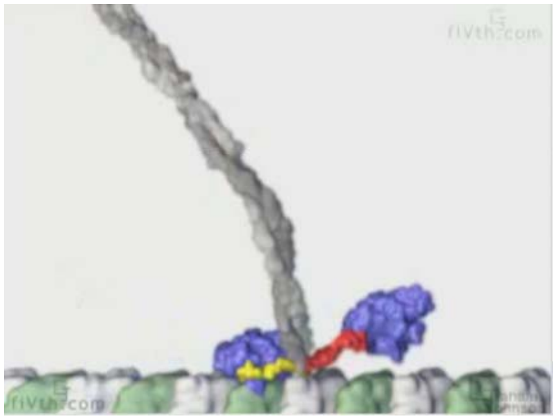
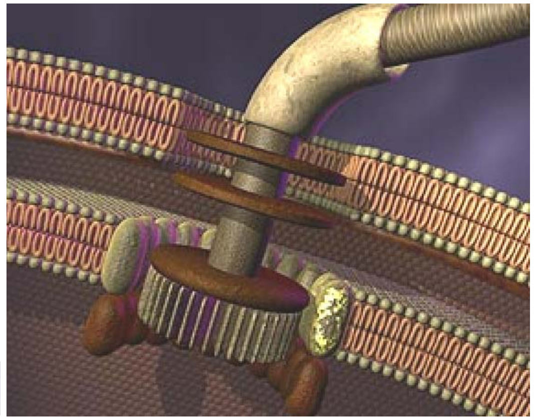
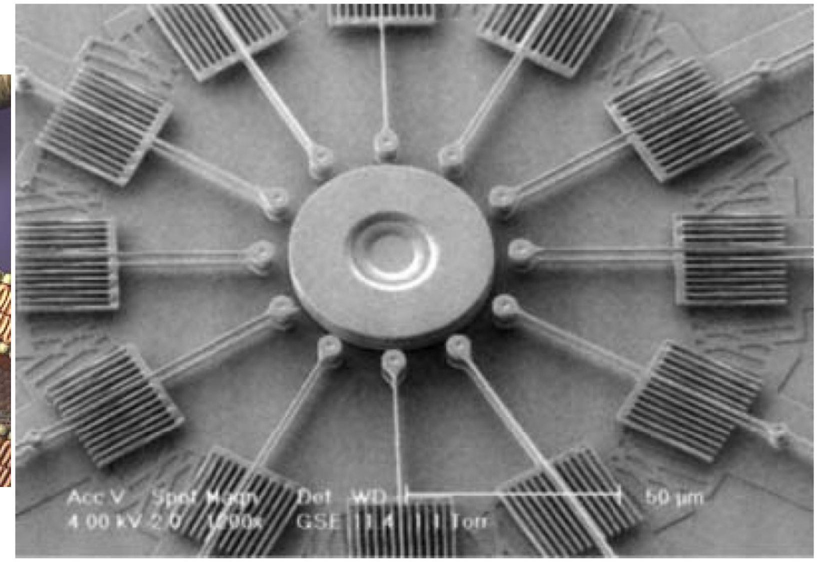
Biological Machines, Cell Mechanics and Nanotechnology







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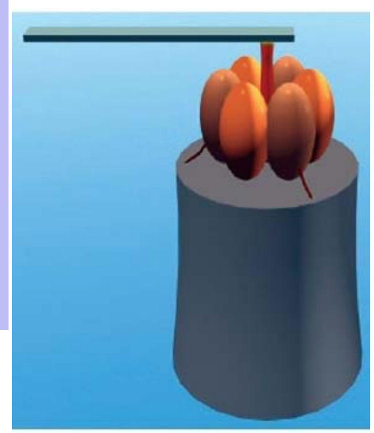
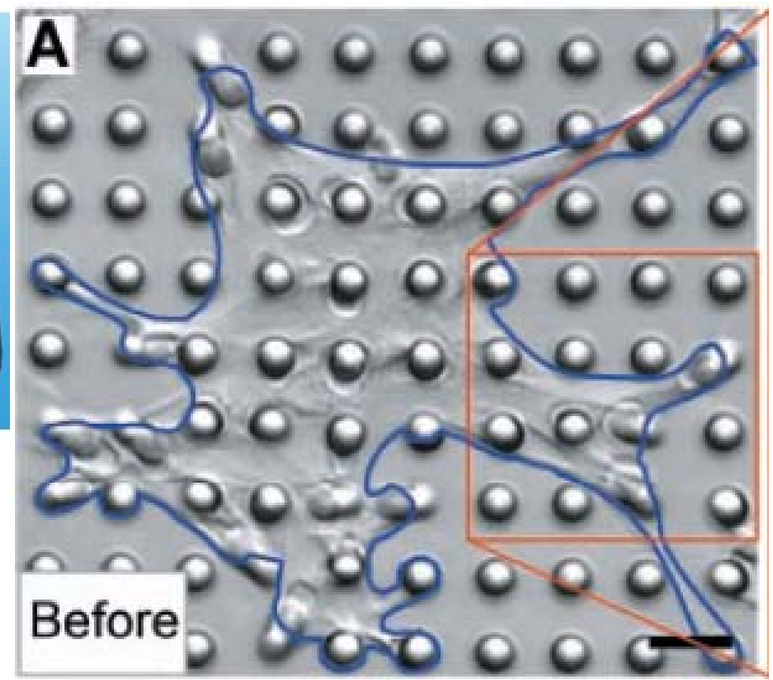
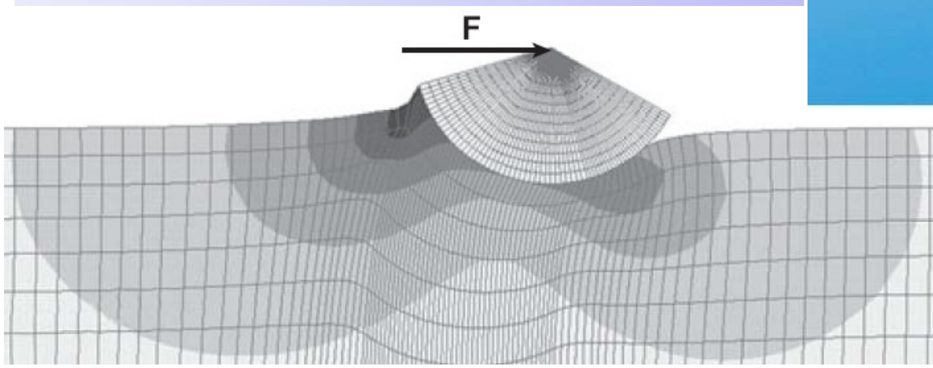






Remaining course overview

4/13	Kinesins, their mechanical properties and MEMS	王歐力
4/20	Myosins, Dynein and an the problems of trafficking	王歐力
4/27	**Midterm Exam** => **only Dr. Perng Ming-Der's Part**	彭明德
5/04	Biological and non-biological nanomachines	王歐力
5/11	Cell mechanics I	王歐力
5/18	Diffusion, friction and entropic forces acting on molecular motors Part I	吳見明
5/25	Diffusion, friction and entropic forces acting on molecular motors Part II	吳見明
6/01	Cell mechanics II	王歐力
6/08	Journal club 1: 張妍, 謝榕, 黃彣軒, 李晳哲	王歐力
6/15	Journal club 2: 蘇子翔, 謝鎔澤, 林淑娟, 陳莉菁	王歐力

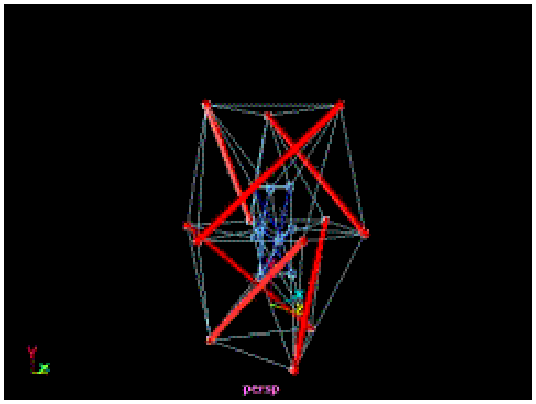
Evaluation:
Presence 25%, Class Performance 40%, Journal Club 35%

Journal Club:
- Pick an article from a journal with IF >5 about **molecular motors** or **cell mechanics**
- Presentation time 20 min. + 10 min discussion (total 2 hours for 4 students)

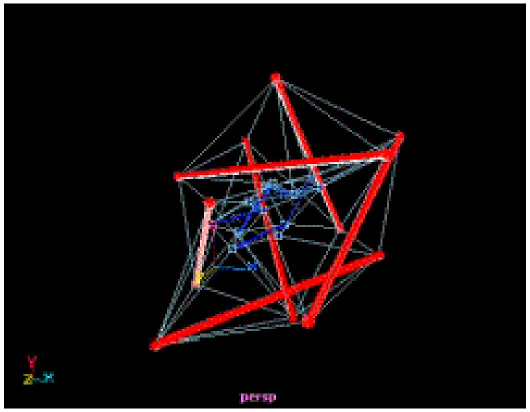
Computer model of cellular tensegrity

Tensegrity is the structural interplay between compression elements (microtubules) and tension elements (actin filaments)

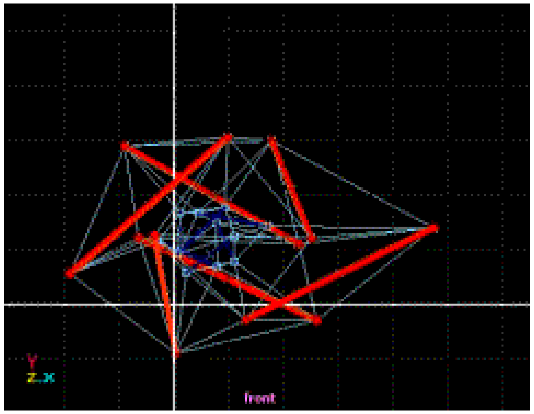
Pull



Shear

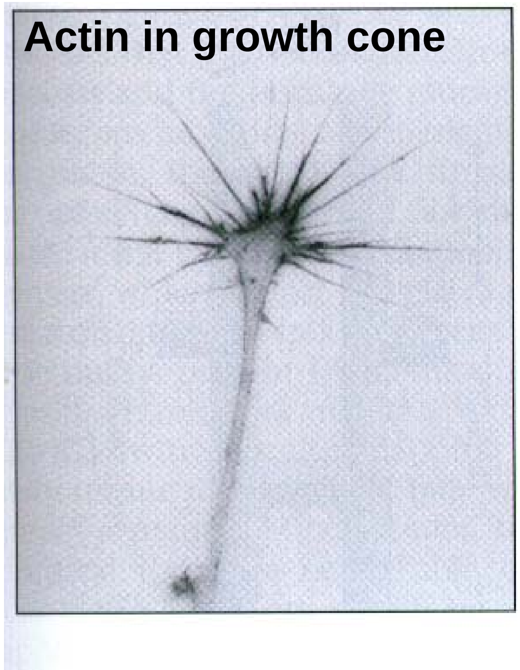


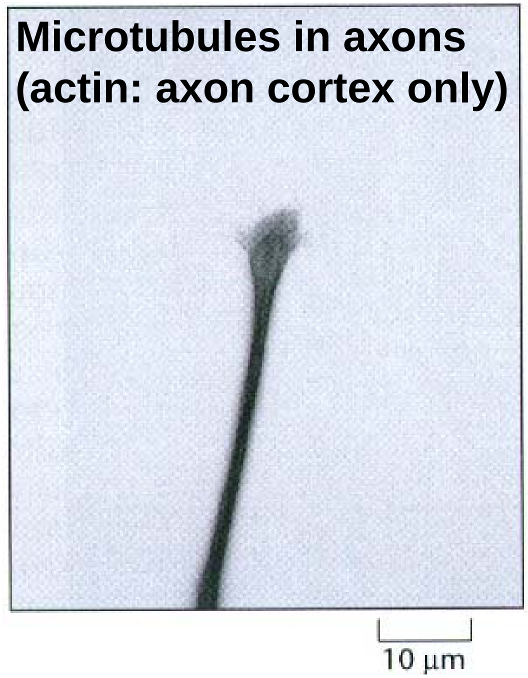
Stretch



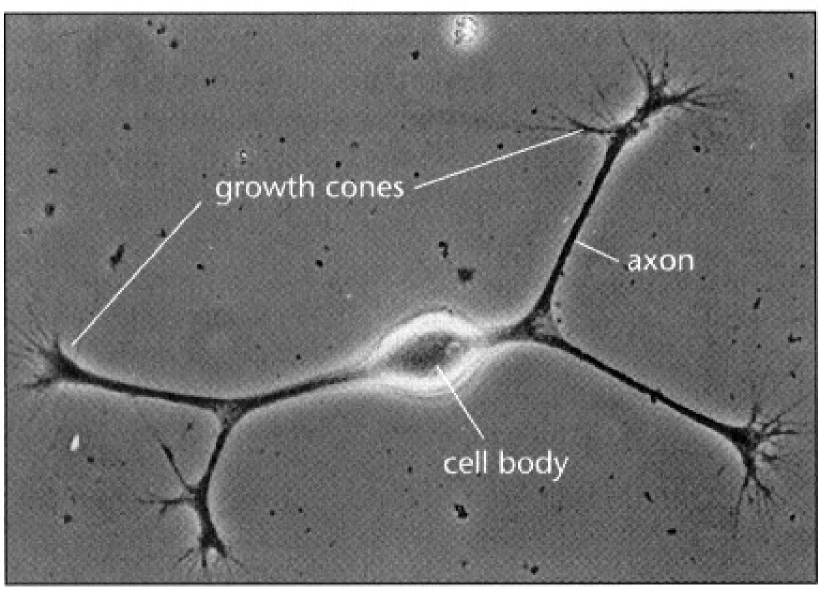
Computer model shows how hierarchical tensegrity structures, such as a cell with a nucleus, behave when pulled, sheared and stretched

Axonal tensegrity: Mechanical properties of neurons

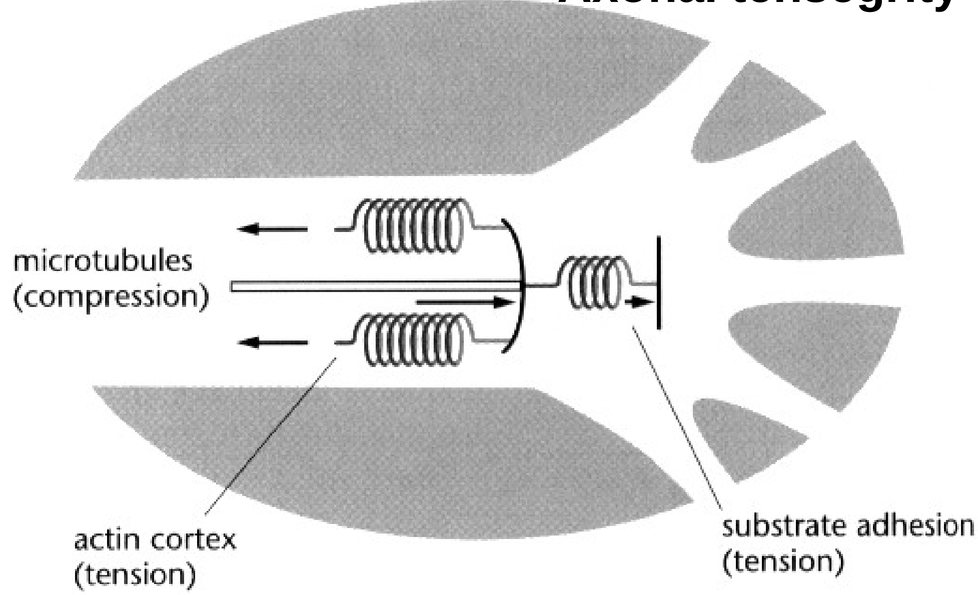




Abrupt axon retraction observed after nocodazole treatment (MT depolymerization):
=> **Mechanical balance** of an axon is provided by creating a **tension** of **actin** along the cortex (as well as substrate adhesion) and antagonistic **compression** forces provided by **microtubules**

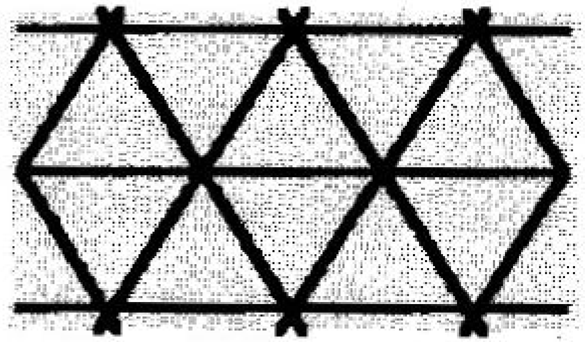


Axonal tensegrity

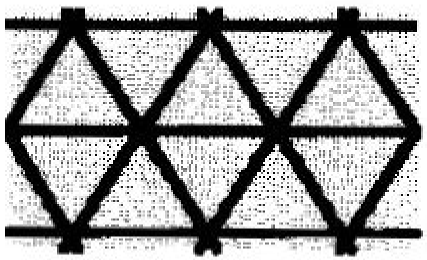


Difference between shear stress and compression

undeformed

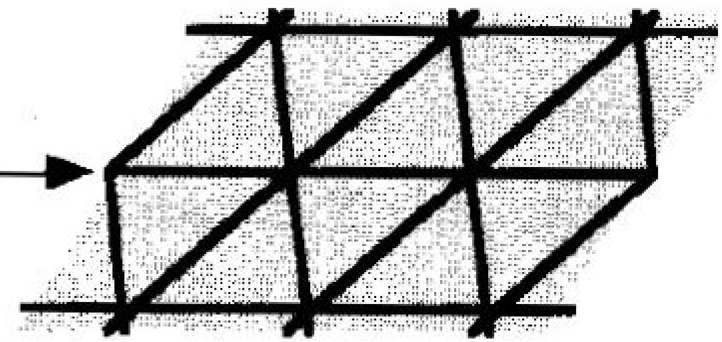


compressed



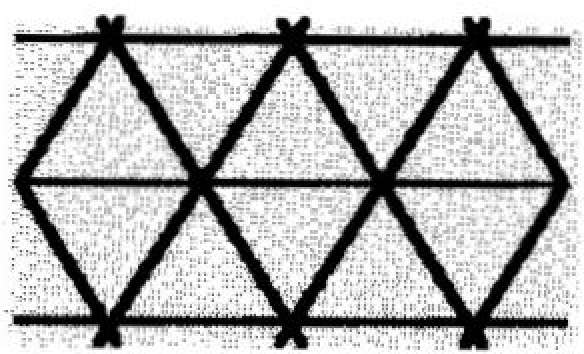
network area changed but <u>no changes in internal angles</u>

sheared

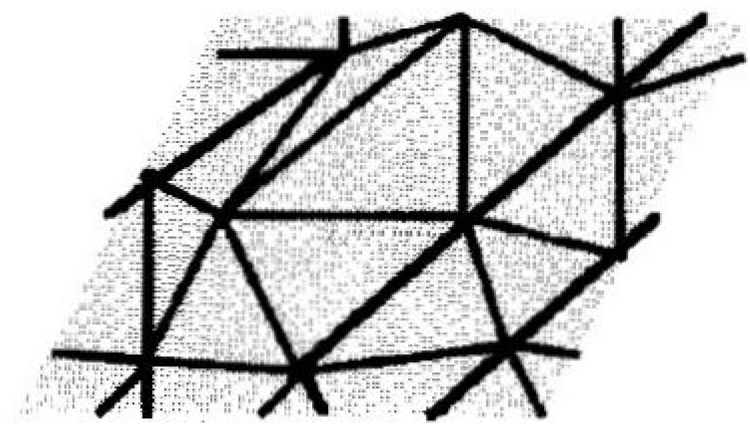


<u>internal network angles changes</u> but **area unchanged**

Effect of **thermal fluctuations**



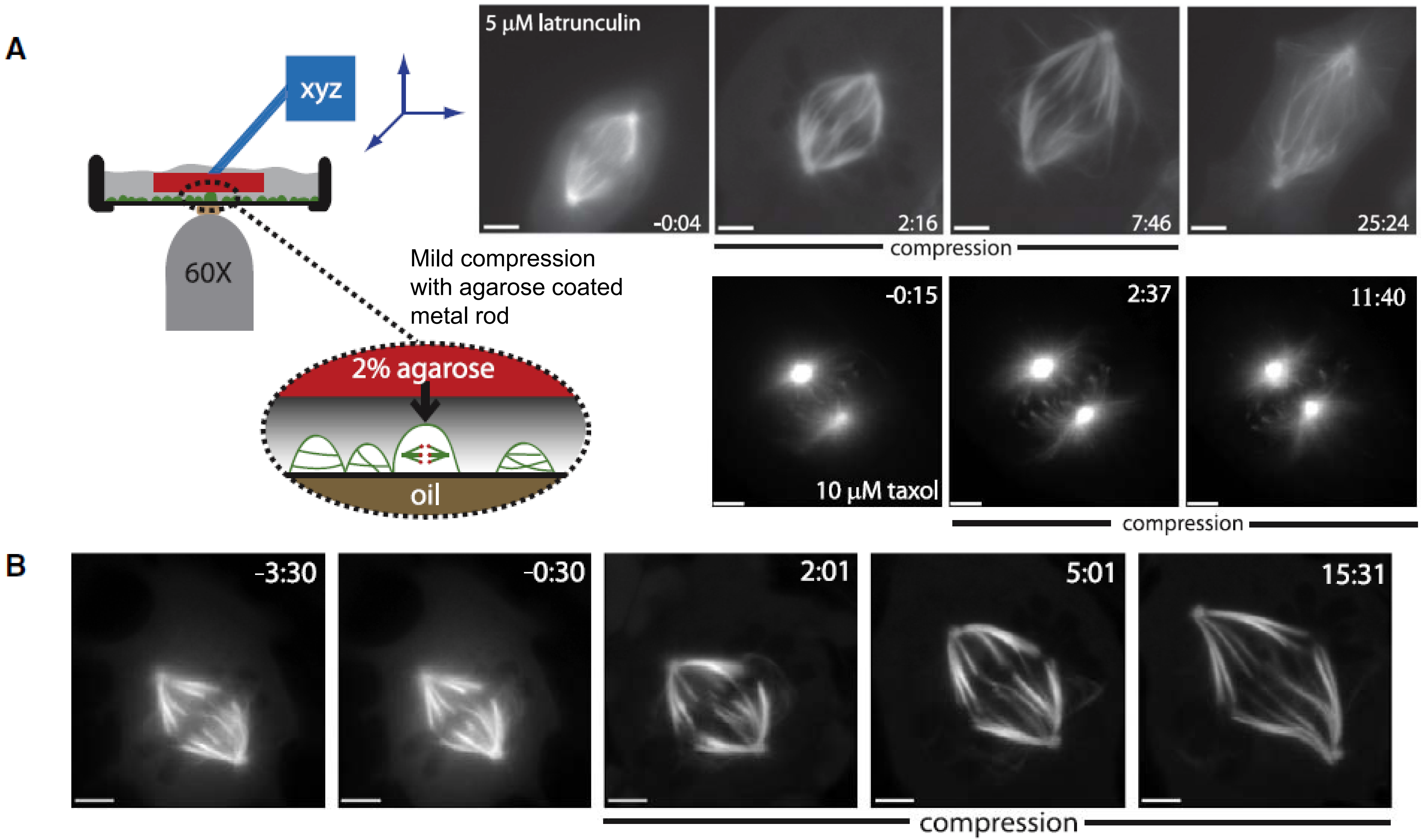
Zero-temperature network



Network becomes more erratic similar after applying a two-dimensional stress

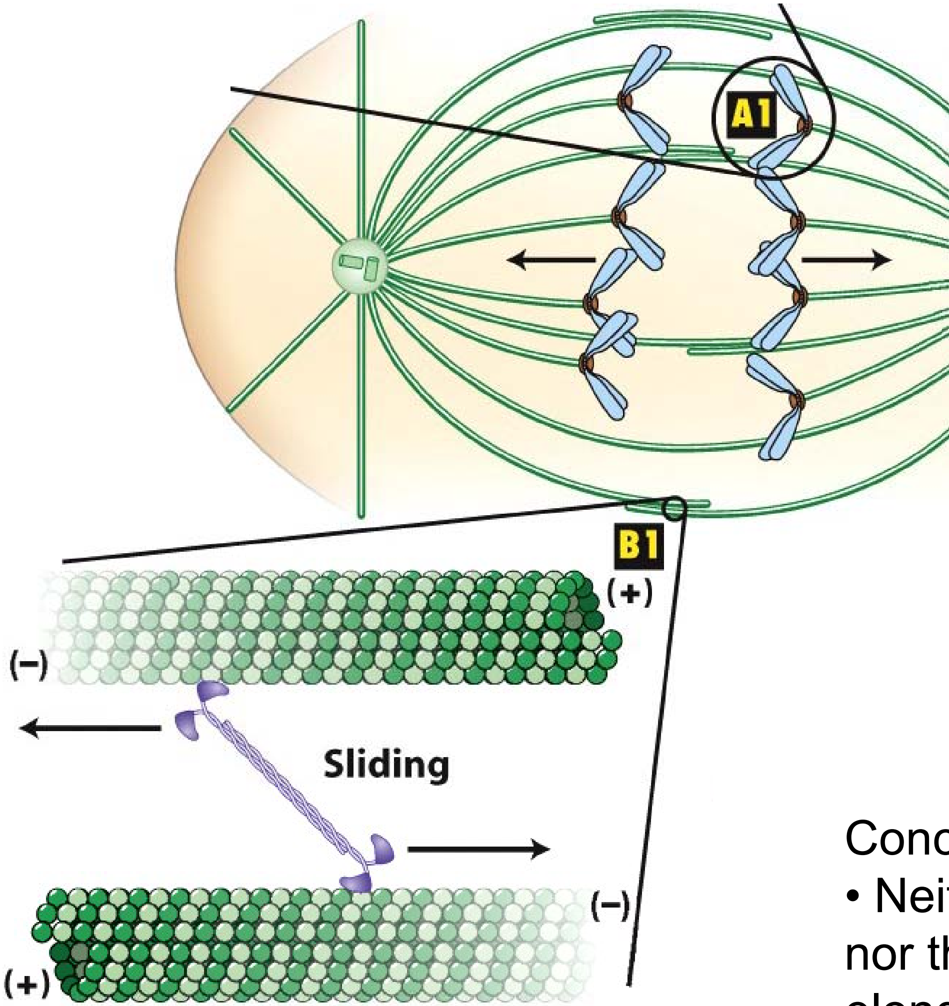
David Boal, Mechanics of the Cell, 1st Ed.

