Cellular Biophysics
Lecture 3: Cell cytoskeleton

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Cellular Mechanotransduction

Contents

1. Recap from previous lecture

2. Focal adhesions

3. Cytoskeleton

    - actins, microtubules, intermediate filaments

4. Force generation in the actin cytoskeleton

5. Tensegrity model of the cell

Cellular Mechanotransduction

Extracellular matrix

Collagens:

- over 25 different collagens have been found
- characteristically triple stranded helical structure

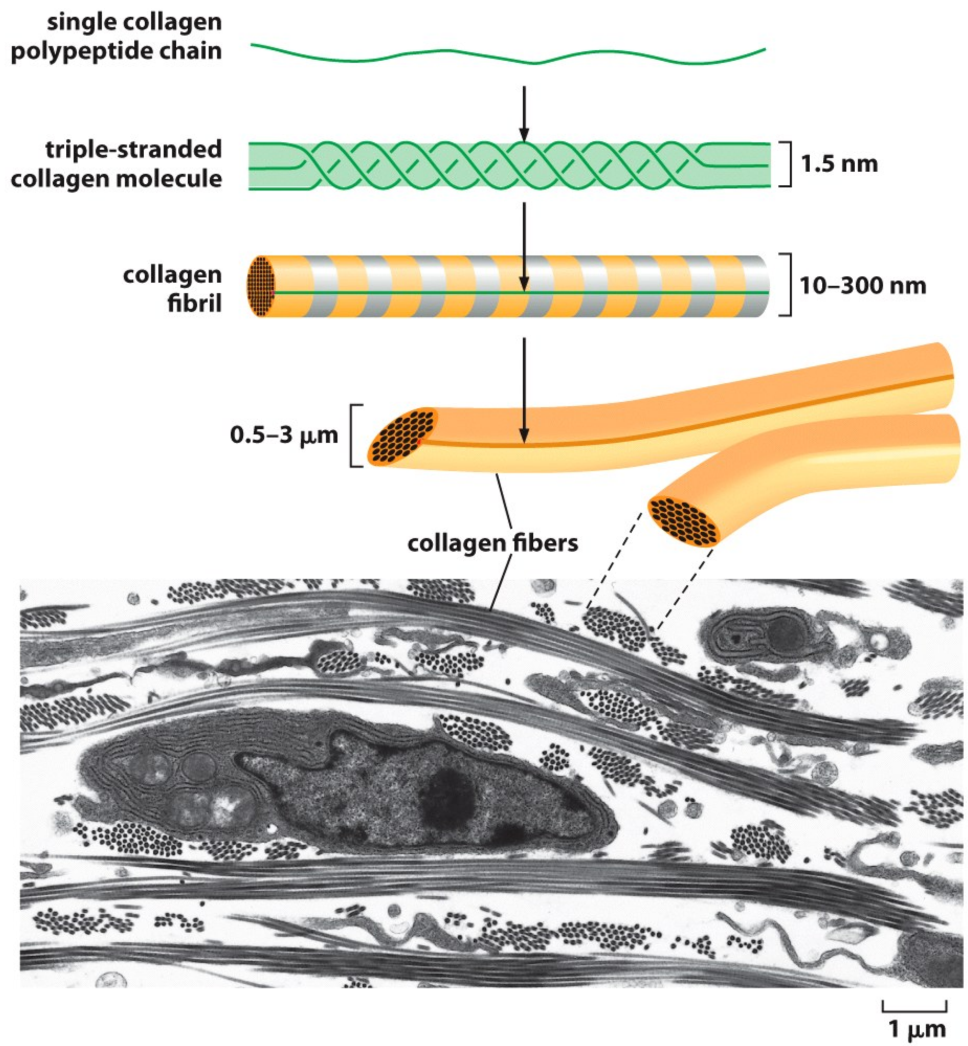


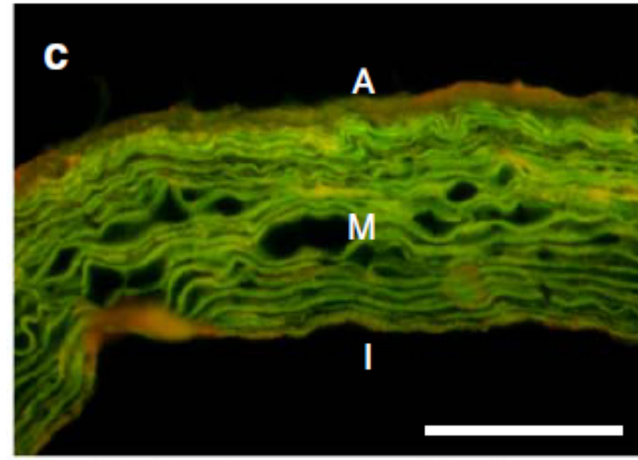
Figure 20-9 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Extracellular matrix

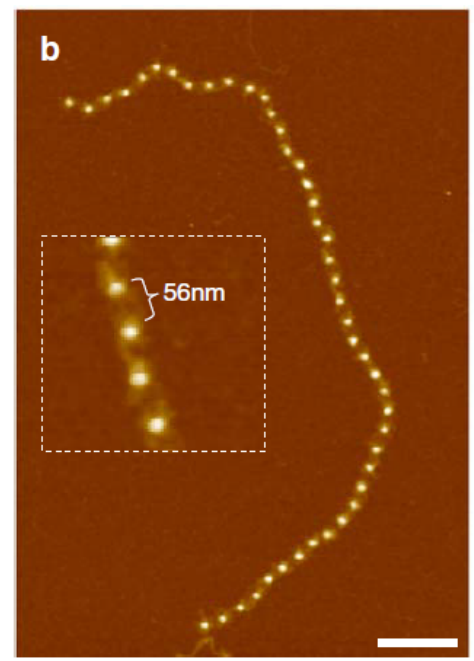
Elastic fibers

Elastin core (90%) which is surrounded by microfibrillar (10%) fibers



Isolated fibrillin fibers have beaded periodicity

Structure and function has not been resolved in detail



Cellular Mechanotransduction

Extracellular Matrix

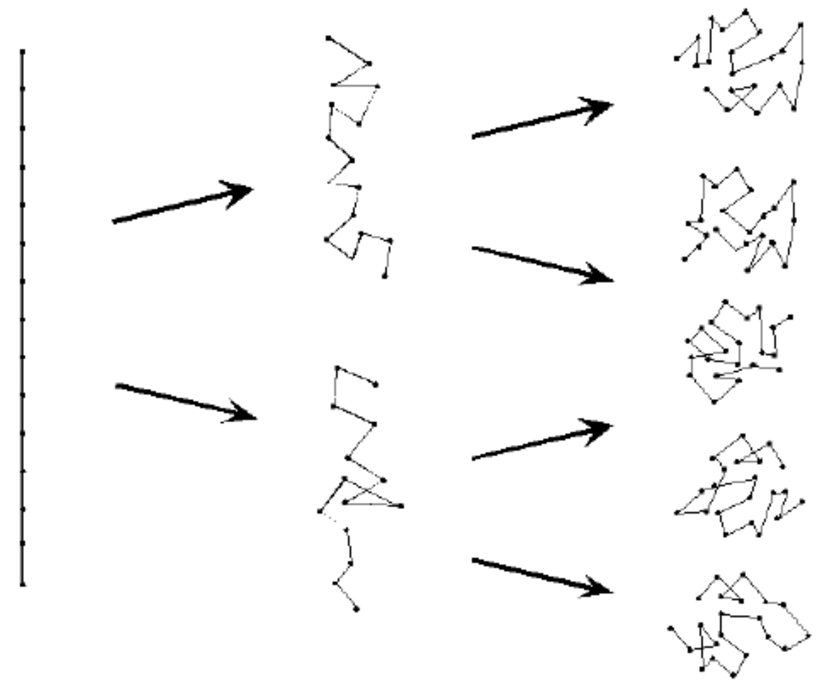
Elastin

Behaves as an entopic spring

$$\Delta G = \Delta U - T\Delta S$$

ΔU can be approximated to 0

During stretching $\Delta S < 0$
→ $\Delta G > 0$
→ non-spontaneous process

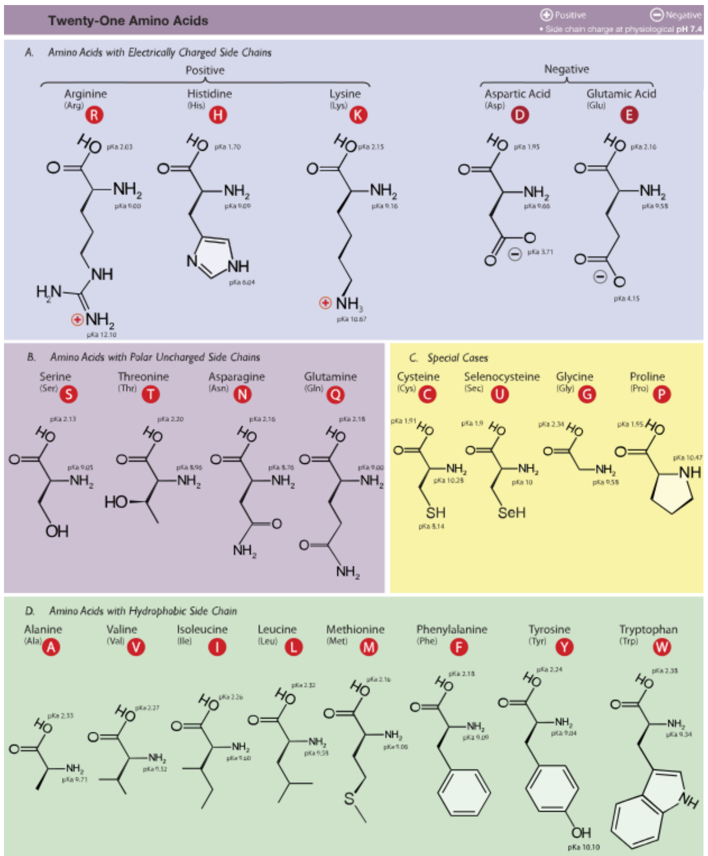


Higher order
Lower entropy

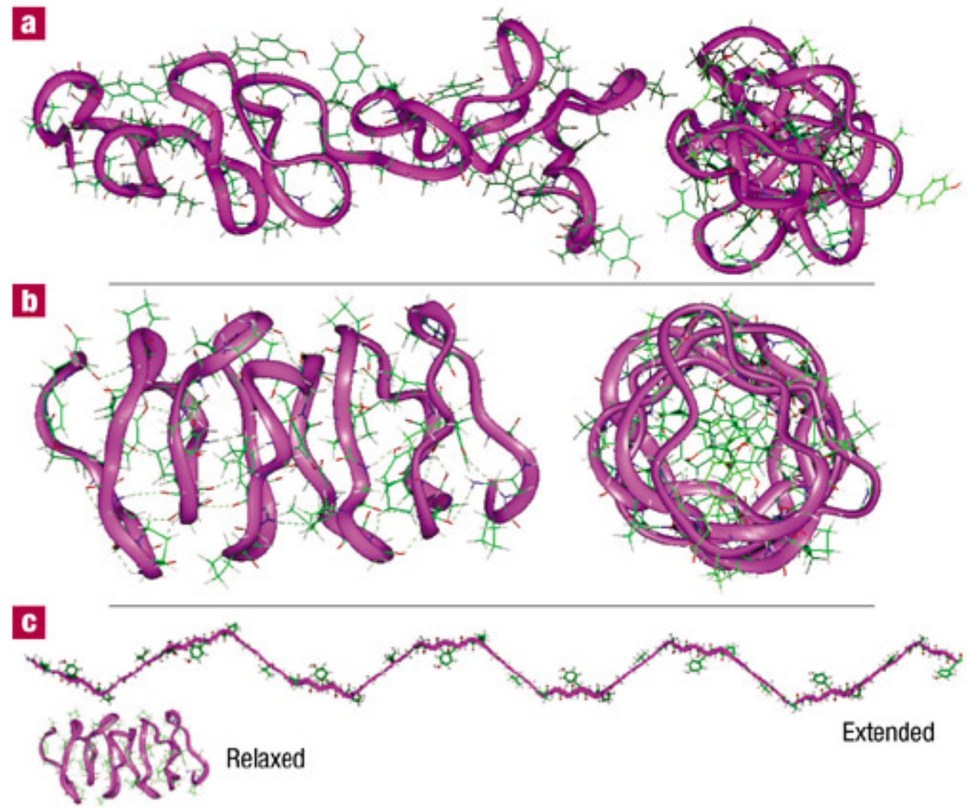
Lower order
high entropy

Cellular Mechanotransduction

Proteins and protein folding



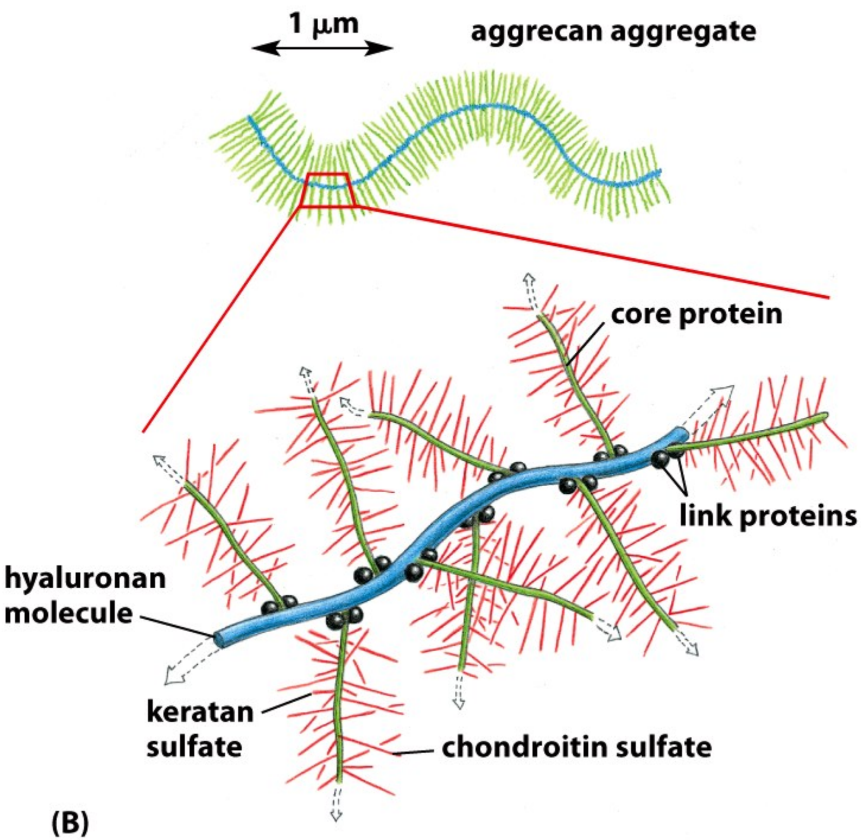
- $(GPGGX)_{15}$ repetition in spider silk (X is V or Y)



Molecular nanosprings in spider capture-silk threads
Nathan Becker, Emin Oroudjev, Stephanie Mutz, Jason P. Cleveland, Paul K. Hansma, Cheryl Y. Hayashi, Dmitrii E. Makarov & Helen G. Hansma
Nature Materials **2**, 278 - 283 (2003) Published online: 23 March 2003
doi:10.1038/nmat858

