THE CYTOSKELETON

PART I: Microfilaments in cell organization and movement

Oliver I. Wagner, PhD
Associate Professor
National Tsing Hua University
Institute of Molecular & Cellular Biology
Department of Life Science
The Cytoskeleton

- F-actin
- Intermediate Filaments
- Microtubules
This lecture is based on the Lodish textbook but also contains material from two other important textbooks.

**Molecular Cell Biology, 7th Edition**
by Harvey Lodish et al.

**Cell Biology, 2nd Edition**
by Thomas D. Pollard et al.

**Molecular Biology of the Cell, 5th Edition**
by Bruce Alberts et al.

VIDEOS, ANIMATIONS, PODCASTS: http://bcs.whfreeman.com/lodish7e
OR @ ILSM
Dr. Flemming: “What are those wispy [filamentous] structures in the cytoplasm?”

Later he described the cytoskeleton of cartilage cells as “threads” (German: “Fäden”).

From: Flemming, W., Arch. Mikrosk. Anat. 16, 302–436 (1879)

Dr. Flemming when he looked at epithelia cells: "On reaching the plasma, one sees nothing at all."
The cytoskeleton enables cells to move

- Microtubuli in flagella
- Microtubuli in cilia
- Actin polymerization and actin-myosin contraction
Why cells need to be motile?

- **Development**: cells migrate inside the embryo to their defined locations
- **Host defense**: motile cells constantly search for pathogens inside the adult animal
- **Wound healing**: injured tissues are immediately invaded by highly motile cells to secret extracellular matrix (ECM) proteins

Why do people need to study the basics of cell movement?

Uncontrolled cell migration contributes to several pathologies:

- **Vascular diseases**
- **Chronic inflammatory diseases**
- **Cancer**: tumor formation and metastasis

What is the basis of cell migration?

A cytoskeleton composed of fibers which dynamically reorient, shrink and grow.

Based on this mechanism:

- axons of neurons can grow and connect to other neurons
- muscle cells can contract and produce force
- cells can send out small filopodia to sense their environment
- cells can divide during mitosis
The cytoskeleton also drives *internal* movements

- **Separation of chromosomes**
- **Streaming of cytosol** (important for plant cells)
- **Transport of membranous vesicles:**
  - Synaptic vesicle transport in neurons
  - **Endo- and exocytosis**
  - Membrane flow (recycling of membranes)

1) **Cell movement**: polymerization/depolymerization of actin drives membrane forward
2) **Cell shape support**: cross-linking proteins bundles and networks actin filaments
3) **Tracks for motor proteins**: myosin walks on actin to transport cellular cargos
4) **Form cell adhesions** & cell-cell contacts to connect to other cells (tissue formation)
5) Lamin (an IF type) **maintains structure of the nucleus**

*Actin* (red) and *IFs* (purple)
The Nobel Prize in Physiology or Medicine 2013 was awarded jointly to James E. Rothman, Randy W. Schekman and Thomas C. Südhof “for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells”.

Photo: © Yale University
James E. Rothman

Photo: © H. Goren. © HHMI
Randy W. Schekman

Photo: © S. Fisch
Thomas C. Südhof
The cytoskeleton occupies lots of space

- Cytoskeleton acts as an underlying **support** for the **cell membrane** (cell cortex)
- Organizes the **cellular content** (positioning of the nucleus, ER, Golgi and organelles)

⇒ occupies lots of space
Cytoskeleton is composed of 3 types of fibers which are all polymers built from globular protein subunits held together by noncovalent bonds. The fibers can be distinguished by their diameter.

**Actin**: twisted, two-stranded (pearl-string like) structure
⇒ cell cortex, microvilli, stereo cilia, adherens belt, filopodia

**MT**: hollow cylinder formed by protofilaments made of tubulin-subunits
⇒ positioning of Golgi, ER, vesicles etc.; cilia/flagella; chromosome separation

**IFs**: rope-like structure
⇒ lamins (nucleus support), keratin (in stiff epithelial cells), vimentin (in soft mesenchymal cells), desmin (bundles myofibers in muscle cells)
All three cytoskeletal elements drawn to scale.
Cytoskeletal organization differs between cell types

Microvilli (epithelia cell)

Leading edge (migrating cell)
How cells move

• Polymerization of actin at the “leading edge” expands the membrane in front of the cell
• This tension force and the contraction of actin/myosin pushes the cell forward
• The effect of the strong tension is seen in the movie when the cell becomes more elongated
• At the “trailing edge” of the cell all organelles are accumulated
Cellular actin organization