Cell compression induces reversible spindle widening and elongation



Spindle elongation is not affected by actions of actin network, but by MT polymerization

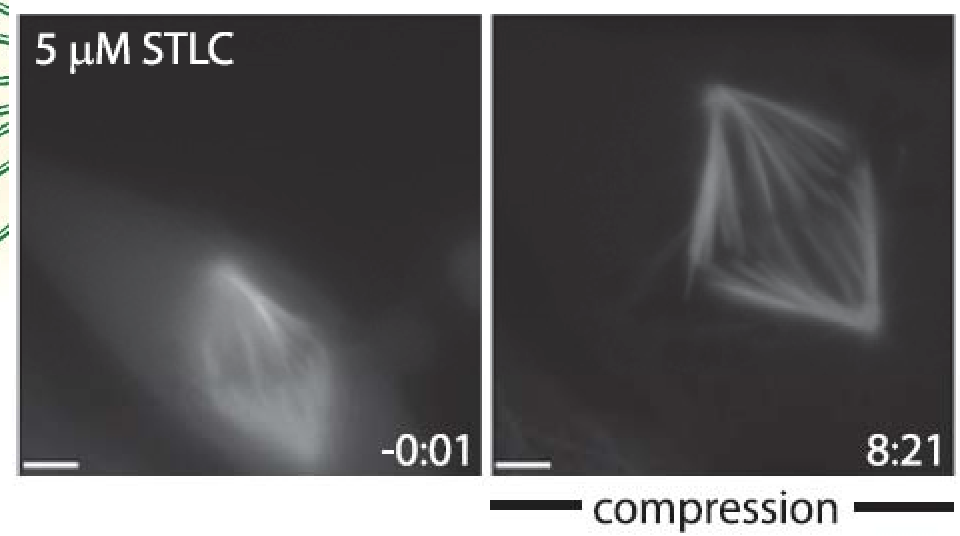
Dumont et al., 2009, Curr. Biol.



Bipolar kinesin 5

The drug STLC inhibits kinesin 5

=> Also here no effect on spindle elongation



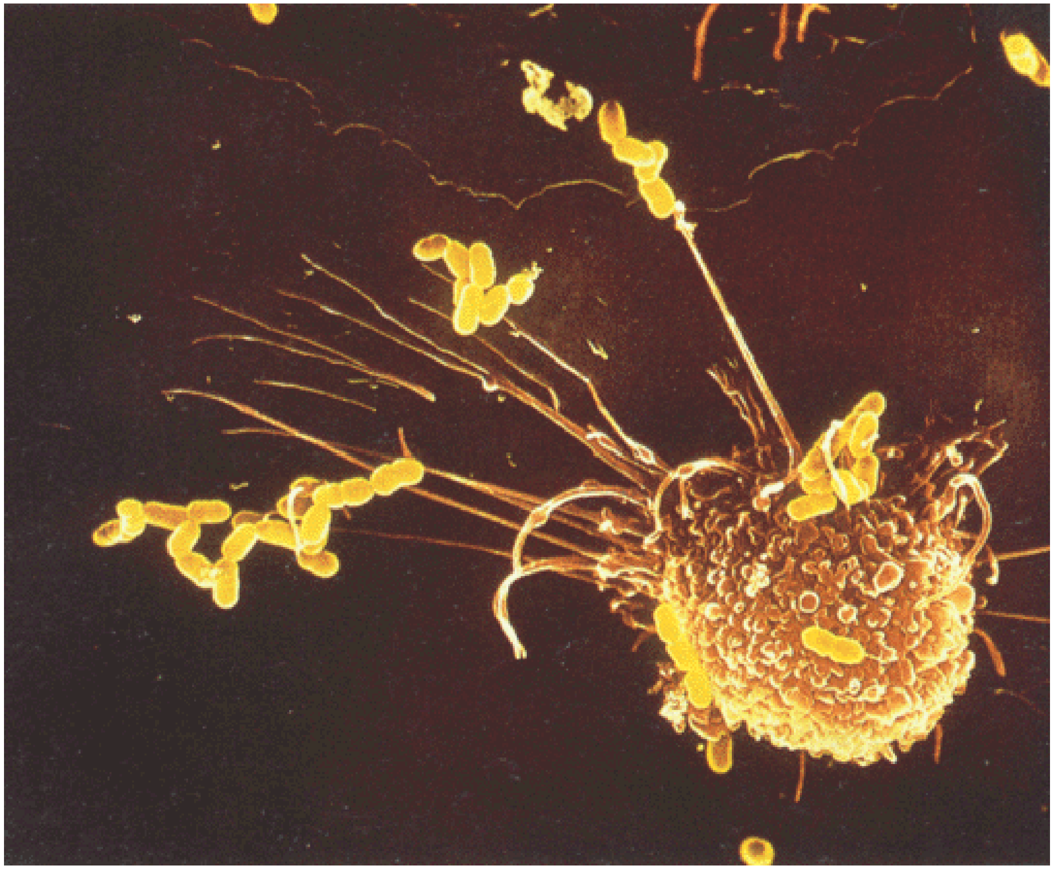
Conclusion:

• Neither the action of the actin network nor that of kinesin 5 affects the spindle elongation upon compression.

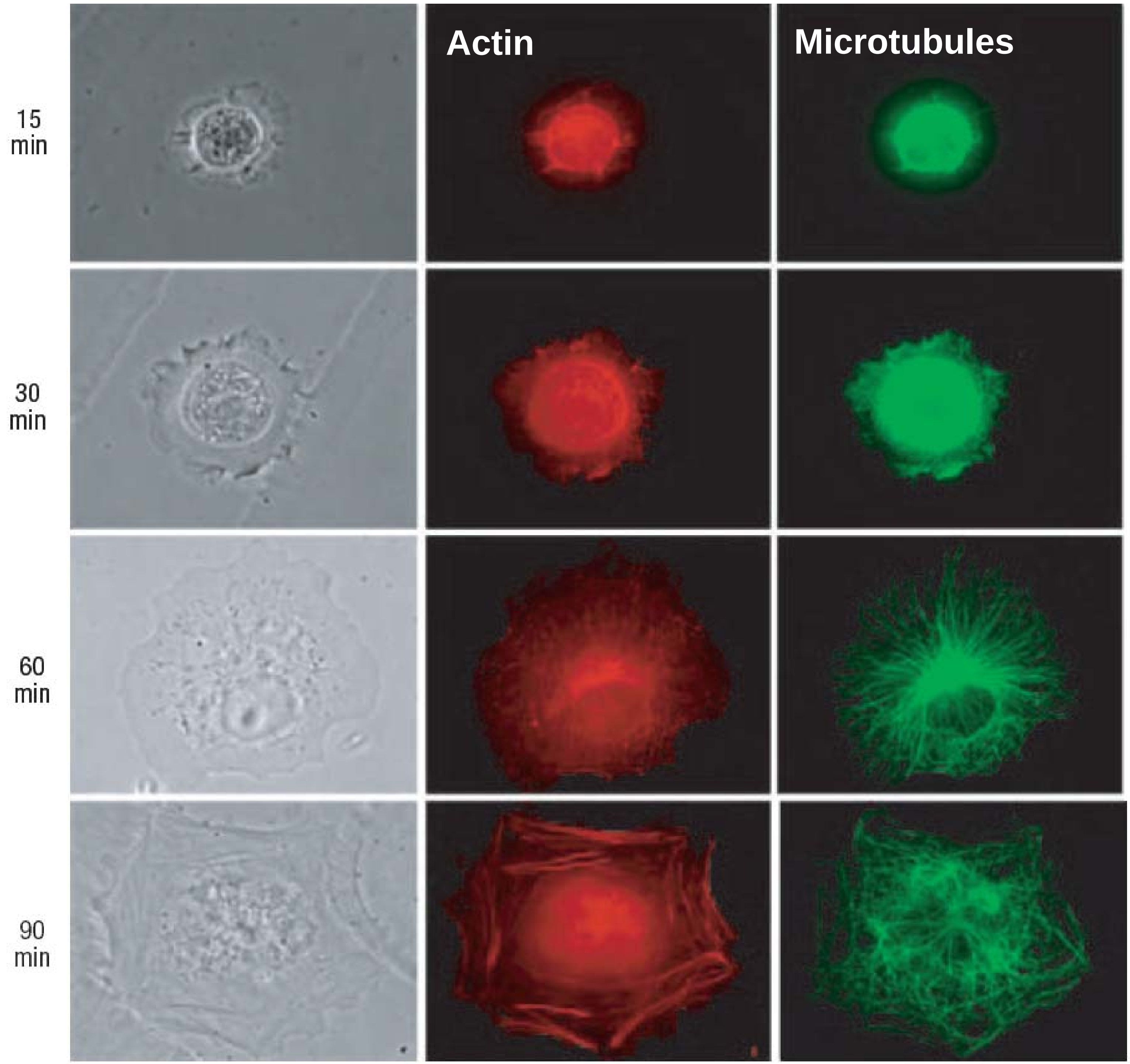
• It is assumed that a <u>mechanochemical switch at the poles regulates the depolymerization rate of kinetochore MTs</u>.

Dumont et al., 2009, Curr. Biol.

Importance of cytoskeleton and cytomechanics in environmental cell responses



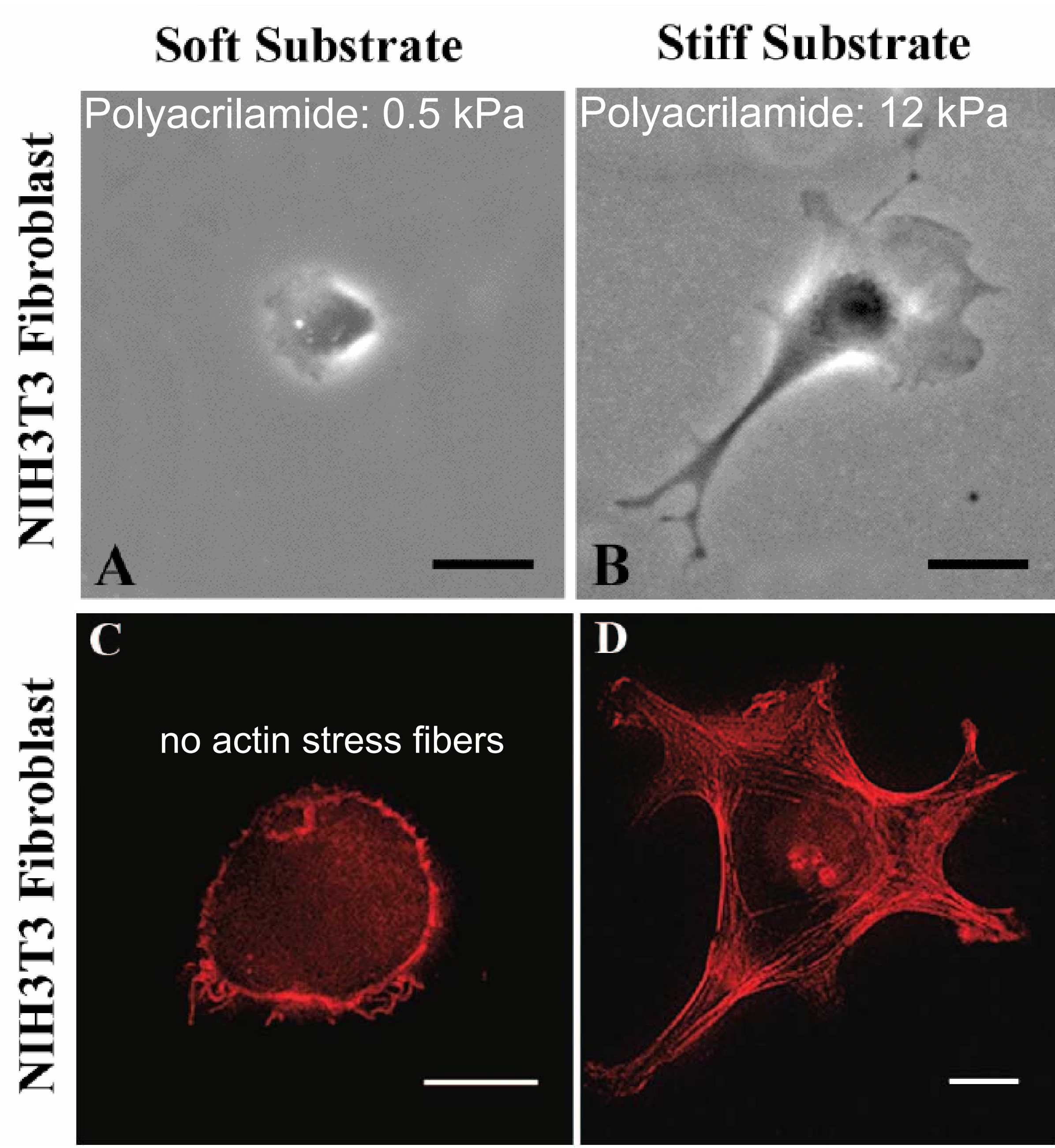
Filopodia (made of thick actin bundles) of white blood cells catching bacteria for lysosomal digestion



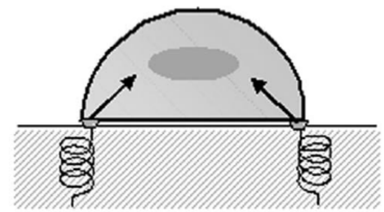
Cytoskeletal response to cell spreading

Stress fiber development upon spreading of a fibroblast on glass

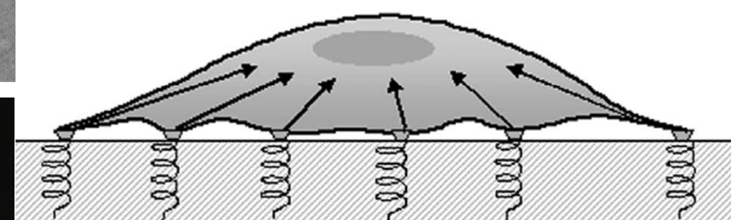
Cellular response to substrate stiffness



Model



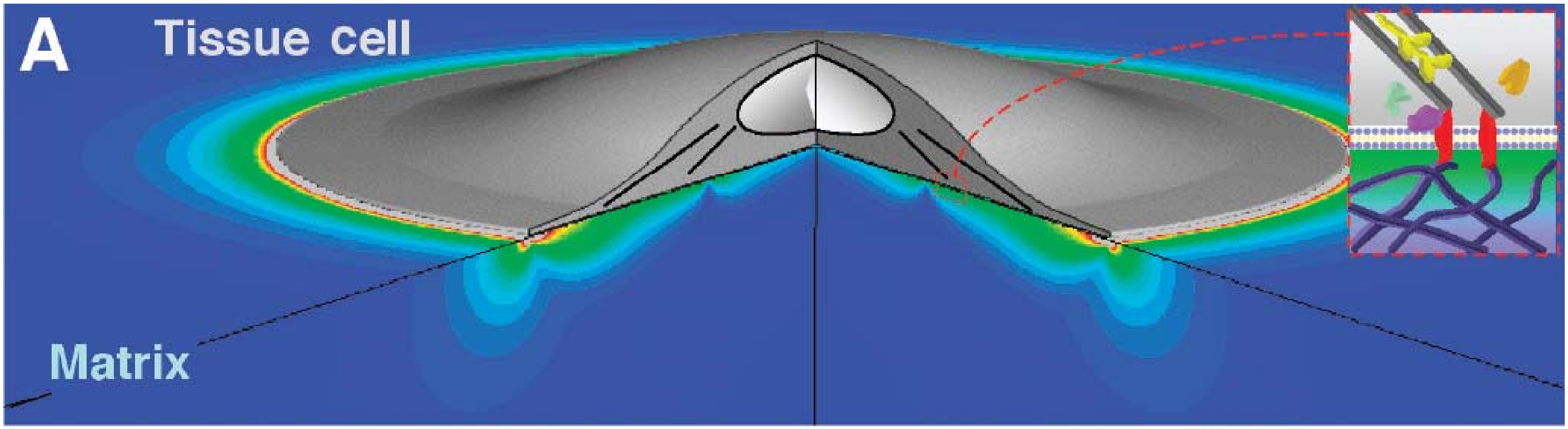
<u>Soft substrate</u> with **small spring constant** (k):
Cell can easily pull on the gel
(no need for stress fibers)



<u>Stiff substrate</u> with **larger spring constant** (k):
Cell need greater force to displace the polyacrilamide gel
(cell need power of stress fibers)

Prestress visualized in a computer model

• A rounded cell on a soft substrate exhibits a **uniform and constant prestress** from the edge (cell border) to the nucleus (cell center)
• Prestress is generated by **actin-myosin contraction** and transmitted to the substrate
• This computed strain distribution is consistent with the tensegrity model

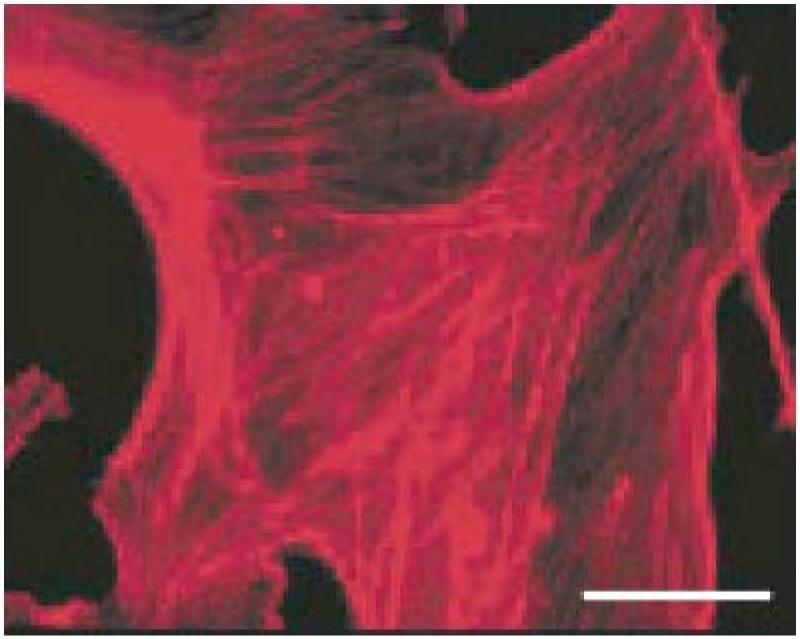


Discher et al., Science, 2005

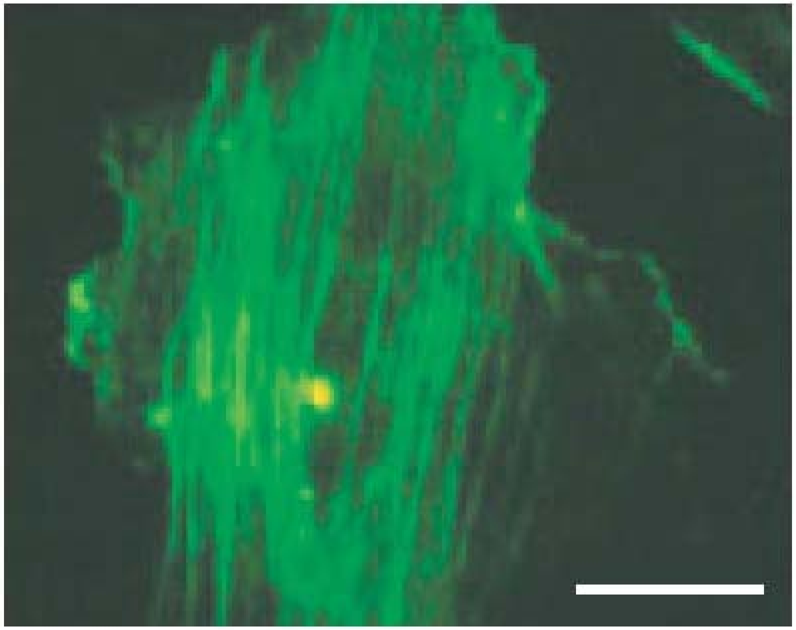
Rearrangement of stress fibers after cyclic cell stretching

How do cells handle mechanical forces generated in organs as the heart or the blood pressure in vessels?

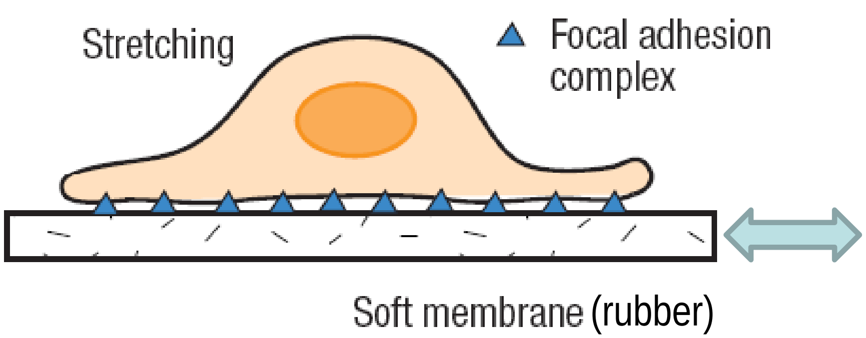
Unstretched human aortic endothelial cell: random distributed stress fibers



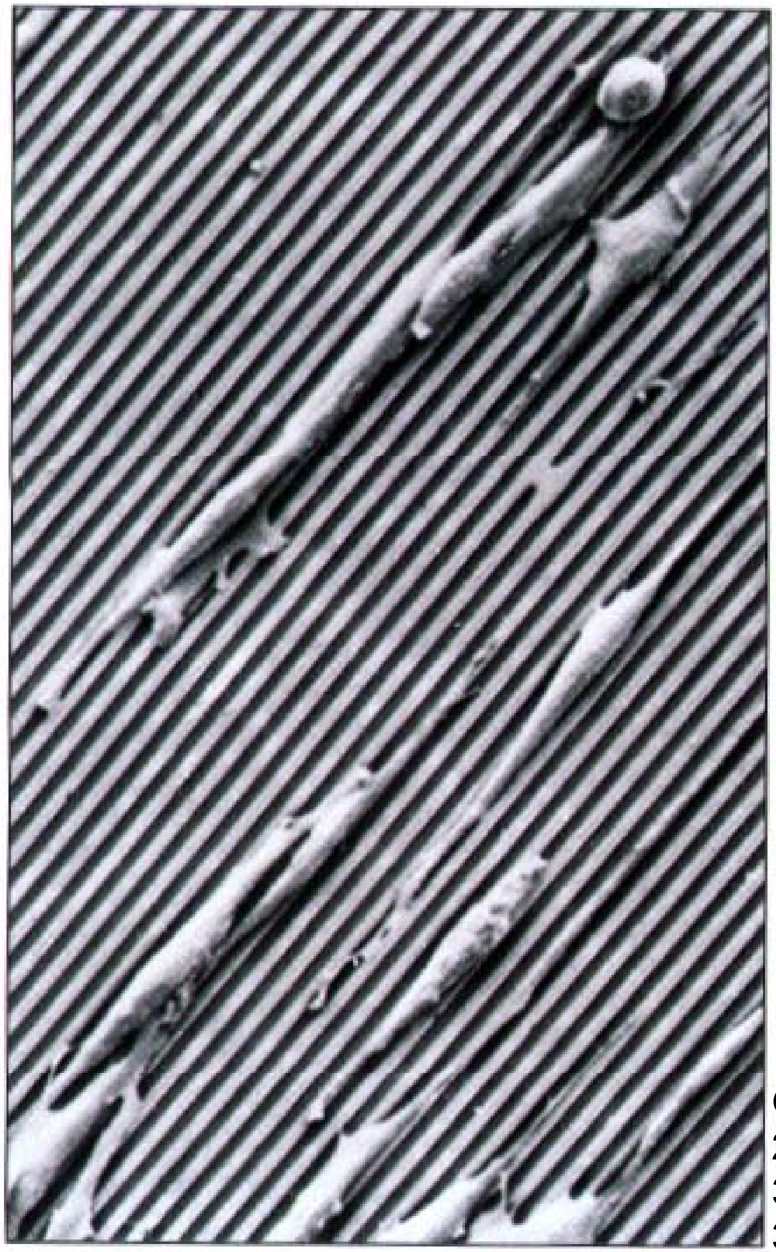
After 3 hours of stretching: stress fibers are oriented into direction of stretching



Very dynamic features of stress fibers are critical for **force sensing** and **force transduction**

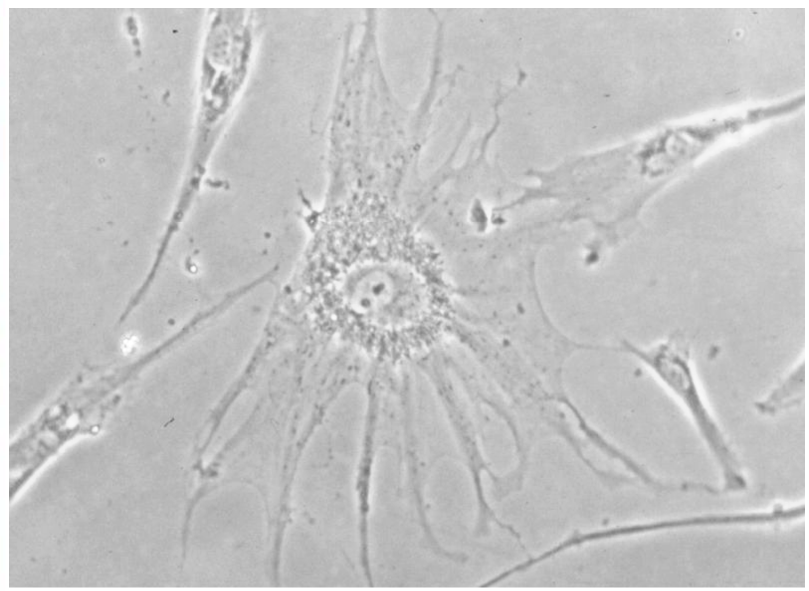


Cellular response to substrate composition



Cultured fibroblast align on a furrowed surface in the direction of the grooves

Preference of the substrate coating is obvious since growing does not occur across the furrows

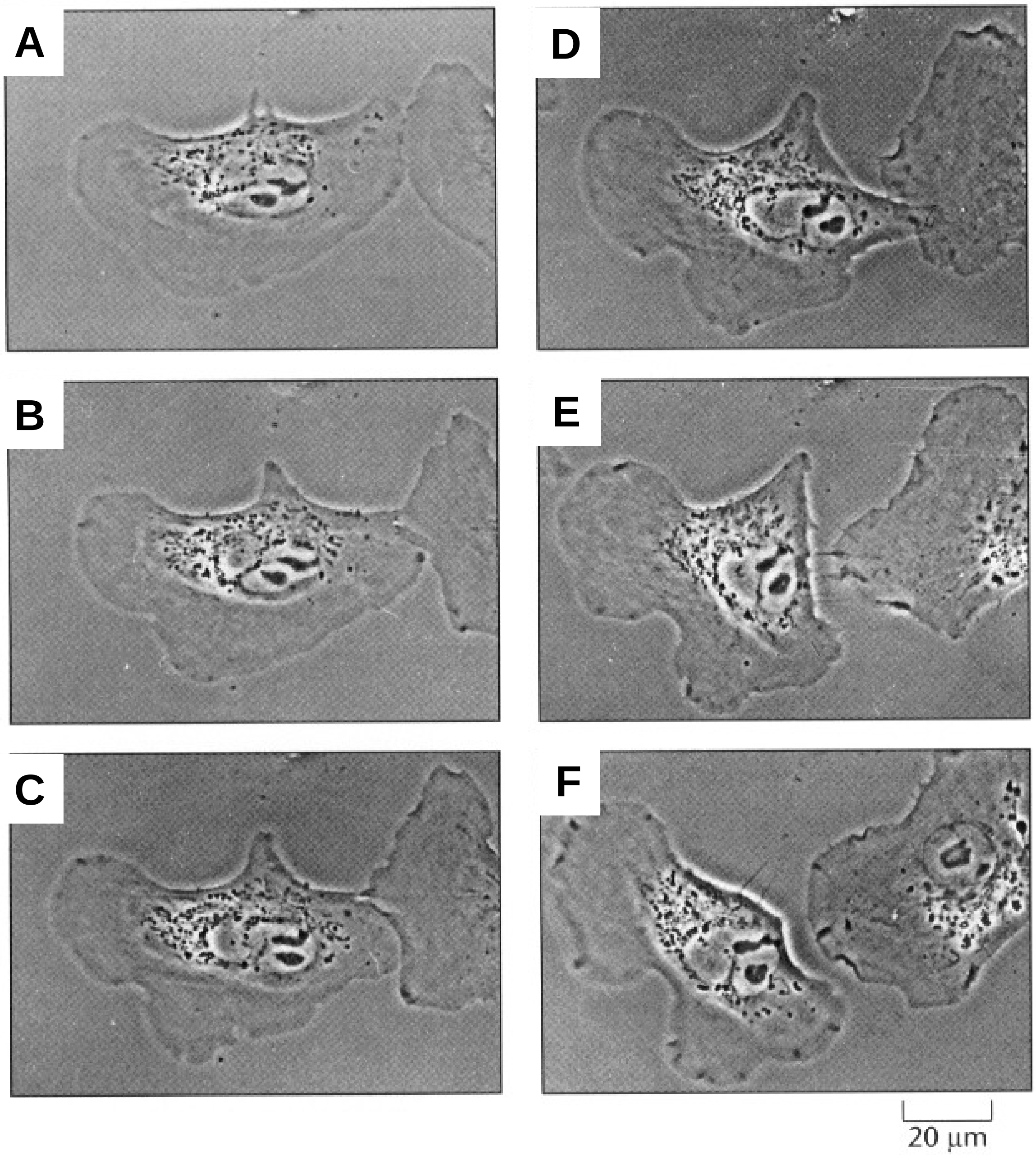


Normal fibroblast cells

Groove dimensions:
2 µm deep
3 µm wide
3 µm spaced apart

Bray, Cell Movements, 2nd Ed.

