School of Medical Sciences, Faculty of Medicine
The University of New South Wales, Sydney, Australia
Lecture Overview

• Microfilaments
  – Structure, function and regulation

• Actin
  – Motility
  – Adhesion, focal adhesions
  – Actin binding proteins, myosin motors
  – Muscle actins

• Microfilament diseases

• UNSW Cell Biology
  • http://cellbiology.med.unsw.edu.au/units/science/lecture07.htm

Image: Dr. Barber at Pikeville College, KY
Microfilament References

• Medline (April) References
  – Actin 62,901 (08)
    • 58,545 (07) 54,273 (06) 50,096 (05)
      46,353 (04)
  – Actin Binding Proteins 63,038 (08)
    • 59,067 (07) 54,711 (06) 50,620 (05)
      46,945 (04)
  – Myosin 30,500 (08)
    • 29,099 (07) 27,683 (06) 26,286 (05)
      24,924 (04)

• Textbooks
  – Essential Cell Biology Ch16 p527-542
  – Molecular Biology of Cell Ch16 p821
  – Molecular Cell Biology Ch19
Cytoskeleton Filaments

microfilaments

intermediate filaments (V)

microtubules

(K)

30 µm
Structural Systems

Microfilaments
- shape
- motility
- contractility
- cytokinesis
- transport
- compartments

Microtubules
- transport
- karyokinesis

Intermediate Filaments
- compression resistance
Actin functional challenge

Diversify function
  • dynamics
  • organisation
  • mechanics

Spatial specialisation
  • pool sizes
  • function

Evolution
  • simple principle
Microfilaments

- Twisted chain 7 nm diameter
- Compared to MT
  - Thinner, more flexible, shorter
- Point in same direction
- Different organisation in different cellular regions
Actin Microfilament Formation

- Globular actin monomer (g actin) polymerise to Filamentous actin (f actin)
  - Cells approx 50:50
  - Monomer can add to either (+ or -) end
    - Faster at + end
- Actin-ATP hydrolysed (ADP) following addition
  - Destabilises (like MT)
Nucleation/Elongation

- **Nucleation**
  - Two actin molecules bind weakly
  - addition of a third (trimer) stabilizes the complex
  - forms a "nucleation site"

- **Elongation**
  - Additional actin molecules form a long helical polymer
    - Initial period of growth
    - Then equilibrium phase reached

- **Dynamic Equilibrium**
  - Elongation $\leftrightarrow$ Depolymerization controls filament length
Actin Types

• 6 Mammalian actin types (isoforms)
  – All are 43 Kd Protein

• 2 cytoskeletal isoforms in all non-muscle cells
  – Beta (β) 7p22-p12
  – Gamma (γ) 17q25

• 4 muscle isoforms in different muscle cells
  – Alpha (α) skeletal
  – Alpha (α) cardiac
  – Alpha (α) smooth
  – Gamma (γ) smooth
Actin Protein

- Conserved in mammals
- Different ratios ($\beta:\gamma$) in different cell types
- 374aa, 43 kD protein
- 4 aa difference between beta and gamma
  - at N-terminal
- Highly expressed gene
  - Promoter used in gene transfections

Gene

5' 3'

Protein

N C
Actin Isoforms are Functionally Distinct

$\beta$- vs $\gamma$-actin in myoblasts

- $\beta$-actin promotes cell spreading and stress fibres
- $\gamma$-actin inhibits cell spreading and stress fibre formation
- $\beta$- and $\gamma$-actin have different preferences for types of tropomyosins
Cell Movement

• Whole or part of cell
  – Amoeba, neutrophil, macrophages
  – Neuron processes
    • axon, dendrites
  – Common structures
  – Contraction
• Intracellular transport
Motile Structures

• Leading/Trailing Edge
  – extension/retraction
  – Actin nucleation

• Lamellipodia
  – Sheet-like extensions

• Filopodia
  – Thin protrusions

• Integrins anchor to ECM

MBoC Figure 16-55
Cell Migration

(A) Non-motile, unpolarized fibroblast

(B) Polarized, motile fibroblast

MT Depolymerization → Growth Factors, ECM gradient → Microtubule Polymerization

FA movement → Fibronectin Adhesion → MT polymerization

Disassembled Focal Adhesion → Stable, Static Focal Adhesion

IV Motile Zone → III Curling Zone → II Persistence Zone → I Formation Zone
Adhesive Functions

