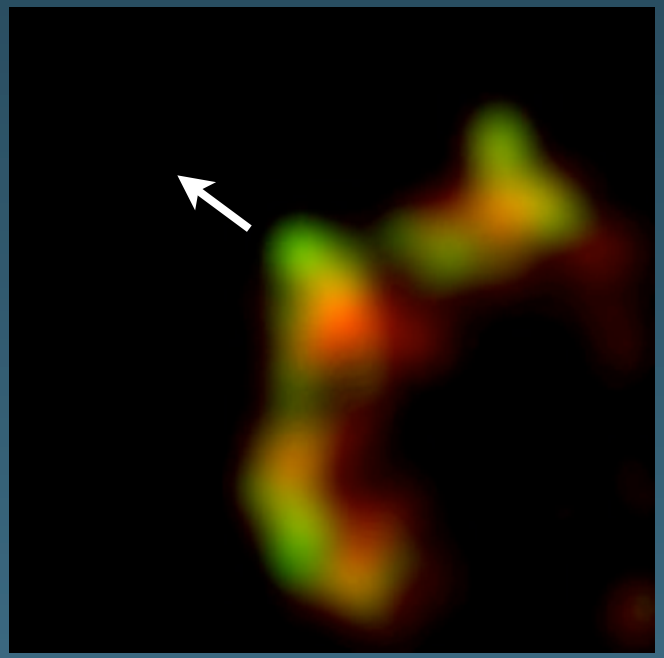


0.2 sec between frames

Treadmilling motion has been seen in FRAP experiments (Bretschneider et al 2009)

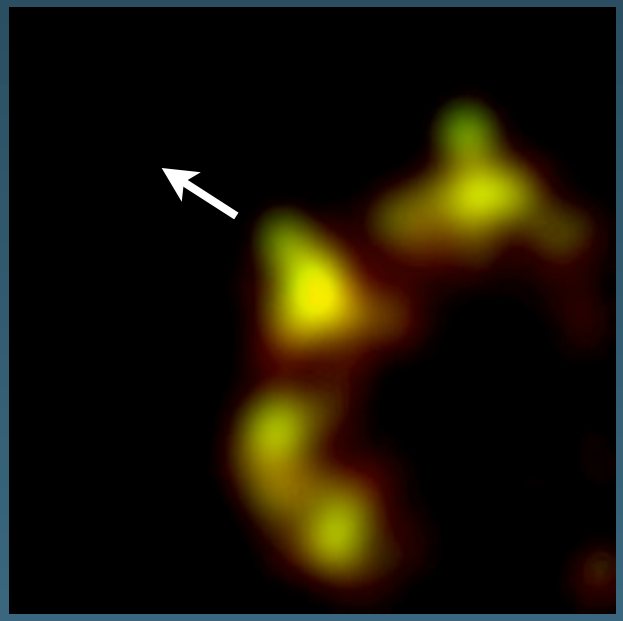
Filament orientations are not strongly polarized, but the distribution of free barbed ends is:

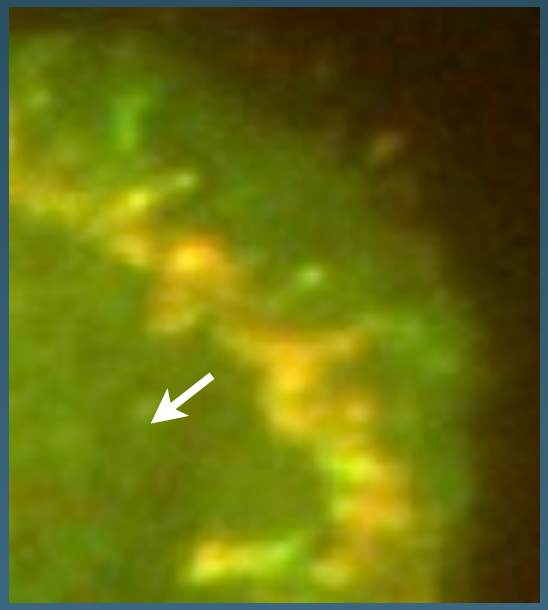




Green: free barbed ends
Red: F-actin
Arrow: direction of wave motion

Distribution of Arp2/3 complex is broadly similar to that of actin



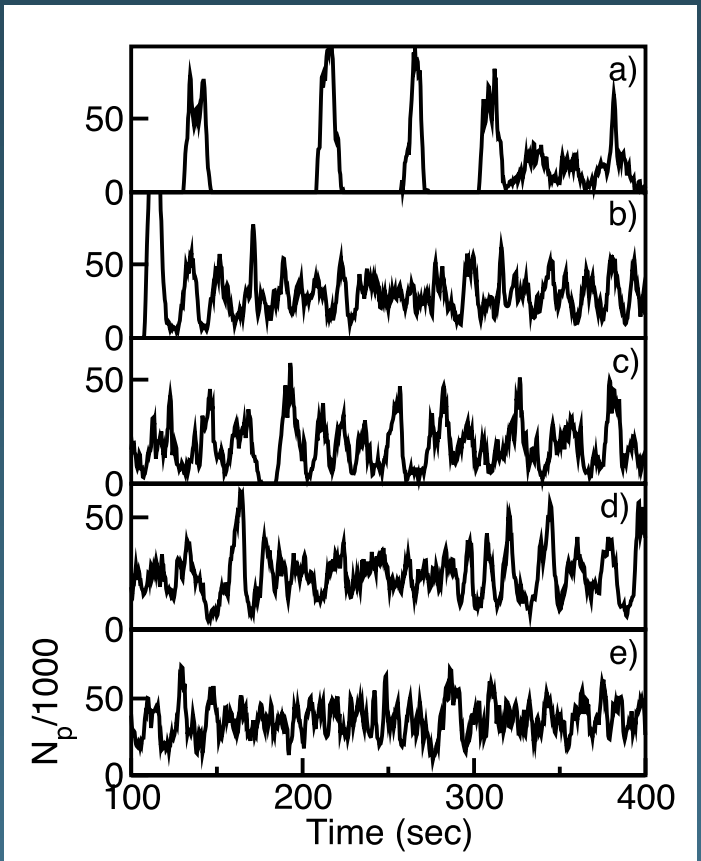


Expt (Bretschneider et al 2009)

Green: Arp2/3 complex
Red: F-actin
Arrow: direction of wave motion

Interventions favoring actin polymerization destroy waves

Wavy state has bursts in # polymerized subunits N_p



Baseline (wavy state)

Reduced capping

Faster nucleation

Increased filament binding

Faster NPF reattachment

These effects can be implemented by manipulating key protein concentrations

Diffusion of NPFs slows, and eventually freezes patch/wave motion, while diffusion of filament clusters accelerates wave motion

- Speedup due to cluster diffusion is a factor of two if they diffuse freely

Attachment of filaments to substrate tunes the behavior in the same way as the actin concentration:

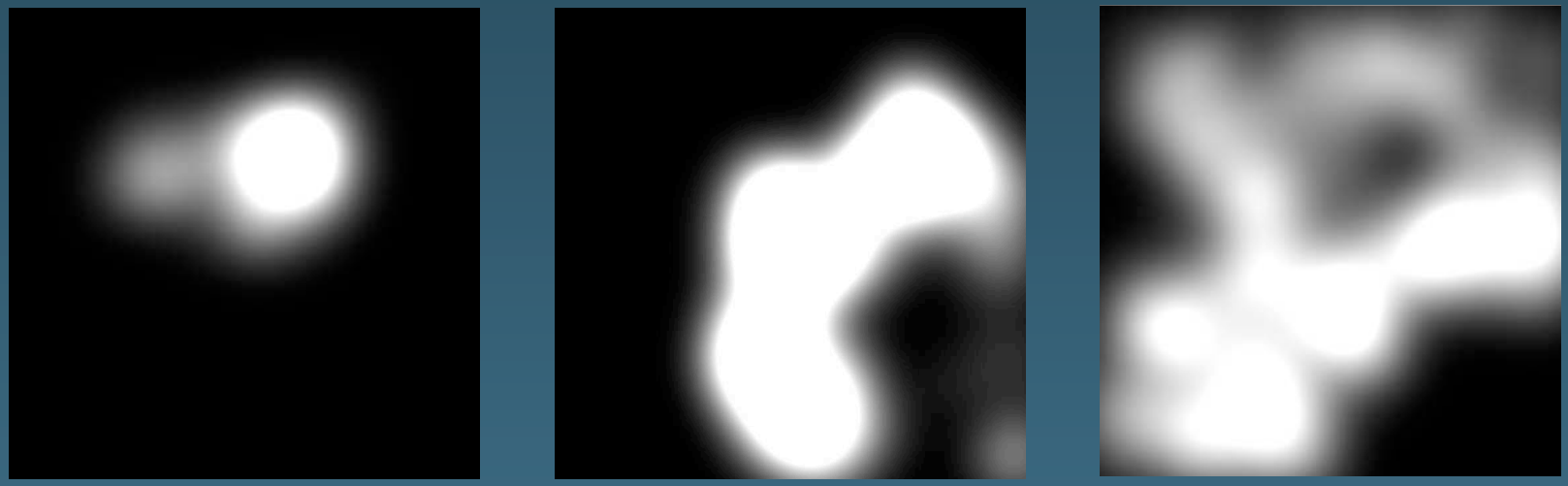
Weak attachment - patches
Medium-strength attachment - waves
Strong attachment - uniform coverage

