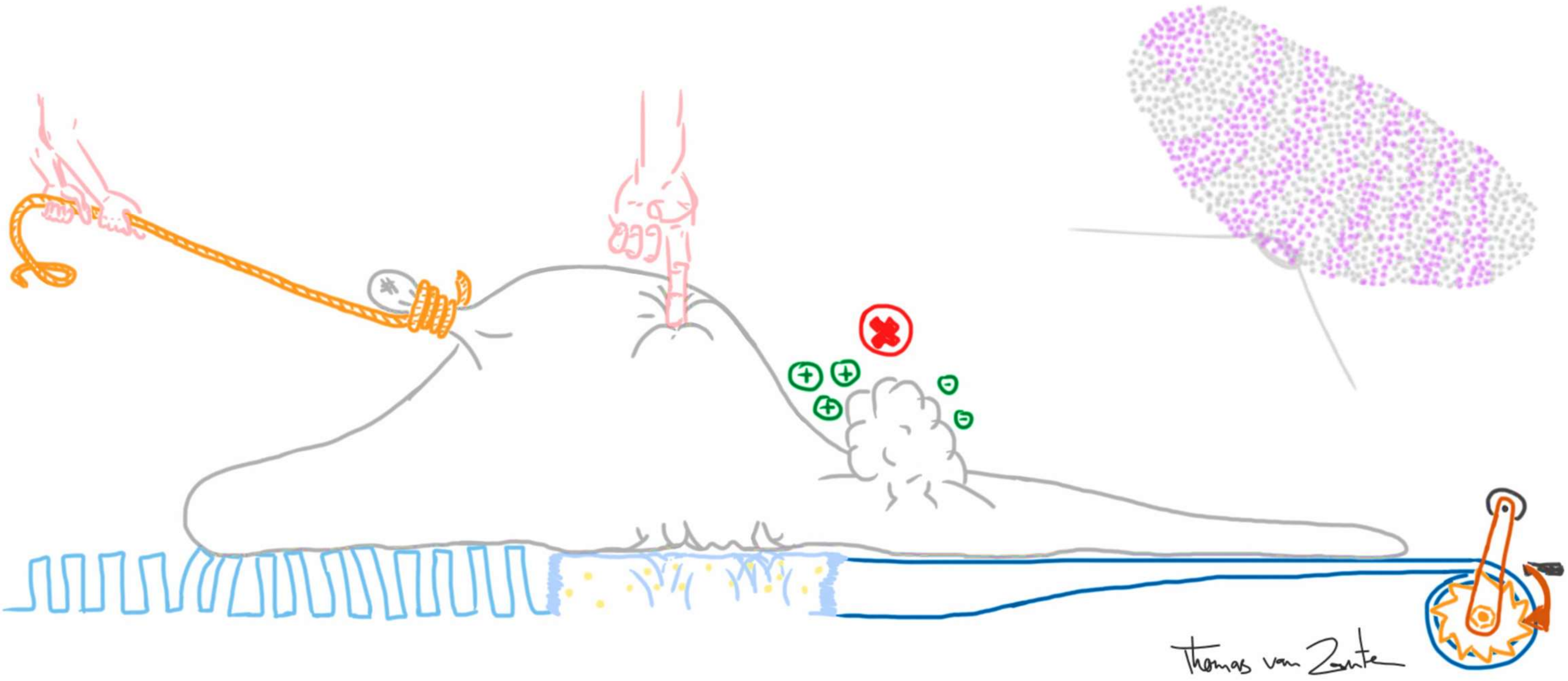
COURSE OPTICS, FORCE and Development _2024

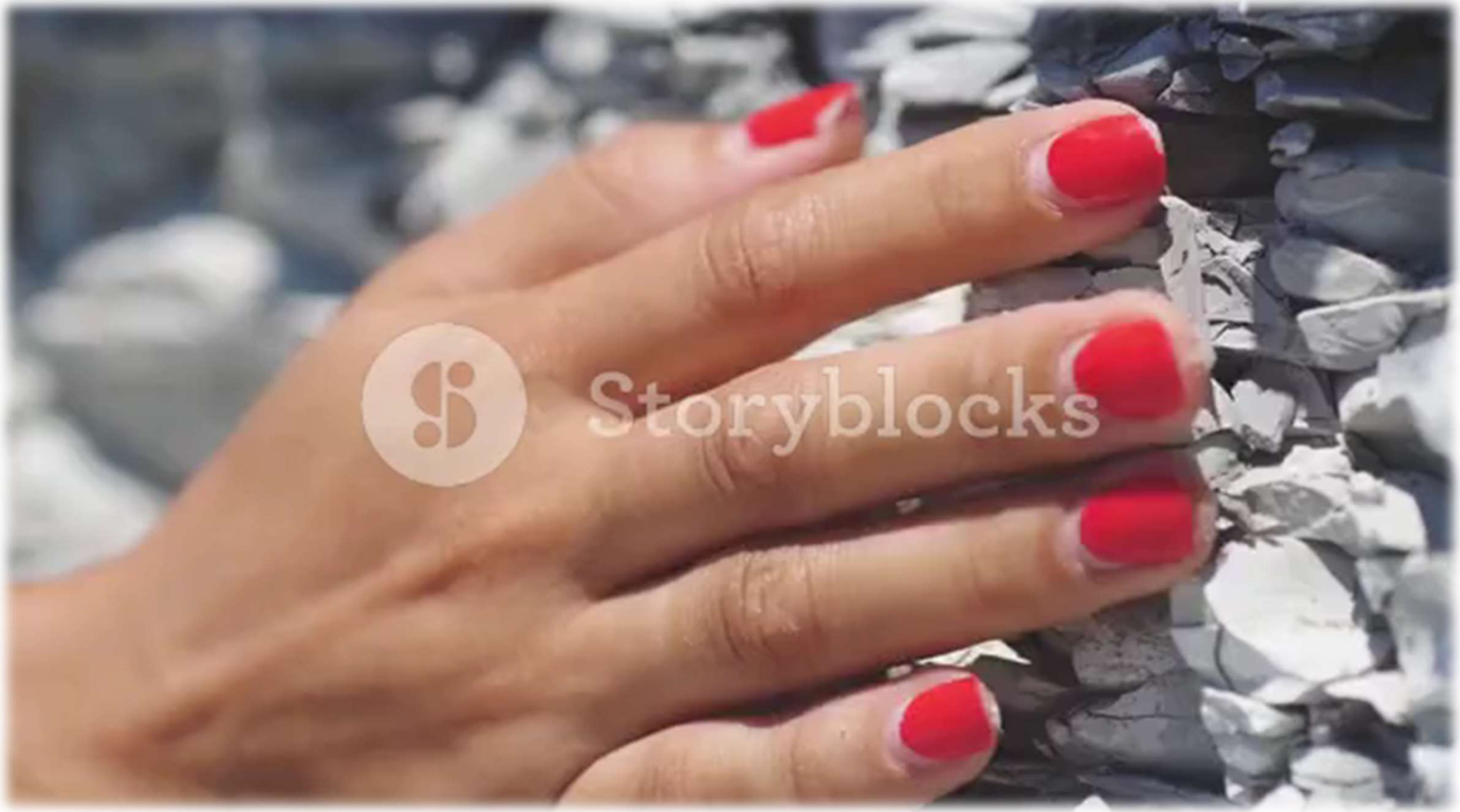
Mechanosensing and mechanotransduction



Dra. Cristina Bertocchi e-mail cbertocchi@uc.cl

Mechanosensing: hearing

Mechanosensing: touch

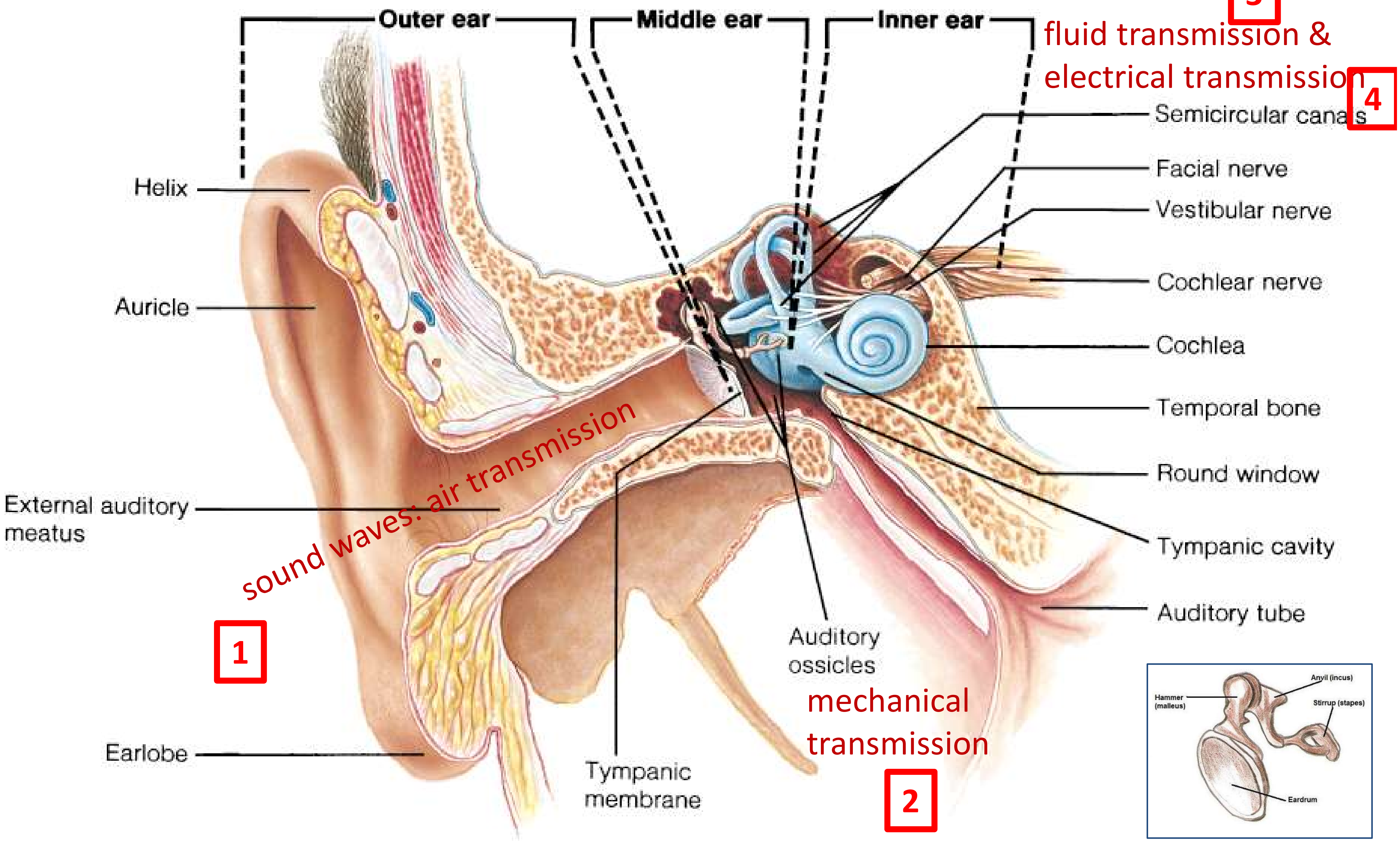


Our senses of hearing and touch are unique in that the translation of physical forces (including air vibrations) into biochemical signals reaches our conscious awareness.

Specialized Mechanosensory Organs and Cells

Mechanotransduction in the ear

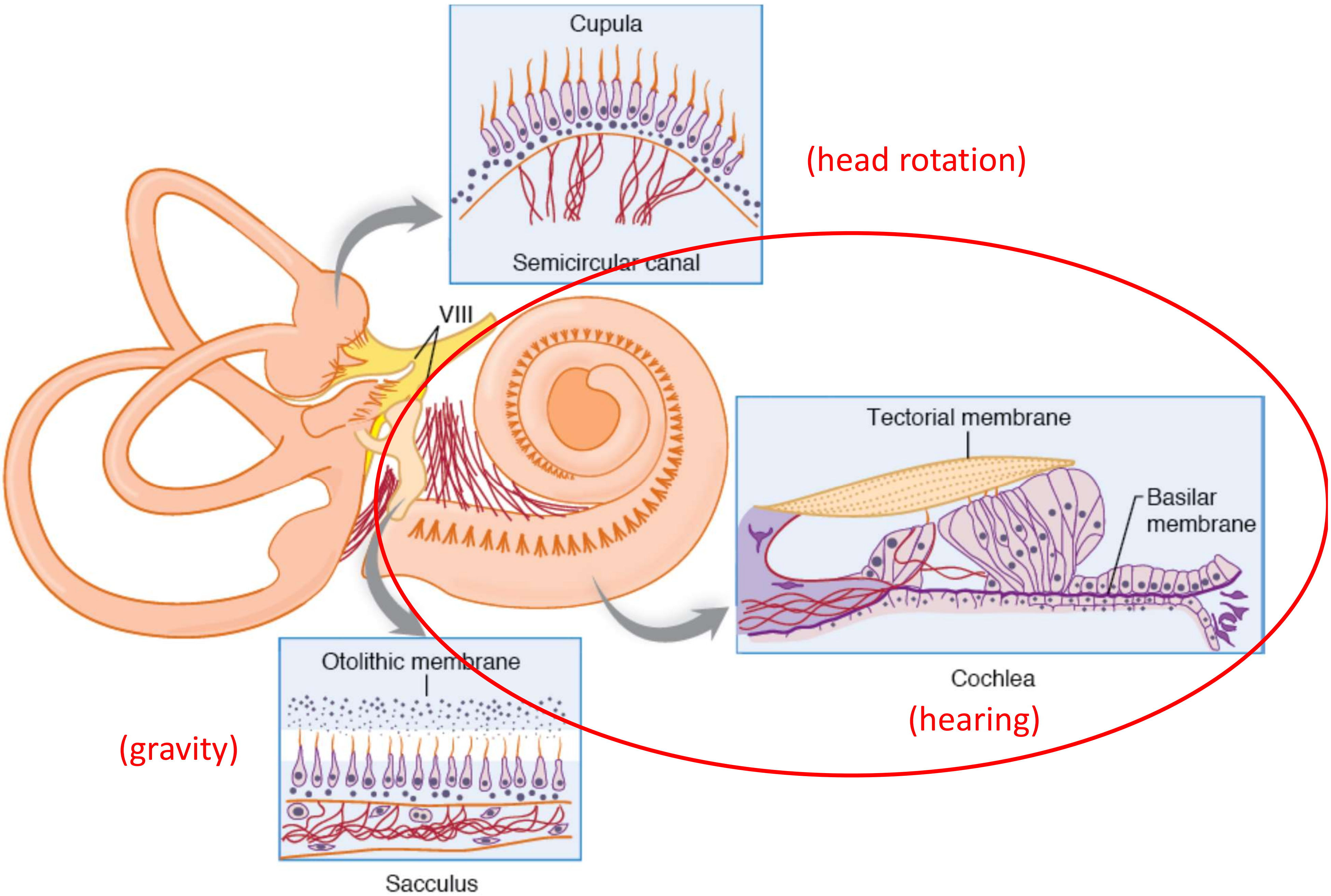
How Sound Signals are Transmitted



tympanic membrane (ear drum) transmits pressure waves in air to movement of Auditory ossicles (bones)

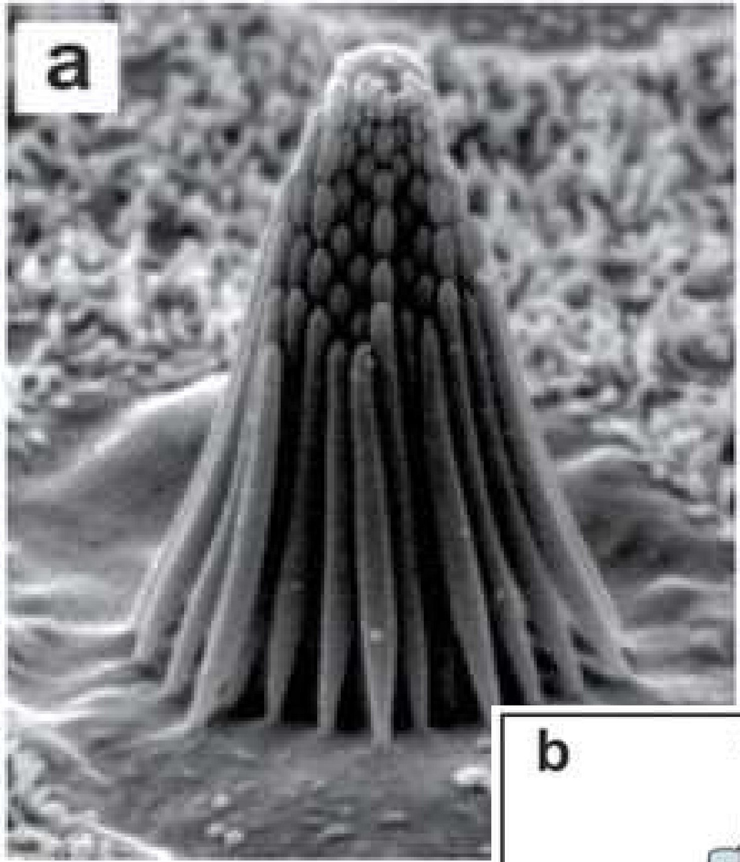
Source: Barrett KE, Barman SM, Boitano S, Brooks HL: *Ganong's Review of Medical Physiology*: www.accessmedicine.com

Inner Ear: hair cells

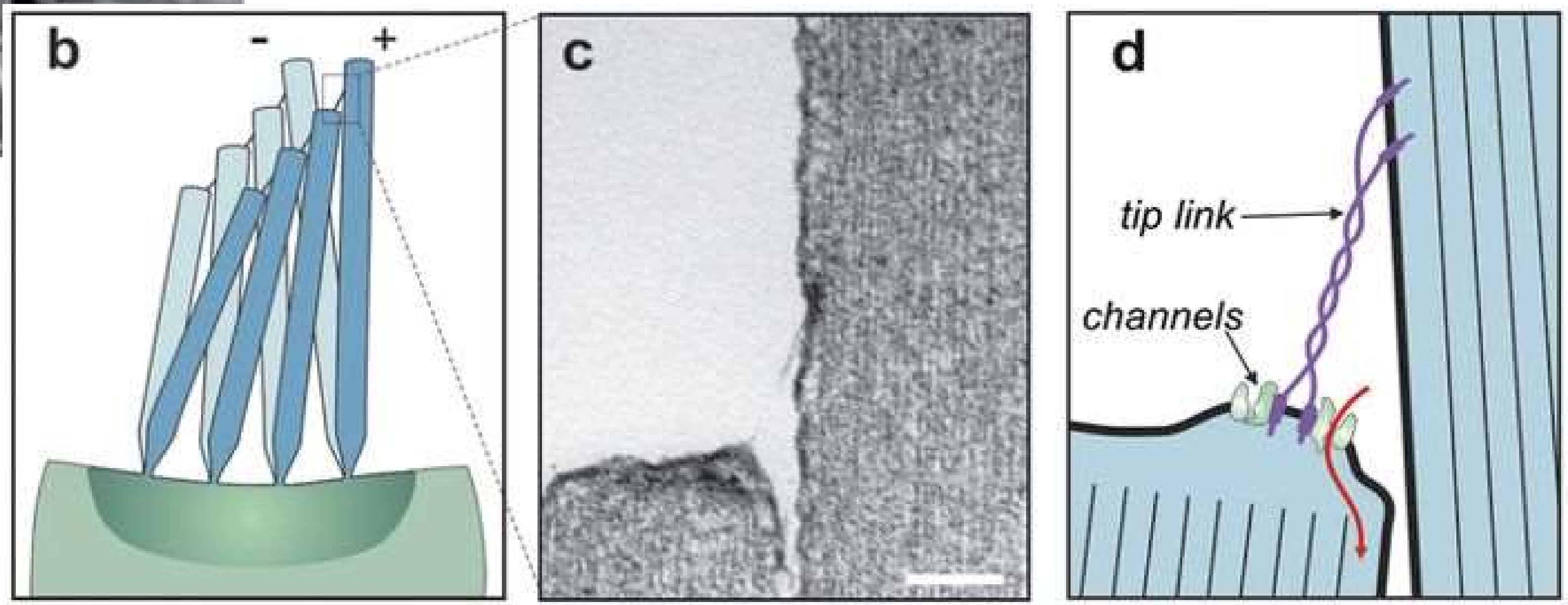


Source: Barrett KE, Barman SM, Boitano S, Brooks HL:
Ganong's Review of Medical Physiology: www.accessmedicine.com