**Stress fibers**: cellular support and **contraction**
**Cell cortex**: **fast-acting** gel-sol transition (cytosol liquefying)
**Filopodia**: **sensing** the **environment**

Alberts, 4th ed.
Cell cortex: elastic actin network to support the fragile plasma membrane

Fibroblast treated with mild detergent to partial remove plasma membrane

Fast-freeze, deep-etch electron microscopy reveals actin-network linked by filamin

Bray, Cell Movements, 2nd Ed.
Summary of cellular actin organization

- Highly polarized epithelial cell
- Migrating cell
- Mitotic cell

- Microvilli
- Cell cortex
- Adherens belt
- Filopodia
- Lamellipodium/leading edge
- Stress fibers
- Phagocytosis
- Moving endocytic vesicles
- Contractile ring

Contractile actin engulfs and internalizes pathogen
Actin polymerization drives vesicles
Actin-bundles in fibroblasts made visible by AFM

Parak et al., *Biophys. J.*, 1999

Atomic Force Microscopy

Tip radius: 20-60 nm
Molecular basics of actin polymerization
“Understand how a cell can change its shape so quickly”

- Actin is the most abundant and one of the highest conserved protein in eukaryotic cells
- The molecular weight is around 43,000 Dalton
- Humans have 6 actin genes encoding for different isoforms:
  - $\alpha$-actin is found in muscle cells (4 isoforms)
  - $\beta$- and $\gamma$-actin in nonmuscle cells: $\beta$-actin = leading edge, $\gamma$-actin = stress fibers

- G-actin is the monomeric (globular) subunit of filamentous F-actin
- G-actin is composed of 4 subunits which form two equally sized lobes divided by a central cleft
- The central cleft binds either ATP or ADP complexed with a $\text{Mg}^{2+}$ ion
  ⇒ four actin states possible:
  ATP-G-actin $\Leftrightarrow$ ADP-G-actin
  ATP-F-actin $\Leftrightarrow$ ADP-F-actin

“A liver cells contains 500 million actin molecules”
Model of F-actin

“negative stained” F-actin in TEM

- Due to the twist of the strands, the F-actin helix appears alternating thinner and thicker
- One repeating unit is **72 nm long** and consists of **28 monomers** covering **13 turns** in the helix
- The cleft acts as a hinge to bring the monomers in the appropriate position causing the helix turn
- After binding ATP or ADP the cleft closes
- Addition of ions as Mg$^{2+}$, K$^+$ or Na$^+$ can **induce polymerization of G-actin** to F-actin
- Lowering the ionic strength **depolymerizes** F-actin to G-actin
- During polymerization ATP is hydrolyzed => however, it affects only the kinetics of polymerization (polymerization of actin can occur **without** ATP-hydrolyzation)

(only 14 G-actin monomers shown)
The polarity of F-actin can be detected via decoration with S1

- The actin-cleft always faces to the (slow growing) minus-end of actin
- EM resolution is not high enough to see the cleft, instead we can coat F-actin with the myosin subfragment 1 (S1 = head of myosin) binding slightly tilted to actin
- S1-decorated actin looks like “arrowheads”
  - pointed end faces to the minus-end
  - barbed end faces to the plus-end

Sequential myosin cleavage by two different proteases
Most actin cross-linking proteins belong to the calponin homology-domain family

- Actin cross-linking proteins can either bundle actin (e.g., microvilli) or form a network of actin (e.g., cell-cortex or leading edge)
- Most of these binding proteins have two actin binding-sites which are homologous to the muscle protein calponin
- Dystrophin is involved in the disease “Duchenne muscular dystrophy” (muscles become weakened in the absence of dystrophin)
**Fimbrin:** actin-binding sites are very close to each other: F-Actin tightly **bundled**

**Filamin:** actin-binding sites are largely spaced apart: F-actin produces a fluffy **network**

Filamin formed actin-networks contribute largely to the **gel-like** character of the **cytoplasm**

*Fast-acting gel/sol transition to power cell movement (specifically in amoeba)*

**Hair cell stereocilium** (= giant microvilli)
Harvard friends Thomas Stossel and John Hartwig were surprised by the “ugly precipitate” in the bottom of their test tube...

What they actually wanted to do was to purify myosin from non-muscle cells...

