

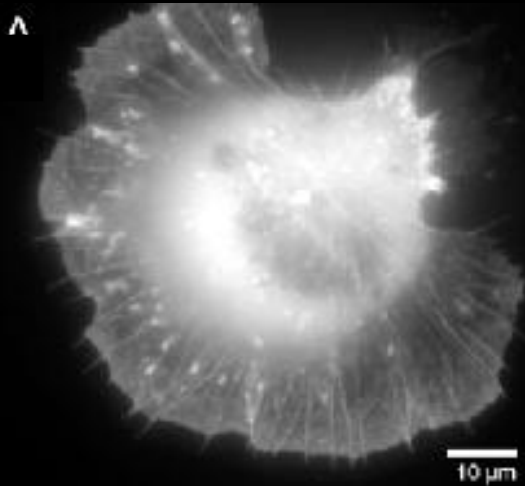
Assembly of the actin cytoskeleton for cell division and motility

Dimitrios Vavylonis

Department of Physics, Lehigh University

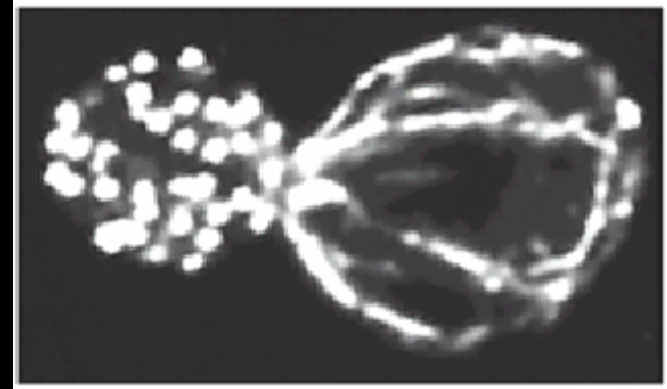
Multidisciplinary Workshop in Biomedical Research
Hellenic Bioscientific Association in the USA
Joseph B. Martin Conference Center, Harvard University
Oct 10, 2009

Actin-driven cell shape changes



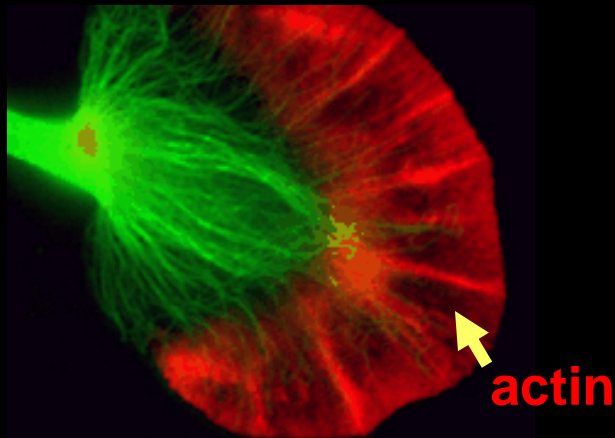
motile fibroblast (GFP-actin)

Watanabe lab, Kyoto Univ.



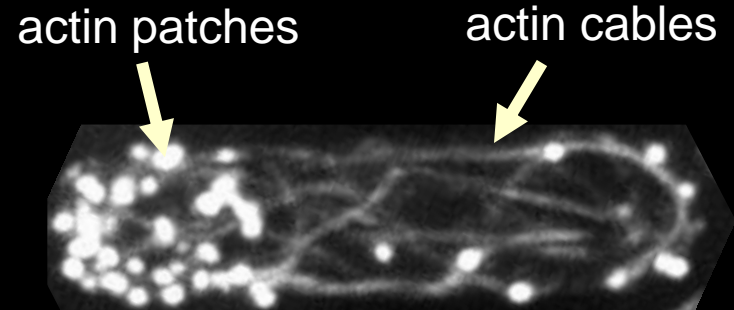
budding yeast

Amberg Mol. Biol. Cell (1998)



Neuron growth

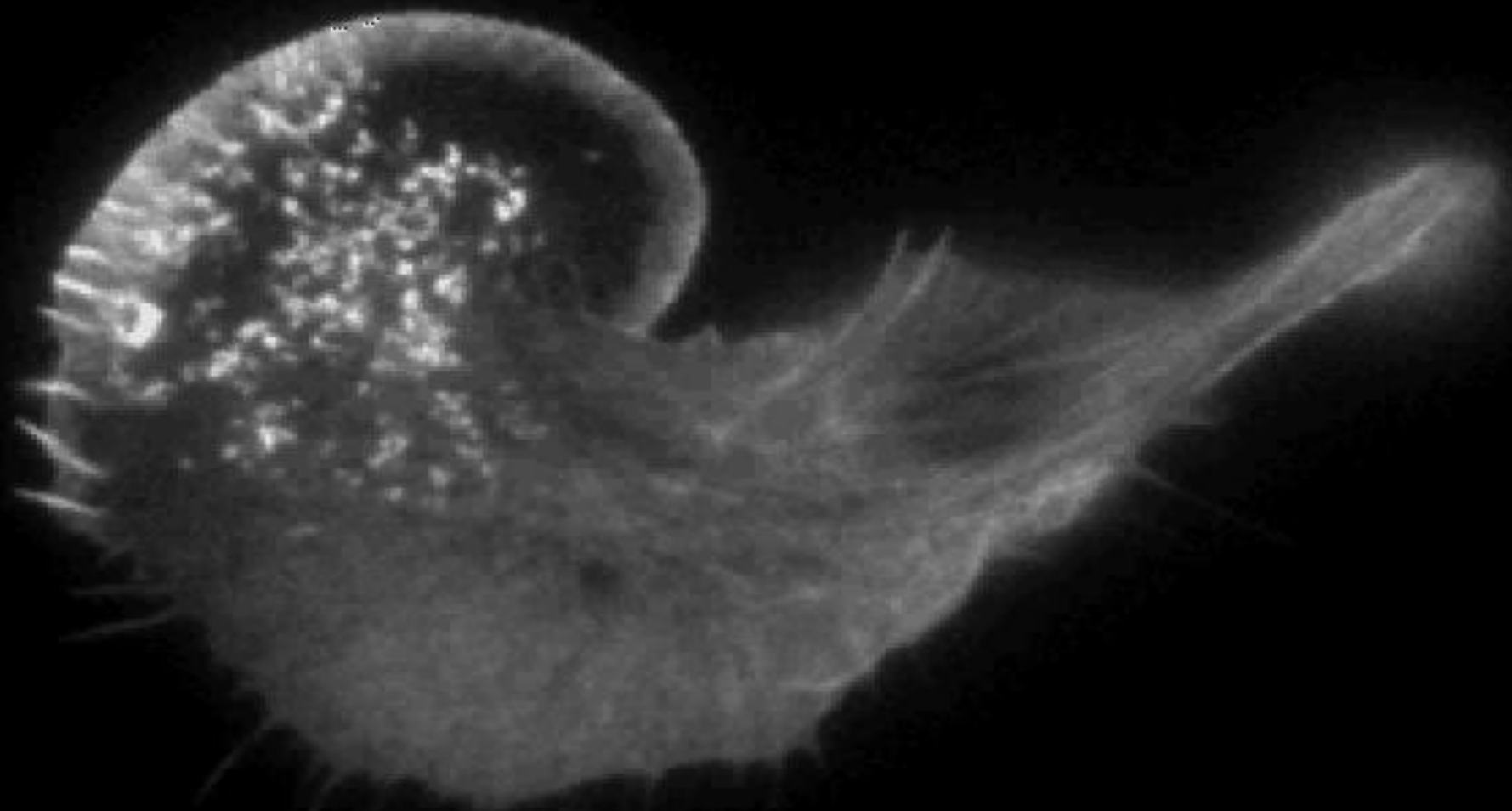
Forscher lab, Yale Univ.