Cellular Mechanotransduction

Extracellular matrix



Proteoglycans

Proteoglycans are highly negatively charged

- Form gels even at low concentrations

High charge leads into high osmotic activity

→ Swelling pressure

Aggregan molecule from cartilage

Sugars are red and blue, proteins green and black

Cellular Mechanotransduction

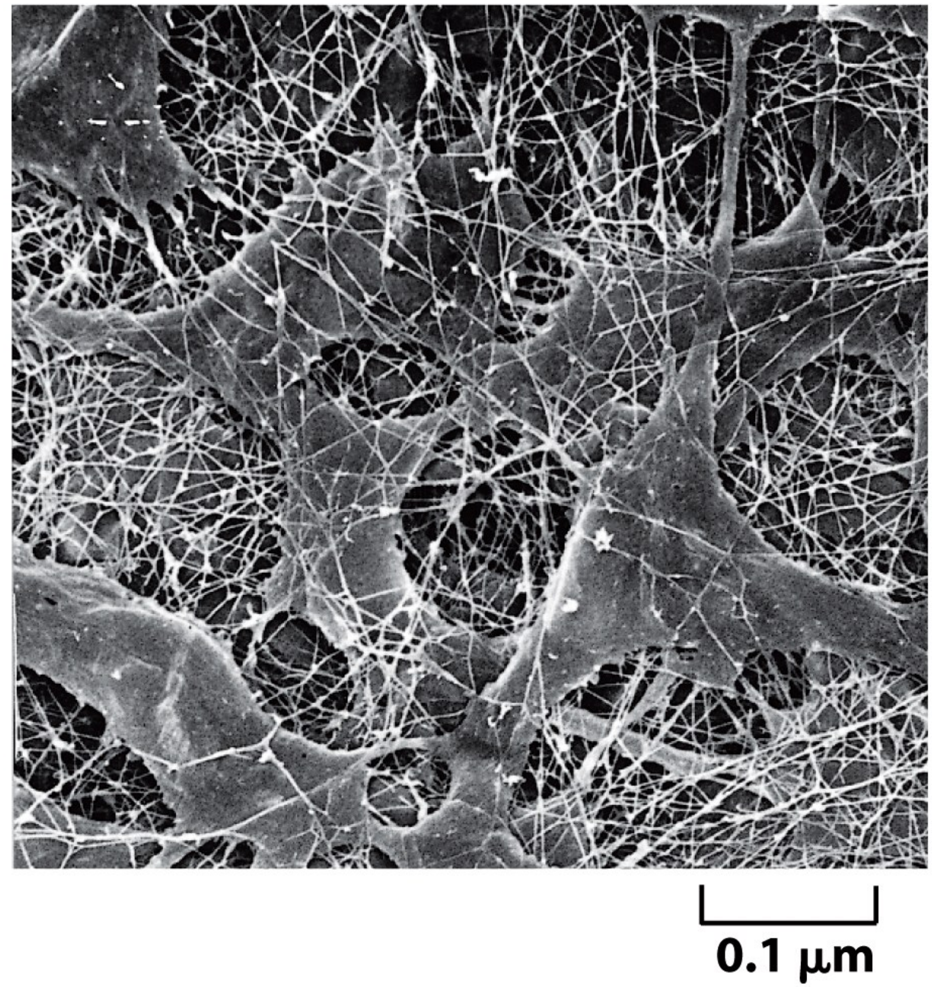
Extracellular matrix

The ECM components are secreted by the cells. The major ECM modifying cells are:

- Fibroblasts (skin, tendon, etc.)
- Osteoblasts (bone)

ECM of collagens + elastic fibers + proteoglycans

- Gel which is “tough” (proteoglycans), “tensed” (collagens) and “elastic” (elastic fibers)



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Cellular Mechanotransduction

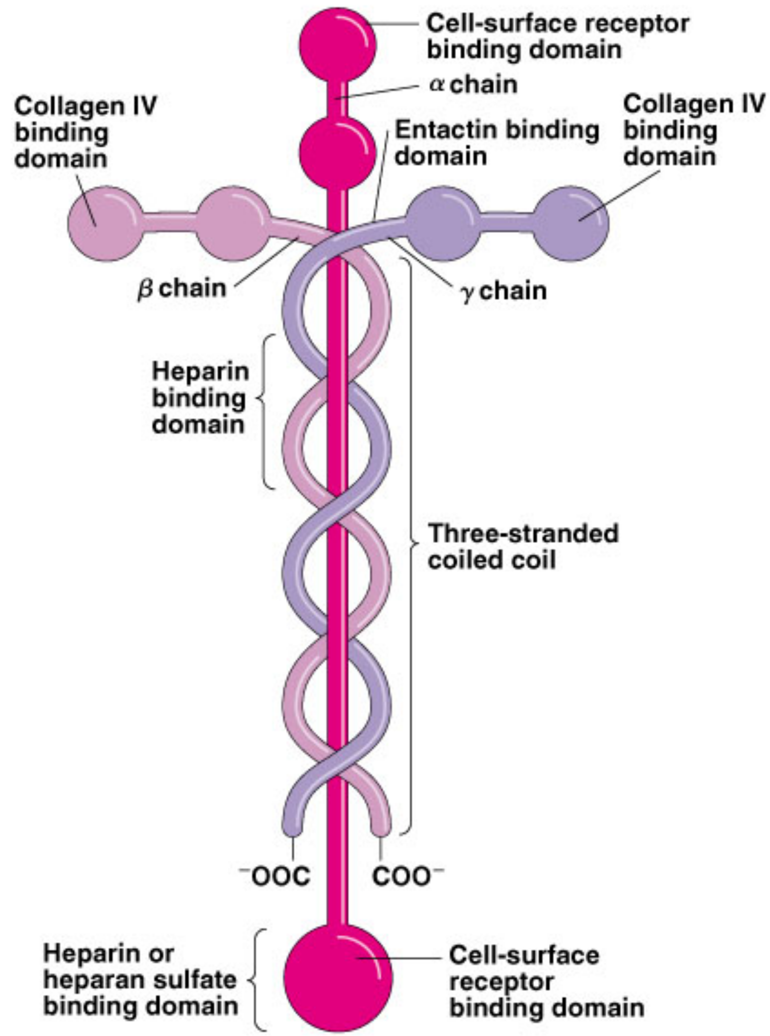
Cell attachment to extracellular matrix

Laminin

-Found in basal lamina and basement membrane

- Binds heparin, Type IV collagen, entactin

- Offers binding sites for cells

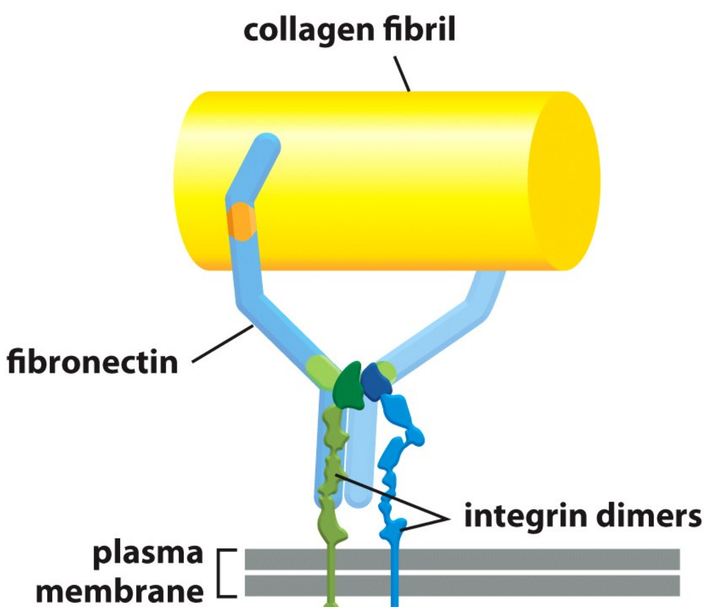


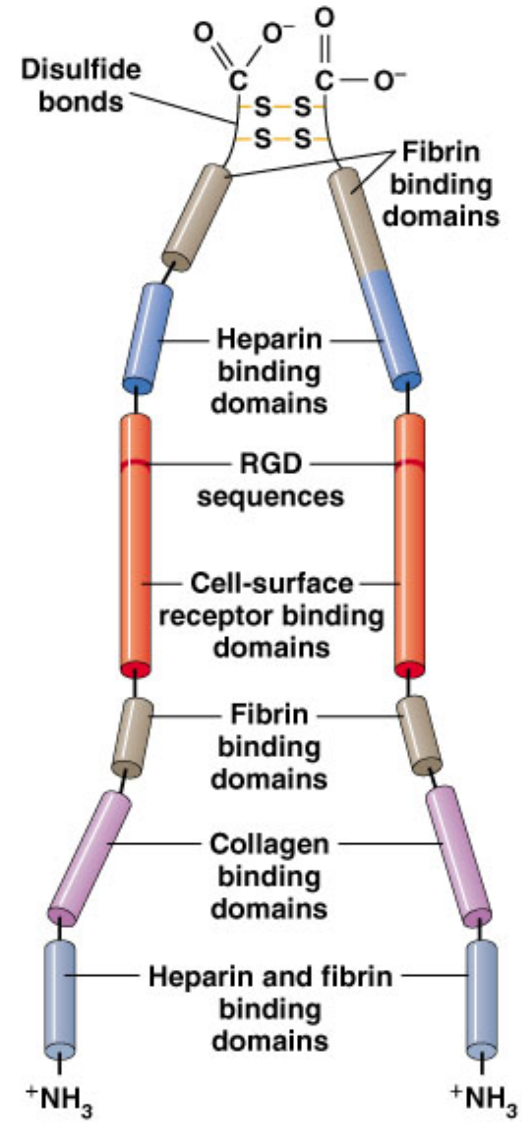
Cellular Mechanotransduction

Cell Attachment to extracellular matrix

Fibronectin

- Dimeric protein, forms fibers
- Can bind collagen, fibrin (converted from fibrinogen by thrombin), proteoglycans
- Offers binding sites for cells





Cellular Mechanotransduction

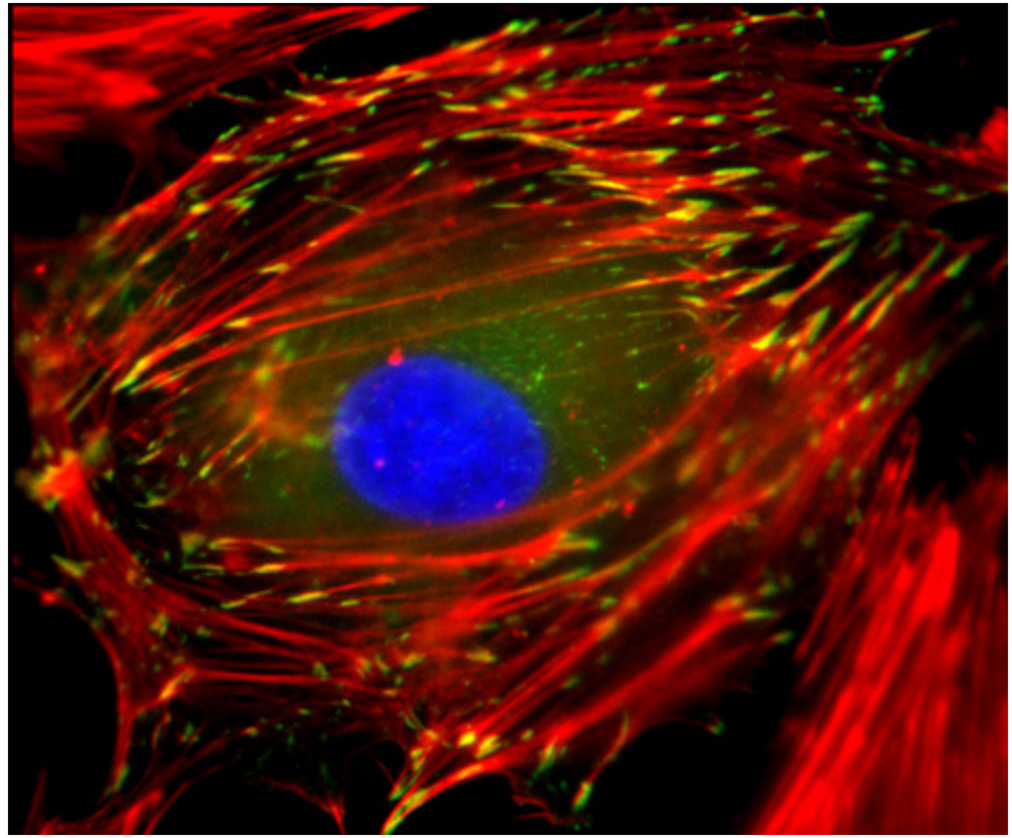
Cell Attachment to Extracellular Matrix

Focal adhesions

- Large protein complexes which physically connect the cells to the ECM

- Contain tens of different proteins (integrins, vinculin, talin, FAK, actin, actinin zyxin, etc.),

-Highly dynamic



http://wyss.harvard.edu/viewmedia/72/focal-adhesions-and-actin-cytoskeleton-1

Cellular Mechanotransduction

“Traditional” view of signaling from cell membrane

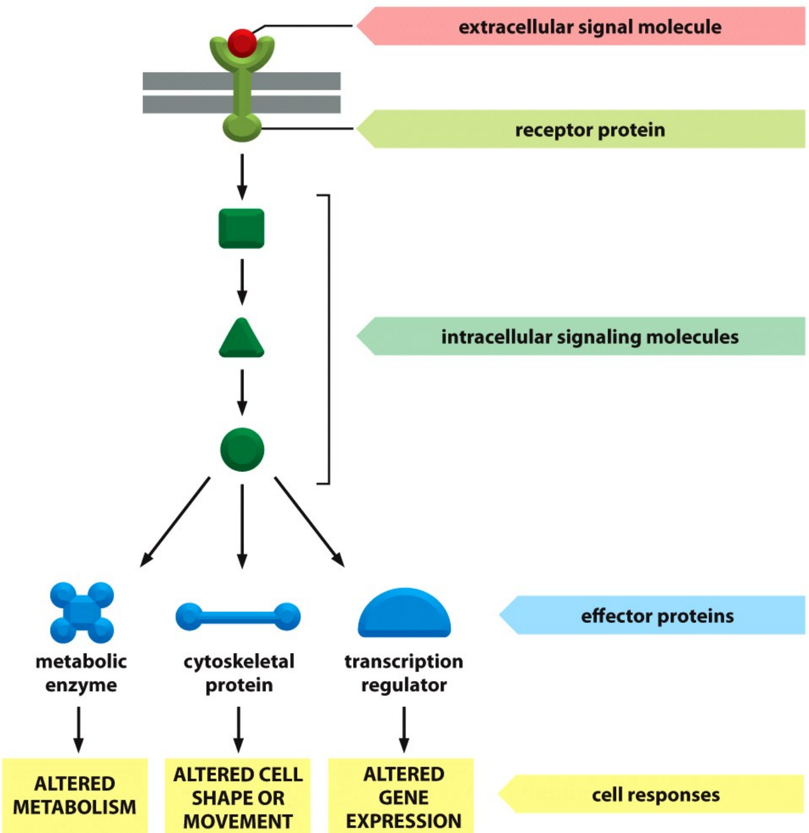


Figure 16-12 Essential Cell Biology 3/e (© Garland Science 2010)

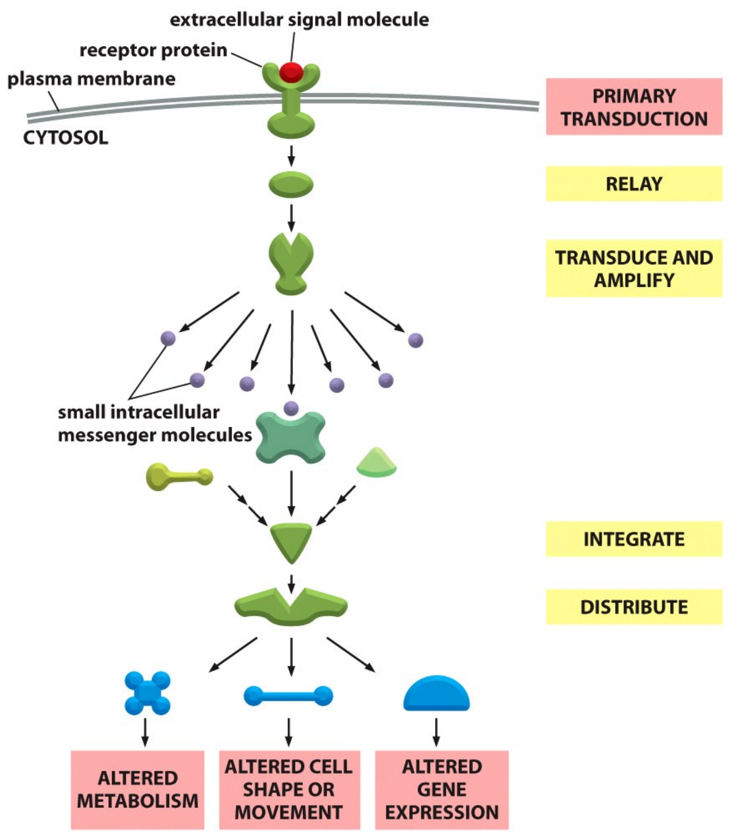


Figure 16-13 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

FAs and Integrins

Integrins link the FA to the ECM

- Heterodimeric transmembrane proteins (alpha and beta chains)

