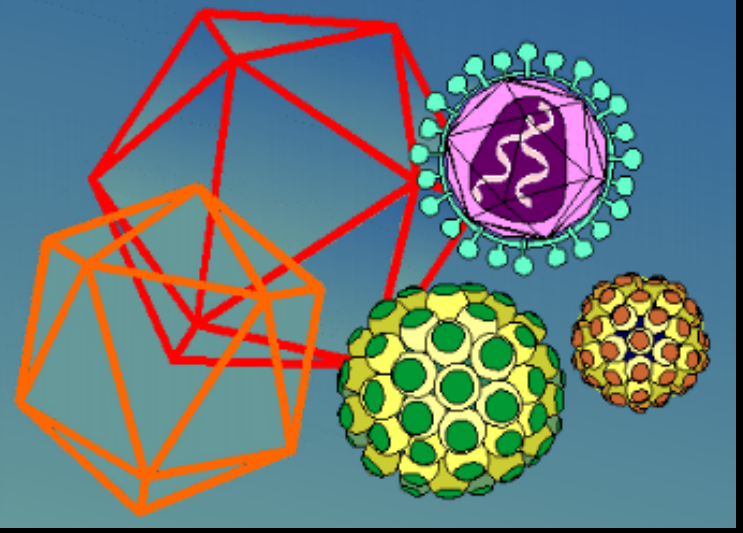


The Architecture of Life

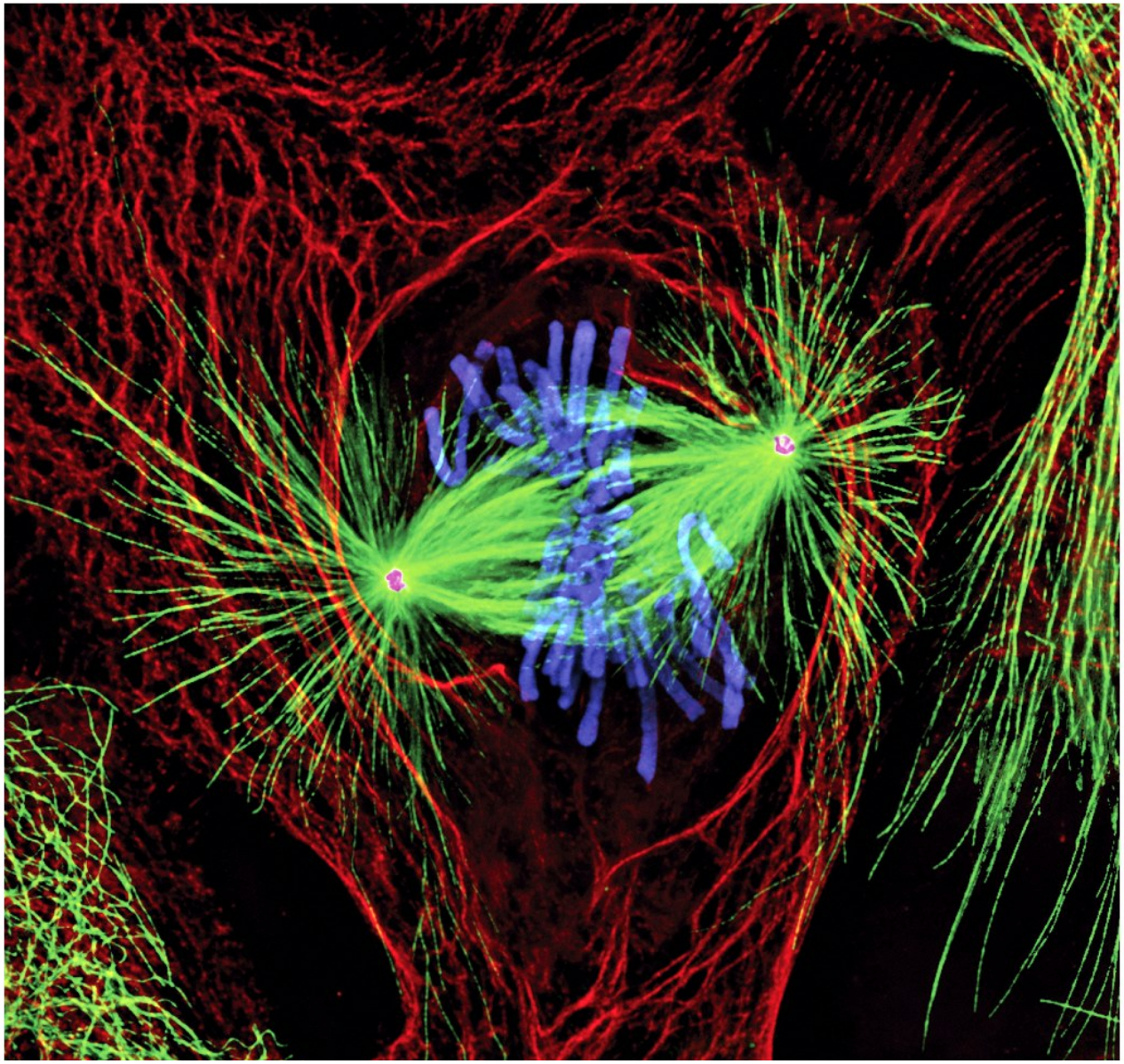
A universal set of building rules seems to guide the design of organic structures - from simple carbon compounds to complex cells and tissues

Donald E. Ingber

Molecules exhibit their own dynamic behavior when alone.

When they are combined into larger unit - such as a cell or tissue - a new and unpredictable properties emerge (ability to move, to change shape and to grow).

Cell architecture correlates with its function



The architecture

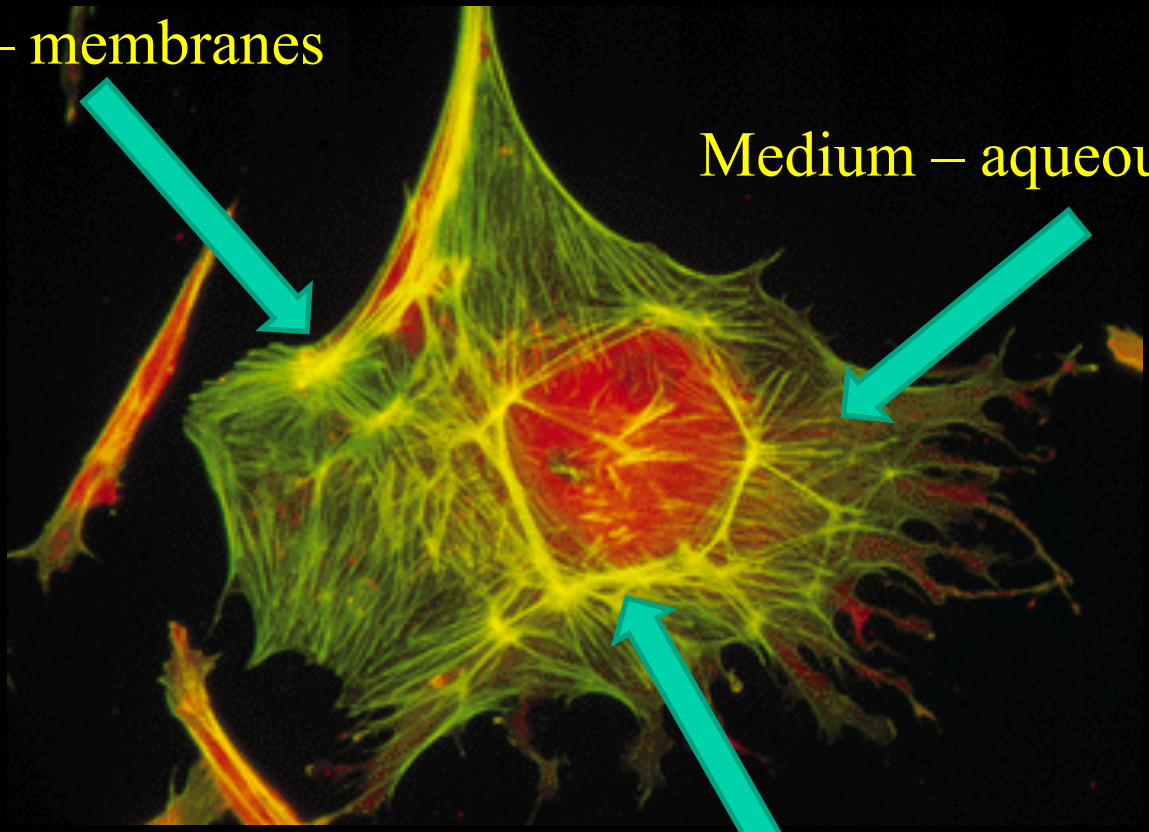
Medium

Surfaces

Construction elements

Architecture of biological system

This perception is static therefore does not explain dynamic character of biological systems

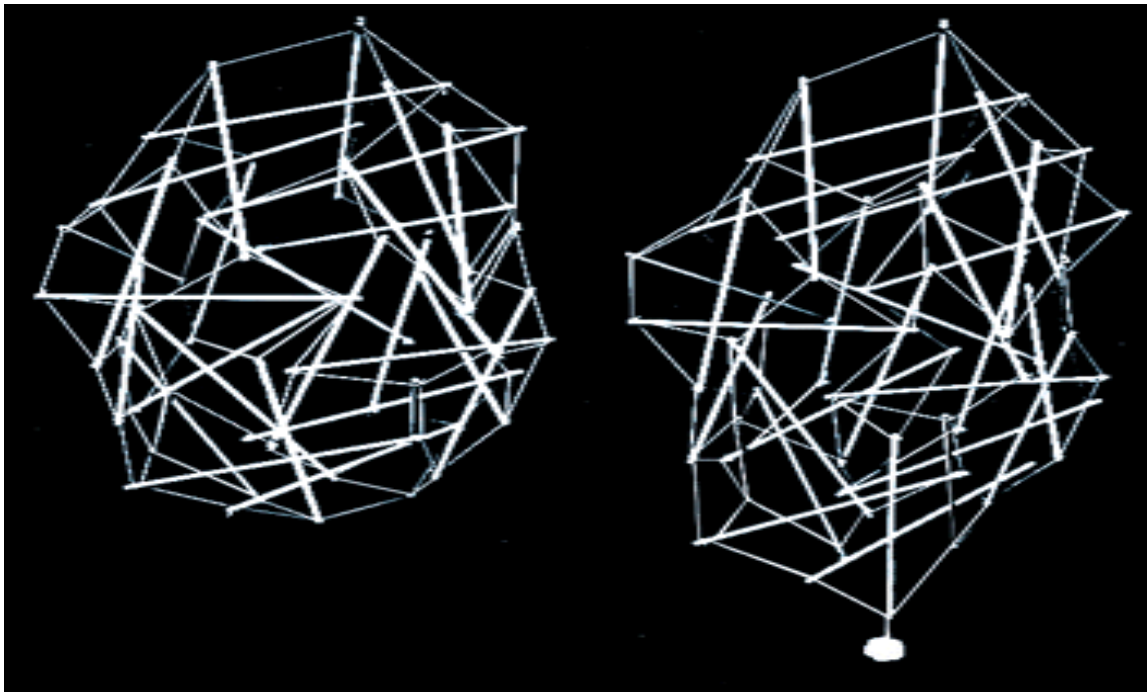


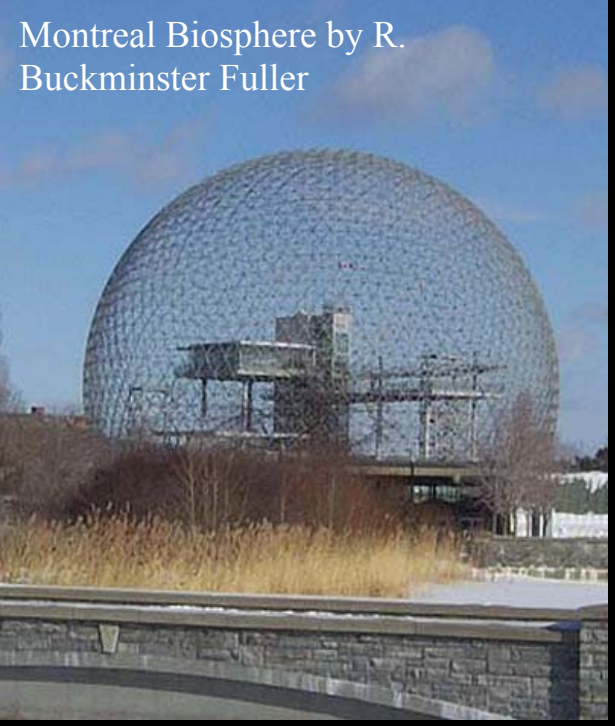
Tensegrity – tensional integrity

Tensegrity is a building principle that was first described by architects.

Fuller definee tensegrity systems as structures that stabilize their shape by continuous tension or "tensional integrity" rather than by continuous compression.

*A **structure distributes stresses** to establish a force balance and stabilize itself against shape distortion that **defines tensegrity.***

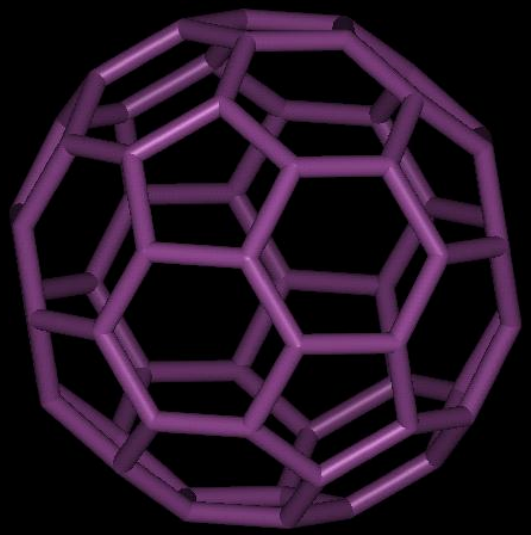



Montreal Biosphere by R. Buckminster Fuller

Tensegrity structures offer a maximum amount of strength for a given amount of building material.

The geodesic domes of Buckminster Fuller

- *Frameworks made up of rigid struts*
- *Each strut can bear tension or compression.*

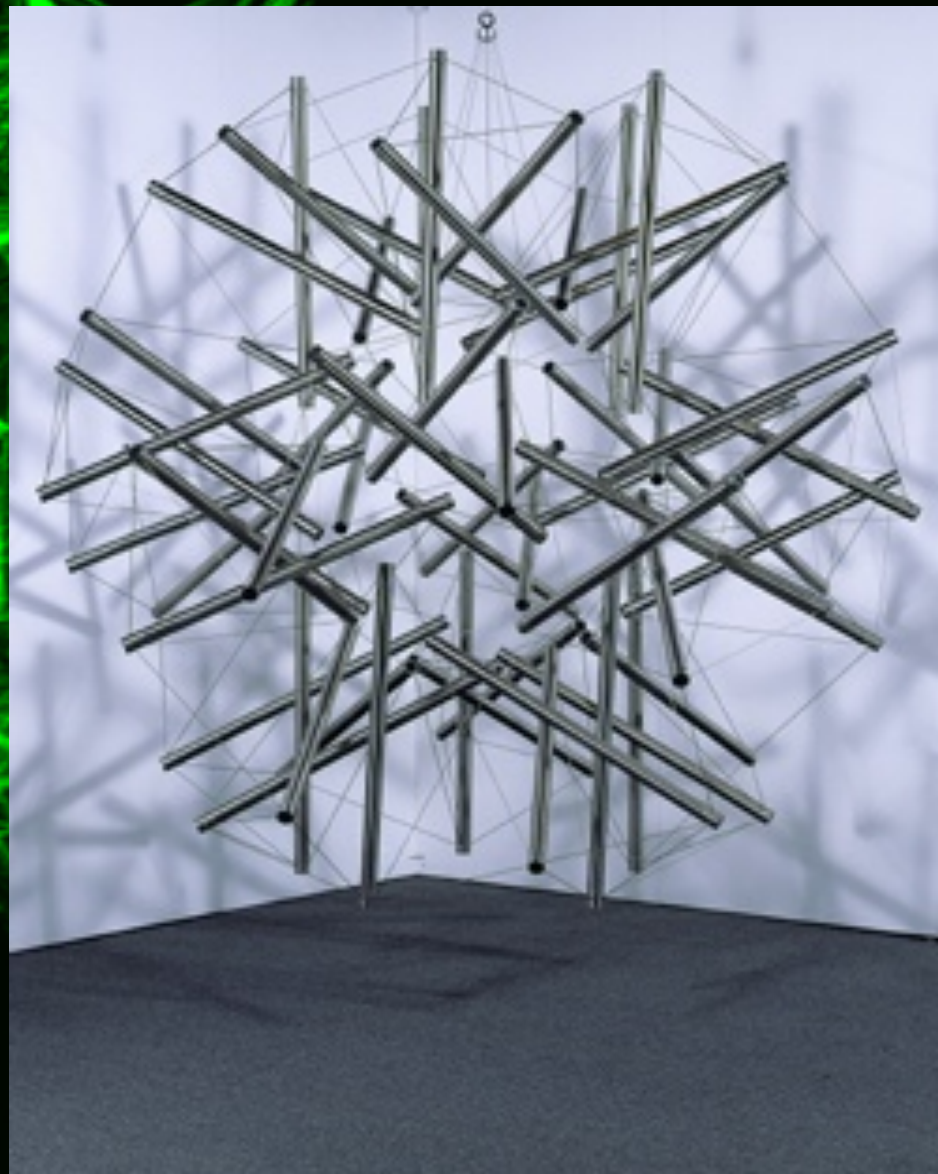


A "geodesic" line on a sphere is the shortest distance between any two points.

Structures that stabilize themselves through a phenomenon known as prestress.

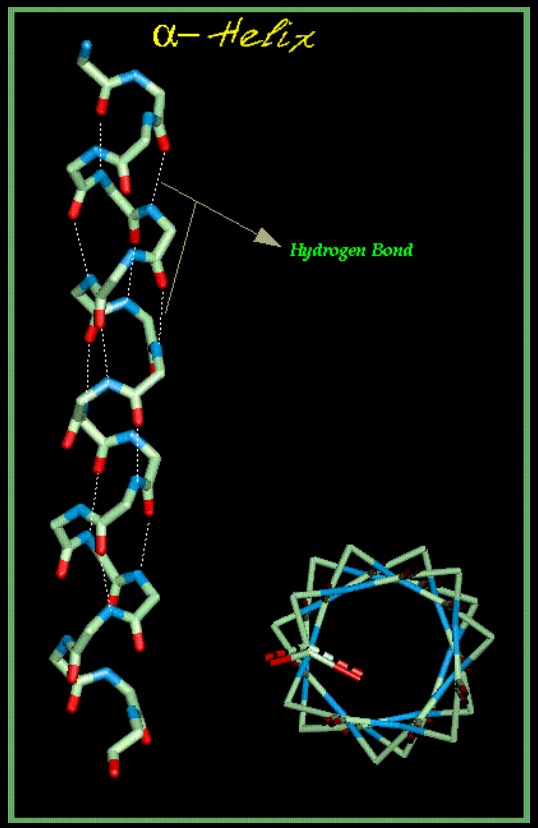
Kenneth Snelson's sculptures

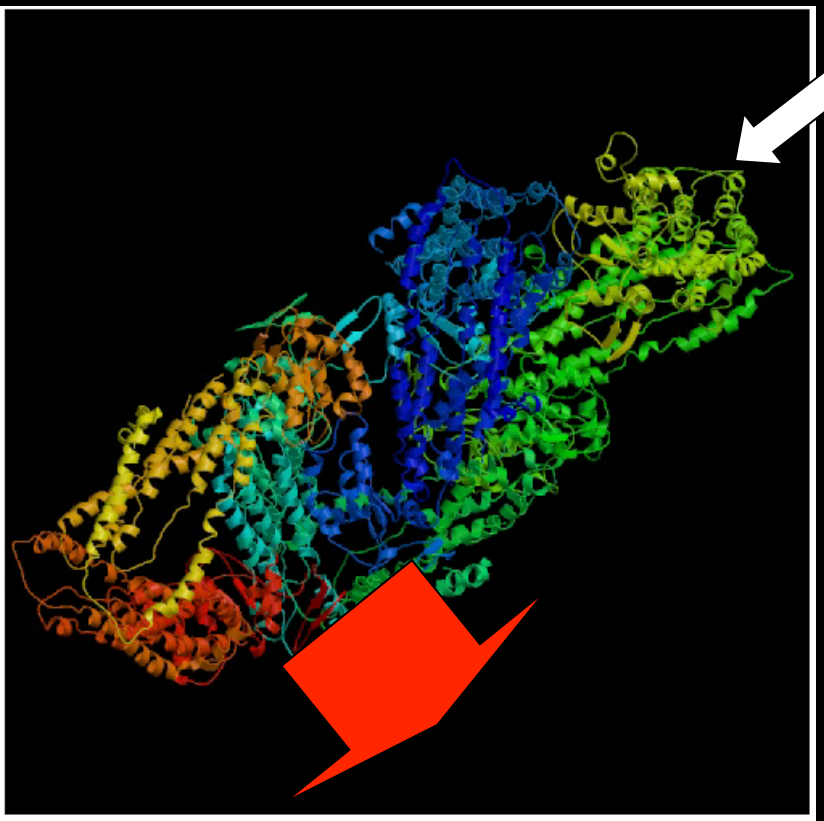
Structural members that can bear only tension are distinct from those that bear compression.



Molecular level

- Protein's backbone may fold into helical forms stabilized through a balance between the attractive force of hydrogen bonds and the ability of the protein coil to resist compression - ***tensegrity***.





Because a local force can change the shape of an entire tensegrity structure, the binding of a molecule to a protein can cause the different regions to change

Cytomechanics

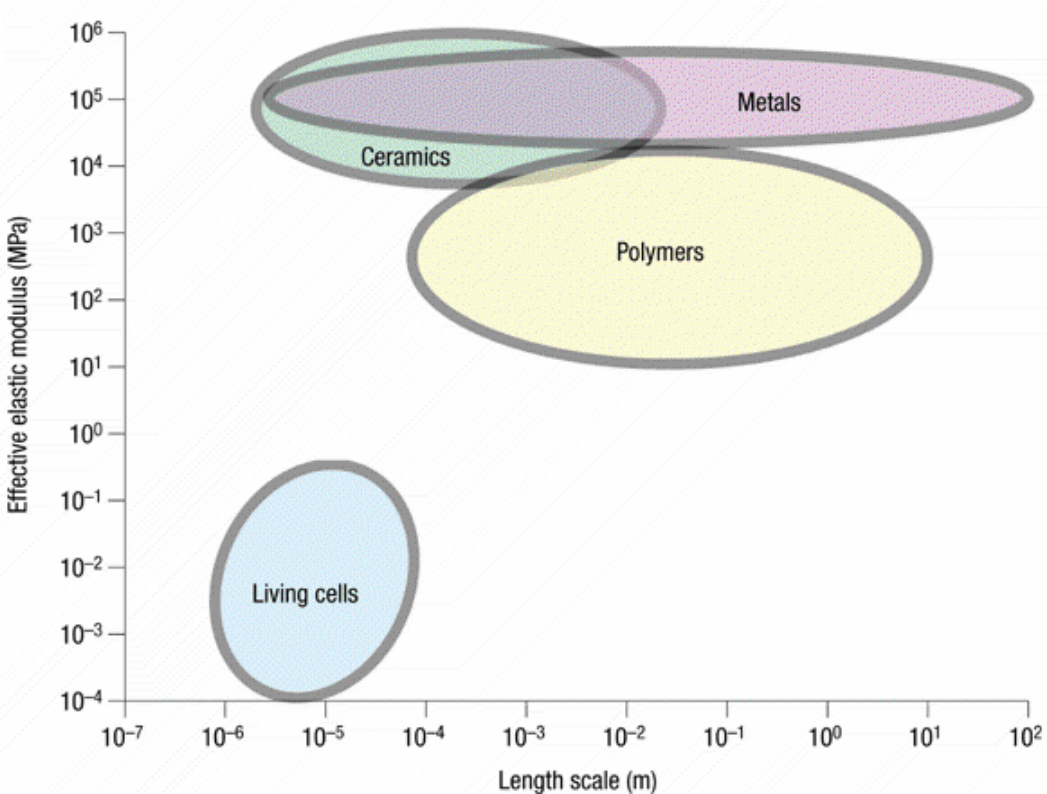
Tensegrity holds call together

- ***Structural components include lipids, and 3 separate filament systems.***

- ***No cell is an island – interactions with others.***

- ***Trans-skeletal molecules regulate the cell.***

Signals travel at speed of sound.