fission yeast

Wu lab, Ohio State Univ. 2007

Actin-GFP



Motility of cancer cells causes metastasis

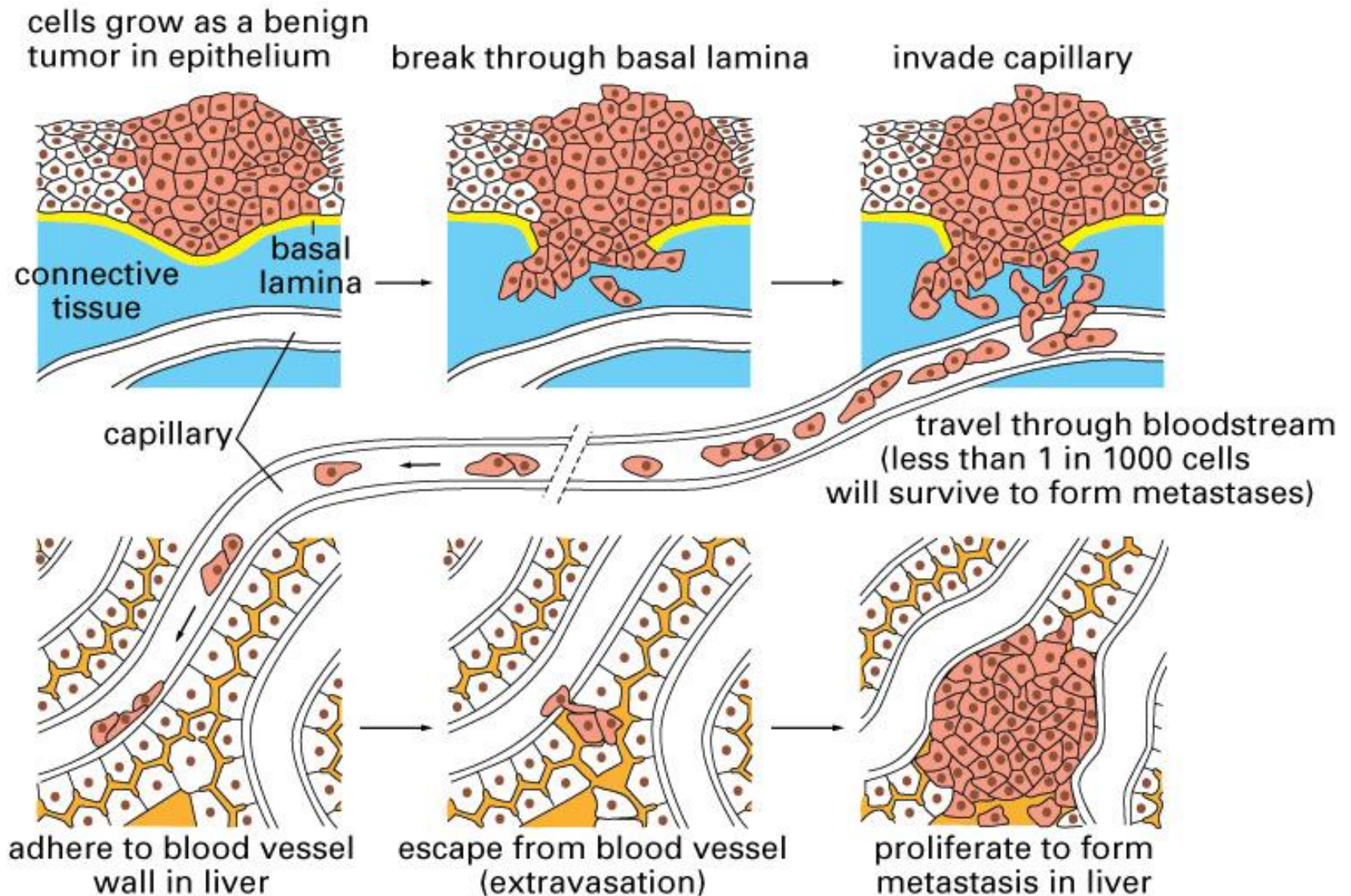
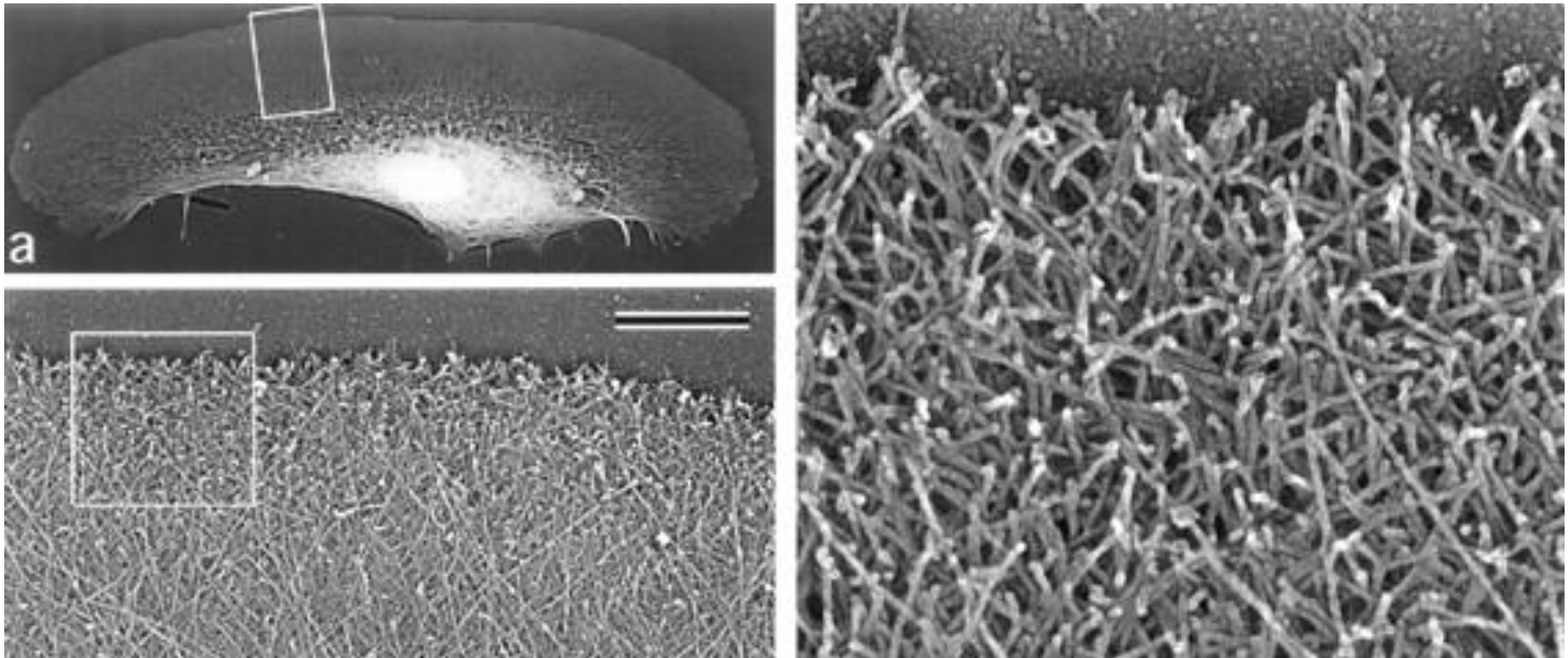


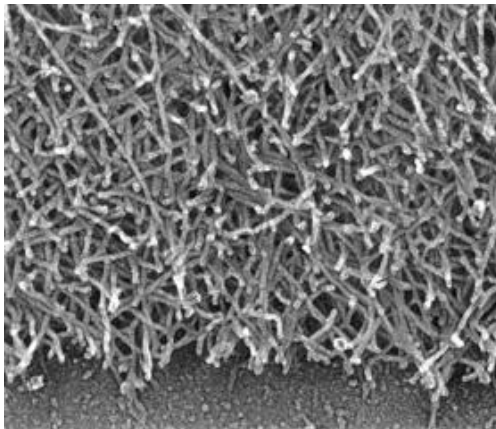
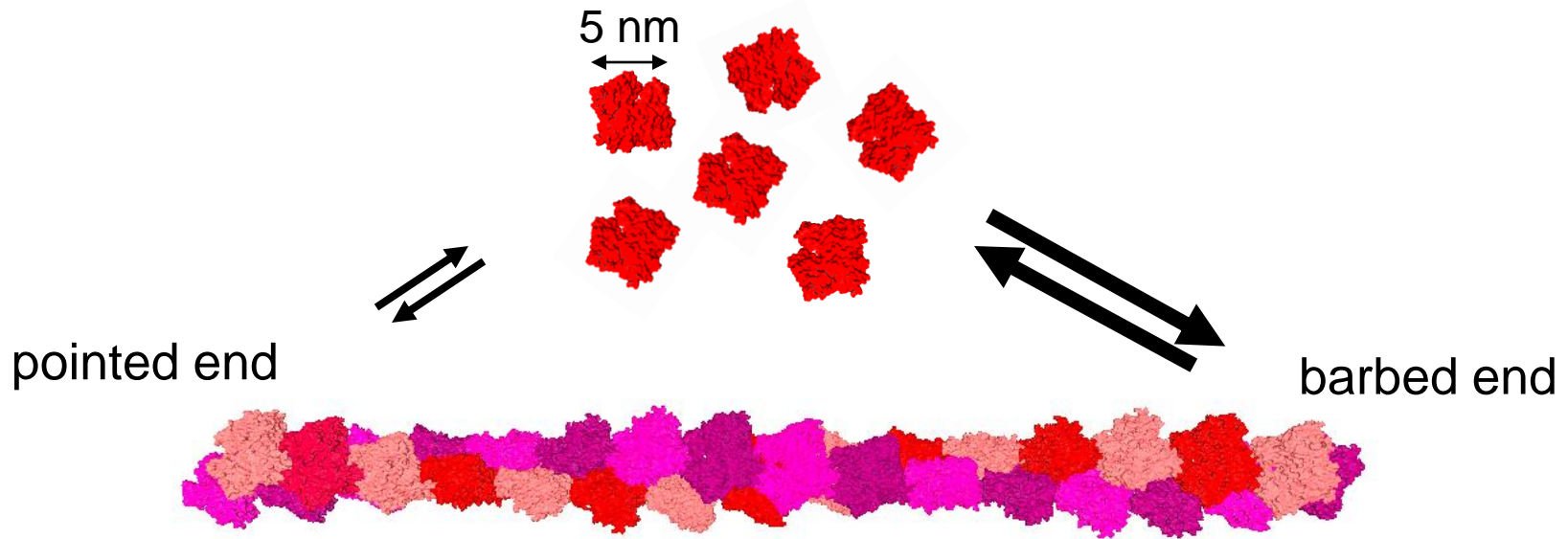
Figure 23-15. Molecular Biology of the Cell, 4th Edition.

Actin network within a motile cell



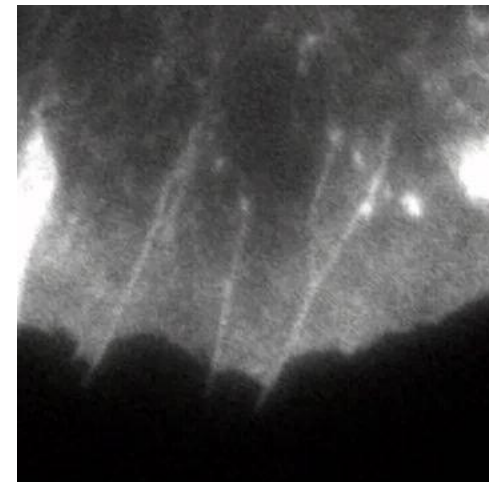
Svitkina et al. *J. Cell Biol.* **139** 397 (1997)

Actin polymerization: driving force for cellular motions



Svitkina et al *J. Cell Biol.* **139** 397 (1997)

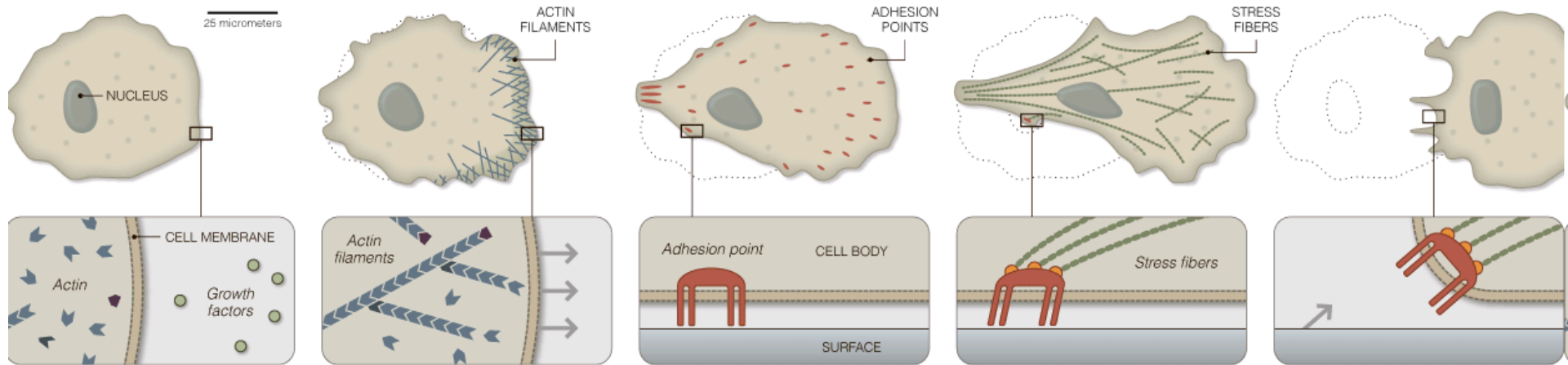
~5 μm



fibroblast
EGFP-actin
movie
duration 156 sec

Watanabe and Mitchison,
Science, **295** 8 (2002)

Stretch, Grab, Pull Cells move by repeatedly extending and contracting their cytoskeleton, a malleable internal structure of tiny filaments and fibers.



