CHAPTER 17
Cell Organization and Movement I:
Microfilaments
Migrating cell stained with fluorescent, a drug bind to F-actin
Microfilaments and actin structures

Dynamics of actin filaments

Mechanism of actin filament assembly

Organization of actin-based cellular structures

Myosin: actin–based motor proteins

Cell migration: signaling and chemotaxis
Overview of the cytoskeletons of an epithelial cell and a migrating cell

(a) TEM of epithelial in intestine

(b) Epithelial cell
- Microvilli
- Cell junctions

(c) Migrating cell

(d) Fibroblast or macrophage
- Microfilaments
- Microtubules
- Intermediate filaments

Extracellular matrix

Apical domain
Basolateral domain

Figure 17-1
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Overview of the cytoskeletons of an epithelial cell and a migrating cell
### The components of the cytoskeleton

<table>
<thead>
<tr>
<th>Structure/Subunit</th>
<th>Microfilaments</th>
<th>Microtubules</th>
<th>Intermediate filaments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Actin</strong></td>
<td>7–9 nm</td>
<td>25 nm</td>
<td>10 nm</td>
</tr>
<tr>
<td><strong>αβ-Tubulin dimer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Various</strong></td>
<td></td>
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</tbody>
</table>

*Figure 17-2
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Cell signaling regulates cytoskeleton function

Signals from soluble factors, other cells, the extracellular matrix

Plasma membrane with receptors

Exterior

Cytosol

Signal transduction pathways

Cytoskeleton

Organization and movement of organelles

Cell shape, movement, and contraction

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Example of microfilament-based structure

- Microvilli
- Cell cortex
- Adherens belt
- Filopodia
- Lamellipodium/leading edge
- Cell cortex
- Stress fibers
- Contractile ring

Figure 17-4a
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**Actin** is ancient (古老), abundant, & highly conserved.

**Most abundant intracellular protein** in most eukaryotic cells; 1-5% of cellular protein in nonmuscle cells. Cytosolic concentration of actin about 0.1-0.5 mM (local can be 5mM).

About 42kDa.

Single-celled organisms: 1 or 2 actin genes; multicellular organisms:
  multiple genes, e.g. human has 6 actin genes: 4 α-actins in various muscle cells, β- & γ-actins in nonmuscle cells (They differ at only 4 or 5 positions).

Actin sequences from amoebas & from mammals are 80% identical.

Humans have 6 genes
  • α actins used in contraction
  • β actins in stress fibers
  • γ actins in leading edge of moving cells

Some plant has 60 actin gene, but most are pseudogene (did not encoding actin)
**G-actin monomers** assemble into long, helical F-actin polymers.

Actin: a globular monomer called G-actin, each molecule contain Mg2+ and 1 ATP (ADP); ATP-G-actin, ADP-G-actin, ATP-F-actin, ADP-F-actin

Filamentous polymer celled **F-actin**

Twisted strands of bead produced thinner 7nm diameter and thicker 9nm.
ATP to ADP and Pi
Polymerization (Mg\(^2+\), K\(^+\) Na\(^+\))
G-actin \(\rightarrow\) F-actin (polymer)
De-polymerization
Ionic strength lowered

**F-actin has structural & functional polarity.**

Myosin S1 heads bind to actin filaments in a polar, spiral (螺旋) fashion, with the barbed (倒勾) ends pointing to the negative end of the actin filament.
F-actin has **structural** and functional polarity

Experimental demonstration of polarity of an actin filament by binding of myosin S1 head domains.

Myosins bind to actin with a slight tilt. When all subunits are bound by myosin, the filaments appear decorated with arrowheads that all point towards one end of the filament. This end is referred to as the “pointed end”.

![Diagram showing polarity of actin filament](image)
Dynamic of actin filaments
**Actin polymerization** in vitro, proceeds in **three** steps

Can be monitored by viscometry, sedimentation, fluorescence spectroscopy.

Each filament is composed of 2 helically intertwined chains of G-actin monomers

To polymerize into filament
- G-monomer must have an ATP bound
- G-ATP hydrolyzes to ADP + Pi (**faster at + end, slower at - end**)
- Become stable ADP-F-actin

This is **reversible**: F-actin depolymerizes into G-actin monomers.