- Outside the cell bind laminin, fibronectin, collagen, …

- Can be activated either from inside or outside of the cell

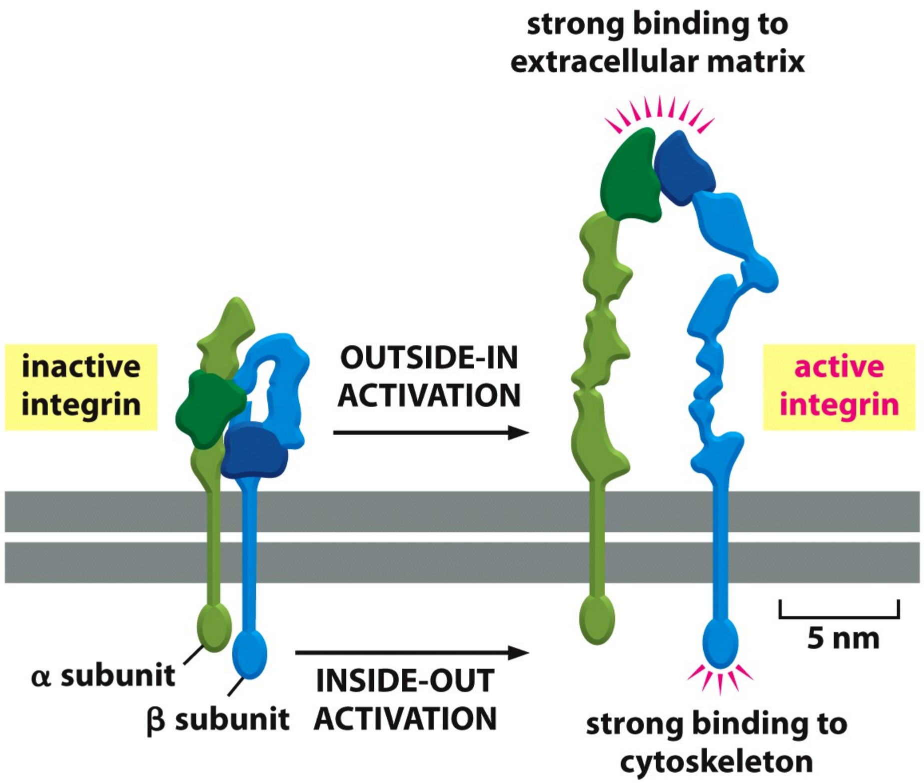


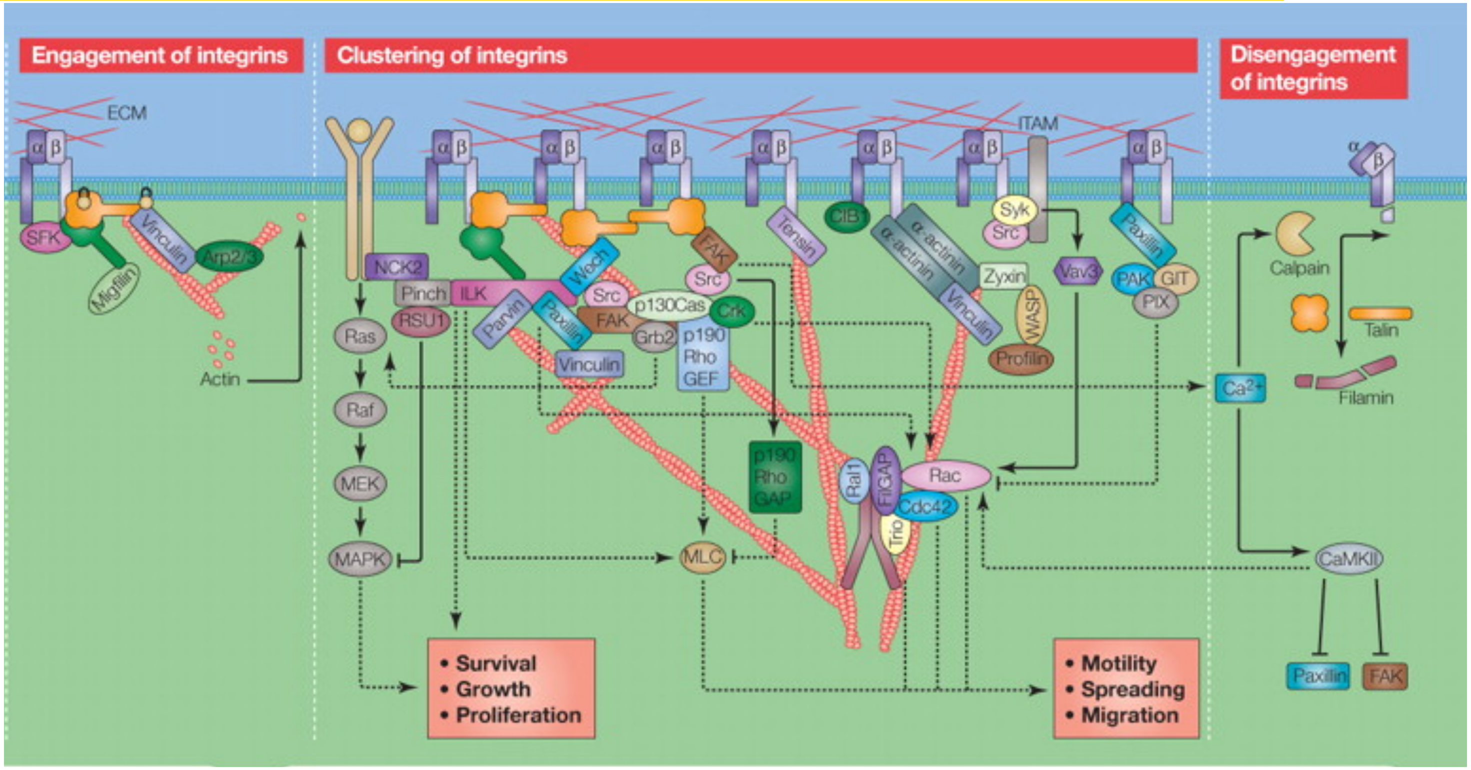
Figure 20-15a Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Integrins

Signaling from the integrins

- Usually requires clustering of integrins
- Variety of different signaling routes can be activated leading to different responses (migration, proliferation etc.)



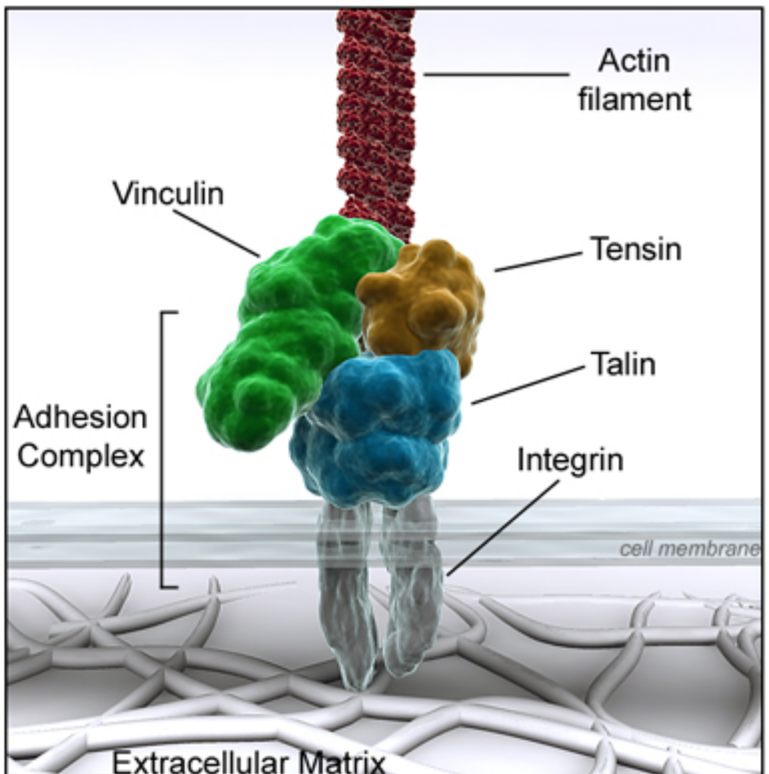
Harburger D S , and Calderwood D A J Cell Sci 2009;122:159-163

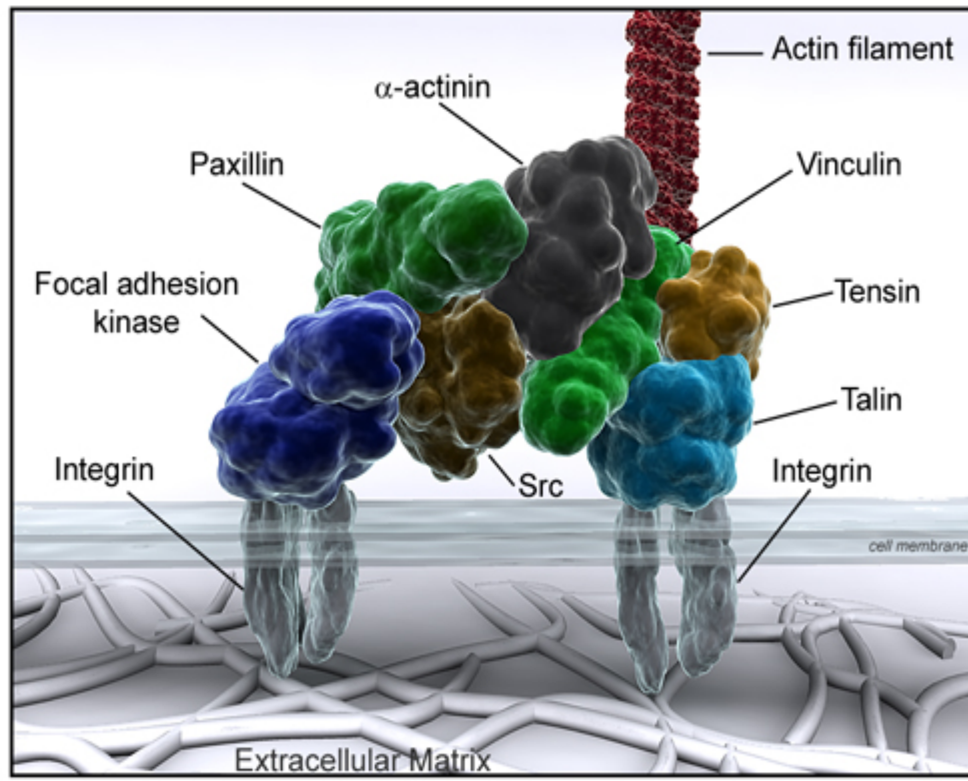
Cellular Mechanotransduction

Focal adhesions

Formation of focal adhesions

- Adhesion complex maturates to focal adhesion





http://www.reading.ac.uk/cellmigration/adhesion.htm

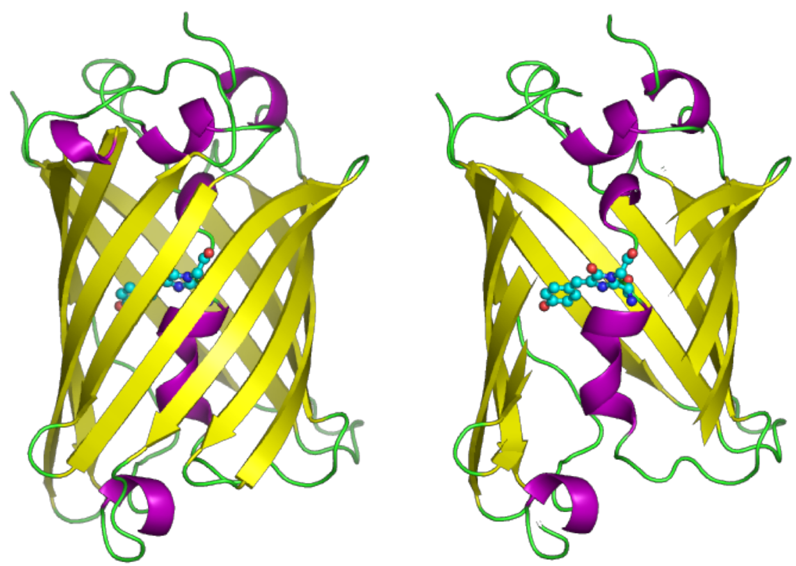
Cellular Mechanotransduction

Fluorescent proteins as a tool to study protein dynamics

Problem in fluorescence microscopy: how to specifically label proteins of interest?

→ usage of fluorescent proteins which can be genetically fused to the target proteins

Enhanced Green Fluorescent Protein (EGFP)



Cellular Mechanotransduction

Fluorescent proteins as a tool to study protein dynamics

The development of EGFP technology was acknowledged with Nobel prize of chemistry at 2008

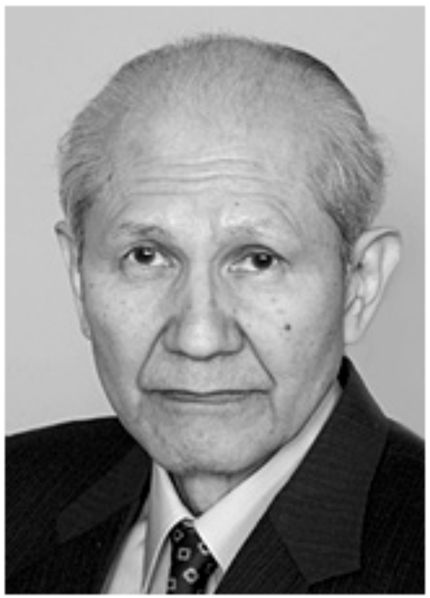


Photo: U. Montan

Osamu Shimomura

Photo: U. Montan

Martin Chalfie

Photo: U. Montan

Roger Y. Tsien

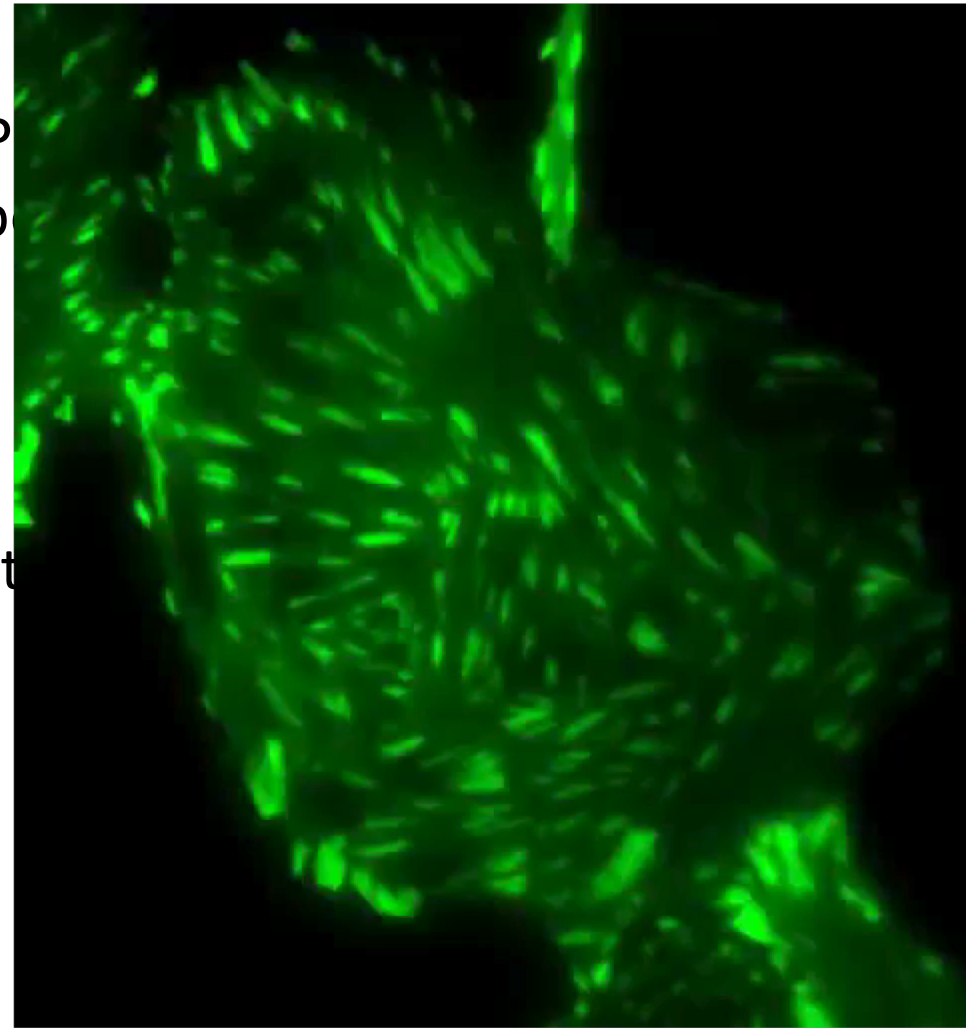
The Nobel Prize in Chemistry 2008 was awarded jointly to Osamu Shimomura, Martin Chalfie and Roger Y. Tsien *"for the discovery and development of the green fluorescent protein, GFP"*.