STIMULATION Above, a single fibroblast cell rests on a flat surface. Fibroblasts are active in wound healing, and can be triggered to move by growth factors released at the site of a wound.

PROTRUSION The cell begins to move by generating a mesh of actin filaments. Actin molecules are added to the tips of each filament faster than they can be removed from the roots, and the combined force pushes the cell membrane forward.

ADHESION As the cell shifts forward, it latches on to its surroundings by forming a series of small adhesion points along its leading edge. The cell also enlarges several adhesion points on its trailing edge, effectively anchoring the rear of the cell in place.

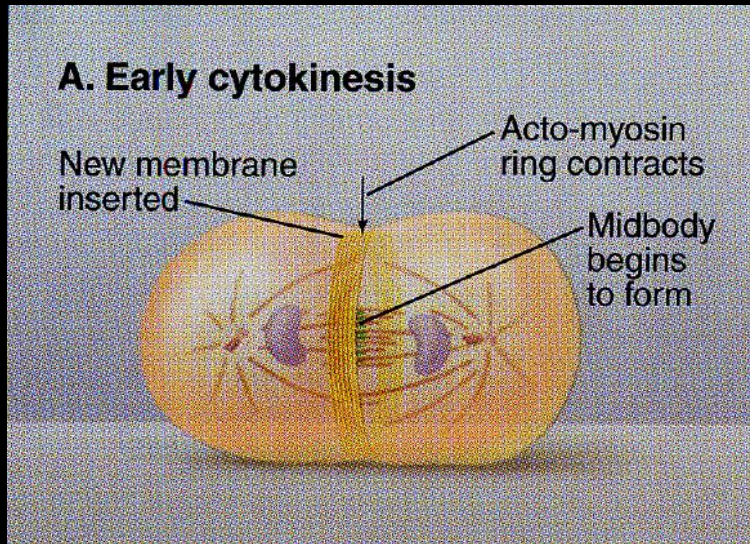
CONTRACTION As the leading edge continues to extend, the cell uses the newly formed adhesion points to pull itself forward with a network of stress fibers, contractable bundles of actin and myosin. Tension continues to build as the cell slides forward.

RETRACTION The cell finally releases its anchored trailing edge, and the stress fibers quickly pull the back of the cell forward. The cell has moved about one cell-width, and can repeat the process to keep crawling forward.

Sources: Clare Waterman, National Heart Lung and Blood Institute; Cell Motility and the Cytoskeleton; Nature

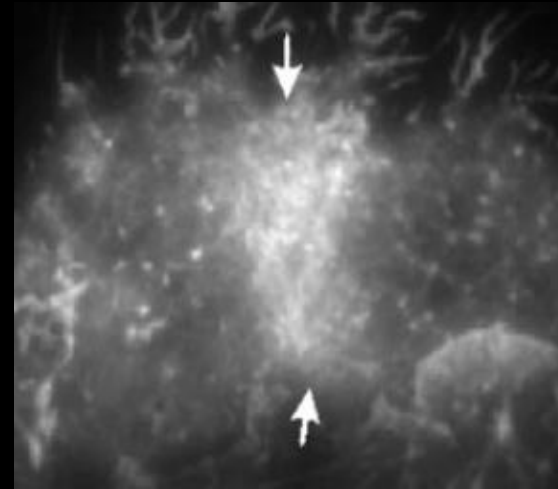
<http://www.nytimes.com/2009/06/09/science/09cell.html>

Actin Cytoskeleton in Cell Division



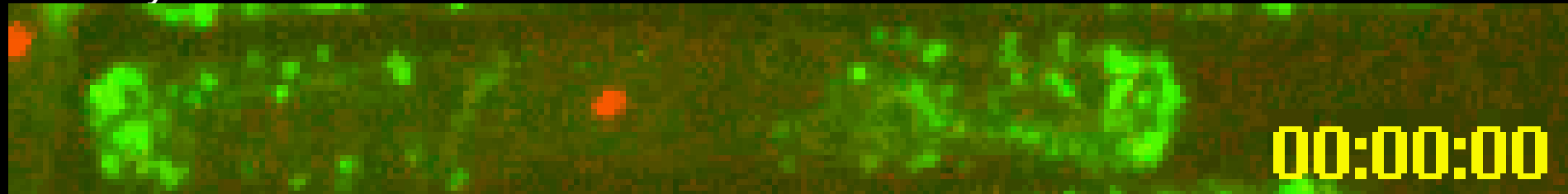
Pollard and Earnshaw, "Cell Biology" (2002)

GFP-actin kidney cell



Zhou and Wang
Mol. Biol. Cell 2008

fission yeast cdc25-22 cell

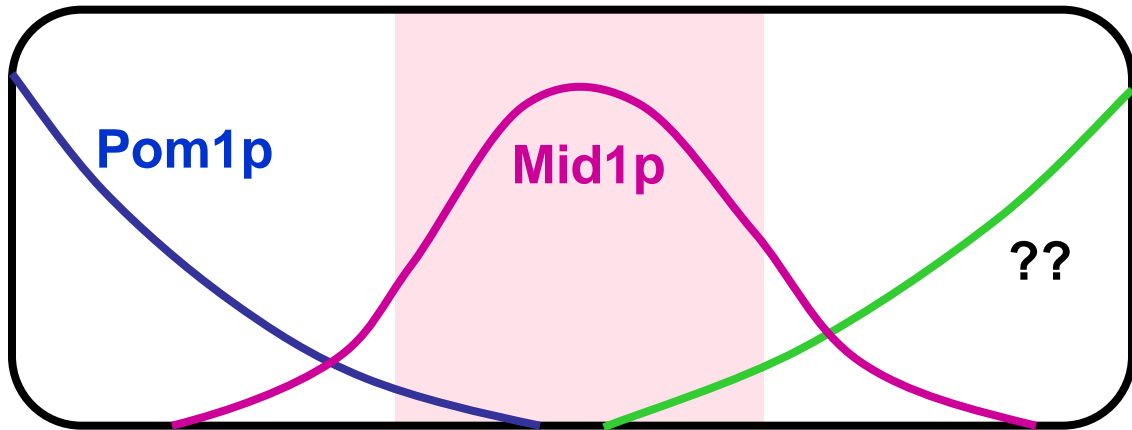
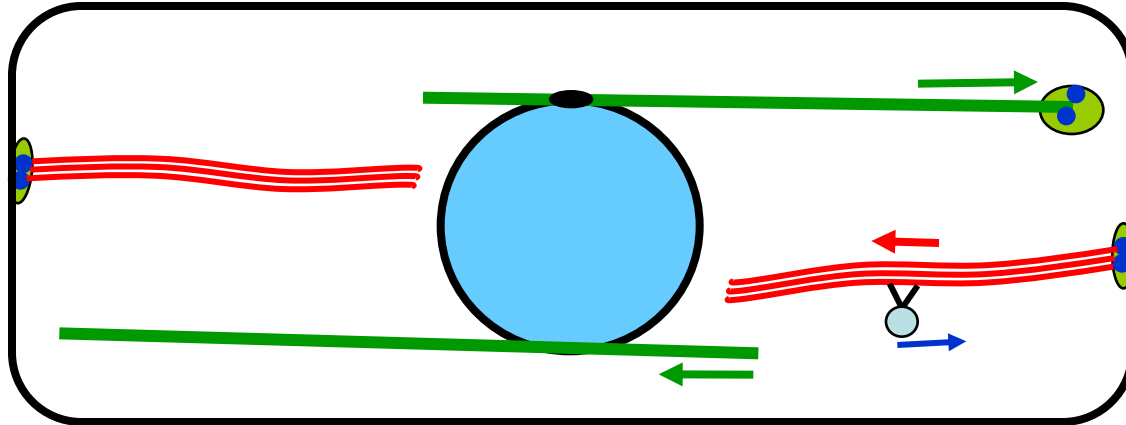


CHD-GFP
binds to sides of
actin filaments

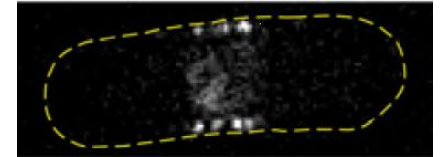
spindle poles
Spb1

Jian-Qiu Wu (Pollard lab, Yale Univ 2007)
Vavylonis, Wu, et al. *Science* 2008

Cell polarization and establishment of cell center

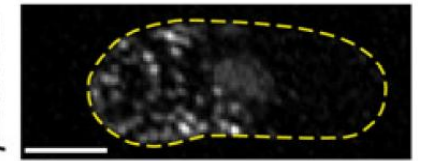


wild type



mid1-GFP

pom1 Δ



Padte et al. Curr. Biol 2006

cytoplasmic
concentration
gradients?