... but what they found was the first actin-binding protein (ABP) named filamin.

Stossel and Hartwig added the ABP protein to an (water-like) G-actin solution that immediately gelled the solution.

Now over one hundred ABPs have been identified.

Actin contributes to the very elastic cell membrane of red blood cells

- To be more flexible (squeezing thru thin capillaries), red blood cells lack of internal actin networks, but contain a very flexible cell membrane supported by a fishing-net type structure
- Several “adaptor proteins” are needed to connect actin to the cell membrane
- Triangular pattern: small actin trunks bind about six spectrin via band 4.1 and ankyrin
- 14 subunits long actin filaments are stabilized along their side by tropomyosin and capped at their minus-end by tropomodulin

Abnormal erythrocyte shapes in sickle cell anemia based on hemoglobin misfolding. Clogged vessels lead to pain and organ damage.

http://www.nhlbi.nih.gov/health/health-topics/topics/sca/
ERM family of proteins link actin to plasma membranes in microvilli

- Besides the dystrophin/dystroglycan complex that links actin to the muscle cell membrane, **ERM proteins** (ezrin-radixin-moesin) provide lateral connectivity of the actin/fimbrin complex to the **microvillus membrane**
- Newly incorporated GFP-actin is mostly obvious at the plus-end tips of microvilli (ABP **formin** anchors actin at the tips)
- At the sides of the microvilli actin is linked **either via ezrin directly** to the membrane or **via an ezrin/EBP50 complex**
- Ezrin exist in a **folded state** but when **phosphorylated** the protein **unfolds** and becomes active

“Transient expression refers to gene expression over a short period of time usually via cell transfection. Transient means the cell lost the plasmid over time mostly during mitosis”
Polymerization of actin proceeds in three steps

1) **Nucleation phase**: G-actin *slowly* aggregates into short oligomers (nuclei/seeds)
2) **Elongation phase**: To both ends of the seed, G-actin monomers *rapidly* added
3) **Steady-state phase**: Equilibrium is reached between filaments and monomers; G-actin adds and falls off on both sides of the filament but no net change in total mass of filaments

- At steady-state, the pool of non-polymerized G-actin is called the **critical-concentration** which is about 0.1 µM (= actin dissociation constant)
  ⇒ a solution of G-actin **above** 0.1 µM polymerizes
  ⇒ lowering the Cc **below** 0.1 µM F-actin would depolymerize
- At the critical concentration the “on rate” of G-actin equals the “off rate”
Falling sphere (ball) viscometer

During polymerization of actin the **viscosity** of the solution **increases** that will slow down the speed of the falling ball.

Put water or honey in the tube: the speed of the falling ball will be different.
Measuring the in vitro polymerization of actin

1. **Viscosimetry:** during polymerization of actin the filaments become more entangled which decreases the **flow rate of the solution** (due to increased viscosity)

2. **Sedimentation assay:** F-actin is **pelleted** upon high centrifugation forces while G-actin remains in the **supernatant**

3. **Fluorescence spectroscopy:** the **fluorescence spectrum** of G-actin **changes during polymerization** into F-actin

4. **Fluorescence microscopy:** **direct observation** of the growth of labeled actin filaments

![Graph showing nucleation, elongation, and steady state phases of actin filament growth](image)
Cytoskeletal fibers also differ in their mechanical properties

Based on their specific structures, the 3 types of cytoskeletal polymers exhibit also different elastic properties

- **Microtubules, actin and intermediate filaments** (all the same concentrations) were exposed to extreme *shear force* and the resulting degree of *stretch* was measured
- With increasing deforming force, **microtubules are the first** which cannot resist the strain and start to break following actin
- **IFs are the most flexible** filaments resisting large deformations

---

Rheometer: liquids are placed between two plates: one is fixed an the other is rotating. The rotating plate applies shear force (**stress**) to the liquid and measures the resulting deformation (**strain**) of the liquid

Viscous liquid deforms under shear stress

P. Janmey, JCB, 1991
The (+) end grows 5-10 times faster than the (-) end

Using myosin S1 as a nuclei to polymerize G-actin (conc. S1 < conc. G-actin), the undecorated (-) end is much shorter in EM compared to the undecorated (+) end.
The (+) end grows 5-10 times faster than the (-) end

- Rate of G-actin **addition** is about 10x faster at the (+) end compared to the (-) end
- Rate of G-actin **dissociation** is about the same at the (+) and (-) end
- Result: Cc(+) is lower than Cc(-)