Mechanotransduction in the ear



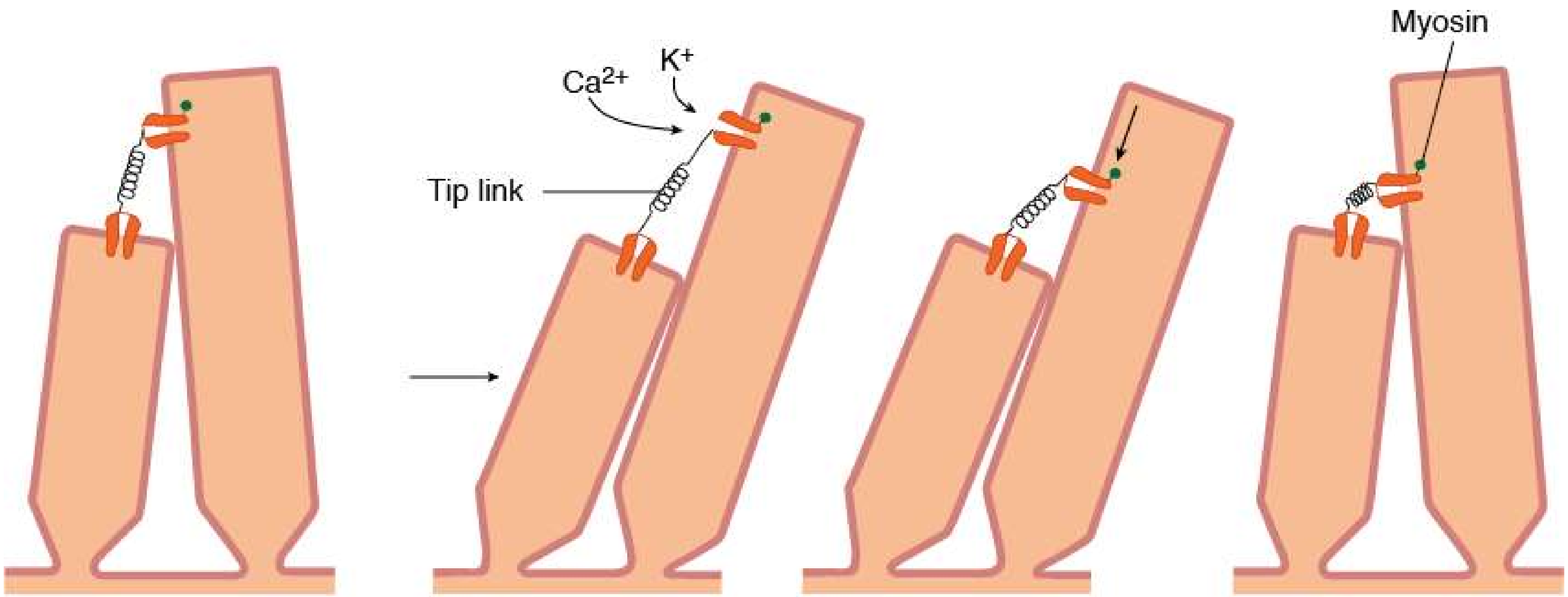
Tension in the tip link activates a stretch-activated ion channel, leading to intracellular calcium ion fluctuations.



Asadnia, M et al; *Scientific Reports_2016*

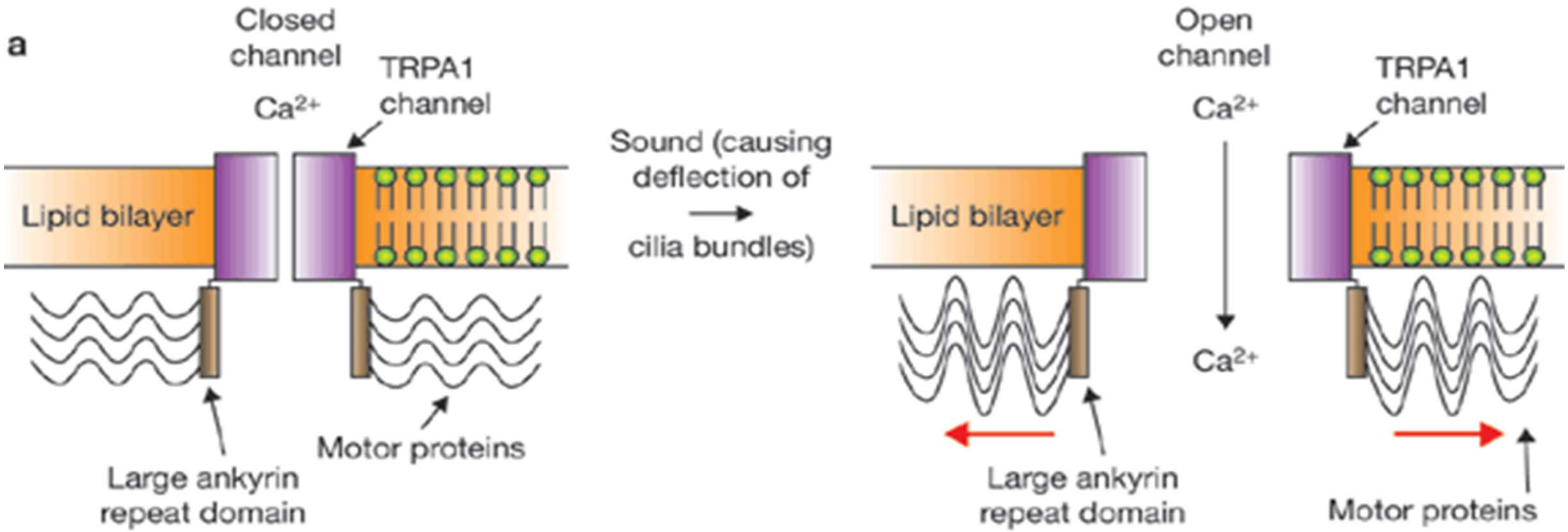
Stereocilia Structure and Function

- Stereocilia are composed of actin filaments and myosin.
- Rows of stereocilia are organized so each is taller than the next.
- A tip link connects the neighboring stereocilia.
- When the stereocilia tilt, the tip links stretch under tensile force
- The tip link opens an ion channel called TrpA on the taller stereocilia, which leads to electrical signaling of nerves.



Source: Barrett KE, Barman SM, Boitano S, Brooks HL:
Ganong's Review of Medical Physiology: www.accessmedicine.com

Models for How Force Opens TRP Channels

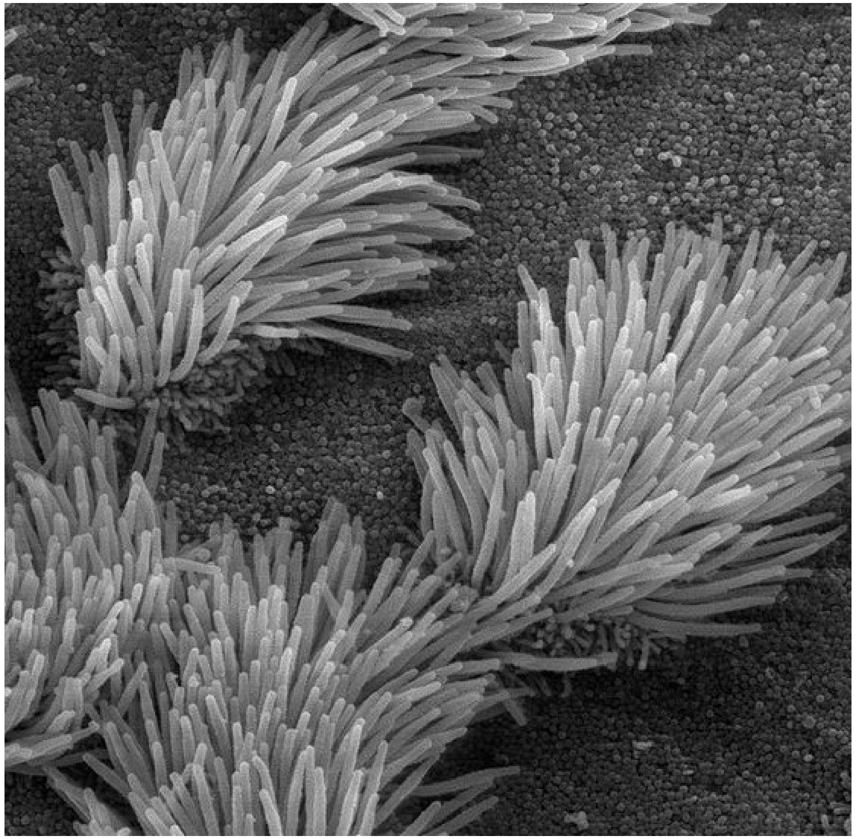


Barritt and Rychkov *Nature Cell Biology*_2005

Specialized Mechanosensory Organelles in Cells

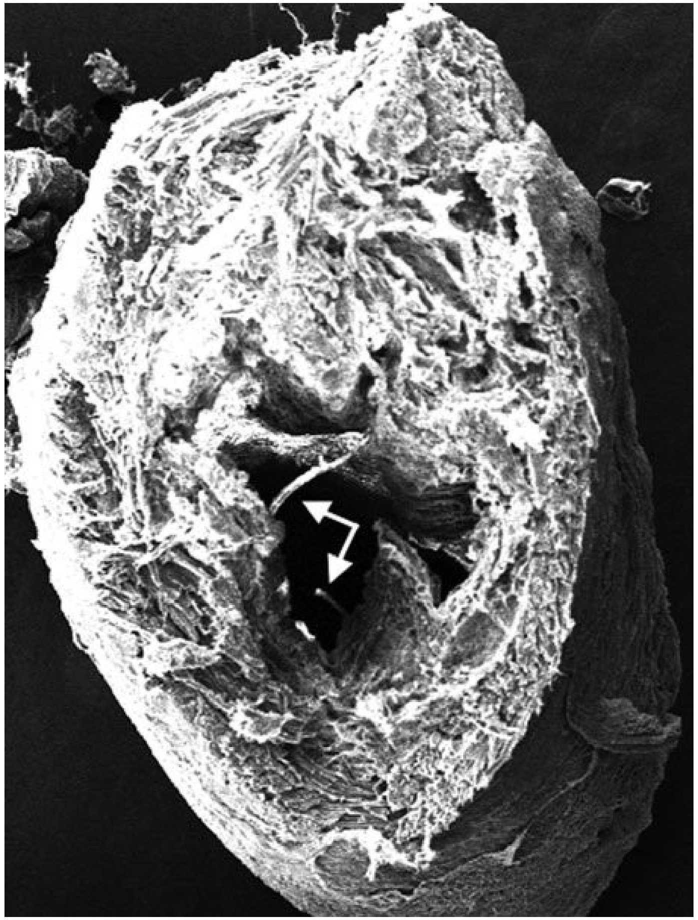
True Cilia

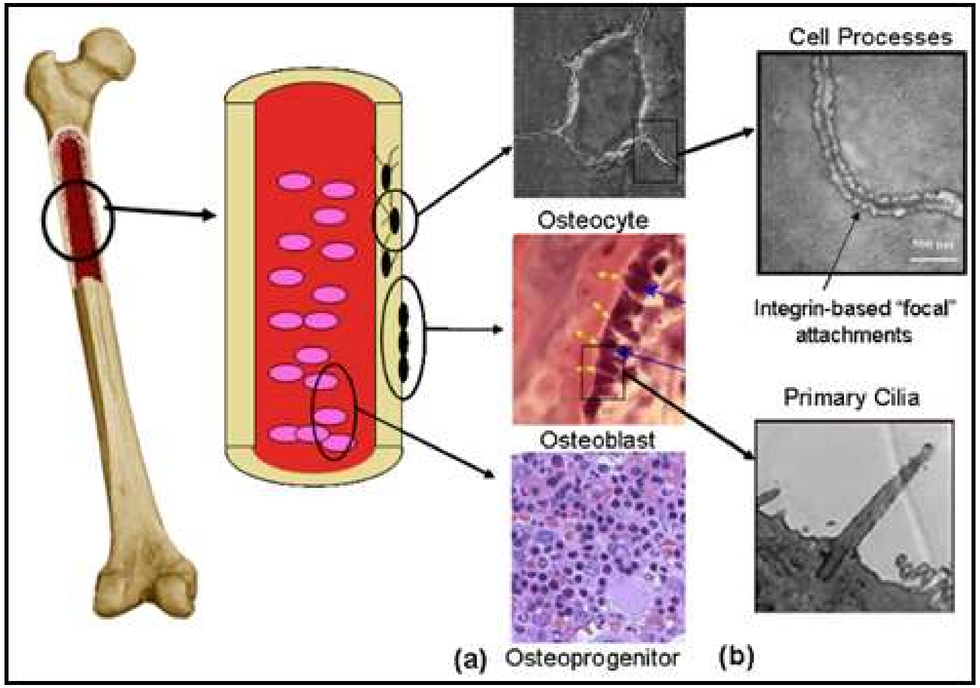
True Cilia



Epithelial cells (respiratory tract, skin, urinary tract, intestines, mouth, etc)

Endothelial cells (blood vessels)

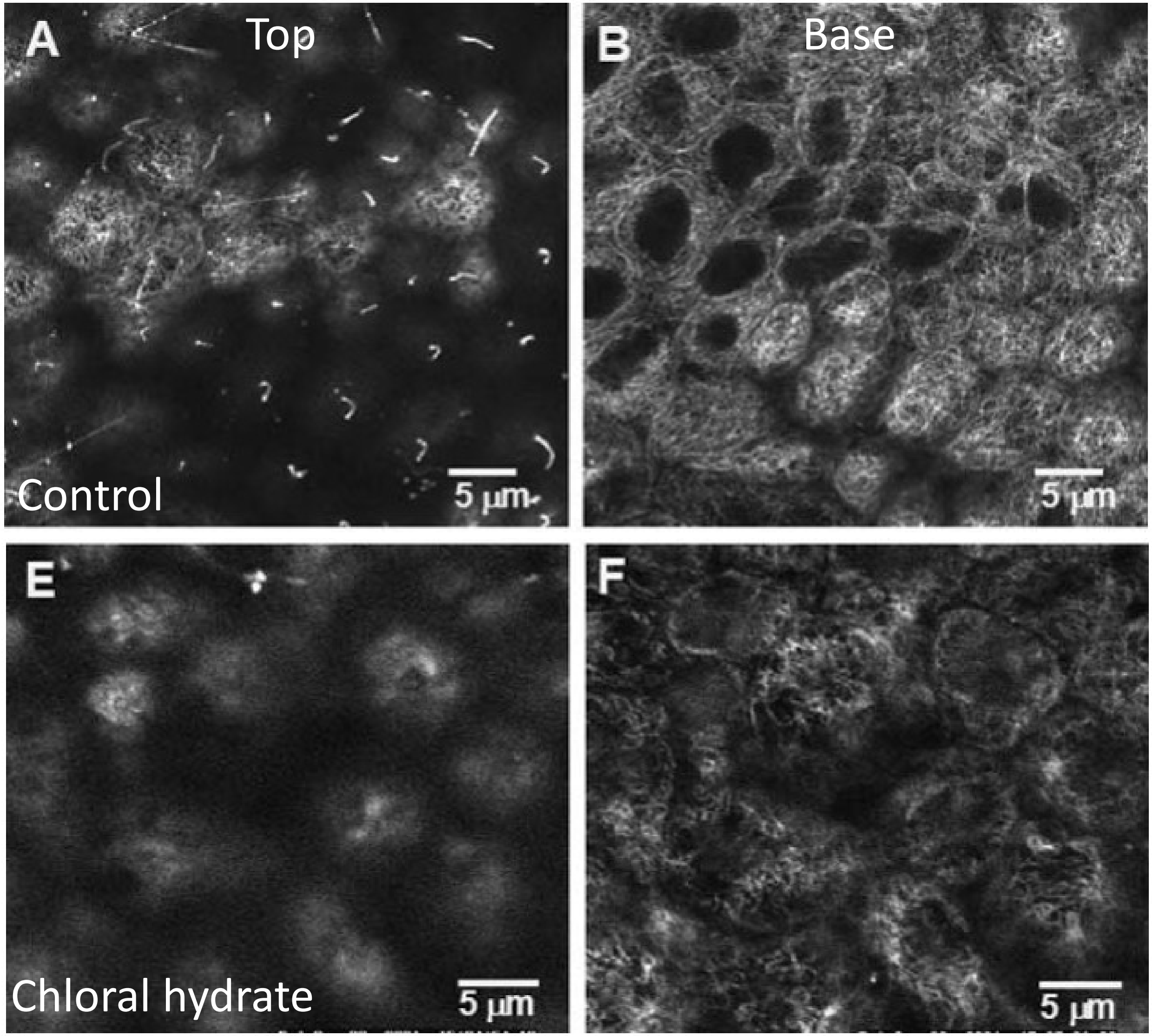


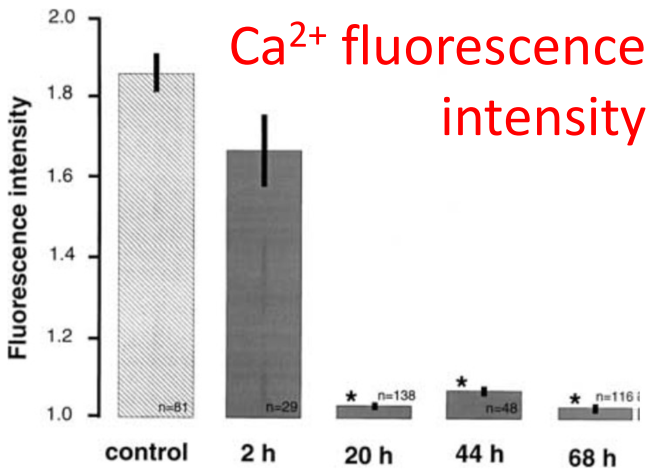


osteocytes (mechanosensors in bone)

