Lecture Overview

- Microfilaments
  - Structure, function and regulation
- Actin
  - Motility
  - Adhesion, focal adhesions
  - Actin binding proteins, myosin
  - Muscle actins
- Microfilament diseases
- UNSW Cell Biology

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 p521
  - Molecular Cell Biology Ch19
Cytoskeleton Filaments

Structural Systems

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

Microtubules
- transport
- karyokinesis

Intermediate Filaments
- compression resistance

Actin functional challenge

Diversity 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

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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 -> 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 (β:γ) 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

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Actin Isoforms are Functionally Distinct

\[ \beta^- \text{ vs } \gamma^- \text{actin in myoblasts} \]
- \( \beta^- \)-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

Cell Migration

Image: MBoC Figure 16-54
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

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Focal Adhesions

Adhesive Signalling

Actin Signaling

- Rho
  - Family of small GTPases
  - 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

Actin Filaments
Tropomyosin slows ‘off-rate’

- Increase tensile strength

Actin functional challenge

- Diversity function
  - dynamics
  - organisation
  - mechanics
- Spatial specialisation
  - pool sizes
  - function
- Evolution
  - simple principle

Distinct subcellular sorting of cytoskeleton Tm isoforms

- Tm1,2,3,5a,5ab,6
- Tm1,2,3
- Tm5a,b
- Tm5NM2
- Tm5NM1,2
- Tm5
- Tm5NM1

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: Filopodia

Small GTPase © Dr M.A. Hill, 2008 Slide 30


Rho activation mimics Tm5NM1 over-expression

Rac activation mimics Tm3 over-expression

Cdc42 activation mimics TmBr3 over-expression

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

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### 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>Scheme of Interaction with Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strengthening function</td>
<td>Actin filament</td>
<td>Tube-like structure</td>
<td>Actin monomer</td>
</tr>
<tr>
<td>Rho family</td>
<td>Actin filament</td>
<td>Star-like structure</td>
<td>Actin filament</td>
</tr>
<tr>
<td>Focal adhesion</td>
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<td>Spoke-like structure</td>
<td>Actin filament</td>
</tr>
<tr>
<td>Migration machinery</td>
<td>Actin filament</td>
<td>Ring-like structure</td>
<td>Actin filament</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

- **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

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 "a" motors.

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 Movement
MBoC Figure 16-71

Muscle Types
• Skeletal, cardiac
  – Striated
  – sarcomeres
• Smooth
  – non-striated

Skeletal Muscle
MBoC Figure 16-83/85
http://www.lab.anhb.uwa.edu.au/mb140/
**Muscle Contraction**
- Sliding of filaments actin against myosin
  - Troponin and tropomyosin
  - Contraction of skeletal and cardiac muscle regulated by 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 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**
  - 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**
Model of function