Cellular Mechanotransduction

Fluorescent proteins as a tool to study protein dynamics

By genetically modifying the EGFP the absorption and emission prope changed

EGFP-Vinculin showing the focal adhesions inside a living fibroblast cell



http://www.olympus-europa.com/microscopy/en/microscopy/media_centre_misd/media_detail_misd_26113.jsp

Cellular Mechanotransduction

Cell Cytoskeleton

Microtubules

Actin

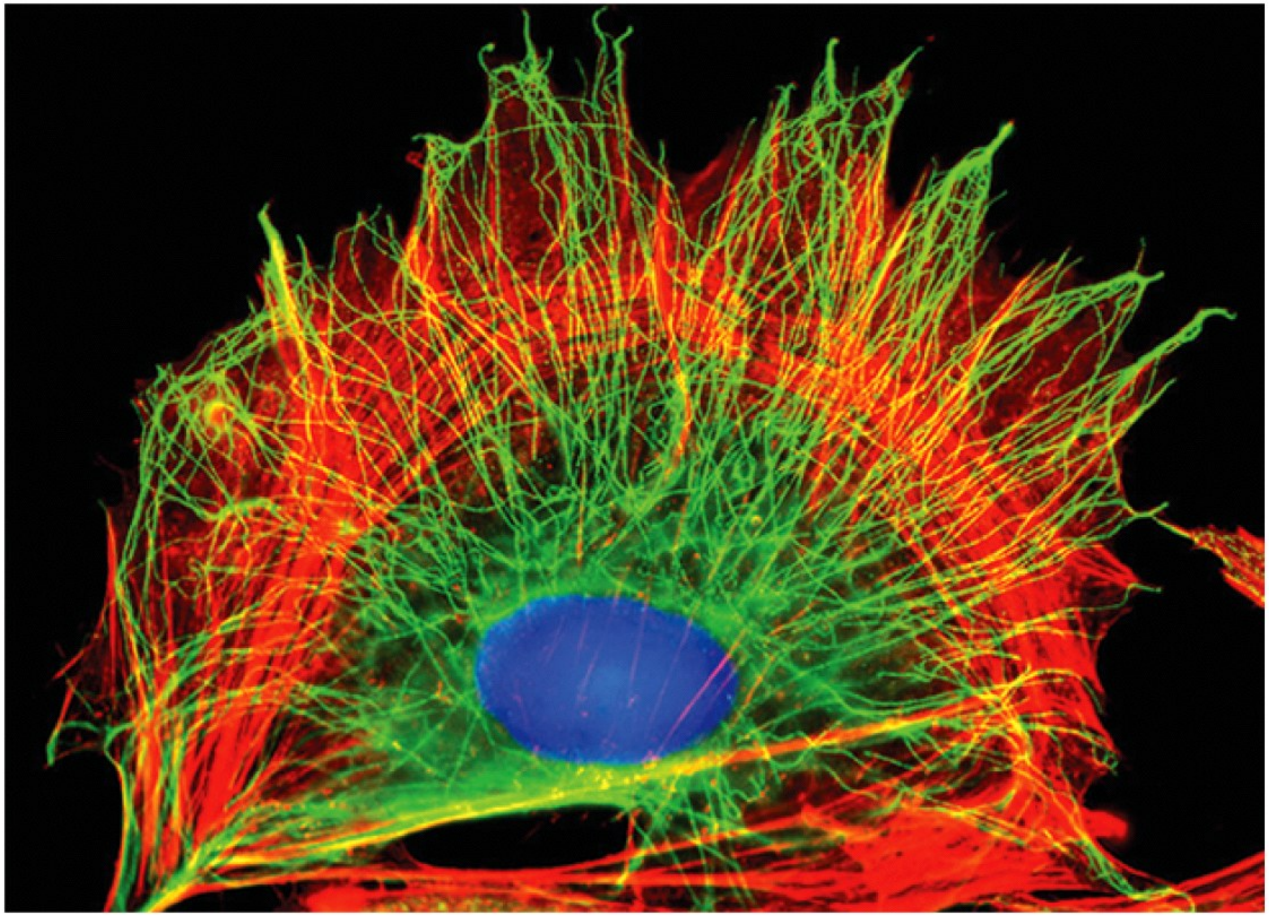


Figure 17-1 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Cell Cytoskeleton

3 Different filamentous proteins

- Actin
    - thin (7nm) filaments
    - composed from G-actin monomers
    - responsible for cell contractility

- Intermediate filaments
    - thicker filaments (approx. 10nm)
    - variety of different proteins (humans have 70 genes)

- Microtubules
    - thickest filaments (approx. 25nm)
    - composed of α- and β-tubulin dimers
    - movement of cargo

Cellular Mechanotransduction

Cell Cytoskeleton

Cytoskeletal proteins

Actin

Microtubules

Intermediate filaments

Motor proteins

Cell contractility and tension

Stabilization of the tension

"Cell structure and shape"

Force generation and movement of cargo

Cellular Mechanotransduction

Cell Cytoskeleton

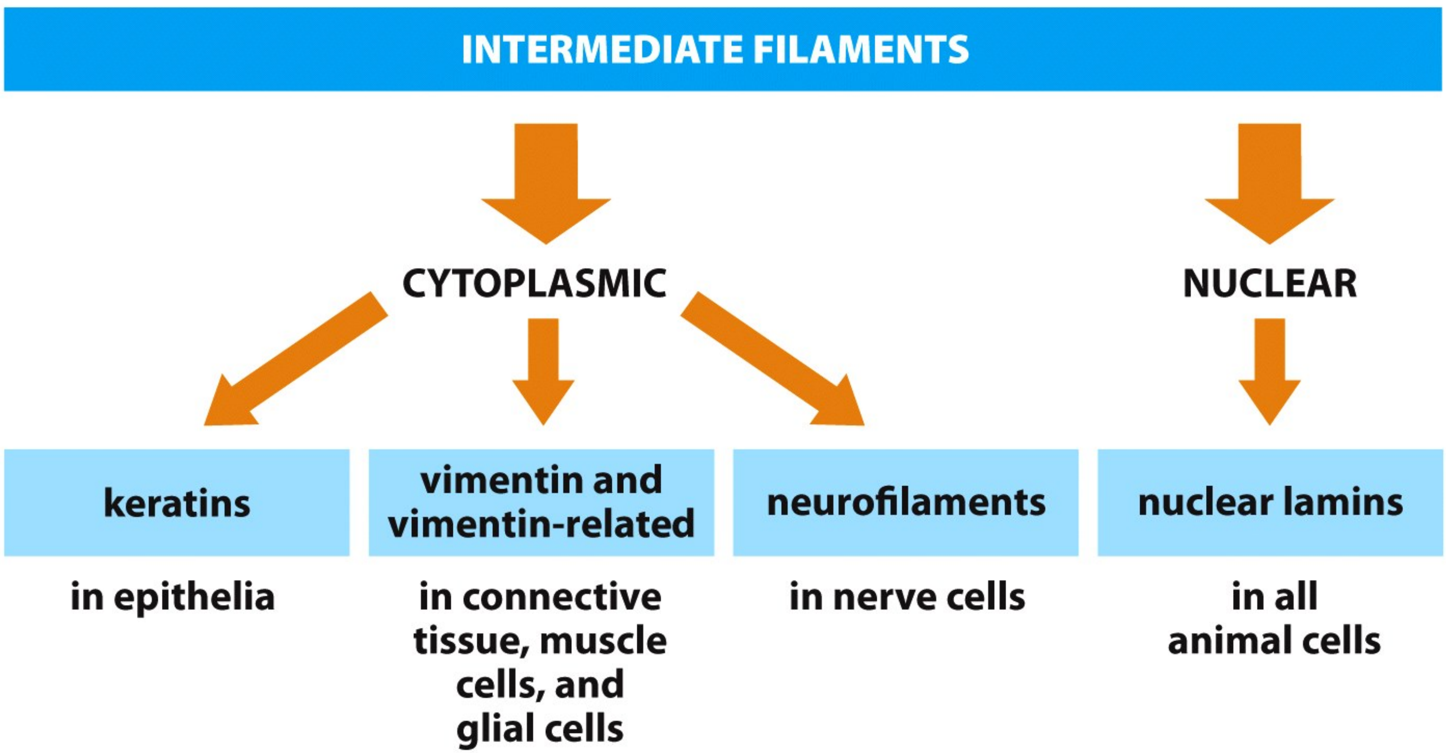
- Cytoskeletal proteins seem to behave as isotropic homogenous material
- Young 's moduli of actin, tubulin and intermediate filaments is in the range of 1000 – 3000 MPa
    - similar to collagen, plastic, silk
    - most rigid proteins
    - close to the Young's moduli of molecular solid
        - structures are stabilized by van der Waals interactions

Cellular Mechanotransduction

Intermediate Filaments

Intermediate filaments differ in location (and structure)

- Prominent in cells which are subjected to mechanical stress
- In nerve cells stabilize the long axons



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Cellular Mechanotransduction

Intermediate Filament Assembly

Intermediate filaments are formed from repeating(?) units

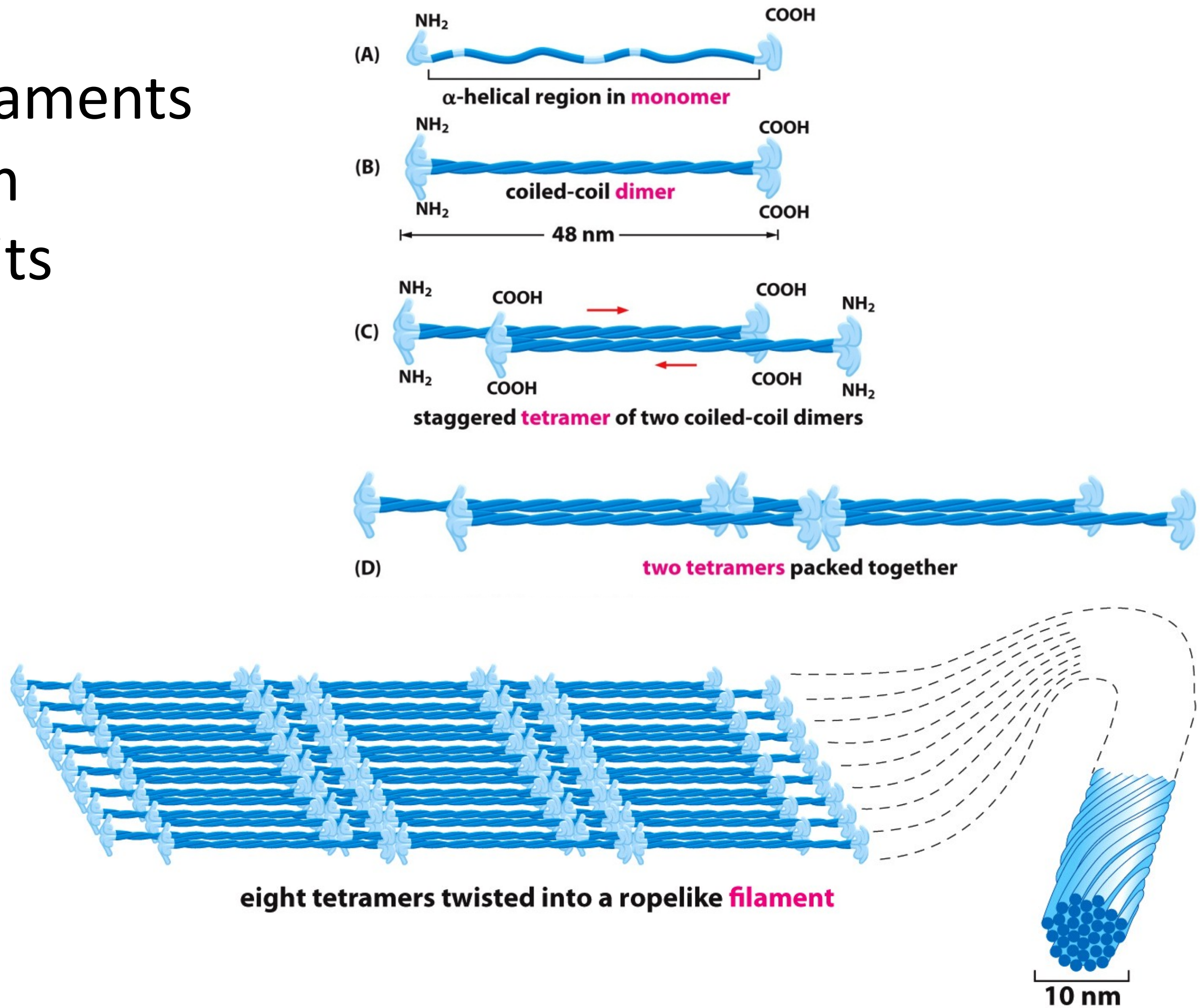


Figure 17-3e Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Intermediate Filament Assembly

Single keratin filaments

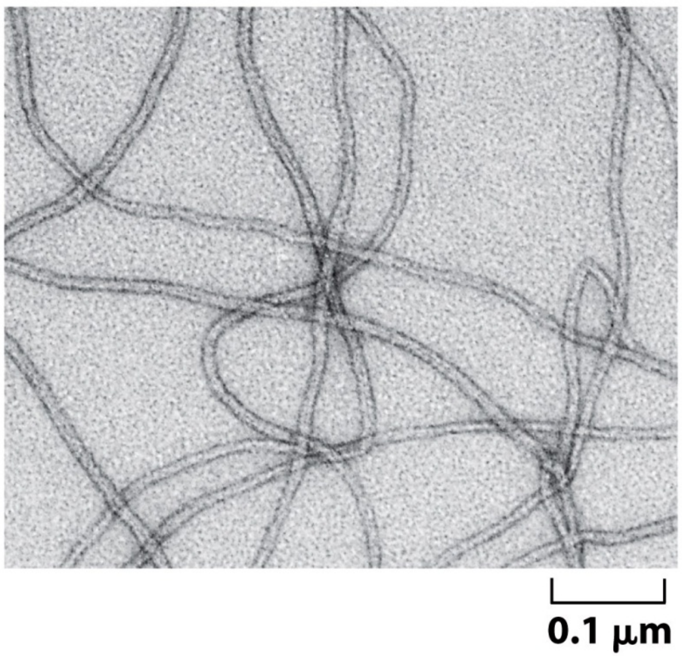
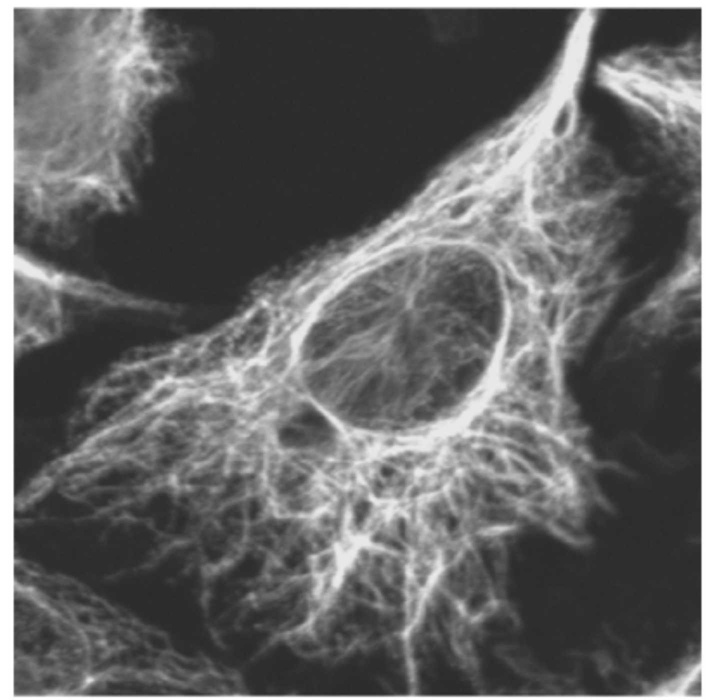