Prestress in cell - Pre-existing tensional stress

Prestress results from the action of tensional forces borne by actin microfilament, trasmitted over intermediate filaments and resisted by both ECM adhesions and internal microtubules

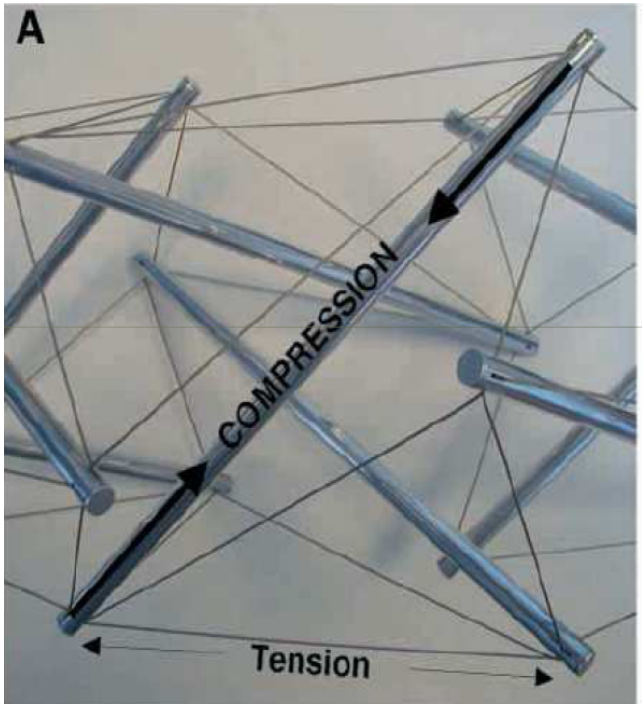
Rules of cellular tensegrity

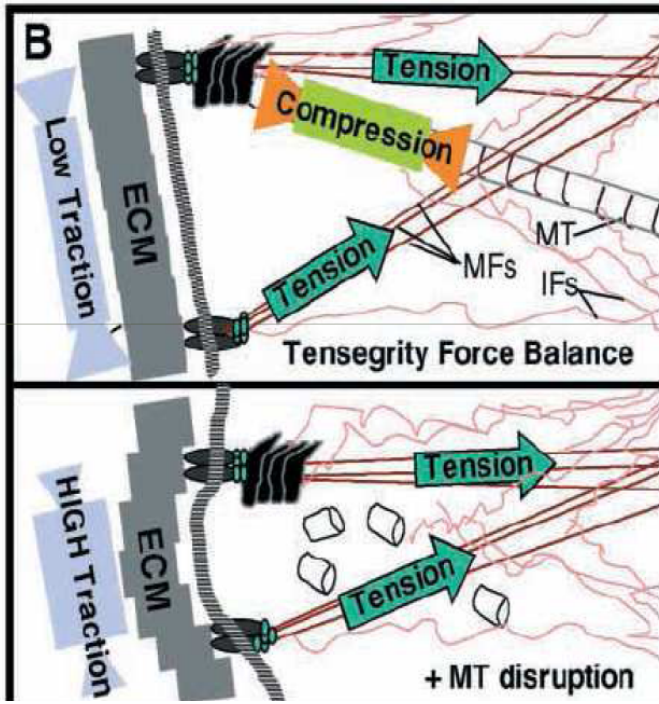
- Cell must behave mechanically as discrete **networks composed of different** interconnected cytoskeletal filament and **not as a mechanical continuum.**

- Cytokeletal **prestress should be a major determinant of cell deformability**

- Microtubules **should function as a** compression struts **and act in a** complementary manner with ECM anchors **to resist cytoskeletal tensional forces and establish a tensegrity force balance at the** whole cell level.

The tensegrity cell model





Forces

Tensions can be generated by:

- The actin-myosin network
- Cellular force generaion through focal adhesion
- Cell-cell adhesion
- Polymerization of cytoskeletal elements

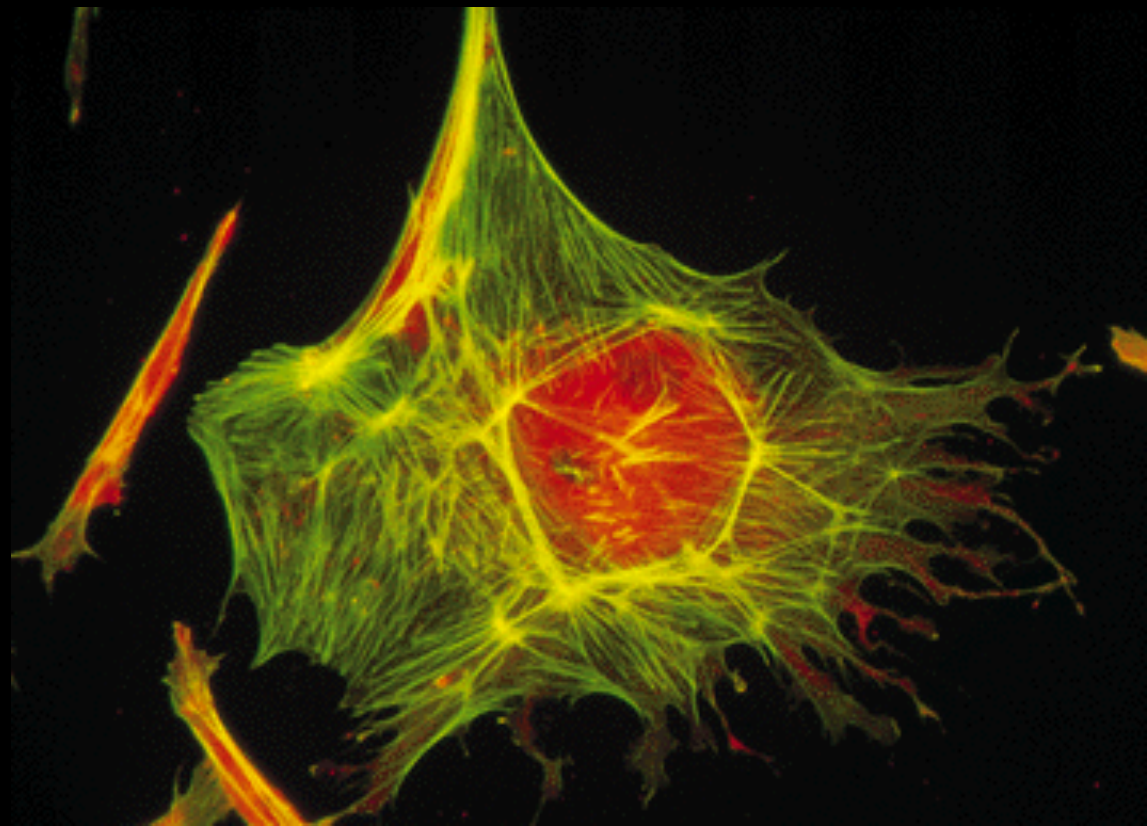
Compressive forces:

- The microtubules
- Adhesion

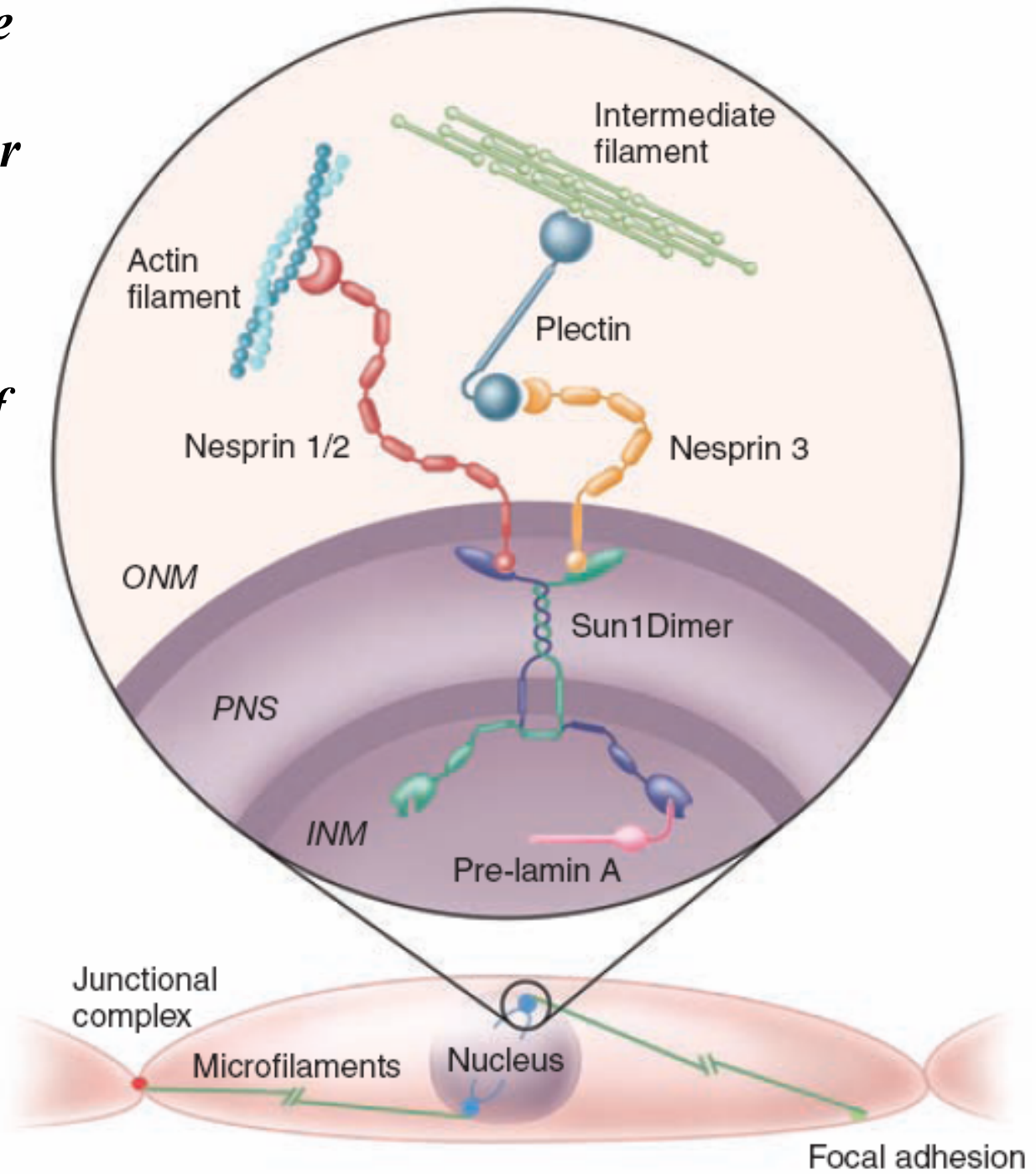
The idea

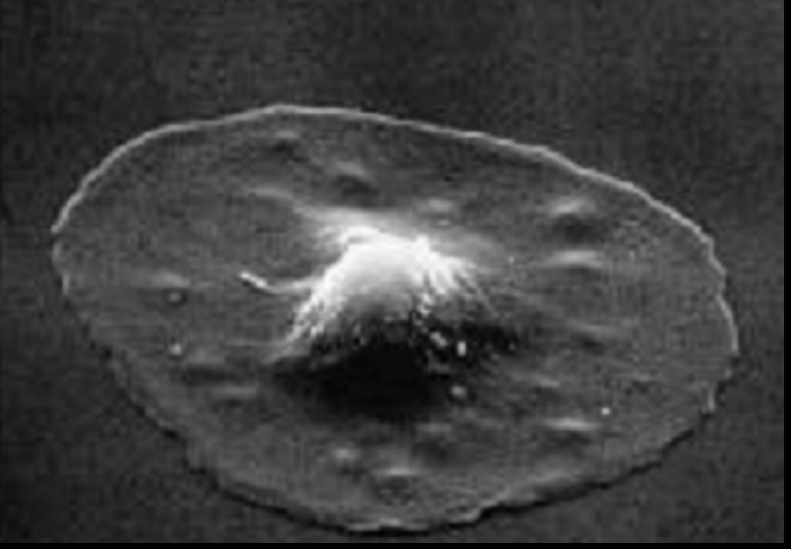
The plasma membrane and nucleus are physically connected by tensile filaments.

Pulling on receptors at the cell surface may produce immediate structural changes deep inside the cell.



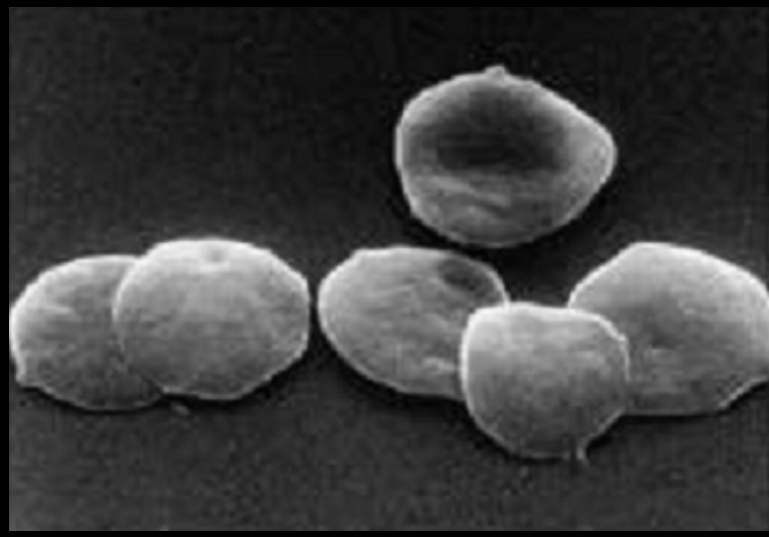
Nuclear components are integrated with cell surface and extracellular structures, including focal adhesions and junctional complexes mediated by elements of the cytoskeleton, including both microfilaments and intermediate filaments.



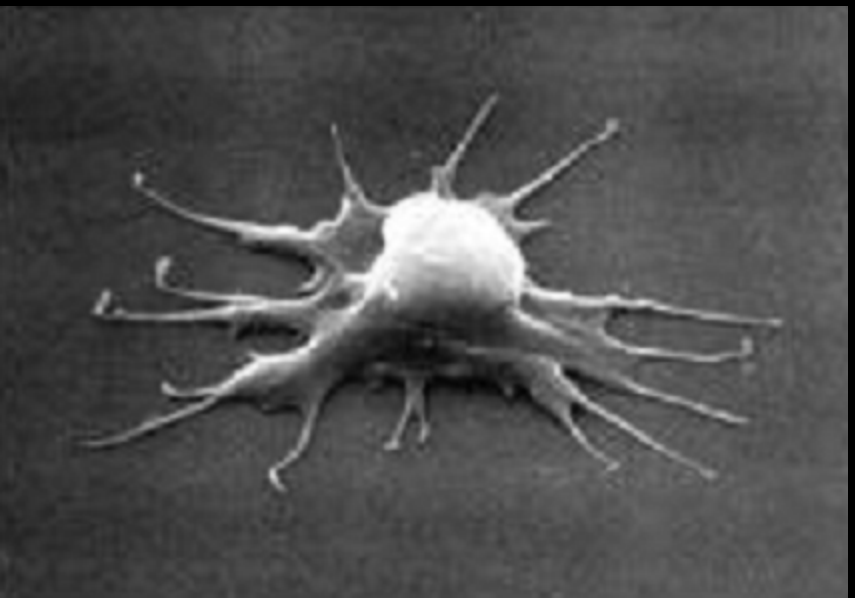


The shape of the cell decides which genetic programs is on.

- Spread flat cells became more likely to divide

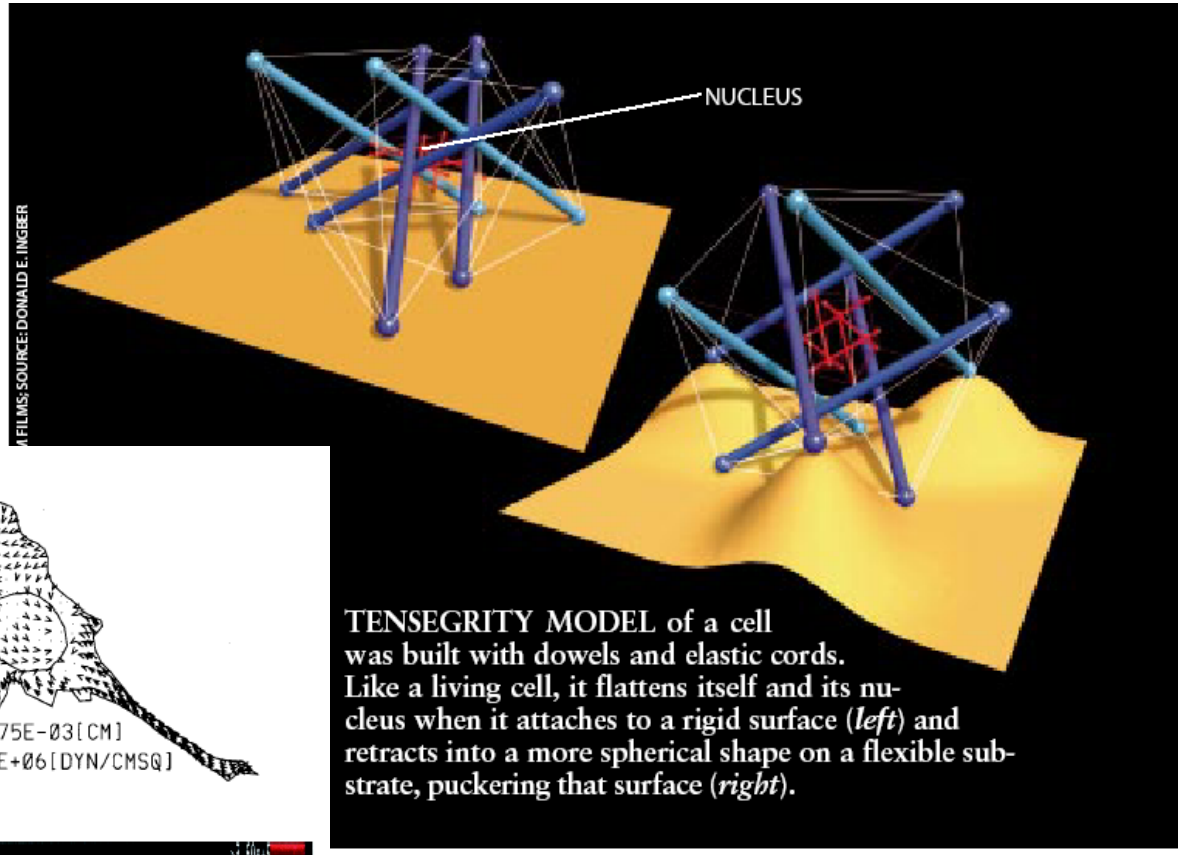


- Round cells that were prevented from spreading activated a death program known as apoptosis,

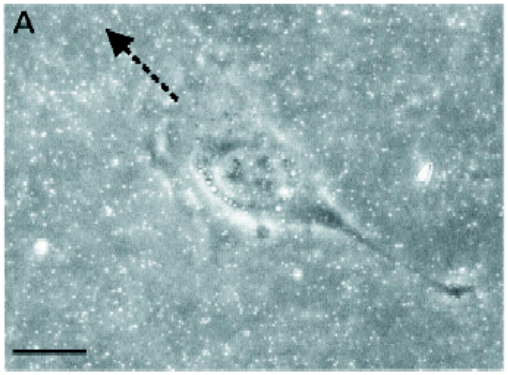


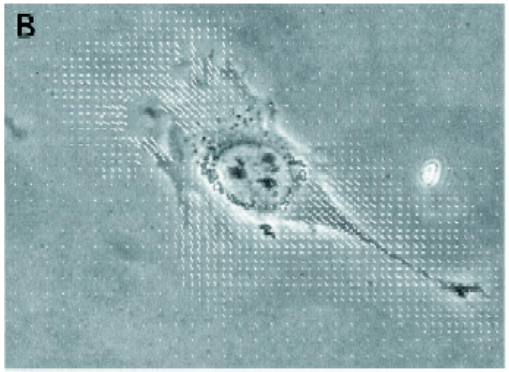
- Cells neither too extended nor too retracted would neither divided nor died (they differentiated themselves in a tissue-specific manner).

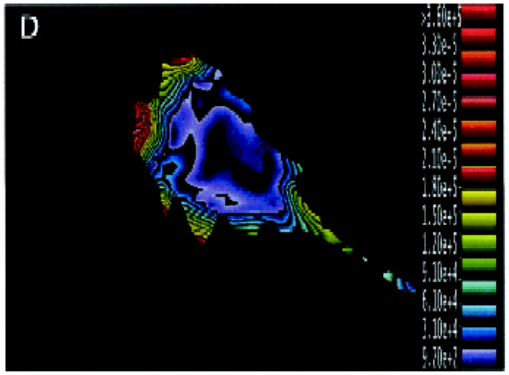
Tensegrity and cell adhesion



TENSEGRITY MODEL of a cell was built with dowels and elastic cords. Like a living cell, it flattens itself and its nucleus when it attaches to a rigid surface (*left*) and retracts into a more spherical shape on a flexible substrate, puckering that surface (*right*).