Dynamic Phases of F-Actin

Patch

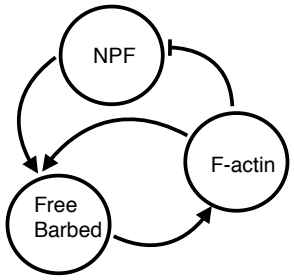
Wave

Random

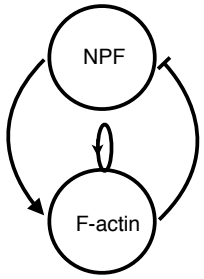


Increasing actin concentration

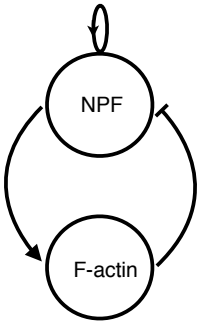
Possible NPF-Actin Feedback Loops



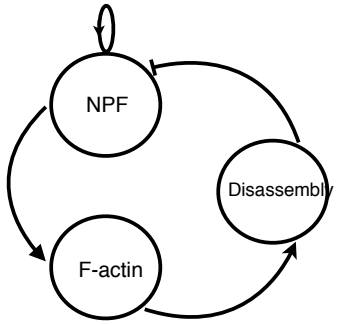
Stochastic-growth simulations



Whitelam et al, 2009

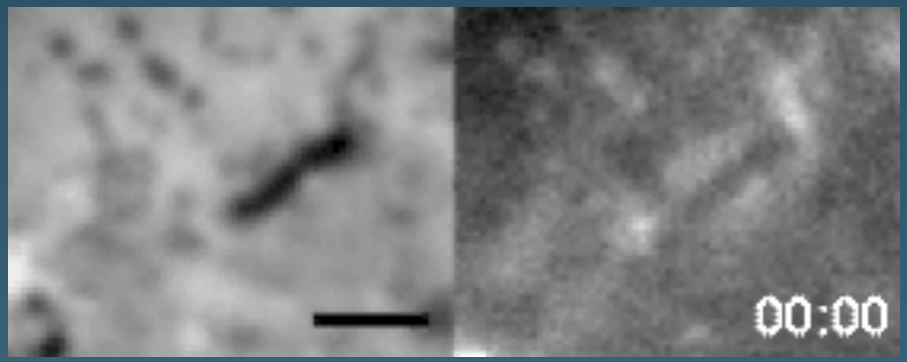


Weiner et al 2007;
Doubrovinski and Kruse 2008



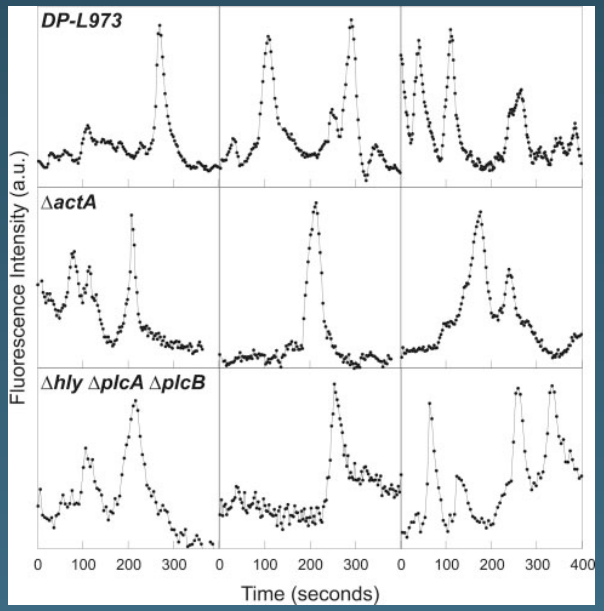
Possible model for actin dynamics
in endocytosis