"A conformational change in actin during ATP hydrolysis is responsible for the different association and dissociation rates"

* 1.4 : 12 = 0.12
** 0.8 : 1.3 = 0.6
Treadmilling occurs in cells at the leading edge

- Treadmilling occurs at steady-state if G-actin concentration is between 0.12 µM and 0.6 µM
- Subunits added to the plus-end mechanically push the membrane forward and then “travel” thru the filament until they fall off at the minus end

\[ Cc(+) < \text{G-actin conc.} < Cc(\neg) \]
Manipulating the Cc is crucial for the cell to control polymerization

- Filament mass only effectively increases above the Cc
- Below Cc filaments depolymerize
- **Adding more monomers above Cc** won't increased the total monomer mass because they will be immediately incorporated into existing filaments (which are then growing and thus their mass increases)

In the cell, plus- and minus-end **capping proteins** control growth rate of filaments
- Using capping proteins, the different Cc at both ends can be determined
Toxins are important for basic research on the cytoskeleton

- **Phalloidin** (from fungus) stabilizes F-actin
- **Jasplakinolide** (from sponge) stabilizes actin nuclei (promotes polymerization)

- **Cytochalasin D** (from fungus) makes a (+)-cap
- **Latrunculin** (from sponge) sequesters G-actin

Phalloidin labeled with a green fluorophore to make the actin cytoskeleton visible (phallodin isolated from the highly poison mushroom *Amanita phalloides* or also named “death cup”
Viscosity of cytoplasm is controlled by F-actin severing proteins

Severing ("cutting") proteins such as **gelsolin** and **cofilin** bind sidewise to actin filaments: induce conformational change in G-actin => **strain** on the filament increases => filament **breaks** => a (+) **cap** remains => (-) end rapidly **depolymerizes**

---

**gelsolin**

Binds to ATP-actin

**cofilin**

Binds to ADP-actin

Pollard, 1st ed.
In the cell, several G-actin binding proteins control actin polymerization

- Due to high salt and high actin concentration (0.5 mM) in the cell, theoretically all G-actin must be fully polymerized => still 40% of all actin is G-actin!
- The G-actin pool is maintained by **G-actin sequestering proteins** as thymosin β4
- **Profilin** acts as both, a G-actin buffer and promotes actin polymerization

Some cellular regulation facts:
- **Profilin** binding to G-actin can be **inhibited by** an interaction with the phospholipid PIP₂
- **Profilin** is targeted to membranes via binding to WASp
- **Thymosin β4** binds in a way to G-actin so that it cannot polymerize
Cellular signals control actin severing proteins

- **PIP$_2$ inhibits** activity of *gelsolin* and *profilin*
- **Phospholipase C** can hydrolyse PIP$_2$ to IP$_3$ which triggers the release of Ca$^{2+}$ from the ER **activating** gelsolin, profilin and coflin
- **Cofilin** is also **activated** by **dephosphorylation** (regulated by LIM kinase under the Control of Rho kinase ROCK and Cdc42)

Muscle sarcomere:
- **CapZ** binds to the (+) end of F-actin (without depolymerizing)
- **PIP$_2$ inhibits** CapZ capping activity
- **Tropomodulin** is a (-) end capping protein (**activated** by *tropomysin*)

"CapZ and tropomodulin keep F-actin at the same length in the muscle sarcomere! “
ABPs that promote actin filament assembly

- Two classes of actin nucleating proteins:
  - **Formins** lead to assembling of **long actin filaments** (filopodia, stress fibers)
  - **Arp2/3** complex leads to **branched actin networks** (leading edge, cell cortex)

**Formin** is a dimer with several regulatory domains:
- **RBD** (rho-binding domain) binds G proteins
- **FH1** (FH = formin homology domain) has prolin-rich domains, thus several profilin-G-actin binding sites
- **FH2** nucleates and binds F-actin

EM micrograph of gold-labeled formin that promoted growth of a long actin filament

The two FH2 monomers have the ability to swing towards the (+) end assisting filament growth
Formins are controlled by a Rho-pathway

- Formins are inactivated by an intramolecular interaction between the FH2 and RBD domain
- RBD binds to activated Rho and formin unfolds (now activated)
- Formins are found in stress-fibers and the contractile ring (mitosis)
- Formins inhibit binding of CapZ to F-actin