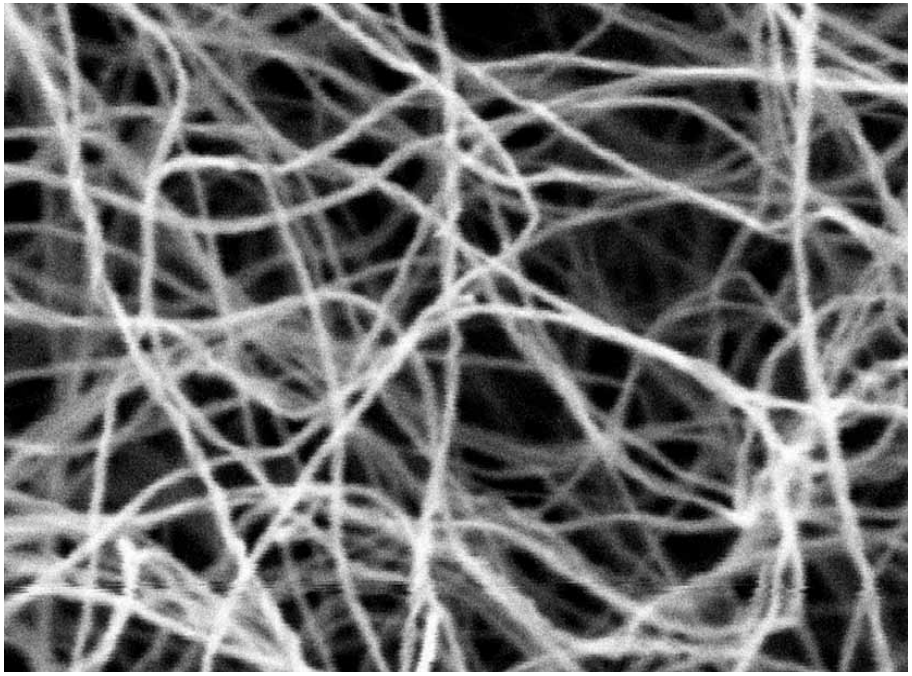


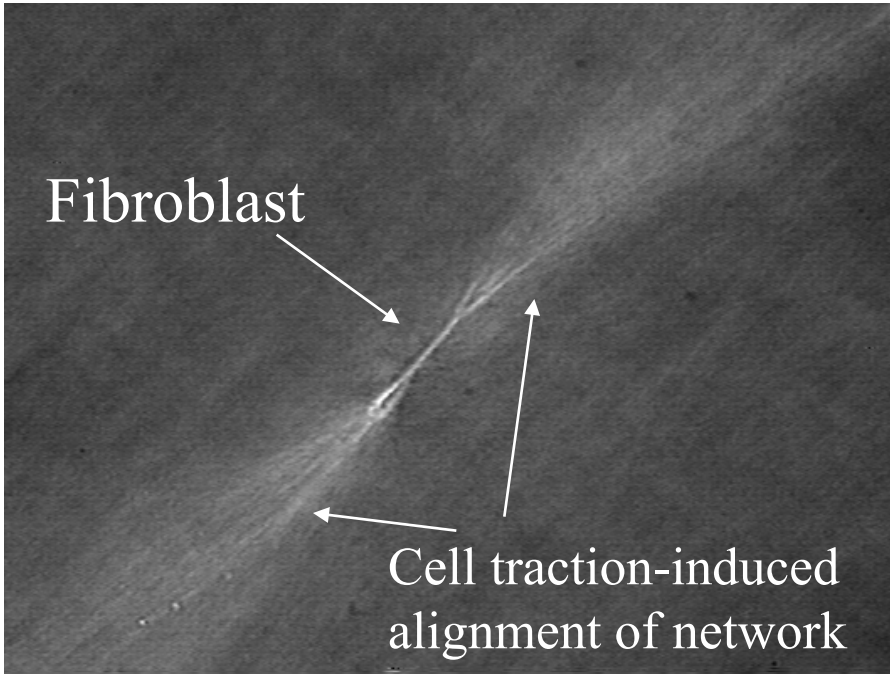




Forces during migration

Tissue equivalents



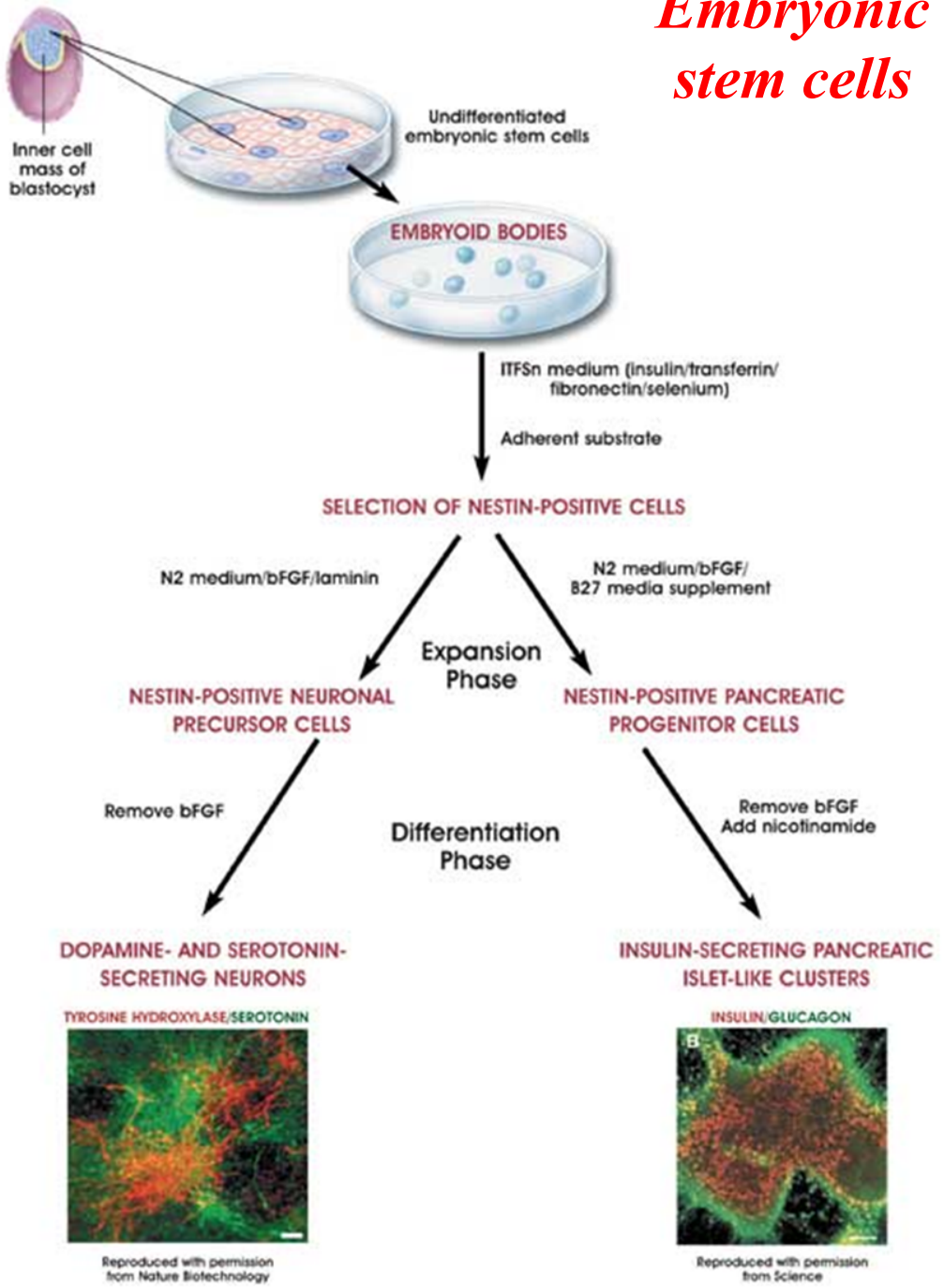


- Self-assembled biopolymer networks with entrapped tissue cells of interest.
- Cells exert traction on network resulting in compaction of the network and/or locomotion of the cells.

Stem cells

- unspecialized cells that renew themselves for long periods through cell division
- under certain physiologic or experimental conditions, they can be induced to become cells with special functions → PLURIPOTENT

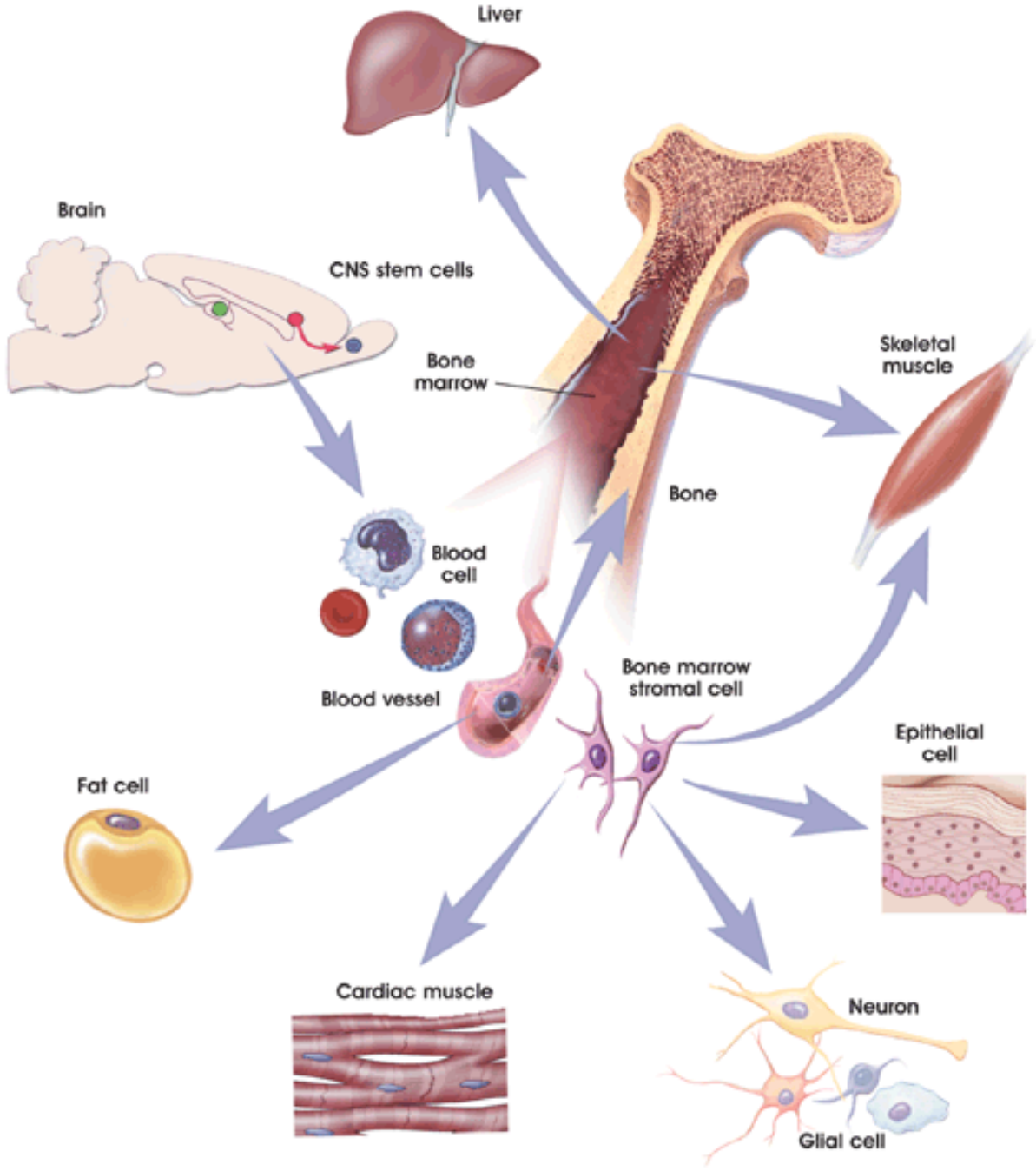
Embryonic stem cells

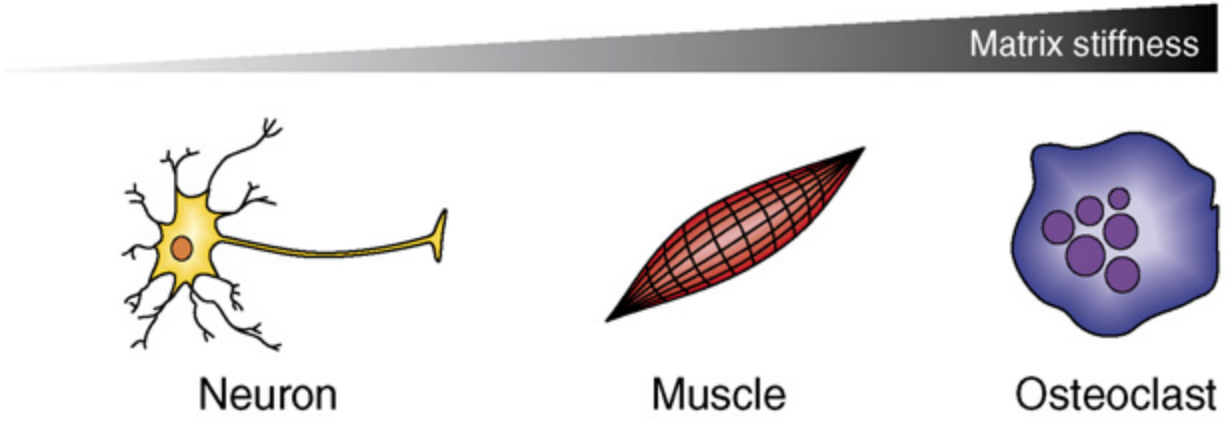


Adult stem cell plasticity

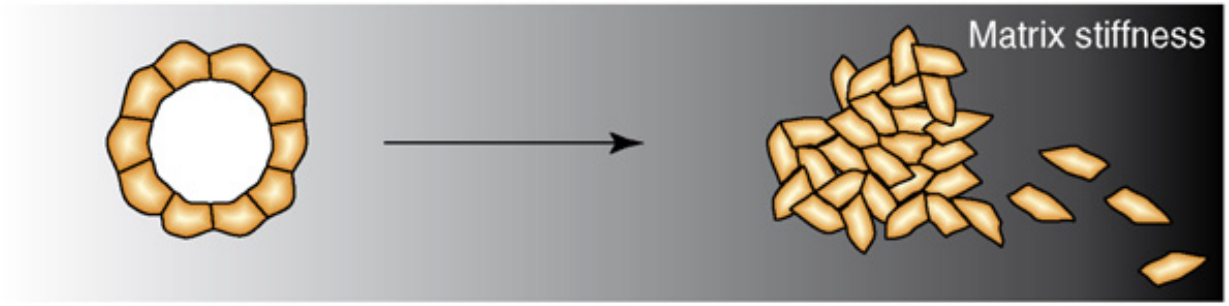
Undifferentiated cell found among differentiated cells in a tissue or organ → differentiate to yield major specialized cell types of the tissue or organ

Primary role: maintain and repair the tissue in which they are found





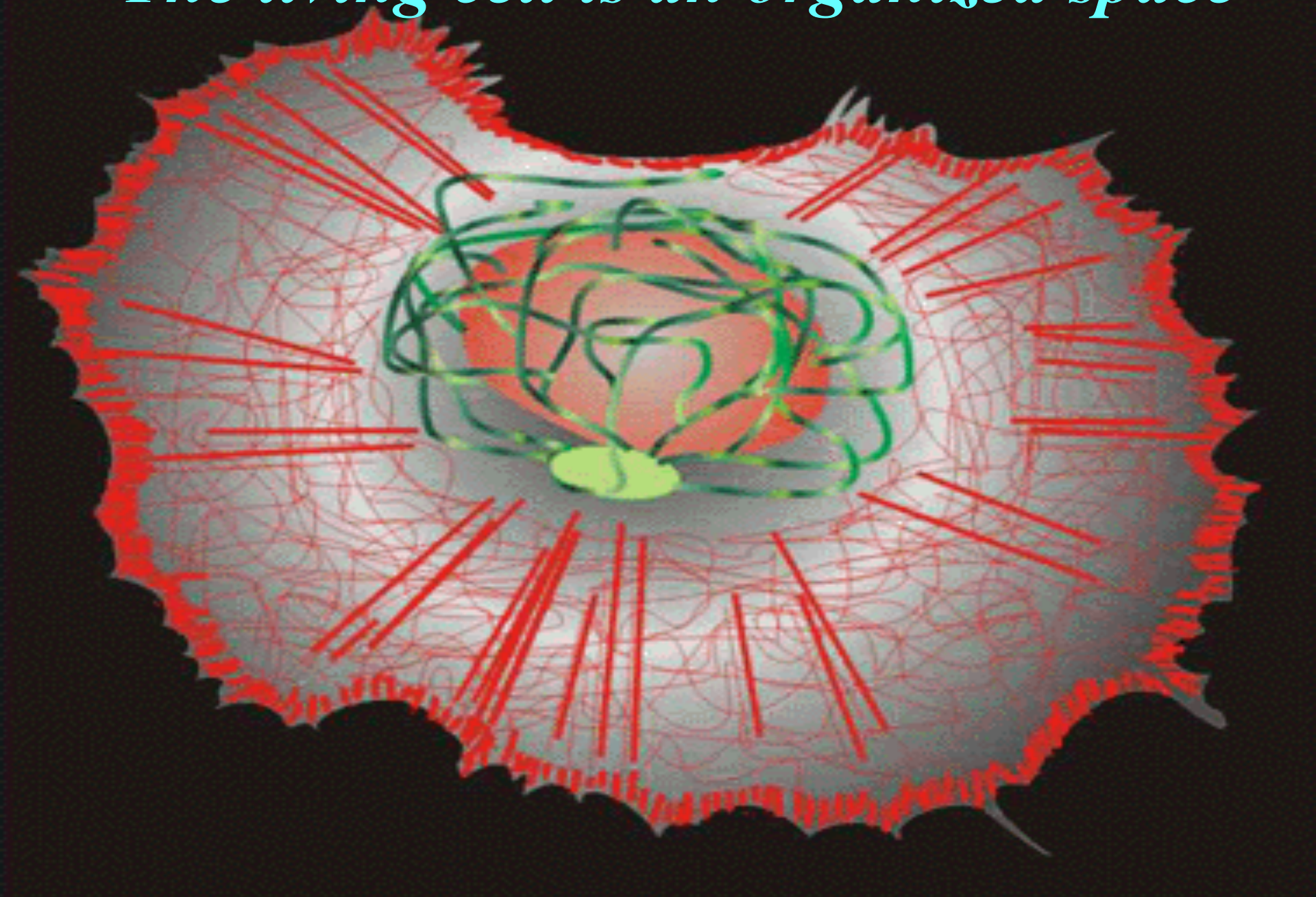
The differentiation program of mesenchymal stem cells grown on different matrices in vitro correlates directly with the tensile properties of native tissues. Soft matrices that mimic brain are neurogenic whereas rigid matrices that mimic bone are osteogenic.

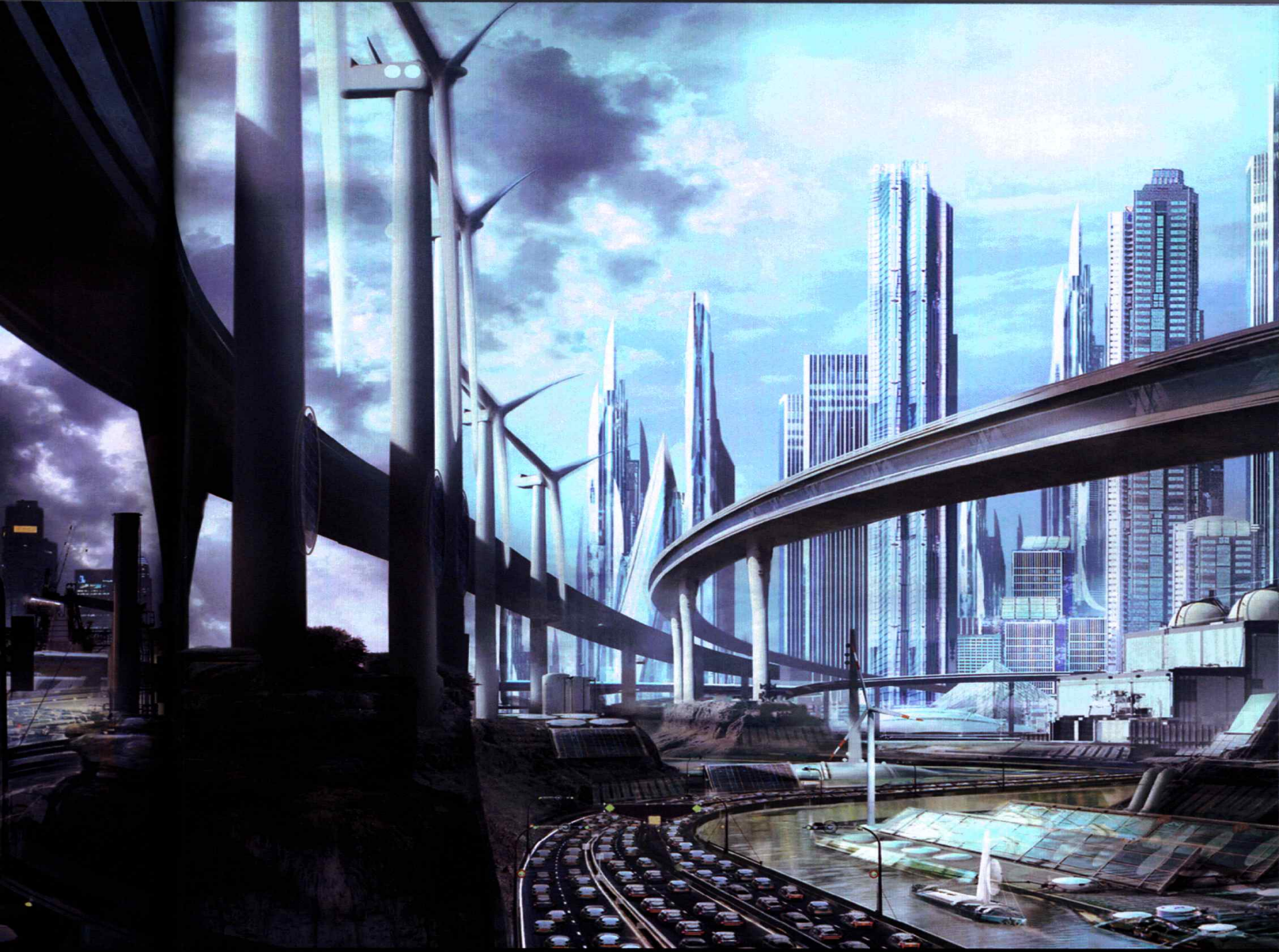


Breast epithelial cells form mammary ducts only on soft and malleable matrices. Mammary carcinoma cells increase actomyosin contractility and matrix stiffness, which maintains the cells in a highly proliferative and undifferentiated state.

The high levels of myosin II-based contractile forces can break cell–cell adhesions promoting metastasis.