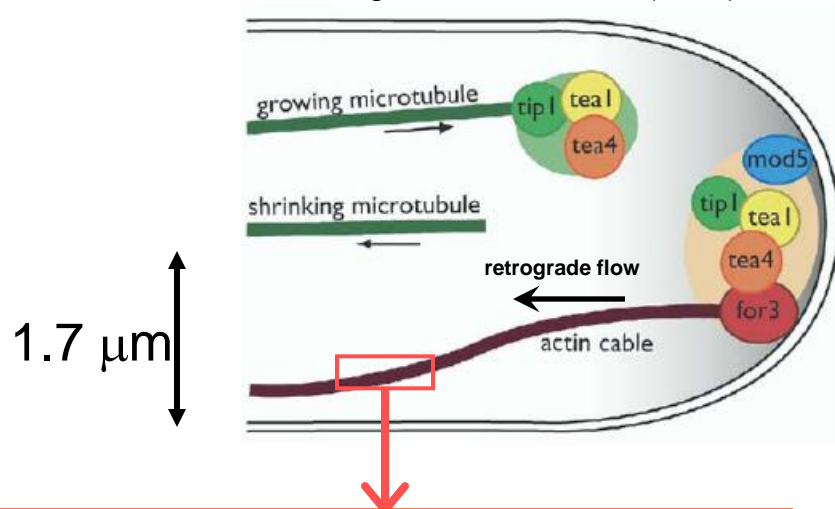
Padte et al. Curr. Biol 2006

Celton-Morizur et al. J. Cell Sci. 2006

Wu et al Dev Cell 2003

Formin For3p and actin cable assembly

Martin and Chang, *Dev. Cell*, **8**, 479 (2006)

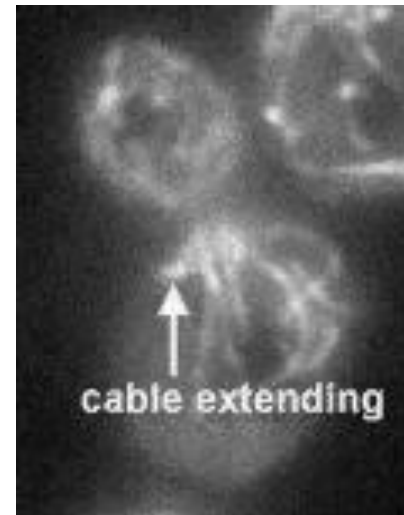


Kamasaki et al., *Nature Cell Biol.*, **7**, 916, (2005).

EM: bundles of ~ 10 actin filaments

short filaments ~ 100 subunits
actin filament: 370 sub/μm

actin cables: bundles of actin filaments
nucleated by formin For3p

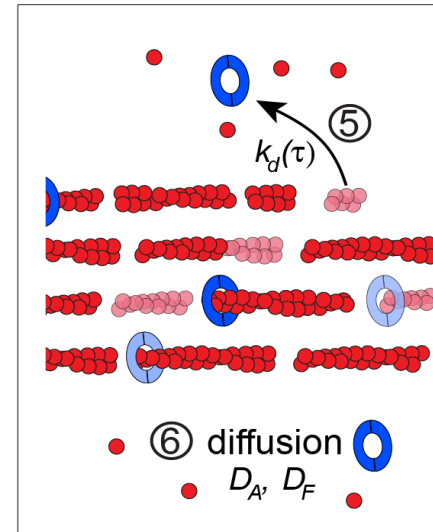
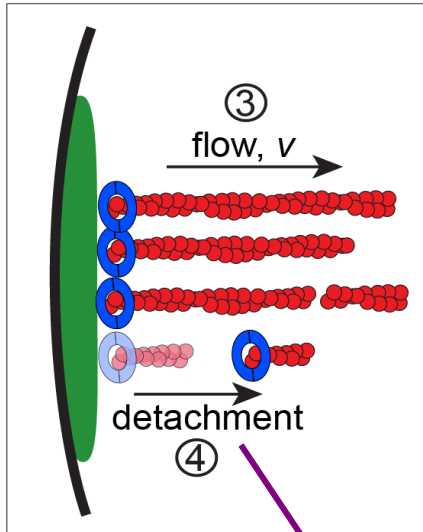
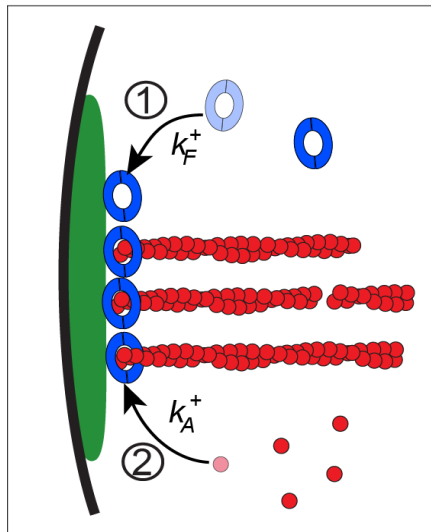
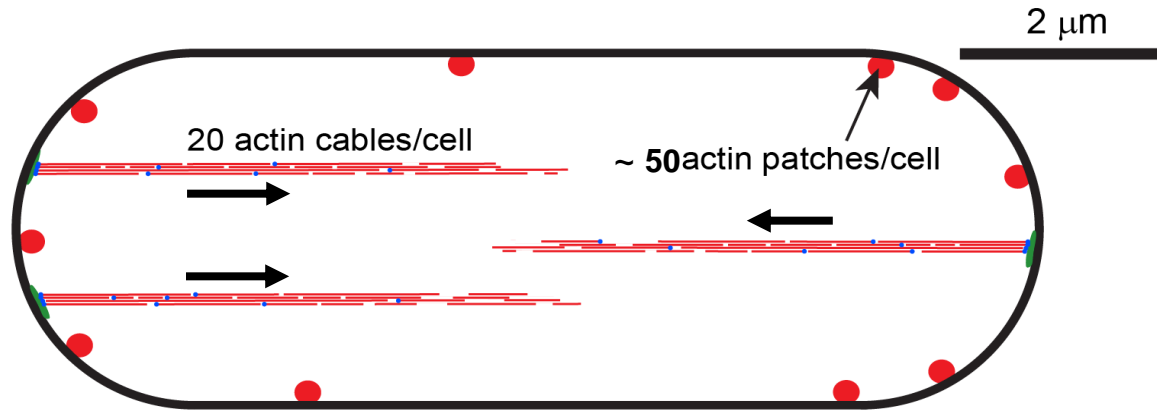


budding yeast cables nucleated by formins Bnr1p, Bni1p

Yang and Pon *PNAS* (2002)

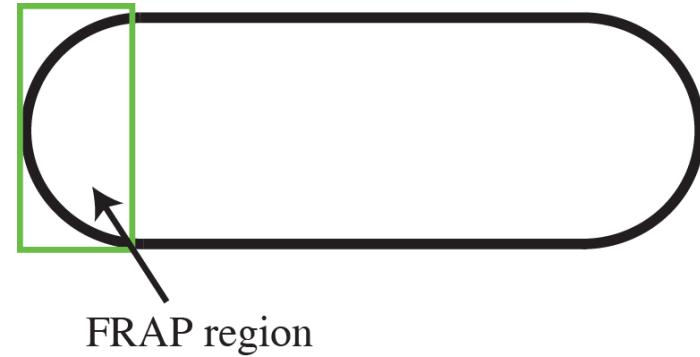
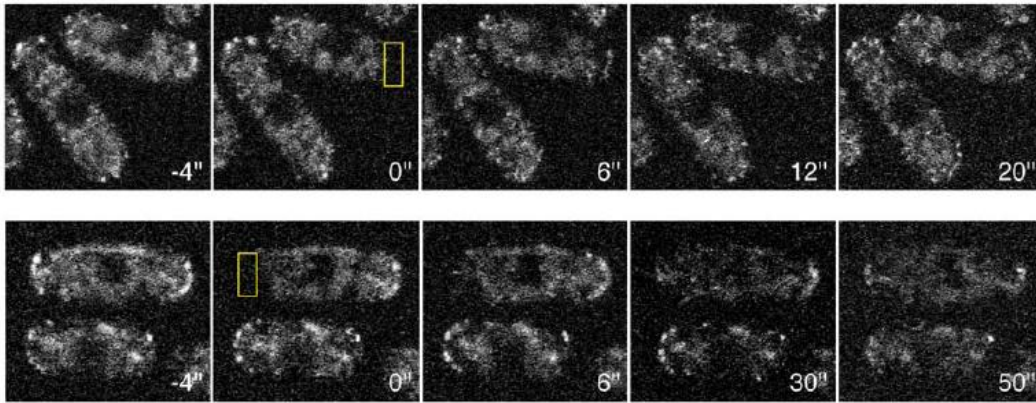
$v \sim 0.3 \mu\text{m}/\text{sec} \sim 110 \text{ actin subunits}/\text{sec}$

Actin cable turnover model



depends on actin polymerization

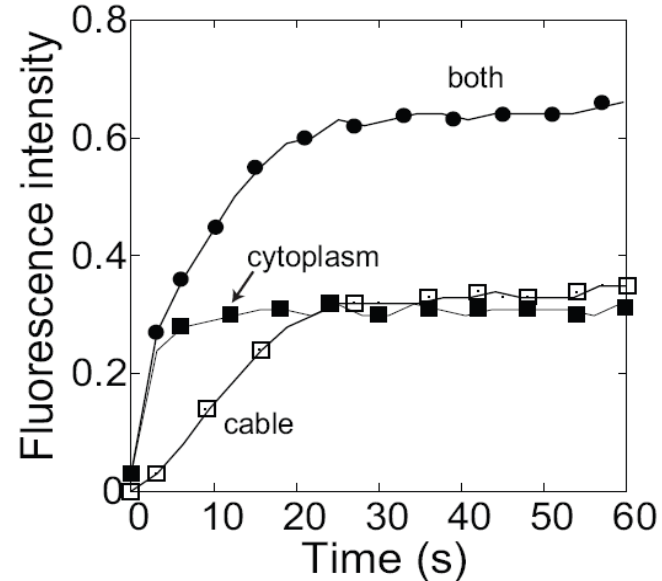
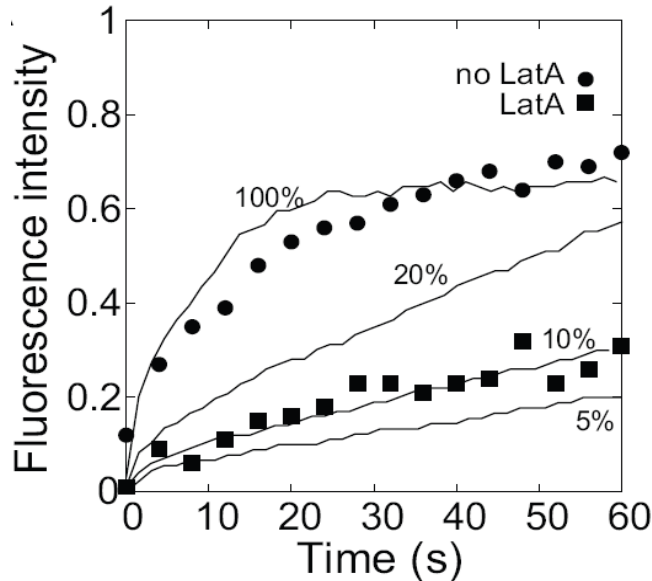
Fluorescence recovery after For3p photobleaching