Cilia are mechanosensory organelles

Renal Epithelial cells





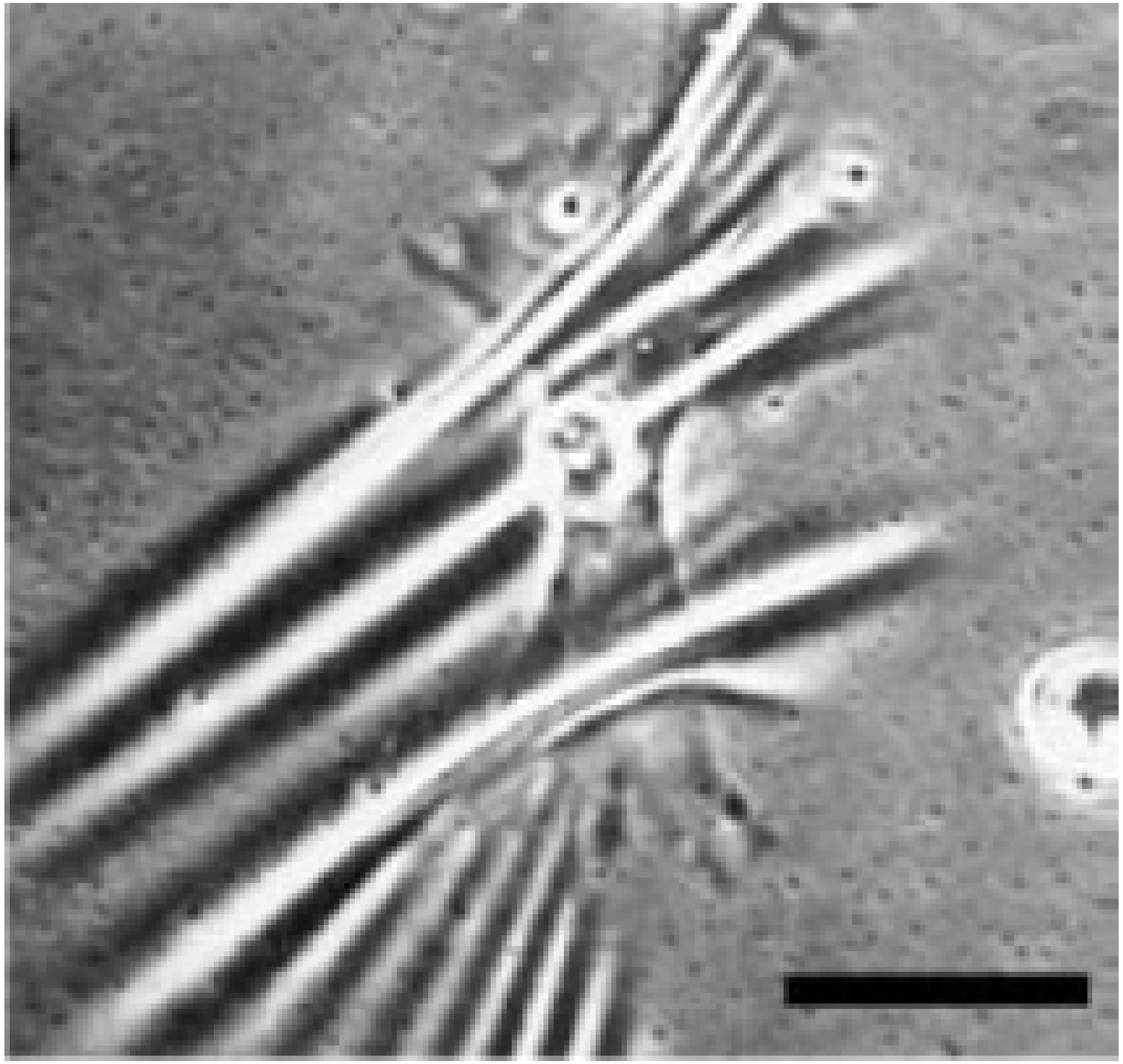
Ca^{2+} fluorescence intensity

Without cilia, cells are unresponsive to fluid-shear stress

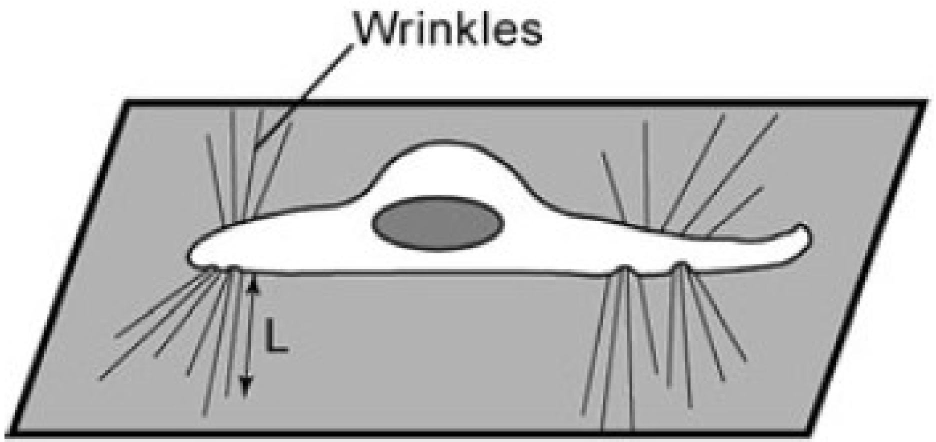
Praetorius HA & Spring KR; J Membr 2003

Cells without specialized mechanosensory organelles

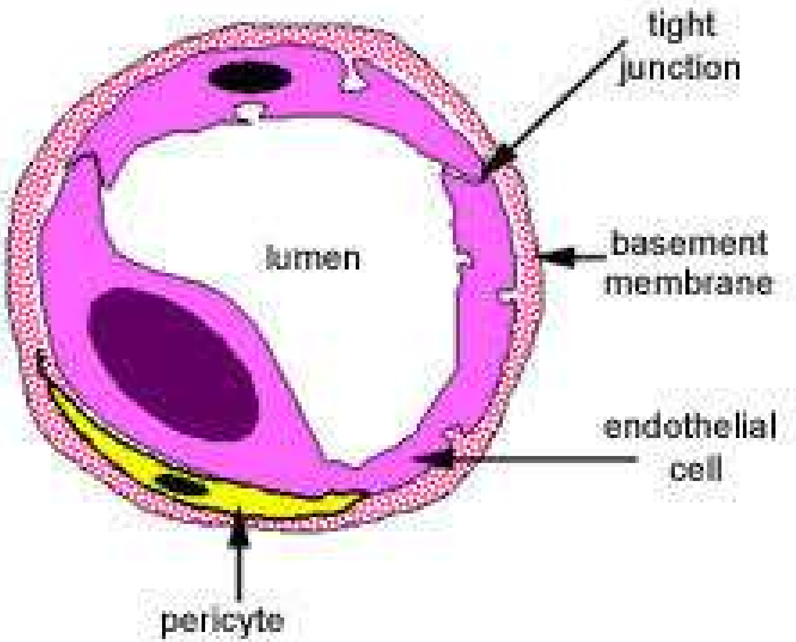
Cells are machines that interact with their physical environment



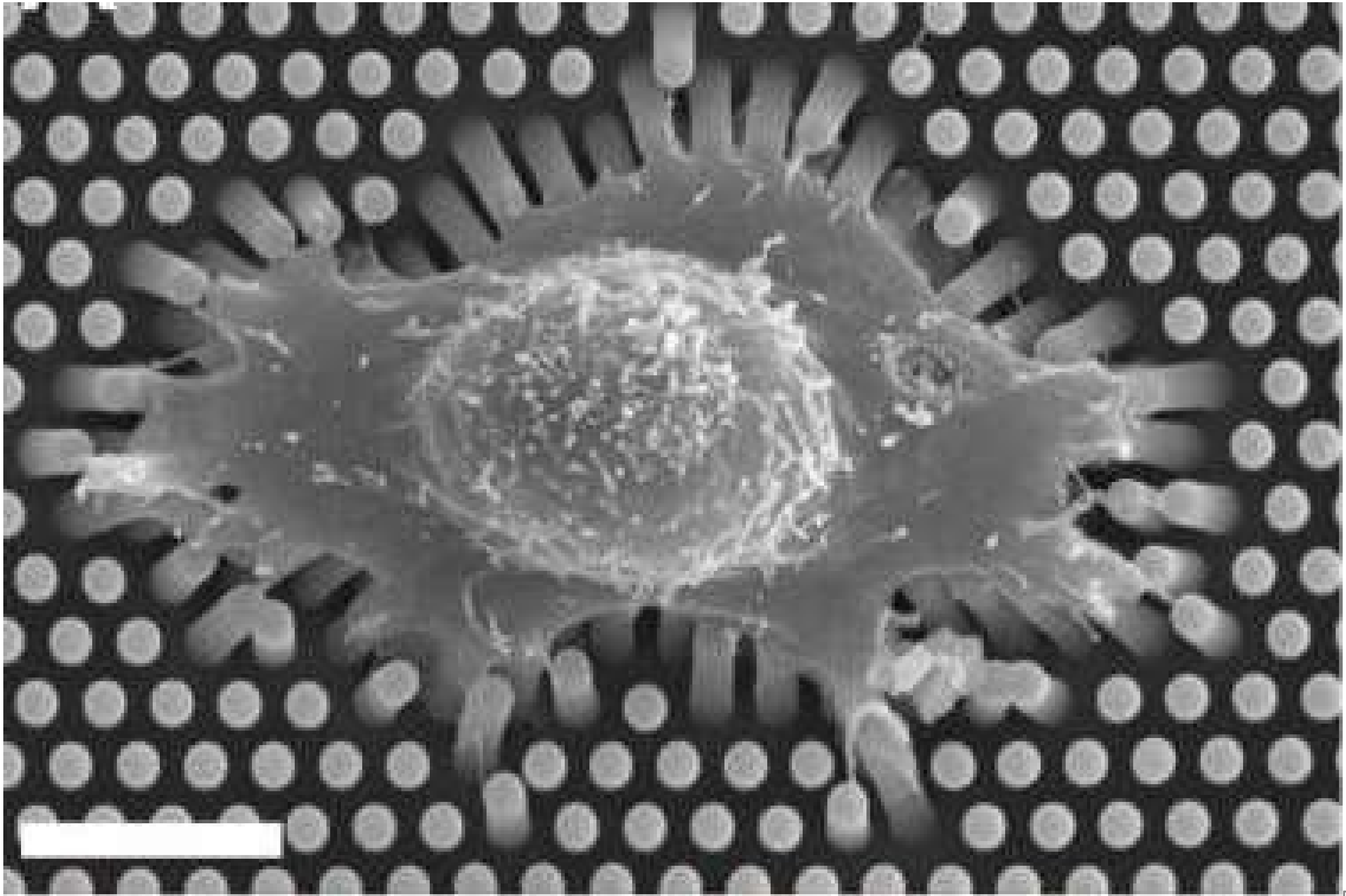
Kelley C. et al; JCB 1987



Pericyte tension development on silicone rubber.

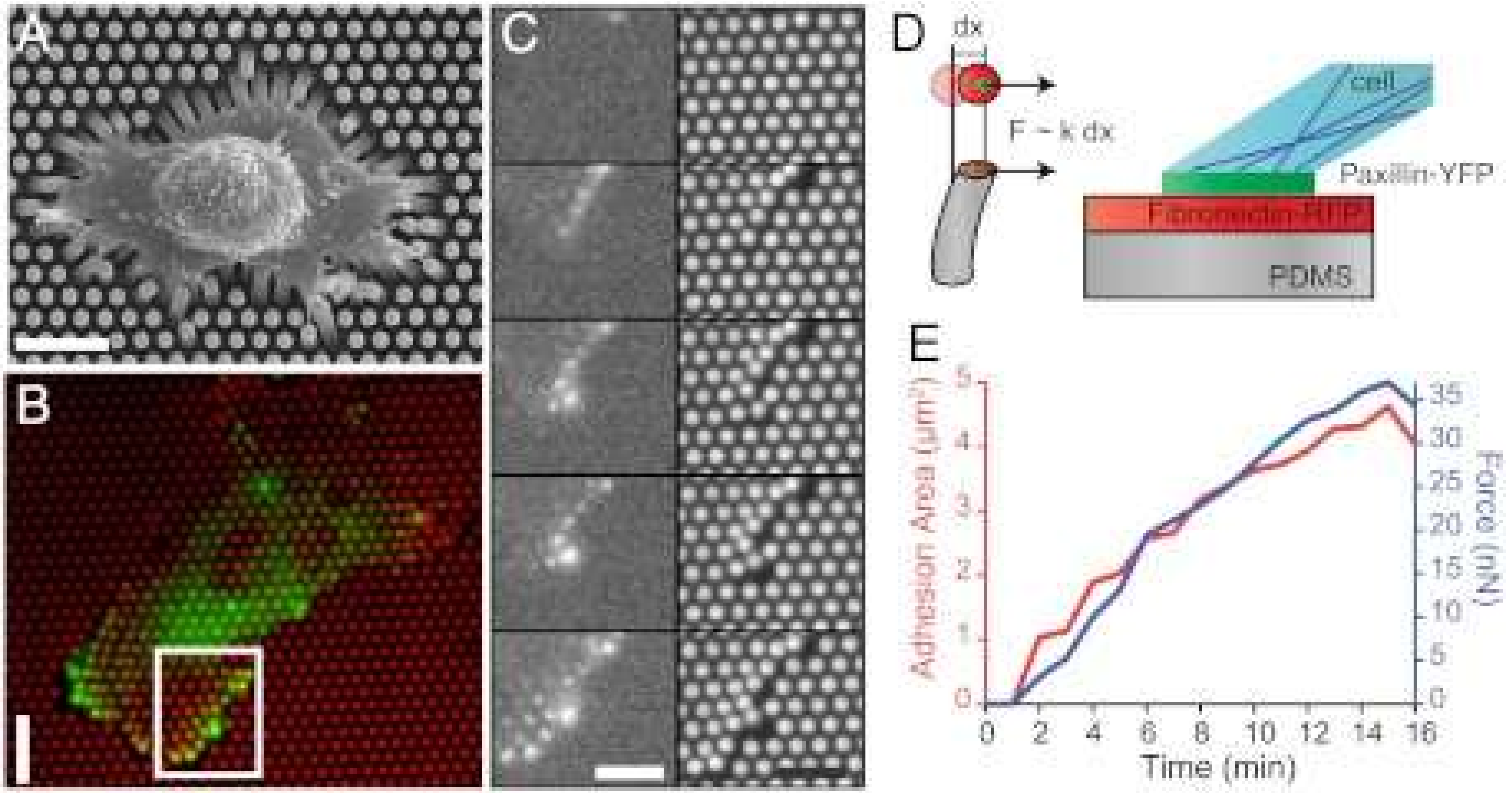


Cells respond to physical cues (topography and stiffness)



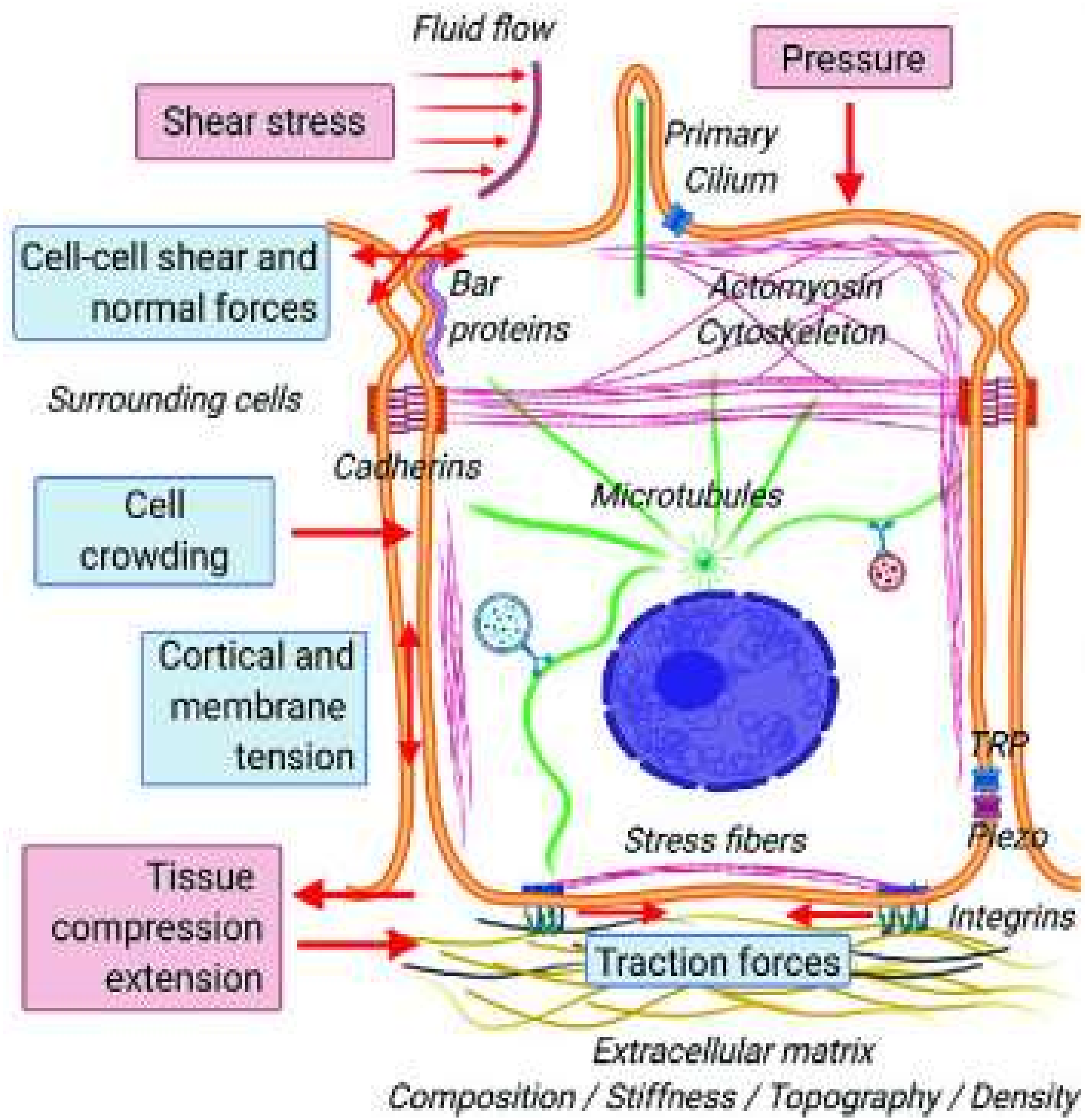
Trichet L. et al; PNAS 2012

Cell adhesion and traction forces



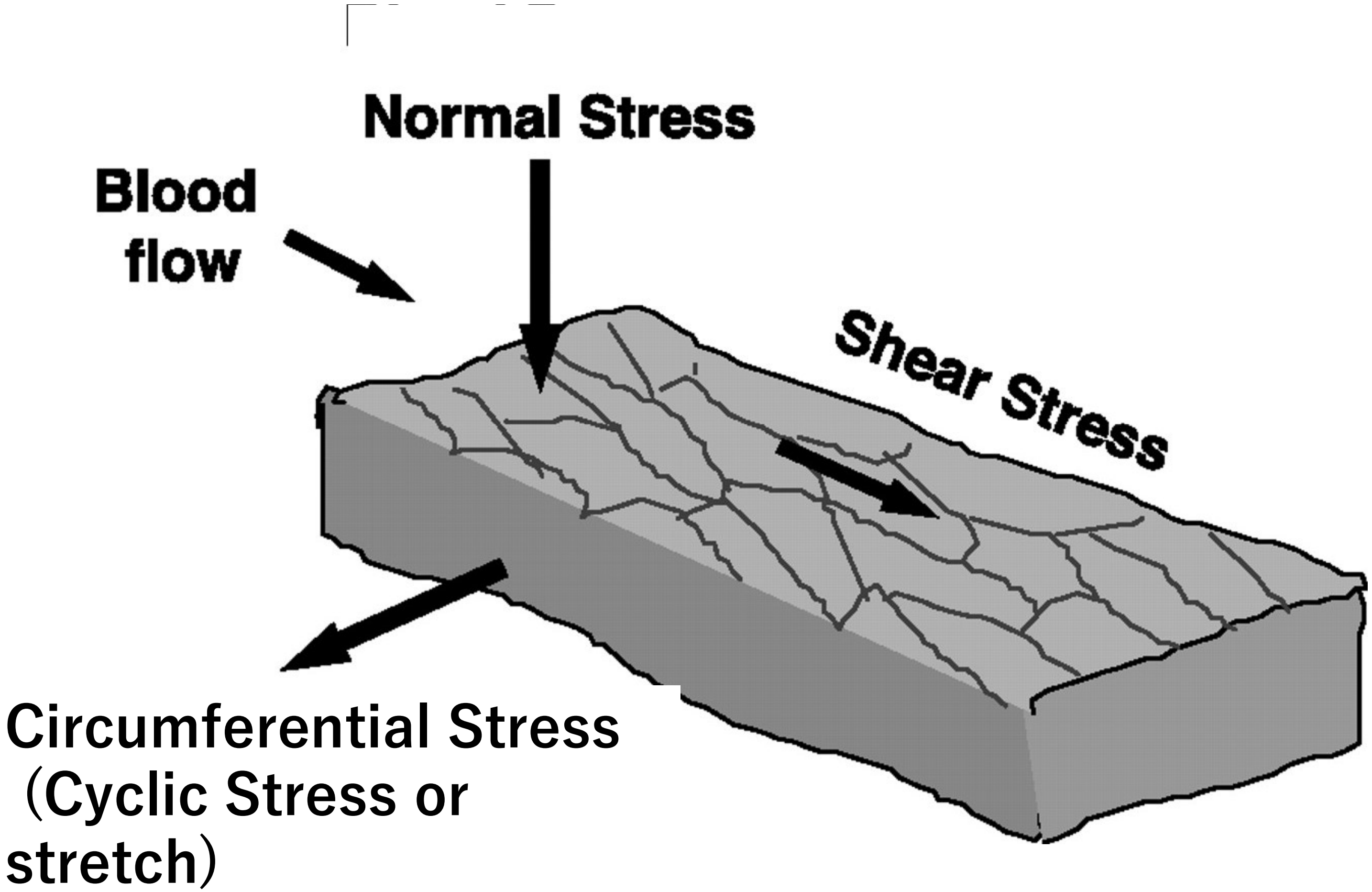
Trichet L. et al; PNAS 2012

Other cells sense…..