Listeria Phagosomes Can Exhibit Spontaneous Oscillations



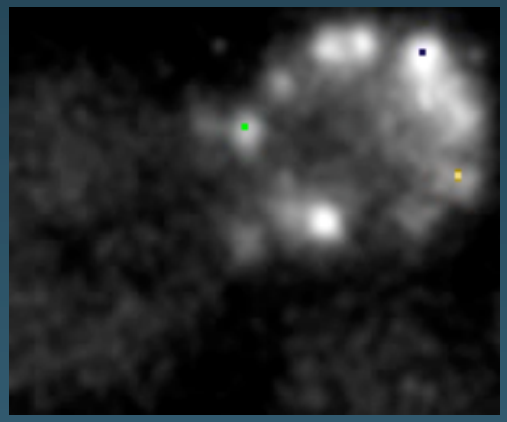
Phase GFP-actin

(Theriot lab 2004)



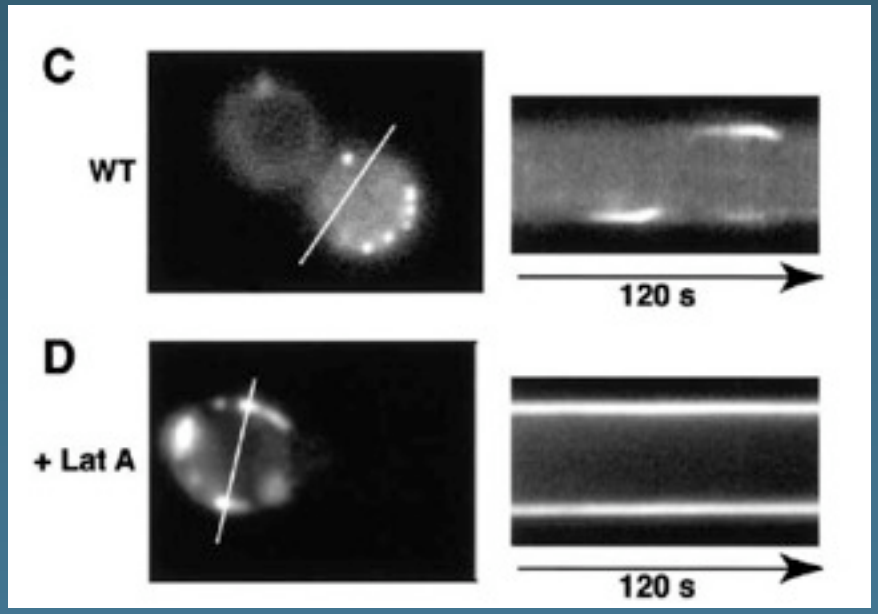
Could these be due to a similar combination of positive and negative feedback?

Does Such a Mechanism Describe Dynamic Actin Patches in Yeast?



(Cooper lab 2006)

Fluorescence of a pre-NPF (Sla1)



(Kaksonen et al 2003)