LatA: sequesters actin

Martin and Chang (2006)

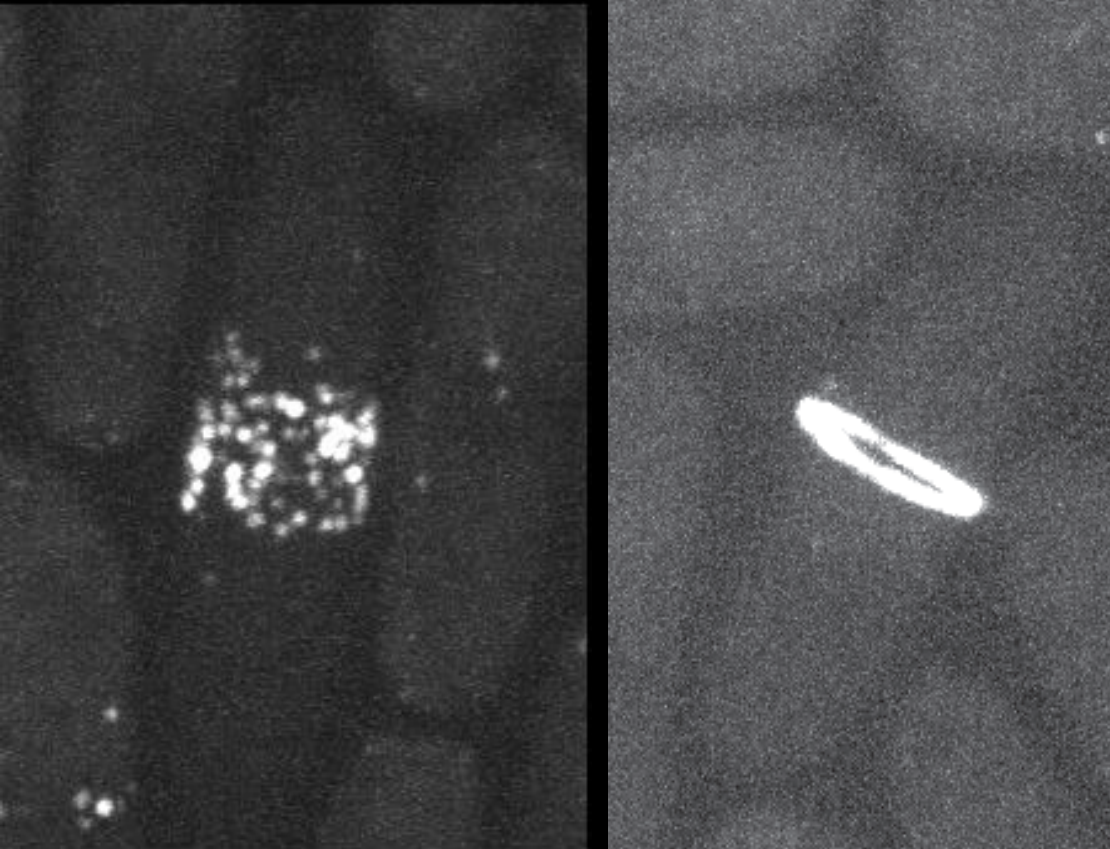
comparison of simulated recovery to experiment:



Contractile ring assembles from ~ 63 myosin II nodes in ~ 10 min

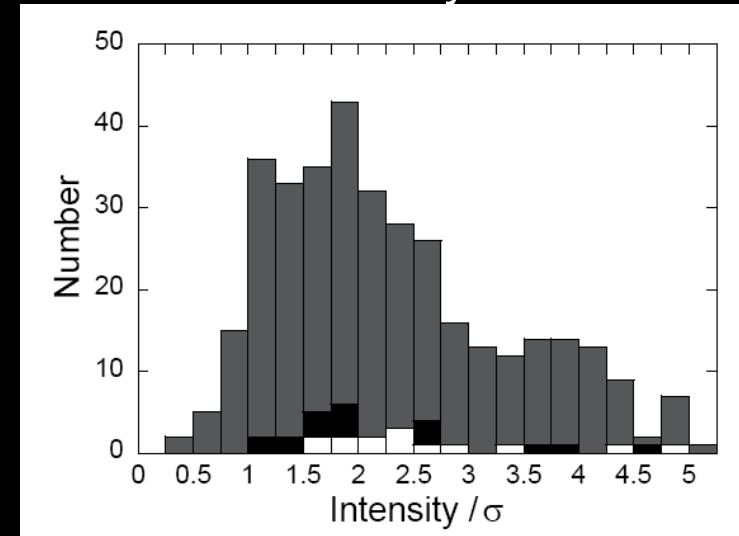
Vavylonis, Wu, Hao, O'Shaughnessy, Pollard, *Science* 2008

Rlc1p-3GFP



spinning disk confocal microscopy

Node intensity distribution

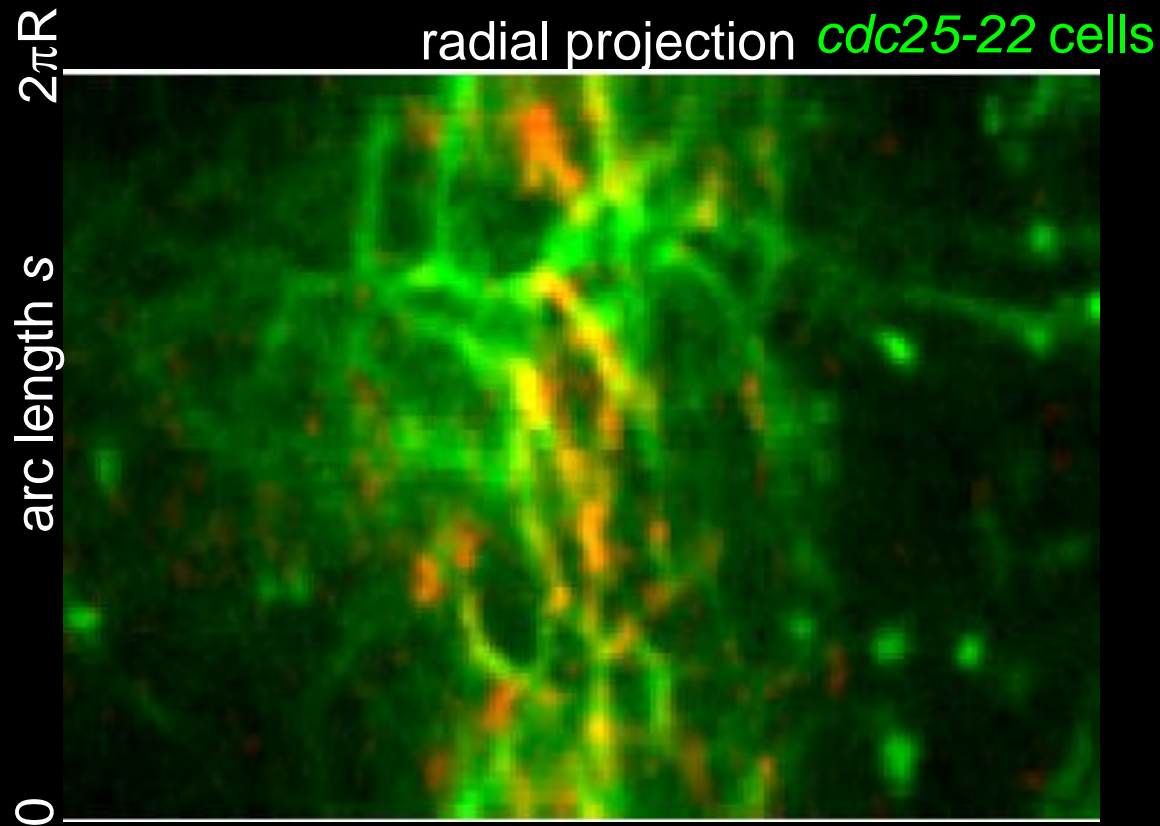
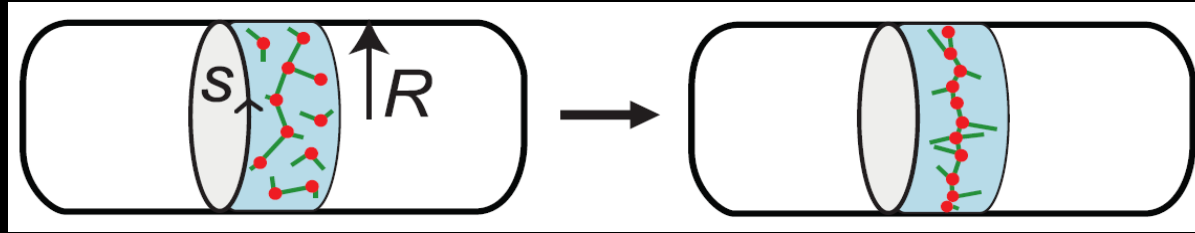