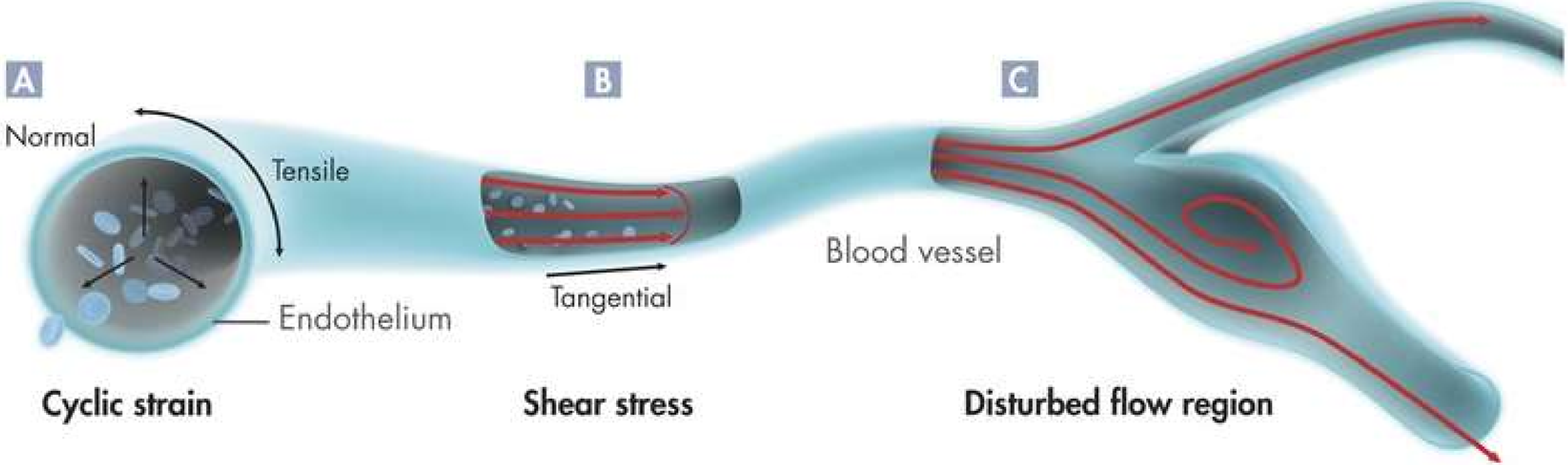
Herndez-Cáceres MPet al; Frontiers Oncol. 2021

Example: Forces acting on blood vessels

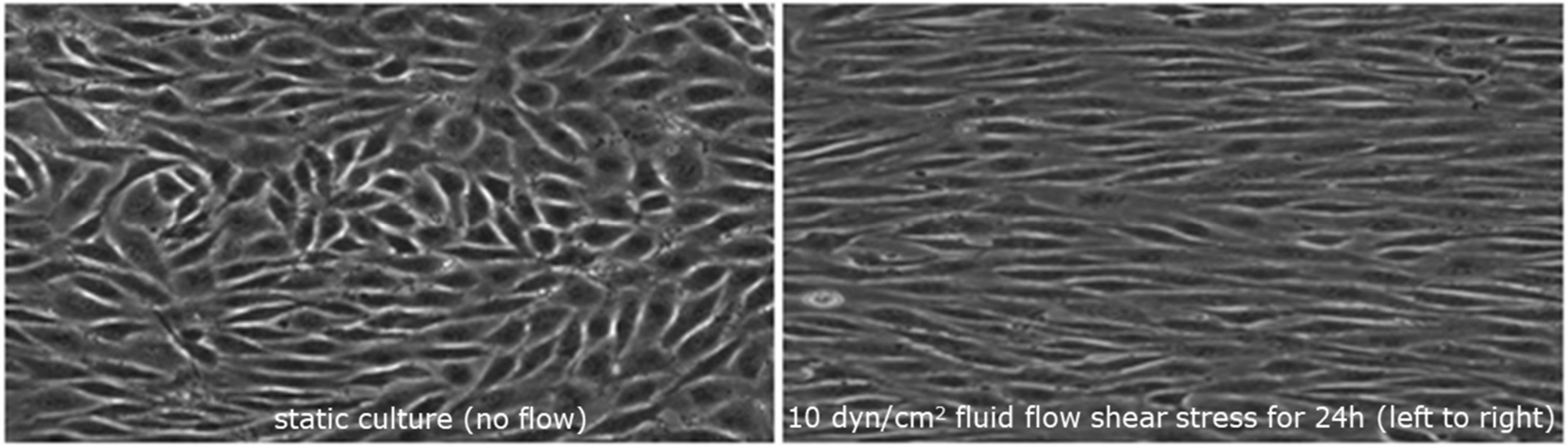


- Shear stress parallel (tangential to the endothelial cell surface) by blood flow and the generations of normal stress (perpendicular to the endothelial cell surface)
- Circumferential stretch due to the action of pressure.

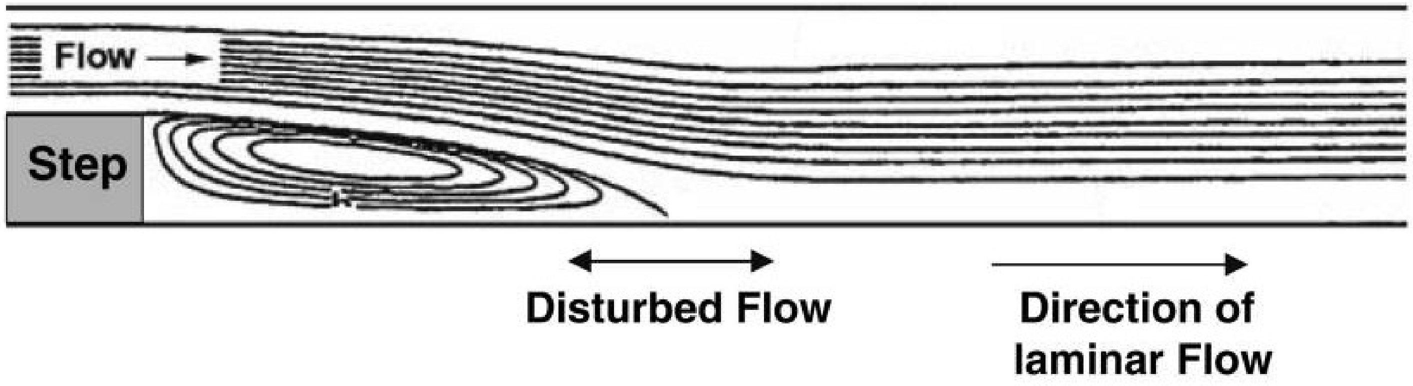
Endothelial Cells sense strain and fluidic shear stress

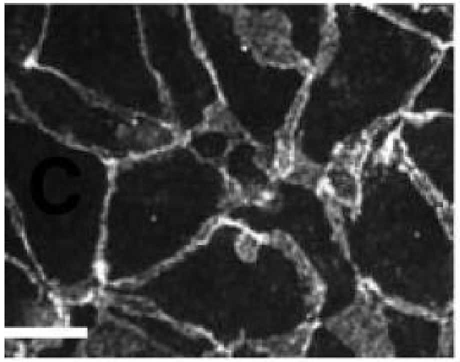


Conway and Schwartz, 2013, Qiagen

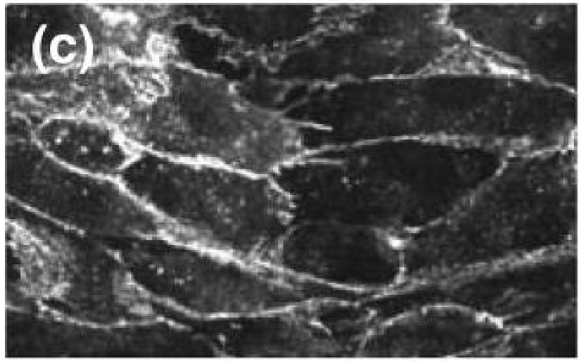


Effect of flow *in vitro* and *in vivo*





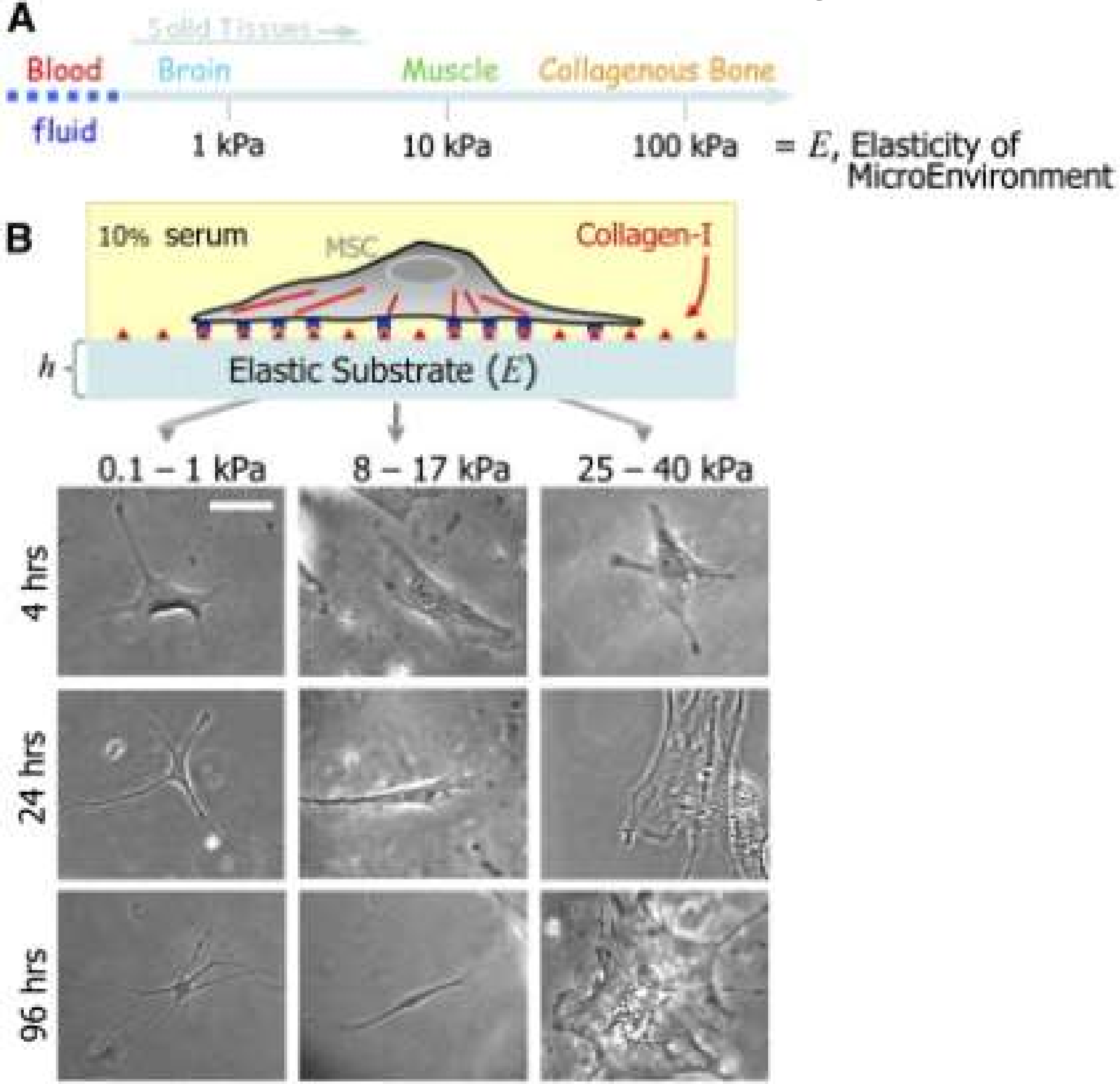
Static Control



Laminar Flow

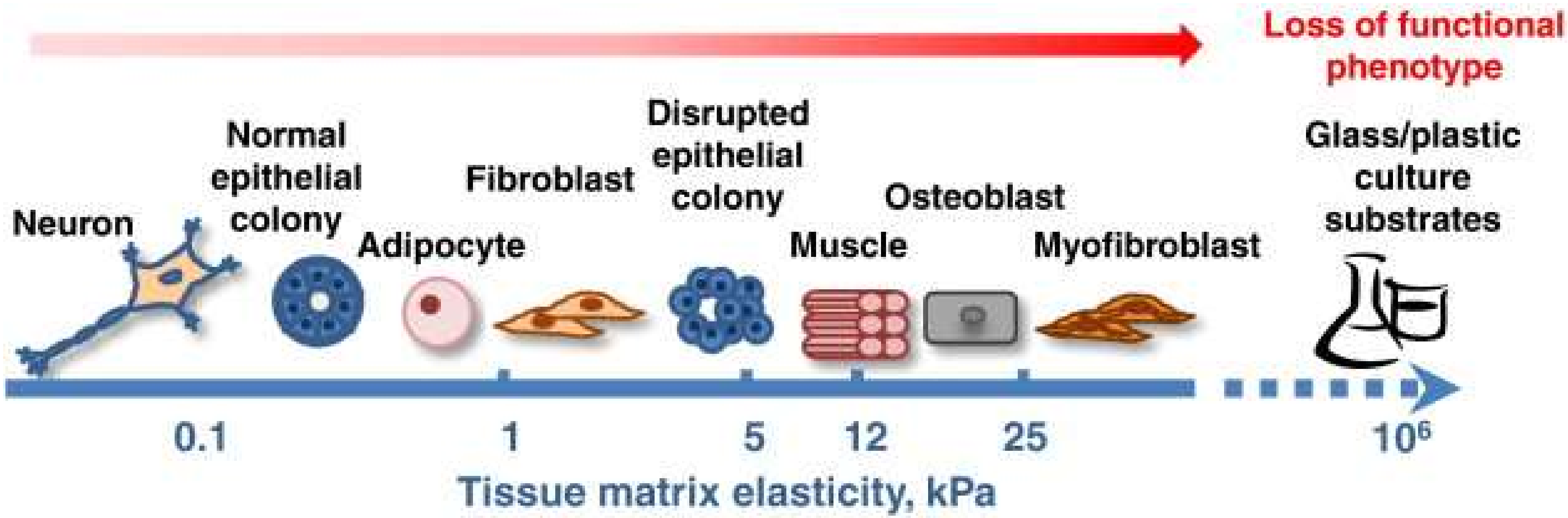
Chien S; Ann Biomed Eng._ *2013*

Example: Tissue elasticity and differentiation of mesenchymal cells



Engler et al; Cell 2006

Example: Substrate stiffness is a key modulator of cell behavior



Mechanotransduction

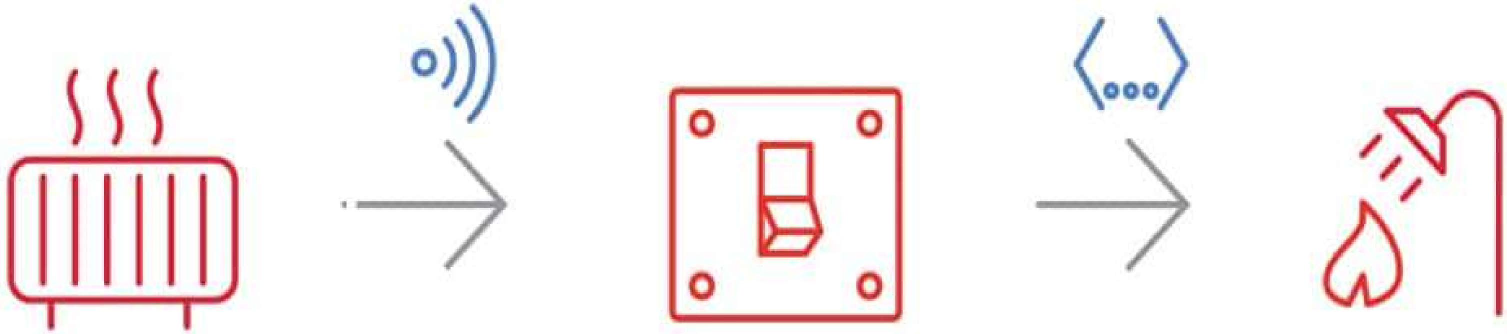
- Conversion of mechanical signal to a biochemical signals
- Specialized cells sense forces in hearing/ balance and touch, and in turn signal the nervous system
- Other eukaryotic cells sense force just like they sense chemical signals.
  - This can lead to localized signal transduction, for example for spatial remodeling or migration.
  - It can also lead to systemic changes in gene expression, for example for differentiation or apoptosis.

From Engineering….

