Cell mechanics

Dan COJOC

Consiglio Nazionale delle Ricerche

CNR-IOM

Area Science Park – Basovizza

Trieste

1. Introduction (1h)

2. Physical principles (7h)

2.1. Forces at molecular and cellular level

2.2. Thermal forces, diffusion, and chemical forces

2.3. Motor proteins (types, working principles)

3. Mechanics of the Cytoskeleton and Mechnaotransduction (6h)

3.1. Cytoskeleton structure

3.2. Force generation by the cytoskeleton and cell motility

3.3. Cellular mechanotransduction (basic principles and examples)

5. Experimental techniques to study cell mechanics (10 h)

5.1. Optical, magnetic and acoustic tweezers

5.2. Super-resolution optical microscopy techniques (STED, PALM)

5.3. Lab visit and experimental optical tweezers – cell mechanics session at CNR-IOM

Tentative plan for 24 h

References:

1. J. Howard, Mechanics of Motor Proteins and the Cytoskeleton, Sinauer Associates Inc., 2001

2. D. Boal, Mechanics of the Cell, Cambridge Univ. Press, 2012

3. C.R. Jacobs, H. Huang, R. Y. Kwon, Introduction to Cell Mechanics and Mechanobiology, Garland Science Taylor & Francis, 2013.

4. Scientific articles – pdf collection; cited with the slides associated to the lectures.

5. Slides presentation for the lectures, pdf.

Useful - Email addresses:

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Introduction

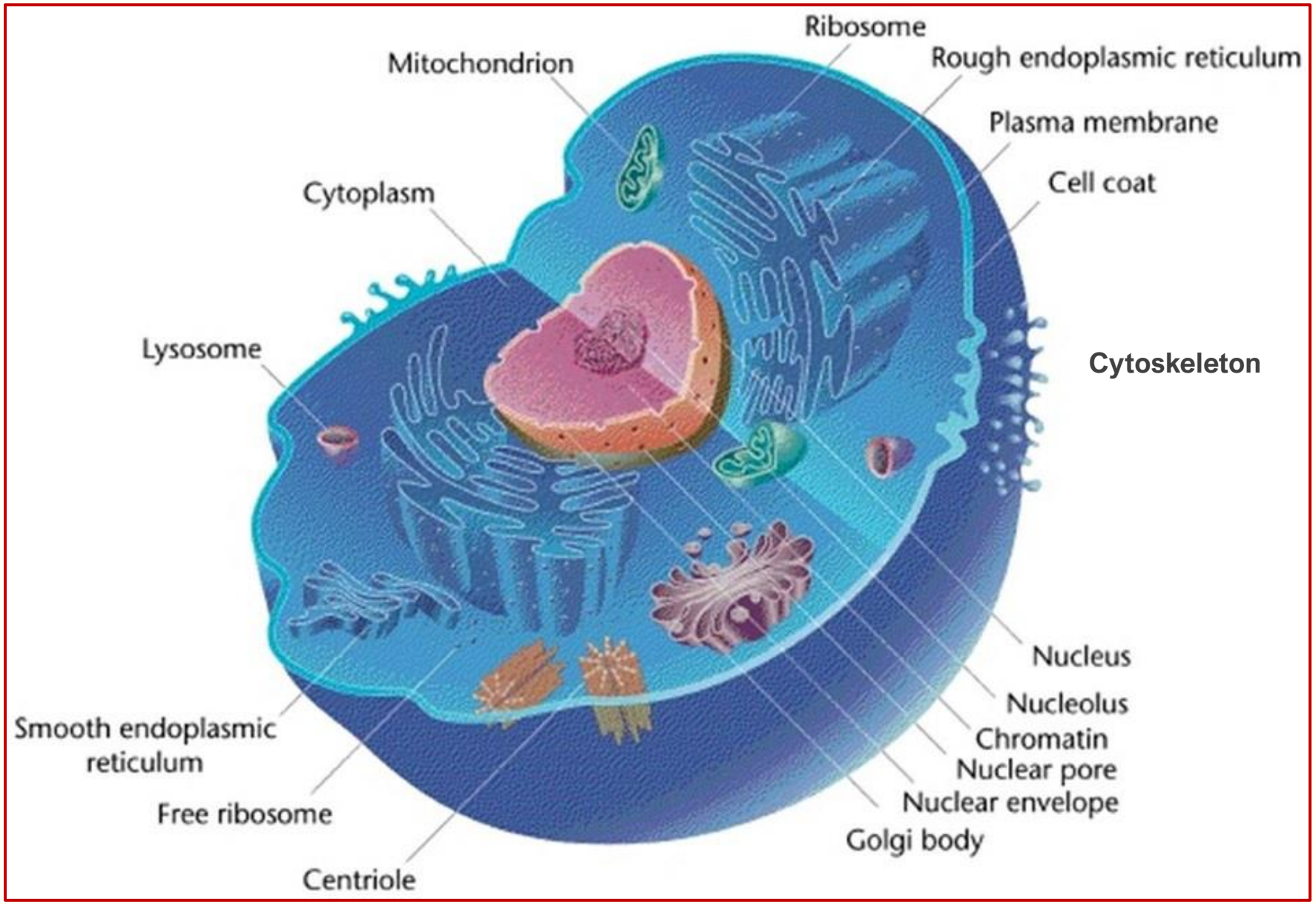
- The cell is the basic unit of life
- Components and structure of a cell
- The importance of cell mechanics and mechanotransduction

The cell is the basic unit of life

- Cells are the basic building blocks of all living things.
- The human body is composed of trillions (10^{12}) of cells.
- Cells provide structure for the body, take in nutrients from food, convert those nutrients into energy, and carry out specialized functions.
- Cells also contain the body's hereditary material and can make copies of themselves.
- Cells have many components, each with a different function. Some of these parts, called organelles, are specialized structures that perform certain tasks within the cell.
- Human cells contain the following major parts:
  - Cytoplasm,
  - Cytoskeleton,
  - Endoplasmic reticulum (ER),
  - Golgi apparatus,
  - Lysosomes and peroxisomes,
  - Mitochondria,
  - Nucleus,
  - Plasma membrane,
  - Ribosomes.

https://ghr.nlm.nih.gov/primer/basics/cell

Components and structure of the cell



https://www.nigms.nih.gov/education/Booklets/Inside-the-Cell/Pages/Home.aspx

Components and structure of the cell

Nucleus

The nucleus serves as the cell's command center, sending directions to the cell to grow, mature, divide, or die. It also houses DNA (deoxyribonucleic acid), the cell's hereditary material. The nucleus is surrounded by a membrane called the nuclear envelope, which protects the DNA and separates the nucleus from the rest of the cell. Diameter : 5 um

Cytoplasm

The cytoplasm is made up of a jelly-like fluid (called the cytosol) and other structures that surround the nucleus.

Plasma membrane

The plasma membrane is the outer lining of the cell. It separates the cell from its environment and allows materials to enter and leave the cell. Thickness: 4-6 nm

Cytoskeleton

The cytoskeleton is a network of long fibers that make up the cell's structural framework. The cytoskeleton has several critical functions, including determining cell shape, participating in cell division, and allowing cells to move. It also provides a track-like system that directs the movement of organelles and other substances within cells.

https://ghr.nlm.nih.gov/primer/basics/cell

D. Boal, Mechanics of the Cell, Cambridge Univ. Press, 2012

Components and structure of the cell

Ribosomes

Ribosomes are organelles that process the cell's genetic instructions to create proteins. These organelles can float freely in the cytoplasm or be connected to the endoplasmic reticulum (see above). Diameter: 20-30 nm .