• Cell signalling
  – Modify cell cytoskeleton
  – Activate intracellular signalling pathways
  – Cell motility
  – **Note** adhesion is covered in detail in later Lecture
Adhesion Junctions

- Adherens Junctions
  - microfilaments anchor the plaque that occurs under the membrane of each cell.
  - plaques not as dense
    - also occur as hemiform
Adherens Junctions

- heart muscle, layers covering body organs, digestive tract.
- transmembrane proteins
- Cadherin
Adhesion Junctions

- **Adherens (cell-cell)**
  - cadherin (E-cadherin)
  - Links to cadherin in neighboring cell

- **Adherens (cell-matrix)**
  - Integrin
  - Links to extracellular matrix
Focal Adhesions
Adhesive Signalling
Actin Signaling

- **Rho**
  - Family of small GTPases organize the actin cytoskeleton
  - Rho, RAC, CDC42
  - Form different actin structures

- **Wasp**
  - Wiskott-Aldrich syndrome protein
  - a downstream effector
  - transfers signal from tyrosine kinase receptors and small GTPases to actin cytoskeleton

Image Source: http://www.zoo.uni-heidelberg.de/gep/k.thelen.htm
Actin Filaments

Actin

Tropomyosin
Tropomyosin slows ‘off-rate’

- Slow ‘off-rate’
- Increase tensile strength
Actin functional challenge

Diversify function
- dynamics
- organisation
- mechanics

Spatial specialisation
- pool sizes
- function

Evolution
- simple principle
Distinct subcellular sorting of cytoskeleton Tm isoforms
Isoforms Define Specific Functional Properties of Actin Filaments

- Spatially segregated filaments contain different tropomyosins.
- Spatially segregated filaments have different functional roles in the cell.
Small GTPase Regulate the Actin Cytoskeleton

Rho → Stress Fibres

Rac → Lamellapodia

Cdc 42 → Filipodia
Tm$_{NM1}^5$ over-expression mimics Rho activation

TmBr3 over-expression mimics Rac activation

Tm3 over-expression mimics Cdc42 activation

Actin Binding Proteins

• Regulate polymerisation and create different structures
  – Monomer binding protein
    • Sequester
    • release
  – Polymer binding proteins
    • Bundling
    • cross-linking
    • Severing
    • contracting
Actin Binding Protein Interactions

- Filamin dimer
- Spectrin tetramer
- Fimbrin monomer
- α-actinin dimer
- Filamin dimer

Filamin binds to filament (strong)

α-actinin

Actin filaments

Tropomyosin

Myosin-II

Network of cross-linked actin filaments

Bundle of contractile actin filaments

Assembly A

Assembly B

Microfilaments, 2010 Sliee 32

MBoC Figure 16-78
## Actin Binding Proteins

<table>
<thead>
<tr>
<th>FUNCTION OF PROTEIN</th>
<th>EXAMPLE OF PROTEIN</th>
<th>COMPARATIVE SHAPES, SIZES, AND MOLECULAR MASS</th>
<th>SCHEMATIC OF INTERACTION WITH ACTIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form filaments</td>
<td>actin</td>
<td><img src="image" alt="Filament" /> 50 nm 370 x 43 kDa/m</td>
<td>minus end plus end preferred subunit addition</td>
</tr>
<tr>
<td>Strengthen filaments</td>
<td>tropomyosin</td>
<td><img src="image" alt="Filament" /> 2 x 35 kDa</td>
<td><img src="image" alt="Interaction" /> 14 nm</td>
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<tr>
<td>Bundle filaments</td>
<td>fimbrin</td>
<td><img src="image" alt="Filament" /> 68 kDa</td>
<td><img src="image" alt="Interaction" /> 40 nm</td>
</tr>
<tr>
<td>Bundle filaments</td>
<td>α-actinin</td>
<td><img src="image" alt="Filament" /> 2 x 100 kDa</td>
<td><img src="image" alt="Interaction" /></td>
</tr>
<tr>
<td>Cross-link filaments</td>
<td>filamin</td>
<td><img src="image" alt="Filament" /> 2 x 270 kDa</td>
<td><img src="image" alt="Interaction" /></td>
</tr>
<tr>
<td>Fragment filaments</td>
<td>gelsolin</td>
<td><img src="image" alt="Filament" /> 90 kDa</td>
<td><img src="image" alt="Interaction" /></td>
</tr>
<tr>
<td>Slide filaments</td>
<td>myosin-II</td>
<td><img src="image" alt="Filament" /> 2 x 260 kDa</td>
<td><img src="image" alt="Interaction" /></td>
</tr>
<tr>
<td>Slide filaments</td>
<td>myosin-I</td>
<td><img src="image" alt="Filament" /> 150 kDa</td>
<td><img src="image" alt="Interaction" /></td>
</tr>
<tr>
<td>Move vesicles on filaments</td>
<td>myosin-I</td>
<td><img src="image" alt="Filament" /> 2 x 265 kDa plus 2 x 260 kDa</td>
<td><img src="image" alt="Interaction" /></td>
</tr>
<tr>
<td>Attach sides of filaments to plasma membrane</td>
<td>spectrin</td>
<td><img src="image" alt="Filament" /> α β β α</td>
<td><img src="image" alt="Interaction" /></td>
</tr>
<tr>
<td>Sequester actin monomers</td>
<td>thymosin</td>
<td><img src="image" alt="Filament" /> 5 kDa</td>
<td><img src="image" alt="Interaction" /></td>
</tr>
</tbody>
</table>
Actin-related proteins (Arp2/3)