Cellular response to "cell traffic": contact inhibition

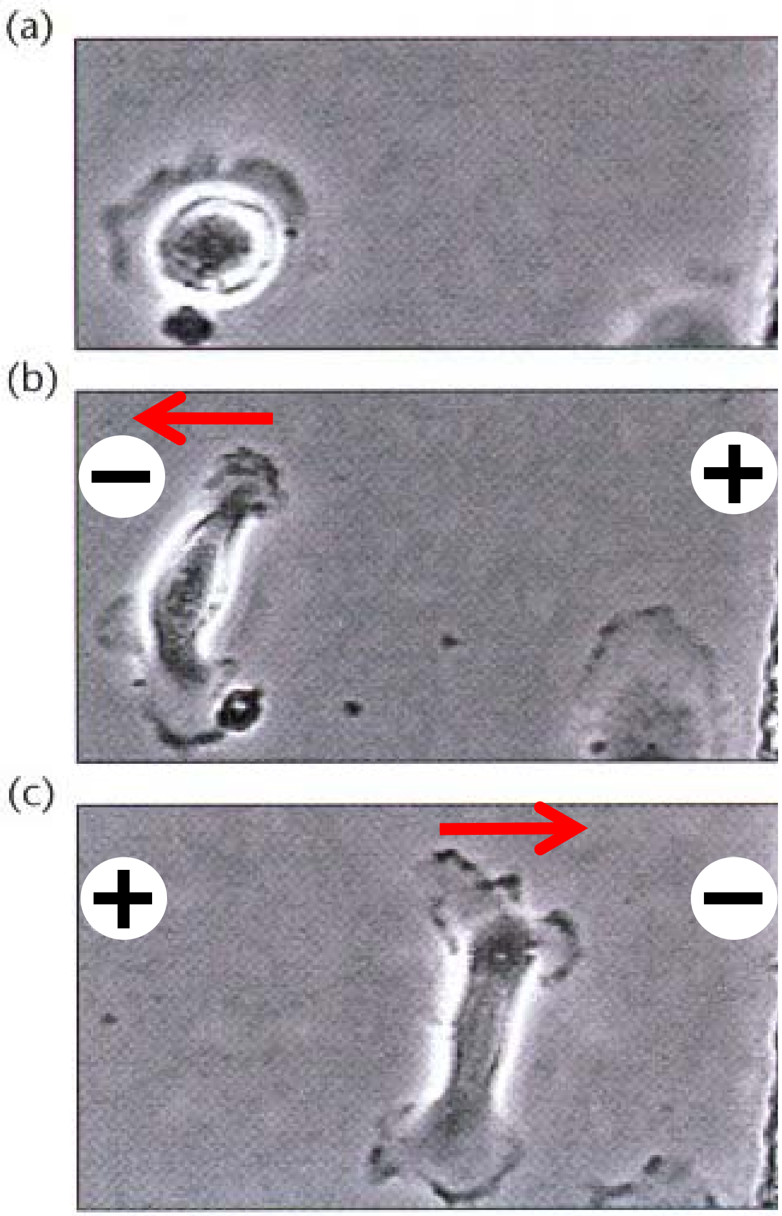


When one cell collides with another a phenomenon named **contact inhibition** occurs:

- At the region of contact (cell's ruffles) a **stationary (quiet) zone** is formed in which cells seemed to form **contact by filopodia**
- Ruffling now occurs in the opposite direction
- Cells are moving away from each other

Bray, Cell Movements, 2nd Ed.

Cellular response to an electric field



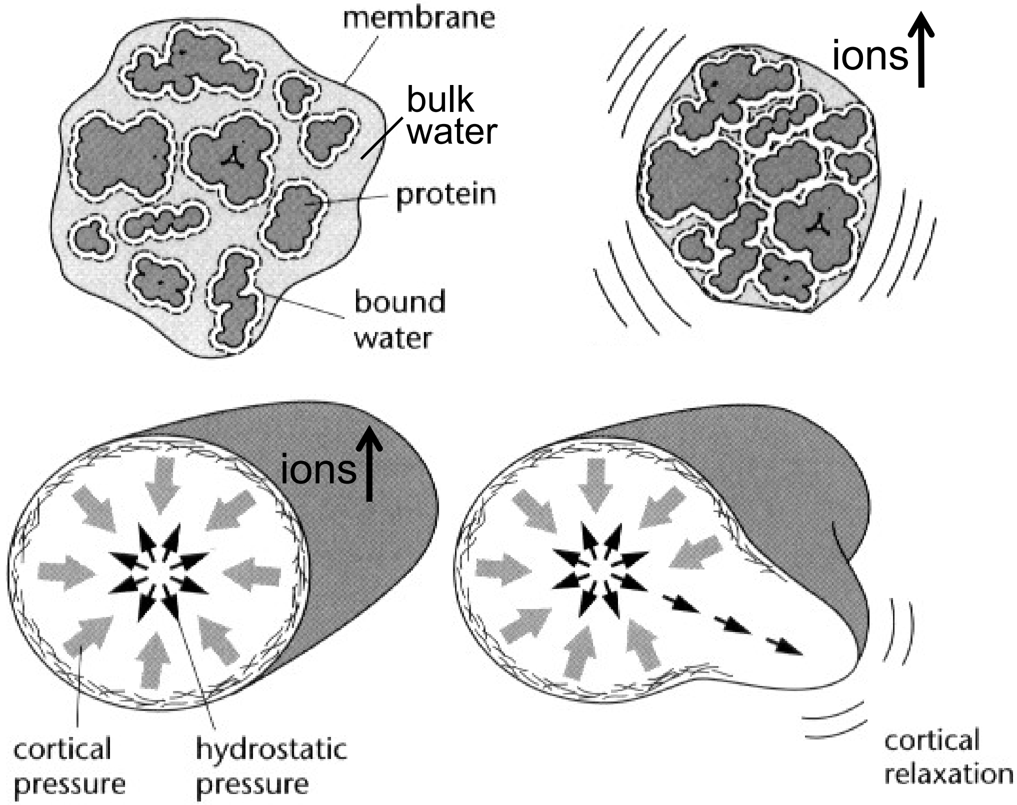
Before the field, the epithelial **cell rounded**

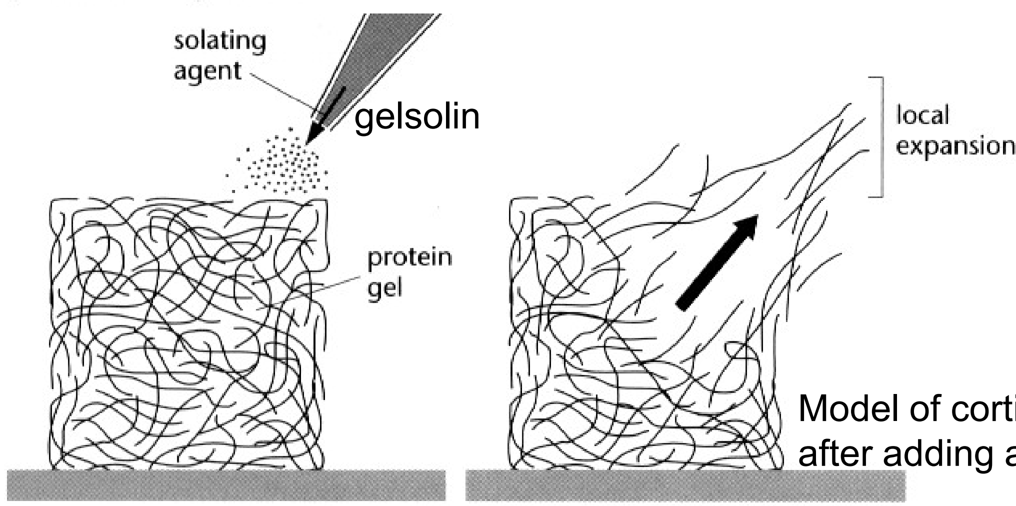
After 1 hour exposure to an electric field of 150 mV/mm **cell becomes elongated** (90° to the field) and starts to move to the minus-pole

Switching the polarity of the field results in a movement to the preferred minus-pole (the cathode)

Bray, Cell Movements, 2nd Ed.

Internal cellular hydrostatic pressure as a cytomechanical factor



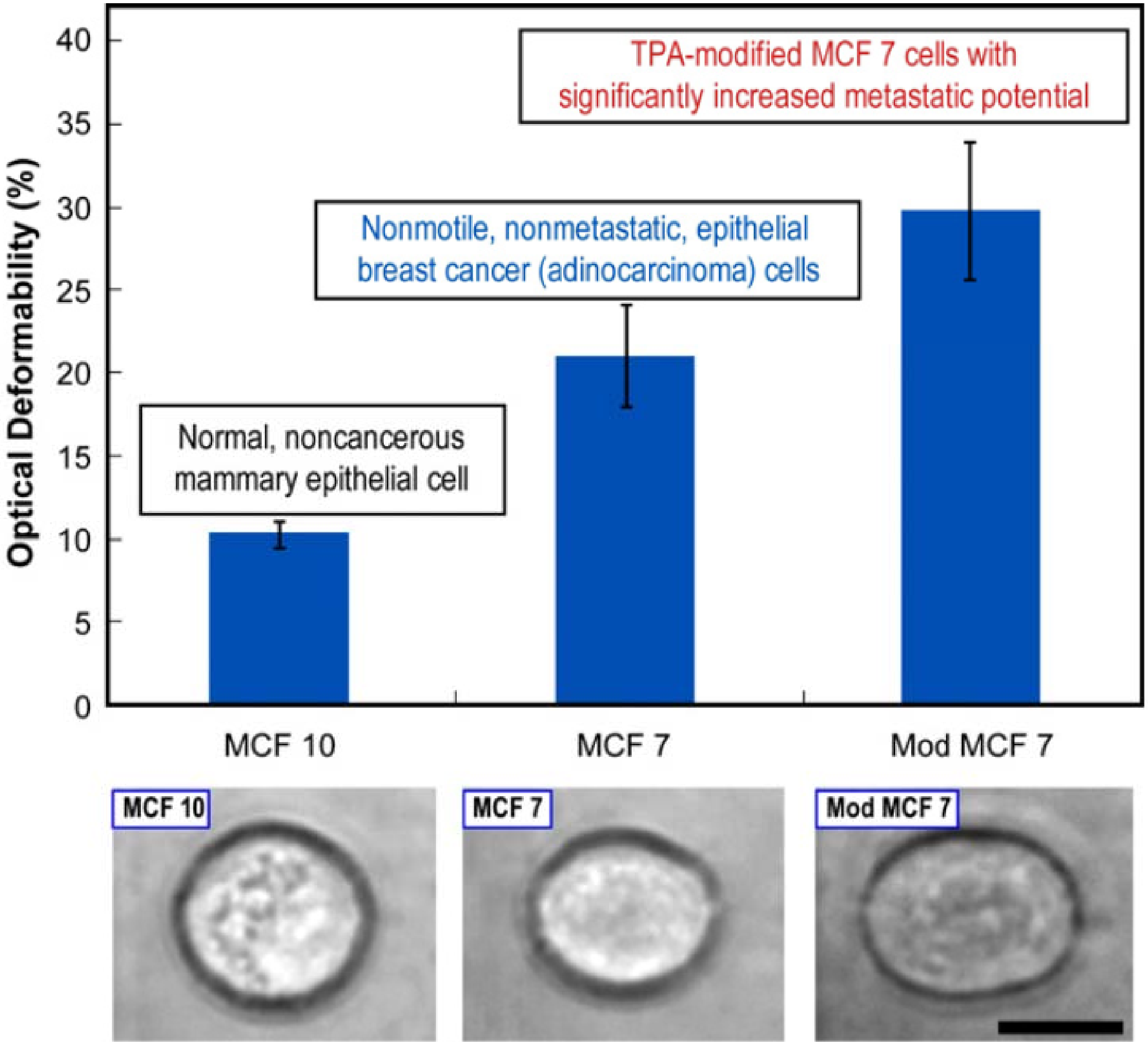


Model of cortical relaxation (based on osmotic forces) after adding an actin depolymerizing factor (gelsolin)

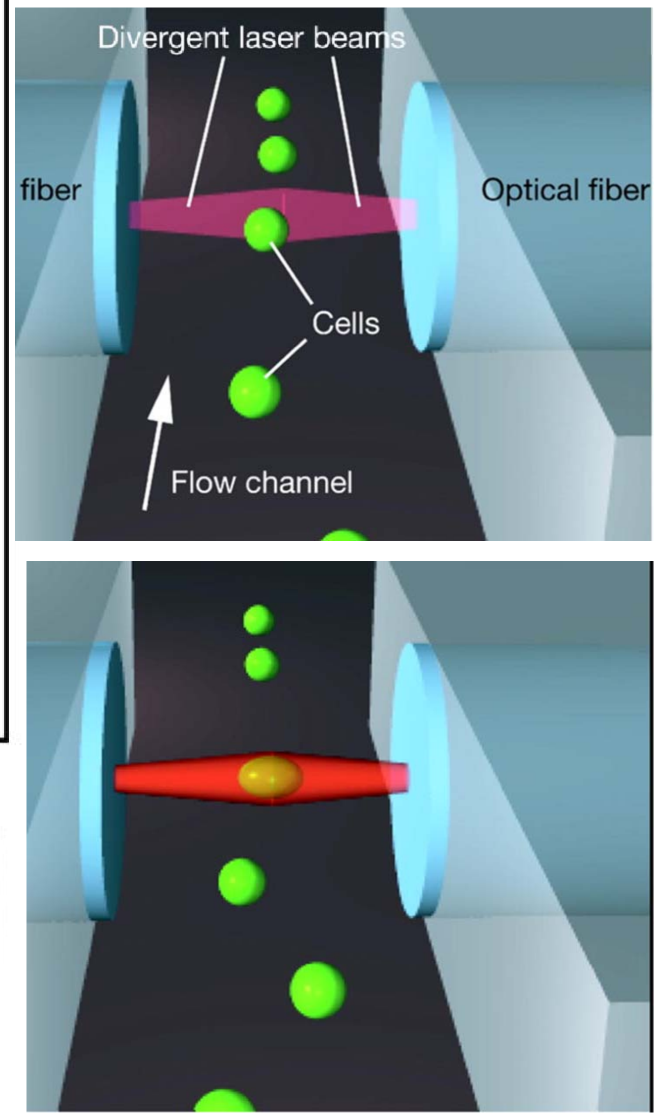
- Cell contains **bulk water** (free water) and **bound water** (bound by proteins)
- Under hyperosmotic conditions, only the bulk water will be lost
- On the other hand, the **high ionic content in the cell** might lead to a **constant flow of water inside** the cell
- To avoid this, the cell develop and maintains a constant hydrostatic pressure to stop water flowing inside
- Some plant cells and bacteria can develop internal pressures up to 10^6Pa
- Relaxation of cortical tension might result in redirecting of internal pressure that may drive cell membrane extension
- Water ingress might also **swell the cytoskeleton** leading to **increased osmotic forces**
- How much does hydrostatic pressure contribute to cell mechanics?

Biomechanics and biophysics of cancer cells

Deformability of breast cancer cell is **increased** (based on f-actin reduction) that also increases metastatic potential



Microfluidic optical stretcher: trapping and stretching cells with two laser beams



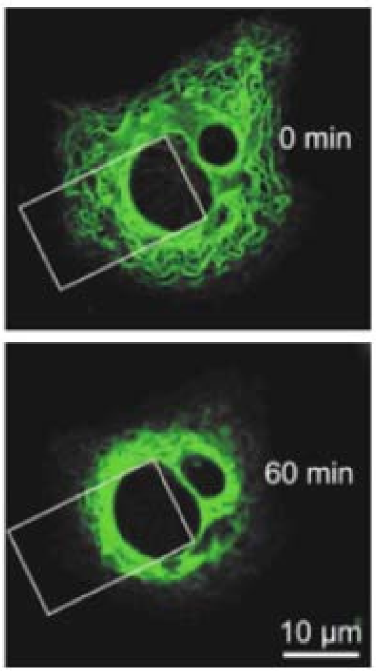
Guck et al., Biophys. J, 2005

TPA is a type of phorbol ester to treat leukemia or lymphoma cancer

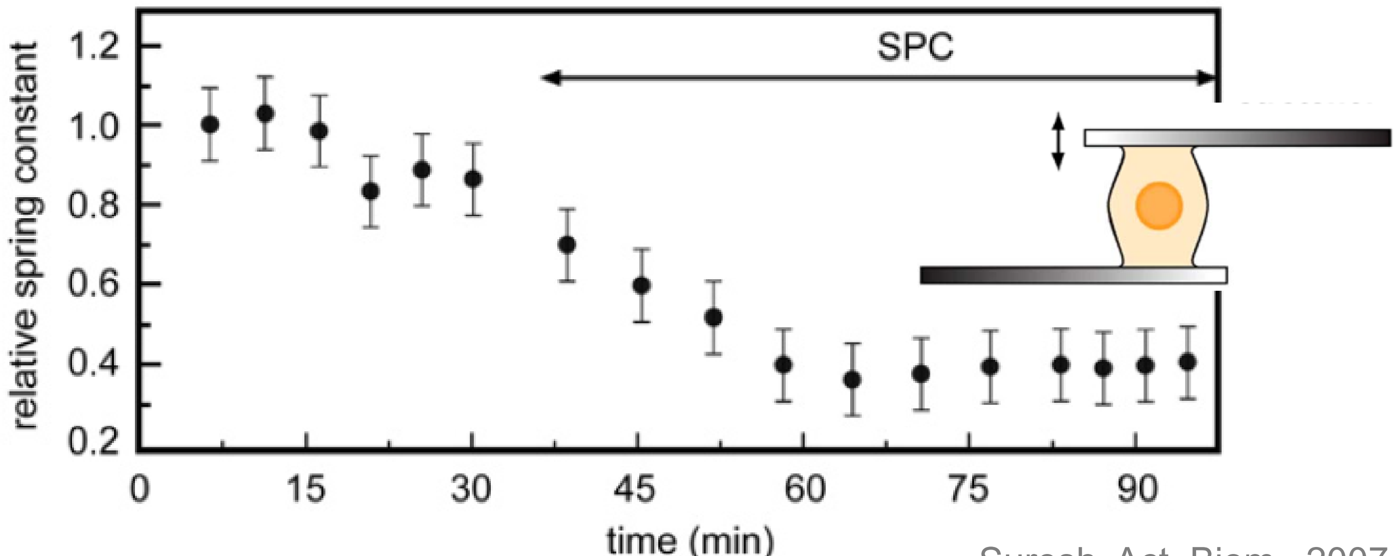
Invasion of Panc-1 epithelial tumor cells in the human pancreas by the bioactive lipid SPC

The substance SPC decreases the IF network which in turn increases metastatic potential

Structure	Property	Disease
Dramatic reorganization of the intermediate filament (keratin) network in the perinuclear region	More than three-fold reduction in Panc-1 cell elastic modulus and increase in hysteretic energy dissipation during cell deformation	Greater motility of tumor cells through size-limiting pores and metastatic invasion?



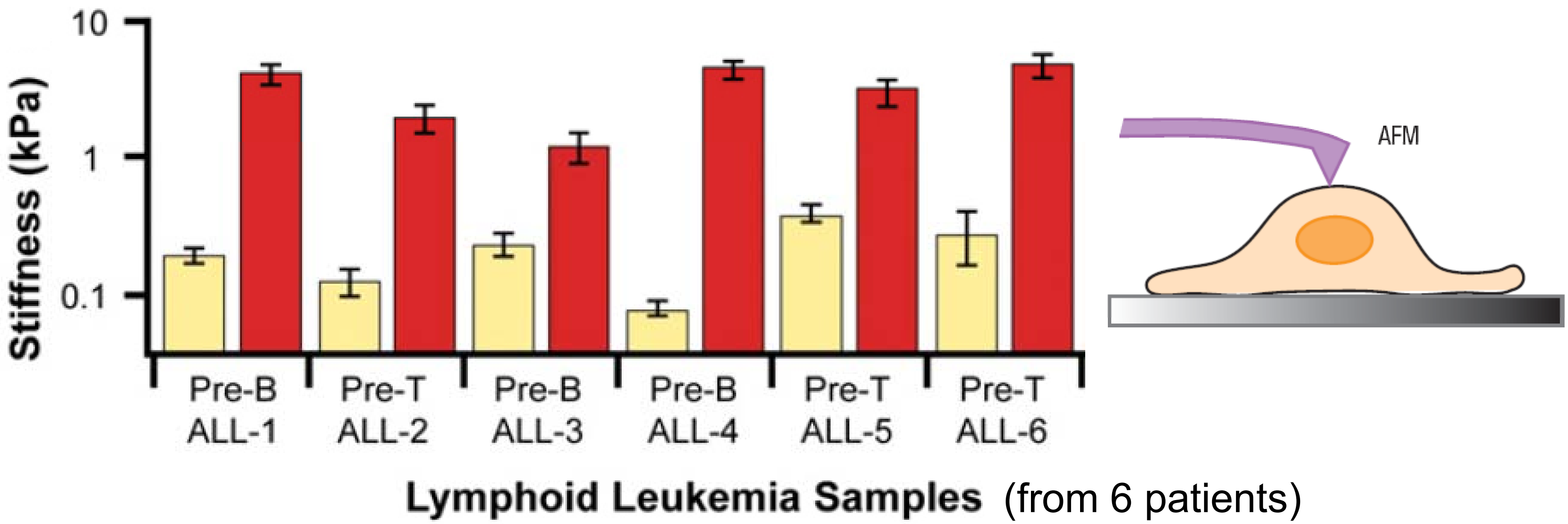
(High SPC levels found in blood in patients with pancreatic tumors)



Suresh, Act. Biom., 2007

Effects of chemotherapy on elastic properties of cancer cells

• Chemotherapy to treat leukemia leads to cell stiffening that might explain observed vascular compilations (atherosclerosis etc.)
• Parallel treatment with cytochalasin D to weaken the actin-network helped to make the dead cells softer for better dead-cell recycling (not shown)

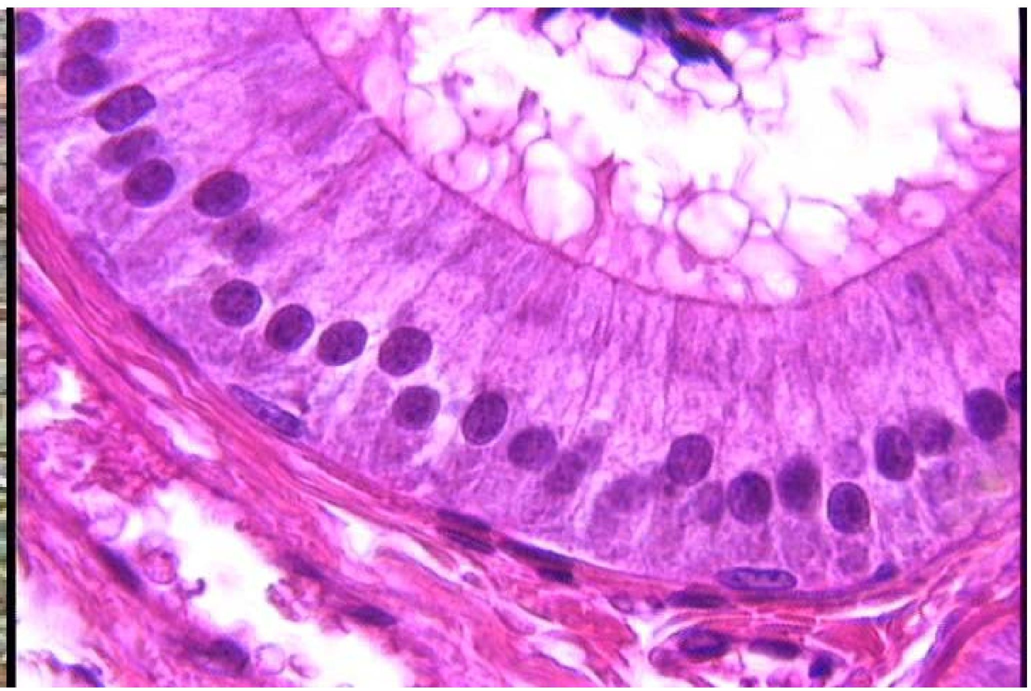


(from 6 patients)

Yellow bars: blood cells **before** chemotherapy
Red bars: dead cells **after** chemotherapy (drug: daunorubicin)