Mitochondria

Mitochondria are complex organelles that convert energy from food into a form that the cell can use. They have their own genetic material, separate from the DNA in the nucleus, and can make copies of themselves. Diameter 1-2 um.

Endoplasmic reticulum (ER)

This organelle helps process molecules created by the cell. The endoplasmic reticulum also transports these molecules to their specific destinations either inside or outside the cell.

Golgi apparatus

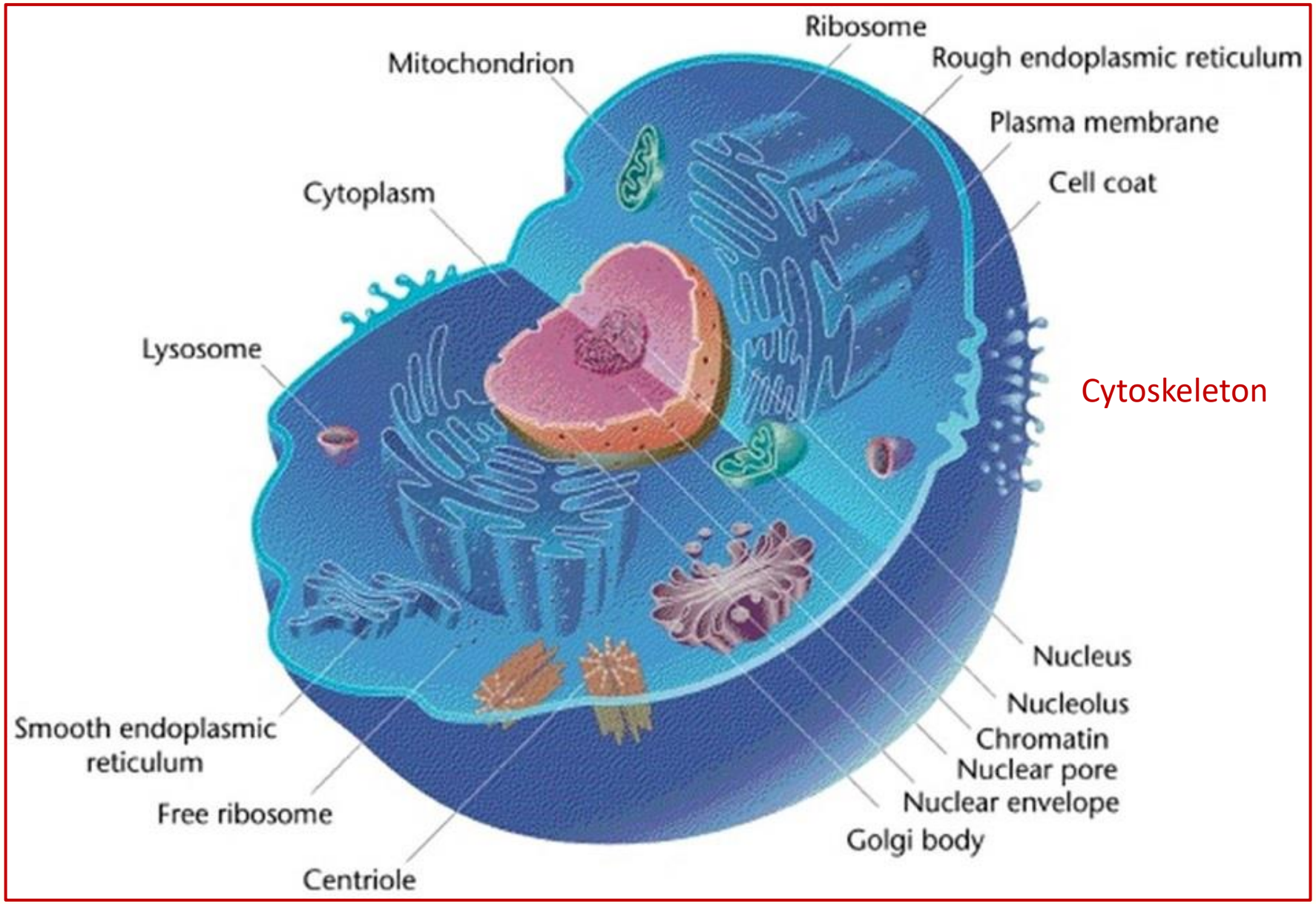
The Golgi apparatus packages molecules processed by the endoplasmic reticulum to be transported out of the cell.

Lysosomes and peroxisomes

These organelles are the recycling center of the cell. They digest foreign bacteria that invade the cell, rid the cell of toxic substances, and recycle worn-out cell components. Diameter: 50 nm – 3 um

https://ghr.nlm.nih.gov/primer/basics/cell

D. Boal, Mechanics of the Cell, Cambridge Univ. Press, 2012



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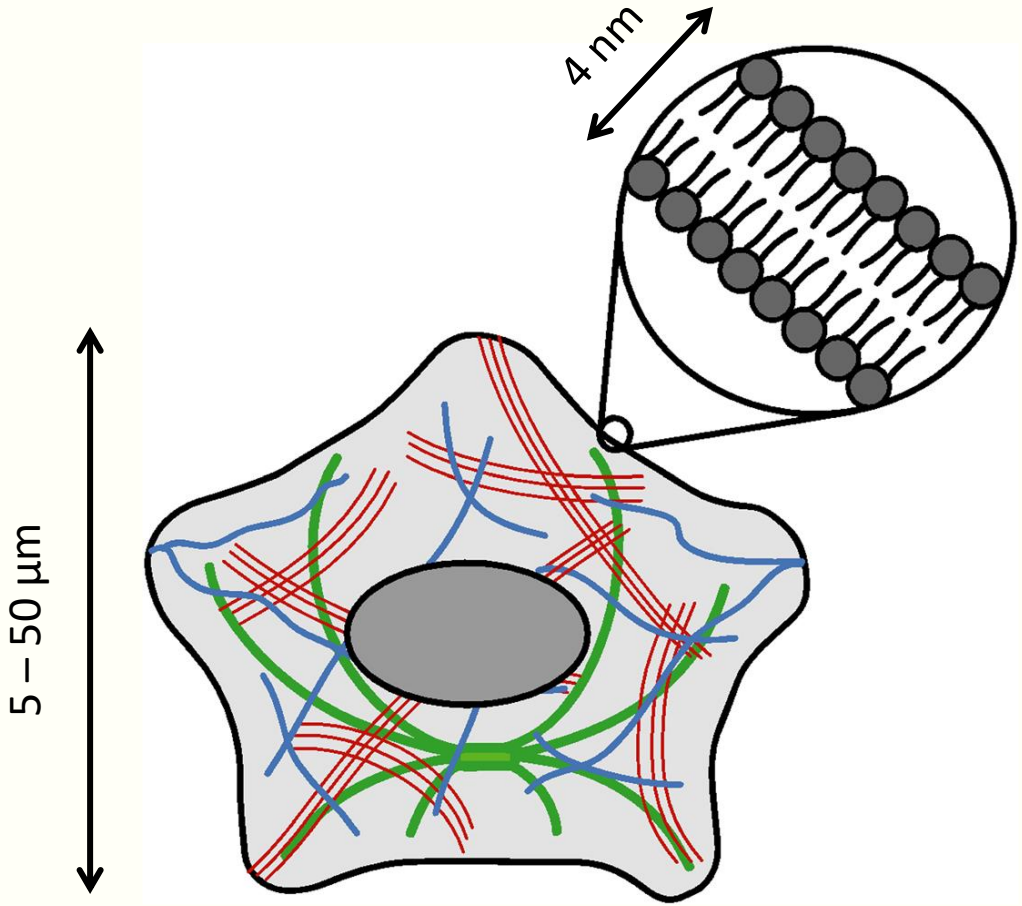
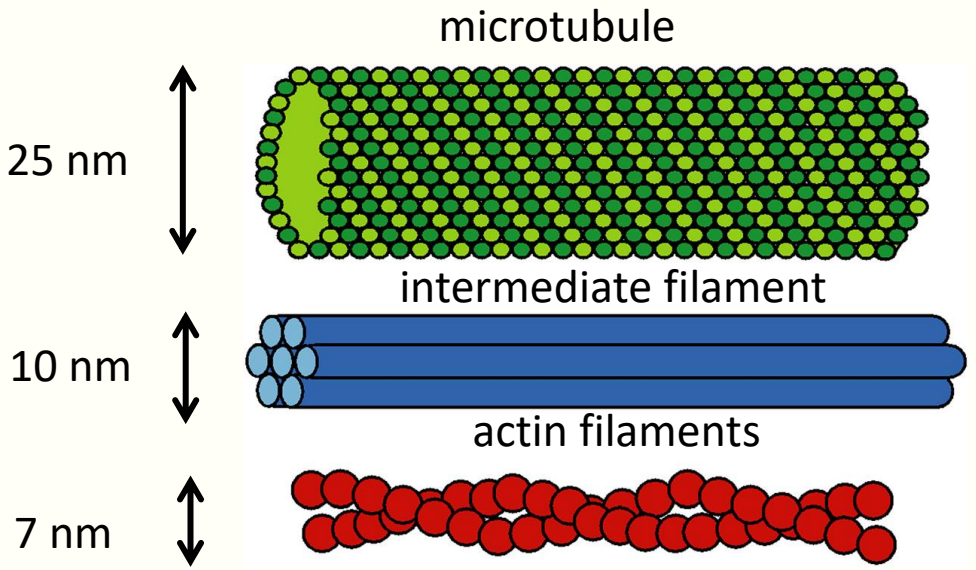
Major structural components of the cytoskeleton