Pollard 2nd Ed.
Arp2/3 binds to the minus ends of actin filaments

- Arp = actin-related protein: has 50% similarity to actin
- Arp 2/3 is a **minus-end capping** protein and **nucleates polymerization**
Arp2/3 branches actin filaments at a defined angle

Arp 2/3 **binds to the sides** an actin filament and **branches** them at an angle of 70°

Alberts, 4th ed.
Arp2/3 branches actin filaments at cell cortex and lamellipodia

- Arp2/3 promotes fast growing of (+) ends pushing the membrane forward at the leading edge
- S1 decoration: filaments face with (+) end towards the leading edge
- Filament density at the leading edge become less dense towards to the cell body
Arp 2/3 action requires WCA domains that are part of NPF (nucleation promoting factor)

[1] First step of Arp 2/3 activation requires an NPF with a WCA (WH2, connector, acidic) domain that binds one G-actin

[2] When the activated NPF binds to Arp 2/3 with it’s acidic A domain, Arp 2 and Arp3 move closer to each other (enabling the binding of a preformed actin filament in a later step)

[3] The G-actin bound to WH2 of the (WCA) acts as a nuclei for polymerization at the plus-end

[4] The plus-end will grow until it is eventually capped
WASp is inactivated by an intramolecular folding (WH2 domain not accessible for G-actin)

- WASp’s RBD domain binds to activated Cdc42 => WASp unfolds and becomes active

WASp = Wiskott-Aldrich syndrome protein (X-linked disease characterized by eczema, low platelet count and immune deficiency)
Arp2/3 is needed for *Listeria* movement in infected cells

- *Listeria monocytogenes* (found in rotten food) is a bacterium which propels thru the cytoplasm using the **power of actin polymerization** stimulated by Arp2/3
- Actin polymerizes into filaments at the base of the bacterium pushing it forward
- Similar to an NPF, **ActA** has an **actin binding site** and an **acidic region** to activate Arp2/3

![Actin rocket tails on bacterium](image1)

![TEM of comet tails](image2)

- ActA binds **VASP** (not shown) which has prolin-rich regions (to recruit profilin-ATP-actin)
- CapZ rapidly caps new filaments and cofilin shortens old filaments
- VASP **inhibits CapZ** binding near the base of the bacteria (so it can be pushed forward by actin polymerization)
Actin rocket tails also push endosomes forward

- Clathrin-mediated endocytosis is a process that involves the uptake of molecules or liquid
- **Endocytosis assembly factors** recruit **WASp** initiating **Arp2/3** dependent actin assembly is initiated
- After invagination and the endosome has pinched off, a short **burst of branched-actin polymerization** drives the endosome forward

Visualization of **actin comet tails** (green) on endocytic **endosomes** (red)
Myosins make up a large family of actin-based motor proteins

- 40 myosin genes found in the human genome
- Loss of specific myosins may cause a particular disease
- 3 important classes:
  - Myosin I (endocytosis)
  - Myosin II (muscle contract.)
  - Myosin V (cargo transport)
Basic structure of myosins

- All myosins consist of several light and one or two heavy chains.
  - **Light chains** have regulatory function (myosins are regulated by Ca\(^{2+}\)).
  - **Heavy chains** consist of a motor head (actin-binding and ATP-binding), a flexible neck, and a tail domain (with “cargo-binding” function).

- Tail consists of \(\alpha\)-helical coiled-coils which forms (the rod-like) myosin dimer.
- Tail defines the function of myosin: myosin I and V have membrane-binding domain.
- **Regulatory light chains** in myosin I and II are calmodulin with Ca\(^{2+}\)-binding sites.
- Myosin II contains essential and regulatory light chains: essential light chains stiffens the neck so it can act as a lever arm.
The myosin-actin cross-bridge cycle

Chemical energy \(\rightarrow\) elastic (mechanical) energy

2. Hydrolysis of ATP to ADP + P\(_i\), myosin head rotates into "cocked" state

Release of P\(_i\) is coupled with release of elastic energy

4. "Power stroke": Release of P\(_i\) and elastic energy straightens myosin; moves actin filament left

Myosin ATPase is actin-activated if no ATP available

"rigor mortis" if no ATP available
Cellular and intracellular movements depend on myosins