Figure 17-3f Essential Cell Biology 3/e (© Garland Science 2010)

Vimentin filaments inside fibroblast cell

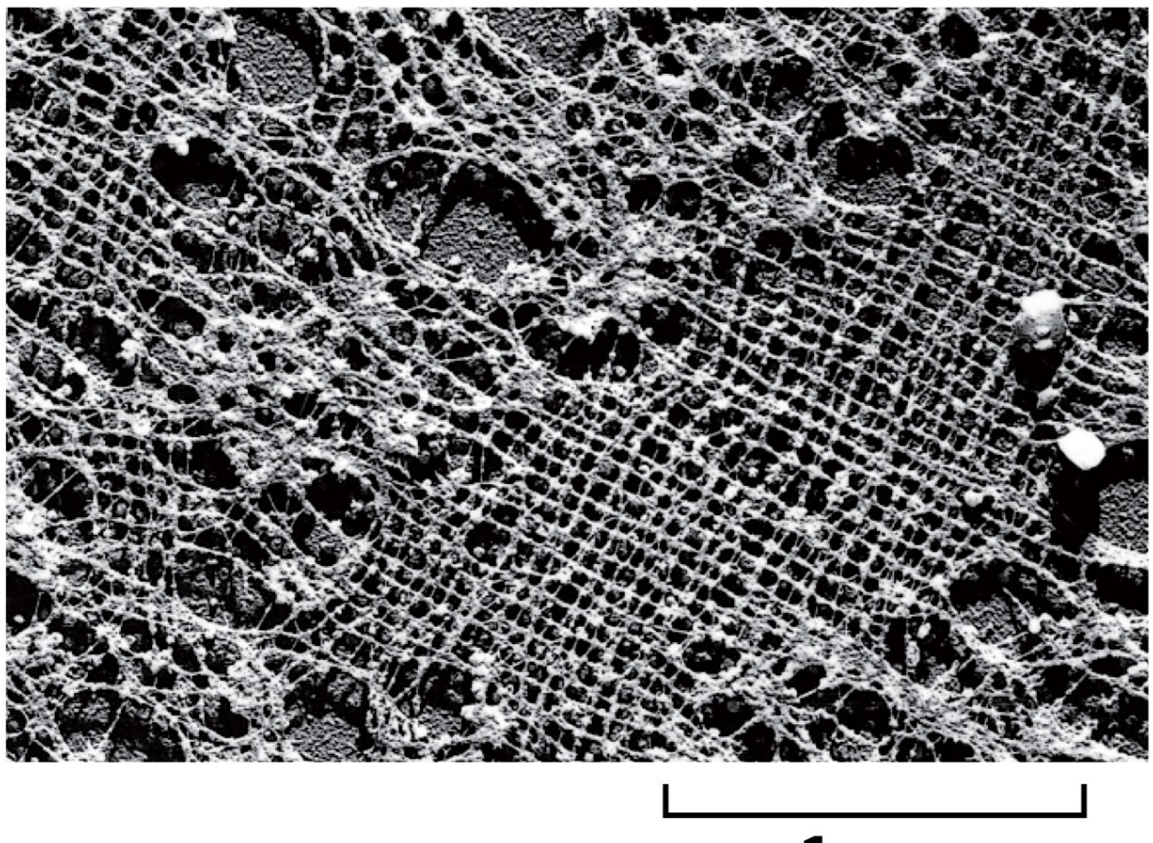


Current Opinion in Cell Biology

Cellular Mechanotransduction

Intermediate Filaments at the Nuclear Envelope

Nuclear lamins on the inner surface of frog (Xenopus) oocyte

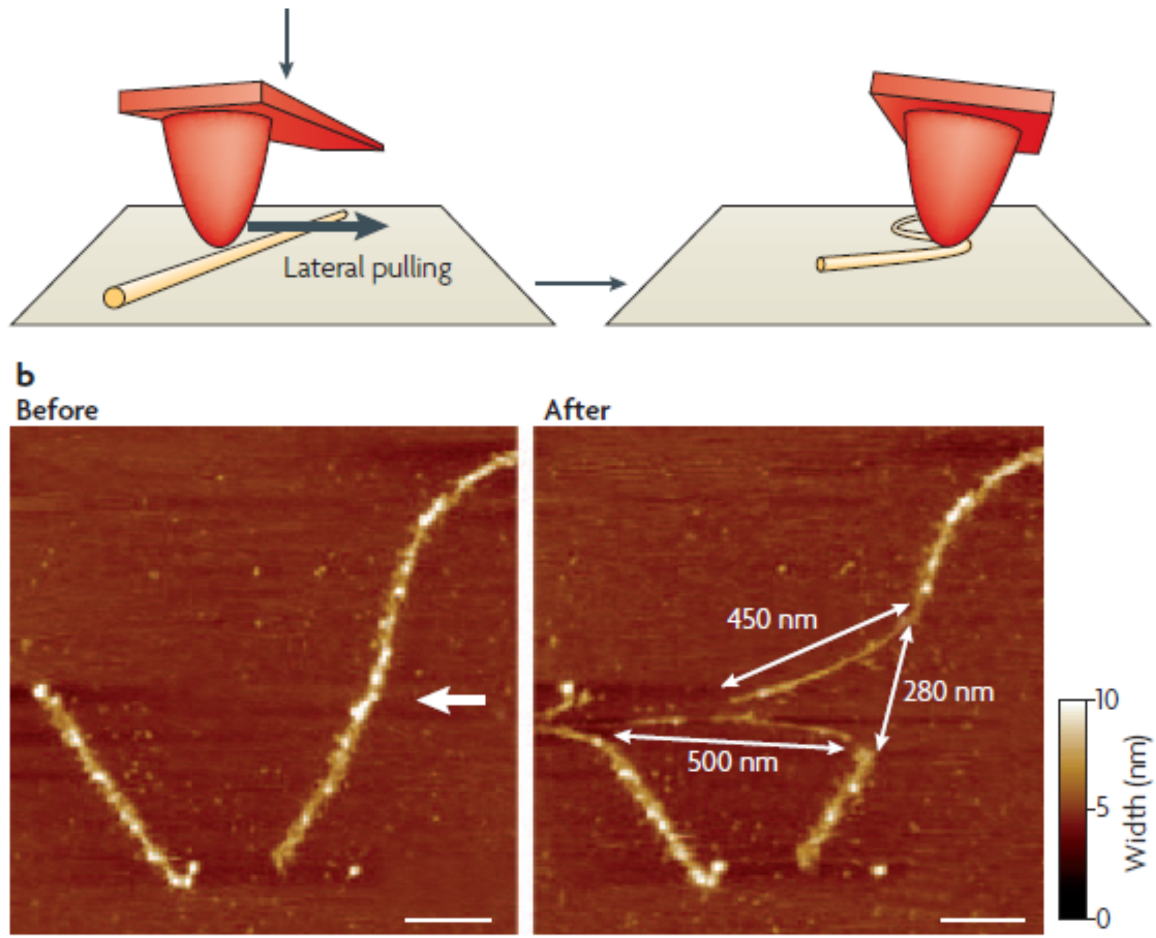


Cellular Mechanotransduction

Intermediate Filaments

Intermediate filaments are flexible

Stretching a single 280-nm-long rat neurofilament fragment by AFM yielded two pieces approximately 450 nm and 500 nm in length (thinner)

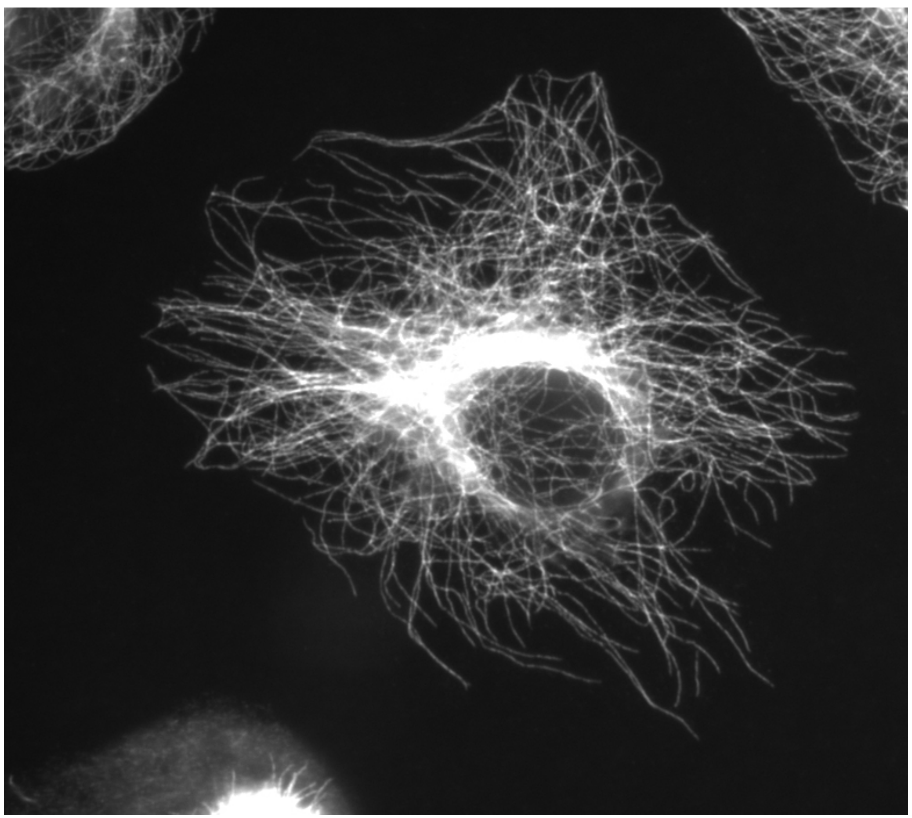


"Intermediate filaments: from cell architecture to nanomechanics"

Cellular Mechanotransduction

Microtubules

- Thick, hollow filaments of the cytoskeleton
- More resistant to bending than the other filaments
- Have structural polarity



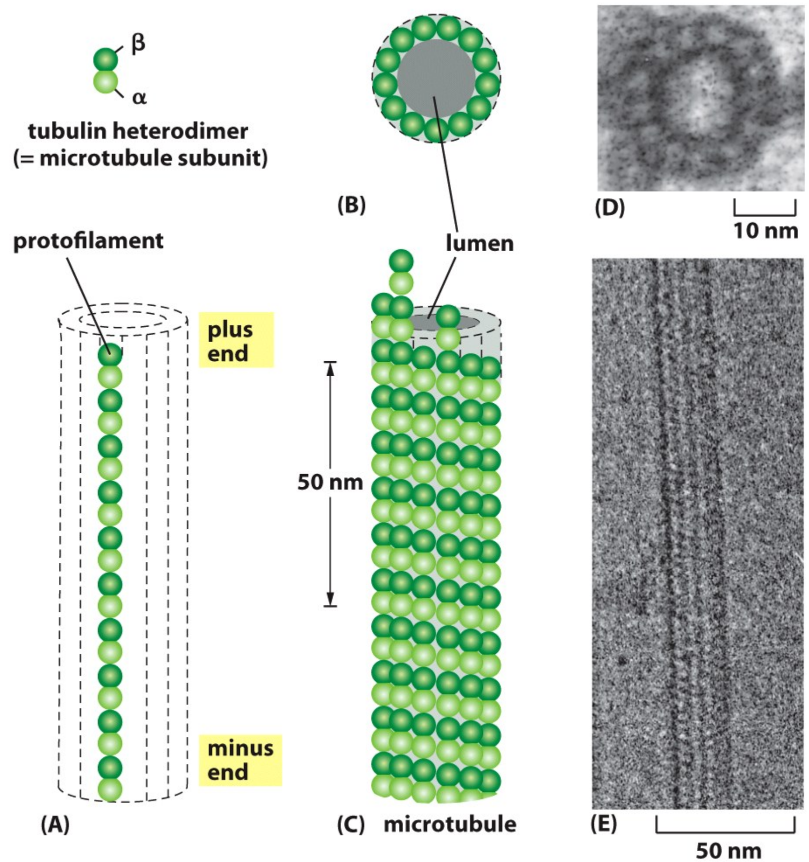


Figure 17-9 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Microtubule Polymerization

- Polymerization from tubulin dimers
- Highly controlled in cells

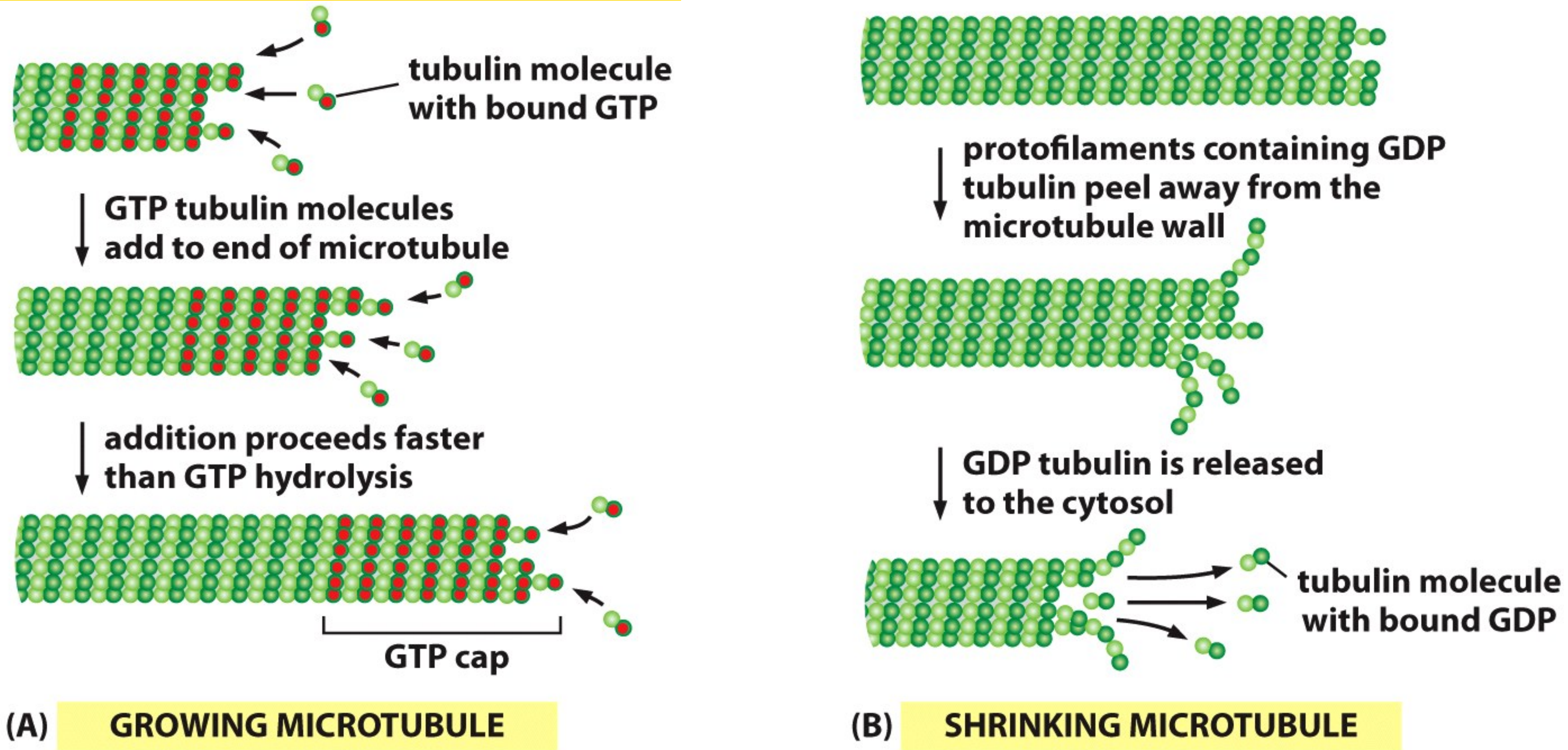


Figure 17-12 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Microtubules in the Cell