The cytoskeleton is composed of **actin filaments**, **intermediate filaments** and **microtubules**.

The mechanics of a cell is also defined by its **membrane**, **nucleus**, and **cytoplasm**.

M.L. Rodriguez, P.J. McGarry, N.J. Sniadecki, *Appl. Mech. Rev.* 65, 060801 (2013)

- **The importance of cell mechanics and mechanotransduction**

MANY BIOLOGICAL PROCESSES ARE DOMINATED BY MECHANICAL FORCES:

- DNA SEPARATION IN CELLULAR MITOSIS
- STEM CELL DIFFERENTIATION
- VIRAL SPREADING
- CELL ADHESION AND MIGRATION

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MANY IMPORTANT DISEASES INVOLVE FORCES:

- CANCER
- MALARIA
- OSTEOPOROSIS

....

THEREFORE OUR BODY DEVELOPED EXTREMELY EFFICIENT STRATEGIES TO SENSE FORCES:

- HEARING
- TOUCH
- SYMPATHETIC CONTROL OF VASCULAR PRESSURE OR CONTROL OF RESPIRATION
- PROPRIOCEPTION

....

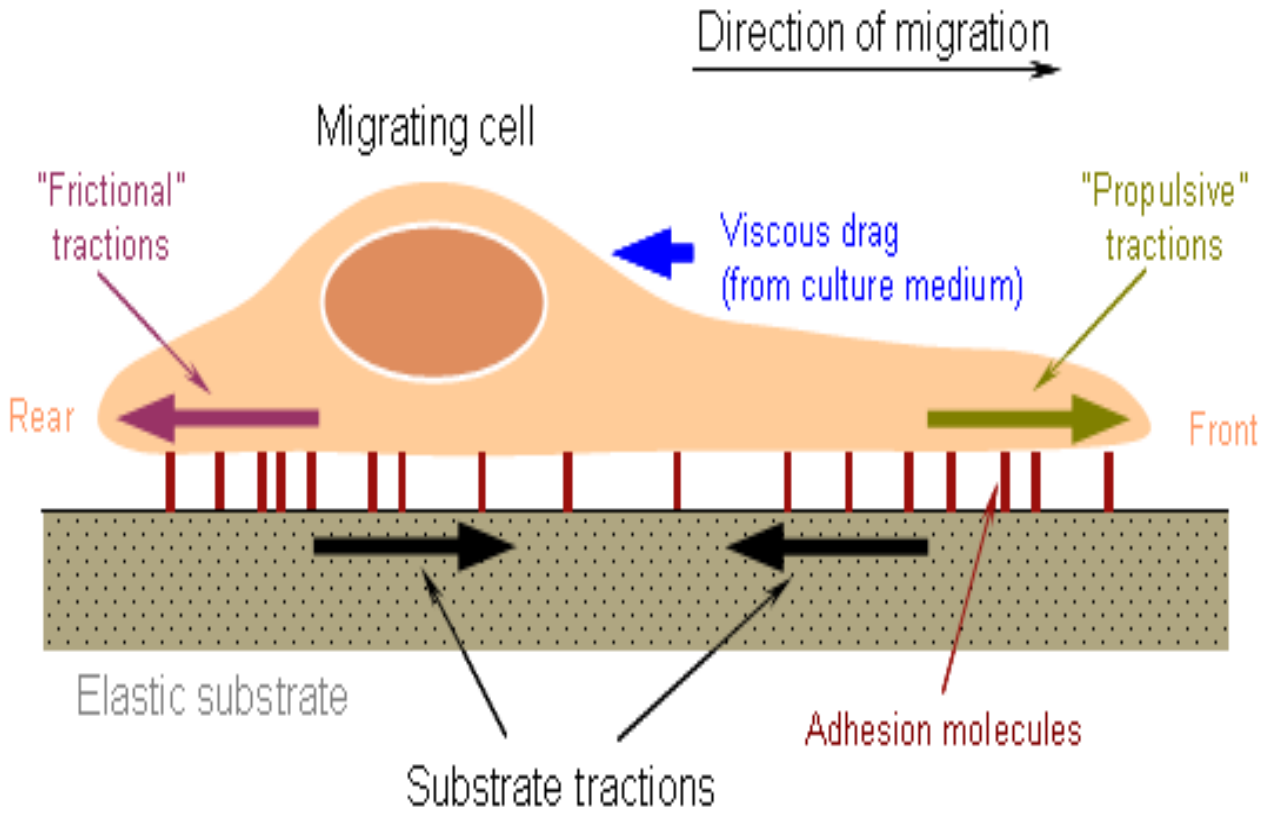
Cell adhesion and migration

Schematic of a migrating cell and the tractions it generates on the substrate.

The thick arrows **(black)** represent forces with which the **cell acts upon the substrate**.

The **elastic substrate reaction** forces acting on the cell are represented by **green** and **purple** forces.

The **"propulsive" tractions** act mainly on the cell front, while **"frictional" tractions** act at the cell rear.



