The interplay between actin dynamics and membrane tension determines the shape of moving cells.

Kinneret Keren
Physics Department
Technion- Israel Institute of Technology
August, 2011
Cell movement is ubiquitous. Nearly all animal cells move with the same basic mechanism: actin based motility.

**Moving cells**

- **Mouse fibroblast** (connective tissue)  
  Movie duration: 3 hours

- **Mouse melanoma cell**  
  Movie duration: 20 minutes

- **Chick fibroblast**  
  Movie duration: 2 hours

- **Fish keratocyte (skin)**  
  Movie duration: 4 minutes

Movie from Vic Small  
“Video tour of cell motility”
Cell movement has important functions

Cell movement is important for various biological phenomena:

- immune response (e.g. white blood cells)
- cancer metastasis
- wound healing

---

**Movie of monolayer of fibroblasts on a coverslip from Sheryl P. Denker and Diane L. Barber**
*JCB, 159(6), 1087-1096 (2002)*

---

**a white blood cell chasing a bacterium**

Movie by David Rogers, Vanderbilt University (taken in the 1950s)
Main player in cell crawling: **actin**

The majority of animal cells move by actin based motility

Actin is a globular protein; forms polarized helical filaments; hydrolyzes ATP

**actin monomer** ~5nm
(g-actin)

**filamentous actin**
(f-actin)

- filament diameter: ~7 nm
- persistence length: ~10 µm
Biochemical model of the polymerization motor

Molecular players involved largely known; constant recycling of molecular building blocks. Motor ultimately powered by ATP hydrolysis that ensures a supply of actin monomers.

Actin network treadmilling:
-assembly primarily at the front
-disassembly toward the rear

A zoom on the polymerization motor

An extended motor: dense cross linked actin meshwork (~$10^{10}$ molecules)

A crawling fish skin cell (keratocyte)
The actin polymerization motor: a paradigm of self organization

- actin concentration ~ $10^{10}$ molecules/cell (500µM)
- actin assembly rate at leading edge ~ $10^6$ molecules/sec
- lifetime of actin monomer in meshwork ~ 30 s
- total length of actin filaments ~ 10 cm filaments/cell
- cell size ~ 10 µm
- cell speed ~ 0.3 µm/s ~ cell diameter/1 minute

A cell moves by rebuilding its entire actin network every ~minute

Data adapted from: Abrahams et al., Biophys. J. 77 1721-1732 (1999)
“Equivalent” problem...

Put the world population in an area the size of Budapest and hope they self-organize to move together at ~600kph

Individual molecule
Size: ~1nm

Individual human being
Size: ~1m

Molecules/cell ~10^{10}
Length: ~10\mu m
Area: ~100\mu m^2
Speed: ~cell diameter/min
Filament length: ~10cm

World population ~10^{10}
Length: ~10km
Area: ~100km^2
Speed: ~10km/min~600kph
Filament length: ~distance to moon

Thickness of human hair
Self organization: from molecules to a moving cell

molecular building blocks → cellular structure and function

length~nm; time~s → length~10μm; time~hours

actin molecules

Biochemistry & Biophysics

Keratocyte: a fish skin cell

10μm  30X real time
Self organization: from molecules to a moving cell

- Can we relate local dynamics at the molecular level to behavior at the cellular level?
- How are global shape and speed determined?
- What role does the surrounding membrane play?
- Can we come up with a mathematical model that quantitatively relates molecular parameters to global cell behavior?

Complex problem....
let's look for the simplest available model system
Model system: fish epithelial keratocytes

Advantages of keratocytes:

- **Persistent motion** - nearly constant shape, steady state motility
- **Fast moving** - up to 1 \( \mu \text{m/s} \); fast turnover at the molecular level.
- **Flat lamellipodium** - 2D molecular machine (ideal for microscopy and modeling)
The lamellipodium is the motility apparatus

Cells can generate fragments which move on their own


Movie: Shlomit Yehudai-Reshef
Lamellipodial fragments as a model system

• Essentially a stand alone lamellipodium; no cell body.
• Speed and persistence similar to cells.
• Keeps going for hours.