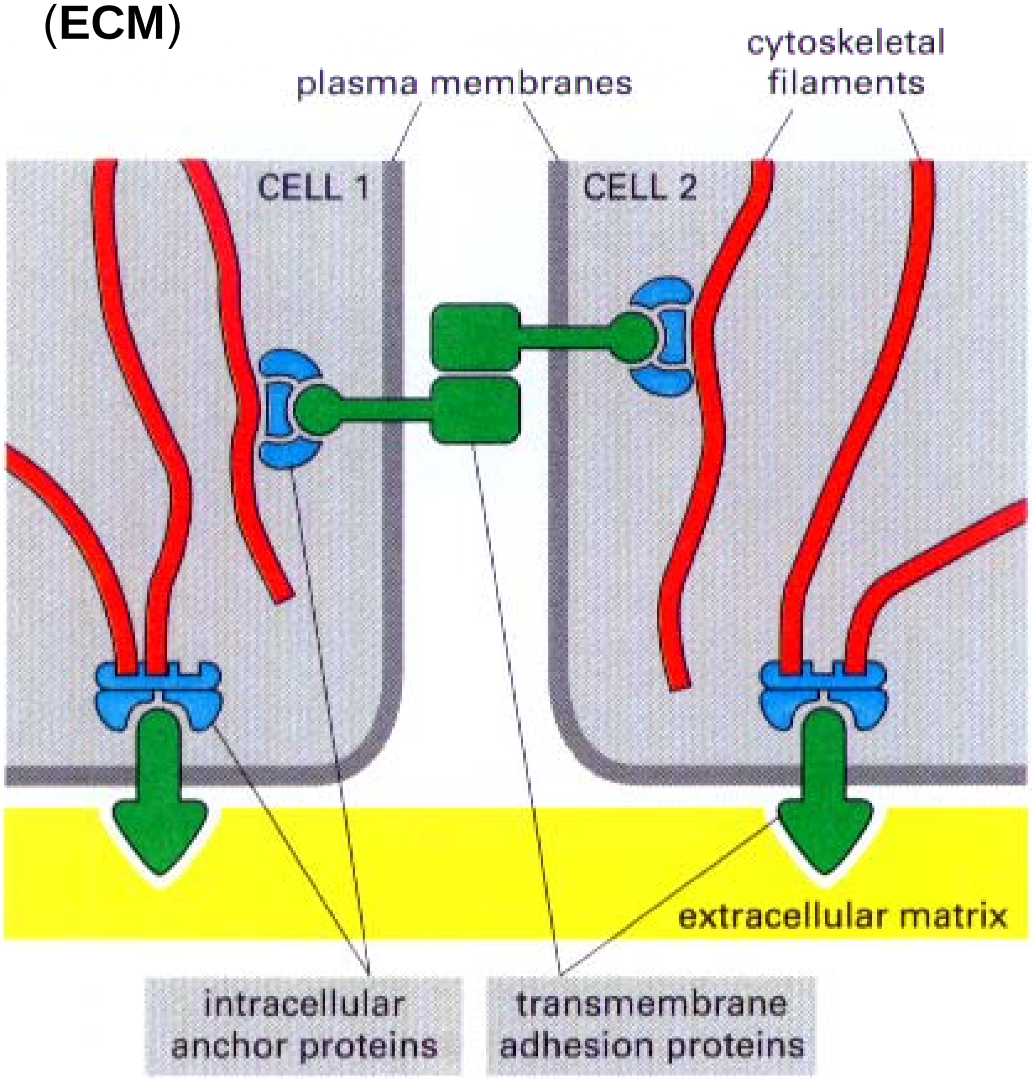
Besides the Cytoskeleton the ECM is important for Cell Mechanics

<u>3 principles act to form a tissue from single cells</u>:
1) **Cytoskeleton** not only acts to stabilize single cells but also helps to <u>connect a cell to a neighbor cell</u>
2) Specialized (polymeric) proteins stabilize cell-cell contacts (<u>cell adhesion molecules</u>, **CAM**)
3) An matrix outside the cell (<u>extracellular matrix</u>, **ECM**) acts as a <u>fibrous filling material</u> and to glue cells to each other

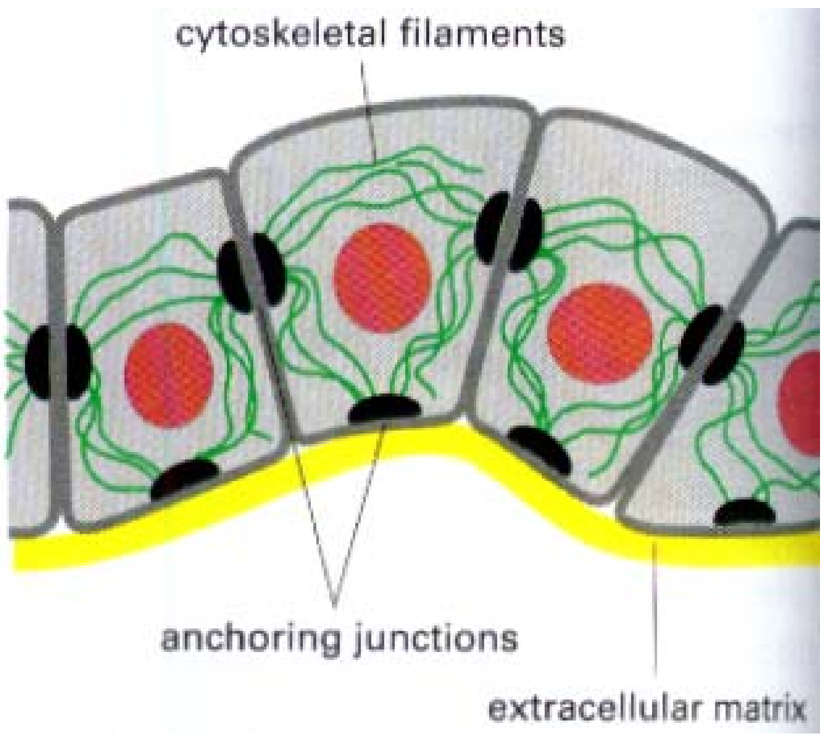



Integrating single cells into stable tissues

• **Intracellular anchor proteins** connect the **cytoskeleton** to **transmembrane adhesion proteins** (**CAM**s)
• **Transmembrane adhesion proteins** are embedded in the **extracellular matrix** (**ECM**)



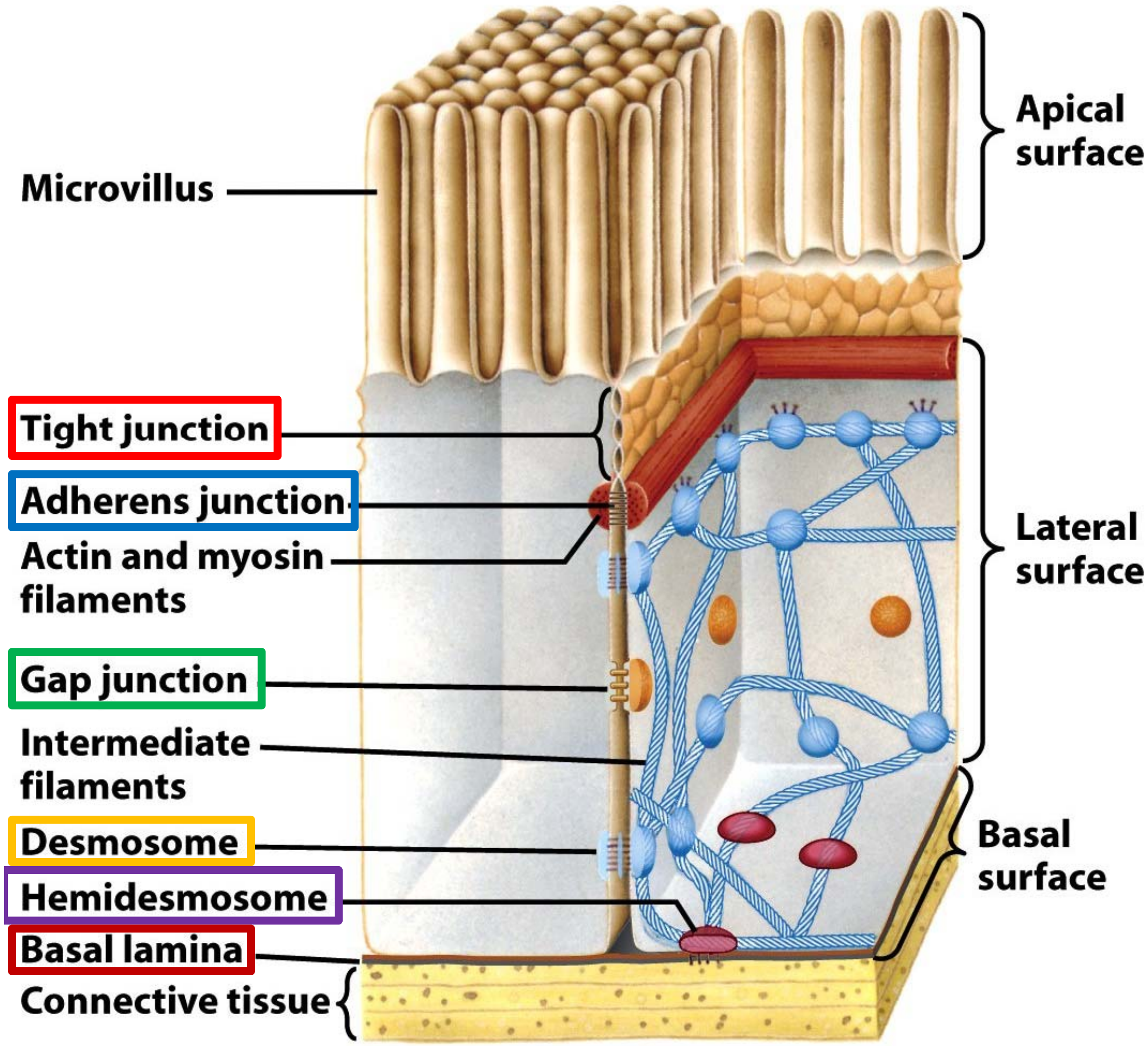
Cytoskeletal filaments are connected to the anchoring junctions



Integrating single cells into stable tissues

A variety of cell-cell contacts functions differently in the tissue:

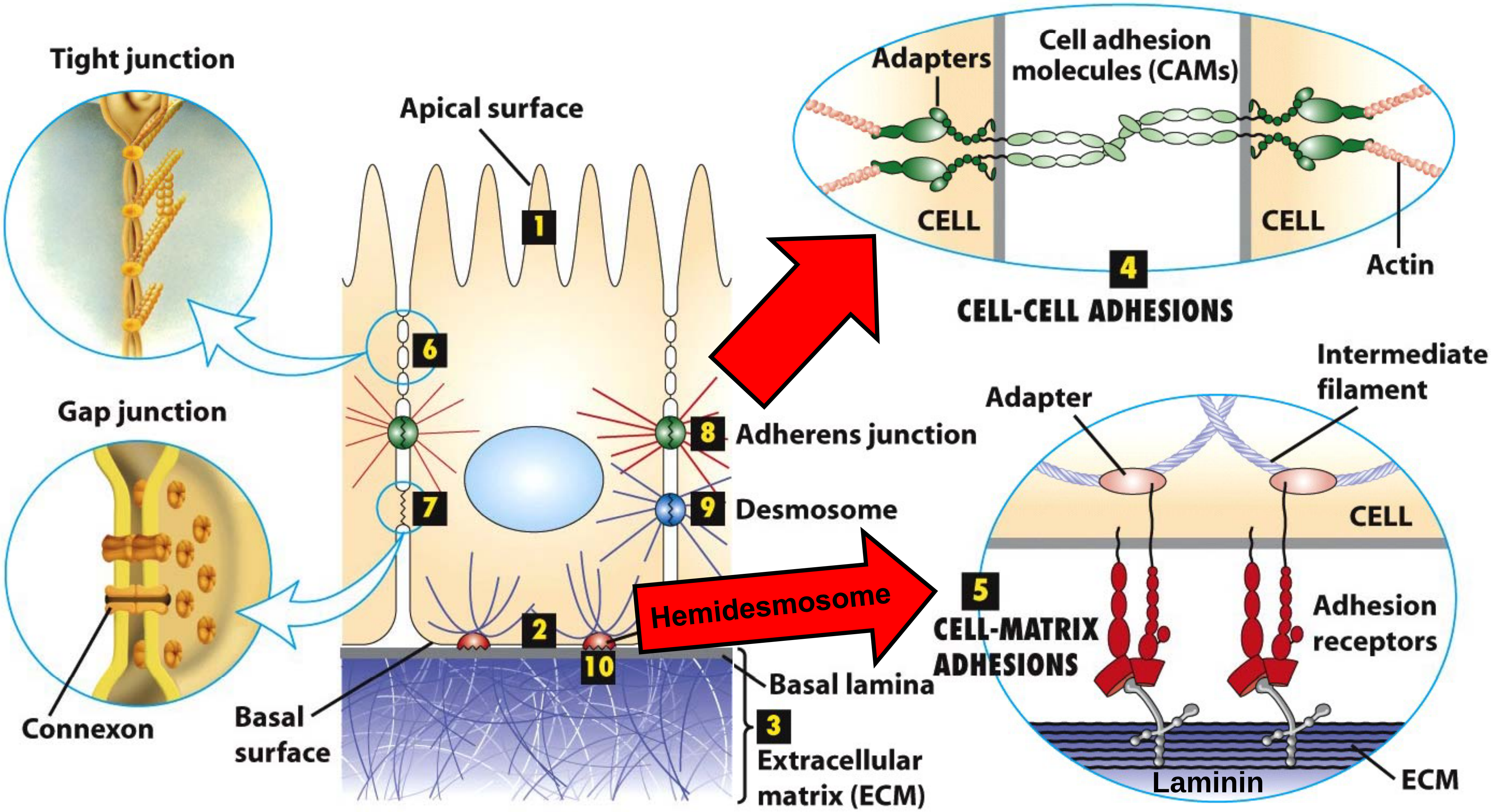
- **Tight junctions**: make part of the membrane almost impermeable (diffusion barrier)
- **Gap junctions** form ion-channels for electrical communication between cells
- **Adherens junctions** are guided by an **elastic actin-myosin cable** to shape the cell



- **Desmosomes** are protein complexes to interconnect the internal intermediate filament network *and* to form contacts to neighboring cells
- **Hemidesmosomes** connect cells to the extracellular matrix (connective tissue)
- The cell is separated from the ECM by an tight and impermeable network-layer ("carpet") named **basal lamina**

Integrating single cells into stable tissues

- While **tight junctions** make the cell almost impermeable, **gap junctions** allow <u>small metabolites and messaging molecules</u> to pass
- The actin or intermediate filaments network first connects to **specific adapter proteins** which in turn connect to the large CAM macromolecules

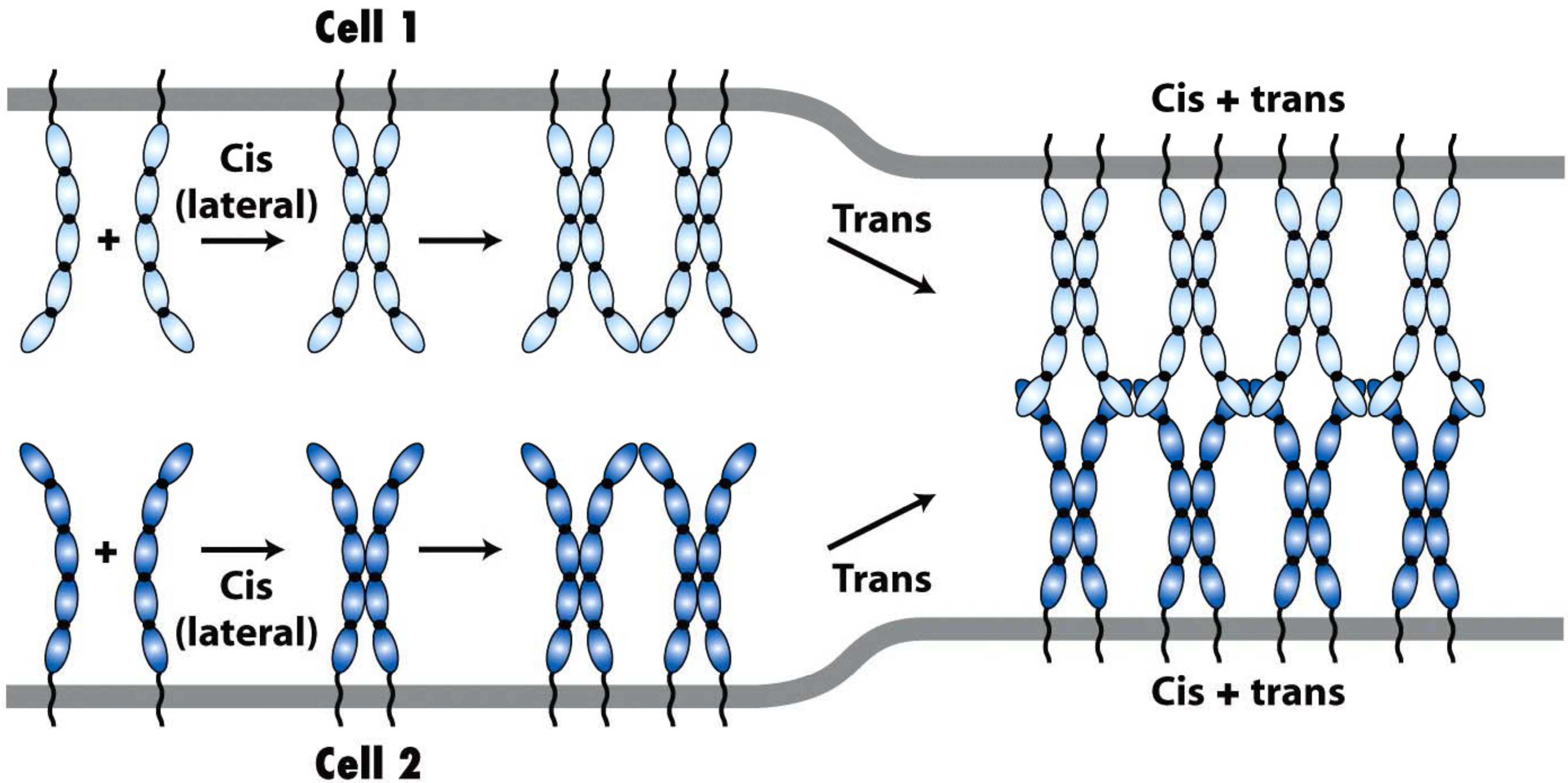


Cell adhesion molecules (CAM) form homophilic (self) cross-bridges

Cell-cell contact is generated in two steps:

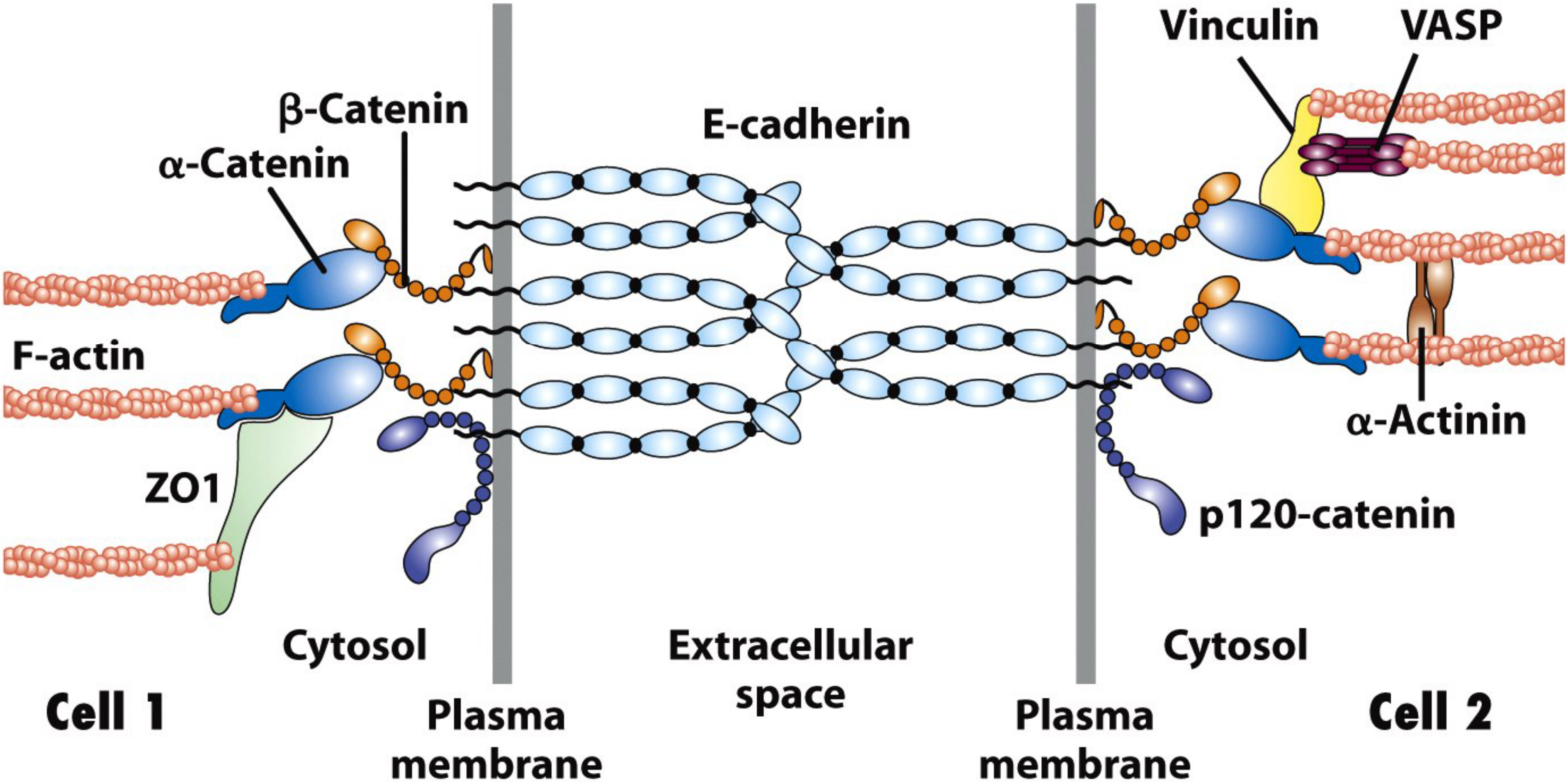
- CAMs first form dimers by **lateral interaction** and **clustering** in the cell membrane
- Strong cell-cell adhesion is generated by **cross-bridging** CAMs to opposite CAMs

(homophilic (self) cross-bridging depends on and is regulated by Ca^{2+})



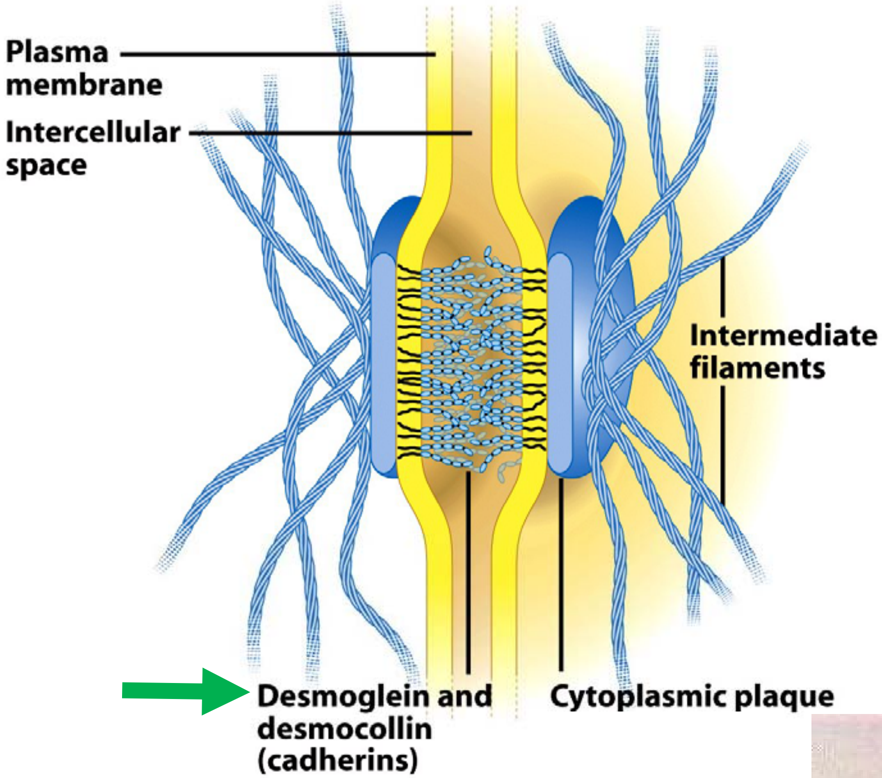
Dissecting an adherens junction

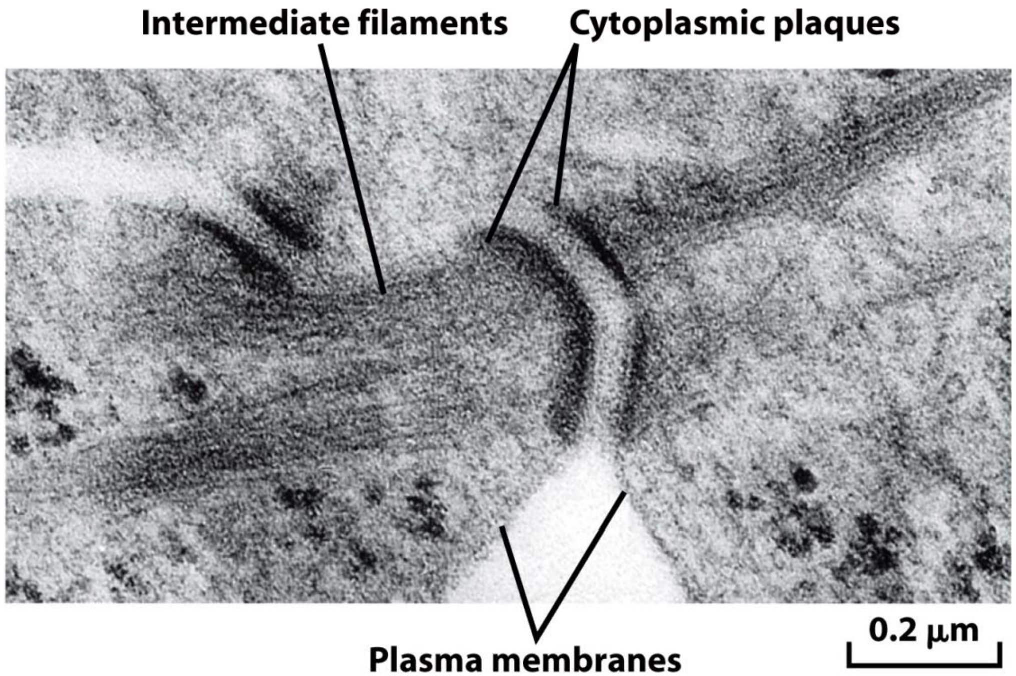
• An adherens junction is a <u>complex apparatus</u> consisting of many components for successful <u>connecting cells to each other and the cytoskeleton to the junction</u>
• Some adapter proteins do **directly participate in intracellular signaling pathways** (for example, β-catenin)