The importance of cell mechanics and mechanotransduction. Examples

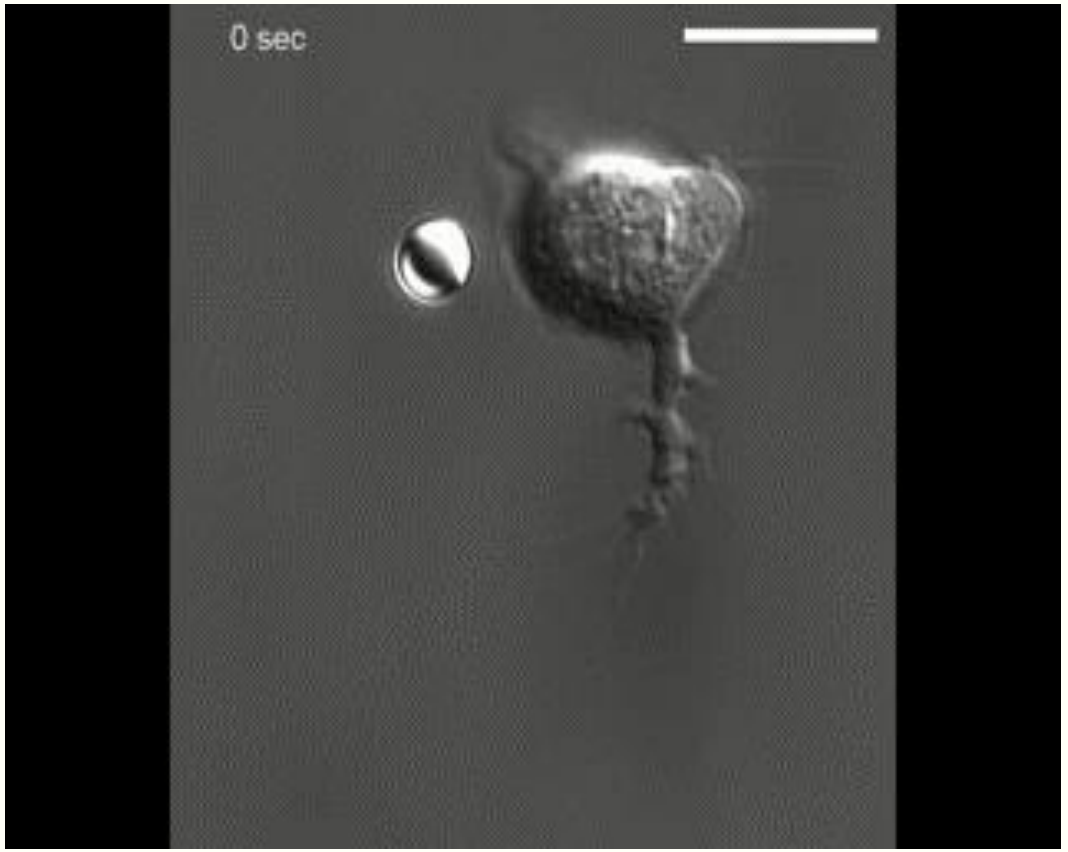
Cell migration

Neutrophil moving under the effect of a chemoactractant: formyl-methionine-leucine-phenylalanine (fMLP), released from a microsource (polylactic-co-glycolic acid (PLGA) bead).

An individual PLGA particle loaded with fMLP is optically micromanipulated and moved close to the membrane of a HL-60 cell (neutrophil). The cell starts to polarize and migrate in the direction of the particle.

Neutrophils (also known as neutrocytes) are the most abundant type of granulocytes and the most abundant (40% to 70%) type of white blood cells in most mammals. They form an essential part of the innate immune system.

Scale bar, 10 µm



H. Kress et al, *Nature Methods* 6, 905 (2009)

Why cell mechanics is important ? Examples

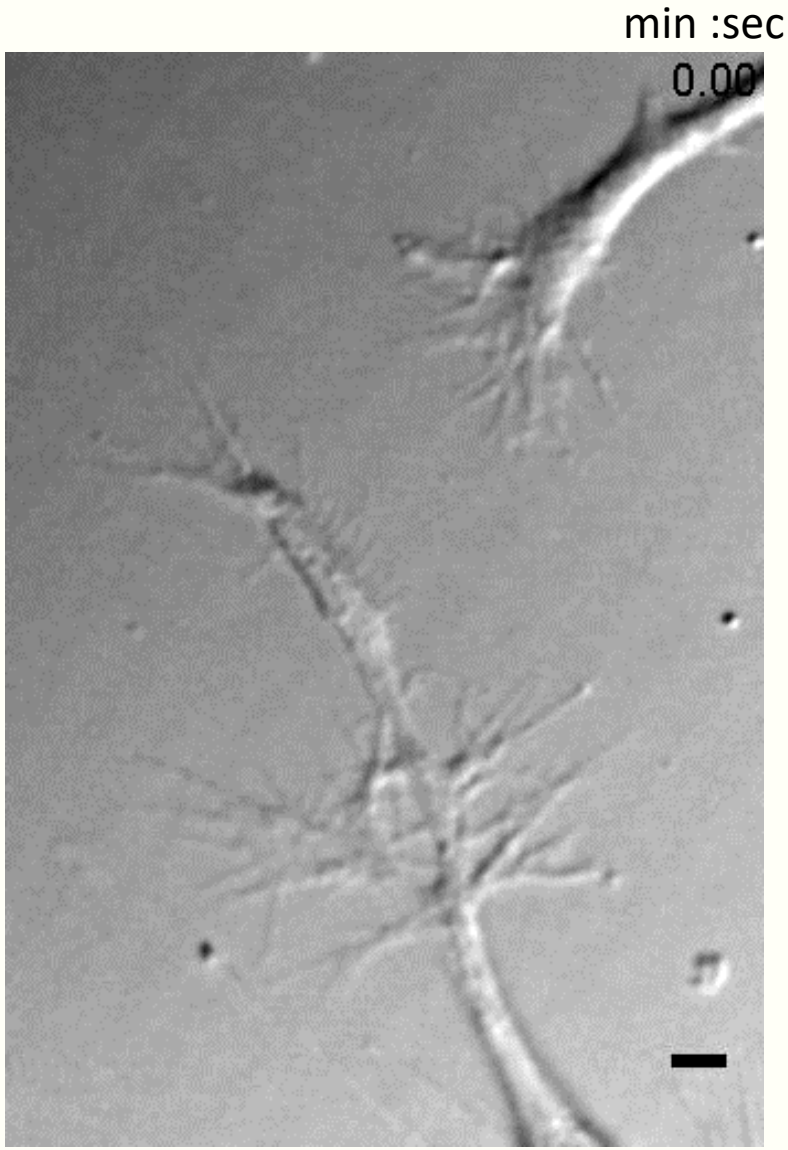
Two neurons interacting each-other during the neuronal development stage.

Molecular cues are released by neurons and induce cell migration

Although the observation is limited to 2 hours, one can see that the neurons get in touch (filopodia), as a result of the growth cone (GC) search.

GC interact bio-mechanically to eventually form synapses.