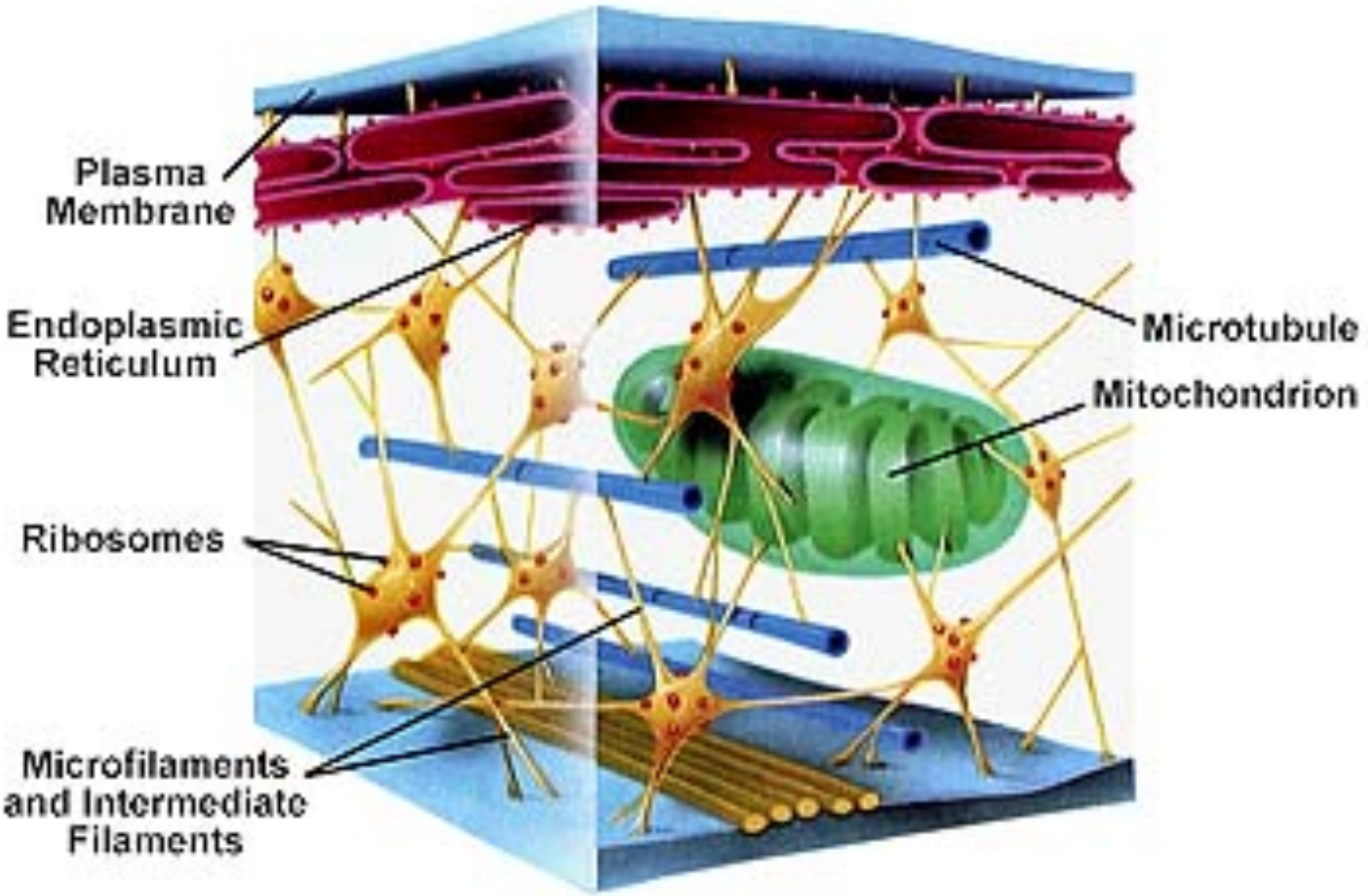
The living cell is an organized space





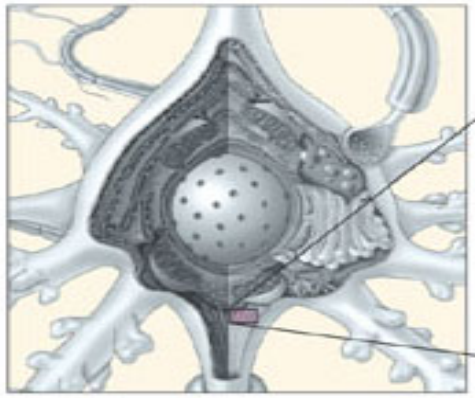
ART BY STEPHAN MARTINIERE (TOP) AND CHARLES FLOYD, NGM ART (BOTTOM)

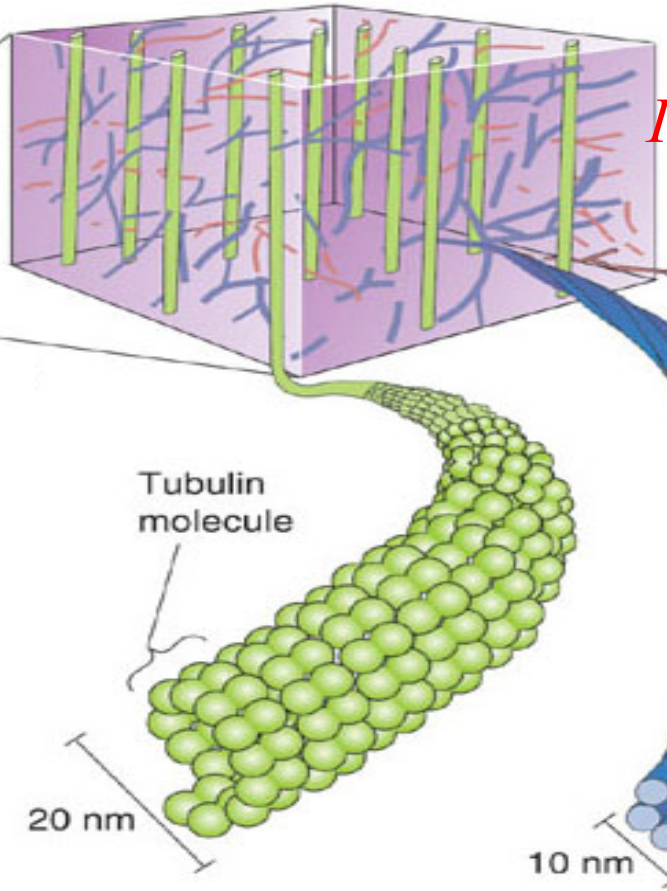
There are two structural elements of the cell: filaments and sheets.



The cytoskeleton

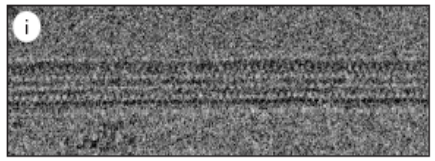
It is a network of filaments

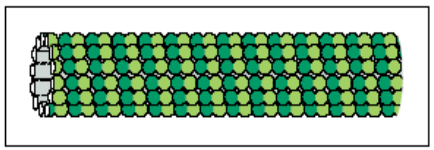


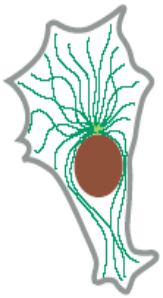


Microtubule Neurofilament Microfilament

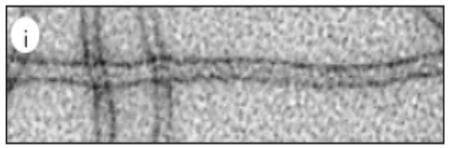
MICROTUBULES

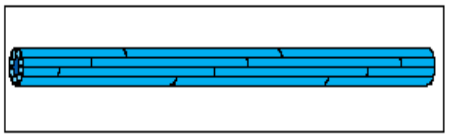


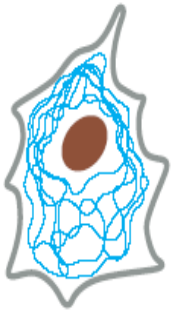




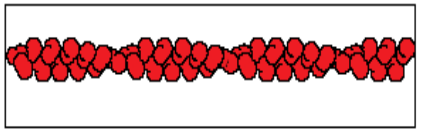
INTERMEDIATE FILAMENTS

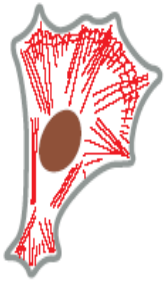


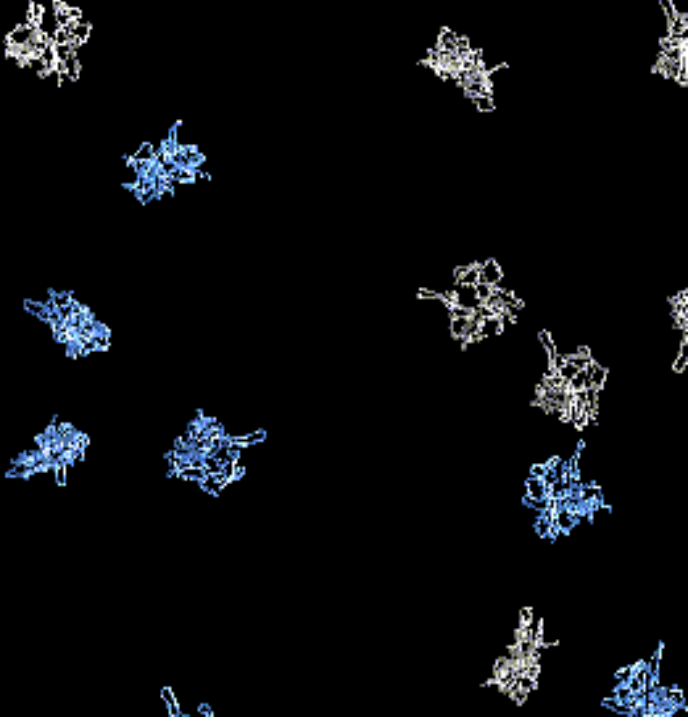




ACTIN FILAMENTS



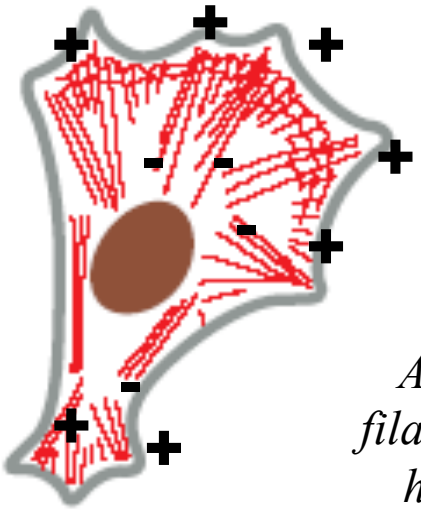




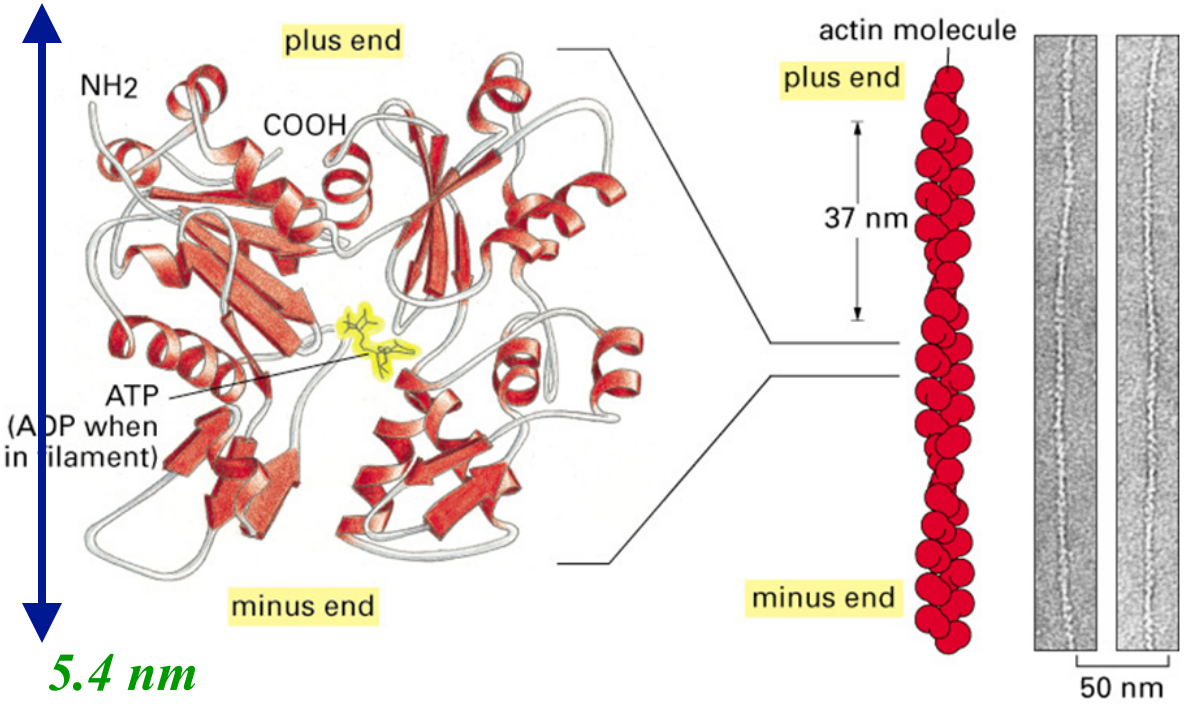
Actin filaments

Protofilaments are wrapped around each other with a 72 nm period (persistance length ~10 μm).

Diameter – 7 nm



Actin filaments have ***polarity****.*



Actin filaments allow cells to adopt different shapes and perform different functions

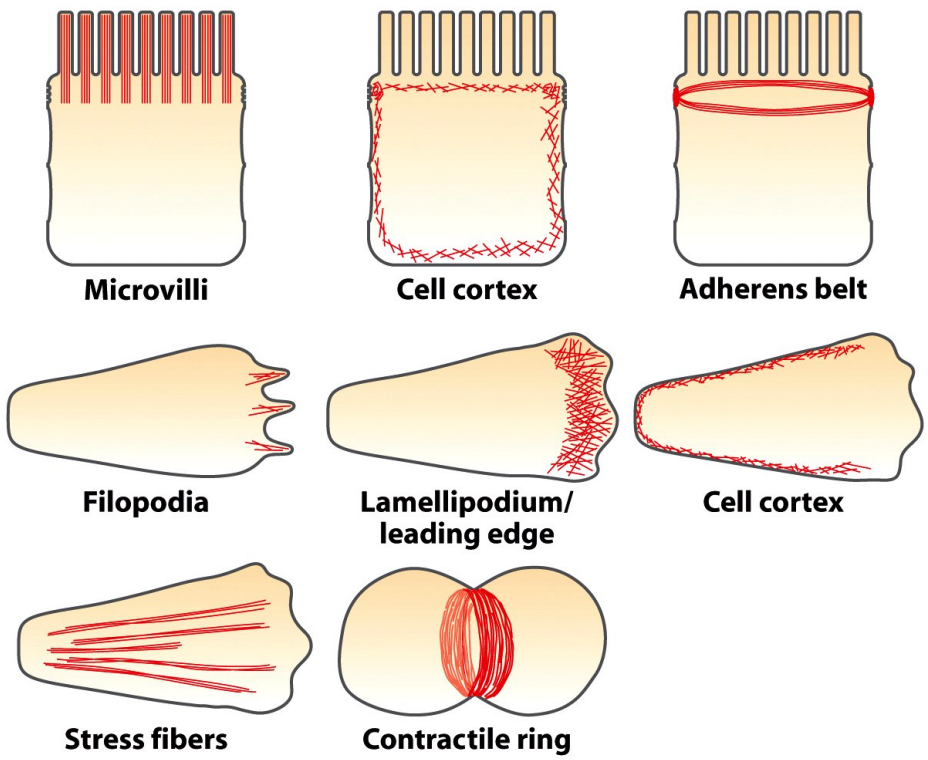
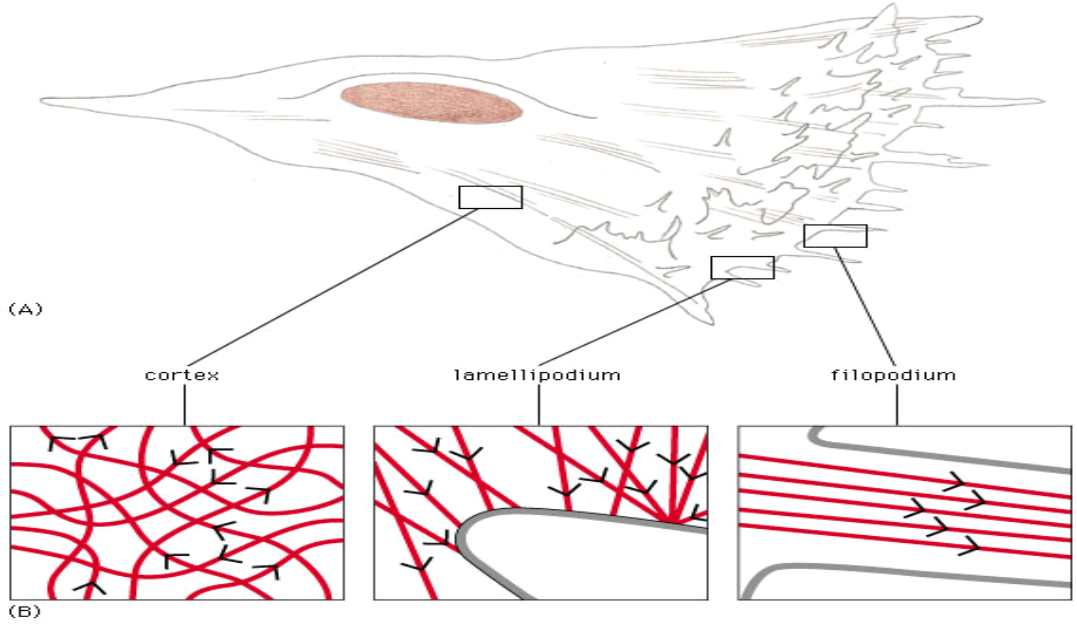
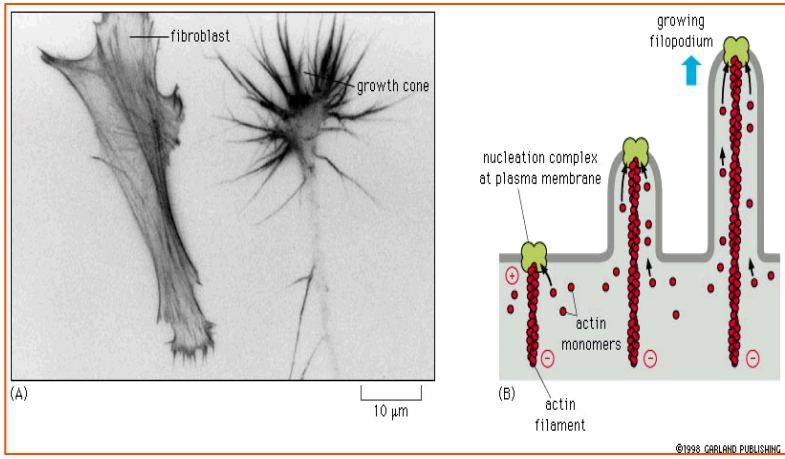


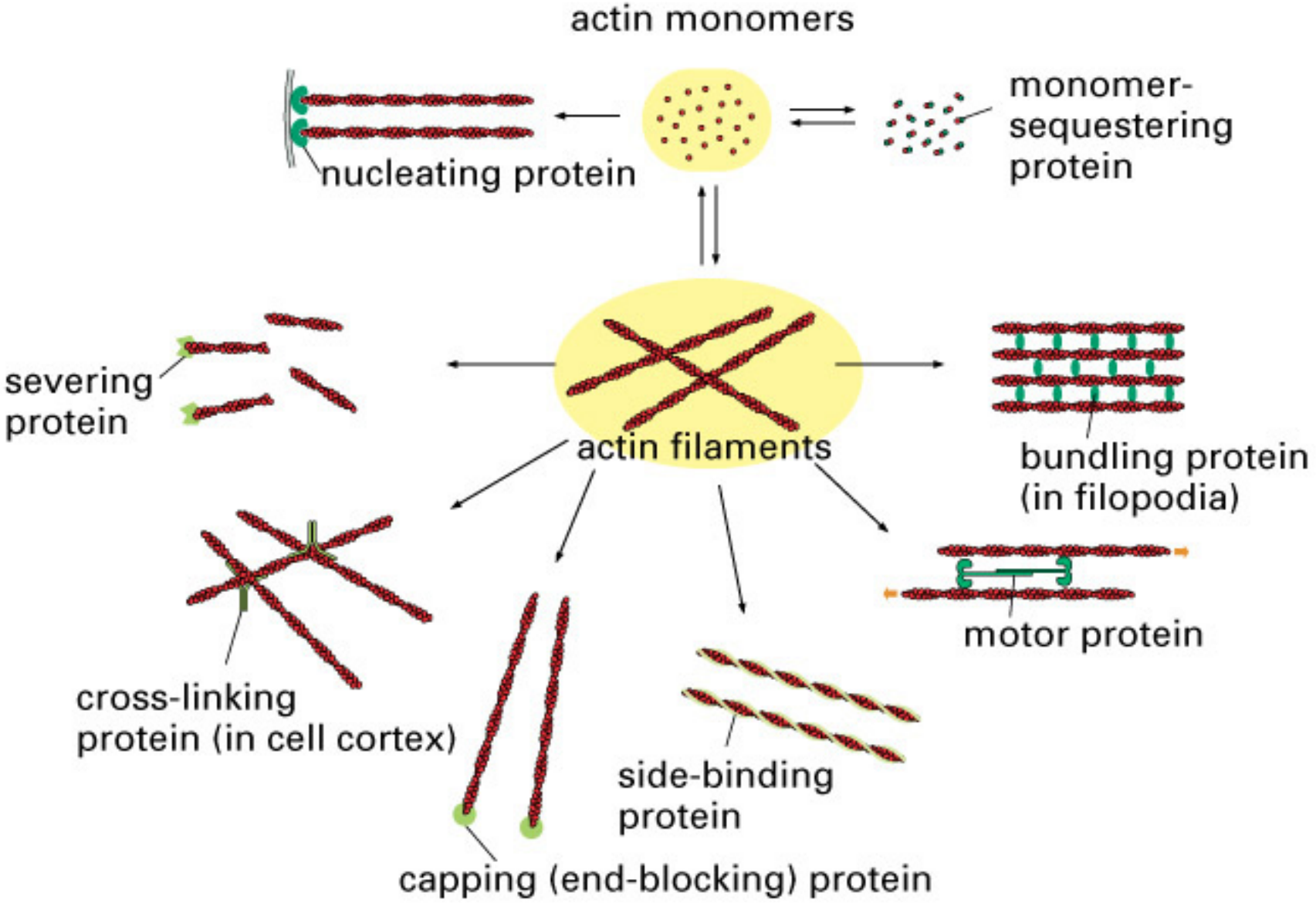
Figure 17-4a
Molecular Cell Biology, Sixth Edition





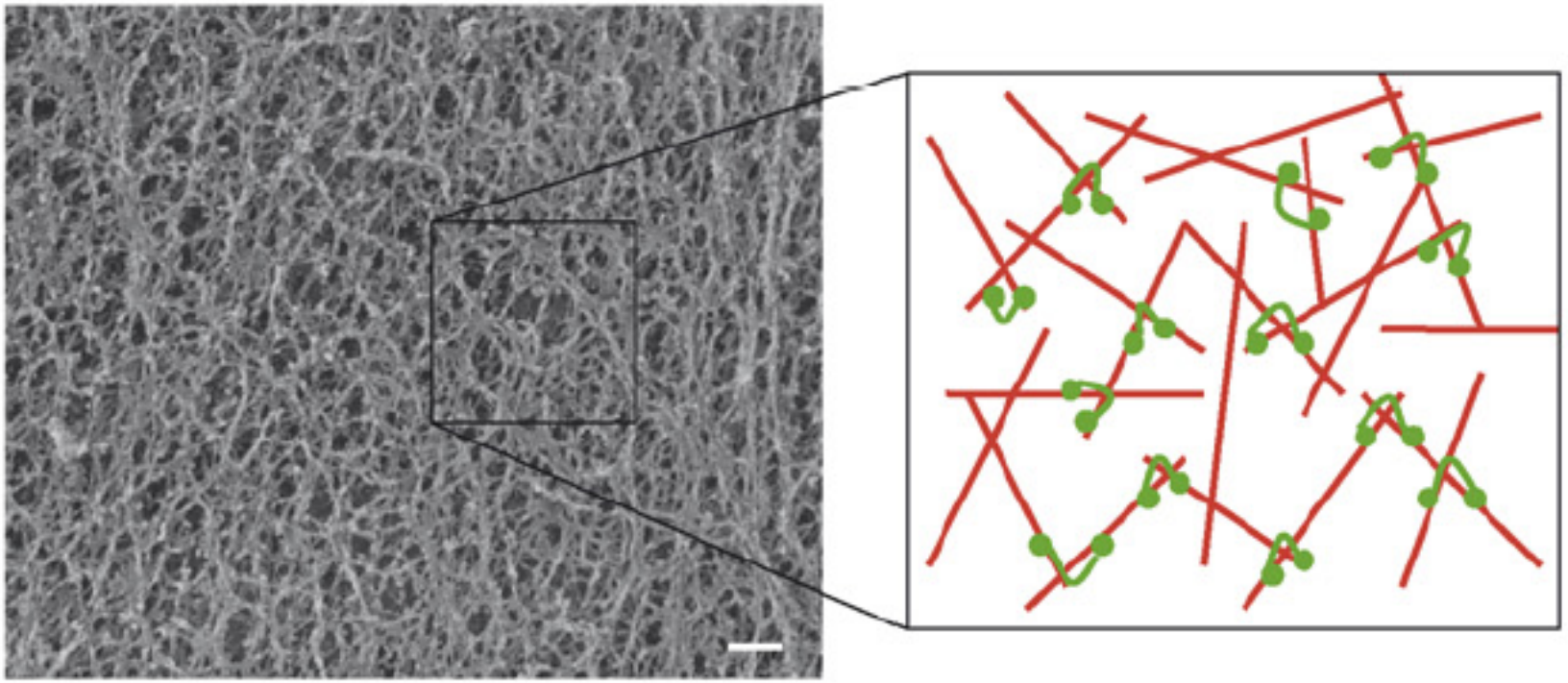
Growth of Filopodia

Actin architecture and function is governed by actin-binding proteins



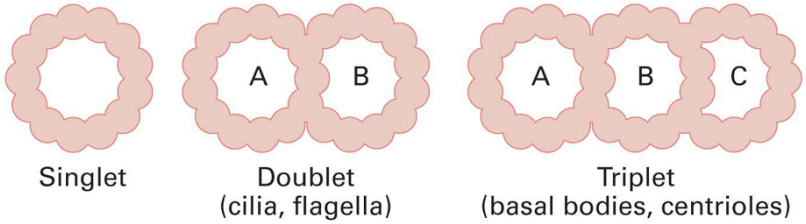
Actin network

The actin filaments are much shorter in length than their persistence length, and hence are nearly rigid. The crosslinkers can themselves be flexible.