~ 40 myosin II (Myo2p) molecules/node

~ 2 formin Cdc12p dimers/node

Wu and Pollard, *Science* 2005

Actin meshwork establishes connections among nodes



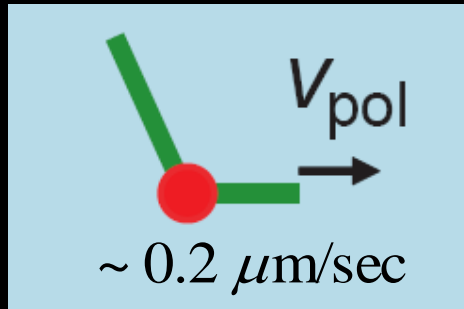
red: nodes (Rlc1-RFP1)

green: actin filaments (GFP-CHD)

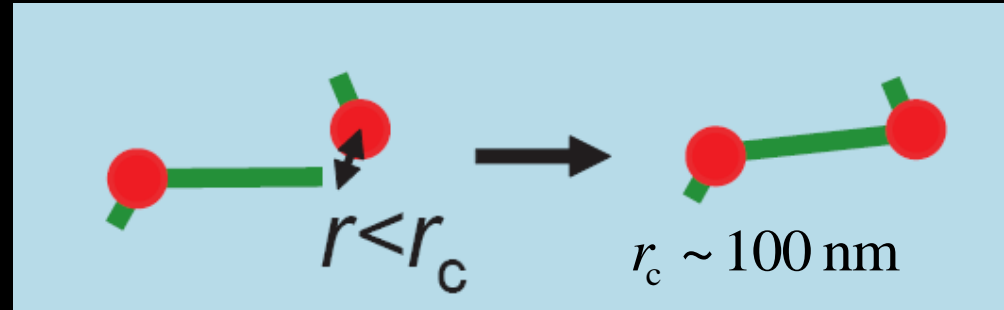
data: Wu (2007, 2008)

Search, capture, pull and release model

actin filament
polymerization



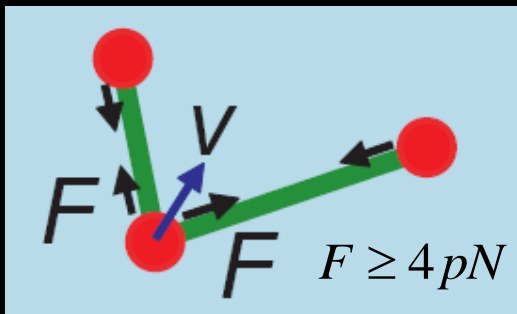
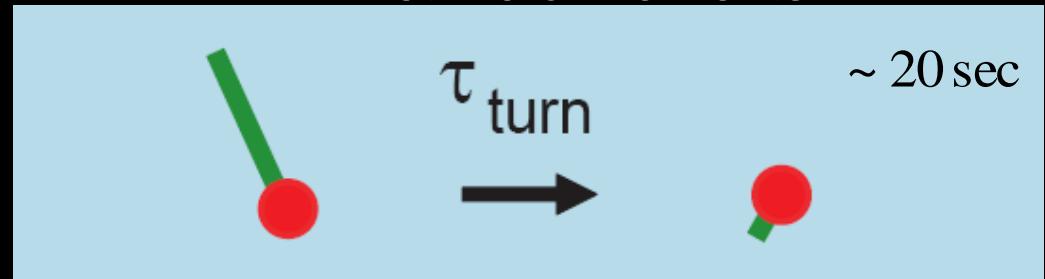
actin filament capture



lifetime of connections



lifetime of filaments



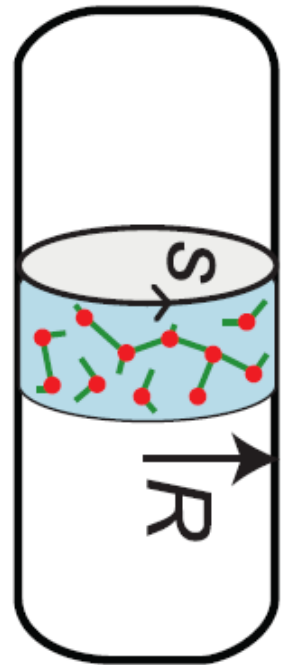
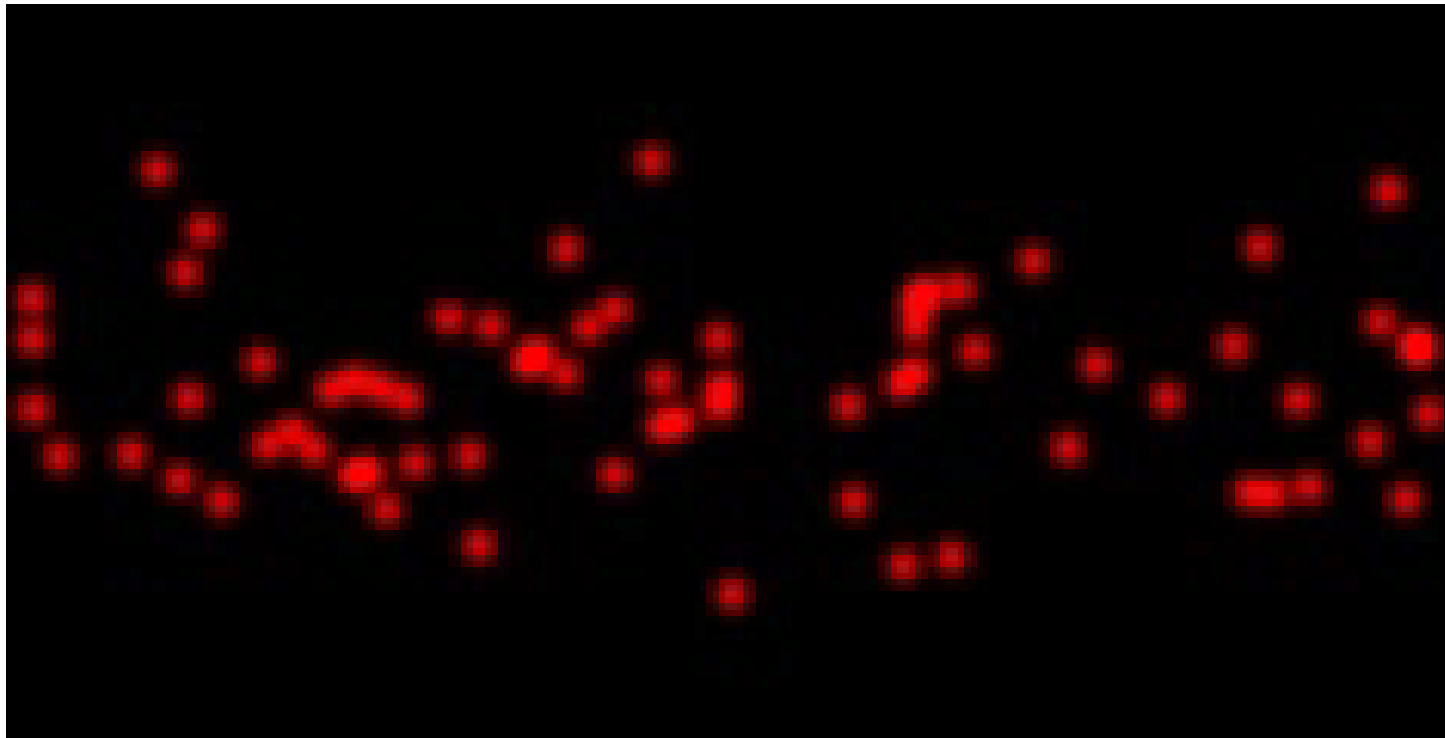
traction on filaments
between nodes

Dynamic reestablishment of connections \rightarrow plasticity of network

Simulations with search, capture, pull and release

Simulated radial projection

red: nodes
green: actin



0

30x time lapse,
20min

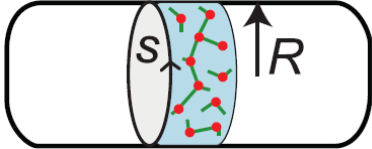
$2\pi R$

experiment:

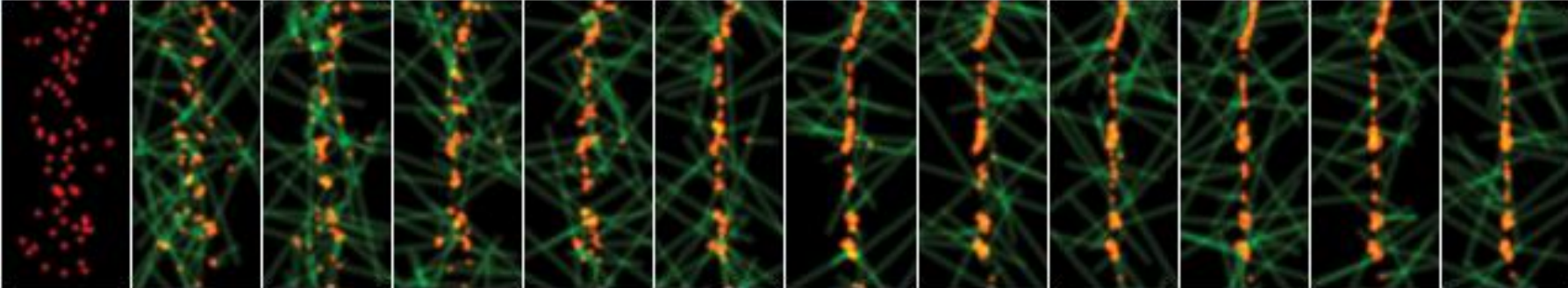


- *model reproduces many observed features*

Dependence on parameter values

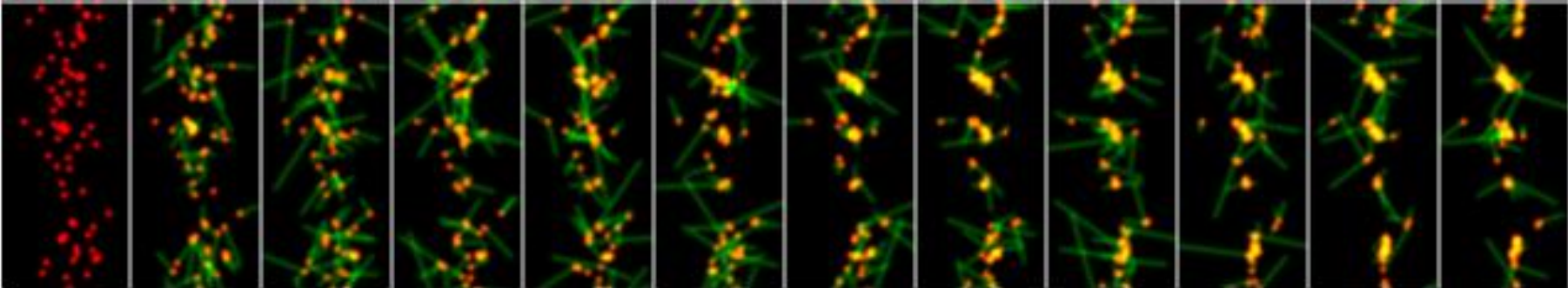


$$v_{pol} = 0.2 \mu\text{m/sec}$$



red: nodes
green: actin filaments

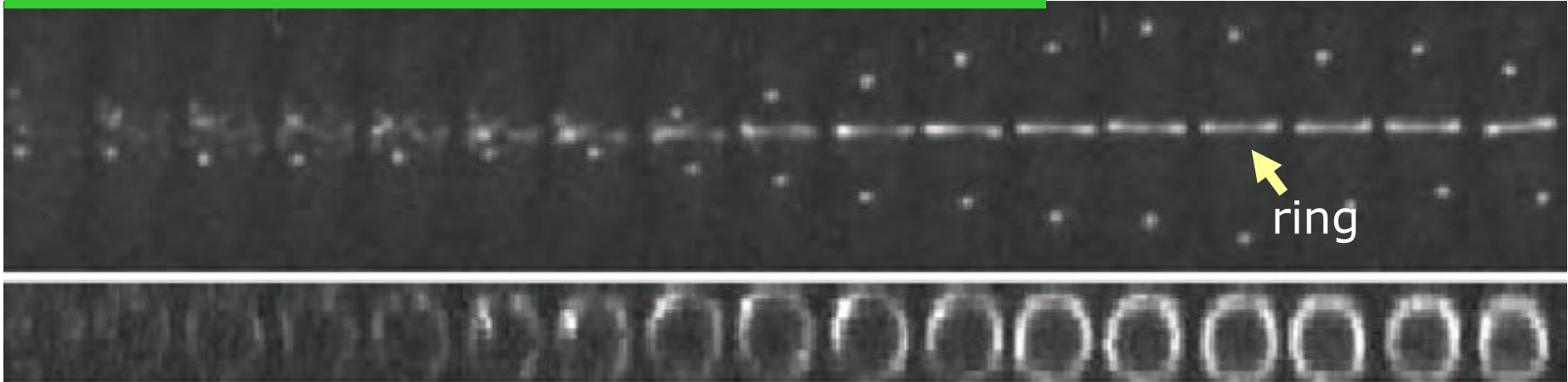
$$v_{pol} = 0.04 \mu\text{m/sec}$$



Some mutant cells form clumps

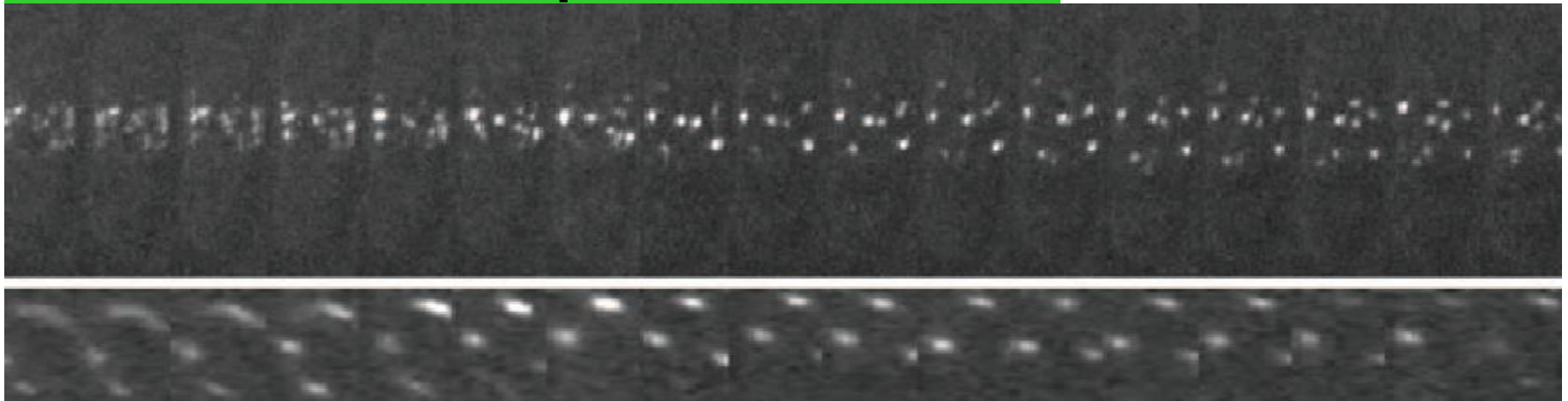
Wild type: robust ring formation

Rlc1p-GFP
Cdc11p-GFP



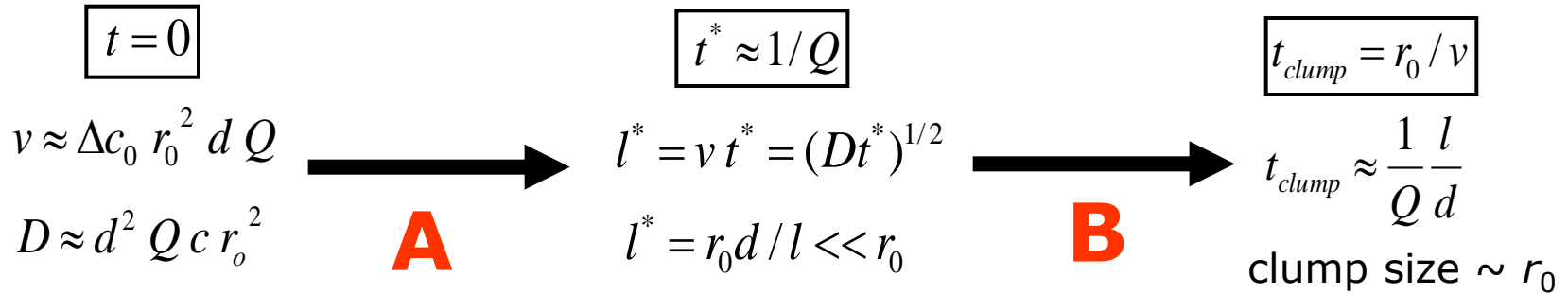
Formin Mutant: clump formation

Rlc1p-GFP
cdc12-112

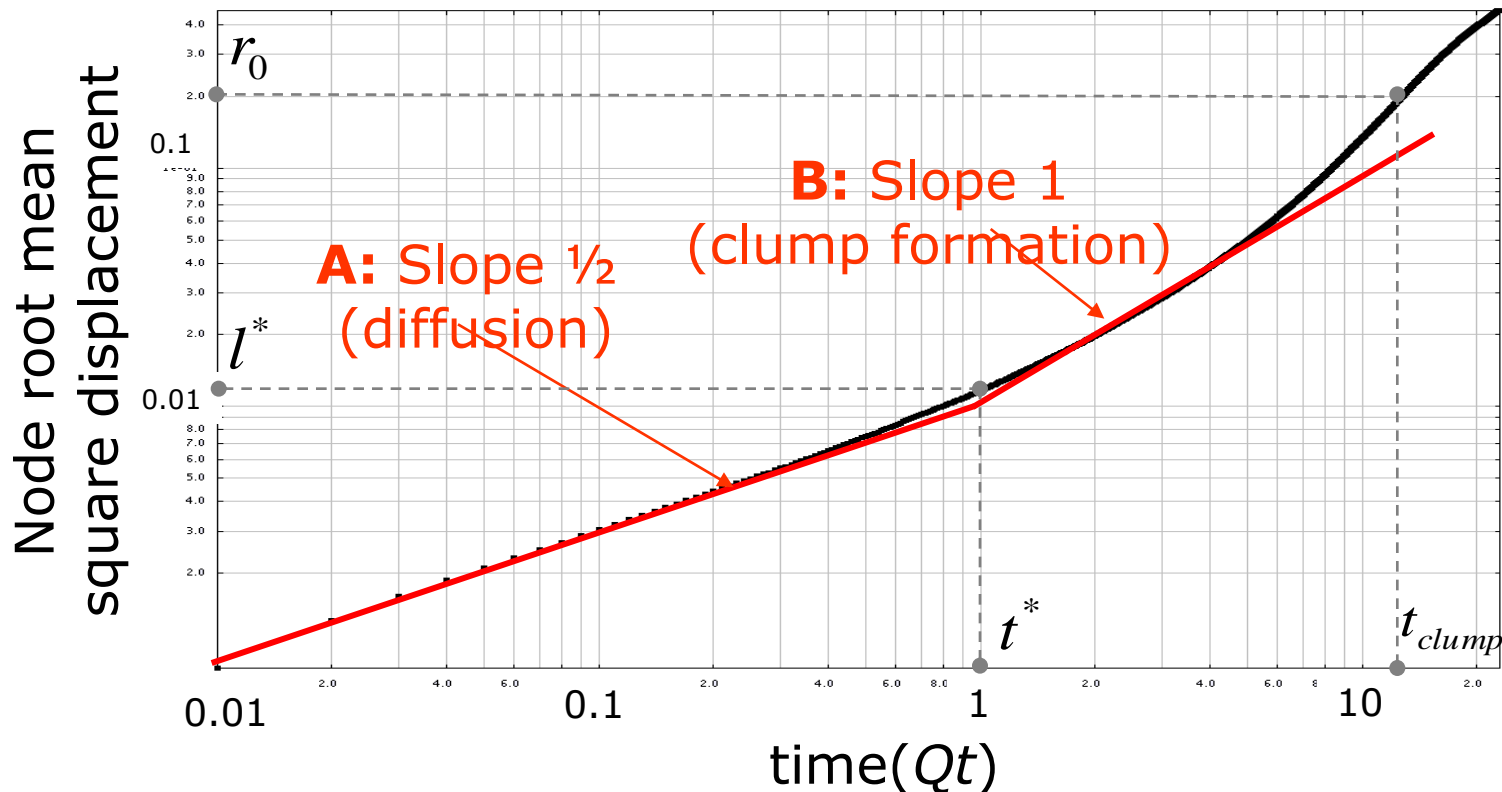


Clump formation kinetics

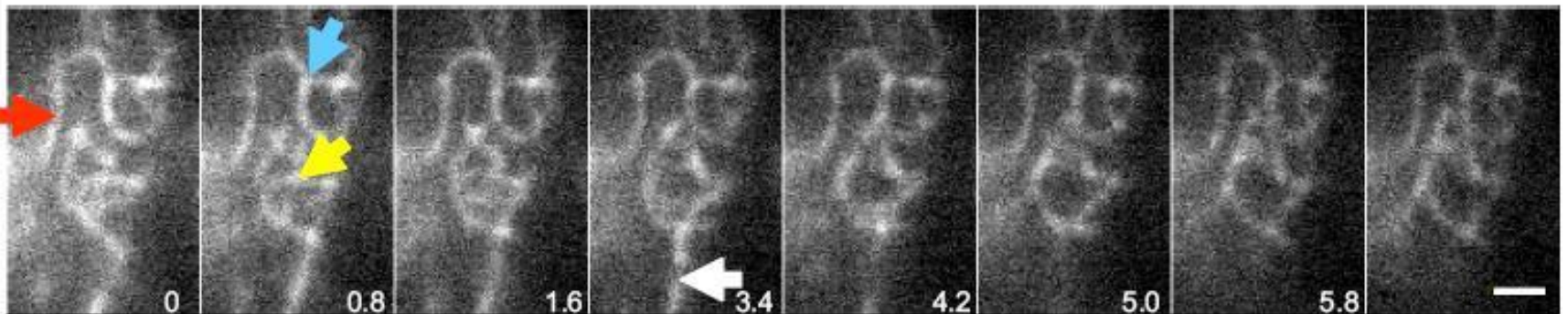
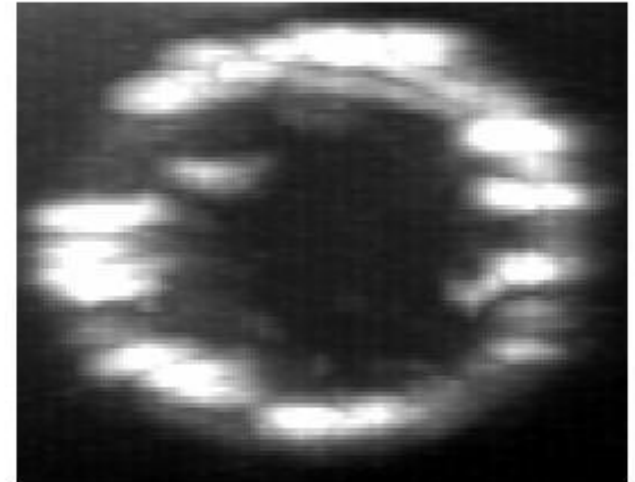
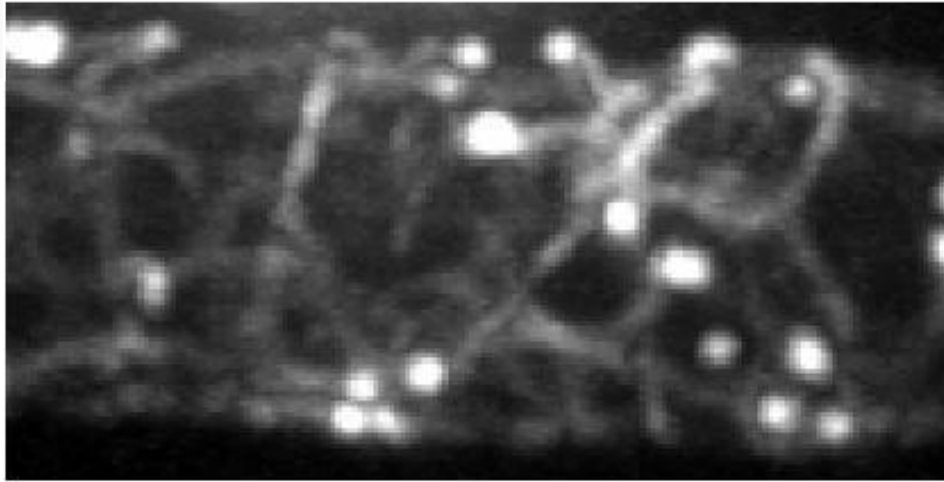
- Scaling arguments:



- Monte Carlo Simulation of 2D bulk of nodes



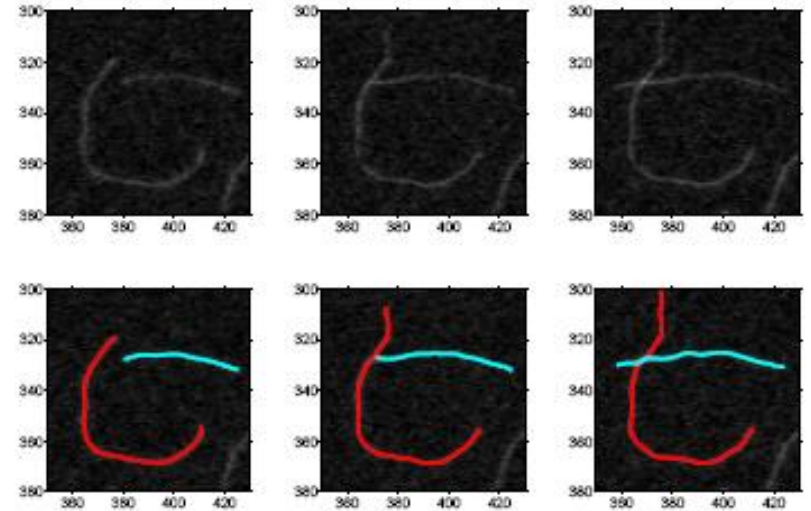
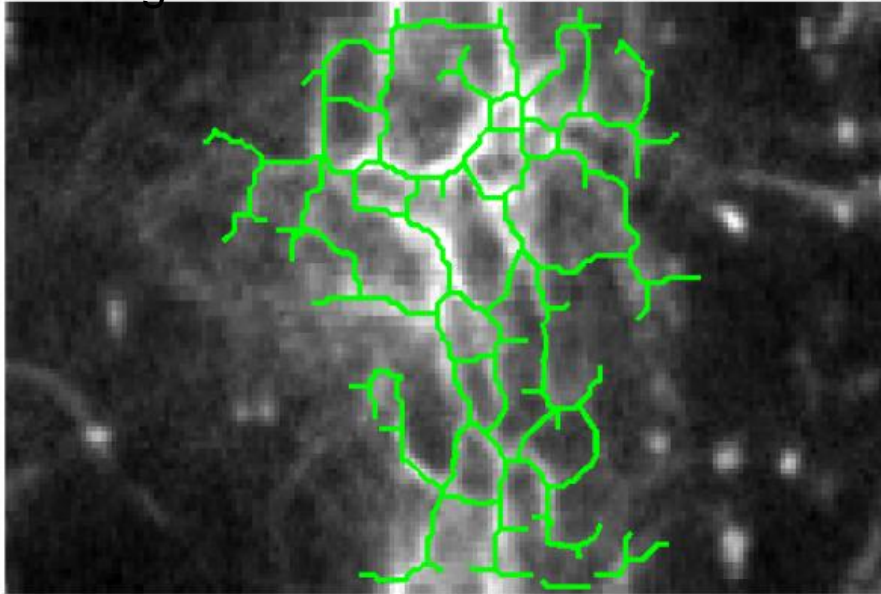
