ANAT3231 - Cell Biology
Lecture 12

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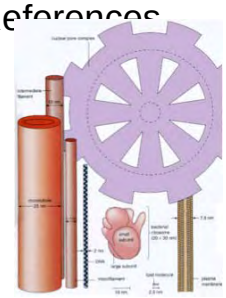
Lecture Overview

- 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

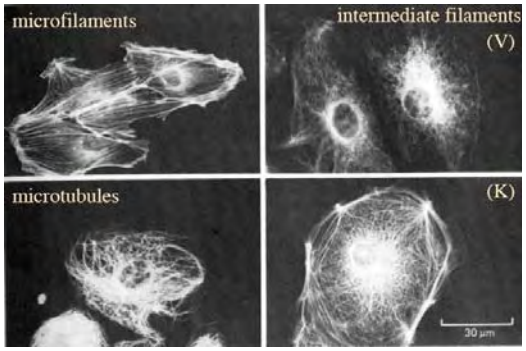
Image: Dr. Barber at Pikeville College, KY

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



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Structural Systems

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

Microtubules
- transport
- karyokinesis

Intermediate Filaments
- compression resistance

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Actin functional challenge

Diversify function
- dynamics
- organisation
- mechanics

Spatial specialisation
- pool sizes
- function

Evolution
- simple principle

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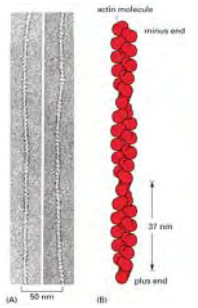
Microfilaments

- 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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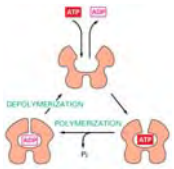
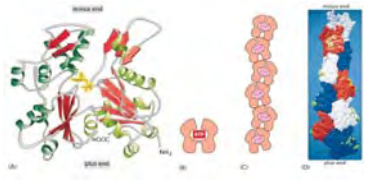
MBoC Figure16-49

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)





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MBoC Figure16-50/51

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

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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

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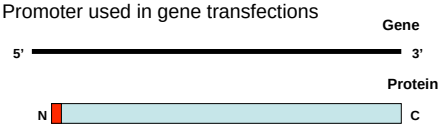
Actin Protein

- 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 Distict

β− vs γ−actin in myoblasts

- β−actin promotes cell spreading and stress fibres
- γ−actin inhibits cell spreading and stress fibre formation
- β− and γ−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

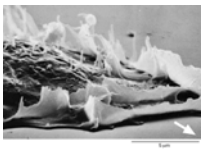
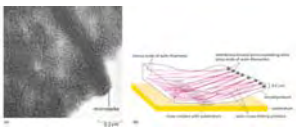


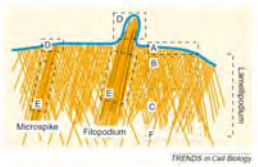
Image: MBoC Figure 16-54

Motile Structures

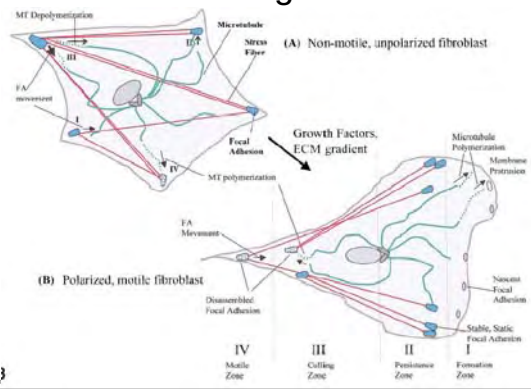
- Leading/Trailing Edge
  - extension/retraction
  - Actin nucleation
- Lamellipodia
  - Sheet-like extensions
- Filopodia
  - Thin protrusions
- Integrins anchor to ECM