In vitro network of F-actin (1 mg/ml) filaments capped with gelsolin (1:555 molar ratio to actin) and crosslinked with filamin A (1:50 molar ratio to actin). The sample is fixed, rotary shadowed, and imaged by transmission electron microscopy. Scale bar = 200 nm.

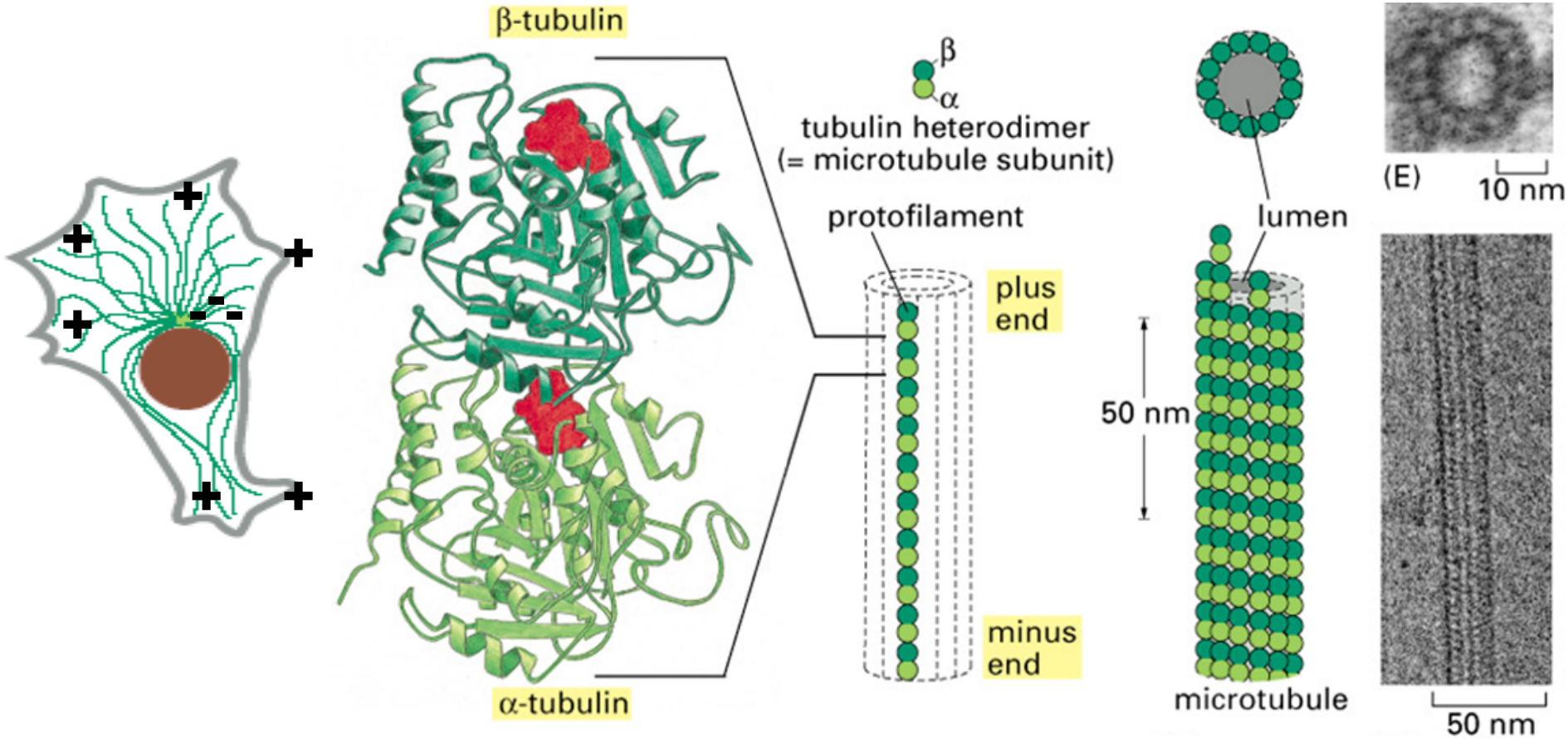
Microtubules

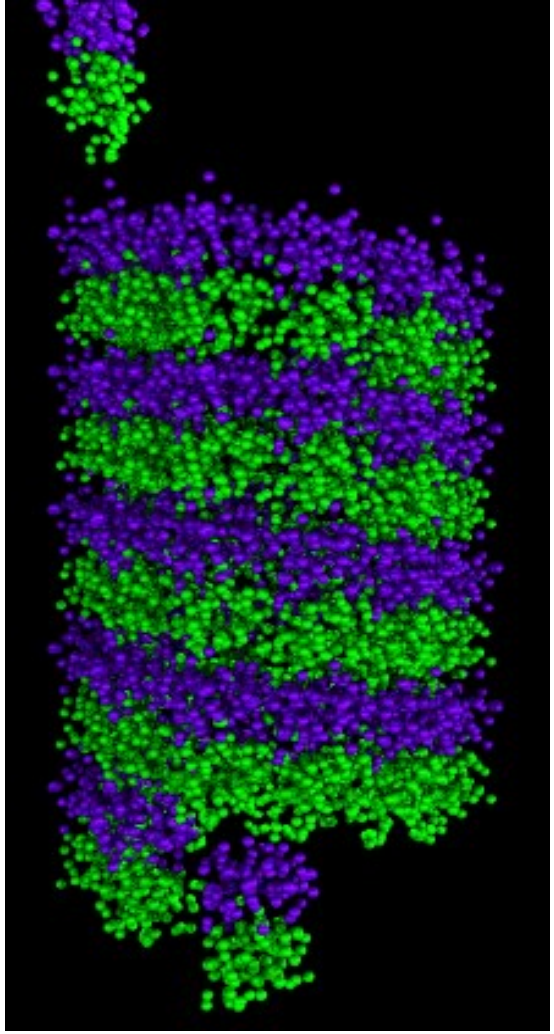


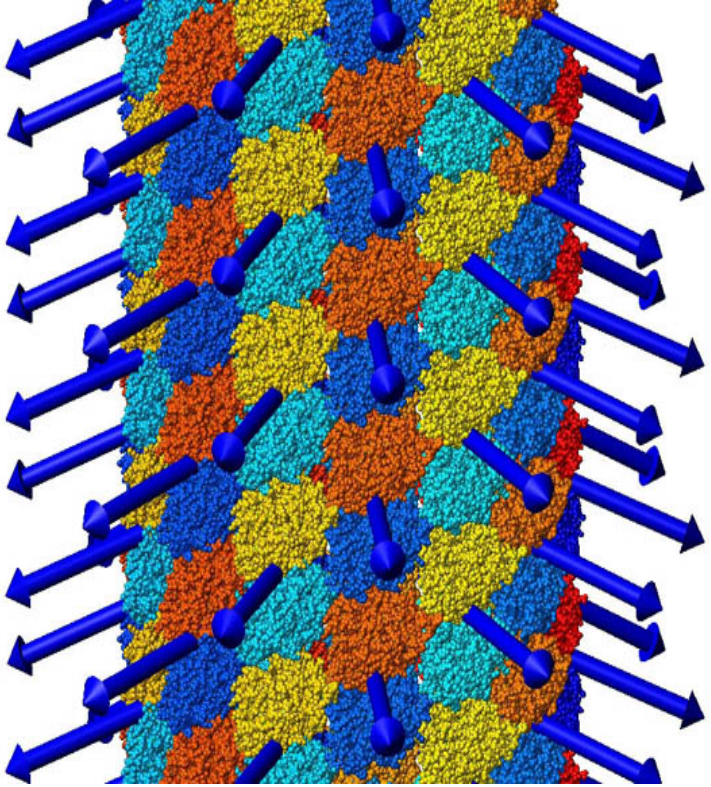
*An **α,β-tubulin heterodimer** does not come apart, once formed.*

***α-Tubulin** has a bound GTP, that does not hydrolyze.*

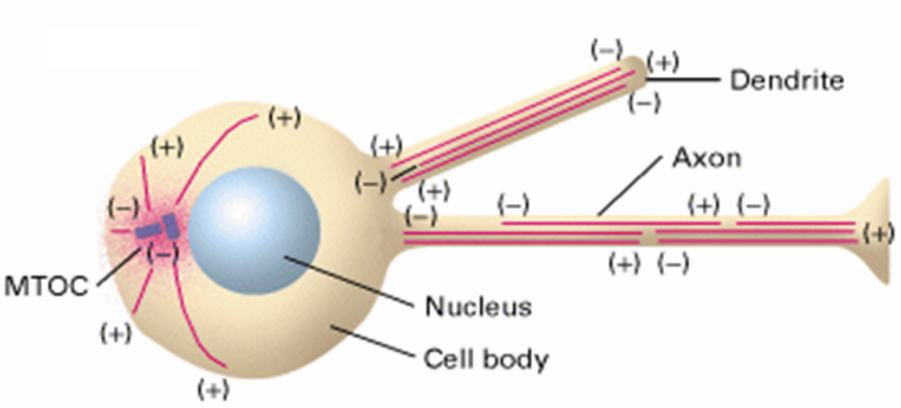
β-Tubulin may have bound GTP or GDP.

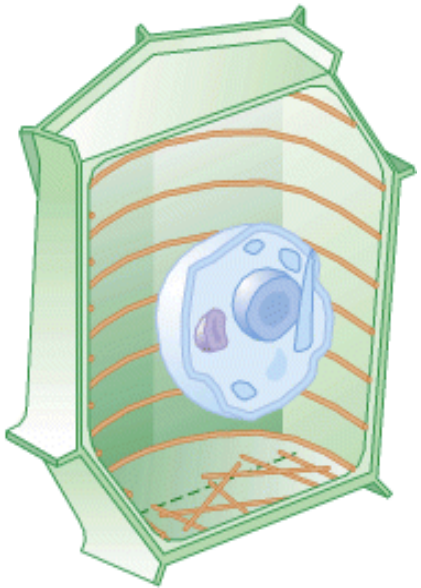




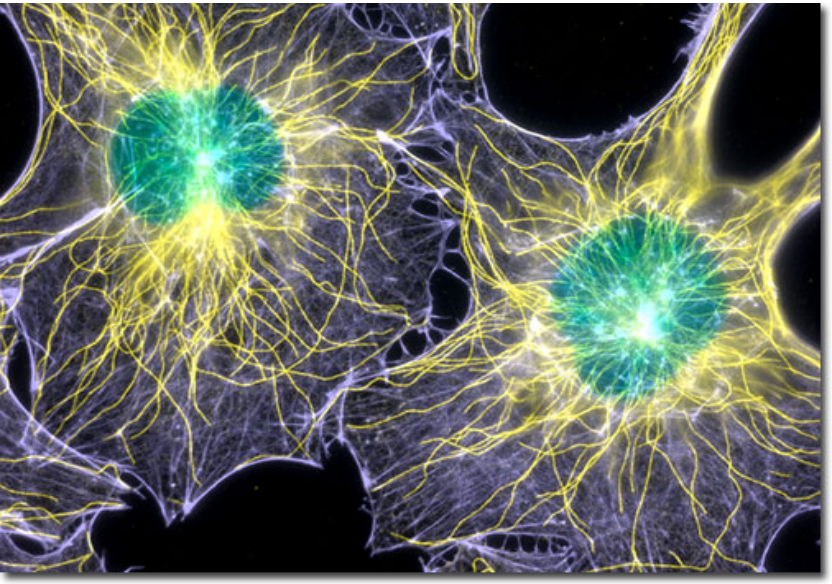


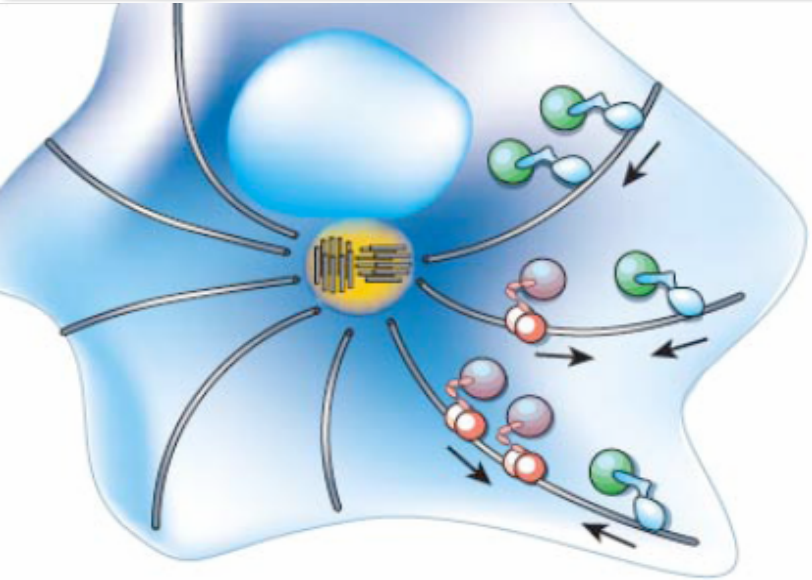
Interphase plant cell



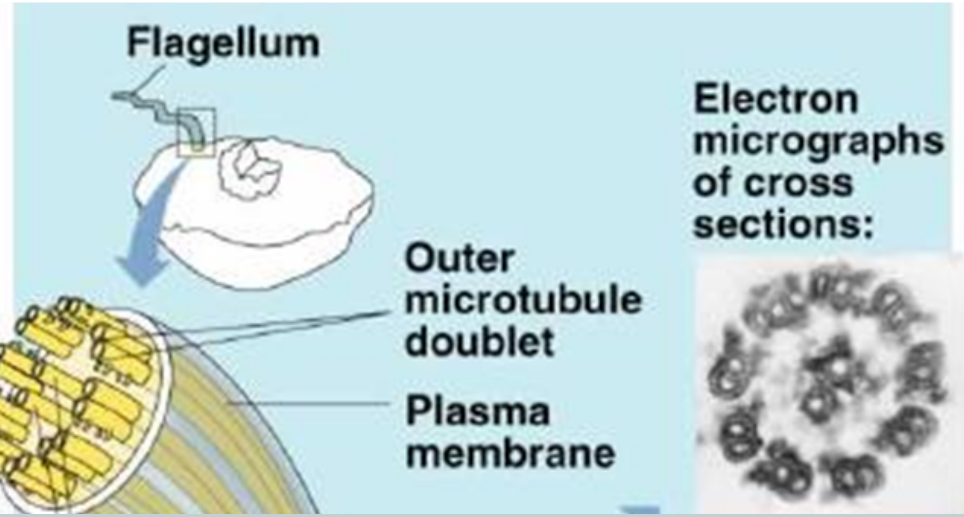


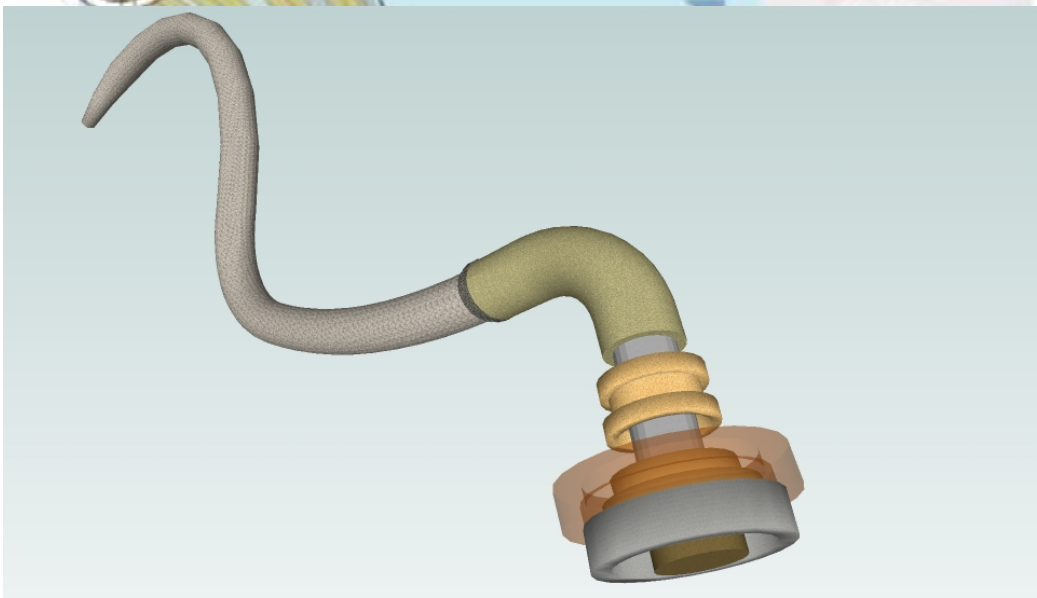
Intracellular communication



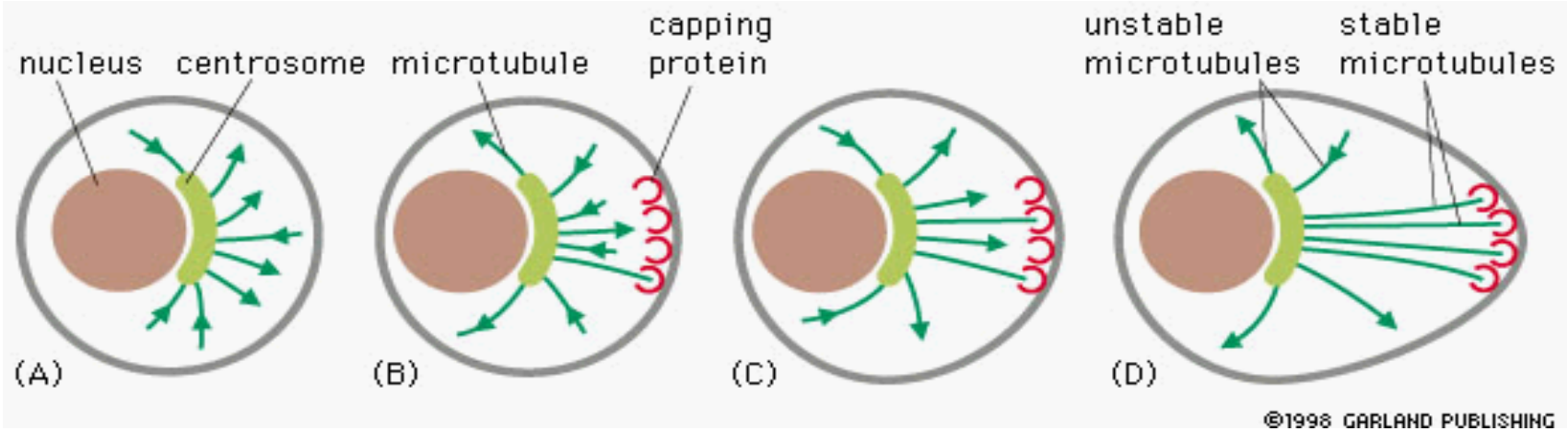


Microtubules form Cilia (Flagella)





Capping proteins bind to the ends of microtubules and provide stabilization by protection from depolymerization.



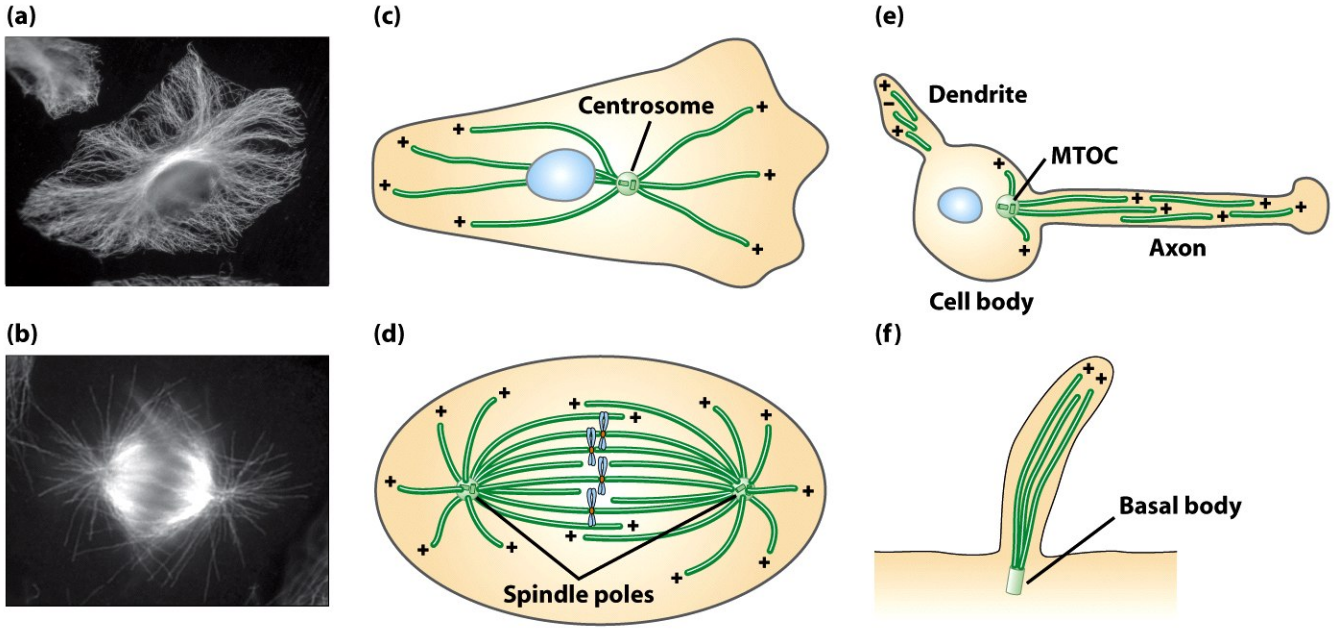
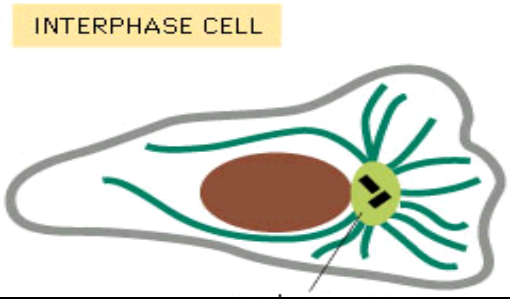
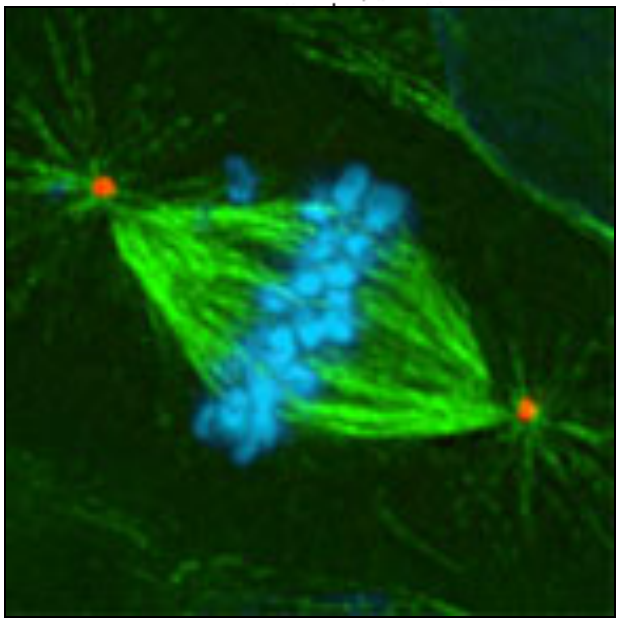


Figure 18-5
Molecular Cell Biology, Sixth Edition

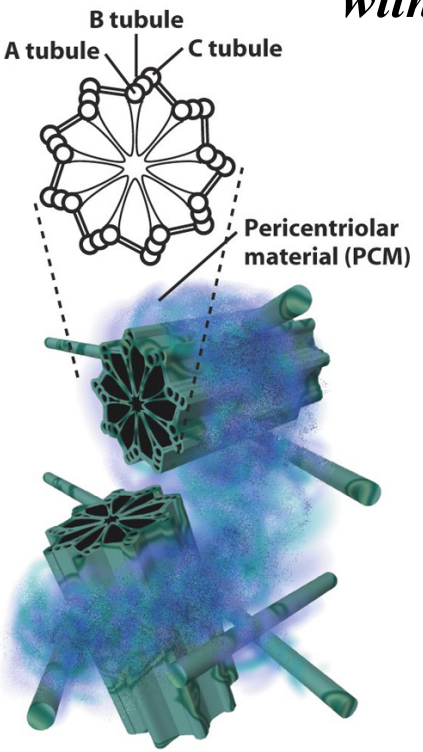
Microtubules provide an organizational structure in an interphase cell and separate chromosomes in a dividing cell.