- **Endocytosis**: monomeric myosin I (3 regulatory light chains)
- **Muscle cells**: contraction based on filament-sliding between F-actin and muscle myosin II (1 essential and 1 regulatory light chain)
- **Non-muscle cells**: non-muscle myosin II (also involved in cytokinesis)
- **Vesicle transport**: major vesicle transporter myosin V (6 regulatory light chains)
Phagocytosis involves branched actin filaments and myosin

- Phagocytosis is an endocytotic process for the **uptake of large particles**
- Antibody produced by the immune system targets bacterium (opsonization)
- Once a white blood cell binds AB with its Fc receptors **internalization starts**
  - Assembled actin filaments together with **backward moving myosin VI** power the engulfment
  - **Lysosomes** fuse with the **phagosome** and **digest** the particle
**In vitro motility assay**

- Movement of fluorescently-labeled **actin filaments** on immobilized **myosin heads** on a cover glass is observed using a fluorescence microscope (TIRF type)
- Addition of ATP triggers the **movement of actin filaments** along the fixed myosins
- Upon ATP-binding myosin heads dissociate from the filament while the **head tilts towards the (+) end** (no power-stroke yet)
- The **upcoming power-stroke** pushes the filament with the (-) end in the lead

Myosin heads spent only 10% of their time attached to actin: **short duty cycle**

Rhodamine-phalloidin labeled actin filaments. One frame each 30s.
Measuring the force generated by single myosin heads using the optical trap

- An infrared beam is used to immobilize a bead attached to an actin filament.
- Upon the myosin’s power stroke, the actin filament is held in position.
- The force generated by the myosin head is determined by measuring the bead displacement. => for myosin II about 3-5 pN (piconewton).
- Step-size, generated force, and velocity depend on the length of the lever arm:
  - myosin II = 8 nm step size
  - myosin V = 36 nm step size
### Myosin V stepping

TIRFM (total internal reflection microscopy) revealed the stepping mechanisms of myosin V: each head spent >50% of their time on actin => long duty cycles

Myosin V **head and neck** on F-actin (visualized by EM)

- Pre-power stroke
- Post-power stroke

Net step size of myo V double head is 36 nm. But each single head makes a 72 nm step.

In 3D, Myosin V stepping

**Hand over hand model**

**Inchworm model**

---

Walker et al., Nature, 2000
Myosins in intracellular trafficking

Vesicles move along actin or microtubule tracks attached to molecular motors as myosins, kinesins and dynein.
Cytoplasmic streaming in *nitella* plant cells

- Near the cell wall actin tracks are immobilized on chloroplasts while myosins are free to move.
- Myosin V tails bind to the endoplasmic reticulum (myosin V also named myosin XI in plant cells).

The pull on the ER caused by myosins results in a flow of (indirectly dragged) cytoplasm.
Contractile and non-contractile actin-bundles

- **Non-contractile actin-bundles**
  - Microvilli (surface extension and nutrient absorption processes)
  - Filopodia (environmental sensing and directing the cell during cell locomotion)

- **Contractile actin-bundles**:
  - Stress fibers important for cell locomotion, mechanical resistance and connectivity to surfaces (via focal adhesions)
  - Adherens belt (circumferential belt) in epithelial cells (tissue integration)
  - Cleavage furrow during cytokinesis: contractile ring forms and splits the cell

*Myosin I* is located at the poles and *myosin II* at the furrow
Cell movement requires contractile bundles and cell adhesions

Cell movement occurs in 4 discrete steps

1. Extension
   - Lamellipodium

2. Adhesion
   - New adhesion

3. Translocation
   - Cell body movement

4. De-adhesion
   - Endocytotic recycling of adhesion molecules and membranes; backflow of actin

Actin polymerization and cross linking of new filaments forms a lamellipodium at the leading edge (Arp2/3 powered similar to actin-based Listeria movement)

The lamellipodium (or filopodia) form new focal adhesions to fix the leading edge to the substratum (interaction between the extracellular matrix and focal adhesion is mediated by integrins)

Contraction of the actin-myosin cortex (near the rear of the cell) leads to translocation of the tail

De-adhesion of focal adhesions at the tail releases the stress caused by contraction of the actin-myosin network
Experimental proof for the action of contractile bundles during cell motility

- As a keratinocyte moves forward, the generated force by the actin-myosin bundle in the center of the cell causes the silicon rubber to wrinkle.
- **Polarity** (front with lamellipodia and back with tail) is a **striking feature** of motile cells: when a cell needs to make a turn the leading edge first forms into the new direction.
Leading edge of a fibroblast showing ruffled membranes of the lamellipodium