Actin meshwork in the middle of a dividing yeast cell



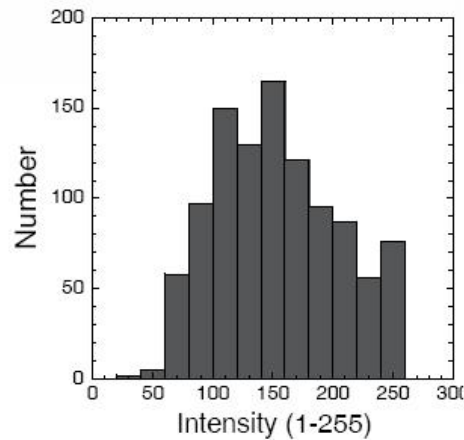
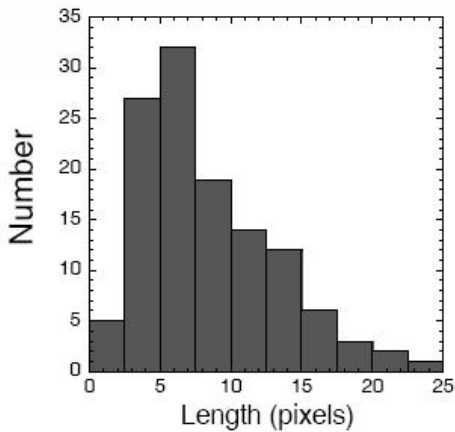
4D confocal microscopy experiments (Jian-Qiu Wu, Ohio State)

Systematic image analysis of actin in cells and in vitro

Actin filament network in the middle of dividing cell



Tracking of polymerizing actin filaments in vitro
Li et al. (ISBI 2009, MICCAI 2009)



skeletonization
(ridge point detection)

Acknowledgments

Hui Wang, Nikola Ojkic, Matthew Smith, Tyler Drake

Department of Physics,
Lehigh University

Xiaolei Huang

Department of Computer Science,
Lehigh University

Jian-Qiu Wu

Department of Molecular Genetics,
Ohio State University

Thomas Pollard

Department of Molecular, Cellular and Developmental Biology,
Yale University

Ben O'Shaughnessy

Department of Chemical Engineering, Columbia University

Support: HFSP, NIH, Lehigh University