Desmosomes are button-like structures connecting two cells

Thick intermediate filament bundles connected to electron dense structures can be seen in EM of **two keratinocytes** (skin cells) firmly connected to each other

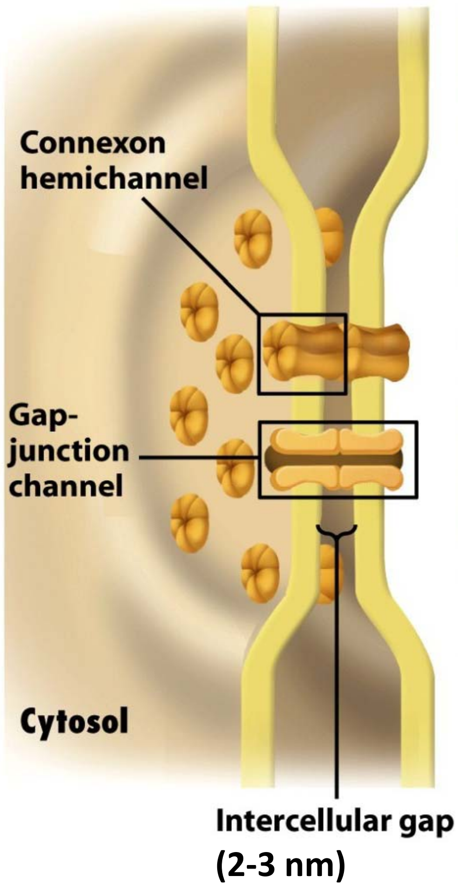


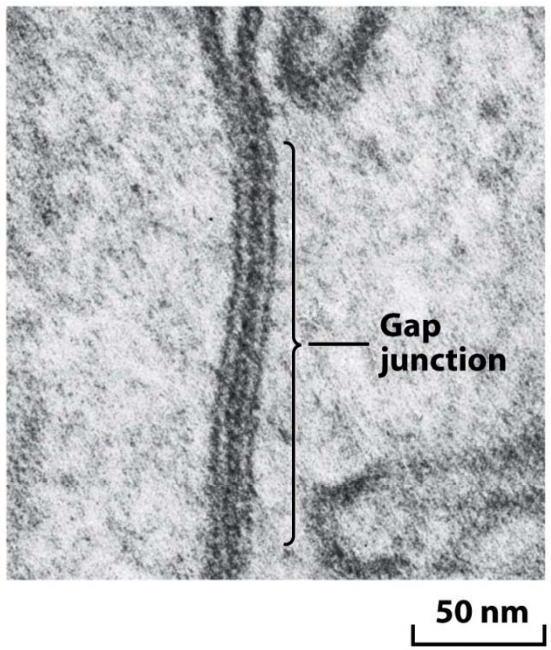


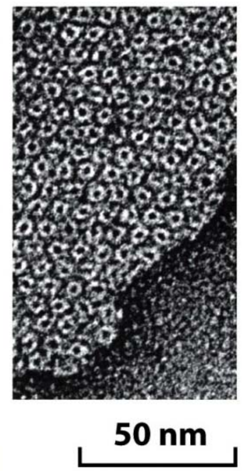
In the skin disease ***pemphigus vulgaris*** the protein **desmoglein** is non-functional, resulting in severe skin blistering

Gap junctions are 2-3 nm wide channels

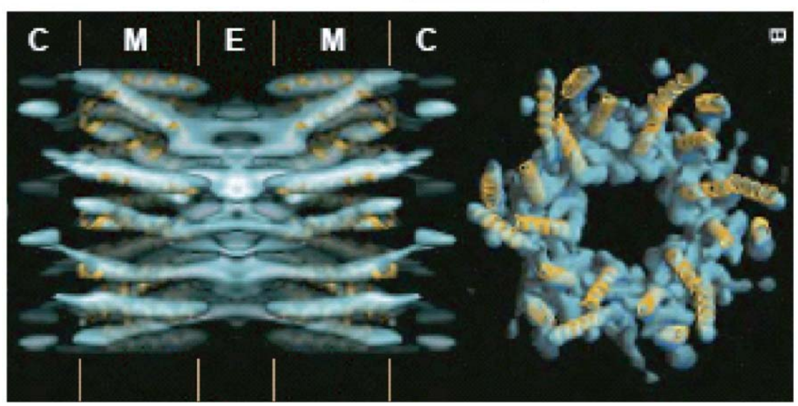
Gap junctions form a channel system for the <u>exchange of small metabolites</u> (as ions, sugars, vitamins, ATP etc.) <u>between two cells</u>







The gap junction channel is formed by the <u>hexagonal protein</u> **connexin**



Atomic structure of gap junctions

C = cytosol, M = membrane bilayer
E = extracellular gap

Cell junctions are crucial for tension and mechanical stability of tissues

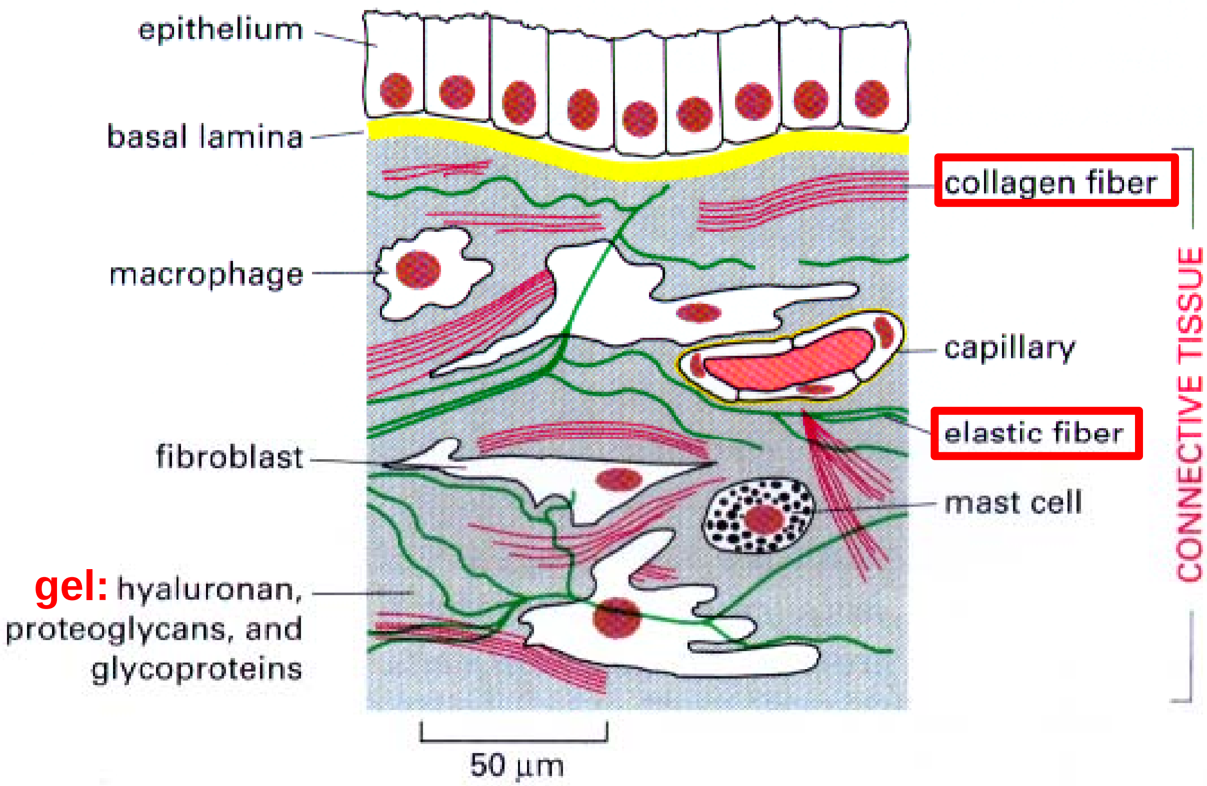
Since **junctions** integrate a cell's cytoskeleton and at the same time strongly connect to neighboring cells, <u>shape, rigidity and cell strength are largely increased</u>

Functions of cell junctions

JUNCTION	ADHESION TYPE	CYTOSKELETAL ATTACHMENT	FUNCTION
Anchoring junctions			
1. Adherens junctions	Cell-cell	Actin filaments	Shape, tension, signaling
2. Desmosomes	Cell-cell	Intermediate filaments	Strength, durability, signaling
3. Hemidesmosomes	Cell-matrix	Intermediate filaments	Shape, rigidity, signaling
Tight junctions	Cell-cell	Actin filaments	Controlling solute flow, signaling
Gap junctions	Cell-cell	Possible indirect connections to cytoskeleton through adapters to other junctions	Communication; small-molecule transport between cells

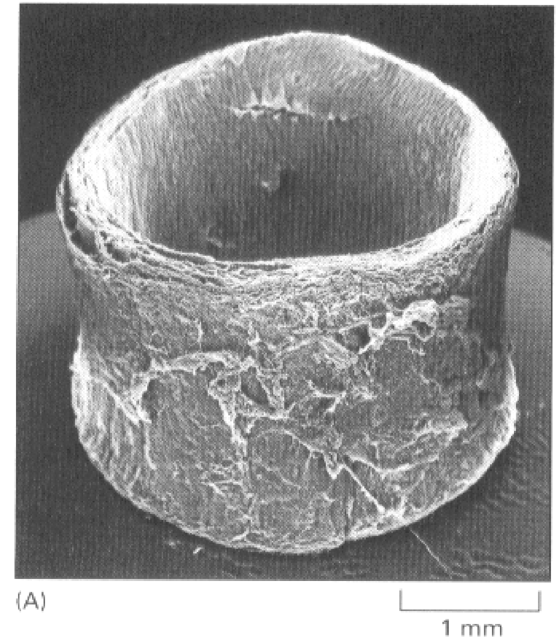
ECM (extracellular matrix)

- Extracellular matrix (**ECM**) is the (connective) tissue below an epithelium
- ECM contains **many highly elastic fibers** but also the cells that secrete these fibers
- These fibers and cells are embedded in a gel (**hyaluronan** and **proteoglycans**)

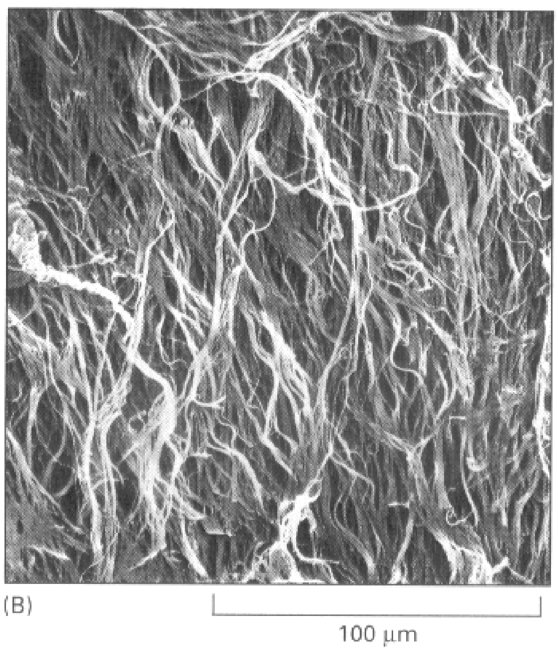


Fibroblasts embedded in the ECM

ECM contains stiff/non-elastic and highly elastic fibers

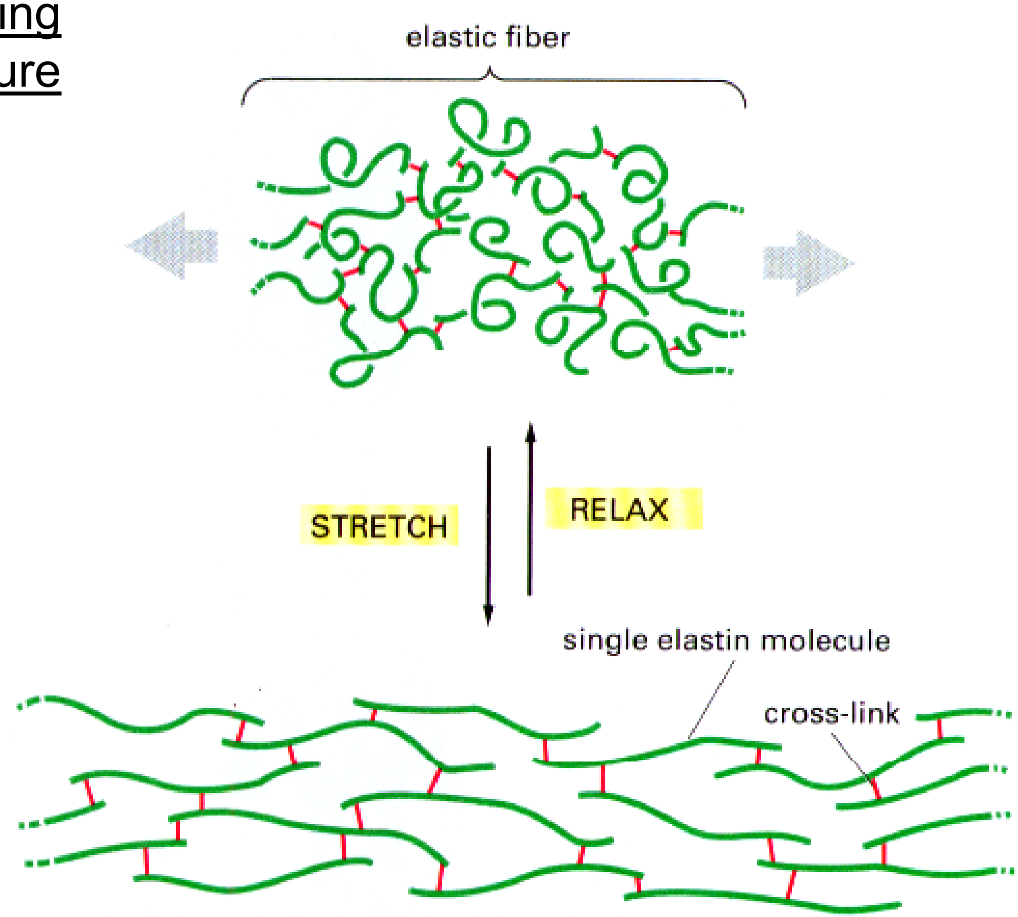


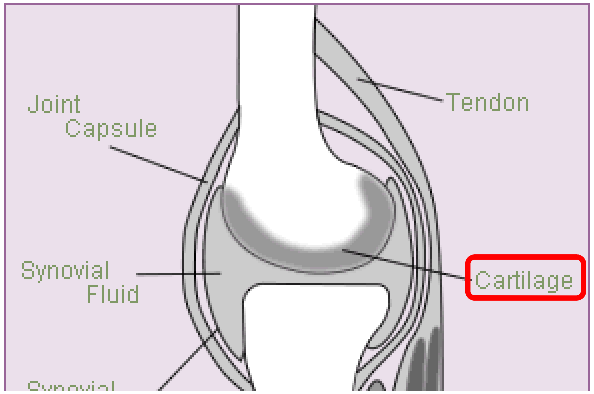
Highly elastic aorta need to resist <u>strong</u> and <u>alternating</u> <u>blood pressure</u>



Elastic fiber (**elastin**) in the outer layer of the aorta

- **Elastin** molecules are <u>highly cross-liked</u> by covalent bonds
- An elastin assembly can <u>stretch and relax</u> like a **rubber-band**

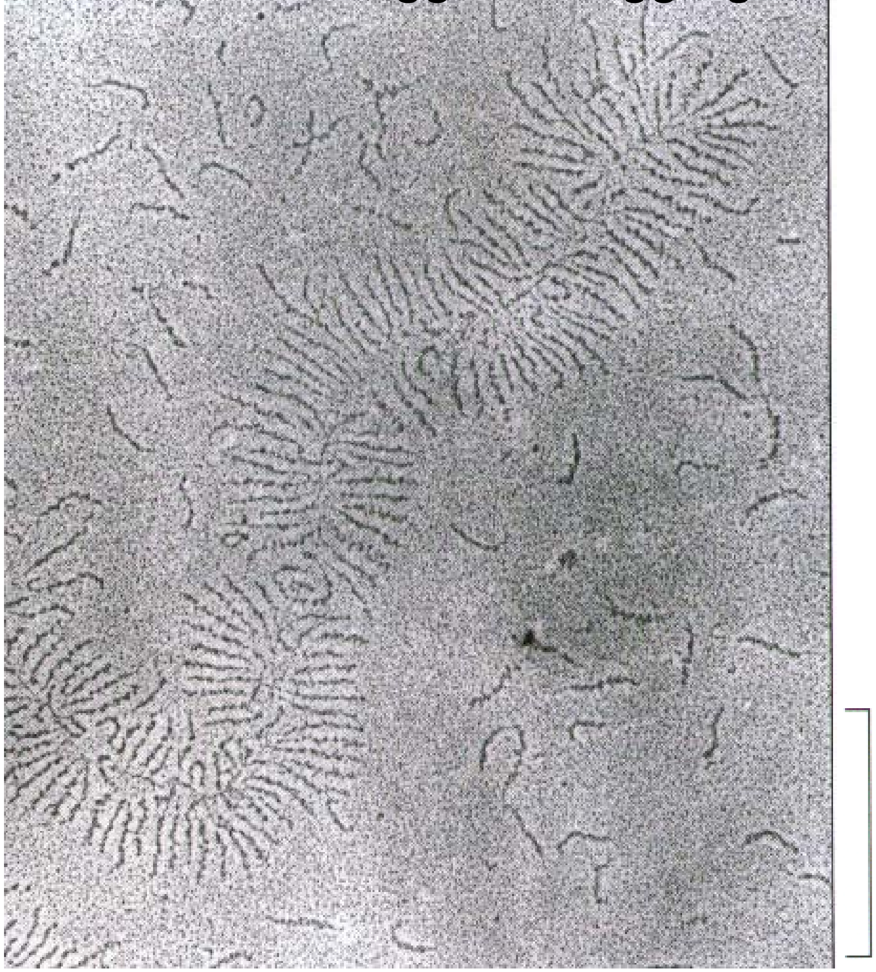


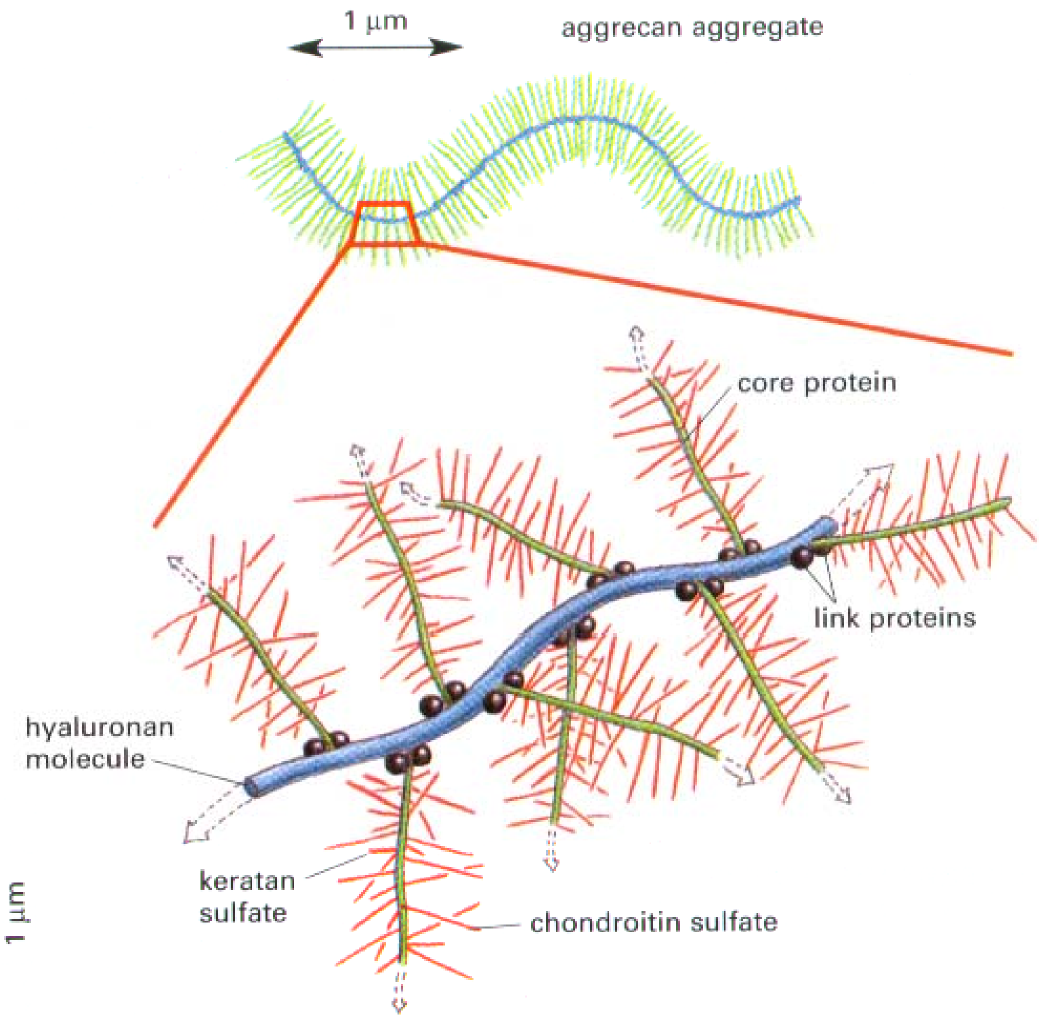


Hyaluronan resists compression and gives cartilage its gel-like properties

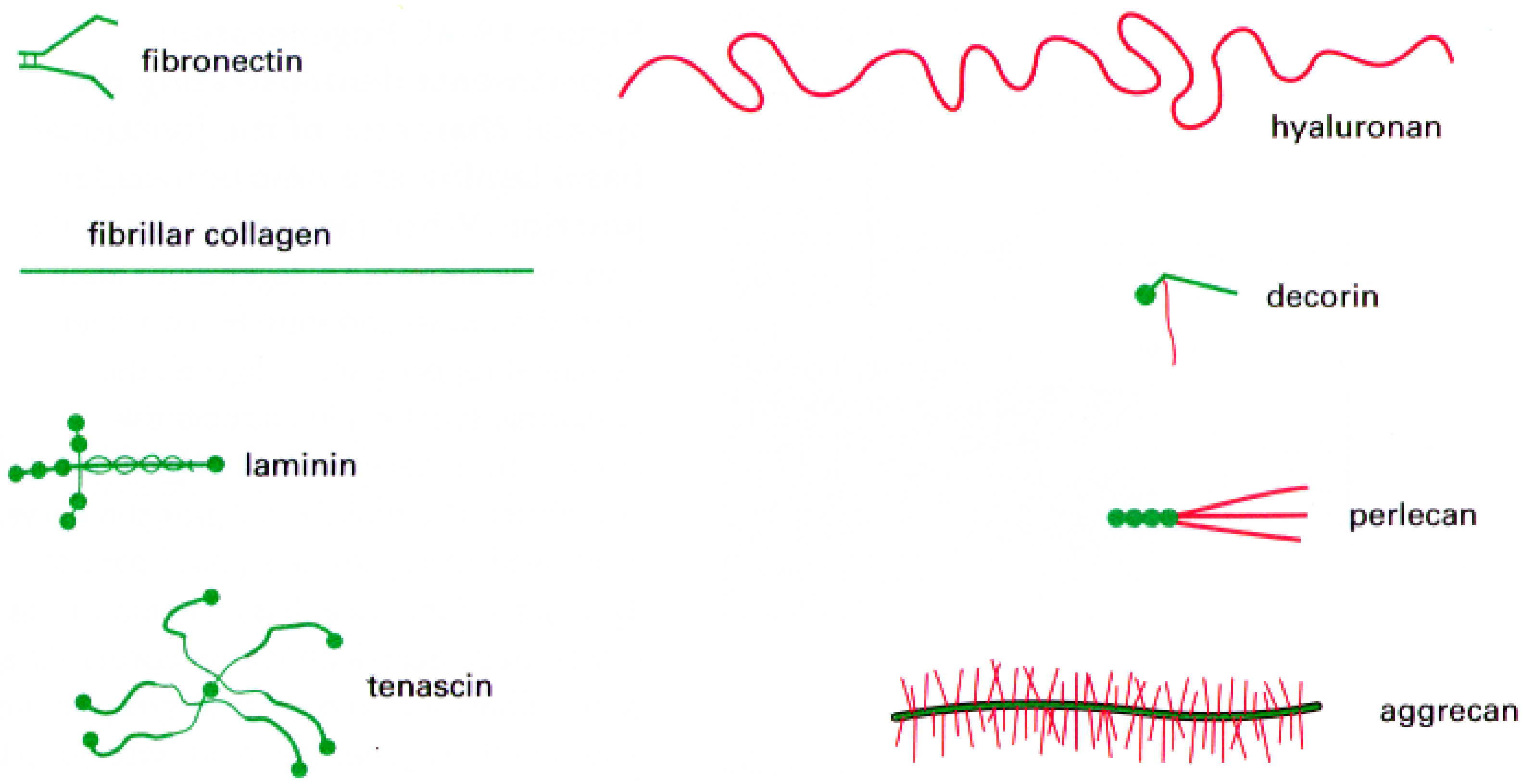
- Major component of cartilage is the **aggrecan aggregate**: <u>huge molecule</u> (MW 2×10^8) with a <u>size of a bacterium</u>
- Up to 100 **aggrecan** molecules are connected to a **hyaluronan** backbone

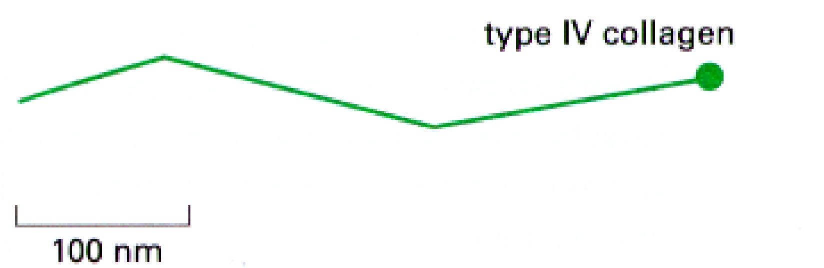
Gel-matrix: the aggrecan aggregate



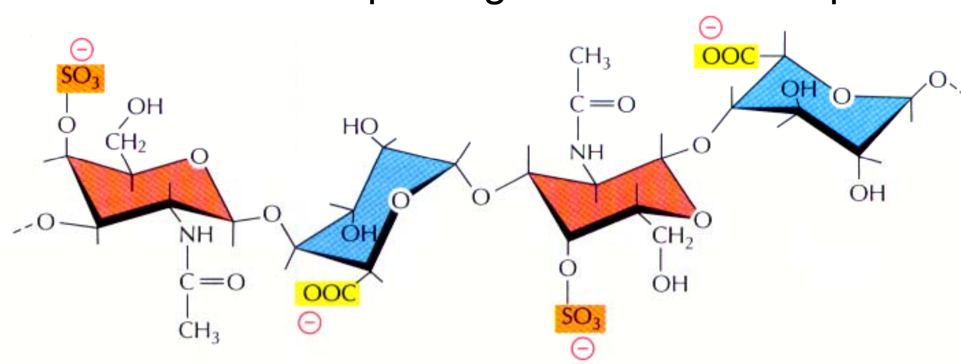


The ECM is composed of a variety of **proteins** and **GAGs** = glycosaminoglycan (or combinations of them)