MBoC Figure 16-55

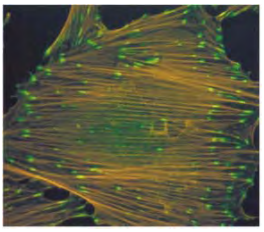


Cell Migration



Adhesive Functions

- Cell signalling
  - Modify cell cytoskeleton
  - Activate intracellular signalling pathways
  - Cell motility
  - **Note** adhesion is covered in detail in later Lecture

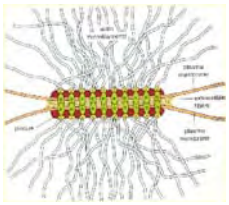


Adhesion Junctions

- 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

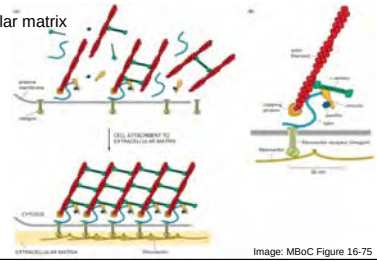
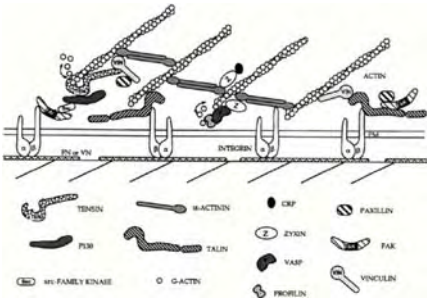
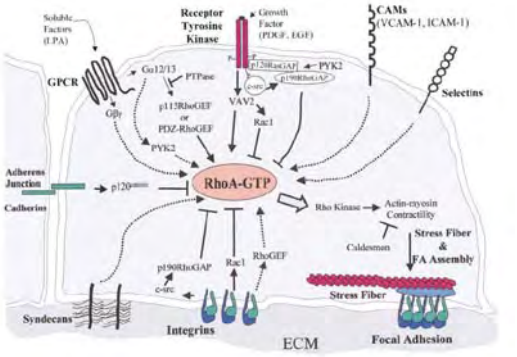


Image: MBoC Figure 16-75

Focal Adhesions



Adhesive Signalling



Actin Signaling

- Rho
  - Family of small GTPases organize the actin cytoskeleton
  - Rho, RAC, CDC42
  - Form different actin structures
    - Cell 1995 Apr 7;81(1):53-62
- Wasp
  - Wiskott-Aldrich syndrome protein
  - a downstream effector
  - transfers signal from tyrosine kinase receptors and small GTPases to actin cytoskeleton

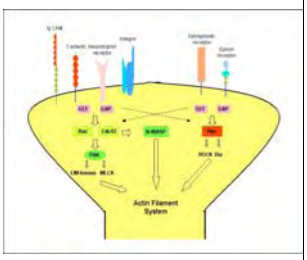
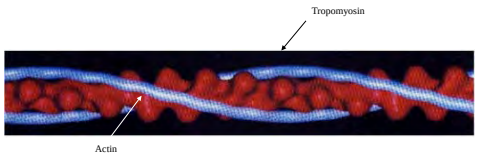
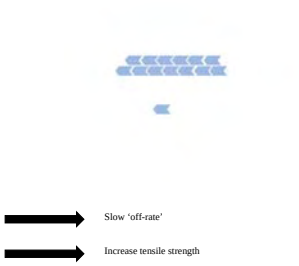


Image Source: http://www.zoo.uni-heidelberg.de/gep/k.thelen.htm

Actin Filaments



Tropomyosin slows 'off-rate'