**Nucleation**
- assembly of trimers
- lag phase can be reduced if polymerized fragments added first

**Elongation**
- ATP-G-actin is hydrolyzed to stable ADP-F-actin;

**Steady State**
- rate of subunit addition = rate of subunit loss
Critical concentration (Cc): concentration of G-actin in equilibrium with actin filaments.

$< C_c = \text{no polymerization;}$

$> C_c = \text{filaments formed.}$
Actin filaments grow faster at (+) end than at (-) end. + end elongates 5-10 times as fast as dose the opposite (-) Can measured by capping protein. -: free site

The value of Cc more lower $\rightarrow$ easy polymerization

$C_c = C_c^+ = 0.1 \, \mu M$

Cc at (+) end is much lower than Cc at (-) end.

$C_c = C_c^- = 0.6 \, \mu M$

Cc: critical concentration
ATP-actin subunits and faster at the (+)end than the (-),

At steady state, polymeration at +, depolymeration at -.
Treadmilling (滑動) of actin filaments:

When critical concentration between -+, → preferentially to the + end.

In steady state phase, G-actin continue to be added at +, and lost from the – end → the length of filament remain constant → can move

Treadmilling (踏車) type mechanism for move.

Treadmilling - if both ends of the polymer are exposed, subunits undergo a net assembly at the (+) end and a net disassembly at the (-) end with the polymer remaining a constant length, even though here is a net flux of the subunits through the polymer.
Monomers
↓
oligomers (nucleus)
↓
assembly
↓
dynamic equilibrium (treadmilling)

ADP

ATP

ADP + ATP + ATP + ATP

ADP + ATP + ATP + ADP + ADP

(b)

Photoactivated actin

Distance traveled by leading edge
Actin filament treadmilling is accelerated by profilin and cofilin

Thymosin $\beta_4$
- Binds ATP-G-actin (1:1)
- Sterically blocks ATP binding site
- Prevents polymerization
- Sequesters $\sim70\%$ of G-actin

Profilin
- Binds ATP-G-actin (1:1)
  - Buffered $\sim20\%$ of free G-actin
- Only actin binding protein that permits exchange of ATP for ADP, thus it likely promotes binding to actin filament.

Cofilin
- Bind to ADP-G-actin

1. ADP-G actin with a profilin and formed complex
1-1. ATP to ADP, ADP-G actin convert to ATP-G actin
1-3. Actin add to the end of a filament
2. Cofilin bind ADP-Actin $\rightarrow$ complex $\rightarrow$ enhanced de-polymerization
3. G-actin bound to thymosin $\rightarrow$ polymerization down
3-1. Free G-actin low $\rightarrow$ G-actin and thymosin complex $\rightarrow$ dissociate $\rightarrow$ polymerization
Cofilin (actin depolymerizing factor) binds, twists, and depolymerizes actin filaments. Binds preferentially to ADP-containing actin filaments, thus promote turnover of actin filaments from (-) end. Regulated by PIP2 binding & phosphorylation.

ADF/cofilin intercalates between subdomains 1 and 2 of two longitudinally associated actin monomers causing twisting of the filament.

*European Journal of Biochemistry* 268 (24), 6426-6434, 2001
Thymosin provides a reservoir of actin for polymerization

G-actin bound to thymosin is “unavailable” for polymerization
Actin polymerization in vivo is regulated by proteins that bind G-actin.

**Thymosin β4**, an actin-sequestering (隔離) protein, keeps cellular G-actin conc. (~200 mM) above its Cc (0.1 mM).

**Profilin**, another G-actin-binding protein, promotes actin filament assembly.

- As a nucleotide-exchange factor (ADP -> ATP).
- Assists actin addition to (+) end of filaments.

Membrane phospholipid PIP2 binds profilin & inhibits its binding to G-actin.

(PIP2 is hydrolyzed in response to certain extracellular signals.)

Membrane-associated signaling proteins (Ena/VASP family) bind profilin & localize profilin-actin complexes to the membrane.
Capping proteins block assembly and disassembly at actin filament

Actin-capping proteins stabilize F-actin