Sensor

Control Center (Amplifier)

Actuator



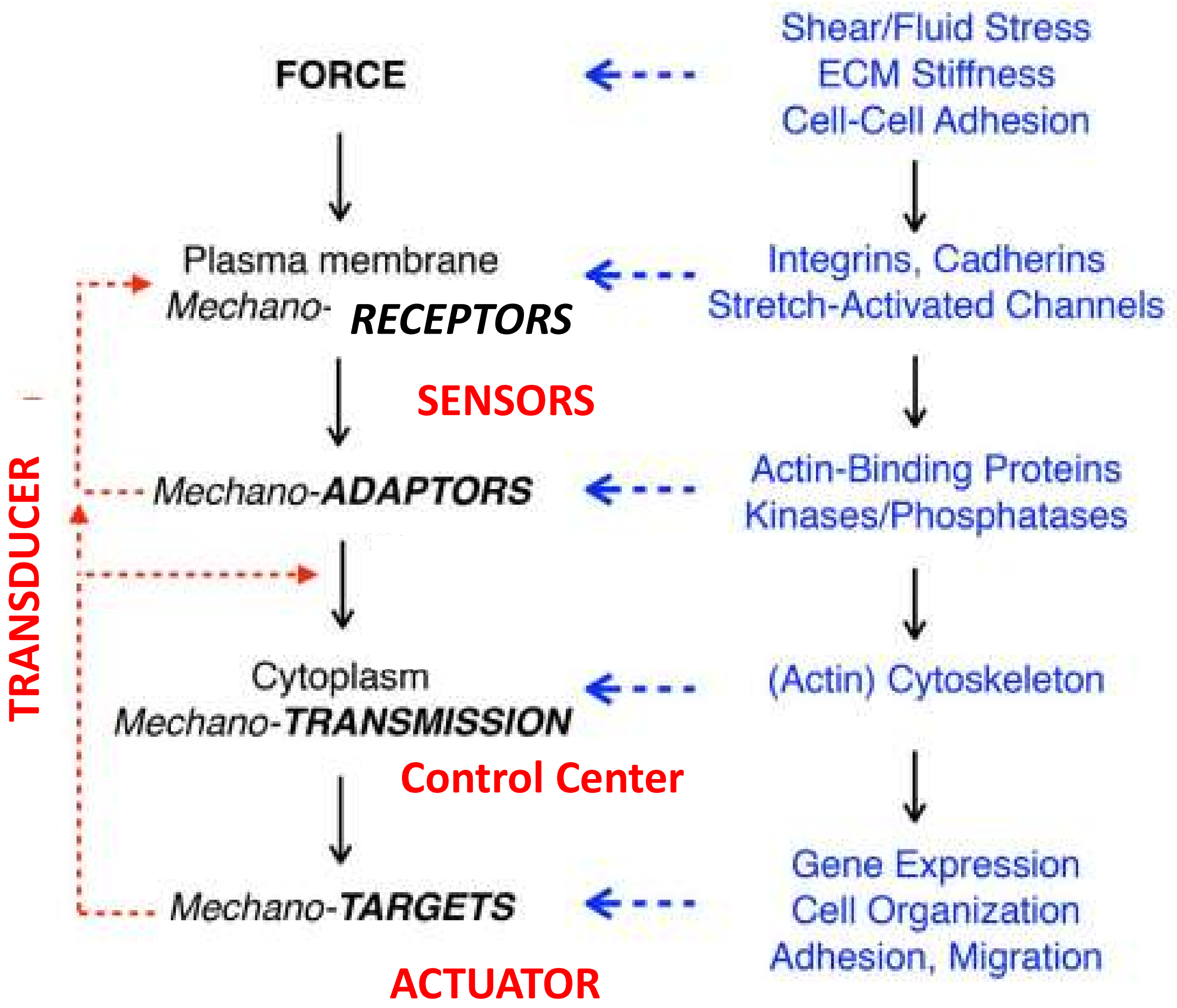
Temperature sensor detects heat.

Control center sends command to sprinkler.

Sprinkler turns on and puts out flame.

Transducer

Transduction of mechanical signals



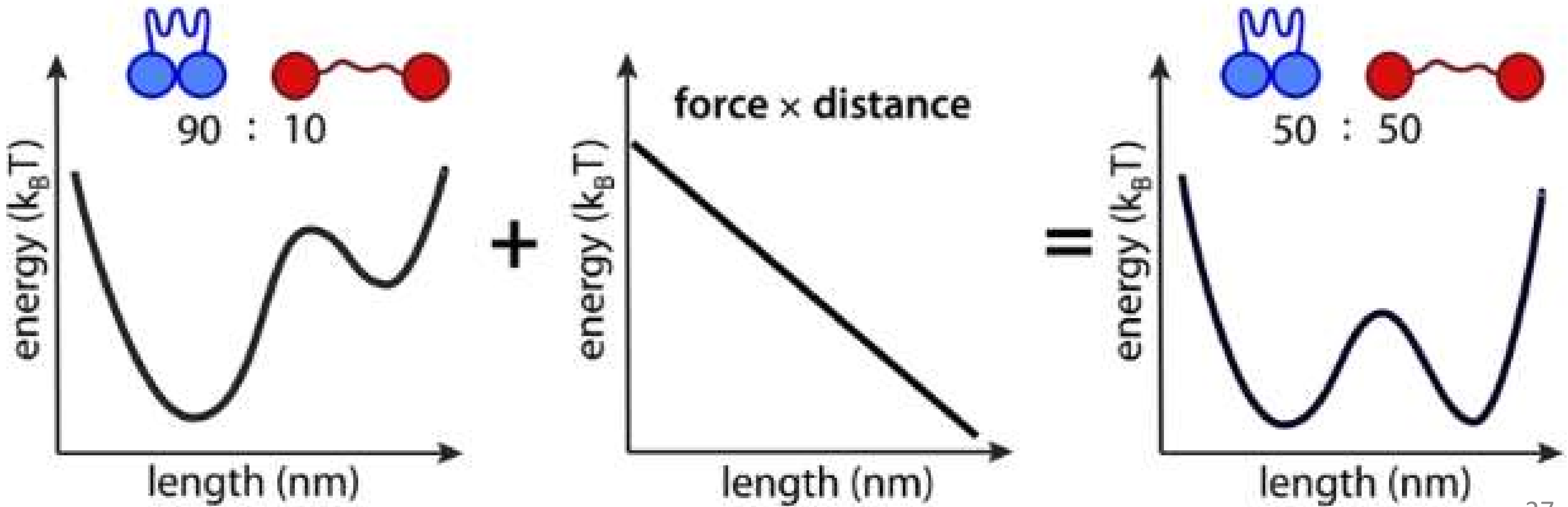
Pruitt, BL et al; *Plos Bology_2014*

Mechanosensor Engineering

- What is needed to make a mechanosensor?
- How much force is necessary to activate a mechanosensor?

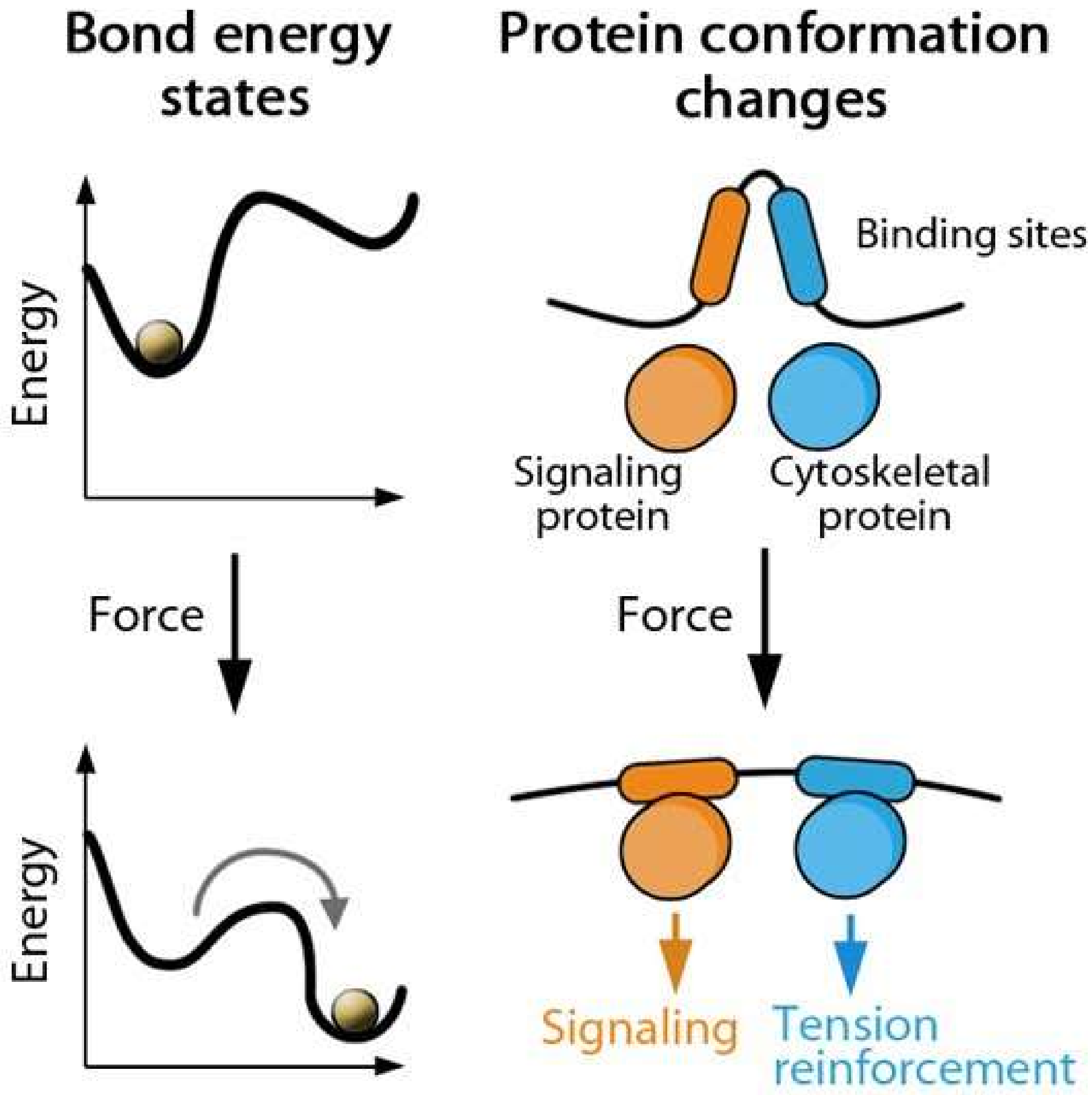
Mechanosensors must have…

- Two distal anchoring sites so it is stretched in the mechanical pathway.
- Two conformations that differ in ability to initiate a biochemical signal.
- One conformation must be both shorter and lower in energy, so force will switch the conformations.

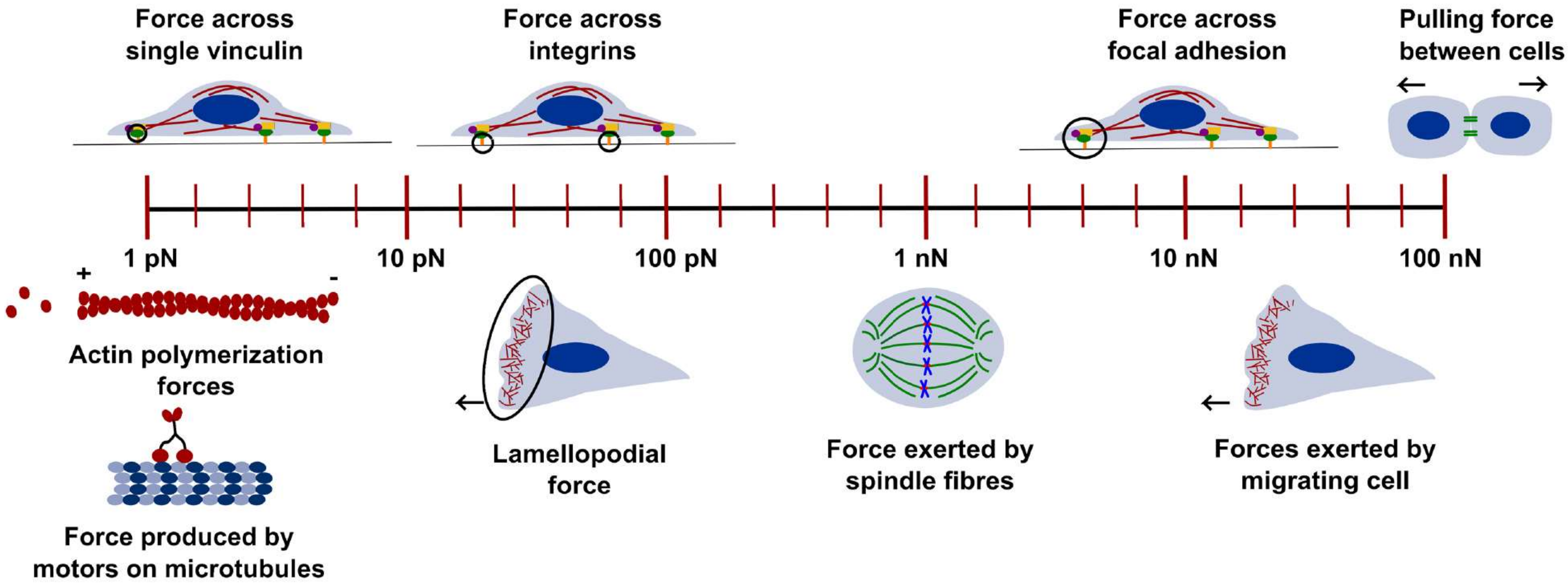


Pruitt, BL et al; *Plos Bology_2014*

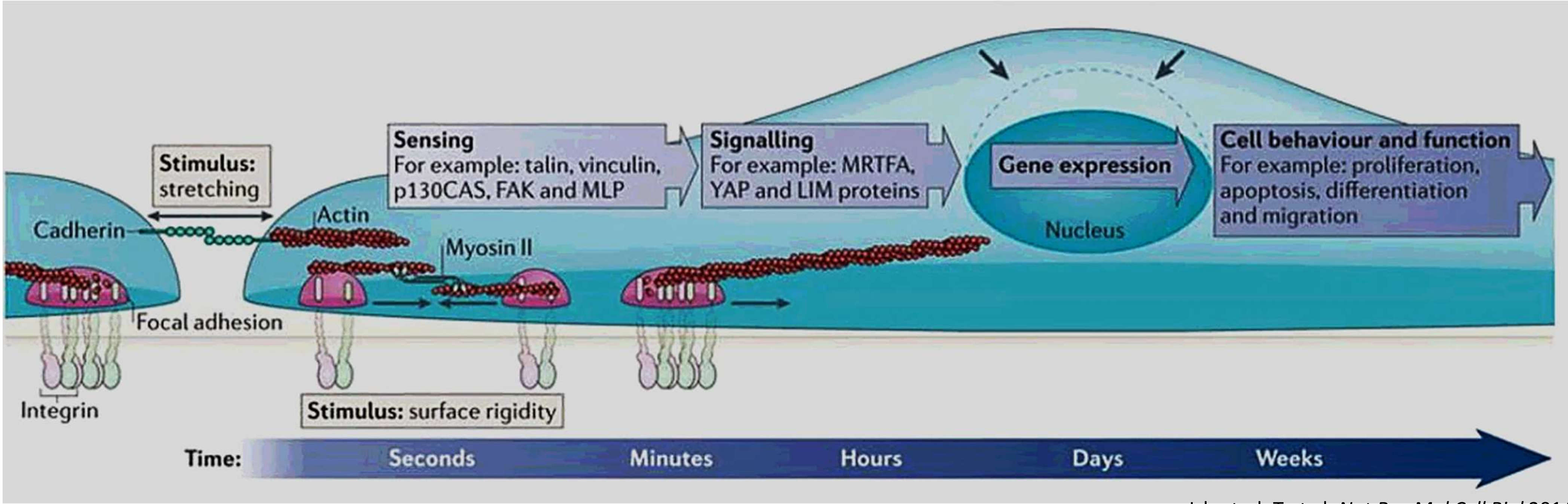
How is Energy Transferred and converted Across the Cellular System?



Range of mechanical forces

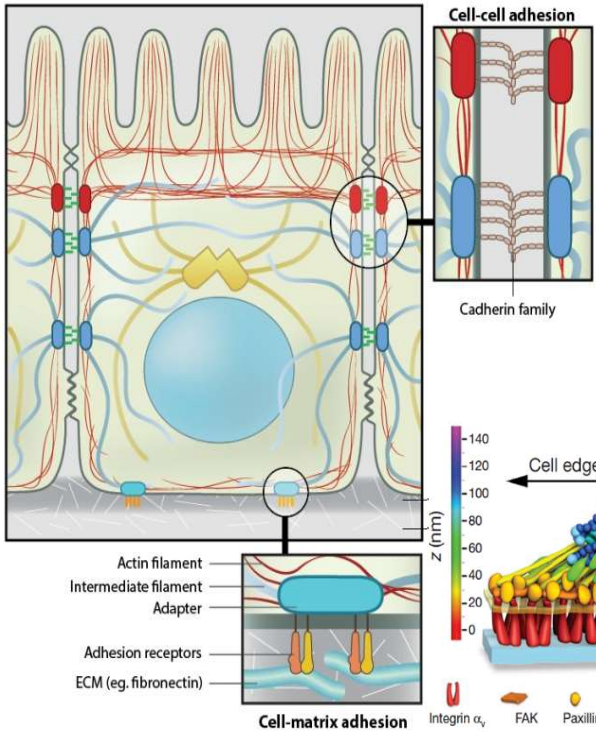


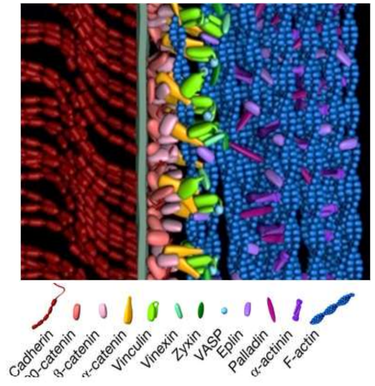
Mechanosensors

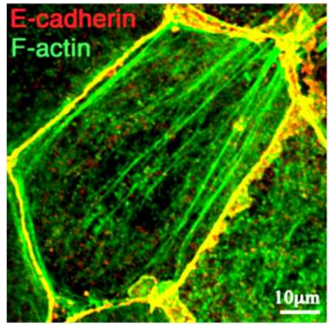


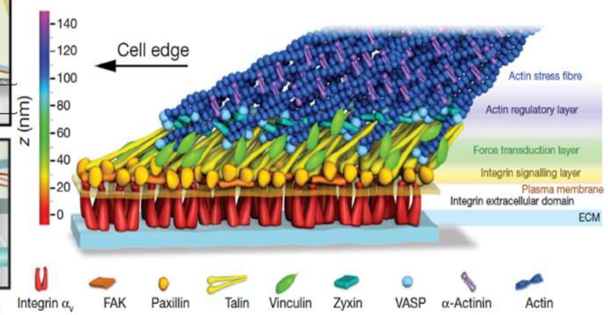
Iskratsch T et al; *Nat Rev Mol Cell Biol* 2014

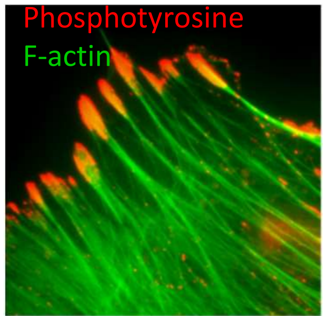
Adherens Junctions and Focal Adhesions have similar nanoscale multilayered architecture