- Microtubules nucleate from Microtubule Organizing Center (MTOC)
- "+" ends grow
  - can be stabilized by some capping proteins

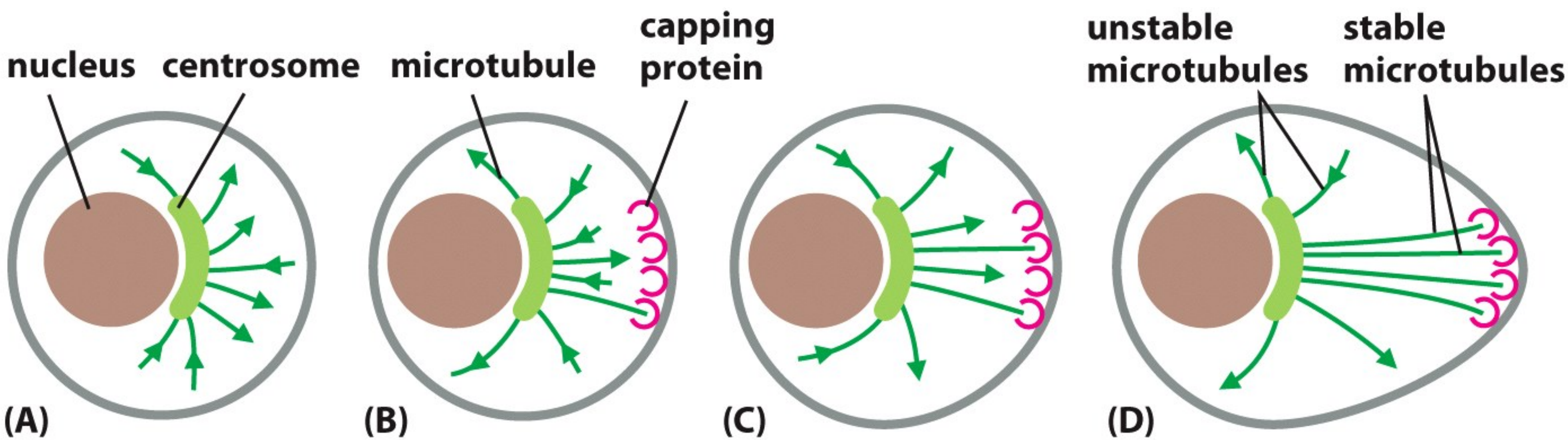


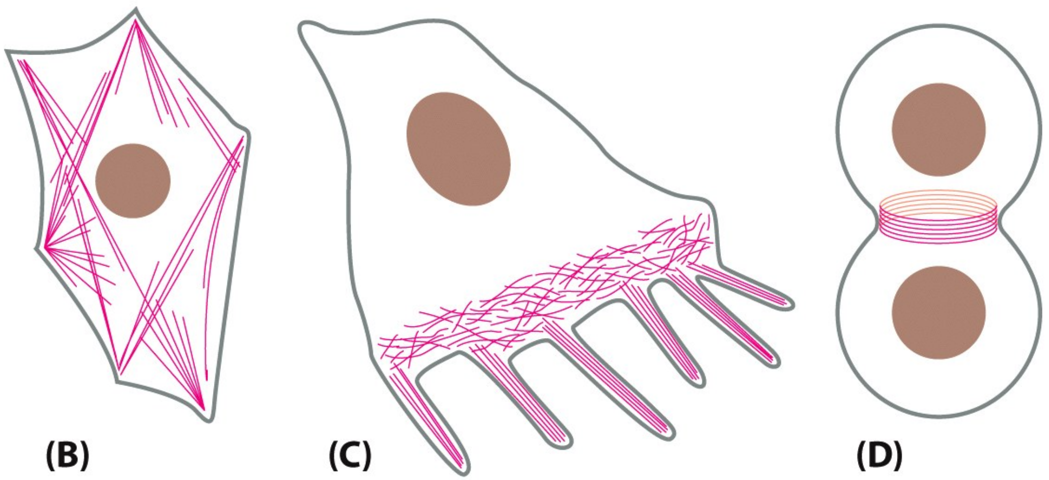
Figure 17-13 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

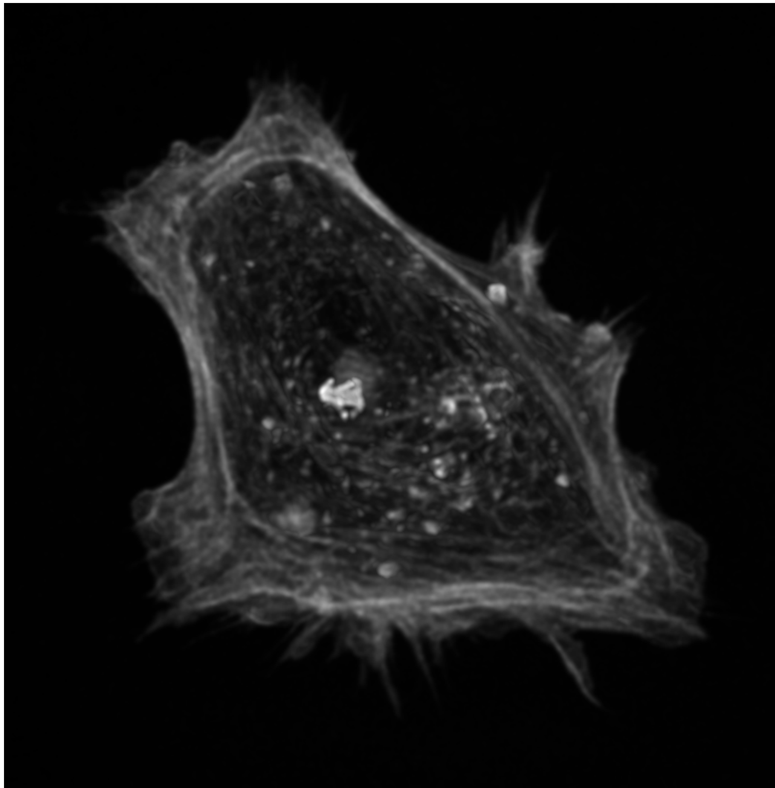
Actin Cytoskeleton

Actin is required for variety of cellular processes

- Cell structural stability
- Cell migration and membrane movement
- Cell division, etc.



Actin filaments in mouse fibroblast cell



Cellular Mechanotransduction

Actin Cytoskeleton

About 5% of total protein mass (mammalians)

Filaments are polymers of actin protein

- α- (muscle), β- and ϒ-actin

Filament is helical, period 37nm

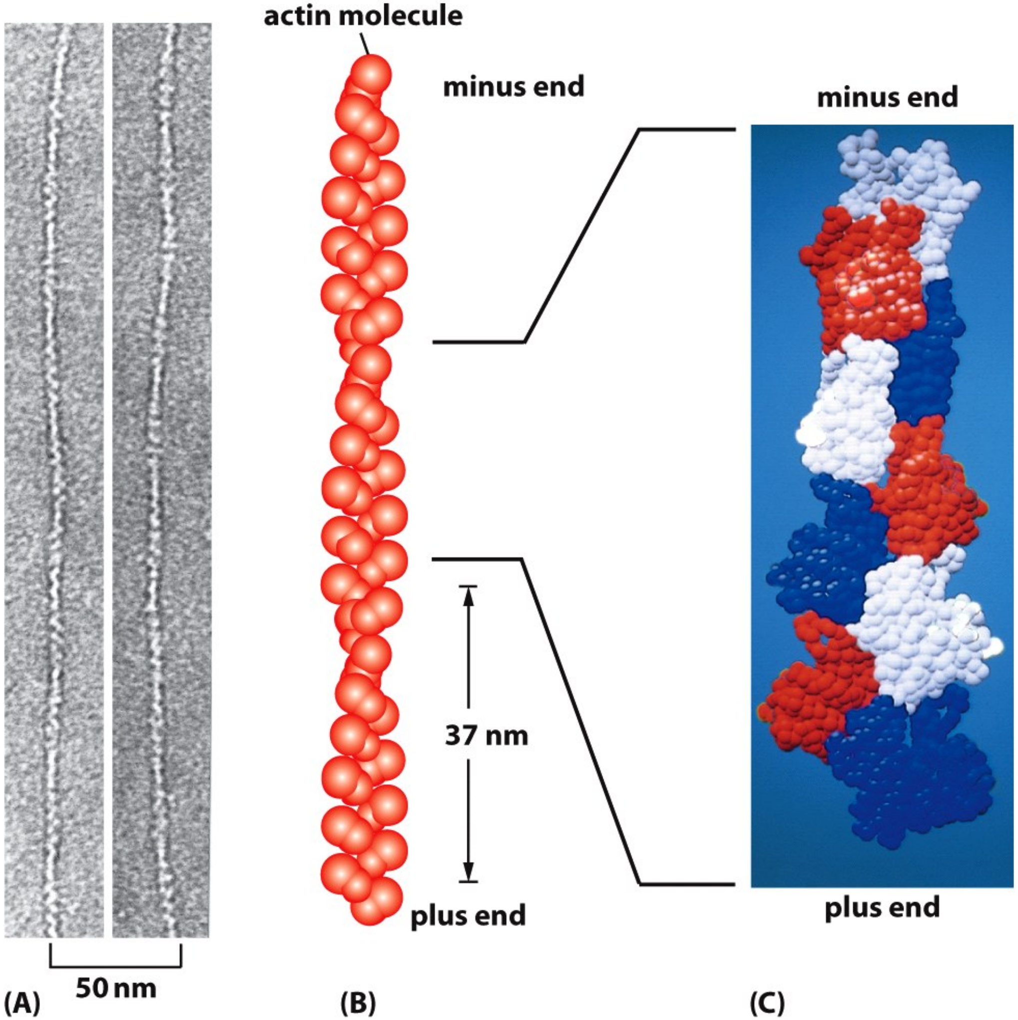
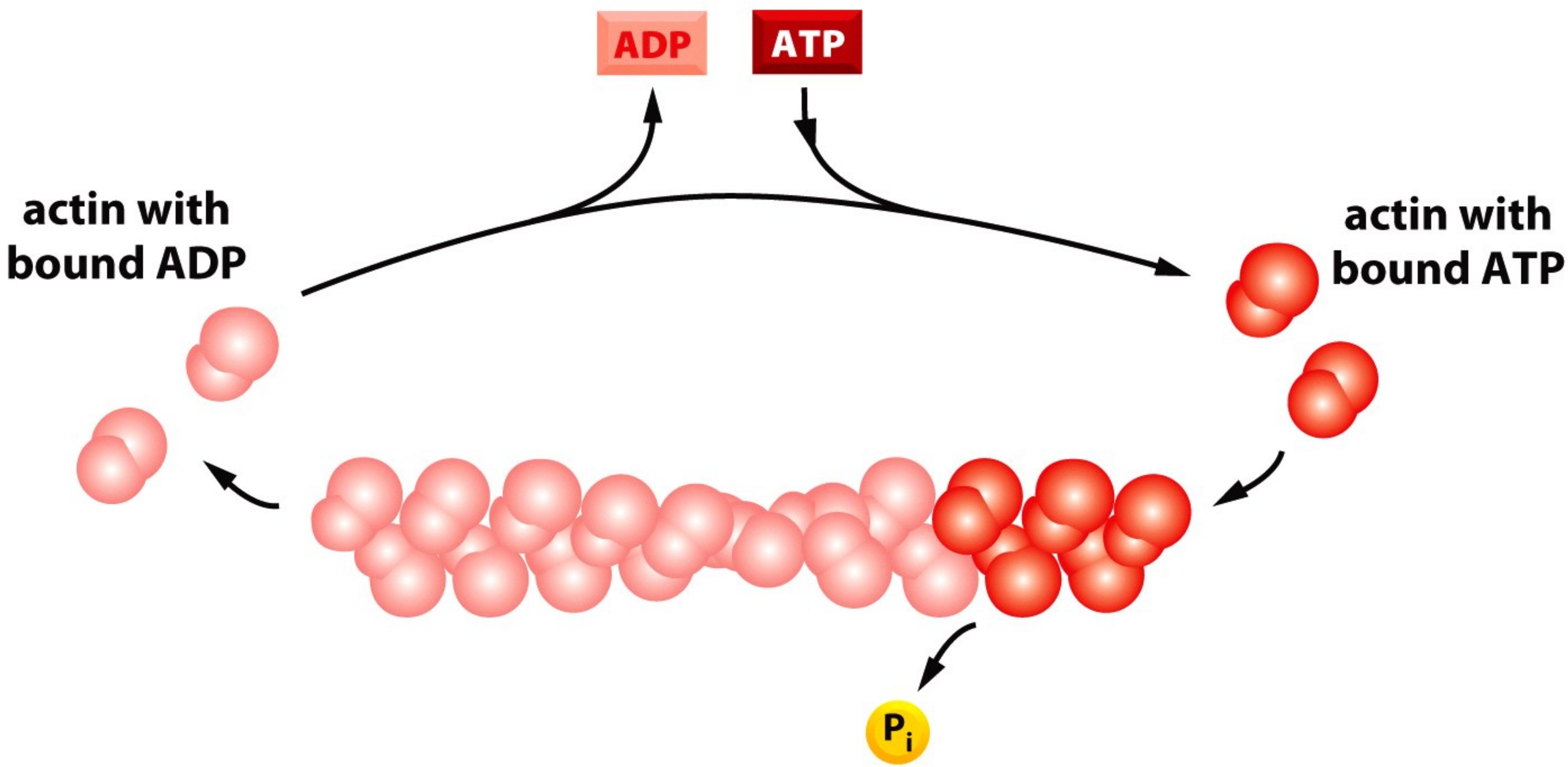


Figure 17-29 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Actin Polymerization

Actin polymerization requires energy



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Cellular Mechanotransduction