- Arp2/3 protein complex
  - control of polymerization
  - lamellipodia localization
  - human complex has 7 subunits
    - ARP2, ARP3, ARC41, ARC34, ARC21, ARC20, and ARC16

- Listeria monocytogenes
  - Induce actin polymerization by Arp2/3 protein complex at Listeria surface

Actin Motors - Myosin
Actin Motors - Myosin

• Myosins
  – Myosin I
    • All cells
    • One head domain
      – Binds actin
  – Myosin II
    • Muscle myosin
      – Also other cells
    • Dimer, 2 heads
    • Bind to each other to form myosin filament
      – Thick filament
Actin Motors- Myosin

Actin (red), Myosin II (green)
Late Philip Presley, MBL:
Fluorescence filter tuning of Zeiss Photomicroscope- III, allowing precise registration for the dual channel exposures.

Myosin I (green), Myosin II (red)
Dr. Edward Korn, Dr. Thomas Lynch, NIH:
Polyclonal anti-Acanthamoeba myosin-I antibody, revealed a unique localization to myosin isoforms

Image Source: http://faculty-web.at.northwestern.edu/med/fukui/04-Cytoskeleton.html
Myosin Movement

MBoC Figure 16-71
Muscle Types

• **Skeletal, cardiac**
  – Striated
  – sarcomeres

• **Smooth**
  – non-striated
Skeletal Muscle

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http://www.lab.anhb.uwa.edu.au/mb140/

MBoC Figure 16-83/85
Muscle Contraction

• sliding of filaments actin against myosin
  – troponin and tropomyosin
    • contraction of skeletal and cardiac muscle regulated by \( \text{Ca}^{2+} \) flux

• smooth muscle cells and non-muscle cells
  – contraction same mechanism
  – contractile units smaller less highly ordered
    • activity and state of assembly controlled by \( \text{Ca}^{2+} \) - regulated phosphorylation of a myosin
Microfilament Binding Molecules

- **Cytochalasin D**
  - Fungal metabolite
  - Binds barbed end
  - Inhibits polymerization and depolymerization
    - Cell permeant
    - Active in low micromolar

- **Phalloidin**
  - Fungal metabolite
  - Binds and stabilizes F-actin
    - Not cell permeant
    - Fluorescent derivatives are used to stain F-actin in situ and in vitro

- **Jasplakinolide**
  - Sea sponge metabolite
  - Binds and stabilizes F-actin competitively with phalloidin
  - Causes nucleation
    - Cell permeant
    - Nanomolar Kd for F-actin

- **Latrunculin**
  - Sea sponge metabolite
  - Binds monomeric actin
  - Inhibits polymerization
    - Cell permeant
    - Active at low nanomolar
A Selection of MF Diseases 1

• Actin
  • So essential to cell that diseases due to mutation of cytoskeletal actin rarely seen

• Cardiac Actin

• Tropomyosin
A Selection of MF Diseases 2

• Myosin

• Wasp
  • Novel mutations in the Wiskott-Aldrich syndrome protein gene and their effects on transcriptional, translational, and clinical phenotypes.

• Destrin

• Filamin