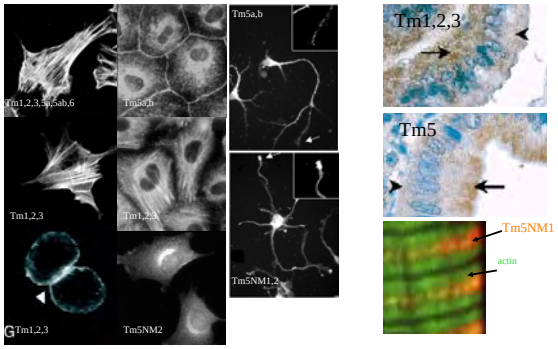
Actin functional challenge

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

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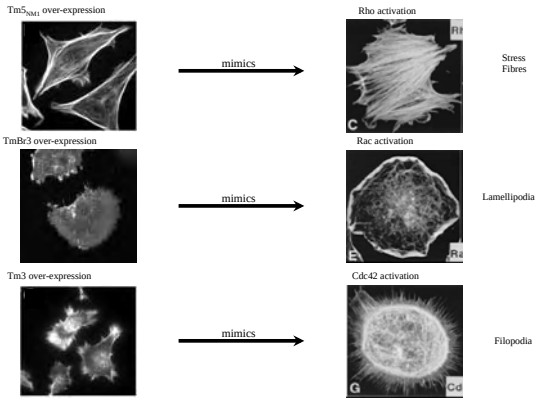

Hall, A. (1998) Science 279:509-514

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Actin Binding Proteins

- Regulate polymerisation and create different structures
  - Monomer binding protein
    - Sequester
    - release
  - Polymer binding proteins
    - Bundling
    - cross-linking
    - Severing
    - contracting

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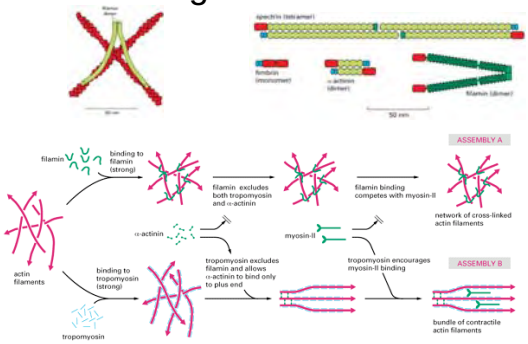
Actin Binding Protein Interactions



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MBoC Fugure 16-78

Actin Binding Proteins

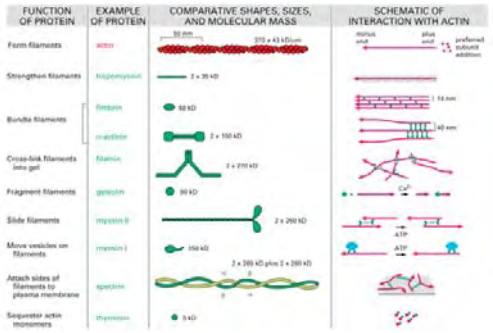

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Image: MBoC Figure 16-79

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

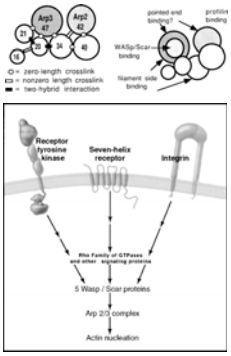

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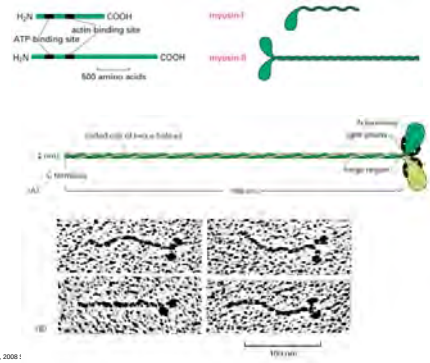
Image Source: Annu. Rev. Biophys. Biomol. Struct. 2000. 29:545-576

Actin Motors - Myosin



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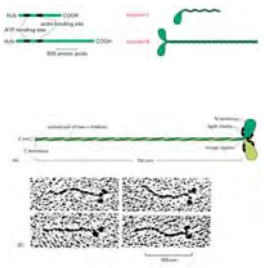
Actin Motors - Myosin

- 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



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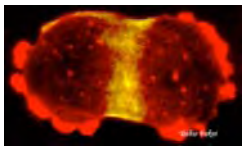
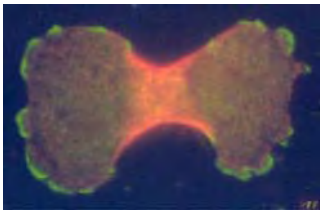
Actin Motors- Myosin