- As the cell moves forward, lamellipodia that fail to attach to the substratum are swept backward over the dorsal surface of the cell, known as **ruffling**
- These membranes are later recycled by endocytosis (and when needed incorporated in newly formed lamellipodia by exocytosis)
The cellular engine that pushes the membrane forward

1) **Profilin** binds **ATP-G-actin** and delivers it to free F-actin (+) ends near the leading edge membrane

2) **Arp2/3** branches and **nucleates** new filaments

3) New branches are **cross linked** by **filamin** while (+) end capping proteins prevent depolymerization

4) **Cofilin** and **gelsolin** sever old ADP-F-actin ends at the (-) end

5) A pool of free ATP-G-actin is maintained by **thymosin β4**

   “Profilin binding to actin induces opening the cleft releasing ADP (and more abundant ATP binds)”

---

**Elastic Brownian ratchet model**

- The elastic actin filament **bends due to thermal fluctuations**
- This allows to **add new monomers** to the (+) end
- The grown filament **pushes the membrane forward** after it flips back (elastic recoil)
Signal transduction pathways control cell movements

- **G proteins** (Ras-like small GTP-binding proteins) mediate cell motility
- G proteins (for example Rho) act as **molecular switches** which are **active** in their GTP-bound state and **inactive** in their GDP-bound state

G proteins are regulated by specific **inhibitors** (**GDI**), **exchange factors** (**GEF**) or **GTPase activity stimulators** (**GAP**)

GEF = guanine nucleotide exchange factor
GAP = GTPase-activating proteins
GDI = guanine nucleotide dissociation inhibitor

Microinjection of constitutively active G proteins in growth-factor starved fibroblasts:
Signal transduction pathways control cell movements

Activation of the different actin structures follows a strict hierarchy:

• **First** event is Cdc42-activated filopodia formation following Rac-activated lamellipodia formation and the **last** is the Rho-activated stress fiber formation.
• However, activated Rho pathways also **inhibits** Rac pathway (to ensure the asymmetry of this process)
Wounded-cell monolayer assay for investigating cell movements

- To investigate cell migration a **scratch** is applied in a petri dish of confluent cells
- Under control conditions cells close the “wound” up to 100%
- When cell **express dominant negative G proteins** the closure is incomplete with Cdc42 > Rac > Rho (reflecting the importance of these G protein in their hierarchy)

(a)

(b)

(c)

Dominant negative Rac
Dominant negative Cdc42
Dominant negative Rho
Control

Percent wound closure
Chemotactic molecules outside the cell control cell directionality

- **Chemotaxis**: cell turns into the direction of a gradient of small chemotactic molecules
- The ameba *Dictyostelium* migrates along increasing conc. of cAMP
- cAMP binds to trimeric G protein-coupled receptors (GPCRs) that activate G protein mediated signaling pathways => cytoskeleton remodeling

**Gradients direct motility:**
- GPCR and G protein gradients are all the same
- PI3 kinase generates PIP₃ => signal to polymerize actin at the leading front
- PTEN dephosphorylates PIP₃ back to PIP₂
- Myosin is important for rear contraction
The Nobel Prize in Chemistry 2012

Robert J. Lefkowitz, Brian K. Kobilka

The Nobel Prize in Chemistry 2012 was awarded jointly to Robert J. Lefkowitz and Brian K. Kobilka "for studies of G-protein-coupled receptors"
MOLECULAR BASIS OF MUSCLE FUNCTION
Structure of striated muscle cells

• Muscle = bundle of **muscle fibers** (aka **muscle cells** aka **myofibers**): 1-40 mm long **syncytium** of >100 nuclei

• **Sarcomere** is a specialized 2 µm long cytoplasmic structure in muscle cells containing densely packed actin and myosin filaments

• Bundled chains of sarcomers form the **myofibril** (and many myofibrils can be found in a muscle cell)

• Upon contraction, the sarcomer shortens to about 70% of its resting length

• The bright I band is entirely composed of thin **F-actin**

• The dark **A band** reflects the **thick myosin filaments**

• The **H zone** is entirely composed of myosin

• Actin filaments are attached with their (+) ends to the **Z disks**

Most of the names for the sarcomere bands derived from early observation with an polarized microscope:

I = **isotropic**

A = **anisotropic**

M = **middle line**

Z = “**Zwischenscheibe**” (German: disks)