Hipocampal Rat Neurons, 2 DIV (two days in vitro)
Scale Bar = 2 µm;
Acquisition freq= 1 frame every 5 s (0.2 Hz)



F. Difato *et al* (2006) OM-Lab & SISSA

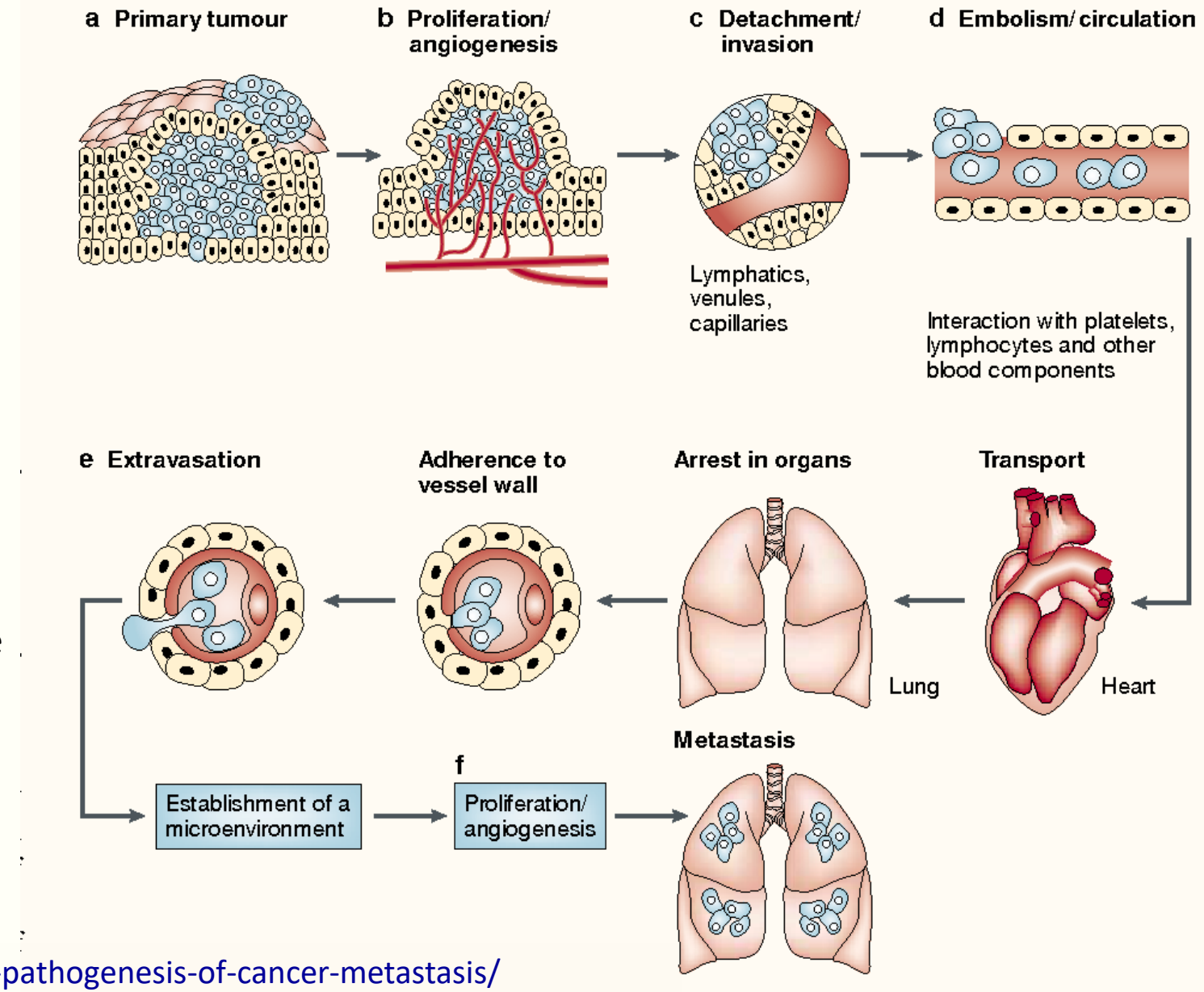
The importance of cell mechanics and mechanotransduction. Examples

Cancer metastasis

The journey of cancer cells from primary tumor to secondary tumors.

Tumor cells proliferate and leave the primary tumor, invading the limphatic and blood circuit.

They adhere to vessel walls and extravase to establish a secondary tumor.



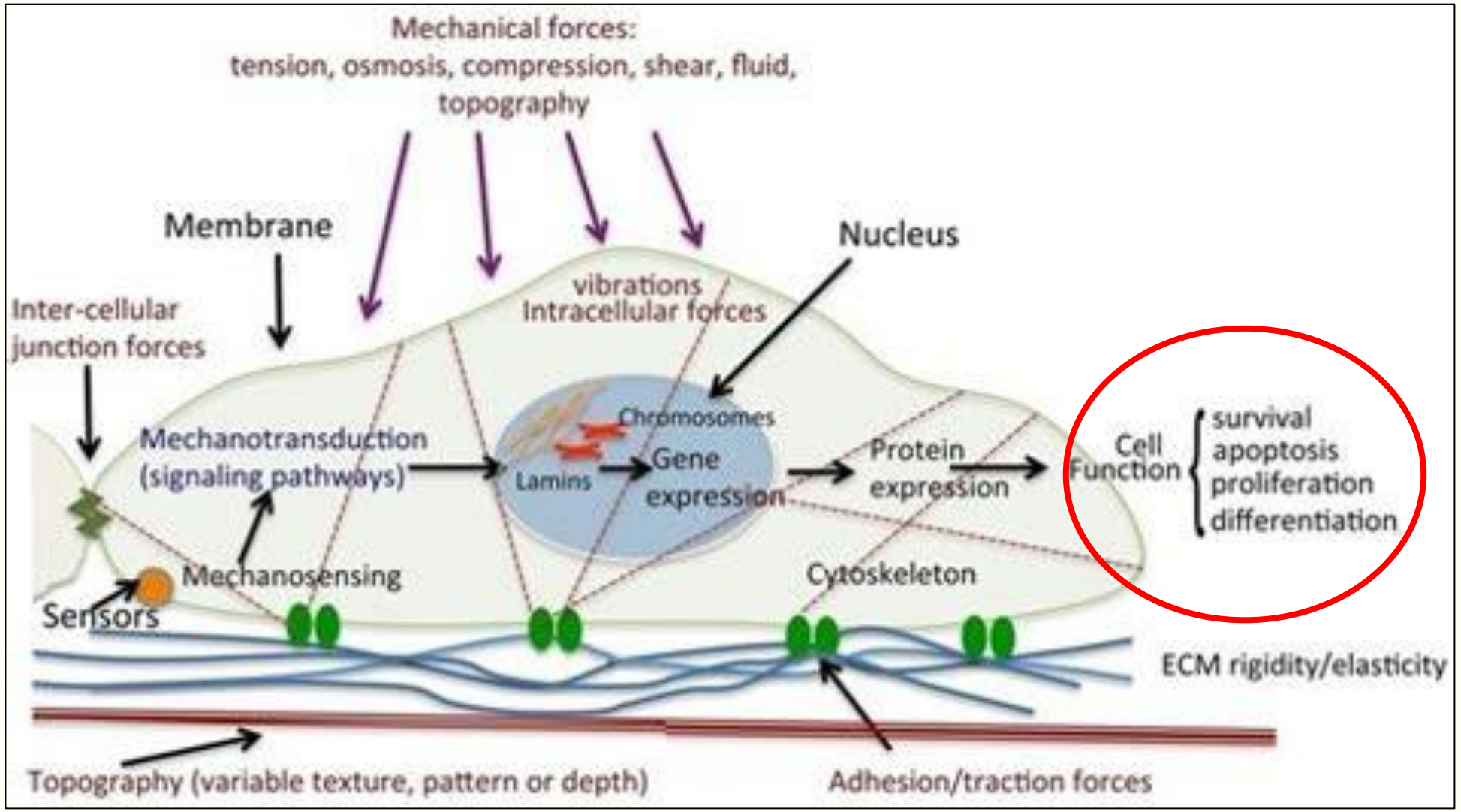
https://www.semanticscholar.org/paper/The-pathogenesis-of-cancer-metastasis/