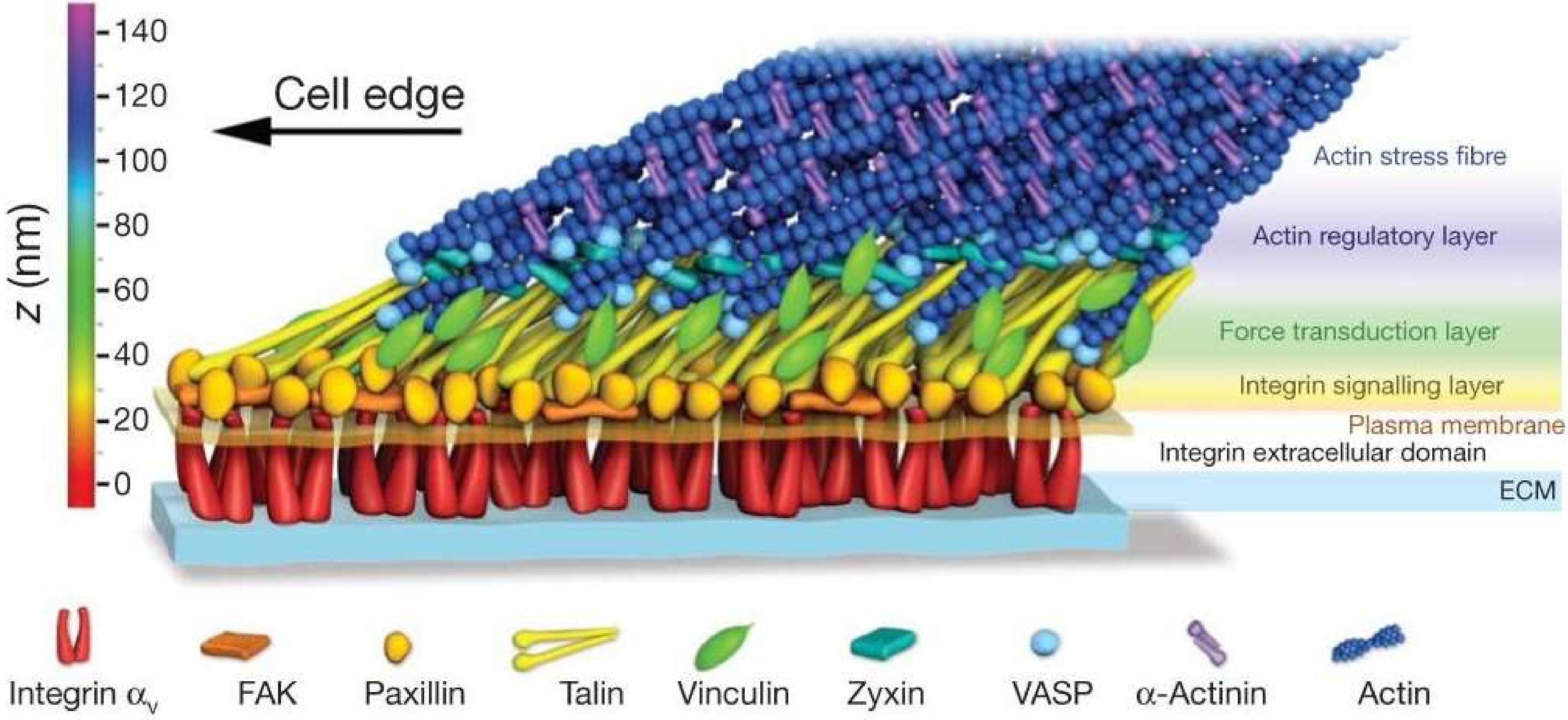


https://www.mechanobio.info

Bertocchi C. et al. *Nature Cell Biology (2017)*

Kanchanawong P. et al *Nature (2010)*

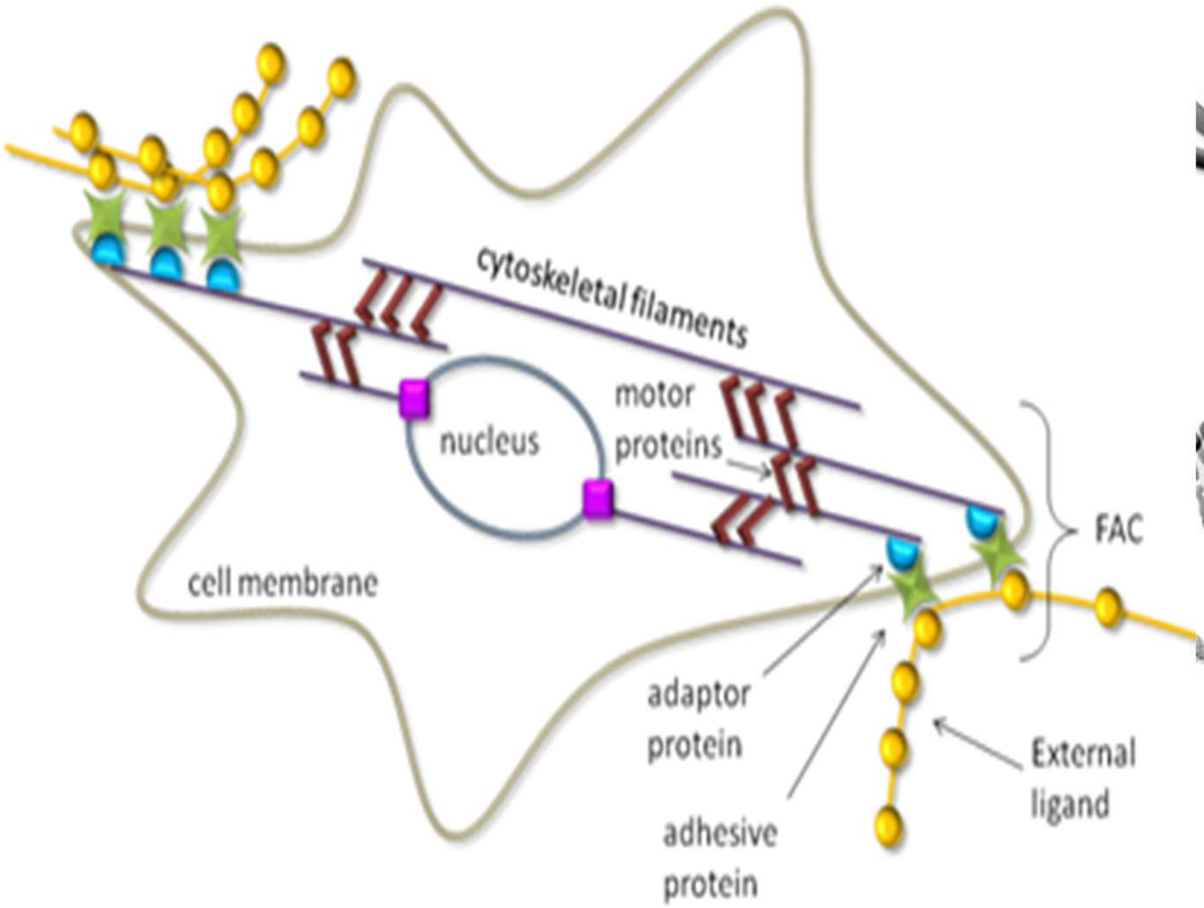
Focal Adhesion Complexes (FA)

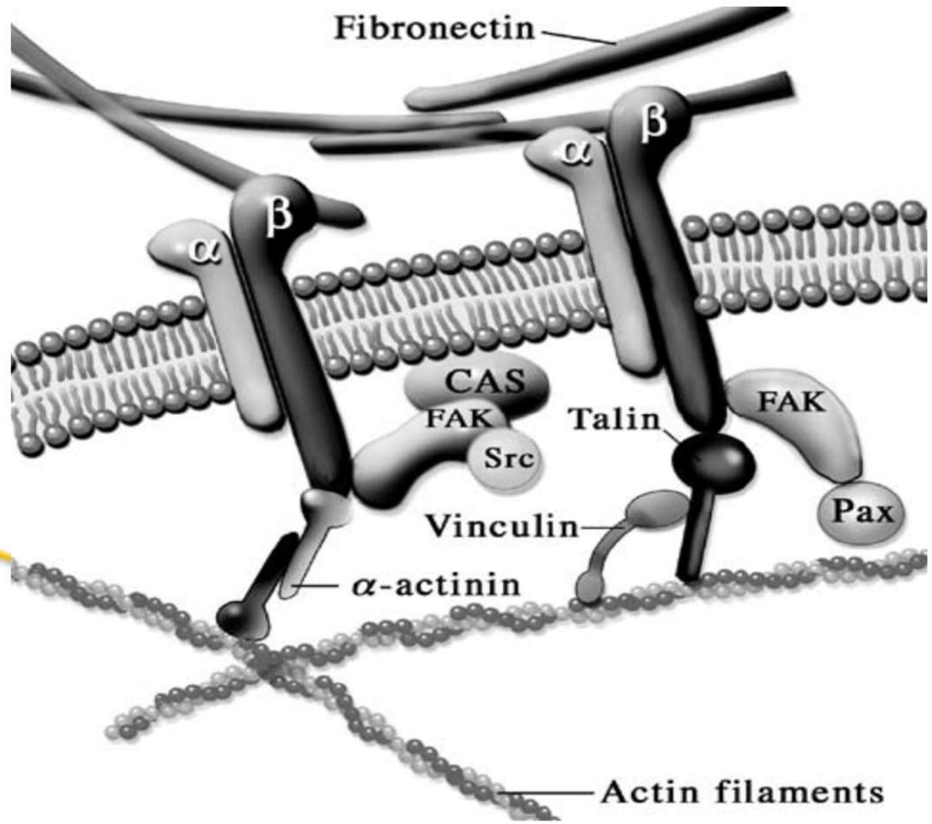


Kanchanawong P. et al; Nature 2010

FA Complexes

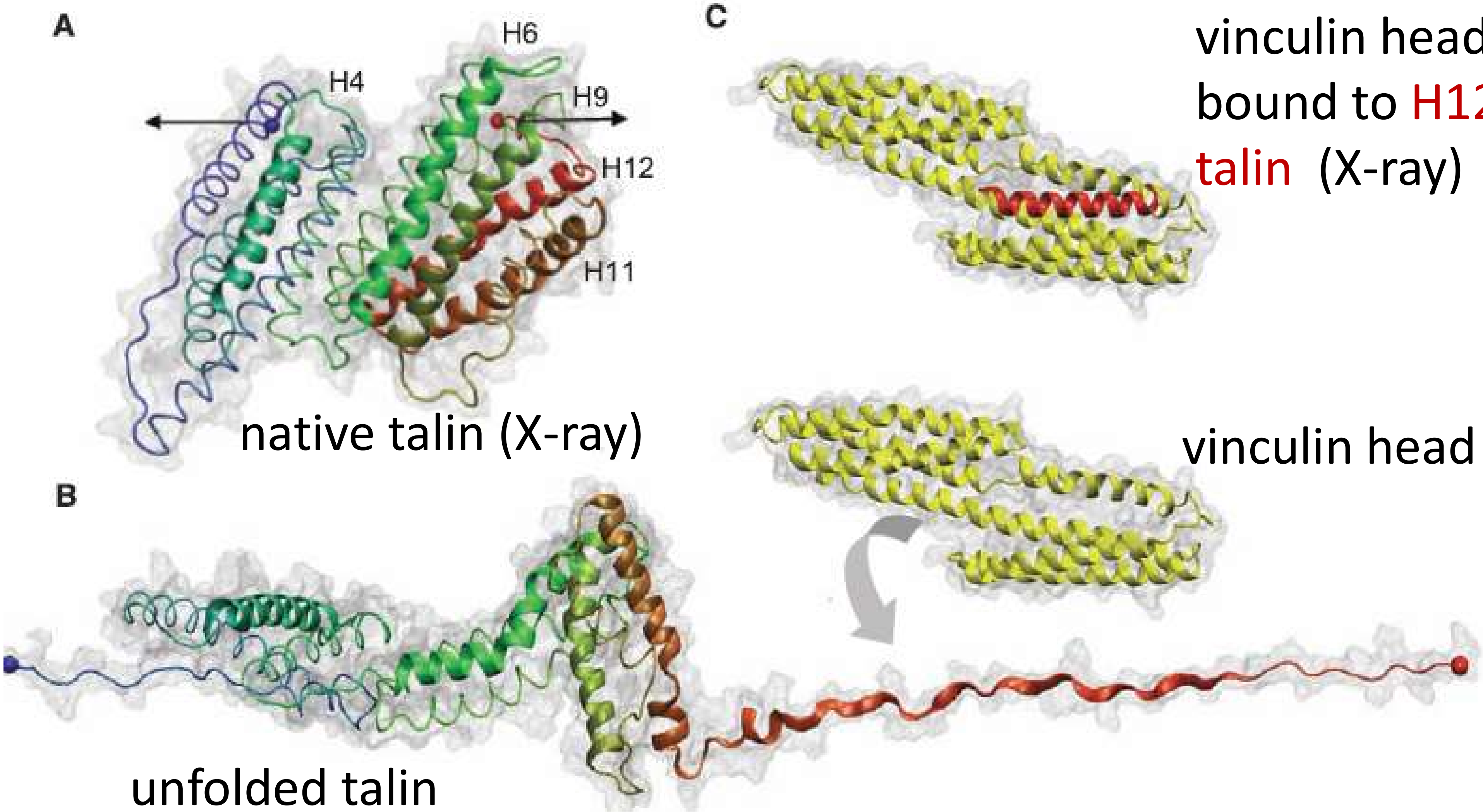
- adhesive proteins (integrin) and adaptors (talin, vinculin, α-actinin) are part of mechanical pathway
- and are coupled to signaling proteins (Src, FAK, Pax)





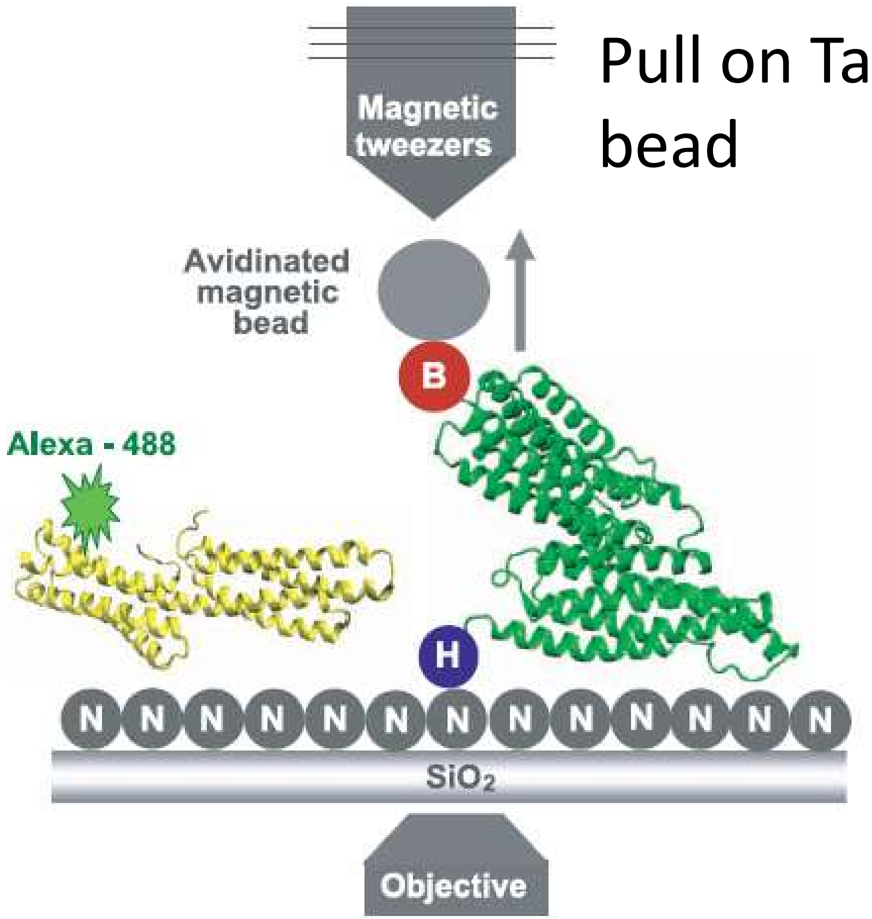
Talin as Mechanosensor

- Talin unfolds under tensile force
- Partially unfolded Talin binds vinculin head domain



del Rio et al; Science 2009

Testing for Mechanosensing



Pull on Talin with magnetic bead

Detect binding of Vinculin

More Vinculin bind at Higher force

del Rio et al; Science 2009

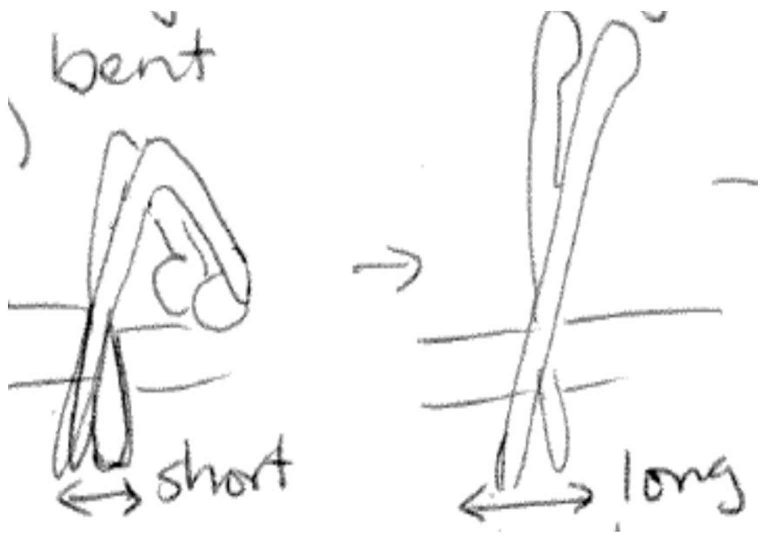
Talin Mechanosensing Conclusions

- Talin tail binds to actin filaments and head to integrins, so it is in mechanical pathway.
- Talin native state is folded but unfolded state can be induced by force.
- Two states have different activity by number of vinculin they bind.
- How does vinculin induce signal transduction? It is known to be activated when it binds talin and leads to reorganization of the cytoskeleton.

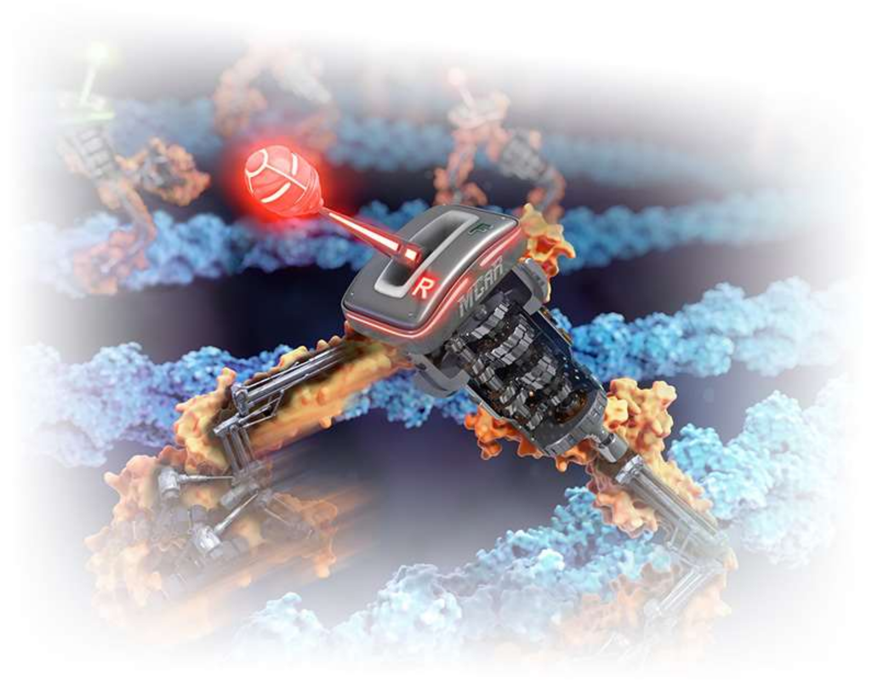
Other suggested Mechanosensors in FA

- Integrins initiate signaling when they bind (linked to signal-transduction pathways).