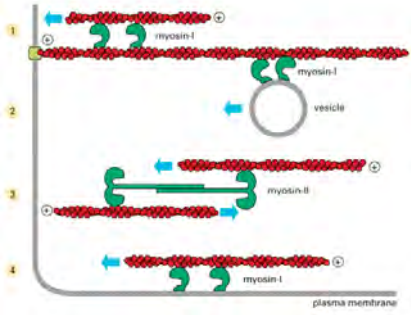
Actin (red), Myosin II (green)
Late Philip Presley, MBL:
Fluorescence filter tuning of Zeiss Photomicroscope- III, allowing precise registration for the dual channel exposures.



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

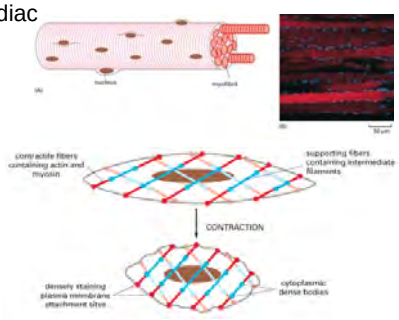
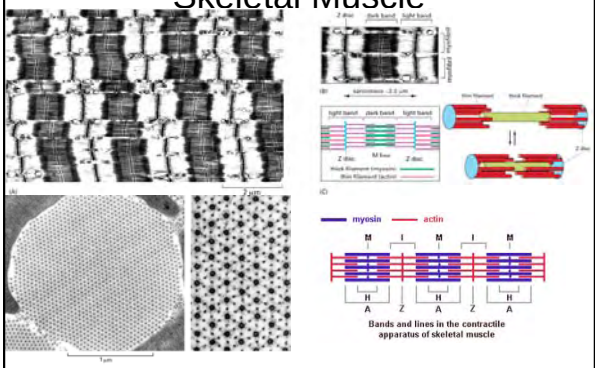


Image: MBoC Figure 16-82

Skeletal Muscle



MBoC Fugure 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

Text modifird from MBoC Muscle Summary

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

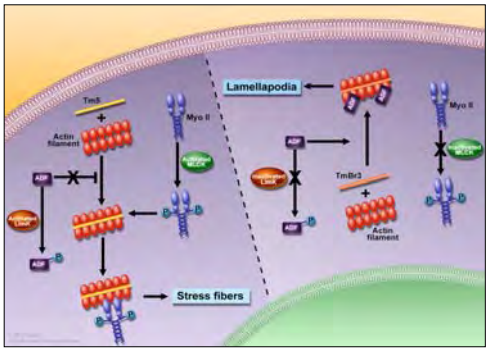
A Selection of MF Diseases 1

- Actin
  - So essential to cell that diseases due to mutation of cytoskeletal actin rarely seen
- Cardiac Actin
  - Mutational analysis of the cardiac actin gene in familial and sporadic dilated cardiomyopathy. Am J Med Genet. 1999 Oct 8;86(4):325-7.
- Tropomyosin
  - Clinical features of hypertrophic cardiomyopathy caused by mutation of a 'hot spot' in the alpha-tropomyosin gene. J. Am. Coll. Cardiol. 29: 635-640, 1997.
  - A mutation in the alpha tropomyosin gene TPM3 associated with autosomal dominant nemaline myopathy. Nature Genet. 9: 75-79, 1995.

A Selection of MF Diseases 2

- Myosin
  - Association of unconventional myosin MYO15 mutations with human non-syndromic deafness DFNB3. Science 280: 1447-1451, 1998.
- Wasp
  - Novel mutations in the Wiskott-Aldrich syndrome protein gene and their effects on transcriptional, translational, and clinical phenotypes.
- Destrin
  - Hum. Mutat. 14: 54-66, 1999. Aberrant actin cytoskeleton leads to accelerated proliferation of corneal epithelial cells in mice deficient for destrin (actin depolymerizing factor). Hum Mol Genet. 2003 May 1;12(9):1029-37.
- Filamin
  - Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. Nat Genet. 2003 Apr;33(4):487-91

Model of function



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