The importance of cell mechanics and mechanotransduction. Examples

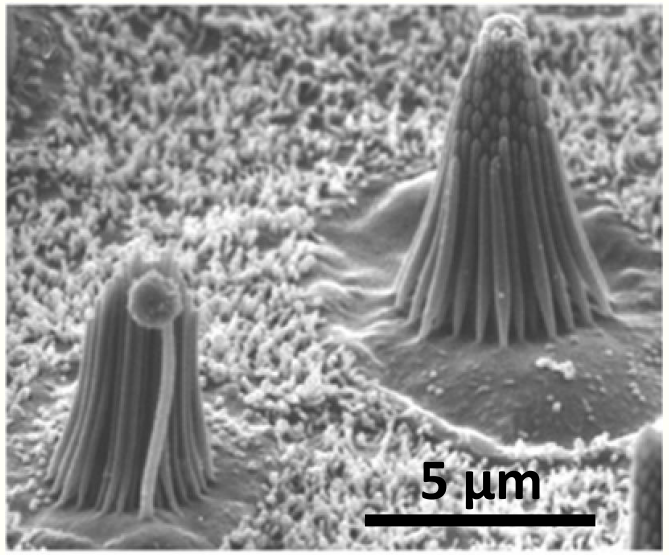
Cell Mechanotransduction

- **Conversion of the mechanical signal into a biochemical signal by the cell**
- **Specialized cells sense forces in HEARING, BALANCE and TOUCH, and in turn signal the nervous system**
- **Other eukariotic cells sense force just like they sense chemical signals**
  - This can lead to localized signal transduction, for example for spatial remodeling or migration
  - It can also lead to systemic changes in gene expression, for example for differentiation or apoptosis.

Cell mechanotransduction – cell function



The importance of cell mechanics and mechanotransduction. Examples



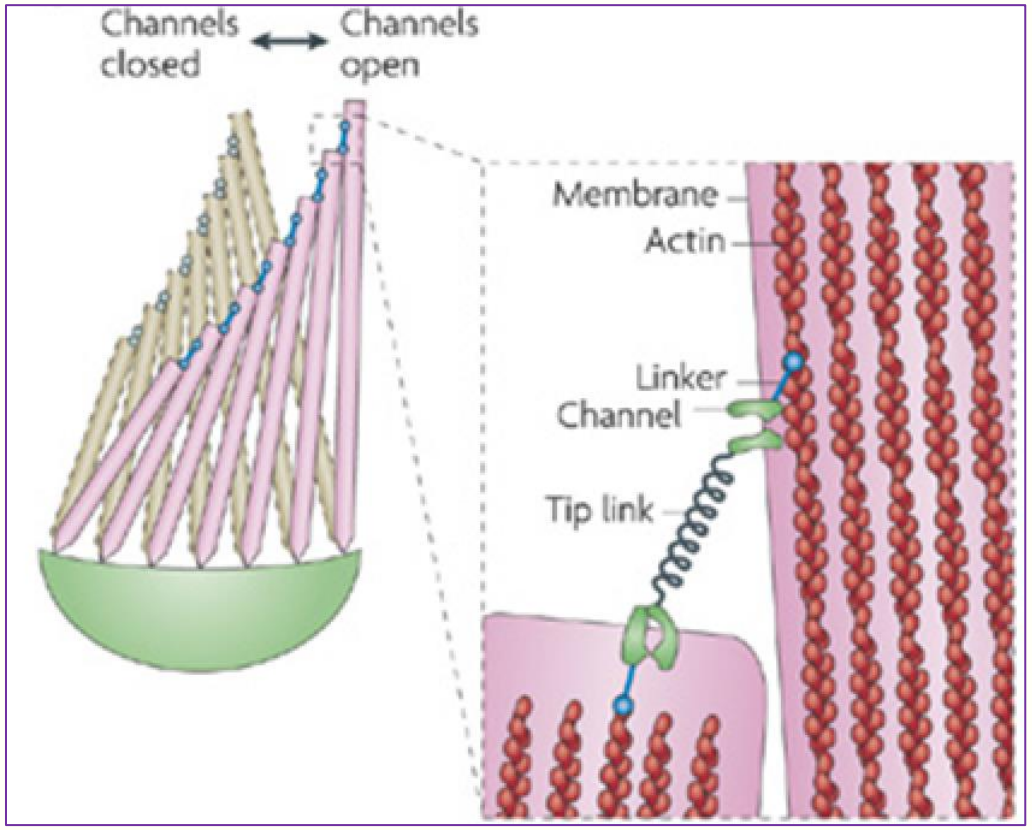
The sensory hair cells of the inner ear underlie the
perception of sound, linear and angular accelerations, and gravity !

SEM of two hair bundles in the sensory macula of the bull frog saccule, showing the stereocilia arranged in bundles with centrally increasing heights.

2014, Nature Reviews / Molecular Cell Biology

Schematic drawing of a hair bundle in resting (yellow) and deflected (pink) configurations.

Deflection, that is shearing of the stereocilia relative to each other, causes the 150–200 nm long tip links to pull directly on K^+ channels in the stereocilia, causing the channels to open.

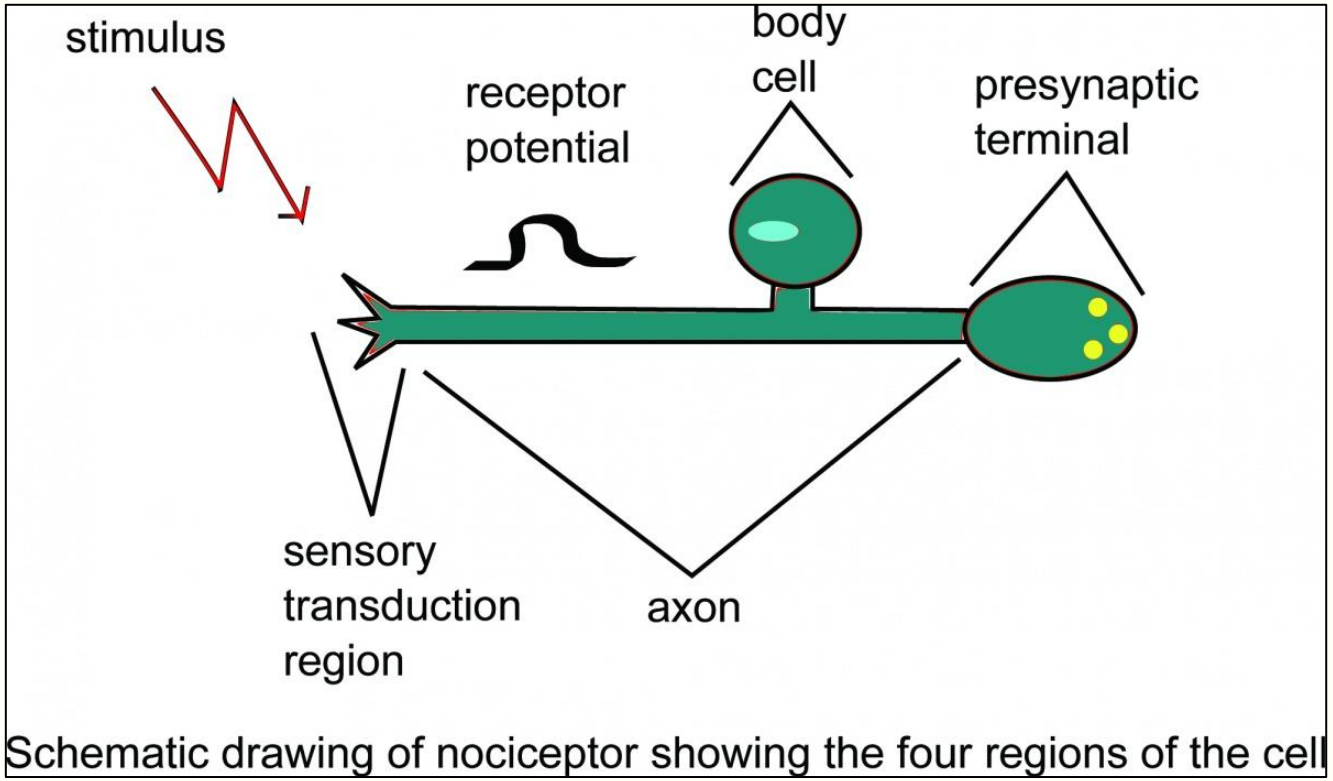


Nociceptor

A **nociceptor** is a sensory neuron that responds to damaging or potentially damaging stimuli by sending “possible threat” signals to the spinal cord and the brain.

