Modeling cytoskeleton self-assembly

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Cell organization

- How does the cell achieve internal organization?
- How is it regulated?
- Can we model cell structure and dynamics?

http://mgl.scripps.edu/people/goodsell/gallery/patterson.html
Thermal and viscous forces are very large on the scale of proteins

\[ v_{\text{protein}} \approx 9 \text{ m/sec} \]

\[ t = 0 \quad t = 3 \text{ psec} = 3 \times 10^{-12} \text{ sec} \]

in this time the protein travels

0.027 nm \ll \text{ protein size } \sim 4 \text{ nm}

The result of this is diffusion
Modeling diffusion: random walk on a lattice

computer picks a random direction (up/down/left/right) with equal probability at each step (a “Monte Carlo” method)

“drunkard’s walk”
Brownian motion, continued

\[ x(t) = \text{constant} \cdot (D \cdot t)^{1/2} \]

Diffusion coefficient \( D \) for a protein in the cell \( \sim 4 \ \mu\text{m}^2/\text{sec} \)

5 \( \mu\text{m} \)

\( t = 0 \) \hspace{1cm} \text{fraction of a sec} \hspace{1cm} \text{several sec} \)
Polymerization of proteins to filaments

+ cross-links \rightarrow \text{rigid, stationary cytoskeleton}

Kovar, Harris, Mahaffy, Higgs, Pollard

Alberts et al, MBOC
Microtubule filaments and cell organization

Alberts et al, MBOC
Search and capture of chromosomes by microtubules during mitosis
GTP-cap model of dynamic instability

Alberts et al, MBOC
GTP-cap “toy model”

GTP-tubulin monomers diffuse in the cytoplasm at concentration $c$.

GTP-tubulin $\rightarrow$ microtubule filament

hydrolysis at blue/red interface, rate $k_{\text{hydro}}$

$GDP$-tubulin

Gillespie algorithm (a Monte Carlo method)
1. pick an event at random according to rate constants (polymerization, depolymerization, hydrolysis)
2. update configuration and time
Numerical simulations of the model

large GTP-tubulin monomer concentrations

small GTP-tubulin monomer concentrations

example of length trajectory at small concentrations:
Insights from simple model

Dynamic Instability of Individual Microtubules
Analyzed by Video Light Microscopy:
Rate Constants and Transition Frequencies
Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599-3280; and * Department of Anatomy, Duke University, Durham, North Carolina 27710

The Journal of Cell Biology, Volume 107, October 1988 1437–1448
Models of chromosome search and capture

Efficient Chromosome Capture Requires a Bias in the ‘Search-and-Capture’ Process during Mitotic-Spindle Assembly

R. Wollman,¹ E.N. Cytrynbaum,² J.T. Jones,³ T. Meyer,³ J.M. Scholey,¹ and A. Mogilner¹,4,*

Current Biology, Vol. 15, 828–832, May 10, 2005,
Actin filaments and cell shape changes

motile fibroblast (GFP-actin)
Watanabe lab, Kyoto Univ.

budding yeast

Neuron growth
Forscher lab, Yale Univ.

actin patches
actin cables

fission yeast
Wu lab, Ohio State Univ. 2007
Actin polymerization: driving force for cellular motions


Actin network within a motile cell

Motility of cancer cells causes metastasis

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

**Stretch** 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.

**Stimulation** Actin filaments are added to the tips of each filament faster than they can be removed from the roots, and the combined forces push the cell membrane forward.

**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 forces push 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. Contractile bundles of actin and myosin tension continue 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 Watersman, National Heart Lung and Blood Institute; Cell Motility and the Cytoskeleton; Nature

Low concentrations of markers: actin speckles

Naoki Watanabe, Kyoto University

Numerical simulations of actin turnover based on analysis of speckle images (ongoing project)
Actin Cytoskeleton in Cell Division

**A. Early cytokinesis**
- New membrane inserted
- Acto-myosin ring contracts
- Midbody begins to form


**fission yeast cdc25-22 cell**

CHD-GFP binds to sides of actin filaments

Jian-Qiu Wu (Pollard lab, Yale Univ 2007)

**GFP-actin kidney cell**

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

Rlc1p-3GFP

spinning disk confocal microscopy

~ 40 myosin II (Myo2p) molecules/node
~ 2 formin Cdc12p dimers/node
Wu and Pollard, Science 2005

Vavylonis, Wu, Hao, O’Shaughnessy, Pollard, Science 2008
Actin meshwork establishes connections among nodes

cdc25-22 cells

data: Wu (2007, 2008)
Search, capture, pull and release model

actin filament polymerization

actin filament capture

v_{pol} \sim 0.2 \ \mu m/sec

r \leq r_c \quad r_c \sim 100 \ nm

traction on filaments between nodes

lifetime of connections

\tau \ \text{break} \quad \sim 20 \ sec

lifetime of filaments

\tau \ \text{turn} \quad \sim 20 \ sec

Dynamic reestablishment of connections \rightarrow \text{plasticity of network}

Simulations with search, capture, pull and release

Simulated radial projection

red: nodes
green: actin

0 30x time lapse, 20min

2πR

experiment:

- model reproduces many observed features
Dependence on parameter values

\[ \nu_{\text{pol}} = 0.2 \, \mu\text{m/sec} \]

\[ \nu_{\text{pol}} = 0.04 \, \mu\text{m/sec} \]
Some mutant cells form clumps

Wild type: robust ring formation

Formin Mutant: clump formation

Hachet and Simanis, *Genes and Development*, 2008
Clump formation kinetics

- Scaling arguments:
  \[ t = 0 \]
  \[ v \approx \Delta c_0 r_0^2 dQ \]
  \[ D \approx d^2 Q c r_0^2 \]
  \[ t^* = v t^* = (D t^*)^{1/2} \]
  \[ l^* = r_0 d / l << r_0 \]
  \[ t_{clump} = r_0 / v \]
  \[ t_{clump} \approx \frac{1}{Q d} \]
  clump size \( \sim r_0 \)

- Monte Carlo Simulation of 2D bulk of nodes

### Graph
- **A**: Slope \( 1/2 \) (clump formation)
- **B**: Slope 1 (diffusion)

**Axes**
- Node root mean square displacement
- time (\( Q t \))
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)
Cell polarization and establishment of cell center

Wu et al Dev Cell 2003

Pom1p

Mid1p

cytoplasmic concentration gradients?

Formin For3p and actin cable assembly

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

v ~ 0.3μm/sec ~110 actin subunits/sec

Yang and Pon PNAS (2002)
3D actin cable turnover model


depends on actin polymerization
Fluorescence recovery after For3p photobleaching

LatA: sequesters actin

comparison of simulated recovery to experiment:
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