

**ANAT3231 - Cell Biology**  
**Lecture 12**  
 School of Medical Sciences  
 versity South Wale

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**Lecture Overview**

- Microfilaments
  - Structure, function and regulation
- Actin
  - Motility
  - Adhesion, focal adhesions
  - Actin binding proteins, myo
  - Muscle actins
- Microfilament diseases
- UNSW Cell Biology
- <http://cellbiology.med.unsw.edu.au/units/science/lecture07.htm>

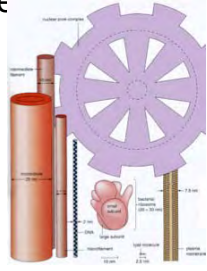


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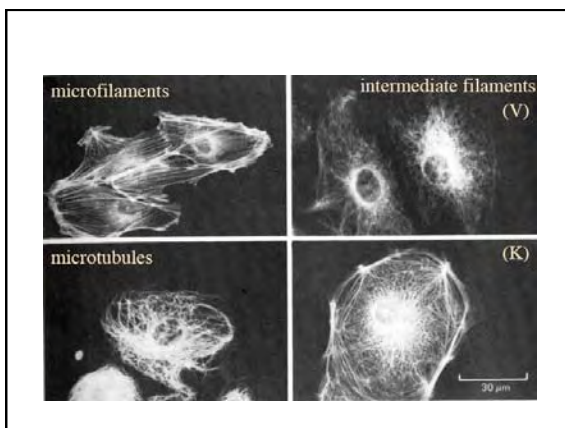
Image: Dr. Barber at Pikeville College, KY

**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



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**Structural Systems**

- Microfilaments**
  - shape
  - motility
  - contractility
  - cytokinesis
  - transport
  - compartments
- Microtubules**
  - transport
  - karyokinesis
- Intermediate Filaments**
  - compression resistance

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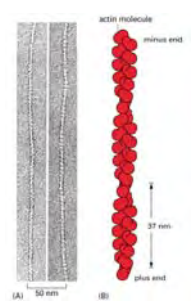
### Actin functional challenge

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

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### Microfilaments

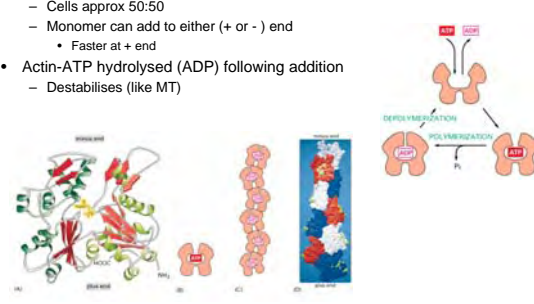
- Twisted chain 7 nm diameter
- Compared to MT
  - Thinner, more flexible, shorter
- Point in same direction
- Different organisation in different cellular regions



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### 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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### 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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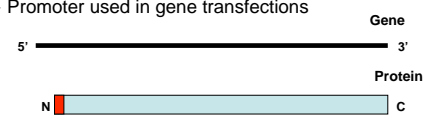
### Actin Types

- 6 Mammalian actin types (isoforms)
  - All are 43 Kd Protein
- 2 **cytoskeletal** isoforms in all non-muscle cells
  - Beta ( $\beta$ ) 7p22-p12
  - Gamma ( $\gamma$ ) 17q25
- 4 **muscle** isoforms in different muscle cells
  - Alpha ( $\alpha$ ) skeletal
  - Alpha ( $\alpha$ ) cardiac
  - Alpha ( $\alpha$ ) smooth
  - Gamma ( $\gamma$ ) smooth

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### 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



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### 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

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### Cell Movement

- Whole or part of cell
  - Amoeba, neutrophil, macrophages
  - Neuron processes
    - axon, dendrites
  - Common structures
  - Contraction
- Intracellular transport

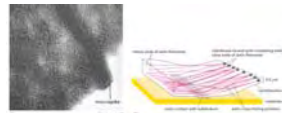


Image: MBOC Figure 16-54

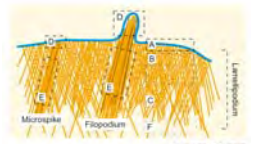
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### Motile Structures