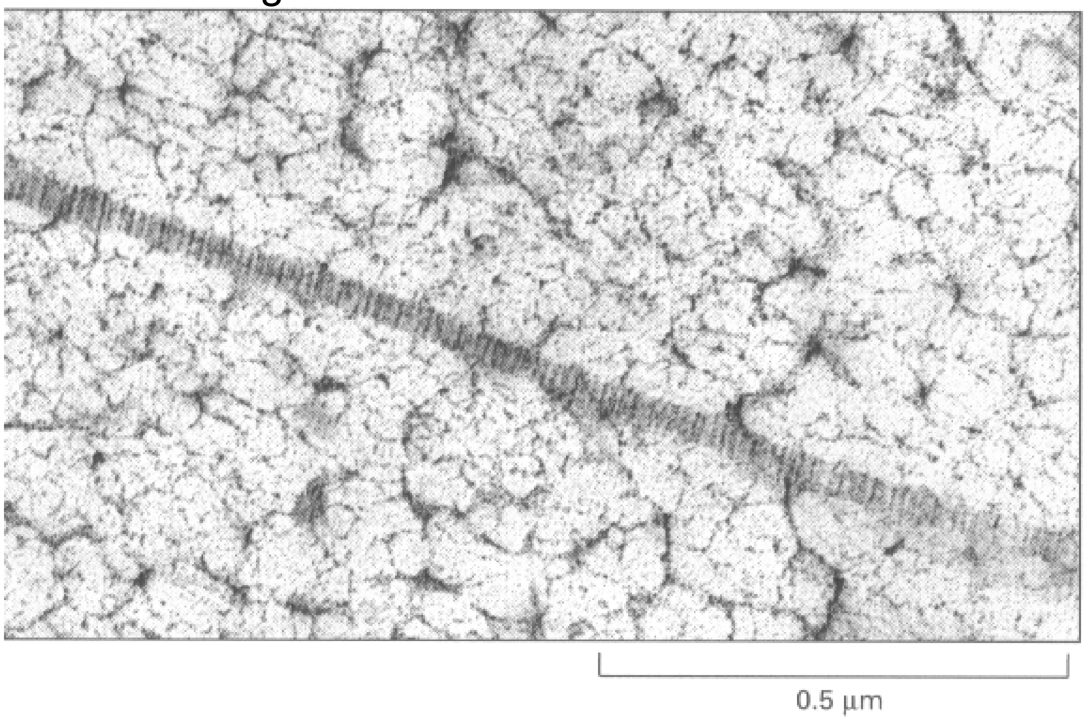
GAG chain is a repeating disaccharide sequence

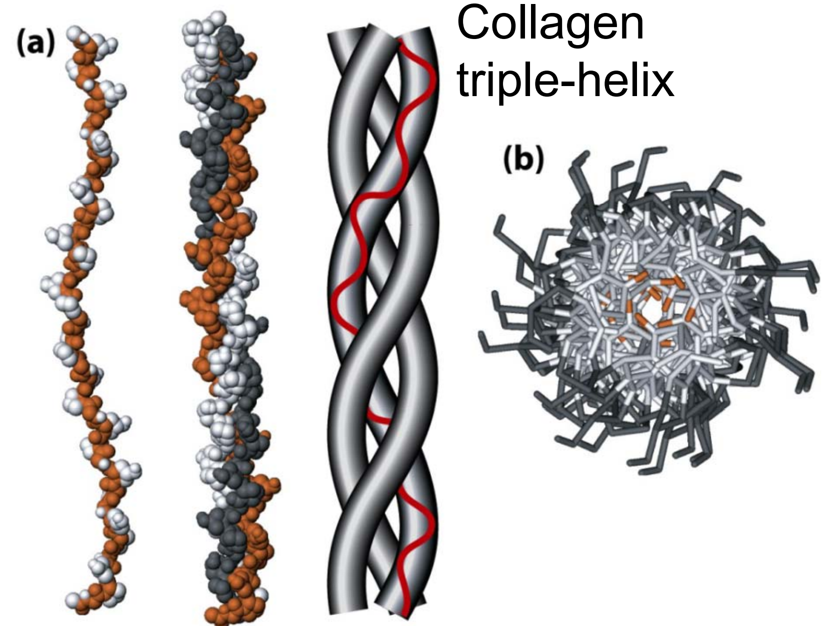


Collagens are rather inelastic fibers found in skin and bone

Collagens are complex molecules embedded in the ECM

EM of cartilage



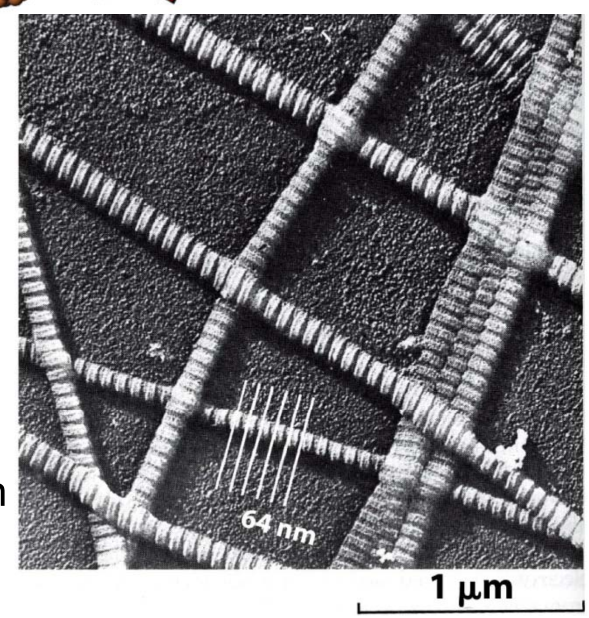


Collagen triple-helix

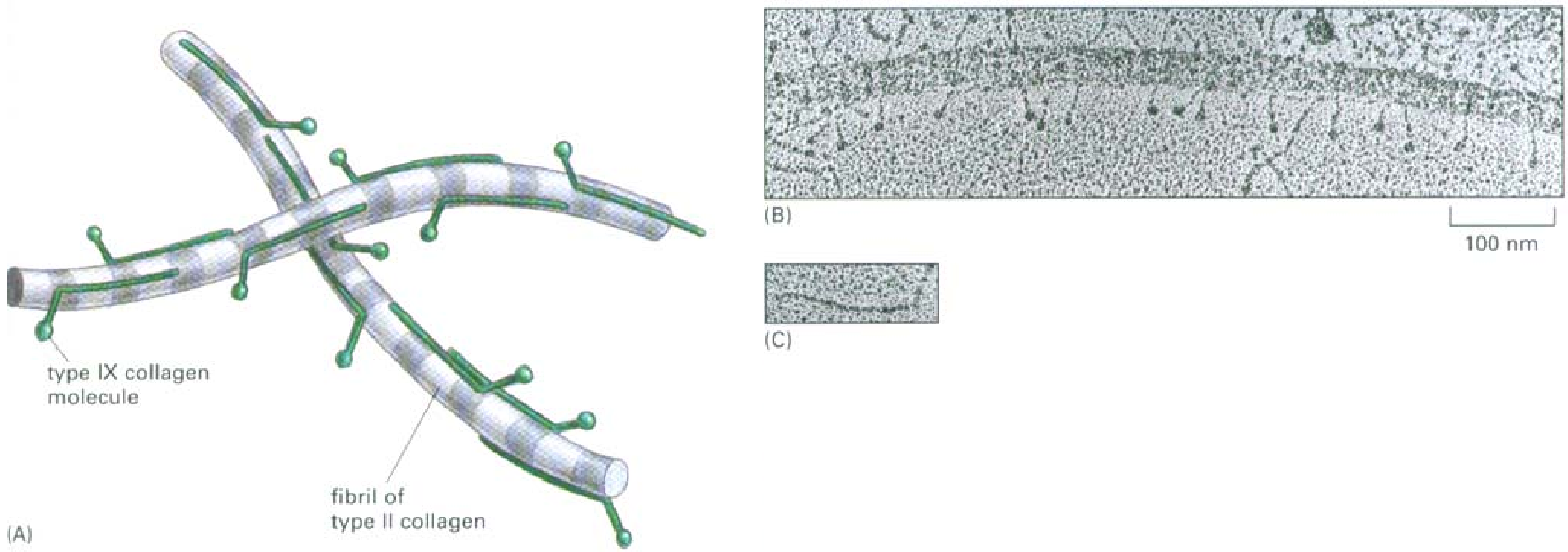
A **single collagen fiber** in the gel-forming matrix of <u>cartilage</u>

=> inflexible but resilient

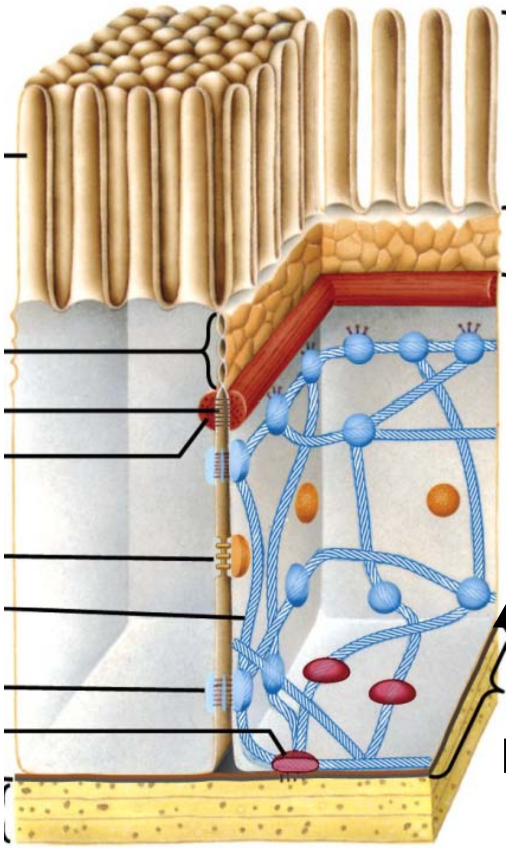
Collagens provide great tensile strength
(high mechanical load capacity)



Collagen fibers can be arrange into a network by type IX collagen



Basal lamina acts as a permeability barrier and integrates cells into tissues



Epithelial cell

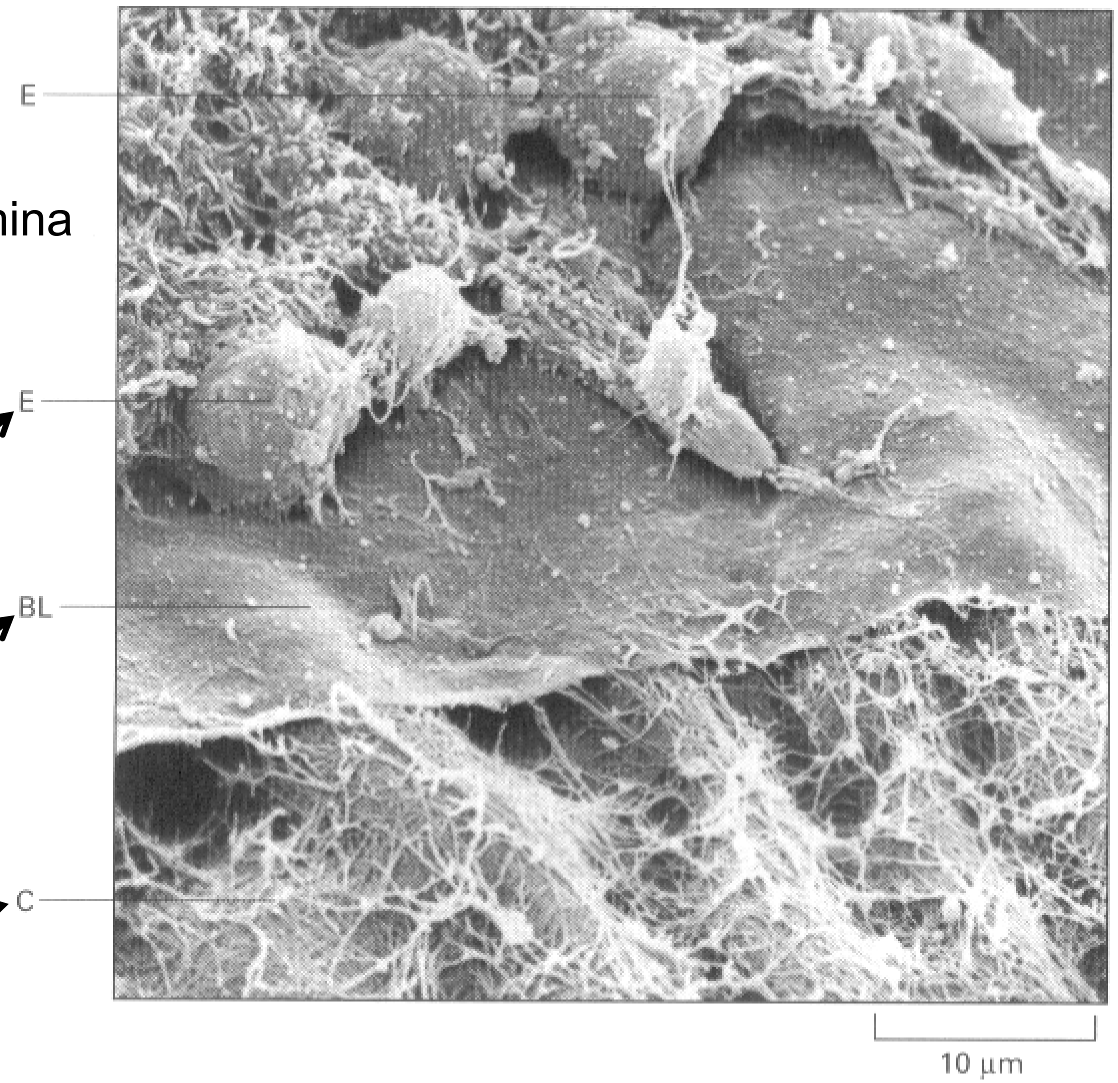
Basal lamina

ECM

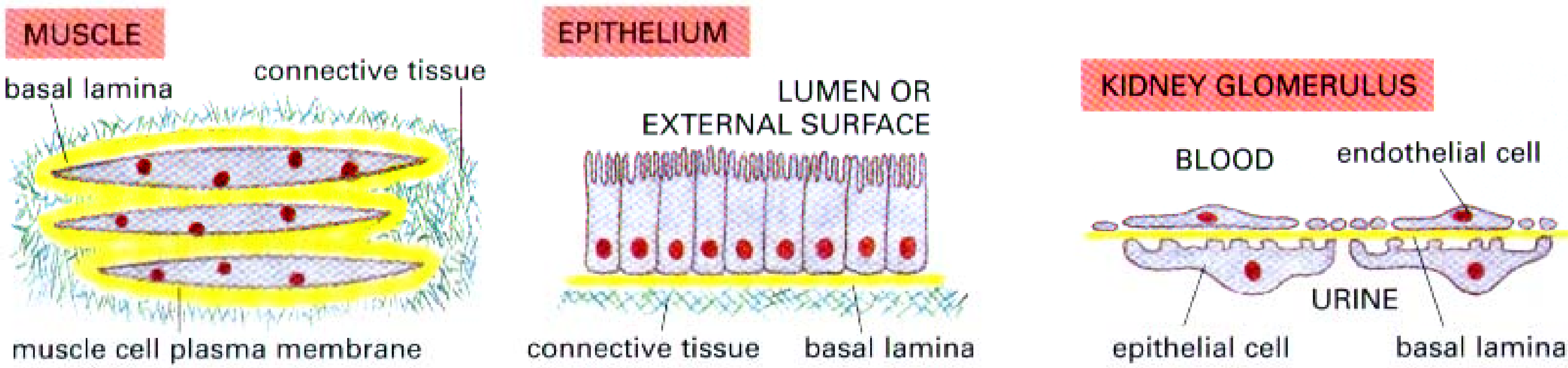
Epithelial cells sitting on top of the basal lamina

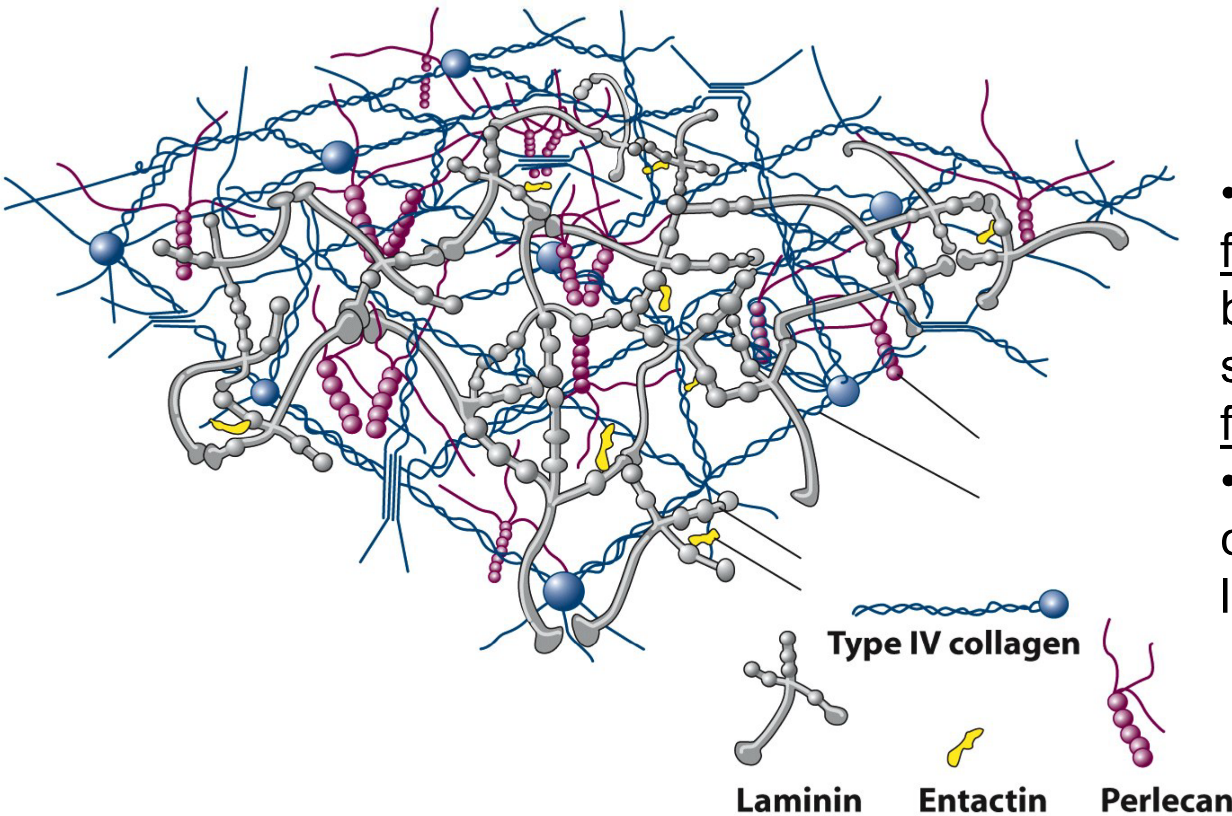
<u>Mat-type</u> basal lamina acting as a **tight barrier** for small molecules

Collagen in the ECM below the basal lamina

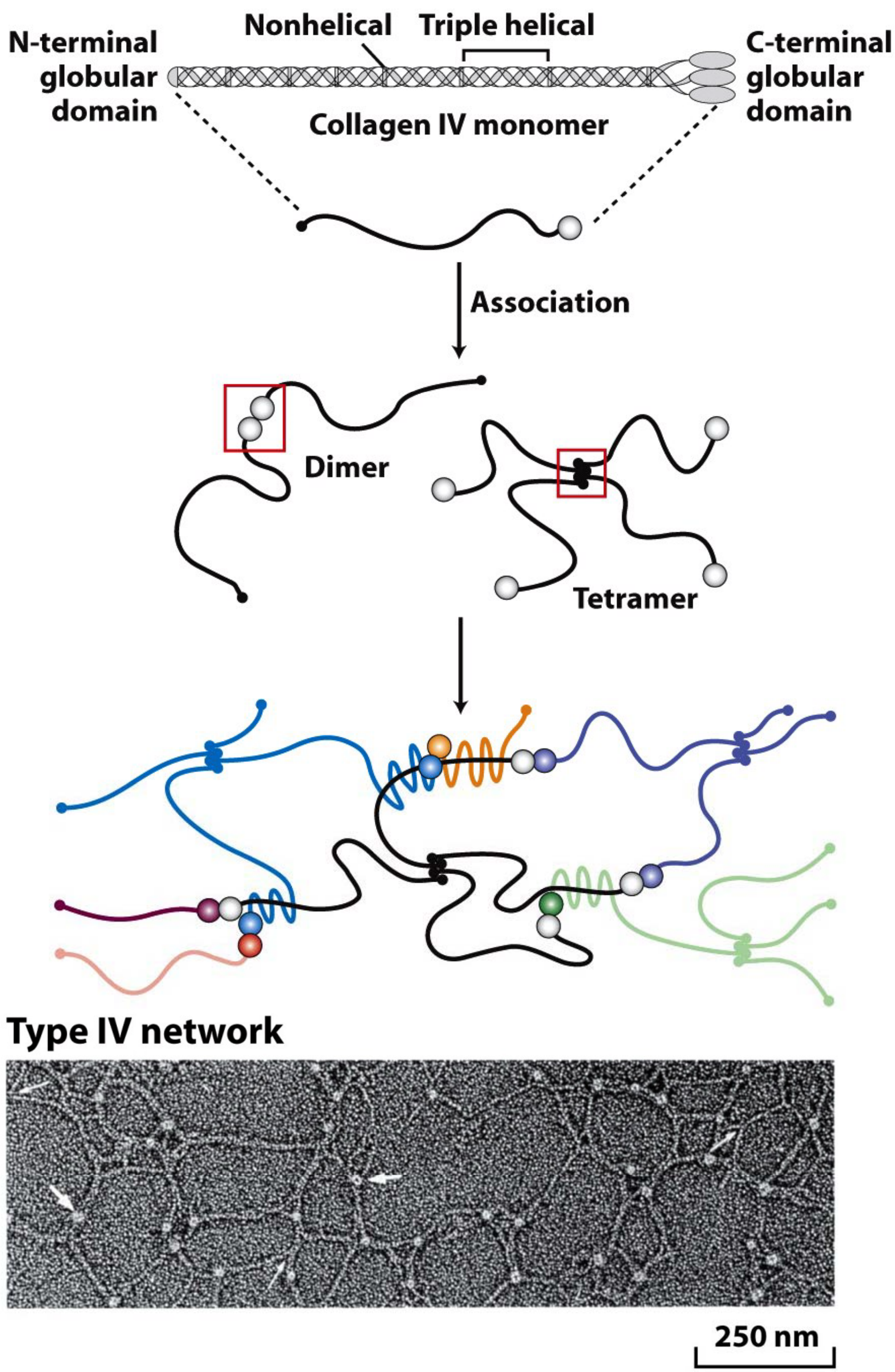


Basal lamina acts as a permeability barrier and integrates cells into tissues

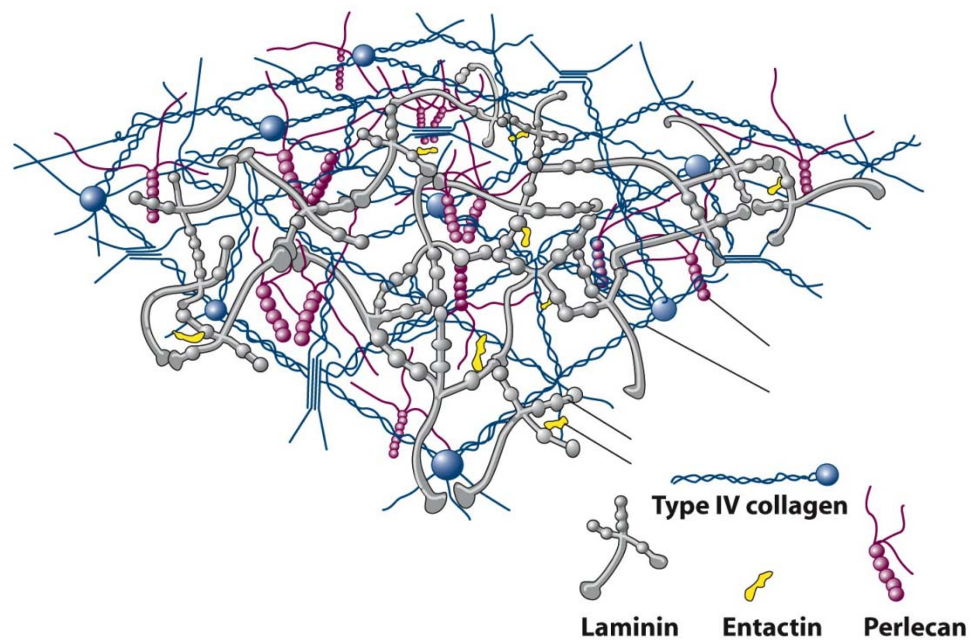




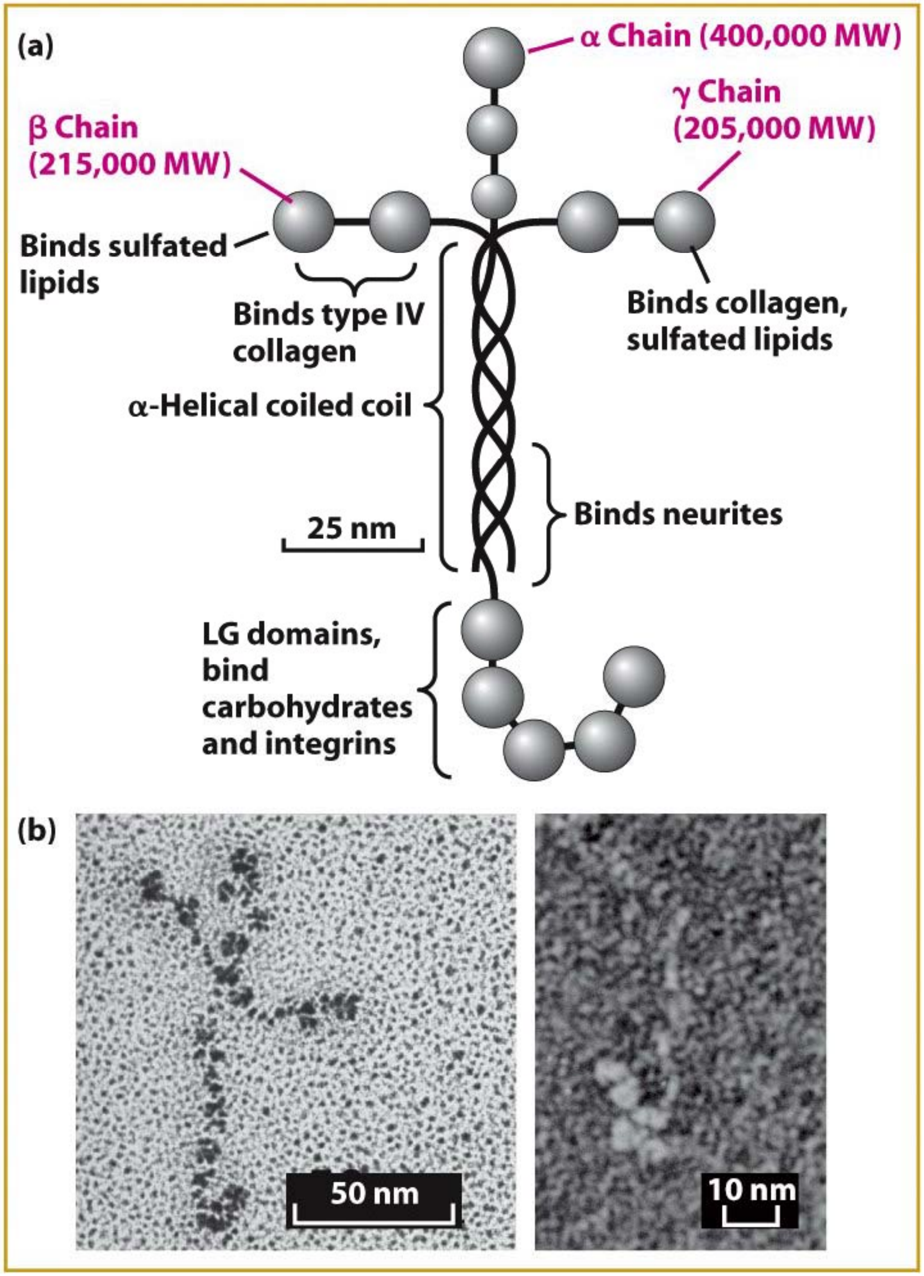
- **Type IV collagen** is a <u>sheet-forming protein</u> making the basal lamina a tight barrier for selecting molecules (<u>filter function/blood brain barrier</u>)
- **Laminin** integrates the major components of the basal lamina