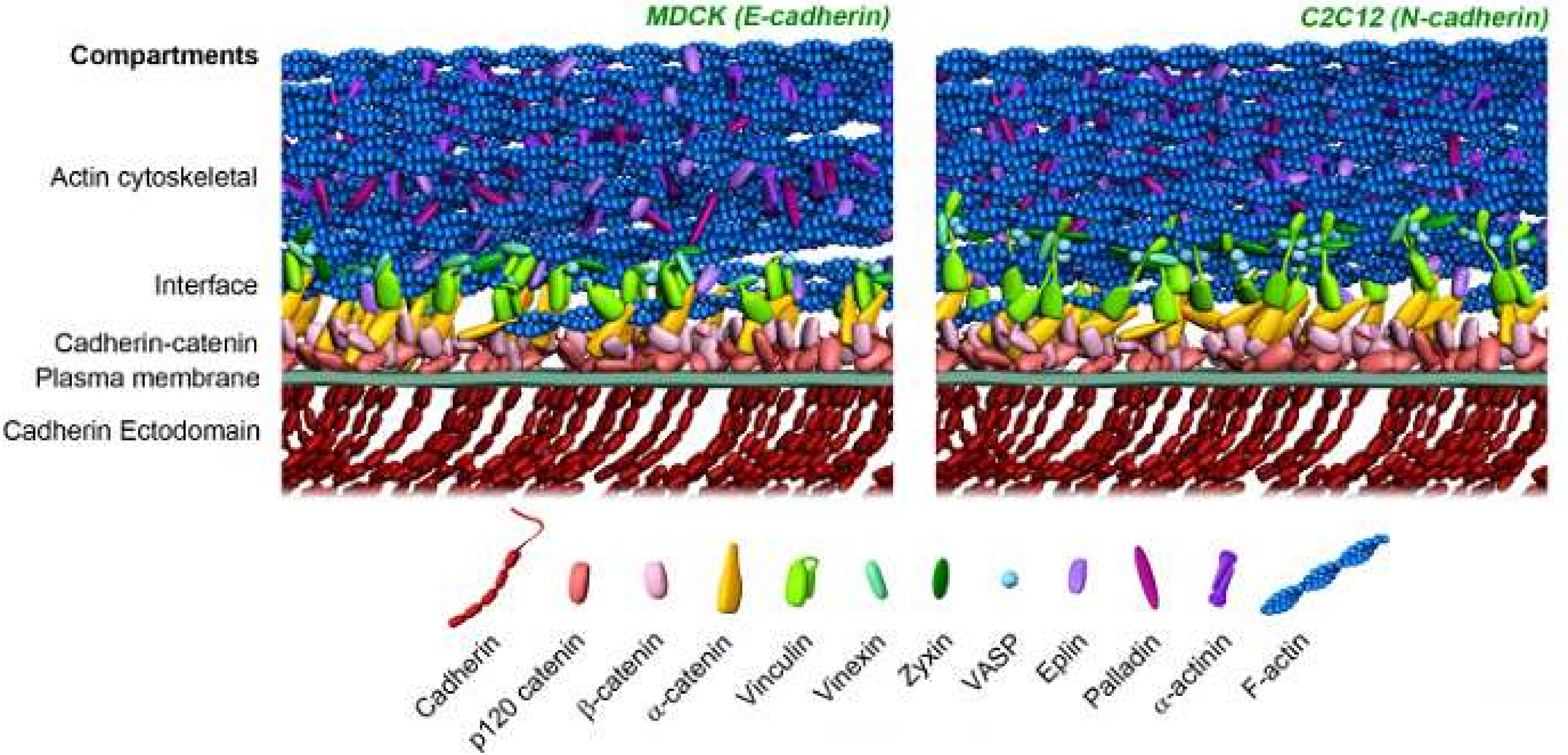
  They are in the line of force, and the active conformation is longer.

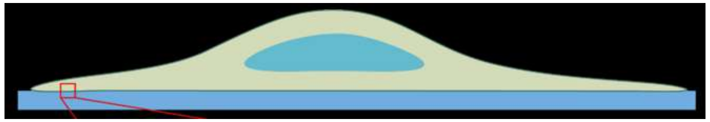


- Vinculin as molecular clutch



Adherens Junction Complex (AJ)

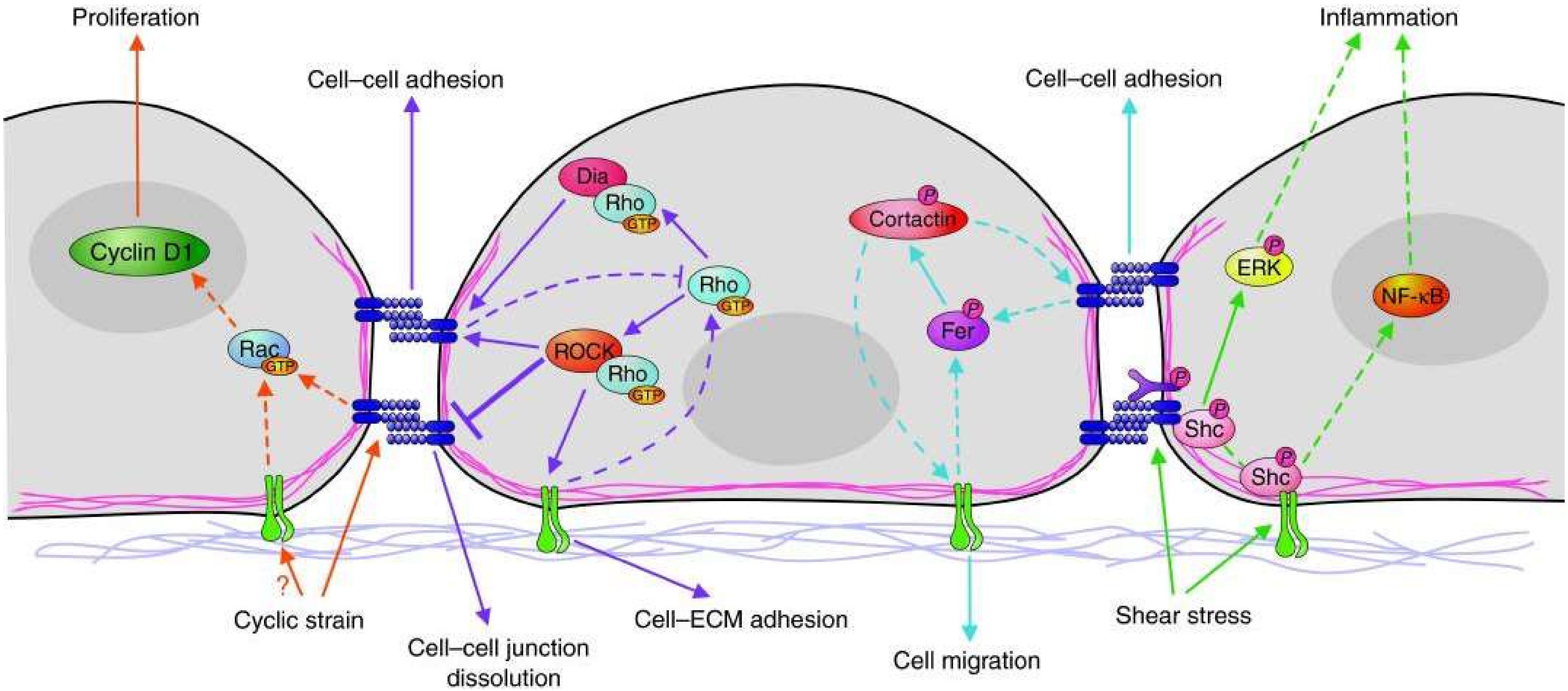




Bertocchi C; Nat Cell Biol 2017

AJ Complex

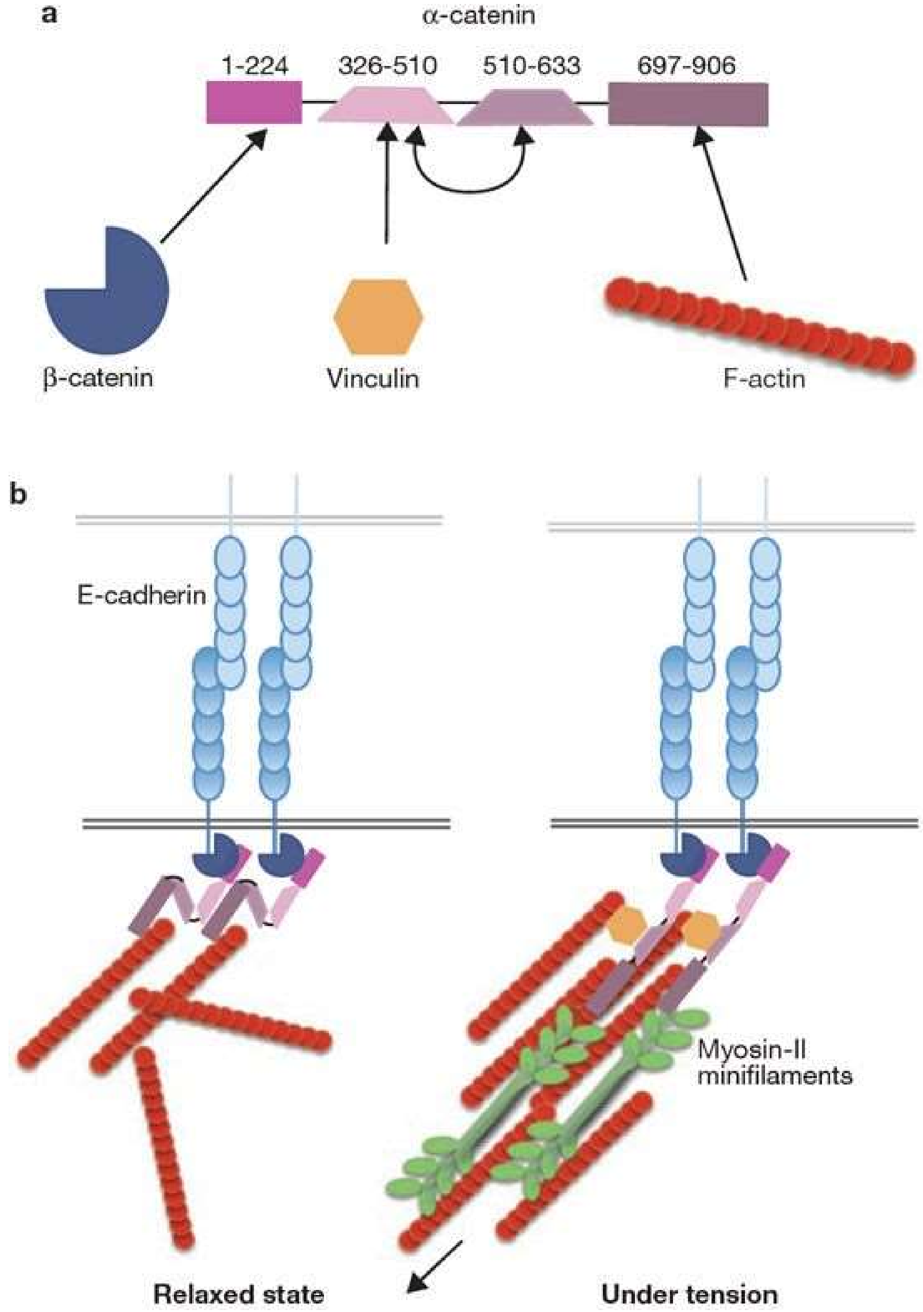
- adhesive proteins (cadherin) and adaptors (catenins, vinculin) are part of mechanical pathway
- and are coupled to signaling proteins (Rho, Rac…)



Weber GF; J. Cell Sci 2011

α-catenin as Mechanosensor

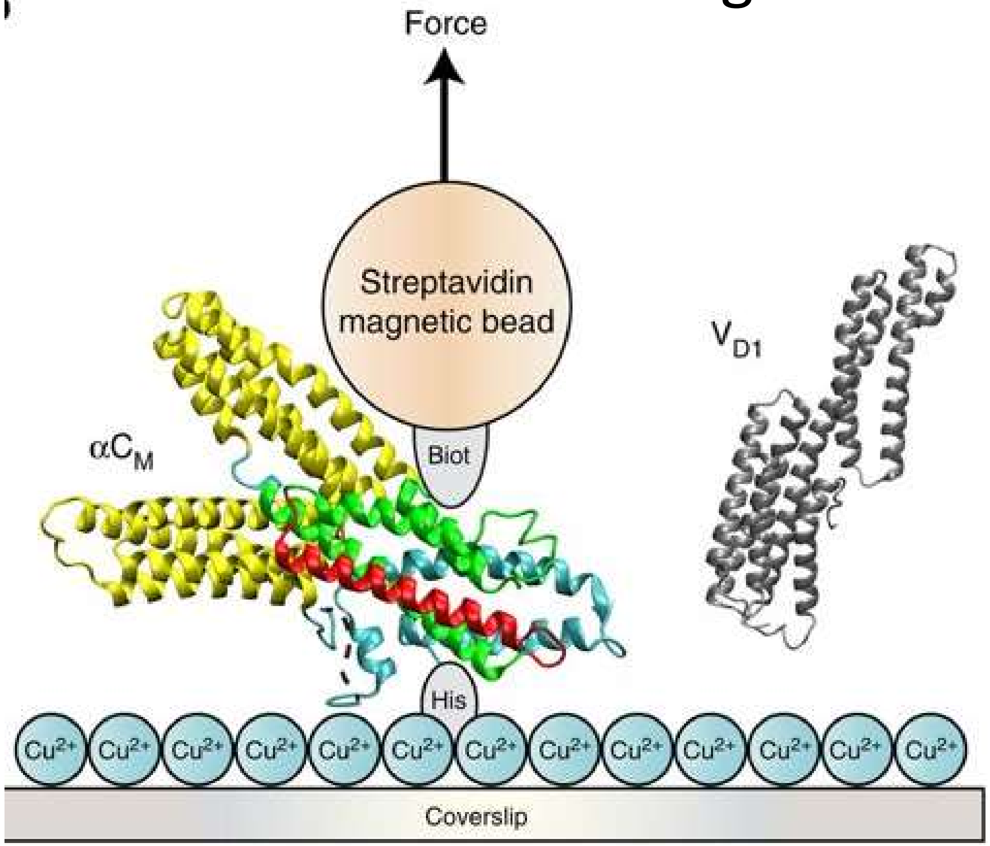
- α-catenin unfolds under tensile force
- Partially unfolded α-catenin binds vinculin head domain

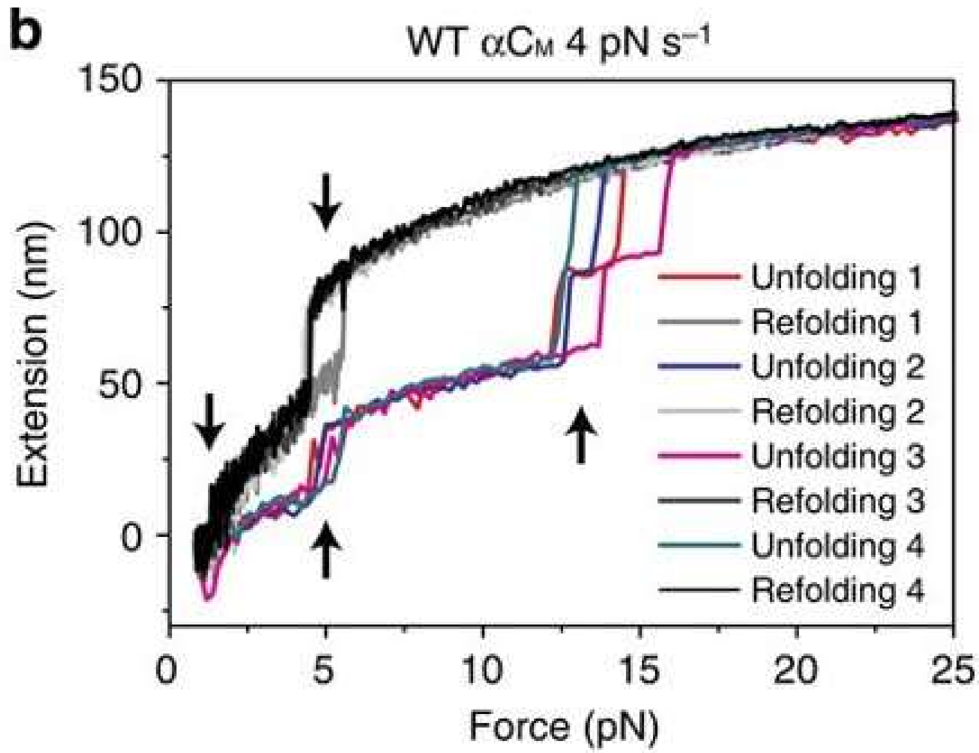


Lecuit T; Nat Cell Biol 2010

Testing for Mechanosensing

Pull on α-catenin with magnetic bead



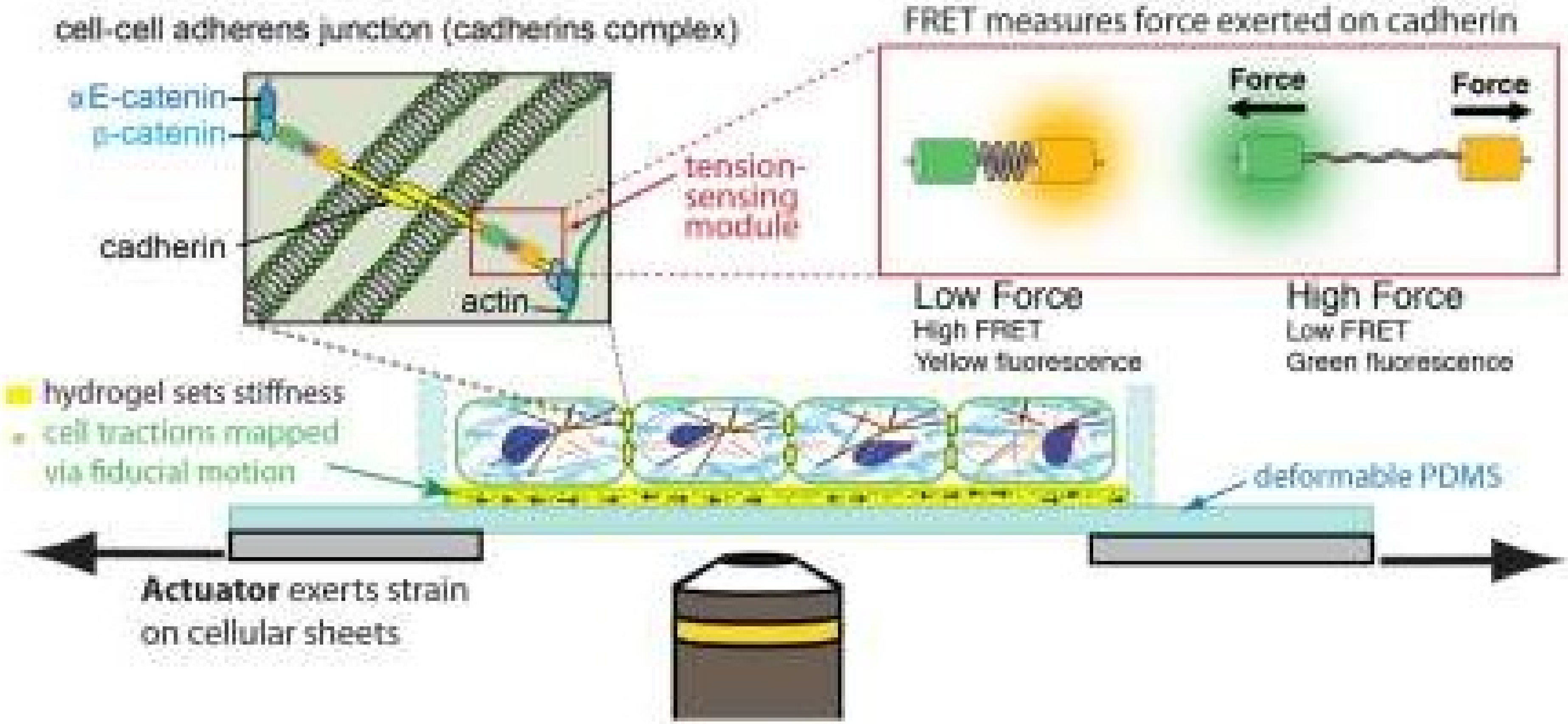


Detect binding of Vinculin

Yao M. et al; *Nat Comm_2014*

Partially unfolded α-catenin binds vinculin

Analysis of force transduction at cell–cell junctions.