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

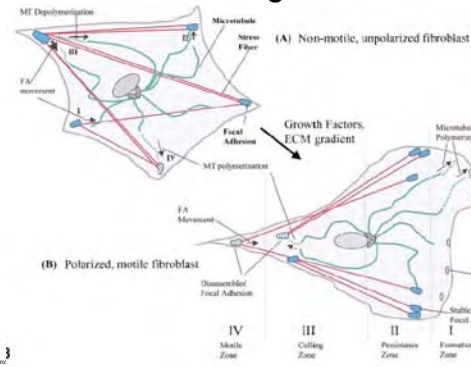


MBOC Figure 16-55



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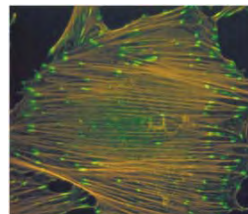
### Cell Migration



Micro 3

### Adhesive Functions

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



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### Adhesion Junctions

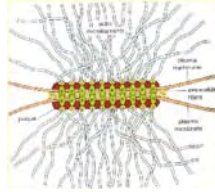
- Adherens Junctions
  - microfilaments anchor the plaque that occurs under the membrane of each cell.
  - plaques not as dense
    - also occur as hemiform



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### Adherens Junctions

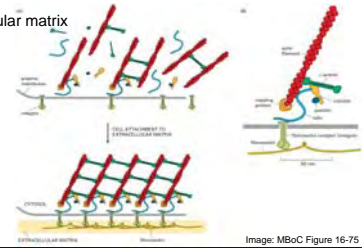
- heart muscle, layers covering body organs, digestive tract.
- transmembrane proteins
- Cadherin



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### Adhesion Junctions

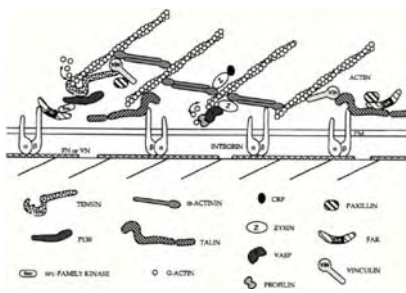
- Adherens (cell-cell)
  - cadherin (E-cadherin)
  - Links to cadherin in neighboring cell
- Adherens (cell-matrix)
  - Integrin
  - Links to extracellular matrix



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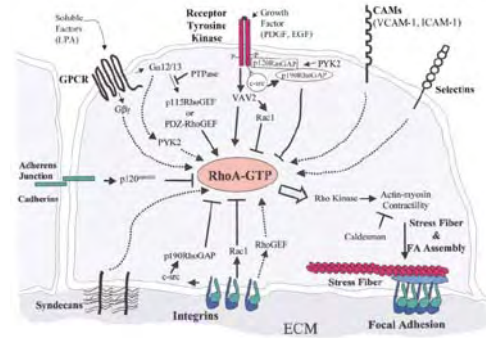
Image: MBoC Figure 16-75

### Focal Adhesions



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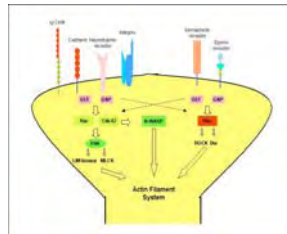
### Adhesive Signalling



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### Actin Signaling

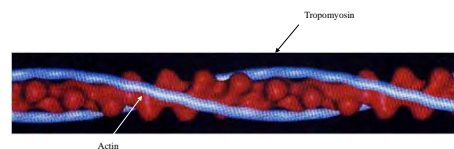
- Rho
  - Family of small GTPases organize the actin cytoskeleton
  - Rho, Rac, CDC42
  - Form different actin structures
    - Cell 1995 Apr 7;81(1):53-62
- Wasp
  - Wiskott-Aldrich syndrome protein
  - a downstream effector
  - transfers signal from tyrosine kinase receptors and small GTPases to actin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



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Image Source: <http://www.zoo.uni-heidelberg.de/cgi-bin/kthelen.htm>

### Actin Filaments



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### Tropomyosin slows 'off-rate'

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### Actin functional challenge

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

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### Distinct subcellular sorting of cytoskeleton Tm isoforms

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### Isoforms Define Specific Functional Properties of Actin Filaments

- Spatially segregated filaments contain different tropomyosins.
- Spatially segregated filaments have different functional roles in the cell.