Sla1 patch disassembly requires F-actin

But patch assembly does not require F-actin

Modeling a Transient Actin Patch

NPF

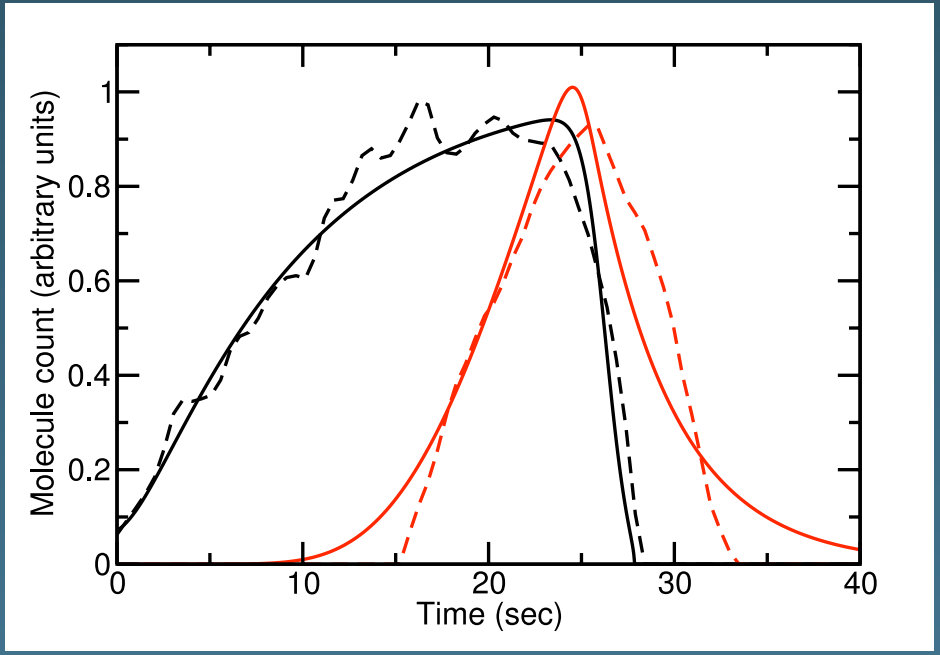
F-actin

Disassembly Proteins

$$\frac{d[N]}{dt} = k_N^+(N_0 - [N]) - k_N^+ N_0 \exp[\varepsilon_s(1/\sqrt{N} - 1/\sqrt{N_c})] - k_N^-[D]^i[N]$$

$$\frac{d[F]}{dt} = k_F^+[N]^j - k_F^-[F]$$

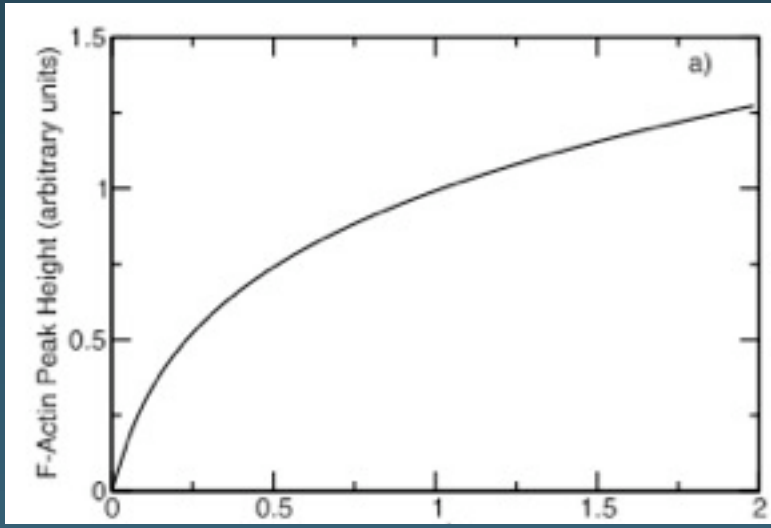
$$\frac{d[D]}{dt} = k_D^+[F] - k_D^-[D]$$



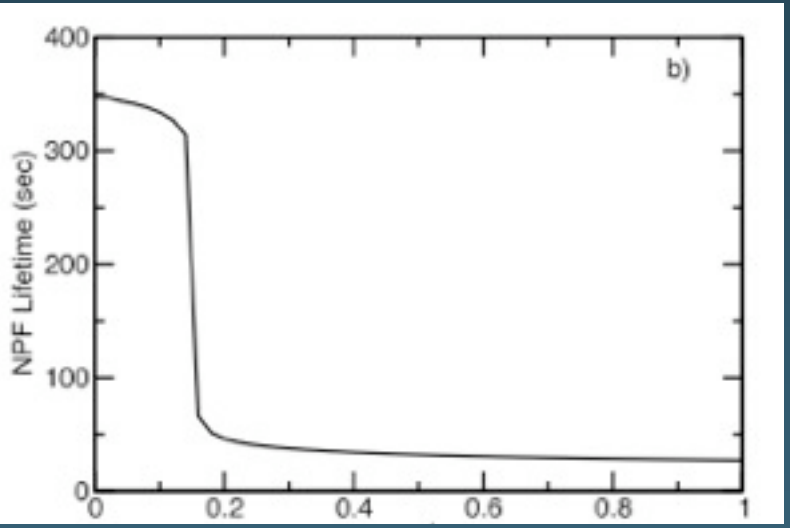
Fit to experimental time courses from Drubin lab

Black: NPF
Red: actin

Effect of Branching Rate on Patch Properties



Branching rate



Branching rate

Actin peak height increases slowly with branching rate - relates to NPF mutation experiments?

NPF lifetime drops abruptly with branching rate

Conclusions

Known actin biochemistry leads to spontaneous formation of waves and patches

Characteristic transitions in dynamic behavior are seen with varying actin concentration

Negative feedback of F-actin on membrane proteins might be a common mechanism leading to oscillating or transient behavior

Currently working on including myosin in network model

Supported by joint DMS/NIGMS initiative in mathematical biology