Actin Filaments

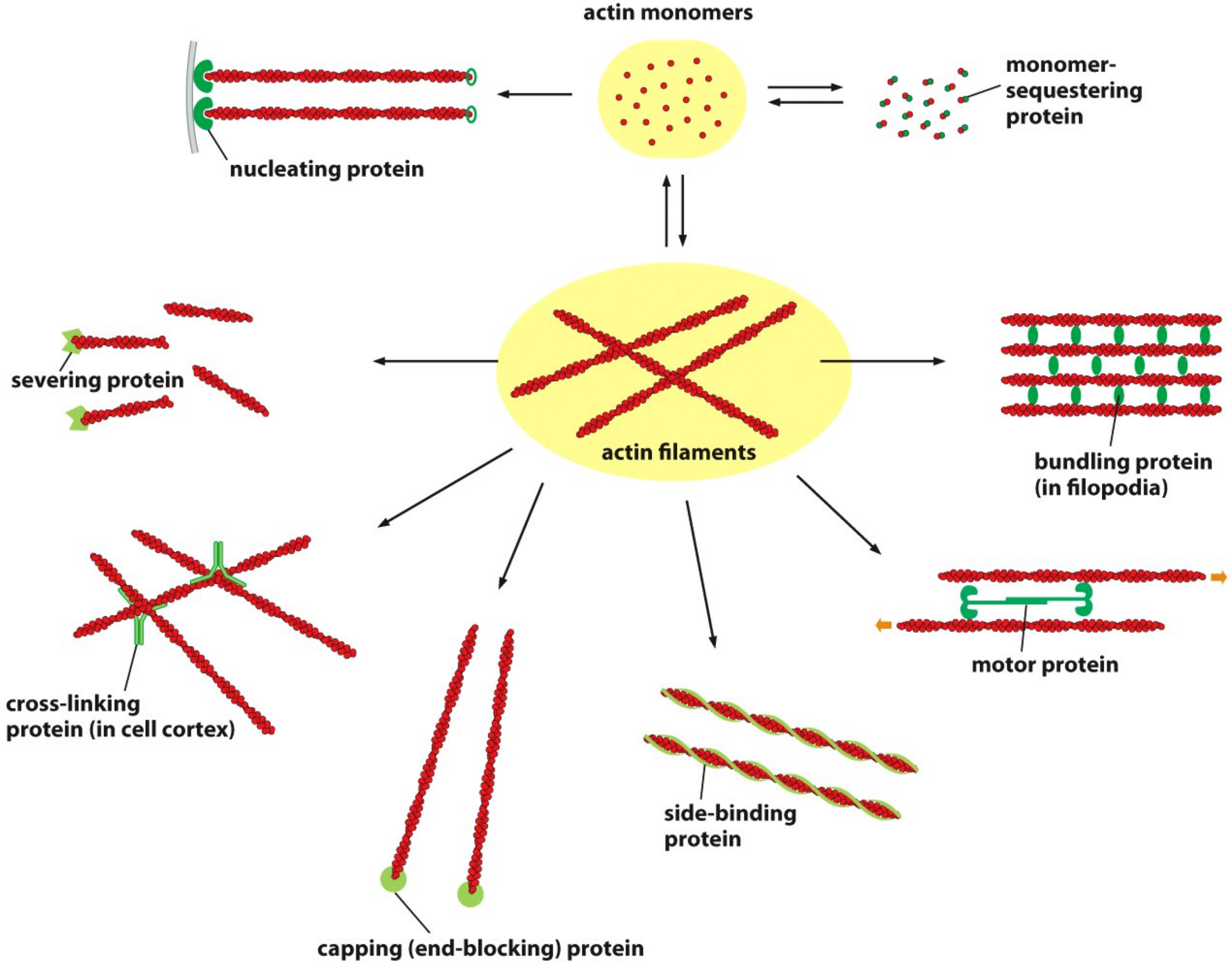


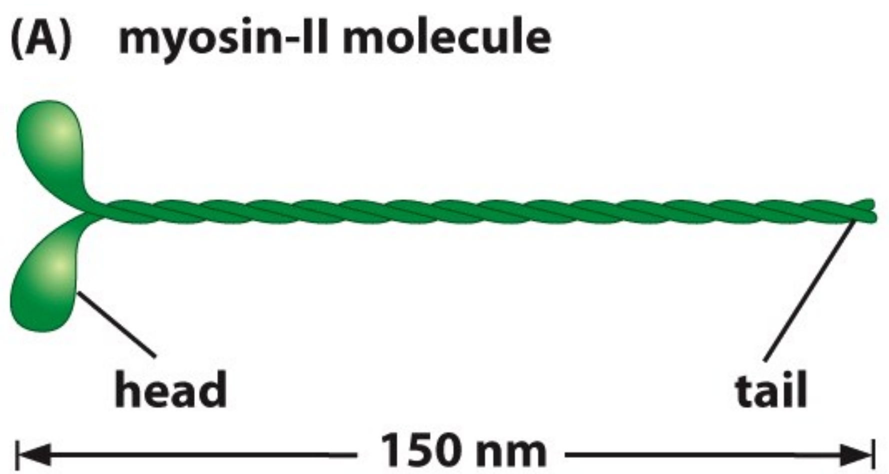
Figure 17-31 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Generation of Tension in Actin Filaments

Motor proteins which bind actin: Myosins

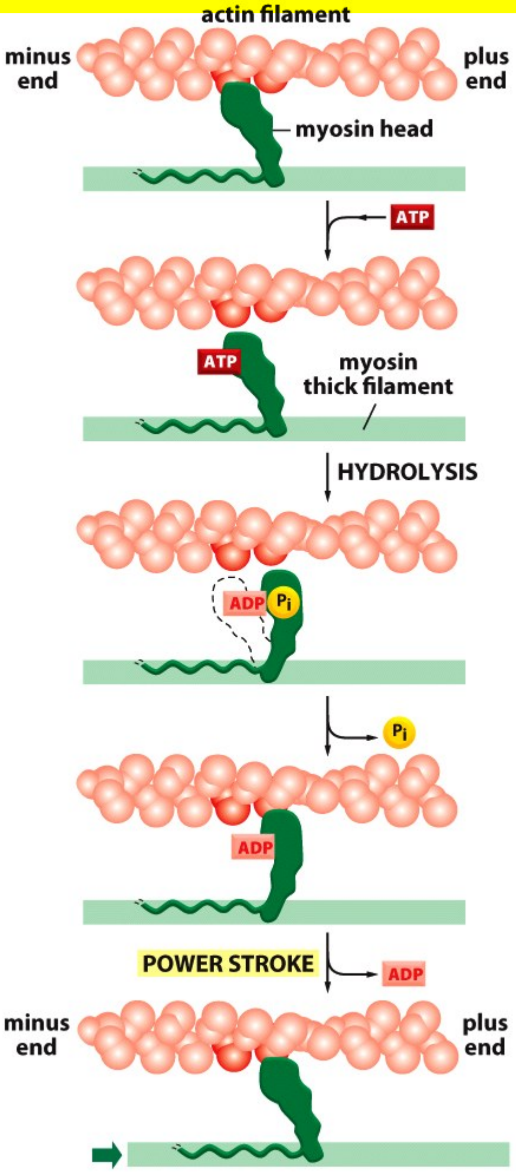
- Large family of structurally different proteins
- Myosin II is responsible for the tension in actin fibers



Single Myosin II can produce a force of approx. 1.5pN

Cellular Mechanotransduction

Generation of Tension in Actin Filaments



No energy → Myosin stays bound to actin

ATP binds → Myosin is released

ATP hydrolysis → Myosin structure changes

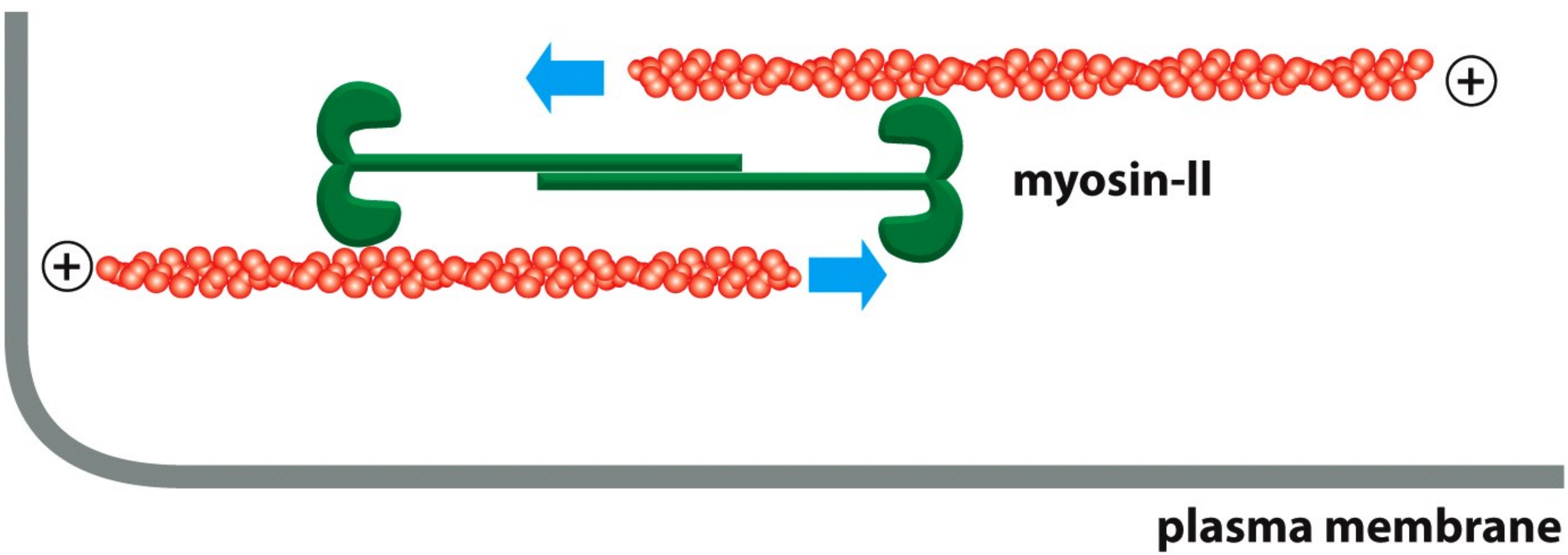
Release of P → Myosin binds actin (“stroke”)

Release of ADP → Return to the resting state

Cellular Mechanotransduction

Motor Proteins of Actin

Sliding filament model



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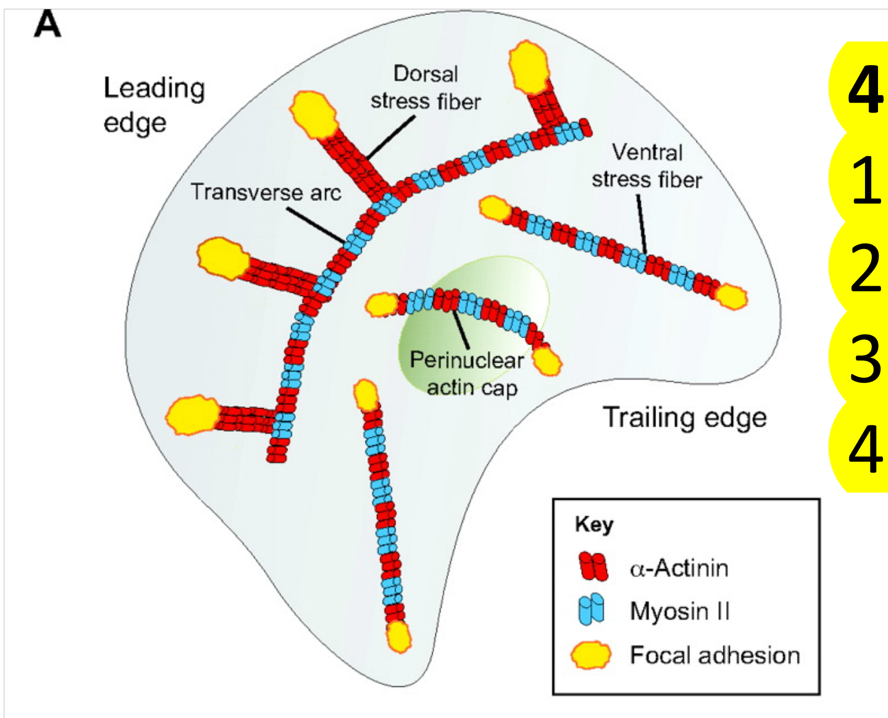
Cellular Mechanotransduction

Actin filaments of the cells, stress fibers

Composed from:

1. Actin
2. Motor proteins and their regulators (myosin, MLCK etc.)
3. Cross-linking proteins (alpha-actinin, filamin etc.)



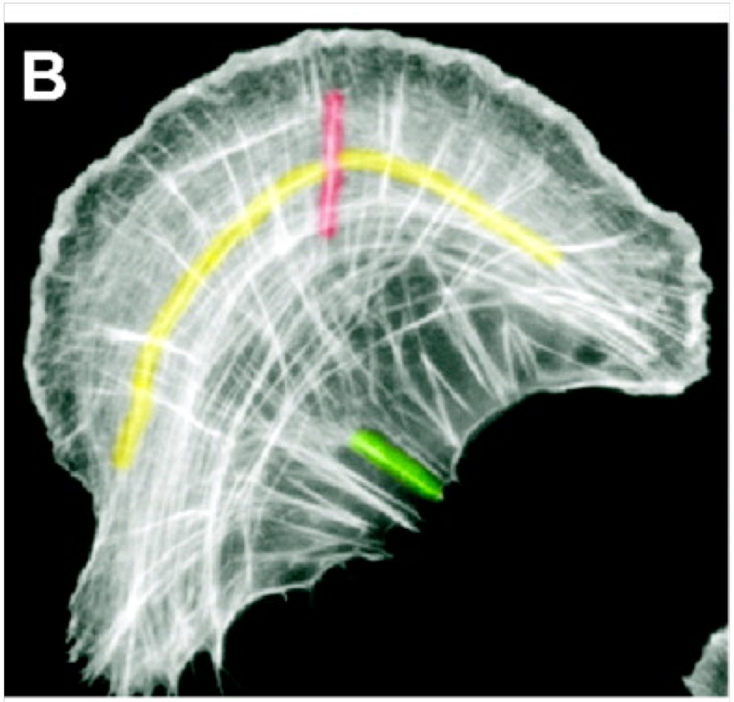
4 different types

1. Dorsal stress fibers
2. Transverse arcs (motile cells)
3. Ventral stress fibers
4. Nuclear actin cap

Actin stress fibers – assembly, dynamics and biological roles
Tojkander, Gateva, Lappalainen
J Cell Sci 2012 125: 1855-1864

Cellular Mechanotransduction

Actin filaments of the cells, stress fibers



Osteosarcoma cell

1. Transverse arcs
2. Dorsal stress fibers
3. Ventral stress fibers

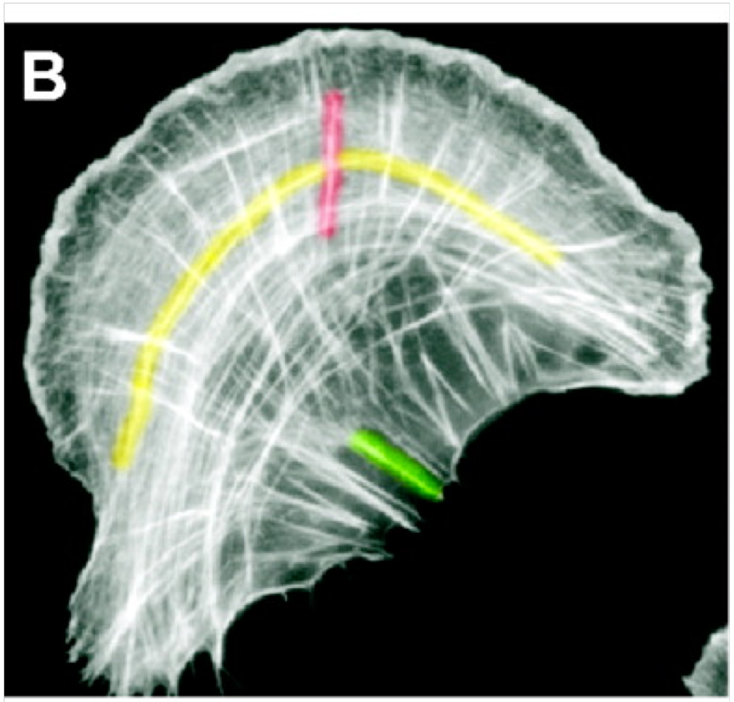
Transverse arcs:

- In actively moving cells
- Not directly associated to focal adhesions
- Retrograde flow to the center

Actin stress fibers – assembly, dynamics and biological roles
Tojkander, Gateva, Lappalainen J Cell Sci 2012 125: 1855-1864

Cellular Mechanotransduction

Actin filaments of the cells, stress fibers



Osteosarcoma cell

1. Transverse arcs
2. Dorsal stress fibers
3. Ventral stress fibers

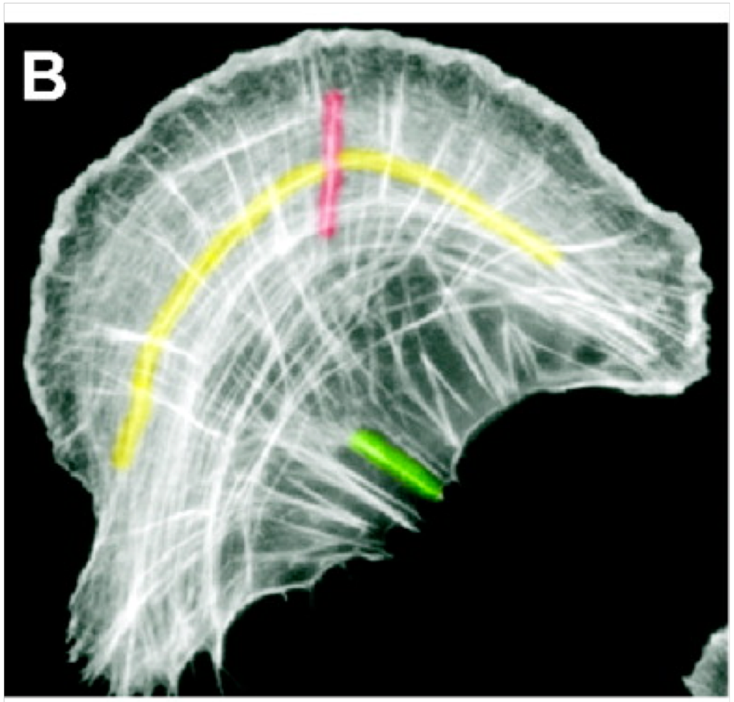
Dorsal stress fibers:

- Start from focal adhesions, often end to transverse arcs
- Transmit forces to the substrate
- Non-contracting

Actin stress fibers – assembly, dynamics and biological roles
Tojkander, Gateva, Lappalainen J Cell Sci 2012 125: 1855-1864

Cellular Mechanotransduction

Actin filaments of the cells, stress fibers



Osteosarcoma cell

1. Transverse arcs
2. Dorsal stress fibers
3. Ventral stress fibers

Ventral stress fibers:

- Start from and end to focal adhesions
- Main producers of contractile force
- Can be really long

Actin stress fibers – assembly, dynamics and biological roles
Tojkander, Gateva, Lappalainen J Cell Sci 2012 125: 1855-1864

Cellular Mechanotransduction

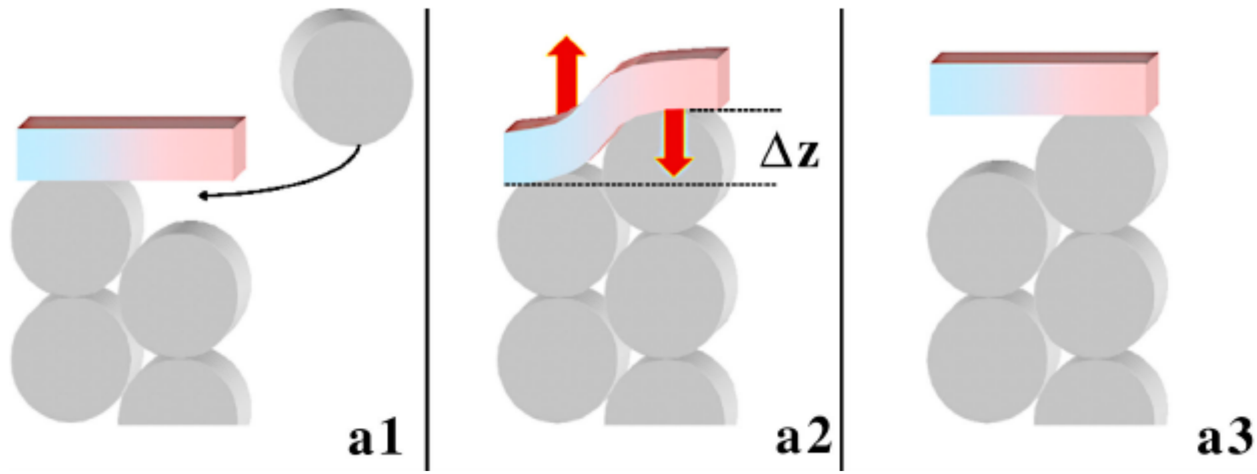
Force Generation Through Polymerization

Polymerization of actin can provide a force for membrane movement

$\Delta U(Z) = F\Delta Z$

Then,

$$\frac{k_f}{k_r} = K_{eq.}e^{\frac{F\Delta Z}{kT}}$$



It can be shown that,

$$F = \frac{kT}{\Delta Z} ln \frac{[monomer]}{K_c}$$

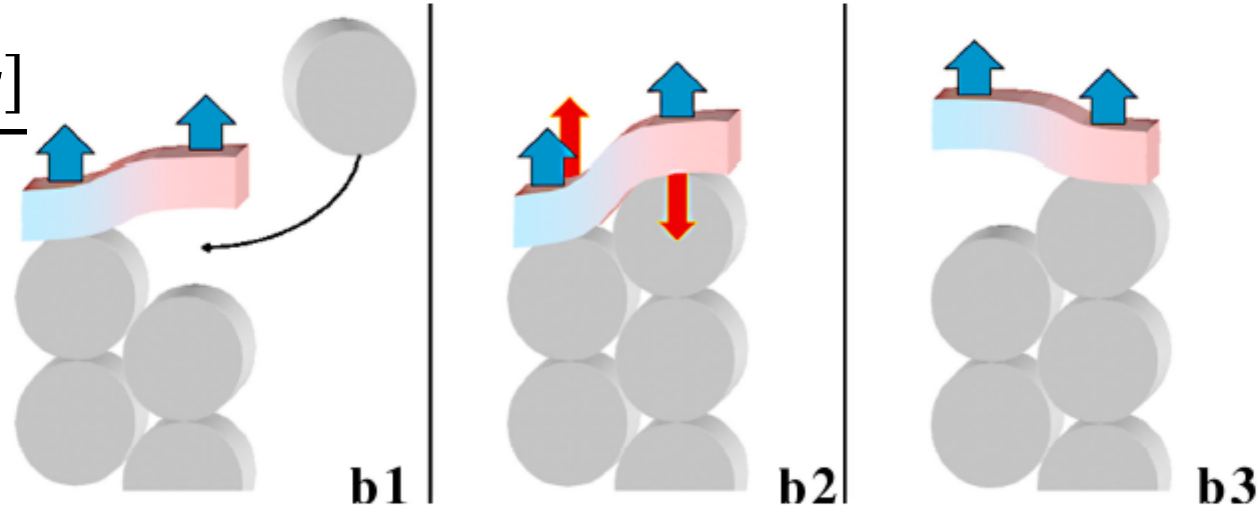
Where K_c = critical concentration, the concentration where polymerization == depolymerization

Cellular Mechanotransduction

Force Generation Through Polymerization

Depolymerization can create pulling forces

$$F = \frac{kT}{\Delta Z} ln \frac{[monomer]}{K_c}$$



In general:
ΔZ increases
→ |F| decreases

Polymerization force is in the range of few pN per single filament

Cellular Mechanotransduction

Cytoskeleton, summary

Different filamentous proteins

- Actin, intermediate filaments and microtubules

- Cell contractility via motor proteins (actin + myosin)

- Movement of cell organelles and vesicles (tubulin + kinesin/dynein)

- Cell elasticity (intermediate filaments)

Motor proteins generate force from ATP hydrolysis

- Hydrolysis leads into structural change

Polymerization creates also force (important in cell movement)

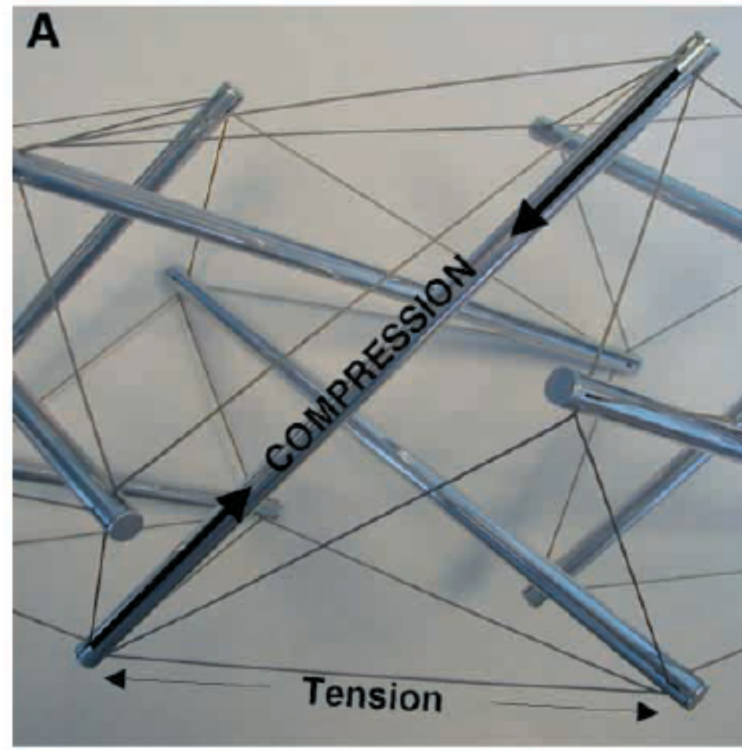
Cellular Mechanotransduction

Tensegrity model of the cell

Tensegrity system:
Structure which is stabilized by "continuous tension"

First described by R. Buckminster Fuller at 1961

The principle is the opposite for "continuous compression" i.e. stone arch etc.



"Tensegrity I:Cell structure and hierarchical systems biology"

Cellular Mechanotransduction

Tensegrity model of the cell

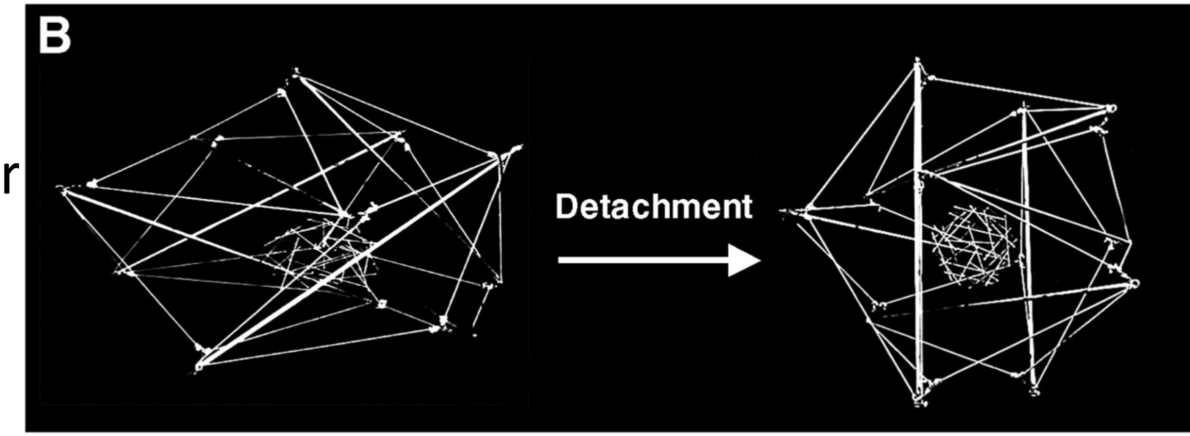
Tensegrity sculpture by K. Nelson

http://raddblog.wordpress.com/2009/10/31/tensegrity-sculptures-by-kenneth-snelson/

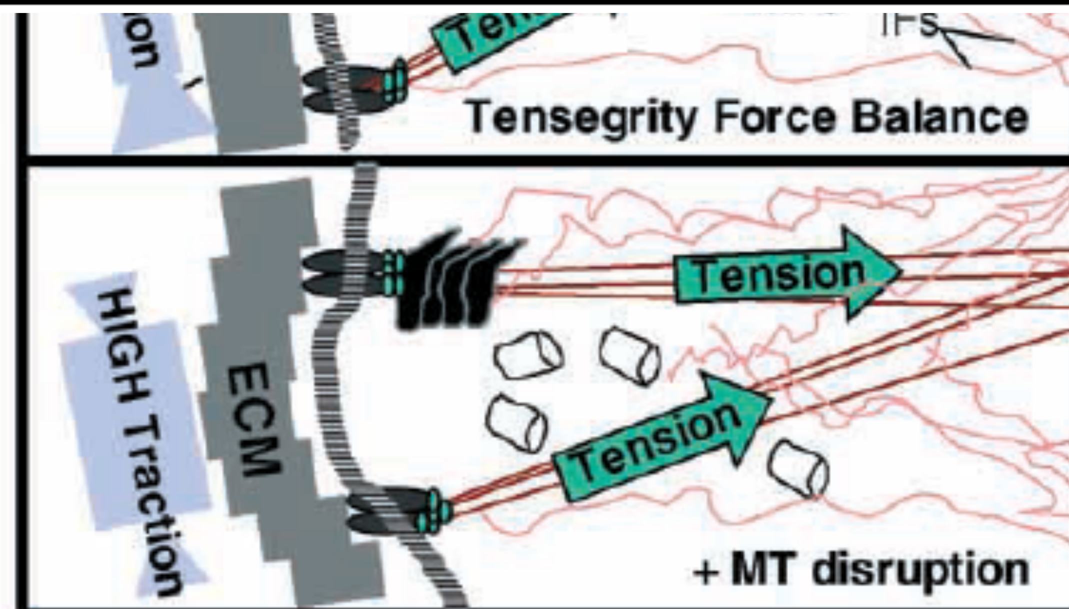
Cellular Mechanotransduction

Tensegrity model of the cell

Introduced for the cells mainly by Donald E. Ingber (Harvard Medical School)



Idea: Actin filaments create tension, which is stabilized by microtubules (until they buckle)



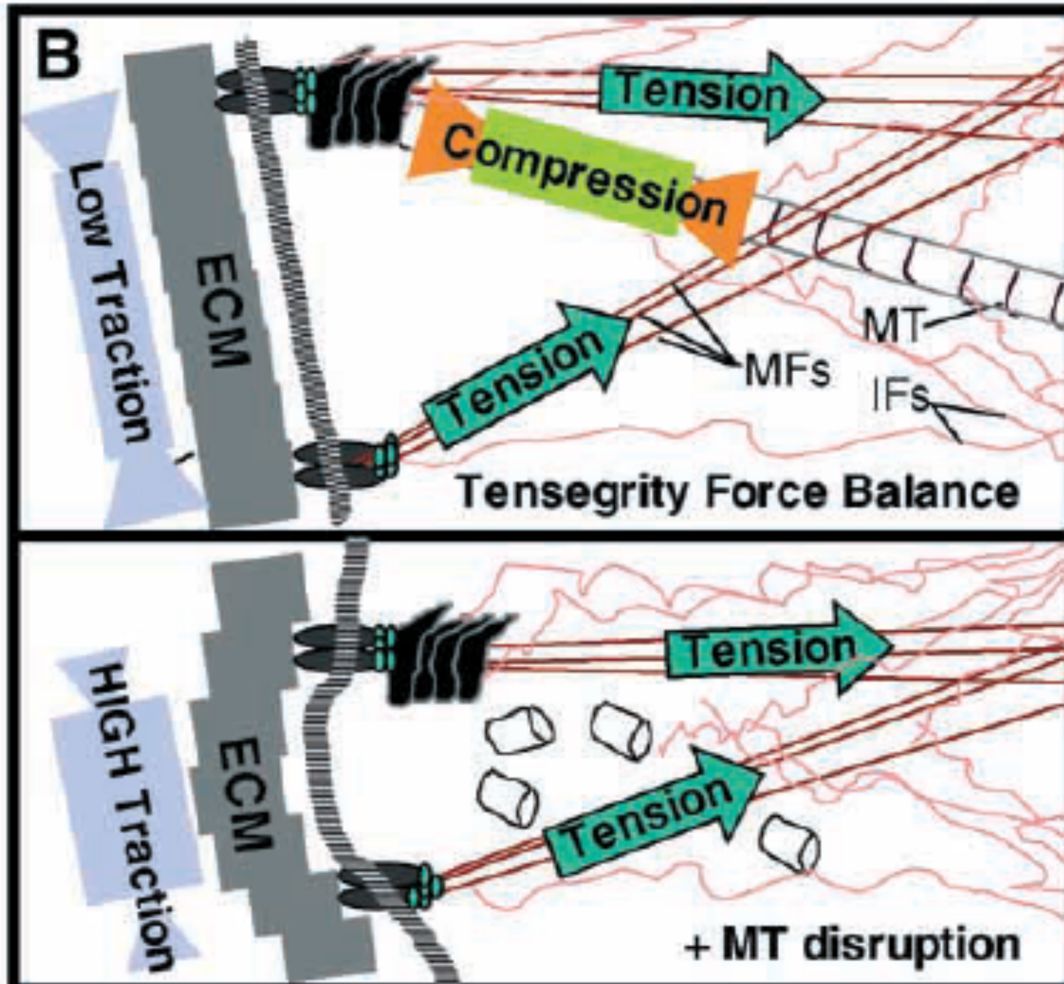
Cellular Mechanotransduction

Tensegrity model of the cell

Hypotheses:

1. When microtubuli are removed cell traction increases and cell "contracts"

2. When actin tension is reduced lower traction and cell "spreads"



Cellular Mechanotransduction

Tensegrity model of the cell

Microtubuli are removed

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Actin tension is reduced