→ Simplest natural system to study lamellipodial motility


First paper on keratocyte fragments:
Outline:

Part 1 - *What determines shape and speed of fragments?*
  • Characterization of the dynamics of keratocyte fragments
  • Theoretical model: treadmilling actin network in a membrane bag

Part 2 - *What determines membrane tension in motile cells?*
  • Measurements of membrane tension in motile keratocytes
  • Perturbations of the motility machinery and their effect on membrane tension
  • Rapid increase in membrane area by fusion with giant vesicles
Actin network distribution in fragments

A fluorescent image of a fragment fixed and stained with phalloidin

Peaked along leading edge

Flat along the rear

Decreases from front to rear

Data from a population of N=115 fragments
Black line- population average
Grey lines- individual fragments
Actin network dynamics in keratocyte fragments

Actin network flow visualized by Fluorescent Speckle Microscopy

Actin network flow maps $v_{cell} = 0.22 \mu m/s$

Actin network is stationary in lab frame

Actin network flow is moving rearward in cell frame
Actin network exhibits net disassembly from front to rear

• Actin density decays exponentially from front to rear

• Actin network is stationary in lab frame

$\frac{\partial B}{\partial t} = -V \frac{\partial B}{\partial s} - \frac{B}{\tau}$

$B(s) = B_c \exp\left(-s/V\tau\right)$

→ Constant actin network disassembly rate
Measurements of the actin disassembly time

Combine time lapse imaging followed by fixation and staining within individual fragments

\[ B(s) = B_0 \exp(-s/\lambda) \]
\[ \tau = \lambda/V = 18 \pm 2 \text{ sec} \]

Disassembly in individual fragments

18s < \tau < 55s

Movie: Noa Ofer
Following individual fragments over time

Area remains constant

→ Plasma membrane area is fixed.

The membrane is stretched around actin network

Fragment area varies between fragments

Time lapse movie of a fragment

Cell frame

Lab frame

30 µm

24X real time

Movie: Noa Ofer
Shape and speed vary over time
Shape and speed vary in a correlated manner.

Cross correlation front-to-rear and speed

N=45
Key experimental observations:

• Area remains constant

• Shape and speed are correlated

• Actin network treadmilling
  - Constant actin flow rearward
  - Constant disassembly rate
  - Graded density along leading edge
  - Flat distribution along rear
Can we relate the underlying molecular processes to global shape and speed?

Actin polymerization drives protrusion

Capping protein terminates elongation

Filament branching generates new filaments

Pollard, Mullins et al.

~\(10^{10}\) molecules
size \(\sim\) nm

The actin network near the leading edge of a moving cell

Svitkina, Borisy et al.

Lamellipodial fragments
size \(\sim\) 10\(\mu\)m

Pollard, Mullins et al.
Theoretical model:
Actin network treadmilling in a membrane bag

Actin network treadmilling
assembly at the front; disassembly toward the rear.

Inextensible membrane $\Rightarrow$ constant area.

Membrane tension is generated by the motility machinery; pushing at the front; resisting retraction at the rear.

- *At the front*: membrane tension applies an opposing force on the polymerizing actin network.
- *At the rear*: retraction is driven by forces due to membrane tension.

\[ \text{adhesions to the substrate} \]
Membrane tension couples front and rear

Local force balance between actin network and membrane tension determines global shape.

Membrane tension is constant along the boundary.

Front: Actin meshwork polymerization force is balanced by membrane tension.

Rear: Actin meshwork resistance to crushing is balanced by membrane tension.

2D model of lamellipodial motility

• Disassembly sets a 'clock' that determines front-to-rear distance.
• Membrane tension mechanically couples protrusion at the front and retraction at the rear.
**Force balance at the front**

between membrane tension and actin network polymerization

---

**Filament density along leading edge is graded**

\[ B(l) = B_0(1 - l^2/L^2) \]

---

**Actin density [a.u.]**

---

**Position along l.e. [μm]**

---

** filament density is graded**

\[ f(l) = \frac{T}{B(l)} \]

---

**Force per filament**

---

**Force balance at the front**

between membrane tension and actin network polymerization

---

**Constant Tension**

force per unit length

---

**Actin density along the leading edge**

**Actin density at front centre**

**Distance between rear corners**

---

\[ L = y + A/2y \]

---

Protrusion is stalled at front corners

Front corners are defined by where protrusion is stalled: \( f = f_{\text{stall}} \)

Force per filament increases toward the sides

\[
f(l) = \frac{T}{B(l)}
\]

\[
T = f_{\text{stall}} B_{\text{sides}}
\]

1 - \[
\frac{1}{1 + 2y^2}
\] = \[
\frac{T}{B_0 f_{\text{stall}}}
\]

**Force balance at the rear**

between membrane tension and actin network resistance

Rear boundary is defined by where actin network has disassembled sufficiently so the membrane tension can crush it

\[ B_{rear} = B(y) = B_0 \exp\left( -\frac{y}{V \tau} \right) \]
Force balance at the rear between membrane tension and actin network resistance

Force needed to crush network \( \propto \) Actin network density

\[
T = kB_{\text{rear}}
\]

\( k \) – Breaking force per filament

\[
B(s) = B_0 \exp(-s/V\tau)
\]

\[
B_{\text{rear}} = B(y) = B_0 \exp(-y/V\tau)
\]
’Actin disassembly’ clock model