If the brain perceives the threat as credible, it creates the sensation of pain to direct attention to the body part, so the threat can hopefully be mitigated; this process is called nociception.

Mechanical nociceptors respond to excess pressure or mechanical deformation. They also respond to incisions that break the skin surface.



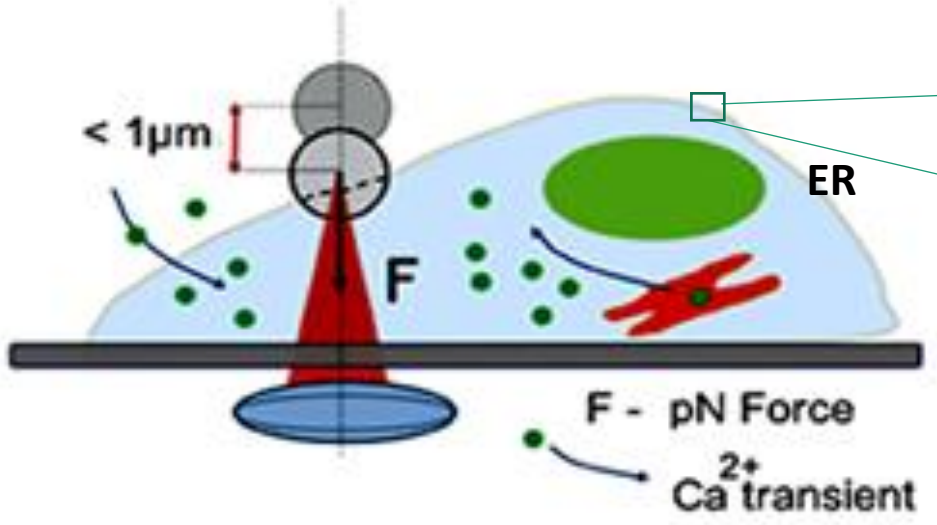
Schematic drawing of nociceptor showing the four regions of the cell

These mechanical nociceptors frequently have polymodal characteristics. So it is possible that some of the transducers for thermal stimuli are the same for mechanical stimuli. The same is true for chemical stimuli, since TRPA1 appears to detect both mechanical and chemical changes.

Mechanotransduction in neurons ?

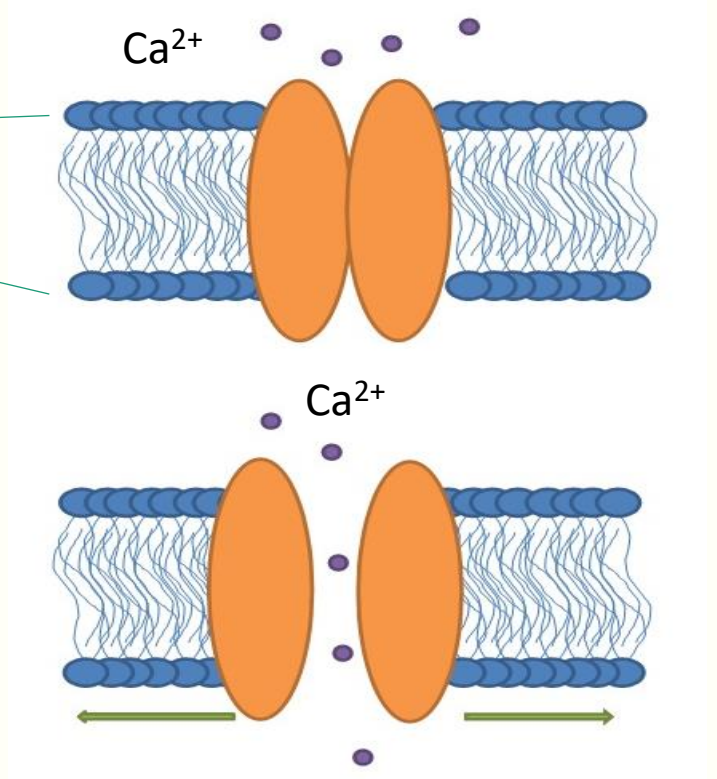
E.g. Ca^{2+} transients induced by small forces

pN Force applied vertically to the cell membrane



Cell membrane and

a mechano sensitive channel MSC



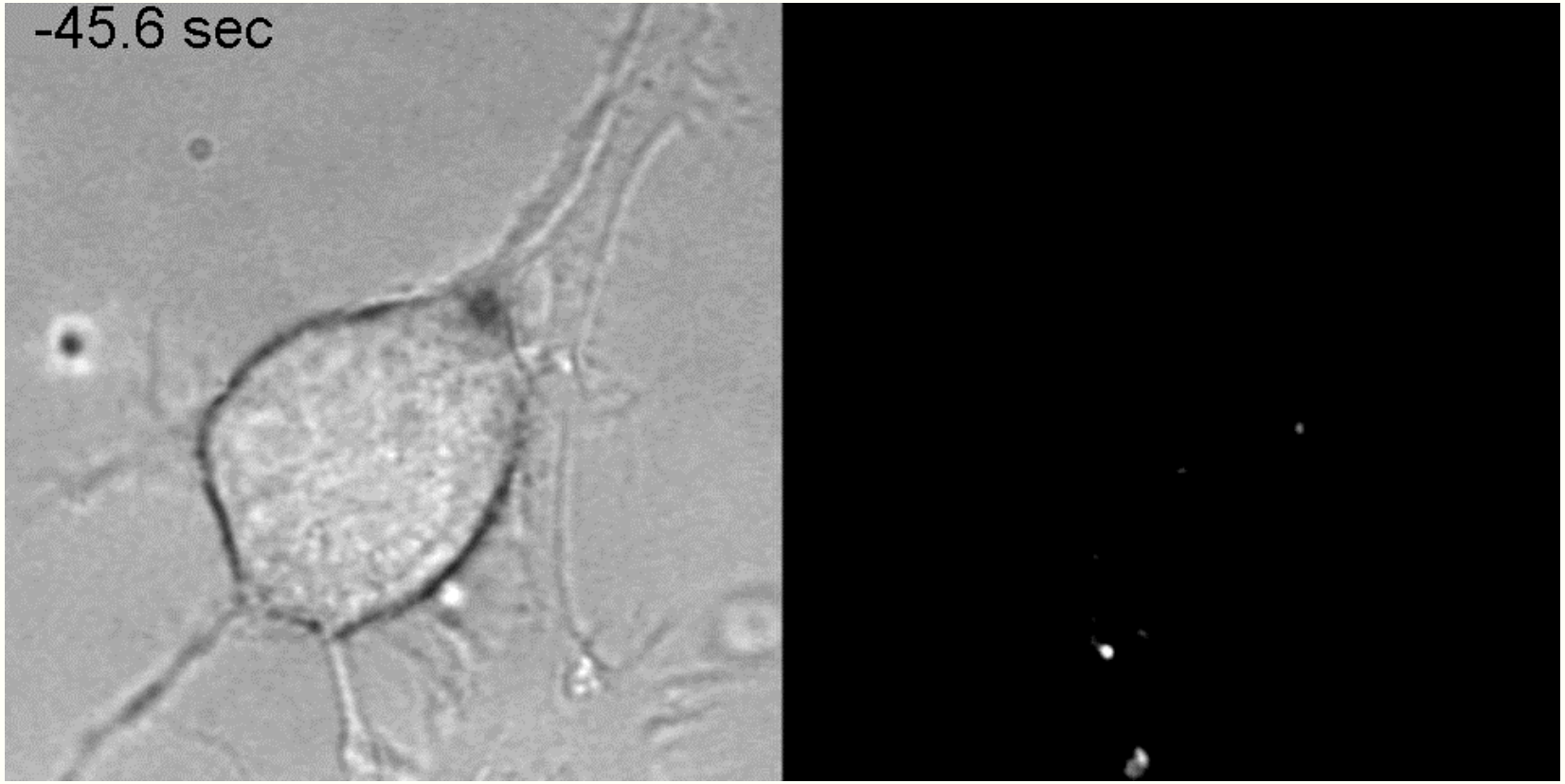
Ca2+ transient can be due to the influx of Ca2+ or internal release from the Endoplasmatic Reticulum (ER)