H = “**heller**” (German: brighter)
Sarcomere accessory proteins

- Actin filaments are stabilized by CapZ at their plus-ends and tropomodulin at their minus-ends
- The thin filaments are also laterally wrapped by the giant protein nebulin (900 kDa)
- Similar, thick filaments are mechanically supported and fixed to the Z disks by giant protein titin (largest known protein with 3800 kDa)
The giant protein titin stretches the sarcomer intra-molecularly

- Modest stretches extend the PEVK domain reversibly
- Extreme stretches unfolds immunoglobulin or fibronectin III domains

Pollard, 1st ed.
Desmin interconnects and bundles the sarcomeres

- **Desmin** surrounds the Z disk and connects sarcomeres to the plasma membrane
- The longitudinal **desmin filaments** interconnect neighboring **myofibrils** and bundle them into a muscle *fiber*

Desmin filaments lie outside the sarcomere and do not participate in muscle contraction

A chain of sarcomers form the myofibrils and many myofibrils can be found in a single muscle cell (muscle fiber)
Contraction of the sarcomere shortens the H zone*

- During the cross-bridge cycle (ATP-hydrolysis), myosin heads move the actin filaments from each side of the sarcomere until their (-) ends overlap
- Contraction requires Ca\(^{2+}\) which is pumped by Ca\(^{2+}\)-ATPases into the sarcoplasmic reticulum (SR)
- An incoming nerve impulse at the neuromuscular junction triggers voltage-gated Ca\(^{2+}\) channels raising the cytoplasmic Ca\(^{2+}\) level from 0.1 µM to above 1 µM

*Kessel & Kardon, 1979
The cross-bridge cycle is controlled by actin binding proteins

- **Troponin** regulates muscle contraction. It consists of three subunits TN-T, TN-I and TN-C (calmodulin homology). Binding of Ca$^{2+}$ to TN-C triggers the movement of tropomyosin $\Rightarrow$ myosin-binding sites on F-actin are now exposed
- In the **off state** myosin still can bind actin, but it cannot move (no power-stroke)
Actin-myosin contraction in non-muscle or smooth muscle cells

• **Smooth muscle** cells are present in the gut, respiratory tract, blood vessels, uterus
• Smooth muscle and non-muscle cell contraction is regulated by **turning myosin on and off** (compare *striated muscle cell*: actin is turned on and off by tropomyosin!)
• Loosely aligned actin-myosin bundles contract when myosin light chain *(LC)* is **phosphorylated by** myosin LC kinase *(MLCK)*

Phosphorylation of myosin LC releases the myosin tail from its “sticky patch” allowing the myosin molecule to assemble into thick filaments.

Alberts, 4th ed.
The MLCK has to be activated by Ca-Calmodulin

- Upon Ca\(^{2+}\) conc. > 1 \(\mu\)M Ca\(^{2+}\) binds to Calmodulin (CaM) which activates MLCK
- A phosphatase (MLCP) can remove the P\(_i\) from the myosin light chains
- The MLC phosphatase can be inactivated by phosphorylation via Rho kinase (ROCK) (leaving the P\(_i\) bound to myosin LC => myosin is active => contraction)
- Ca\(^{2+}\) has to diffuse over long distances that makes the process slow (compared to fast contraction in skeletal muscles with immediate available Ca\(^{2+}\) storages)
- Ca\(^{2+}\) levels are also mostly controlled by externals signals as hormones and not fast action potentials

**Regulation of myosin’s light chains**

**Regulation of MLCP**

“Thick filament regulation”
Dense bodies and dense plaques anchor actin-myosin filaments in smooth muscle cells.

Smooth muscle cells have a spindle-like appearance.
Cardiac muscle cells also appear with striated pattern

- Cardiac (heart) muscle cells also have striated pattern based on I bands, A bands and Z disks
- The difference to striated skeletal muscle is that cardiac muscle cells are not multinucleate
- Mono- or bi-nucleated cells are connected to each other (linear or in branches) via intercalated disks
- Intercalated disks are rich of desmosomes and gap junctions
- The energy used for contraction comes from fat metabolism rather than from glucose metabolism (skeletal muscle)
- Heart attack happen when blood flow to cardiac muscle cells is disturbed and cells die. Permanent heart dysfunction is the result because heart cells do not regenerate (stem cell therapy is thought to partially cure dysfunction)
The end of part 1