Front and rear coupled by the membrane; Membrane tension is the same everywhere

front corners:
\[ T = f_{\text{stall}} B(x) = f_{\text{stall}} B_0 (1 - (1 + 2y^2/A)^2) \]

front-to-rear distance determined by the time needed for disassembly

\[ y \sim V \tau \]

V - speed
\( \tau \) - disassembly time
'Actin disassembly' clock model

Membrane tension is the same at the front and at the rear

\[
\exp \left( -\frac{y}{V\tau} \right) = \varepsilon \left( 1 - \left( 1 + \frac{2y^2}{A} \right)^{-2} \right)
\]

\[
\varepsilon = \frac{f_{\text{stall}}}{k} < 1
\]

Model parameters:

- \( A \) area
- \( \tau \) disassembly time
- \( B_0 \) barbed end density
- \( \nu \) cell speed
- \( \varepsilon = \frac{f_{\text{stall}}}{k} \) stall force (per filament)
- \( k \) breaking force (per filament)

Simple solution for \( \varepsilon \ll 1 \)

\[
V = \frac{-1}{\log \varepsilon} \frac{y}{\tau}
\]
Model predicts observed correlation between shape and speed

Model prediction

\[ V = \frac{y}{\tau} \left( \log \varepsilon + \log \left( 1 - \left( 1 + \frac{2y^2}{A} \right)^{-2} \right) \right) \]

Look at individual fragments
use measured \( A, \tau \); fit \( \varepsilon \)

Model prediction for time series of individual fragment

Model parameters:
- \( A \): area
- \( \tau \): disassembly time
- \( B_0 \): barbed end density
- \( v \): cell speed
- \( \varepsilon = \frac{f_{\text{stall}}}{k} \)
- \( f_{\text{stall}} \): stall force (per filament)
- \( k \): breaking force (per filament)
Direct test of 'disassembly clock' model
What happens if we slow down actin disassembly?

Biochemically slow down disassembly by adding jasplakinolide
(stabilizes filaments and slows disassembly)

→ expect $\tau \sim y / V_{cell}$ will increase
The model also predicts the shape of the leading edge.

Curvature of the leading edge

Contours of from a time lapse movie of a fragments (dt=48s)

Leading edge curvature varies between fragments and over time.
Front-to-rear distance is highly correlated with curvature of the leading edge.
Model predicts correlation between front-to-back distance and front curvature

\[ B(l) = B_0 \left( 1 - \left( \frac{l}{L} \right)^2 \right) \rightarrow f(l) = \frac{T}{B(l)} \rightarrow V(l) \rightarrow \text{shape} \]

\[ R_{le} \approx \frac{l}{\theta} \approx \frac{L}{2} \sqrt{\frac{V}{B_0}} \]

\[ \sqrt{A} : \text{length unit} \]

\[ \tilde{R}_{le} = \frac{R_{le}}{\sqrt{A}} \quad \tilde{y} = \frac{y}{\sqrt{A}} \]
Summary I

• Fragment area remains constant while front-to-rear distance and speed vary in a correlated manner.

• Global shape and speed are determined by local force balances.

• Membrane tension arises from a dynamic interplay between the actin cytoskeleton and the cell membrane. Tension mechanically coordinates protrusion at the front with retraction at the rear.

• Model of actin network treadmill coupled to membrane tension explains observed shapes in a quantitative manner: force balance between actin polymerization and membrane tension along leading edge; “Disassembly clock” defines rear.
Summary I

• Fragments are the simplest natural model system for actin-based motility.

• Fragment area remains constant while front-to-rear distance and speed vary in a correlated manner.

• Membrane tension arises from a dynamic interplay between the actin cytoskeleton and the cell membrane. Tension mechanically coordinates protrusion at the front with retraction at the rear.

• Global shape and speed are determined by local force balances.

• Model of actin network treadmill coupled to membrane tension explains observed shapes in a quantitative manner: force balance between actin polymerization and membrane tension along leading edge; “Disassembly clock“ defines rear.
Membrane tension measurements in keratocytes

Membrane tension can be measured by pulling a membrane tether

\[ T = T_m + \gamma = \frac{F_{\text{tether}}^2}{8B\pi^2} \]

- \( F_{\text{tether}} \): tether force
- \( B \): membrane bending modulus
- \( T \): apparent membrane tension

Hochmuth, Sheetz et al. (1996)
Dai and Sheetz (1998)

Nambiar et al, 2009
A conA-coated bead is attached to a motile keratocyte. Cell movement (at ~0.5μm/s) leads to tether formation.

Movie: Arnon Lieber
Thanks!

Technion lab:
- Enas Abu-Shah
- Arnon Lieber
- Noa Ofer
- Shlomit Yehudai-Reshef

Alex Mogilner
(UC Davis)

Julie Theriot, Erin Barnhart
(Stanford)

Michael Kozlov (Tel-Aviv Univ.)