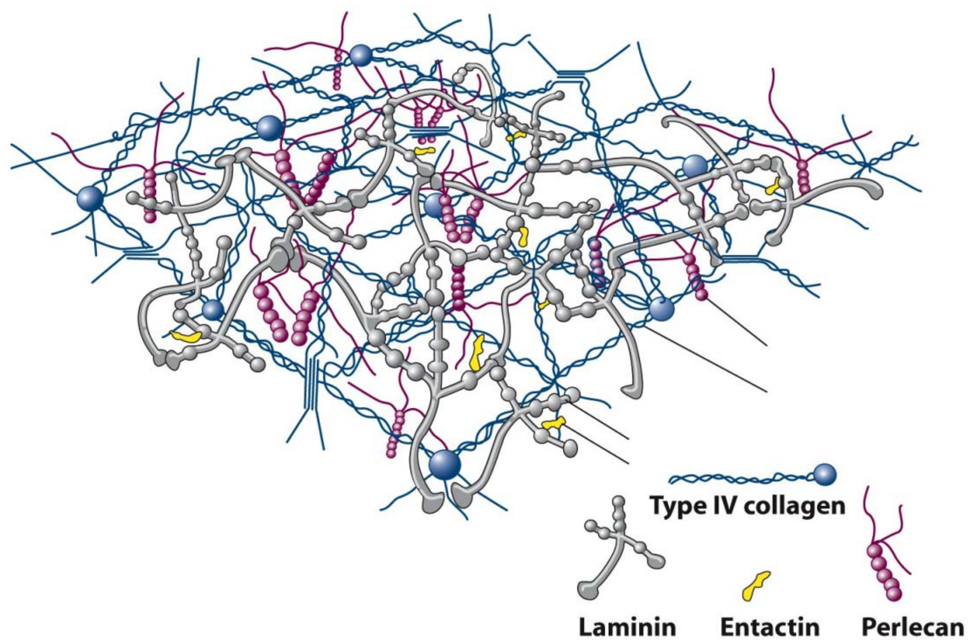
Type IV collagen can form a complex network by forming **dimer** (tail-tail) and **tetramer** (head-head)



Laminin is a multiadhesive matrix protein found in all basal lamina

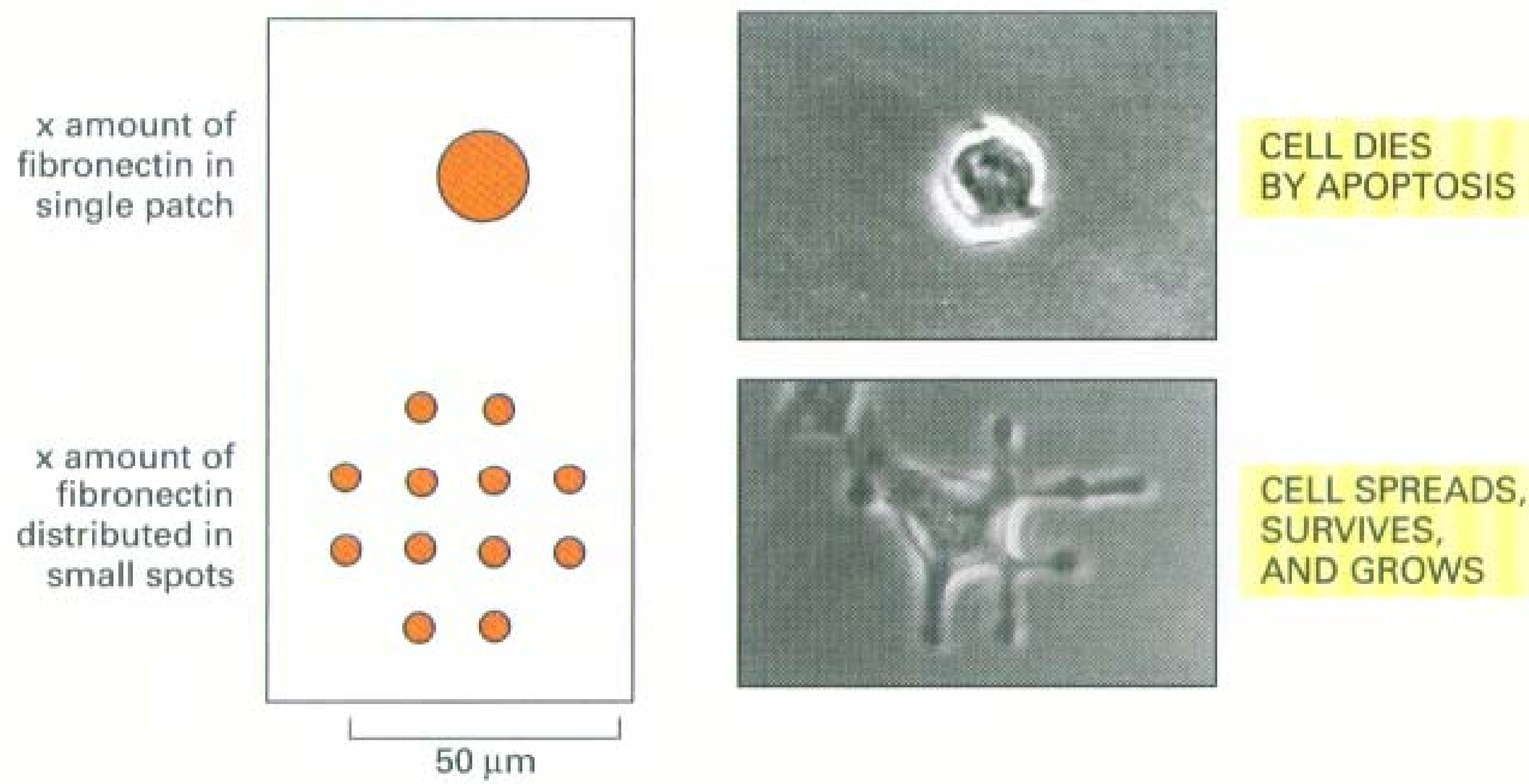


- Structure of laminin: it is a complex molecule build from <u>three chains</u> containing several **globular domains** and **coiled-coils**
- It can bind collagen, integrins, lipids, carbohydrates and even neurites



Cell contact with the ECM is important for growth, proliferation and survival

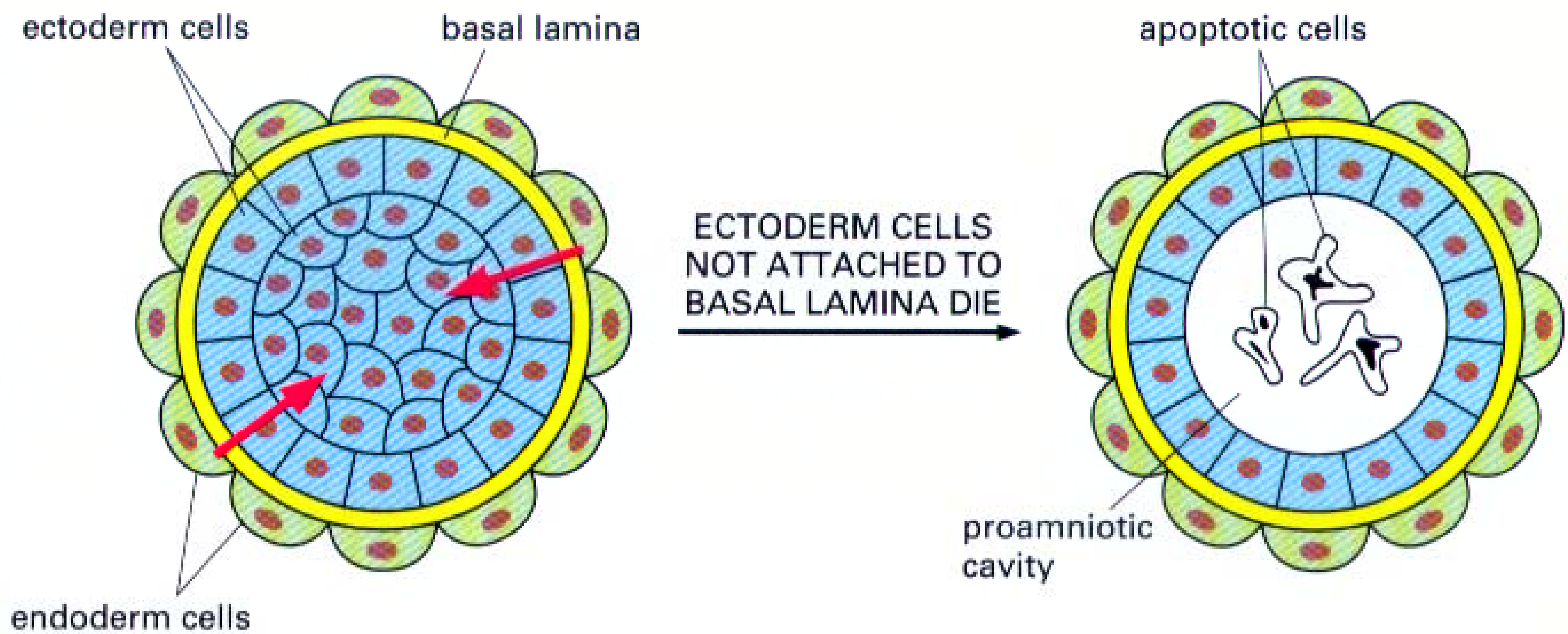
If a cell cannot nicely spread on a substrate it will eventually die



The extent of cell spreading is more important than the amount of molecules the cell interacts with

The basal lamina is important for cell survival

Embryonic development of bodies cavities:
- **Endoderm cells** send out signals that **make ectoderm cells die** (forming a cavity)
- However, those with direct contact to the basal lamina survive



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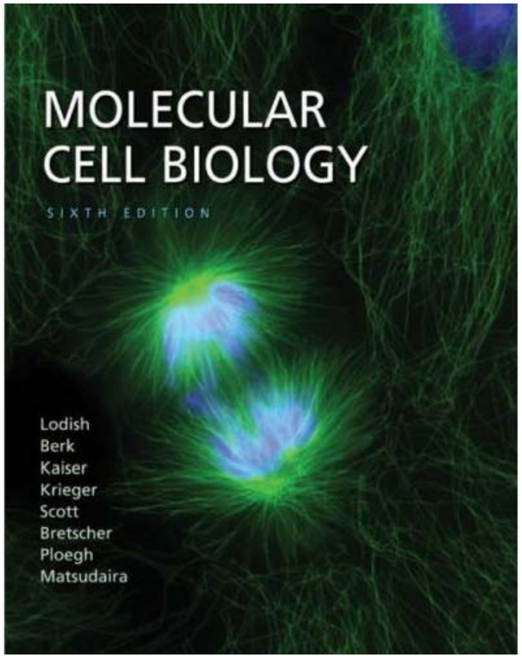

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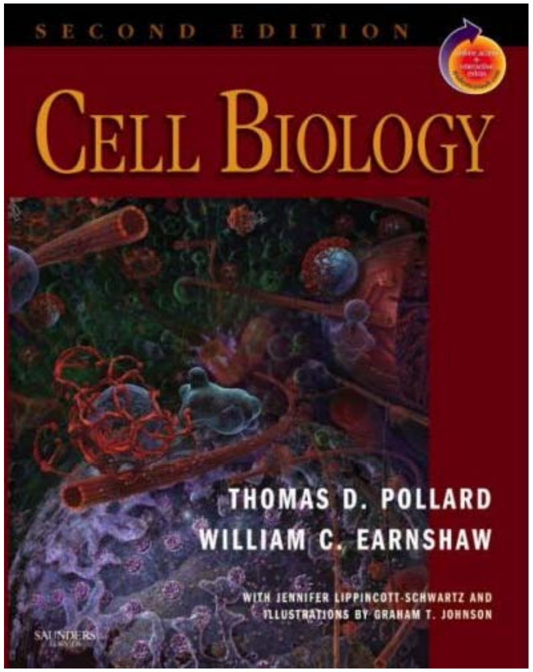

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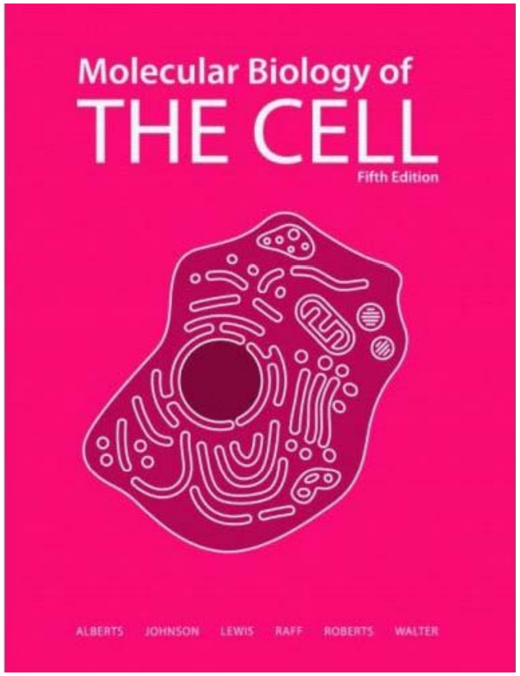

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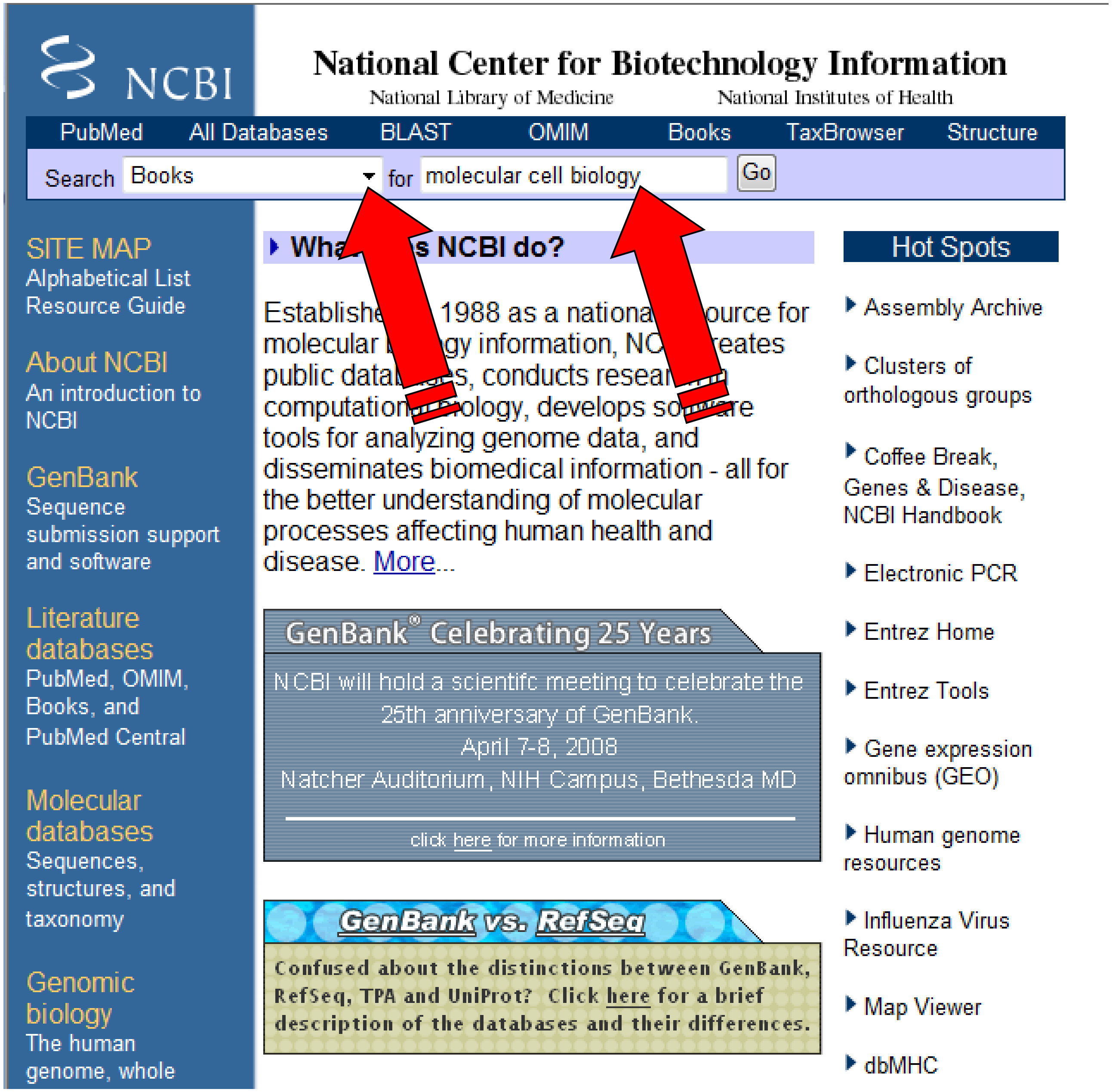

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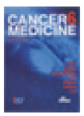

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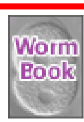

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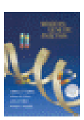

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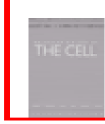

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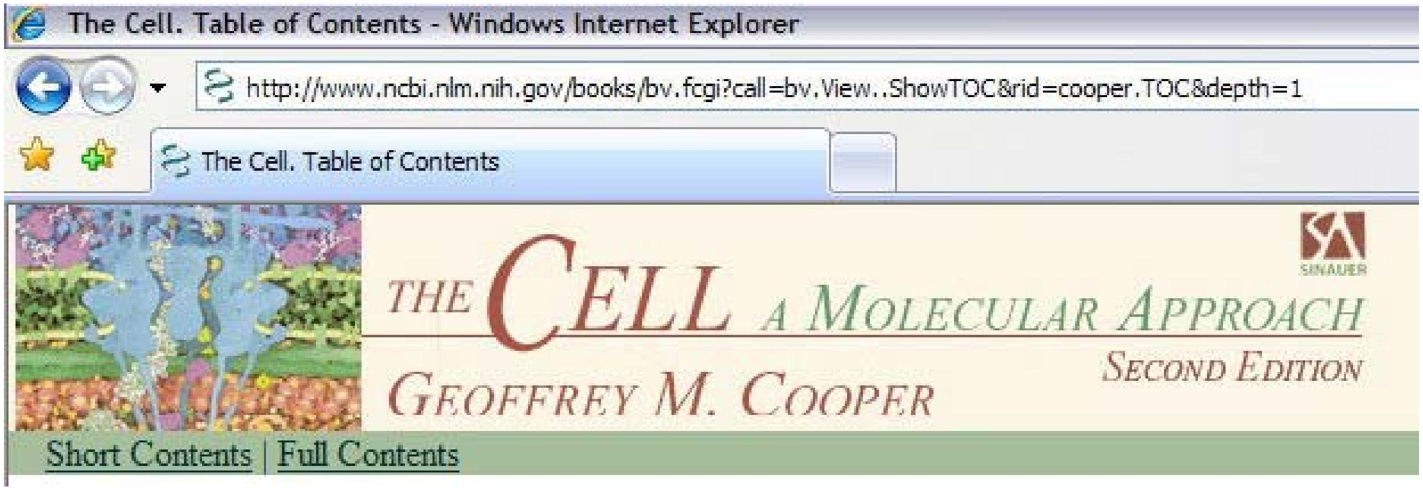
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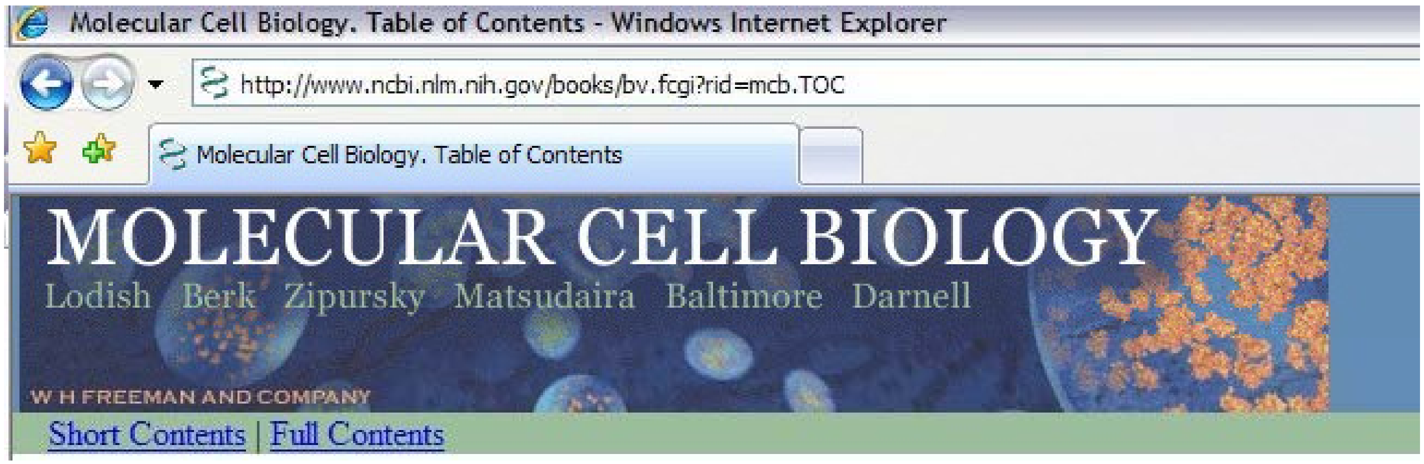
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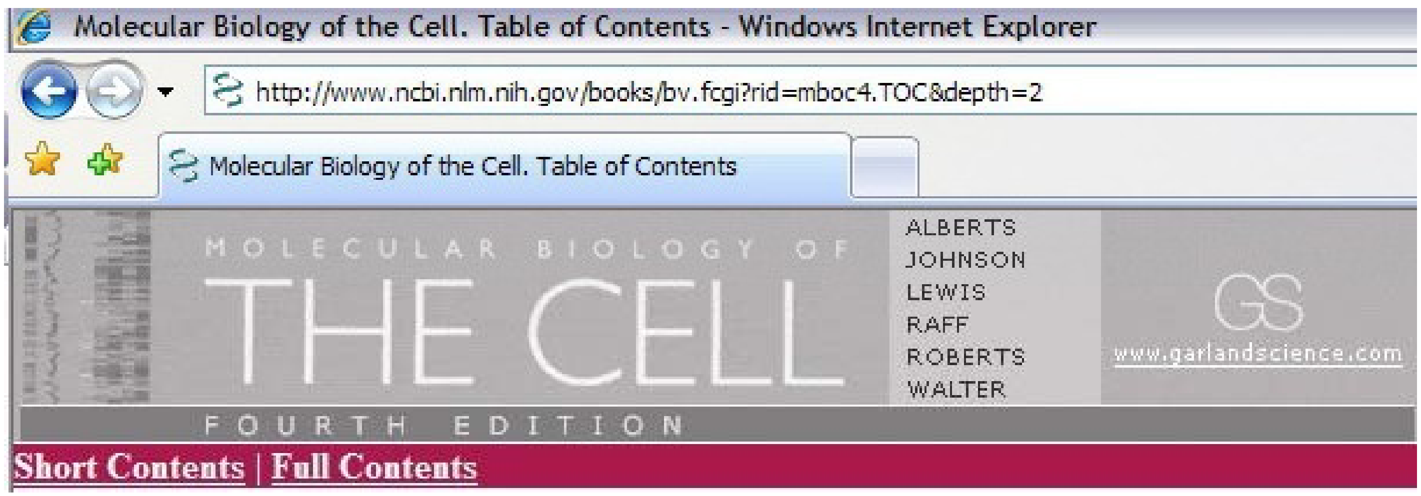
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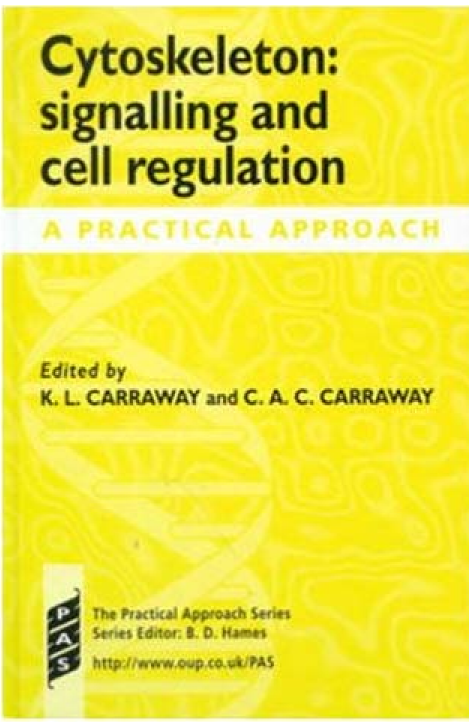