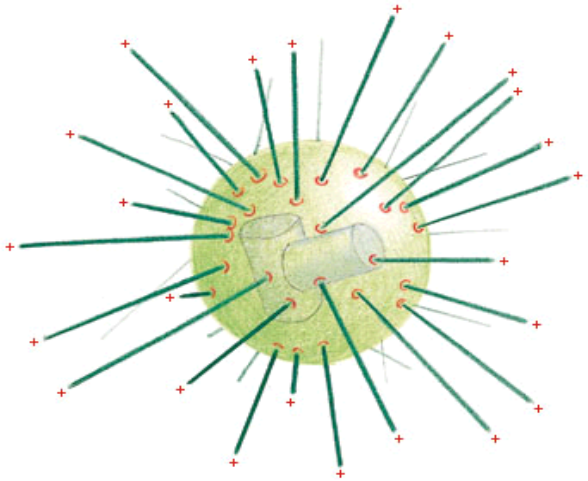
Centrosome and spindle fibers





0.2 µm in diameter and about 0.4 µm long. They are composed of 9 fibrils, with each fibril composed of 3 fused microtubules





(B) microtubules growing from nucleating sites on centrosome

The centrosome provides nucleating sites from which microtubules can grow.

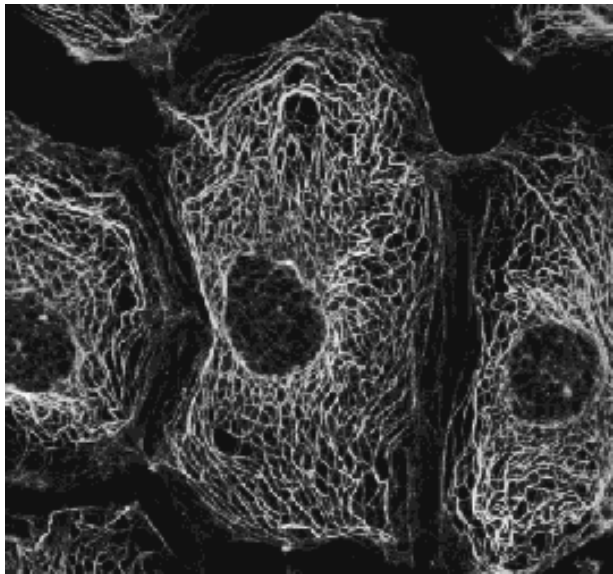
Intermediate filaments

Rope-like network of filaments in the cell

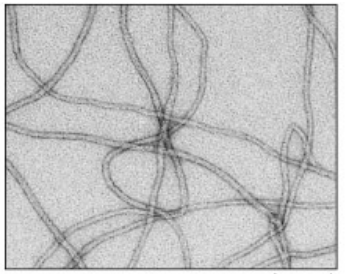
Principle function is maintenance of cell structure - provide tensile strength to the cell

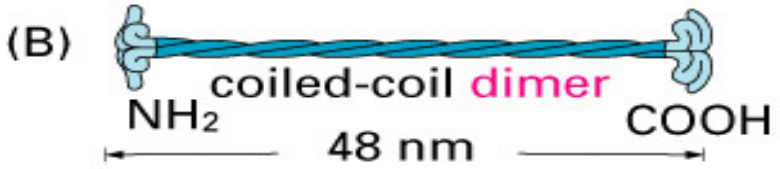
Intermediate filaments differ from actin & microtubules

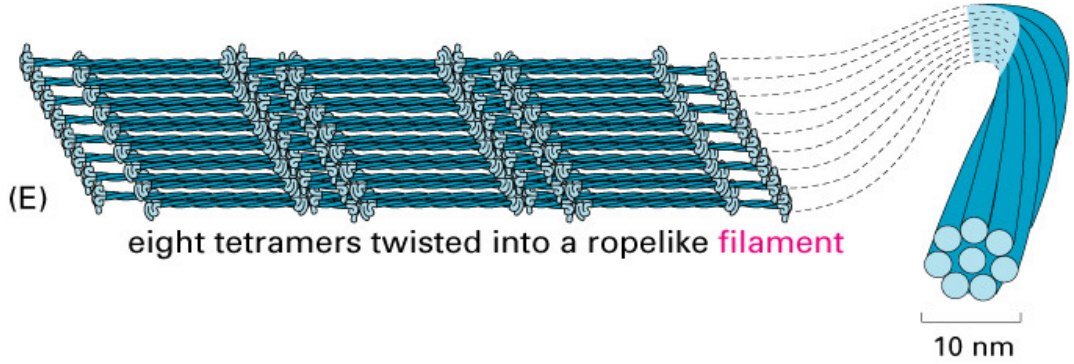
- I.F.s do not have a defined polarity (i.e. no plus or minus ends)
- I.F.s have no associated motor proteins
- I.F.s do not bind to nucleotides (ATP or GTP)
- I.F.s are very stable compared to actin or microtubules

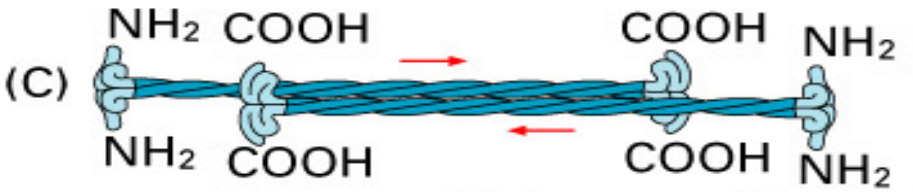


Intermediate filaments share a common structure

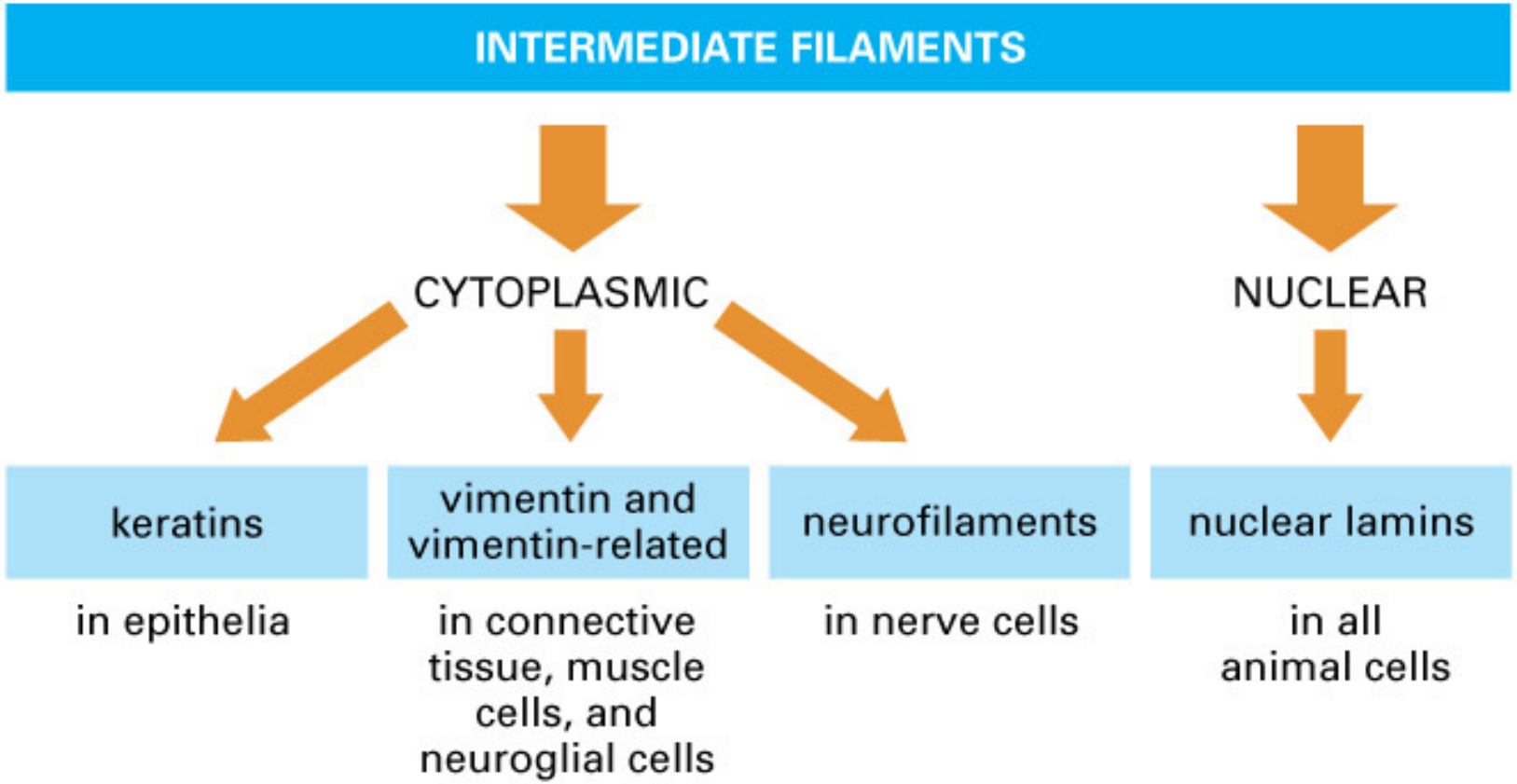


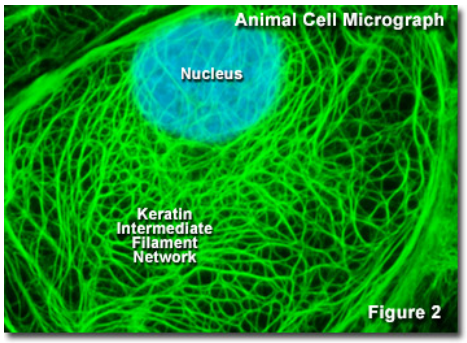


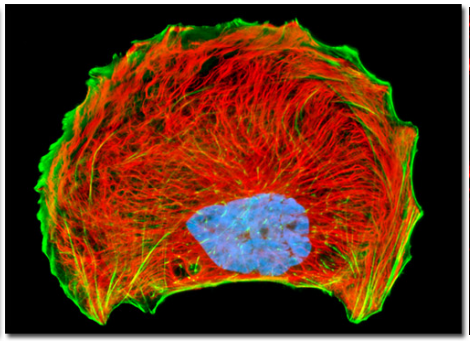


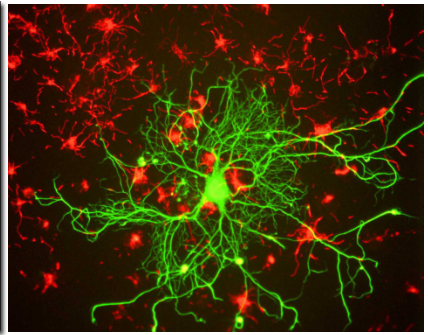


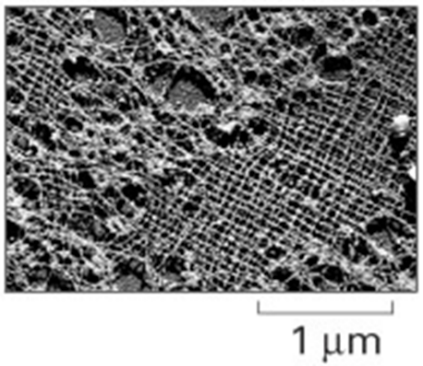
Classes of intermediate filament proteins



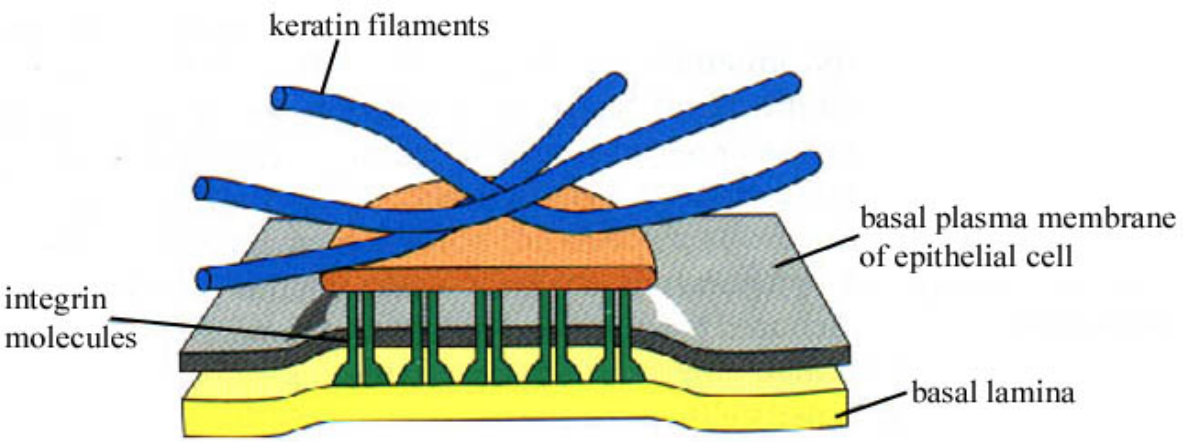


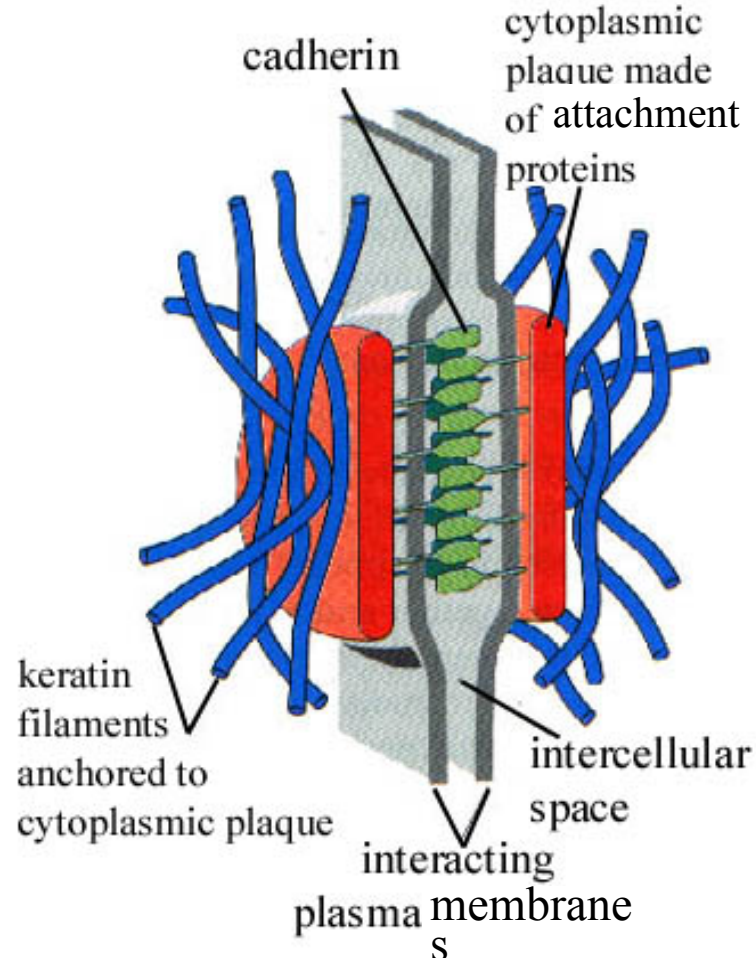






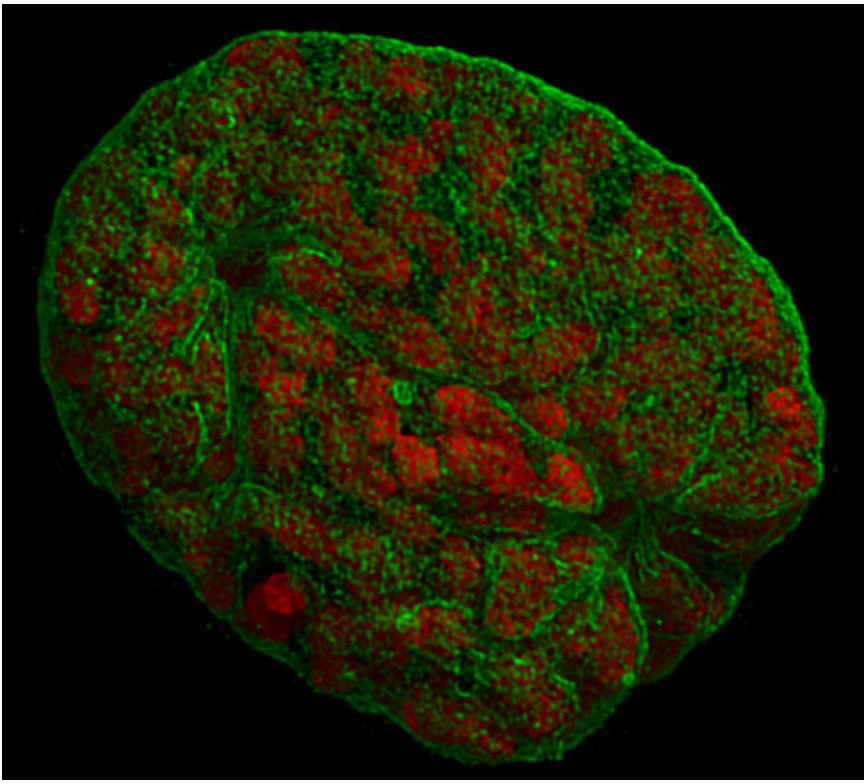
Hemidesmosomes mediate cell-matrix adhesion between epithelial cells and basal lamina