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### Small GTPase Regulate the Actin Cytoskeleton

Rho → Stress Fibres  
 Rac → Lamellapodia  
 Cdc 42 → Filipodia

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

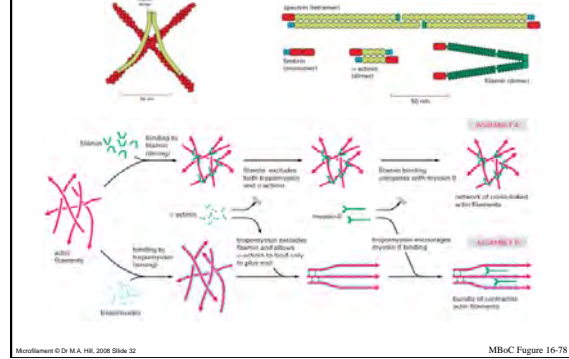
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### Actin Binding Proteins

- 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 Figure 16-78

### Actin Binding Proteins

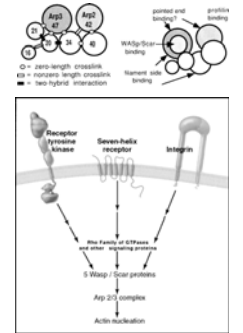
FUNCTION OF PROTEIN	EXAMPLE OF PROTEIN	COMPARATIVE SHAPES, SIZES, AND MOLECULAR MASS	SCHEMATIC OF INTERACTION WITH ACTIN
Form filaments	actin	42 kD, 375 x 43 kDaum	actin monomer, actin filament, polymerized actin filament
Strengthen filaments	formin	2 x 35 kD	actin filament, actin filament with formin
Bundle filaments	alpha-actinin	66 kD	actin filament, actin filament bundle
Cross-link filaments into gel	filipin	2 x 100 kD	actin filament, actin filament network
Fragment filaments	gelsolin	80 kD	actin filament, actin filament with gelsolin
Slide filaments	myosin II	2 x 200 kD	actin filament, actin filament with myosin II, ATP
Move vesicles on filaments	myosin V	180 kD	actin filament, actin filament with myosin V, ATP
Attach sides of filaments to plasma membrane	spectrin	2 x 180 kD plus 2 x 260 kD	actin filament, actin filament with spectrin
Sequester actin monomers	thymosin beta 4	9 kD	actin monomer, actin monomer with thymosin beta 4

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Image: MBoc Figure 16-79

### Actin-related proteins (Arp2/3)

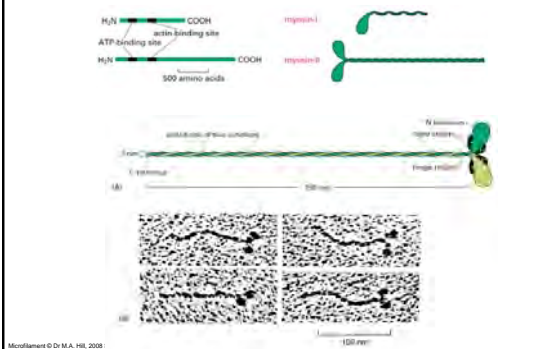
- Arp2/3 protein complex
  - control of polymerization
  - lamellipodia localization
  - human complex has 7 subunits
    - ARP2, ARP3, ARC41, ARC34, ARC21, ARC20, and ARC16
- Listeria monocytogenes
  - Induce actin polymerization by Arp2/3 protein complex at Listeria surface