F. Falleroni *et al*, *Frontiers Cell Neurosci*, 2018

Ca^{2+} transients evoked by calibrated mechanical stimulations

Brightfield

Calcium Imaging



mouse neuroblastoma NG108-15

F. Falleroni *et al*, *Frontiers Cell Neurosci*, 2018

Molecular level - Protein as a machine

A) A protein as an assembly of mechanical components including a lever, a hinge (cardine), a spring, a latch (serratura), and various binding sites. The constituent amino acids give the protein a rigidity similat to that of hard plastic.

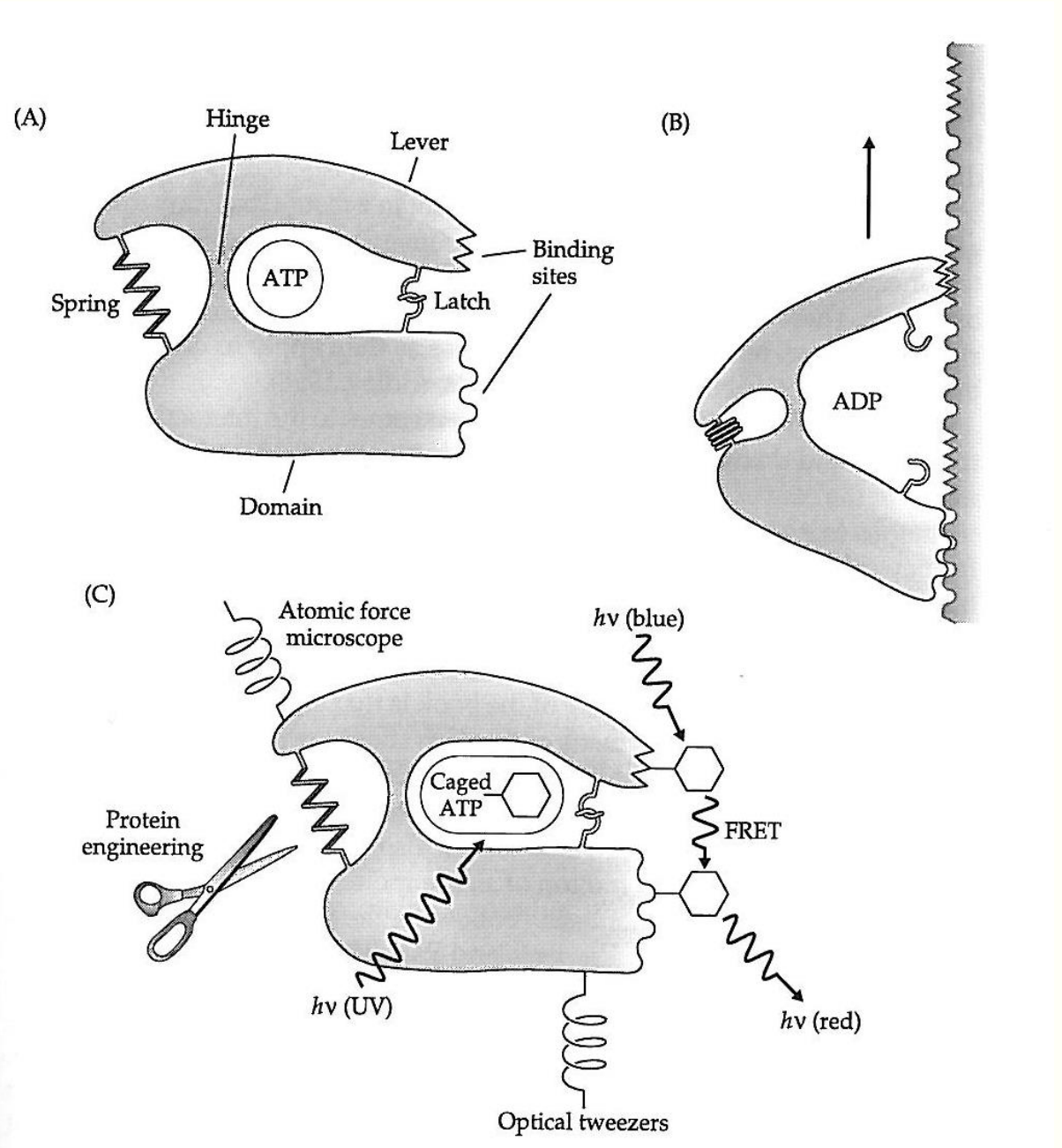
B) The opening and closing of a cleft, together with the coordinated binding and unbinding from a surface, can produce directed motion.

C) Single molecule techniques.

Optical techniques can be used to release a caged ligand such as ATP an measure its binding to protein. The relative movement of protein domains can be monitored by FRET.

Optical tweezers and AFM can exert forces on the protein and therefore can be used to measure stiffness.

Site directed utagenesis can be used to make specific alterations to a protein in order to identify the mechancial components and to facilitate the binding of chemical probes.



J. Howard, Mechanics of motor proteins and the cytoskeleton, 2001

HOW TO MESURE CELLULAR FORCES ? – Techniques and instruments

Method	Typical force range
Atomic force microscopy	10 pN–100 nN
Microindenter	1–100 nN
Microplate stretcher	1–100 nN
Magnetic bead microrheology (twisting)	10 pN–1 nN
Magnetic bead microrheology (pulling)	100 pN–10 nN
Optical traps	1–500 pN
Micropipette aspiration	1–100 nN
Substrate strain	1–30% strain
Shear flow	1–100 Pa
MEMS devices	0.5–1500 nN

Table 35.3 Typical force ranges in cell biology

Biological force	Force range
Force generated by motor proteins (e.g., kinesin, myosin)	≈ 1–10 pN
Force transmitted by protein–protein interactions	≈ 1–200 pN (rate dependent)
Force required for (partial) protein unfolding	≈ 100 pN
Force generated by migrating or contracting cells	≈ 1 nN–10 μN