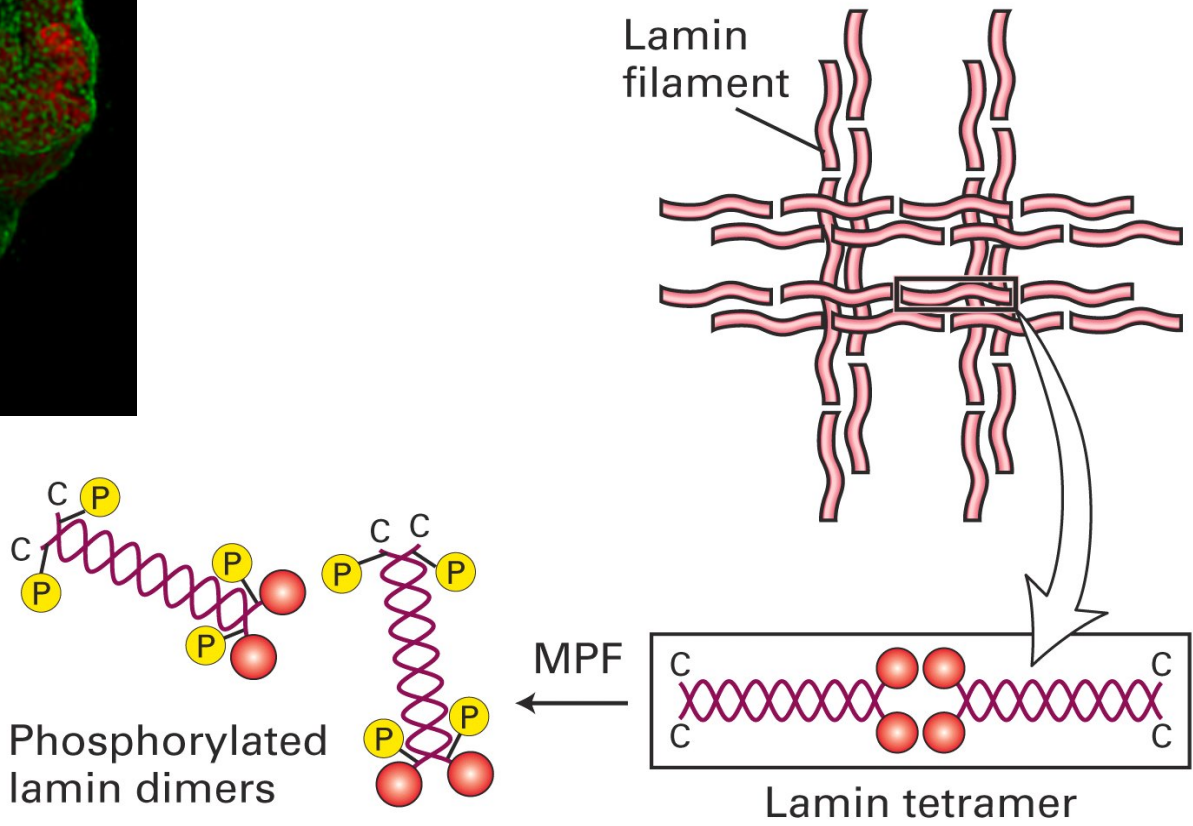


Desmosomes mediate cell-cell adhesion between epithelial cells

Nuclear lamins disassemble when cells enter mitosis



Red: DNA, Green: lamin (3D SIM imaging



Cytoskeleton function

Structure and support

Provides a scaffolding to give cells their shape

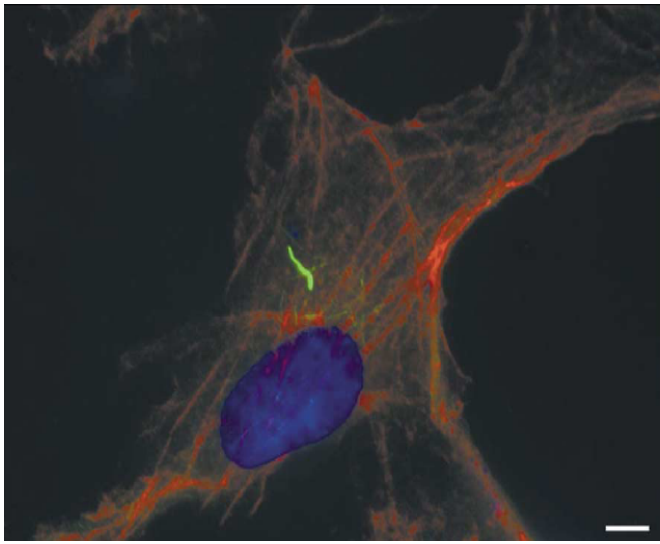
Intracellular transport

Moves organelles and molecules inside cell

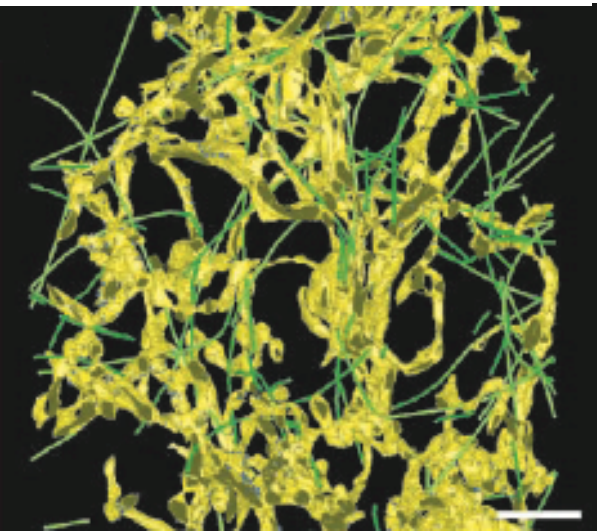
Contractility and motility

Movement of cells in their environment

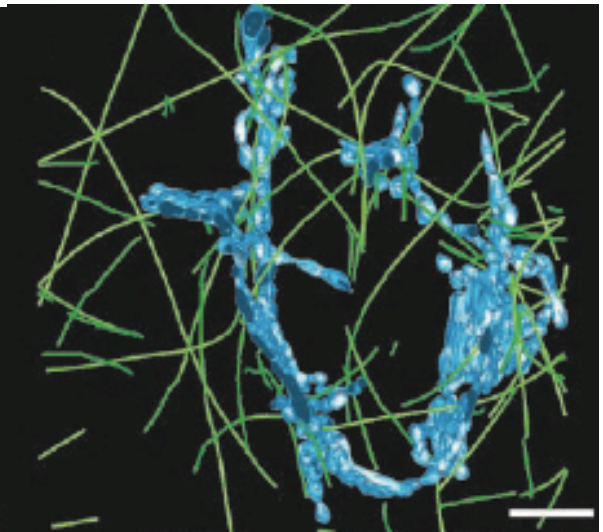
Mechnaosensor



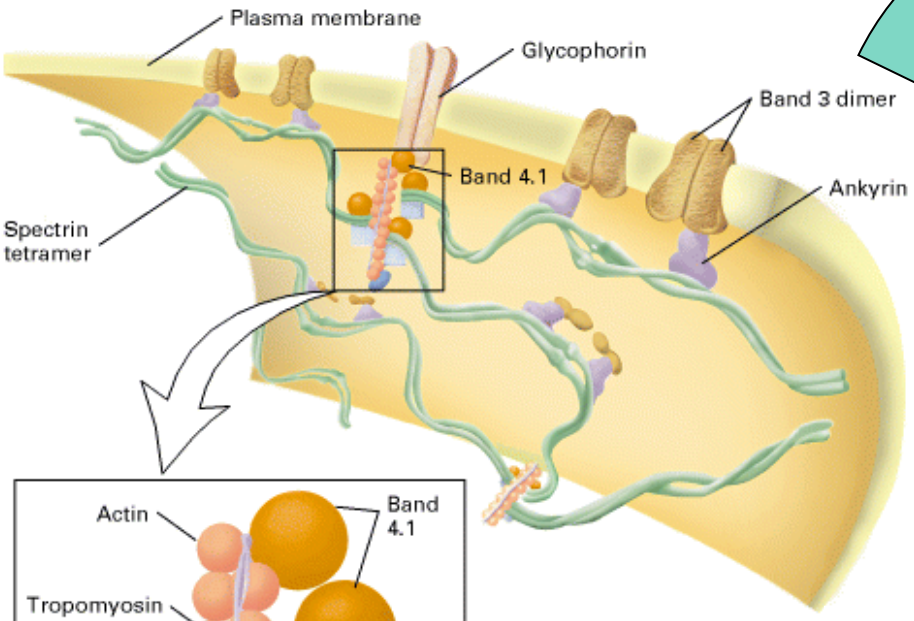
Endoplasmic reticulum

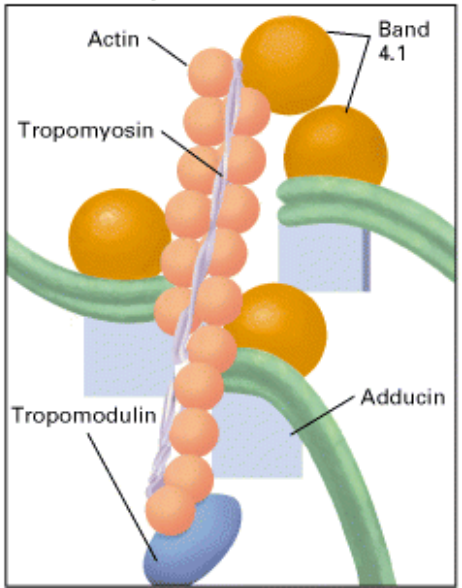


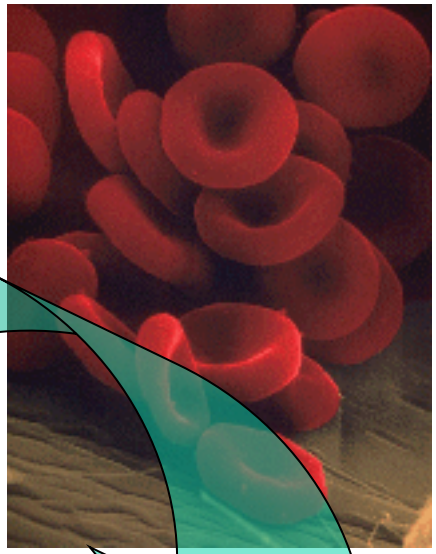
Golgi



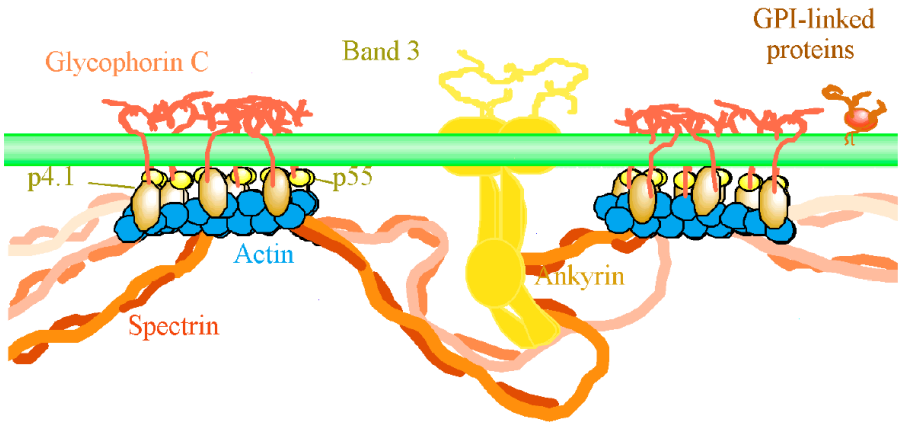
Erythrocyte cytosceleton







Spectrin *is an actin-binding protein that forms an elongated* ***tetrameric*** *complex.*



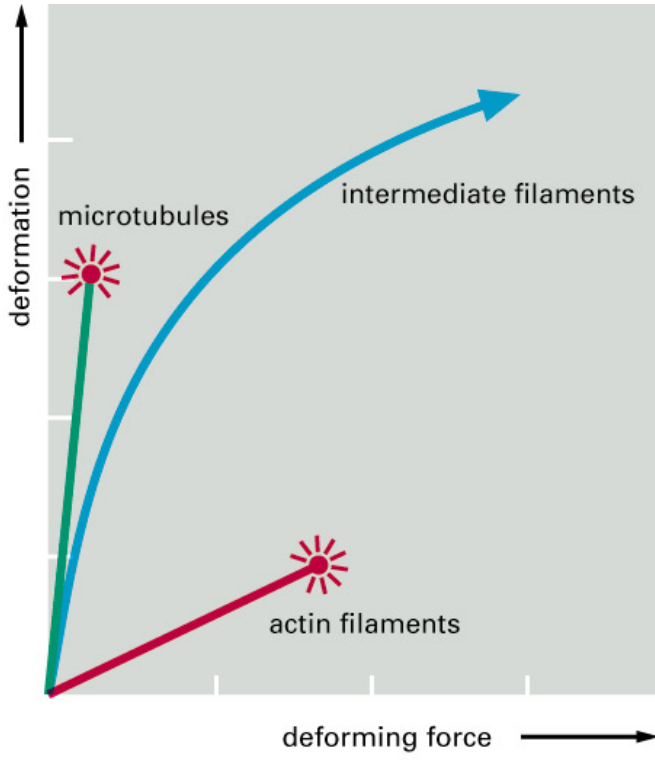
Persistence and entanglement length

For a single filament, the persistence length or the length of thermal flexibility l_p is the length-scale over which thermal bending fluctuations become appreciable and can change the direction of the filament,

$$\mathbf{l_p = \kappa / k_B T}$$

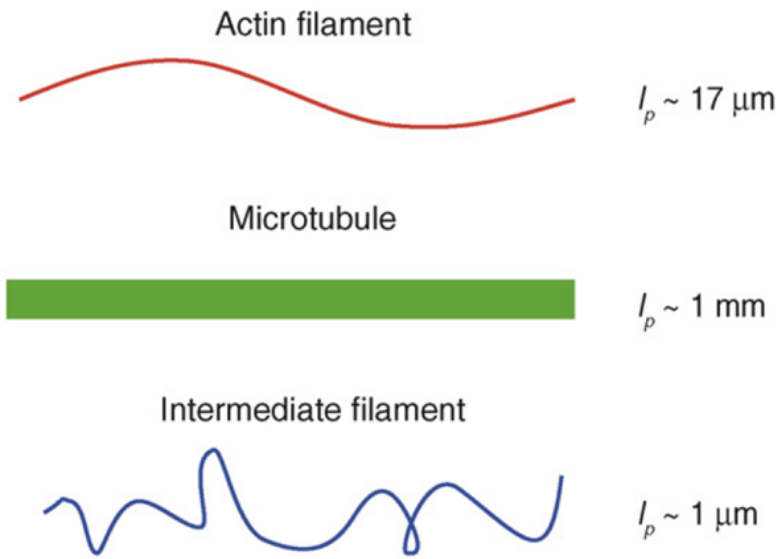
where κ is the bending modulus of a single filament, $k_B T$ is the thermal energy.

For network of filaments the entanglement length l is the confining length-scale over which the motion of each filament is restricted via topological constraints from neighboring filaments.



Comparison of the cytoskeletal networks

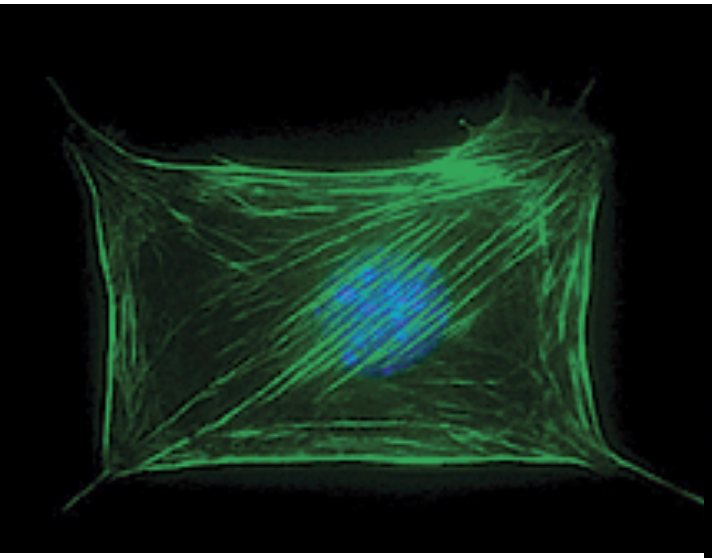
	Microtubules	Actin	I.F.s
Nucleotide	GTP	ATP	none
Polarity	yes	yes	no
Motors	kinesin & dynein	myosins	none
Subunits	Tubulin (globular)	G-actin (globular)	Elongated fibrous proteins
Subunit gain & loss	From ends	From ends	Subunits exchange throughout
Rate of dynamics	Fast	Fast	Slow (except mitosis)
Primary functions	Polarity & transport	Cell shape & transport	Tensile strength

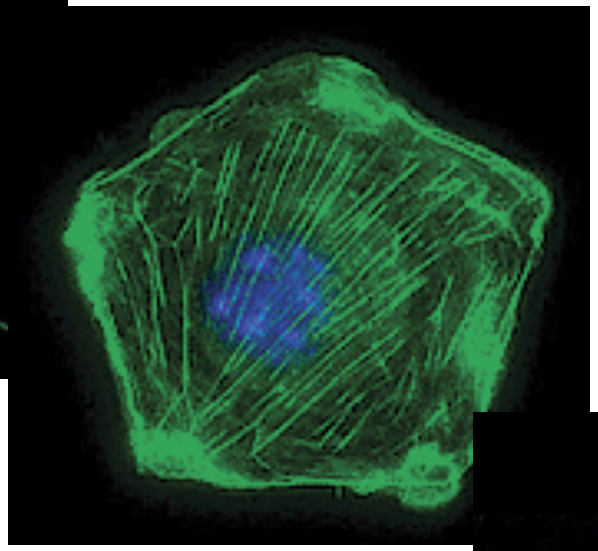


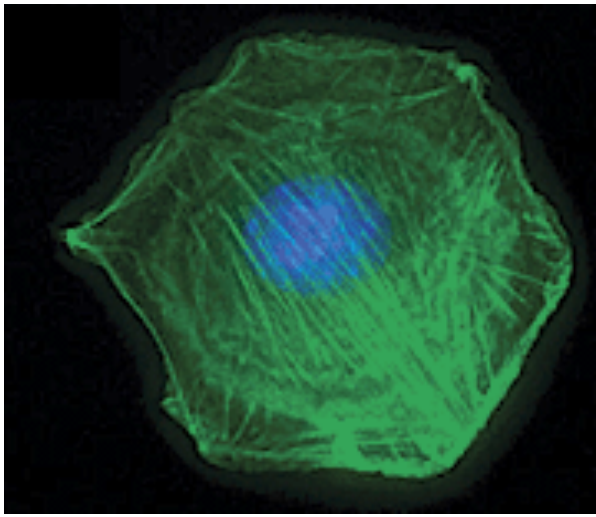
The filaments that constitute the cytoskeleton with respective the persistence length, l_p (the length scale above which these thermally fluctuating filaments appear to be floppy).

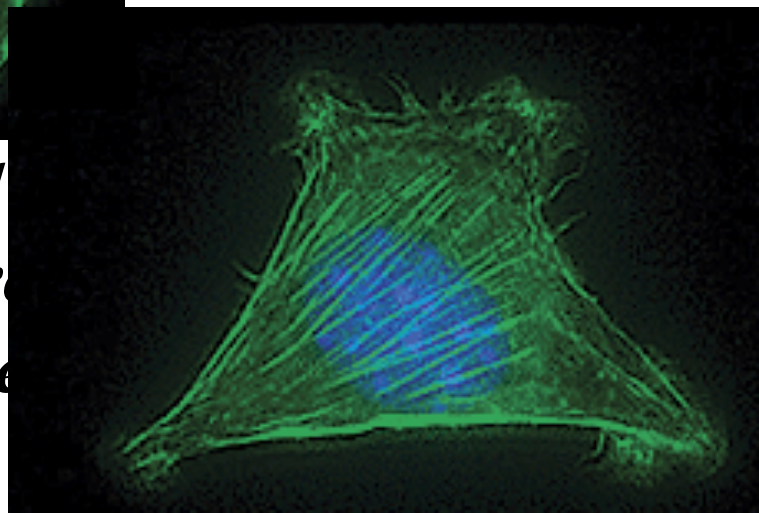
Tension + compression hold the cell together

A tensegrity provides a means to integrate mechanics and biochemistry.



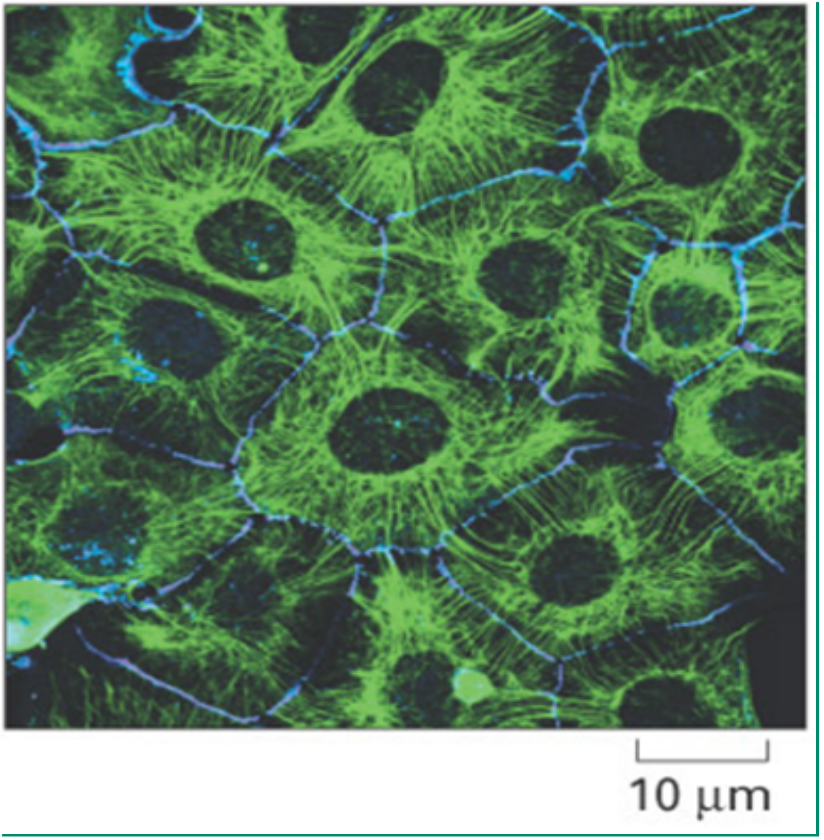


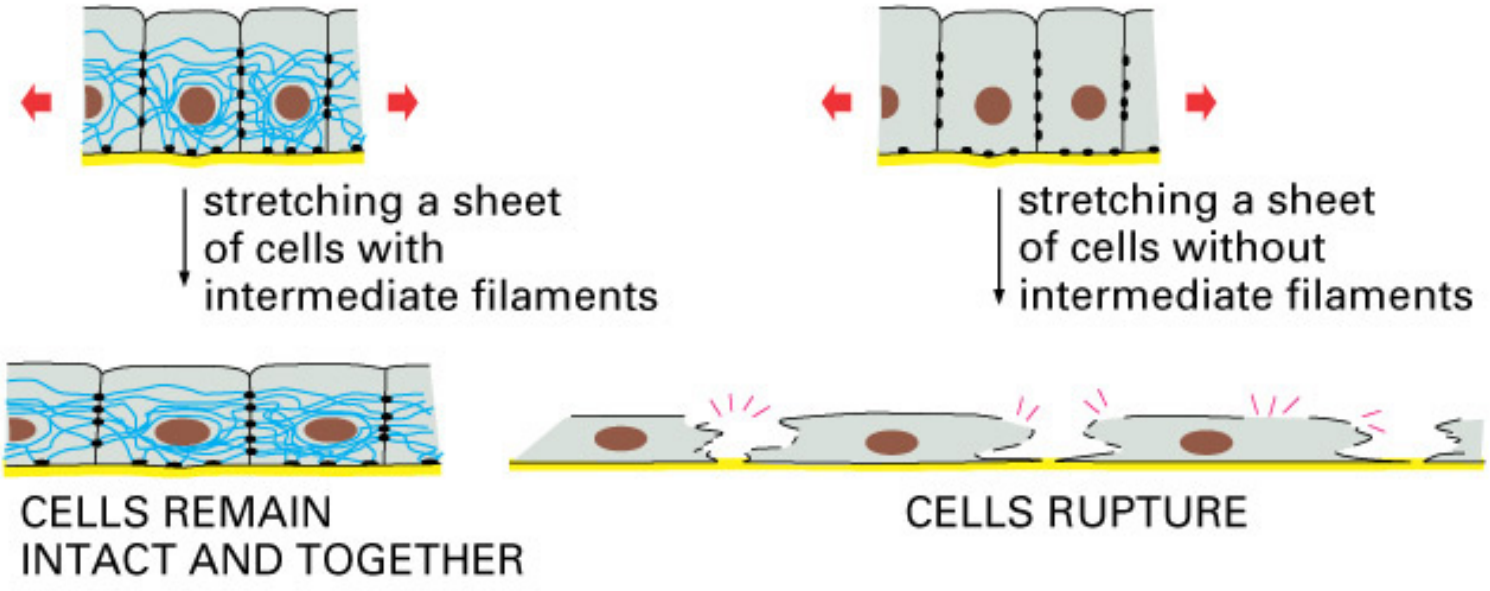




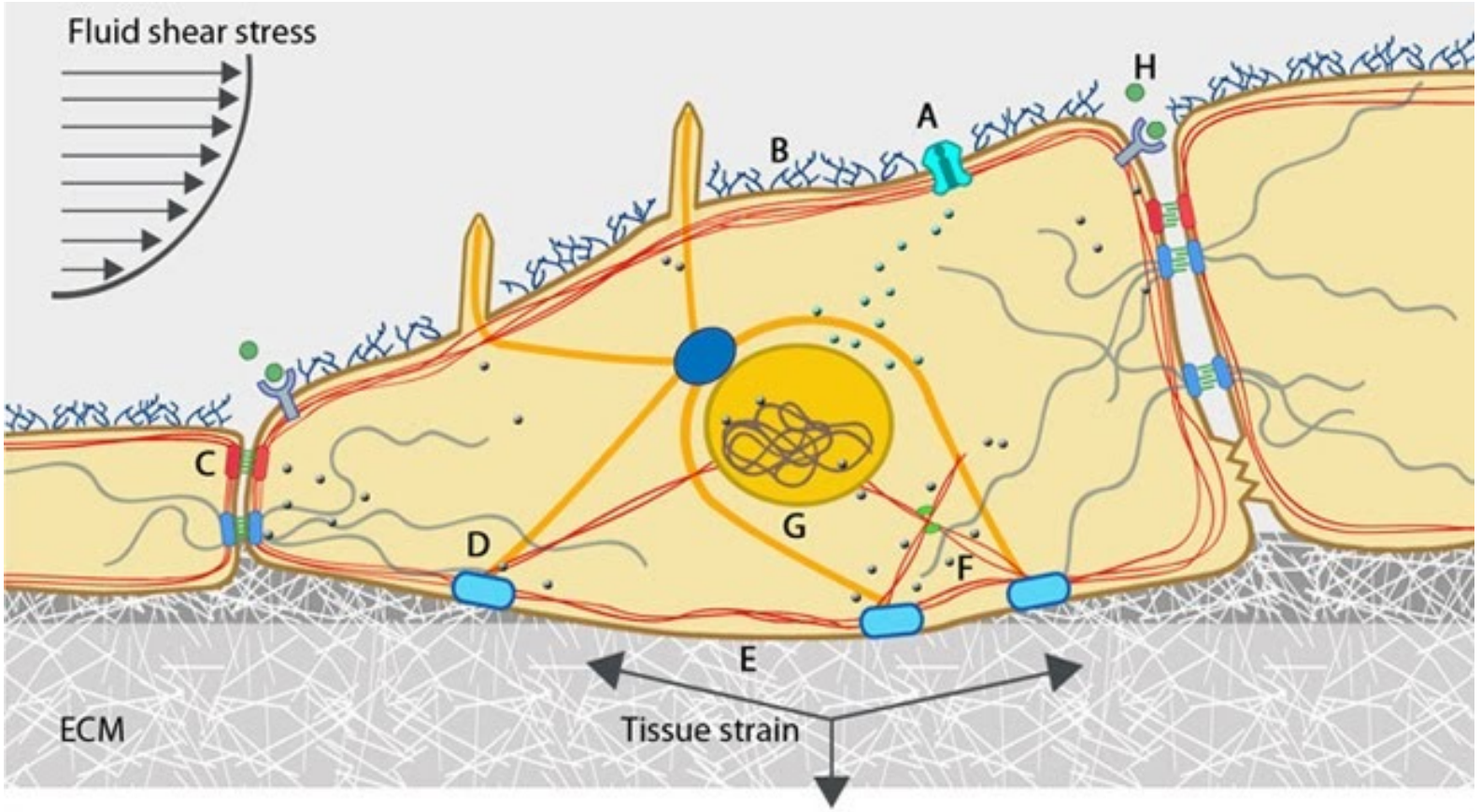
Cell's cytoskeleton can be changed altering the balance of physical for transmitted across the cell surface