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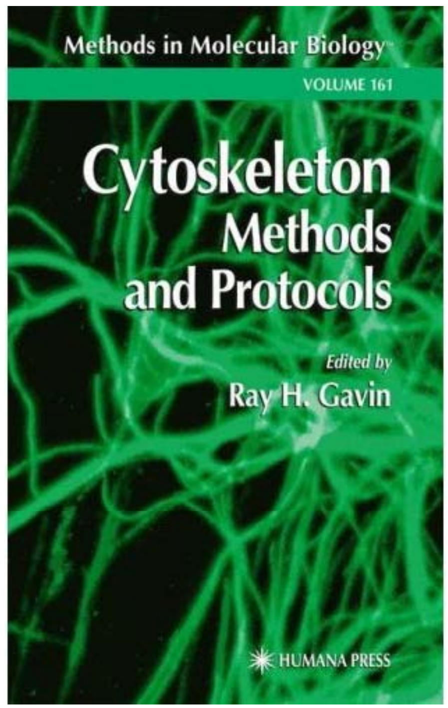

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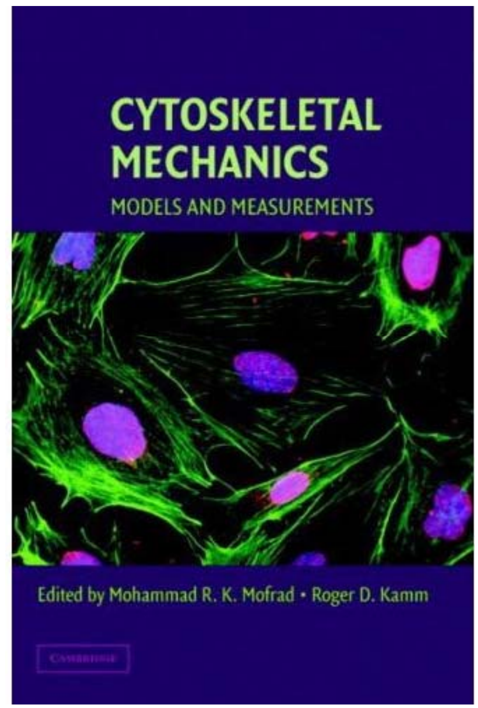

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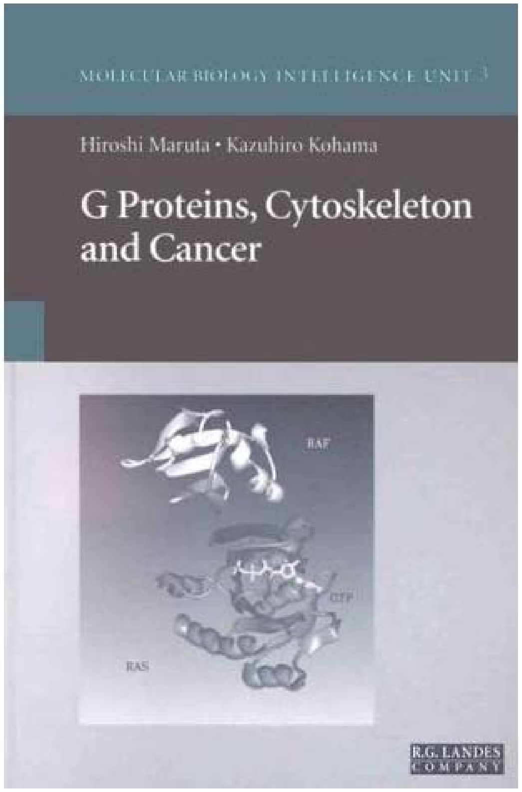

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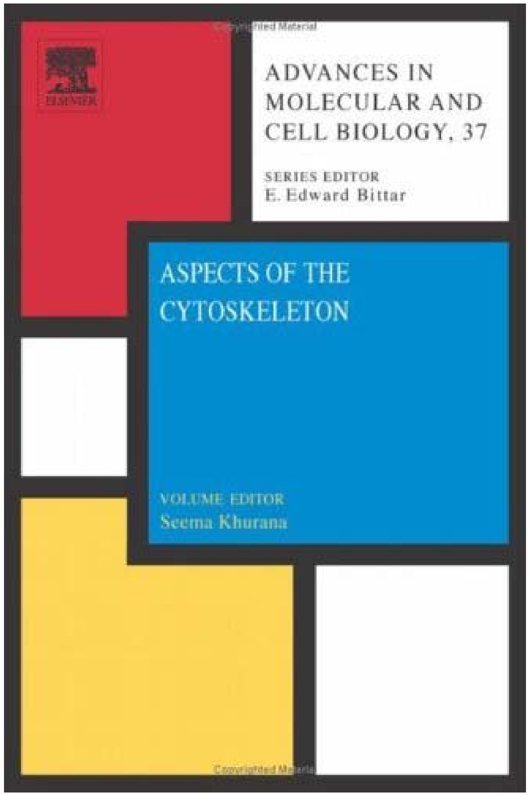

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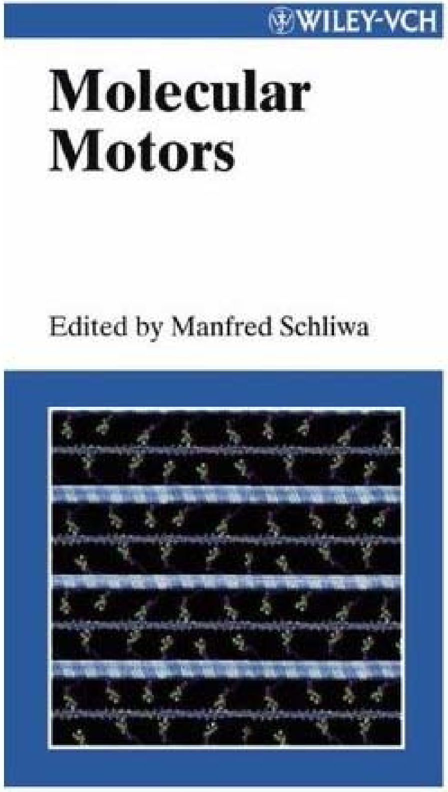

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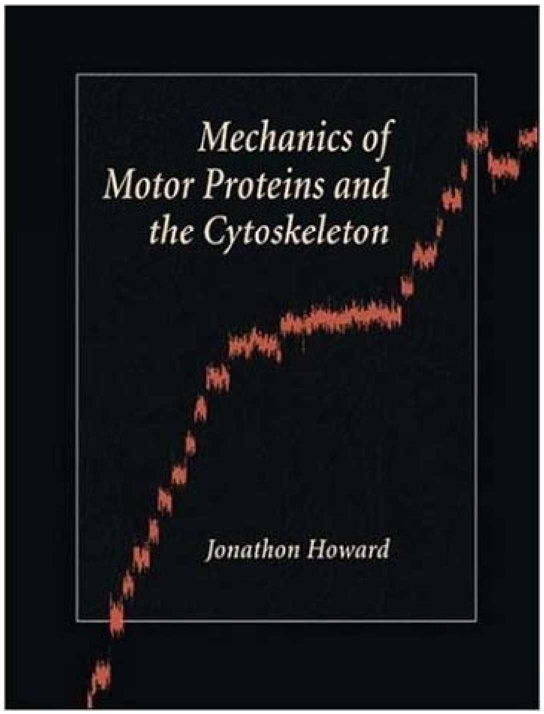

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Molecular Motors: Methods and Protocols
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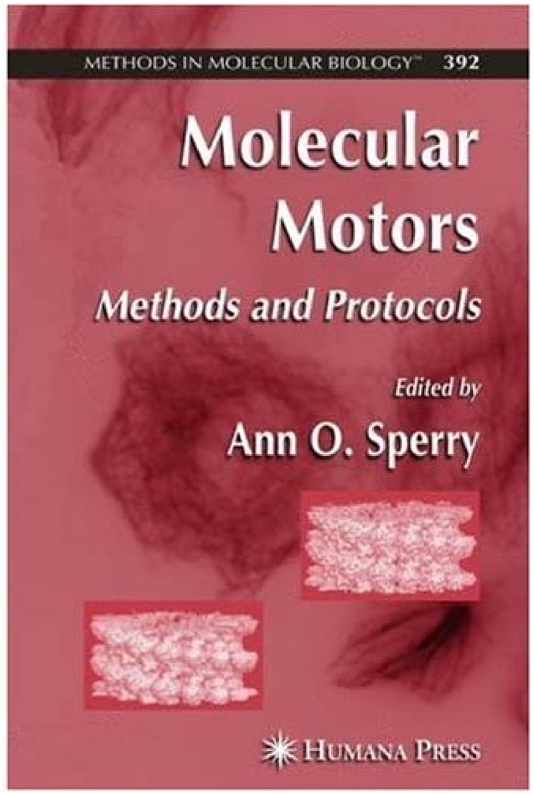

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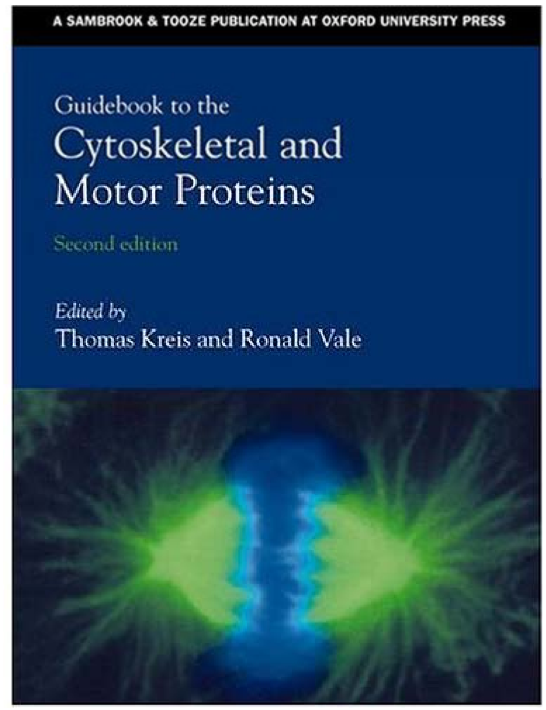

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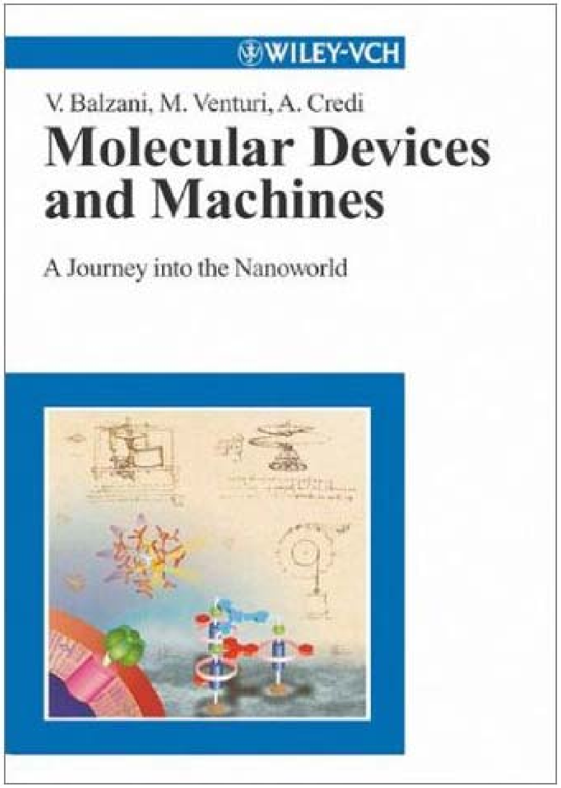

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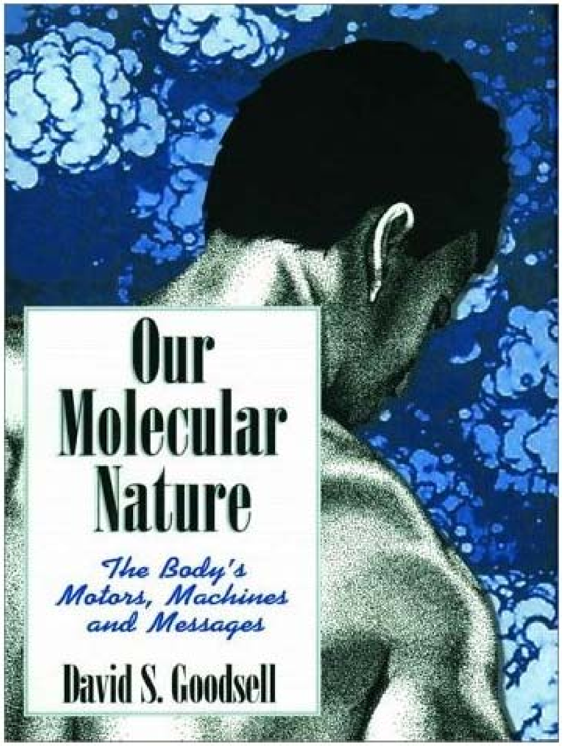

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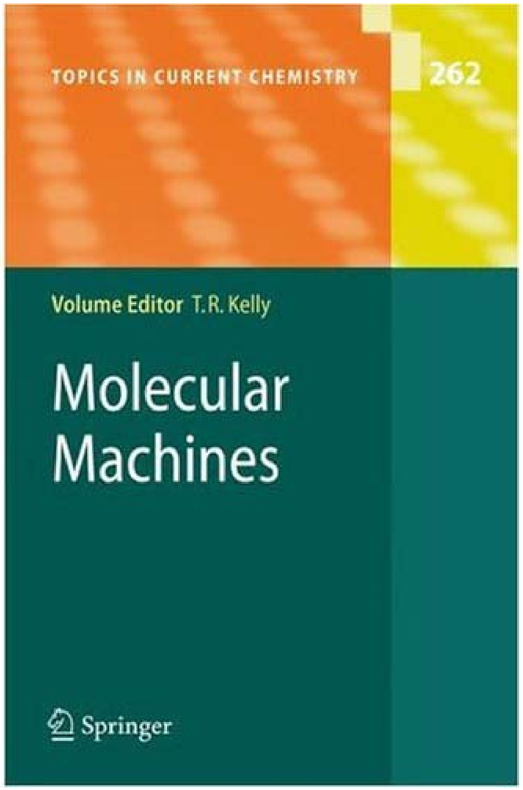

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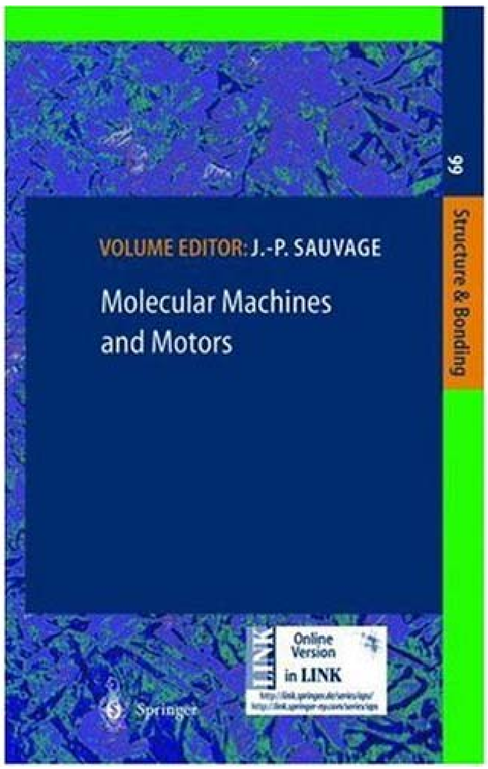

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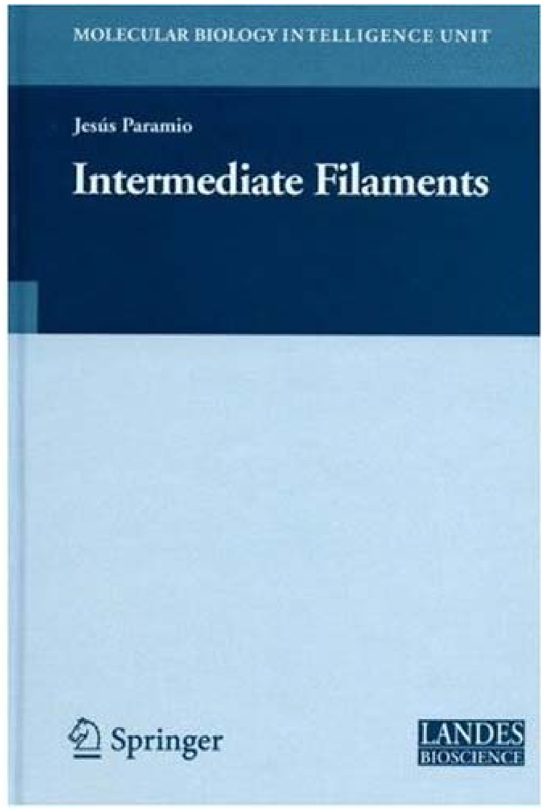

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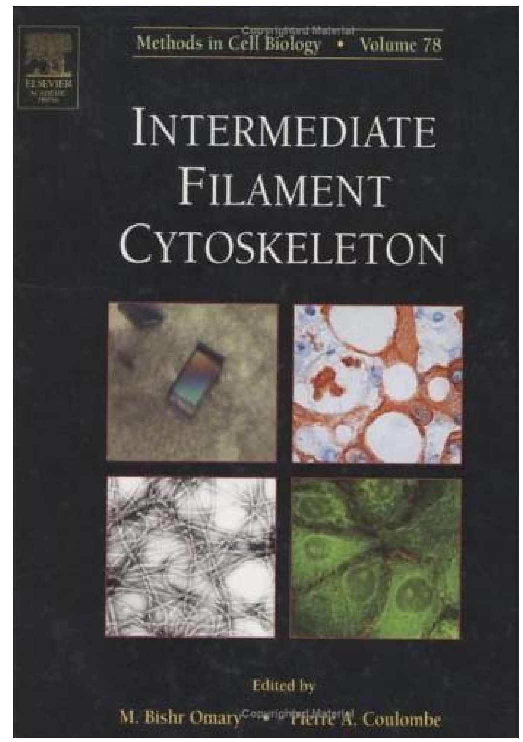

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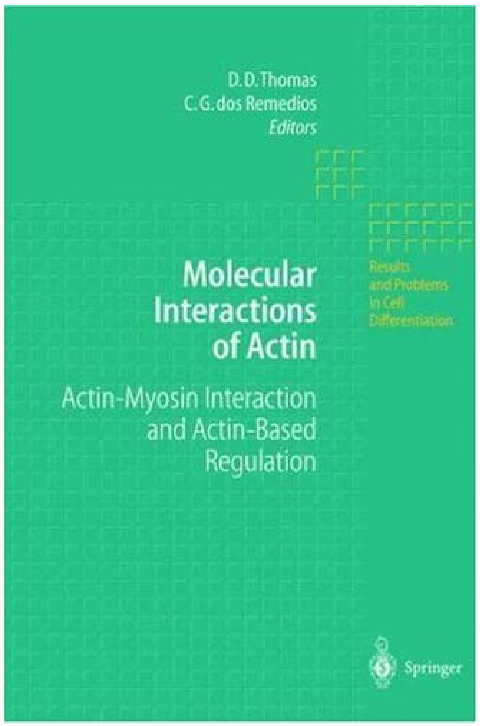

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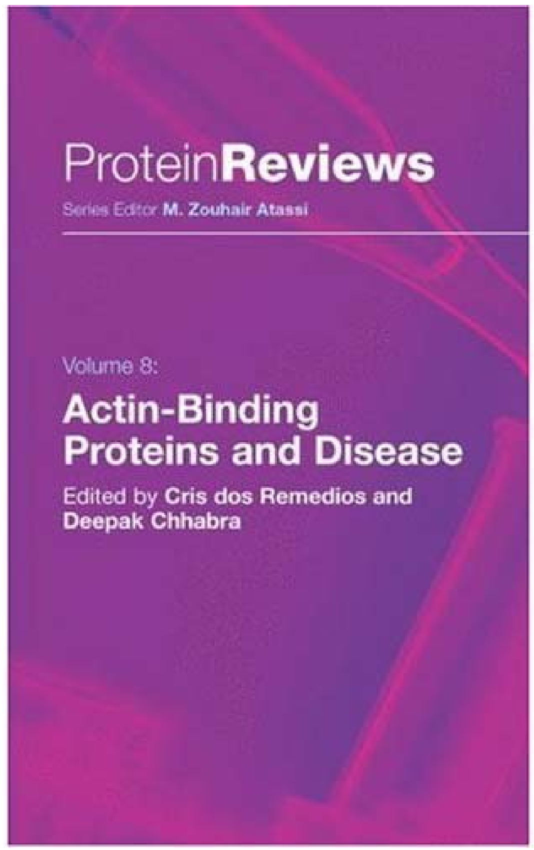
Actin-Binding Proteins and Disease by Cris dos Remedios and Deepak Chhabra



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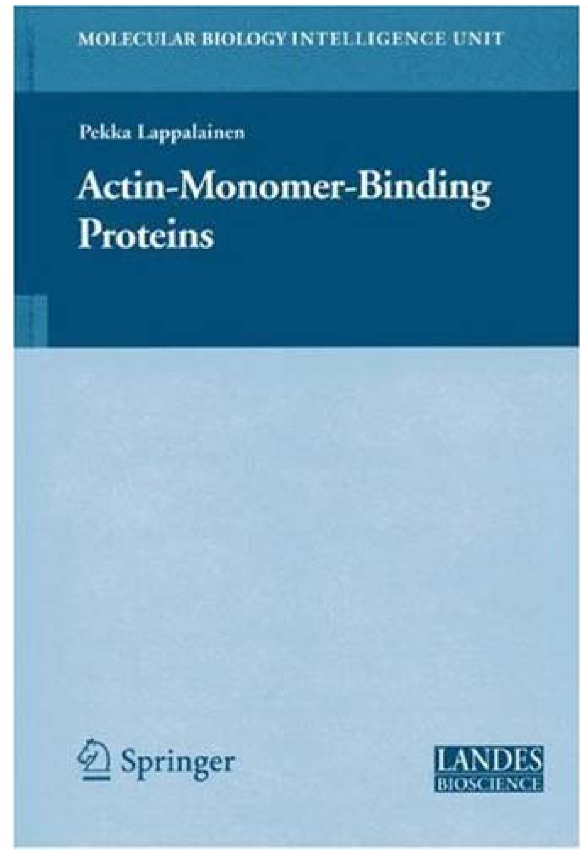
Actin-Monomer-Binding
by Pekka Lappalainen



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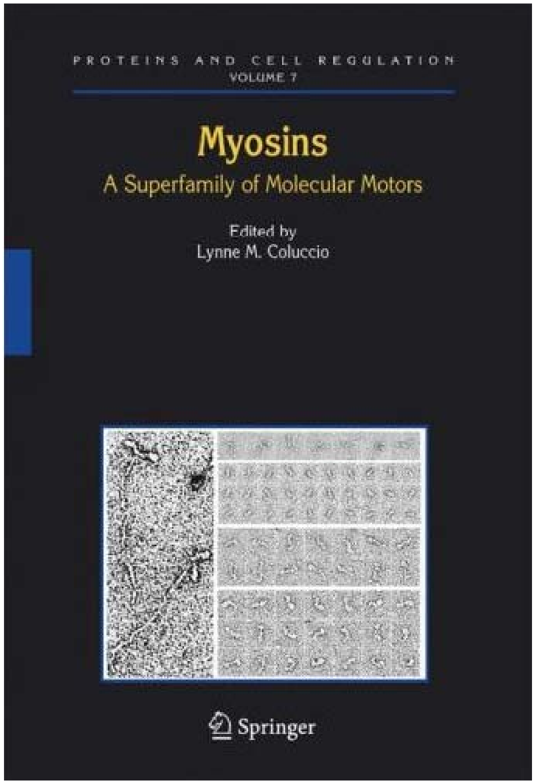
Focused/specialized literature

Myosins
by Lynne M. Coluccio



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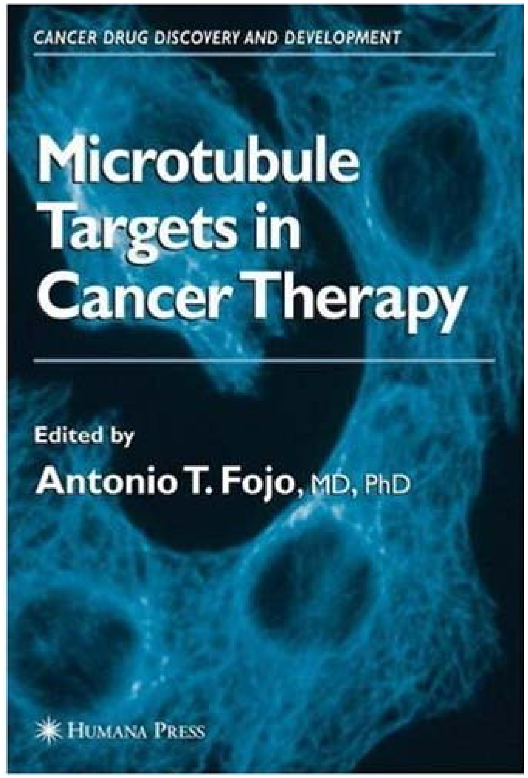
The Role of Microtubules in Cell Biology, Neurobiology, and Oncology
by Antonio Tito Fojo



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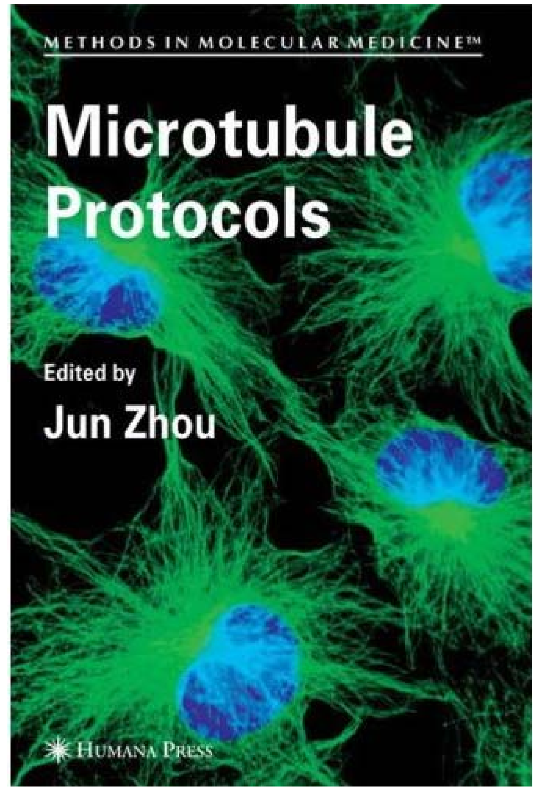
Microtubule Protocols
by Jun Zhou



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