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

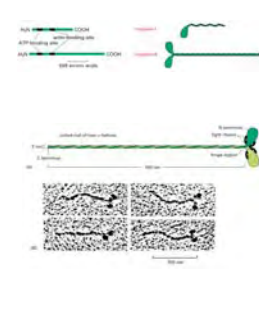
### Actin Motors - Myosin



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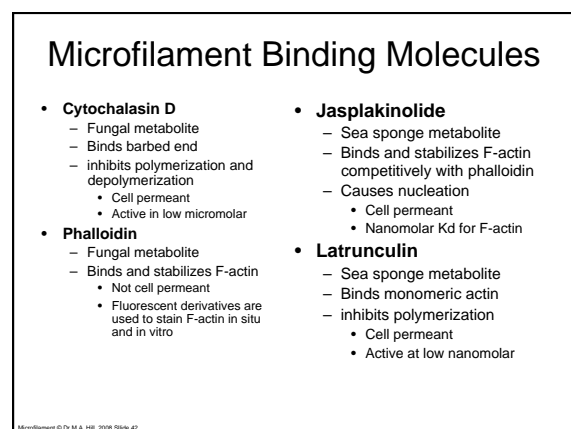
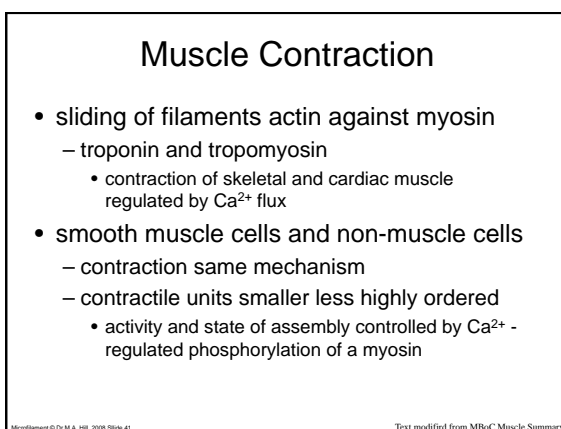
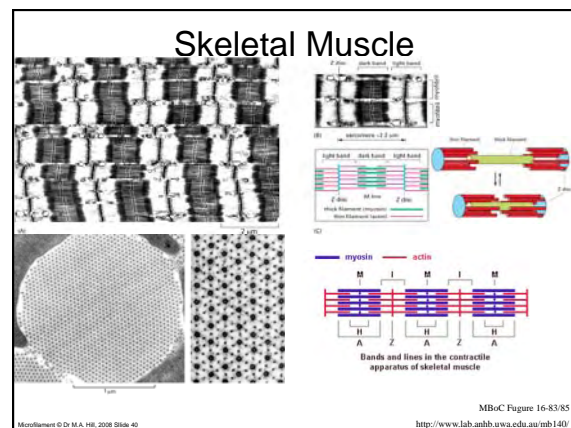
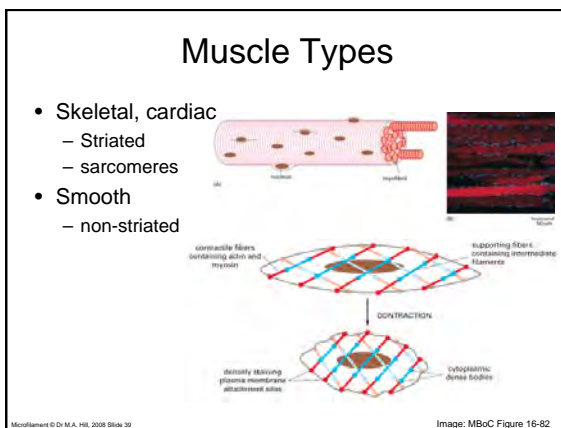
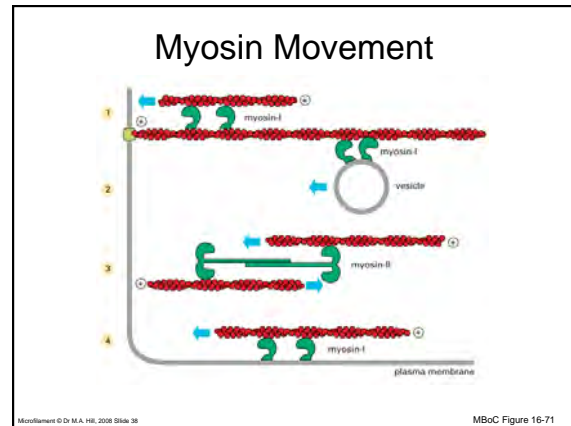
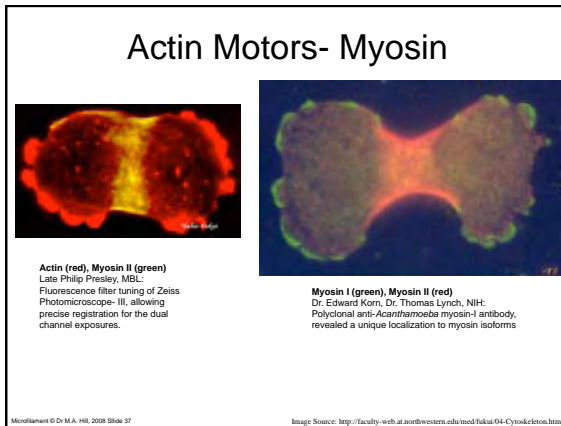
### Actin Motors - Myosin

- 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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### 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.

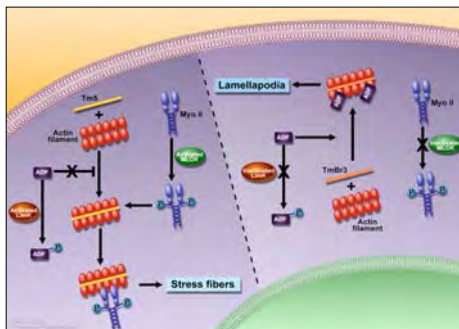
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### A Selection of MF Diseases 2

- **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

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### Model of function



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