Keratins allow sheets of cells to resist mechanical stresses

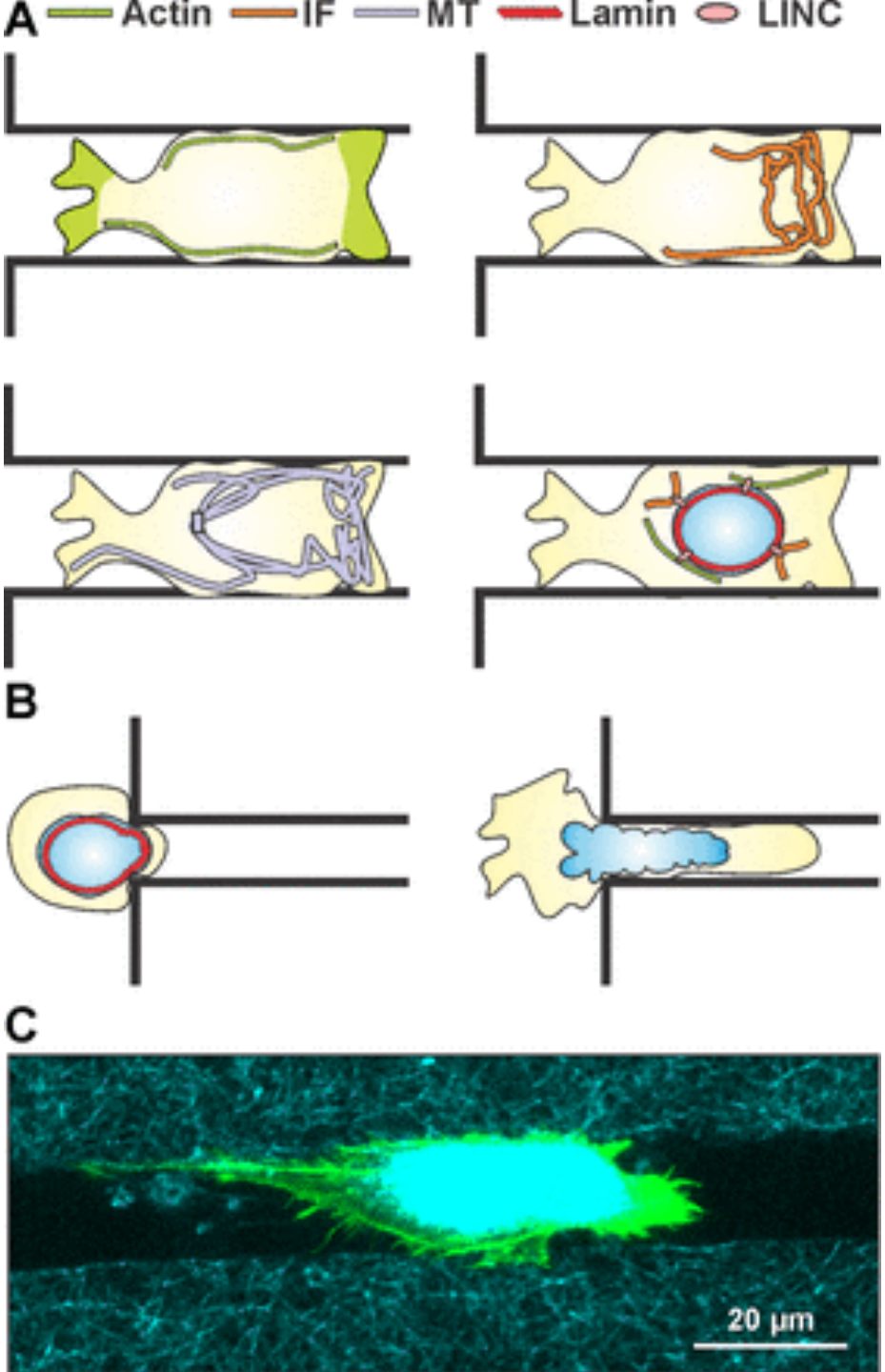




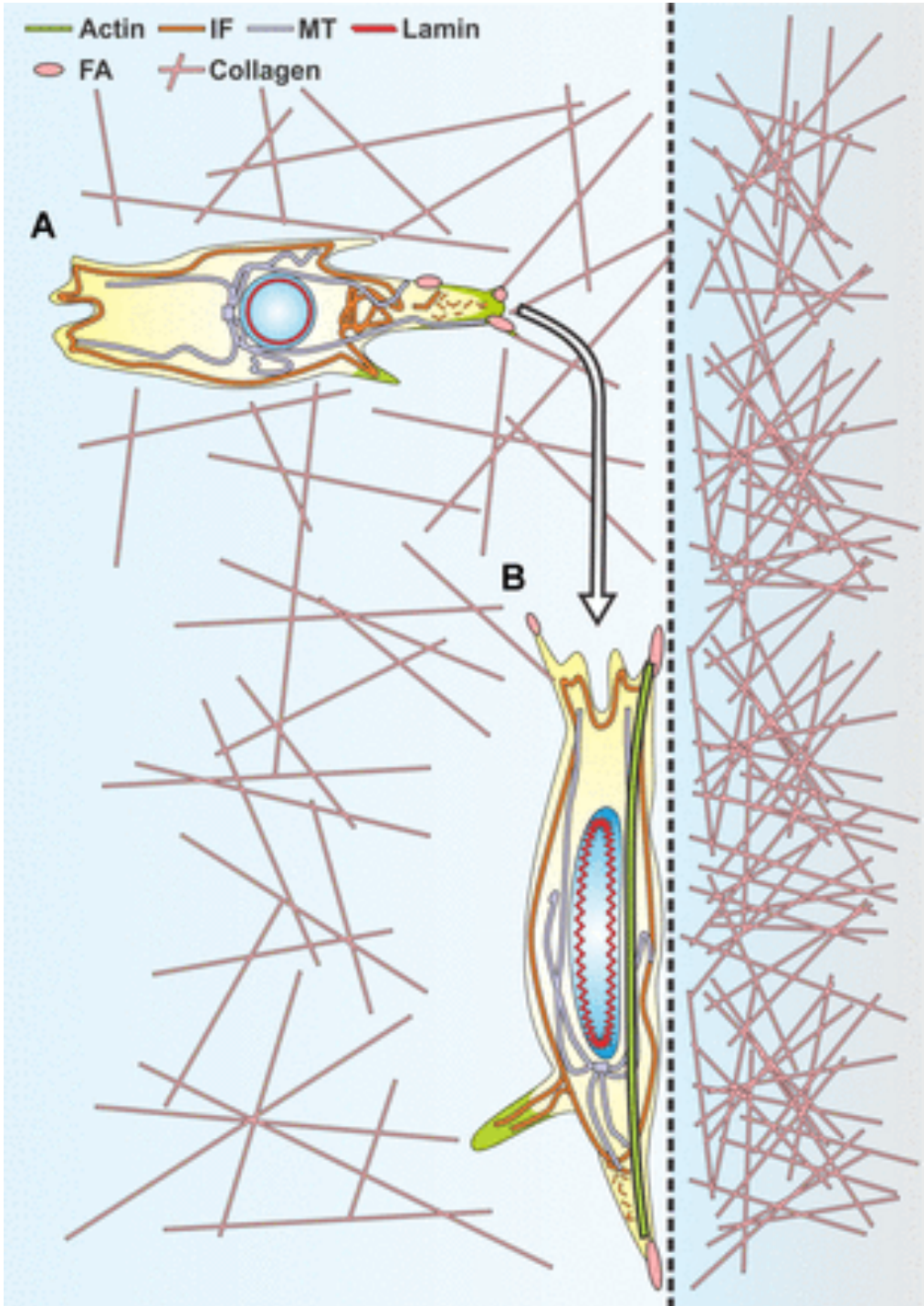
Overview of mechanotransduction in a cell



Review of key mechanical features governing cell migration in restrictive 3-dimensional channels.



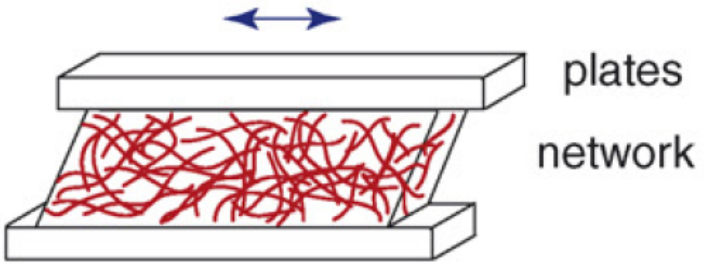
A migrating cell relies on the coordinated effort of the cytoskeleton for its mechanosensing ability in a 3D matrix.



Physical methods to probe mechanical properties of cells.

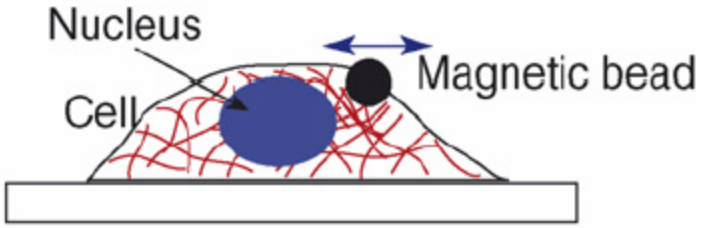
Bulk rheology

A material is sheared between two plates using an oscillatory stress to probe the shear elastic, G', (in-phase) and viscous, G'', (out-of-phase) moduli.



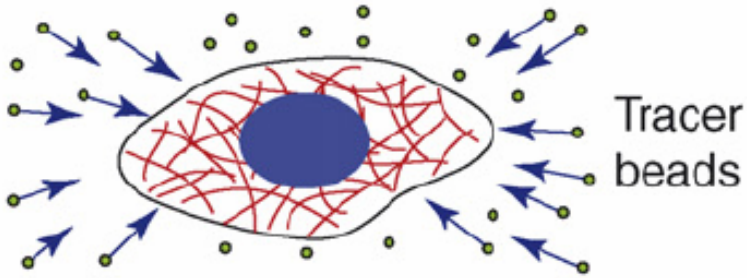
Magnetic bead cytometry

An external magnetic field applies a stress to a magnetic bead. The bead is position tracked to determine the response.



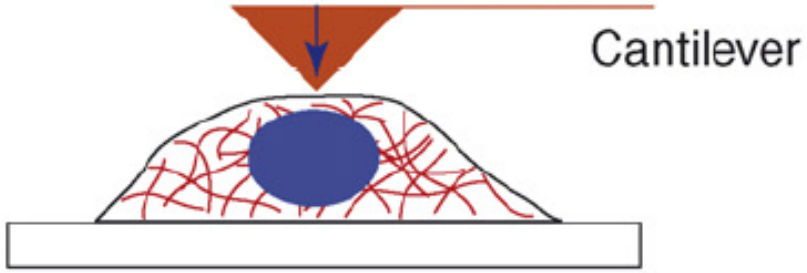
Traction force microscopy

Cell contractions deform a flexible substrate. Forces are estimated from bead displacements.



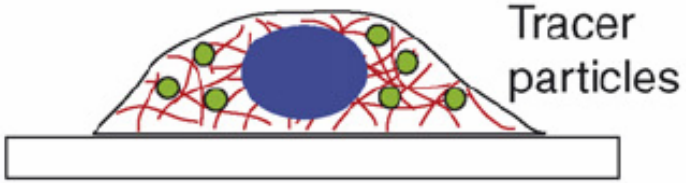
Atomic force microscopy

A cantilever applies stress to the cell. The cantilever deflection is measured by laser reflection.



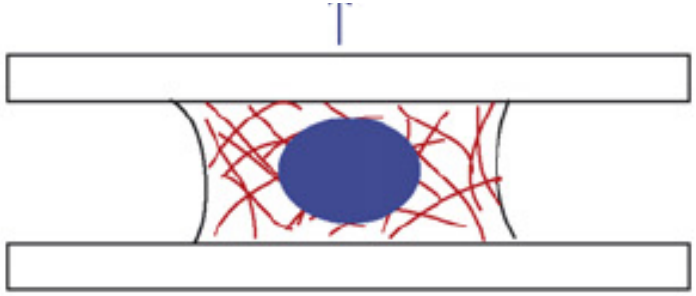
Microrheology

The motion of probe particles is measured using either video or laser tracking techniques. Particle motion is either driven externally or thermally induced and is interpreted to yield the viscoelastic modulus.

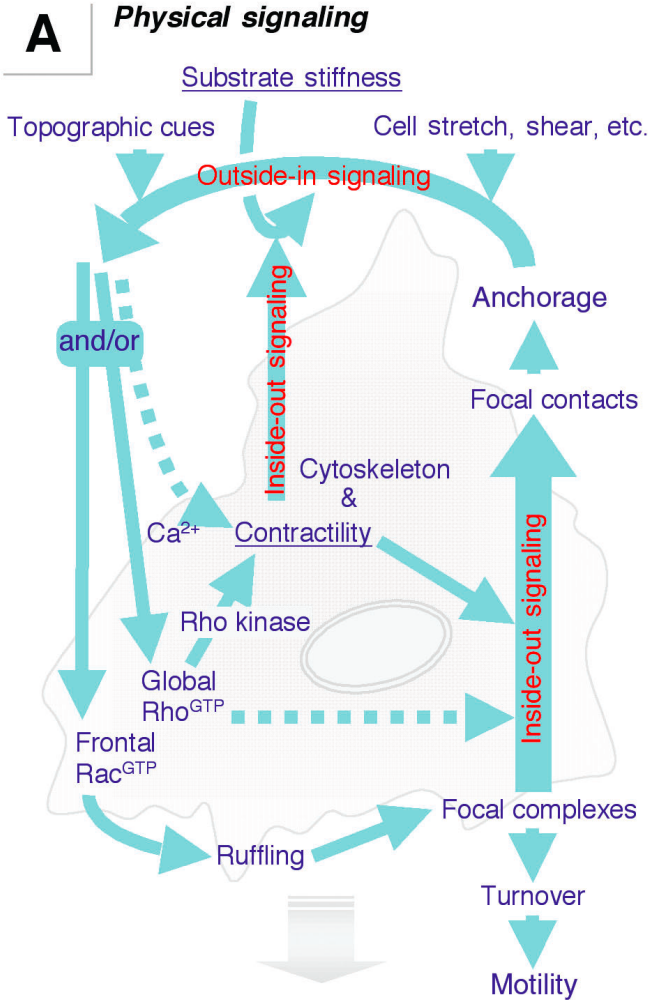


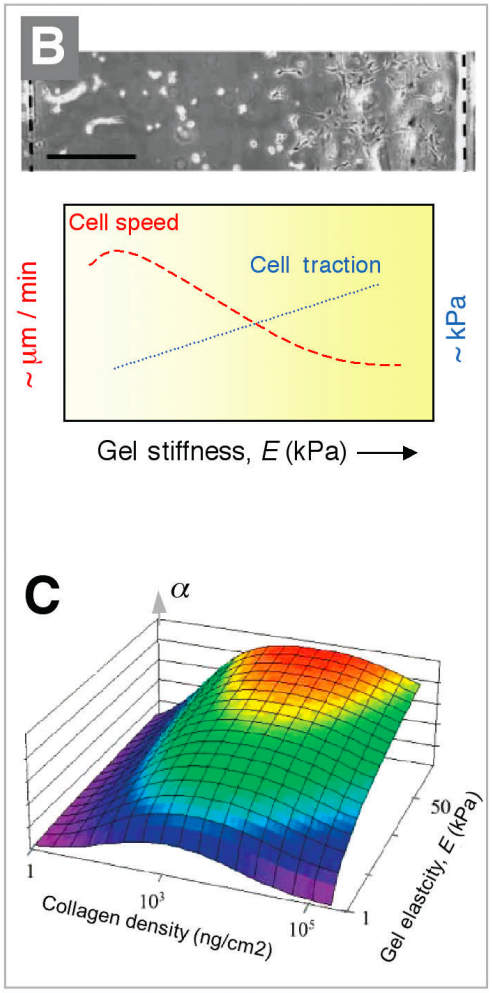
Whole cell stretching

A cell is attached to two surfaces. A force is applied to one surface and the plate separation is measured.



Substrate stiffness influences contractility, motility, and spreading.

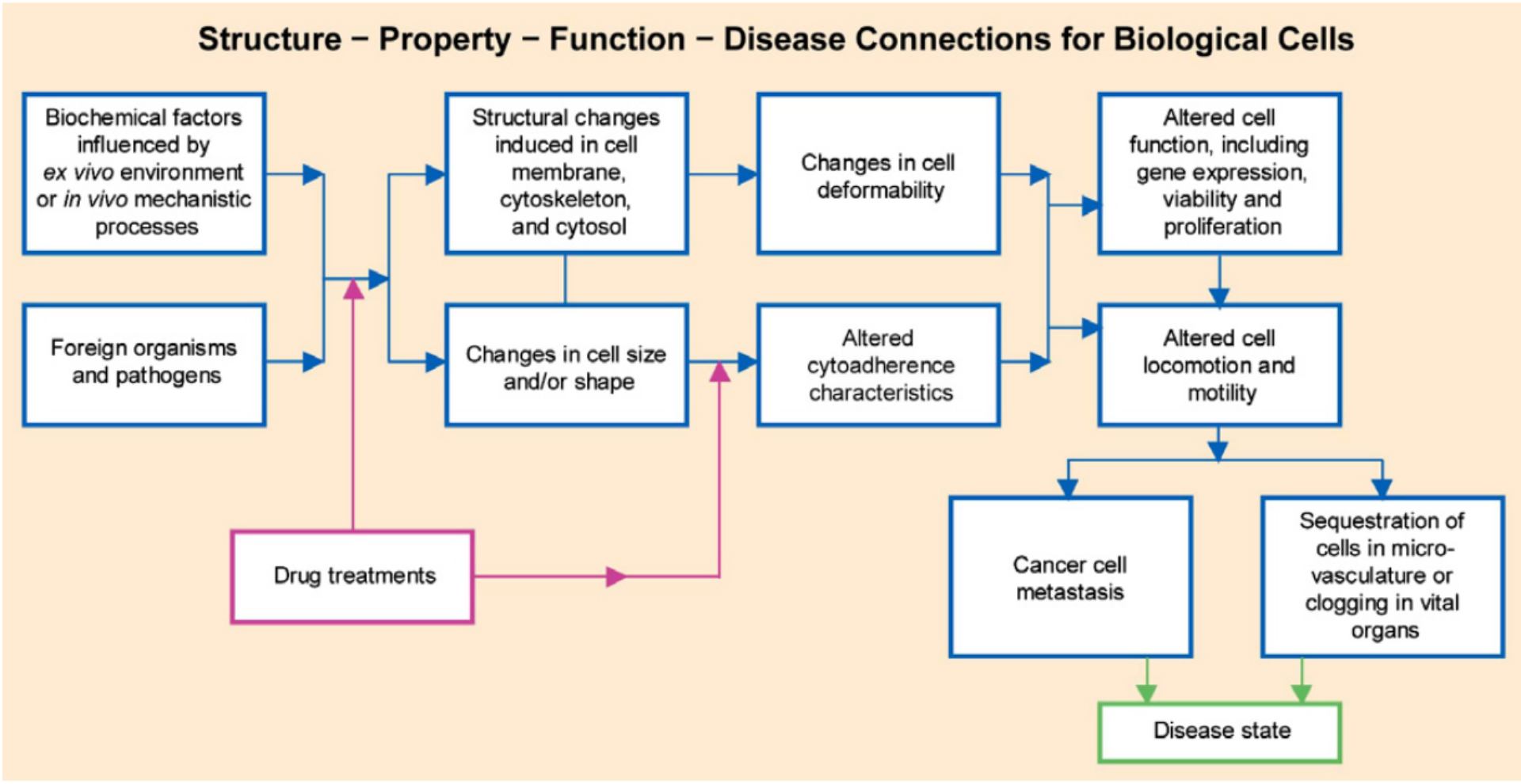




(A) Interplay of physical and biochemical signals in the feedback of matrix stiffness on contractility and cell signaling.

(B) Cells exert less tension on softer, collagen-coated gels but crawl faster, causing an accumulation of cells toward the stiff end of a soft-to-stiff gradient gel.
(C) Spread area, α, of smooth muscle cell versus ligand density and matrix stiffness, based on measurements fitted by a thermodynamic model. Similar nonlinear responses are also seen for adhesions, cytoskeleton organization, tractions exerted on the substrate, and other cellular processes.

Chemo-biomechanical pathways influencing connections among subcellular structure, cell biomechanics, motility and disease state.



The macroscopic level → ***Skeleton***

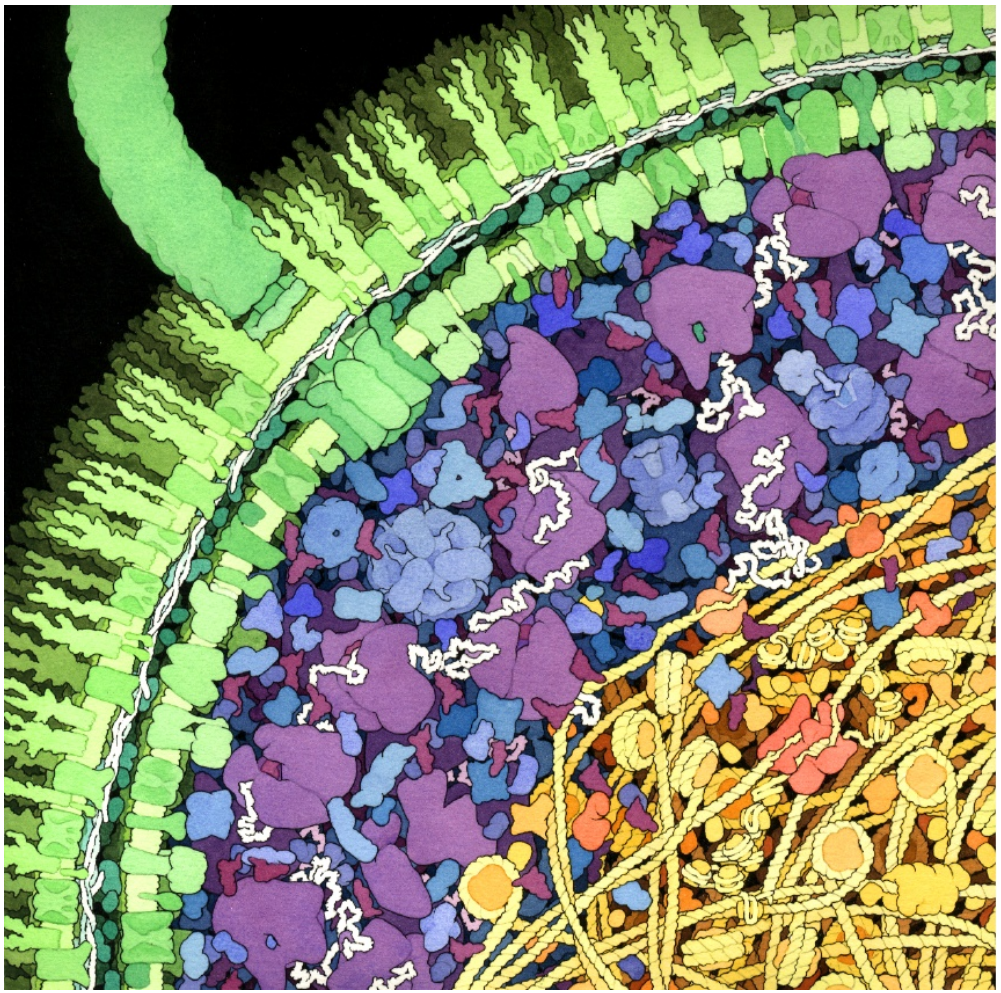
The 206 bones of skeleton are pulled up against the force of gravity and stabilized in a vertical form by the tensile muscles, tendons and ligaments – ***Snelson's sculptures***.

A "minimal" organism

"We are wondering if we can come up with a molecular definition of life"

"The goal is to fundamentally understand the components of the most basic living cell"

Craig Venter, founder of Celera Genomics, IBEA and several other gene tech companies



E. coli membrane region

Hutchinson et al. Science 286, 1999, 2165