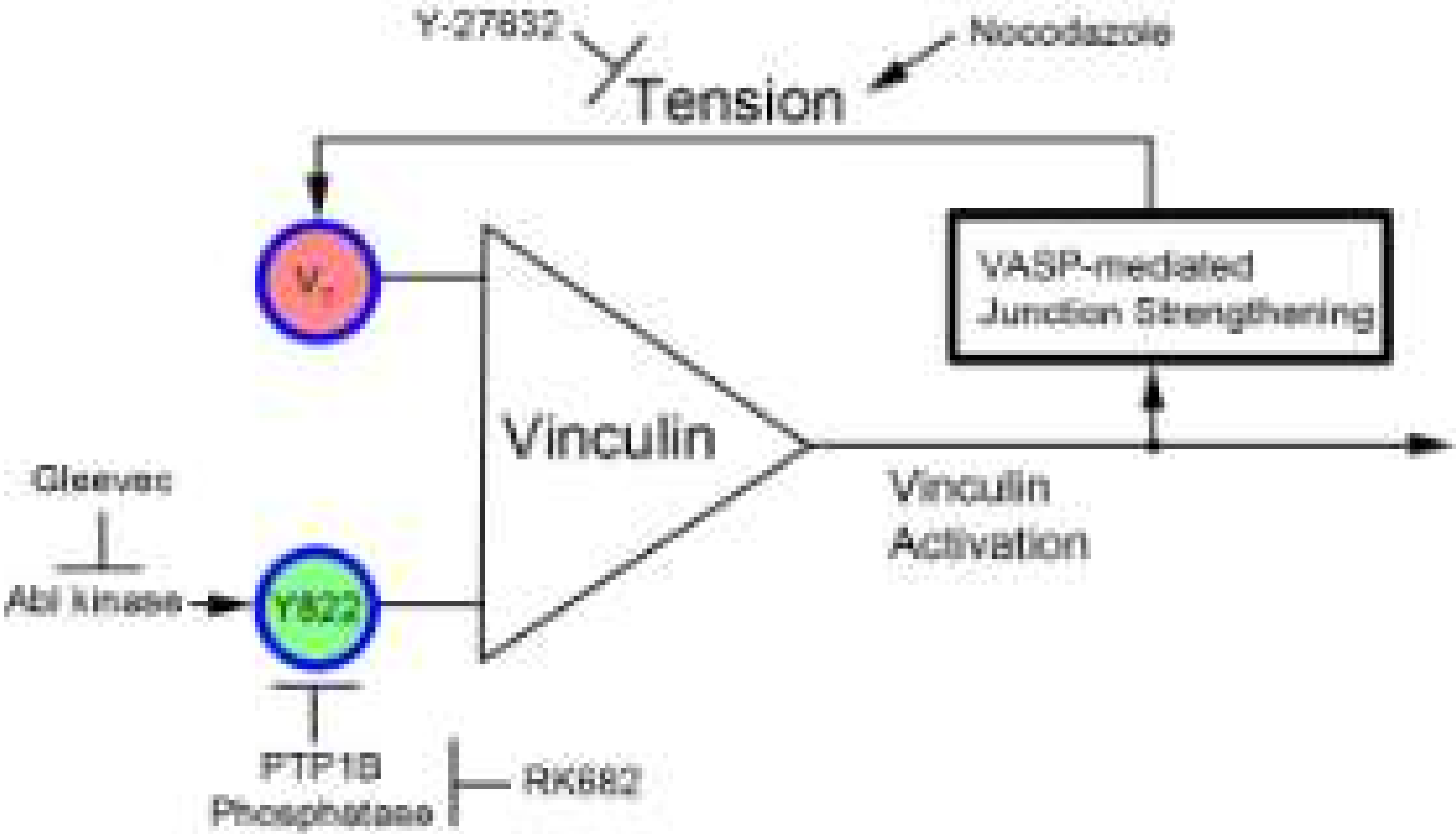
Pruitt, BL et al; *Plos Bology_2014*

α-catenin Mechanosensing Conclusions

- α-catenin tail binds to actin filaments and head to β-catenin, so it is in mechanical pathway.
- α-catenin native state is folded but unfolded state can be induced by force.
- Two states have different activity by force applied (2 steps)
- How does vinculin induce signal transduction? It is known to be activated when it binds α-catenin and leads to reinforcement of adherens junction.

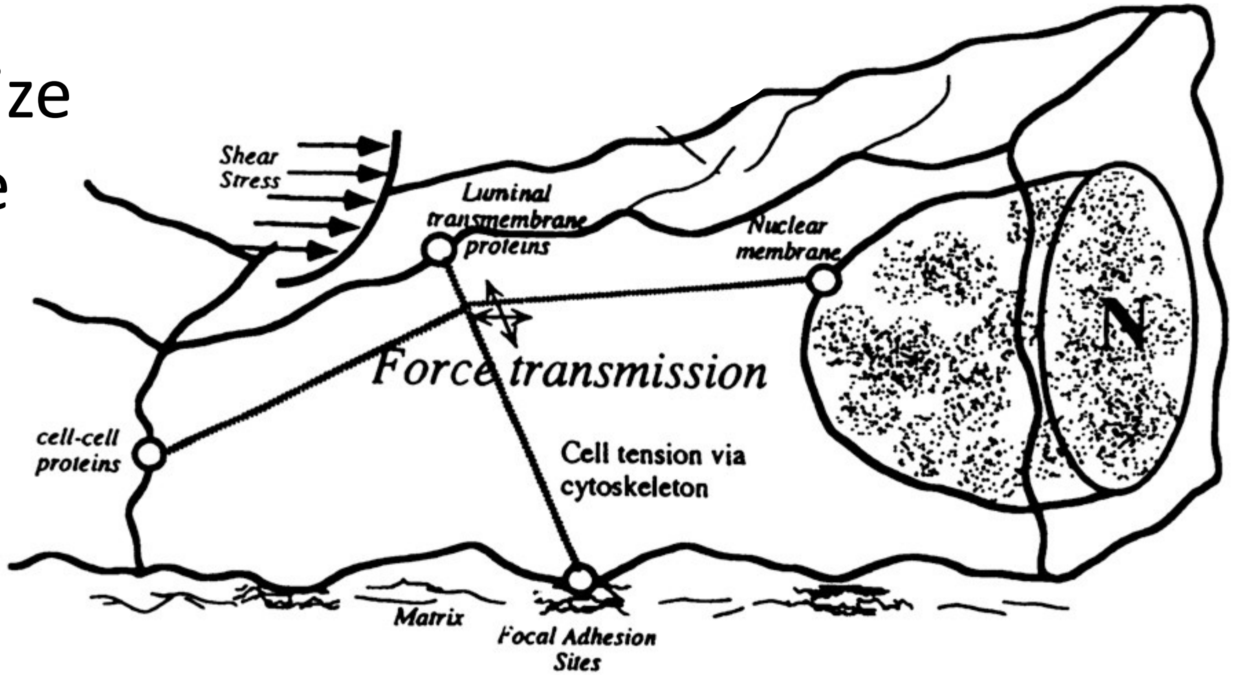
Other suggested Mechanosensors in AJ

- Vinculin as molecular clutch.



Forces applied at one point are transmitted via cytoskeletal network

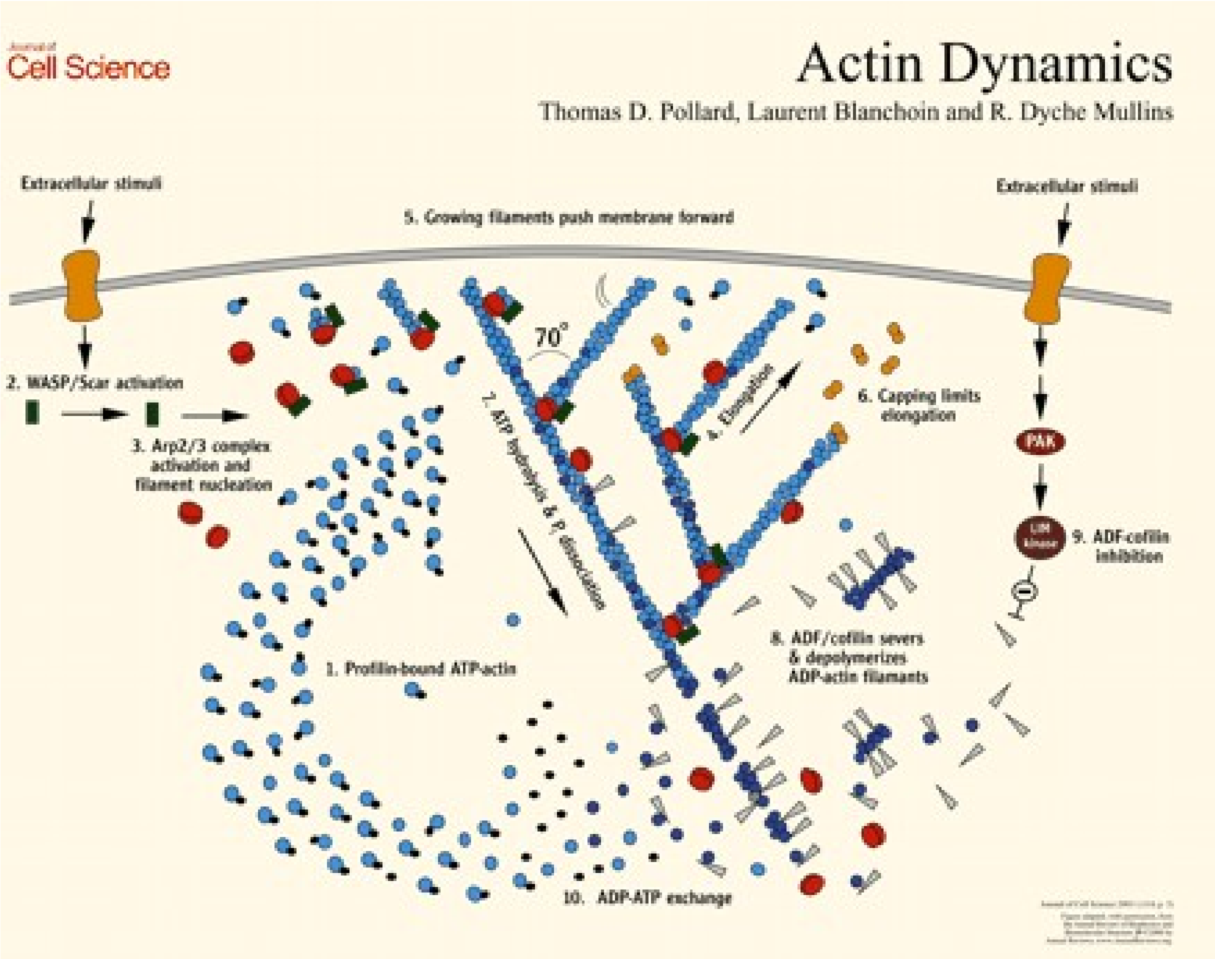
- Mechanical and structural cues influence cell behavior by regulating signaling processes
- These signals alter gene expression and re-organize the cytoskeleton and the extracellular matrix resulting into an orchestrated cellular response.

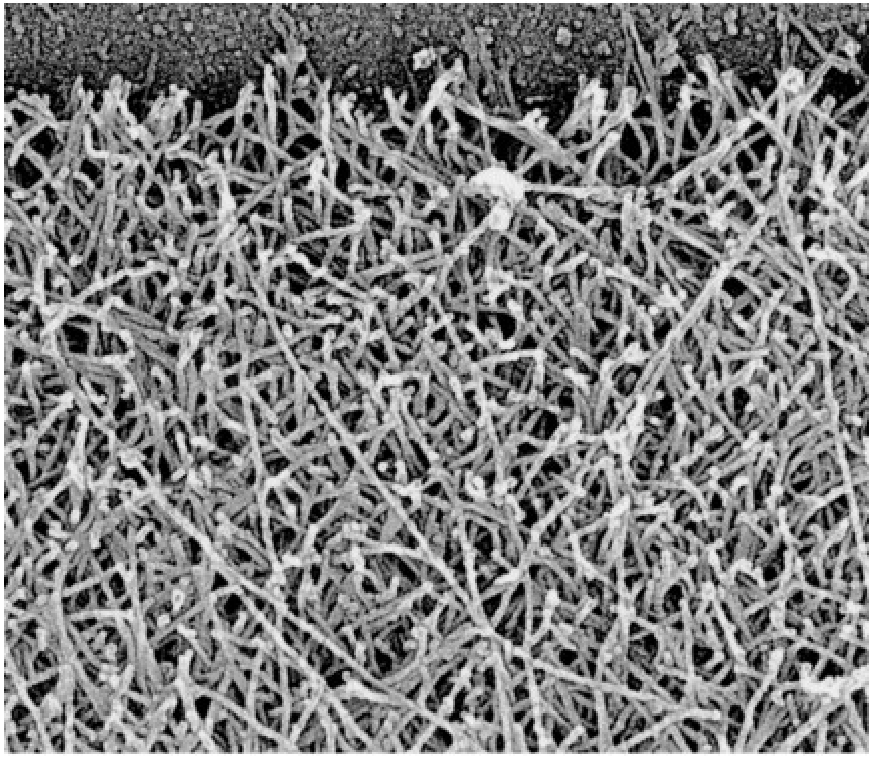


Davies P. ; Physiological Reviews _1995

Intracellular structure: Actin

- It is believed to be the primary structural component of most cells (1-10% of all the proteins)
- Responds rapidly and dramatically to external forces
- Play active role in the formation of the leading edge (migration)





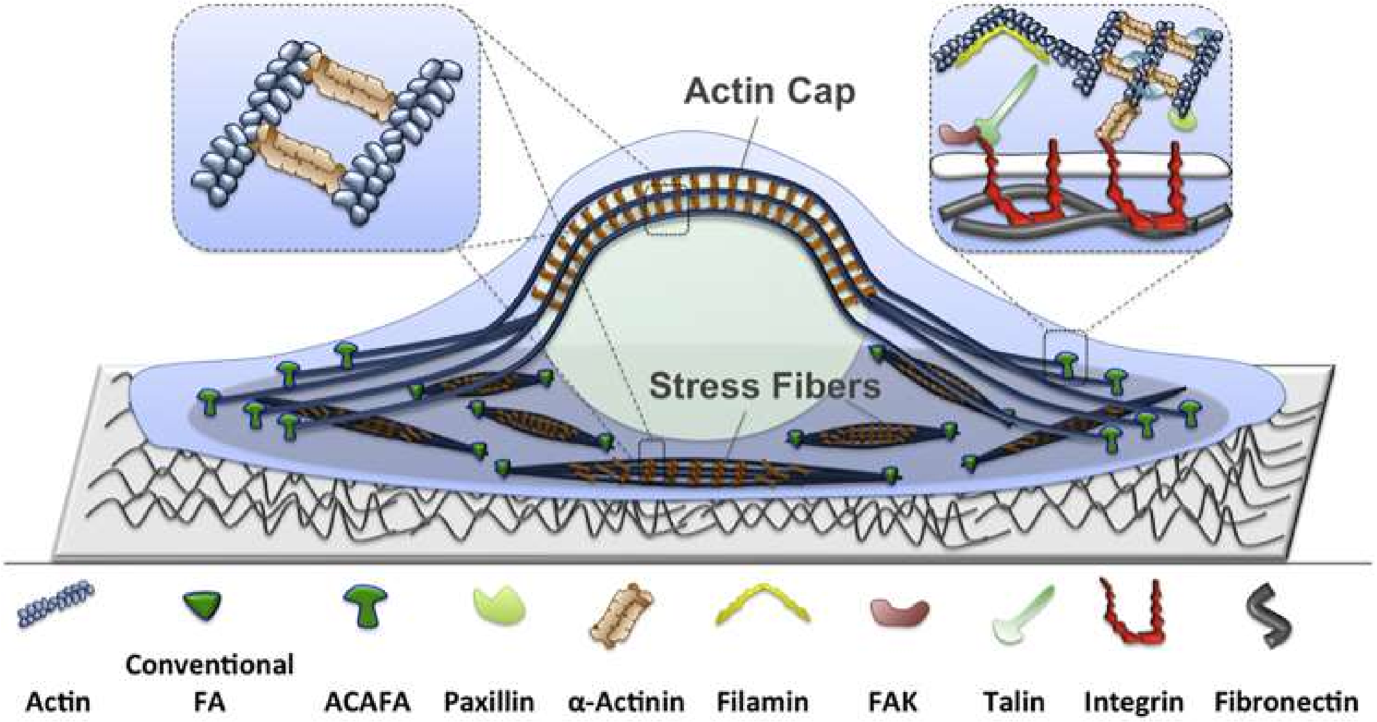
Pollard TD, et al;
Ann Rev Biophys Biomol Str 2000

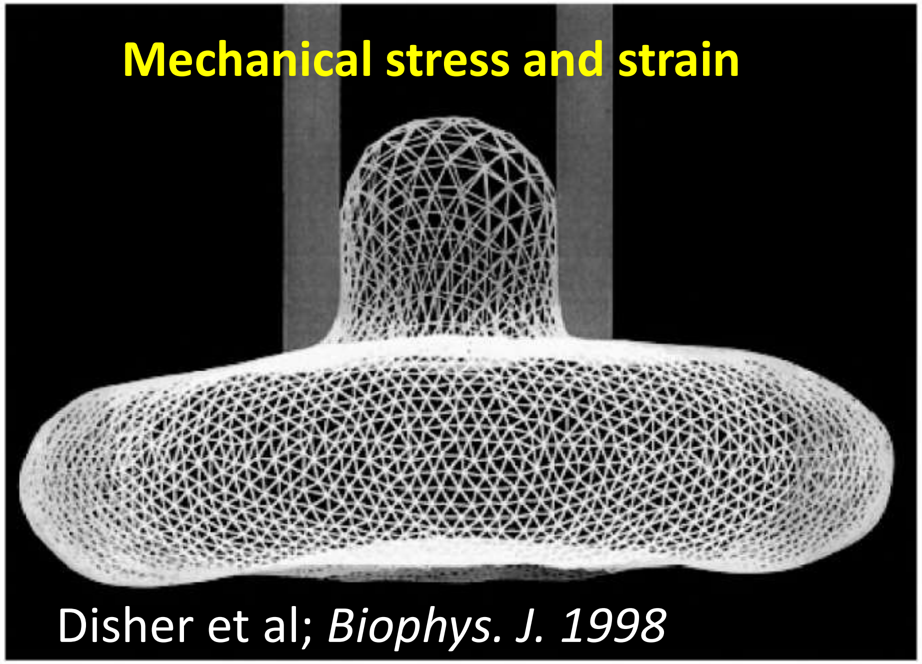
Cytoskeleton: Diversity in unity

A wide range of models proposed for cytoskeletal biomechanics:

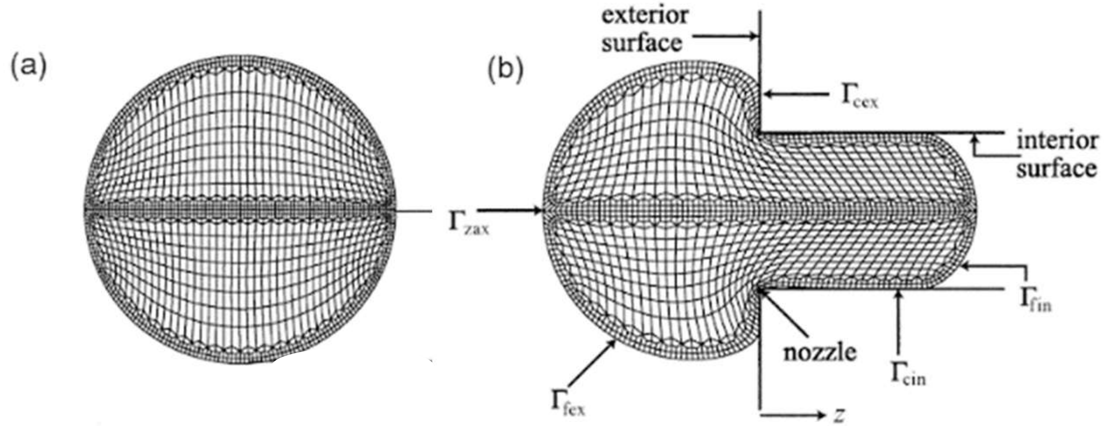
- Continuum models describing the cytoskeleton as a simple mechanical elastic or viscoelastic continuum
- Tensegrity (tension integrity) network incorporating discrete structural elements that bear compression
- Active gel theory where Actin is considered an active gel maintained in a non-equilibrium state by constant consumption of energy.
- ….

Continuum description of the cell



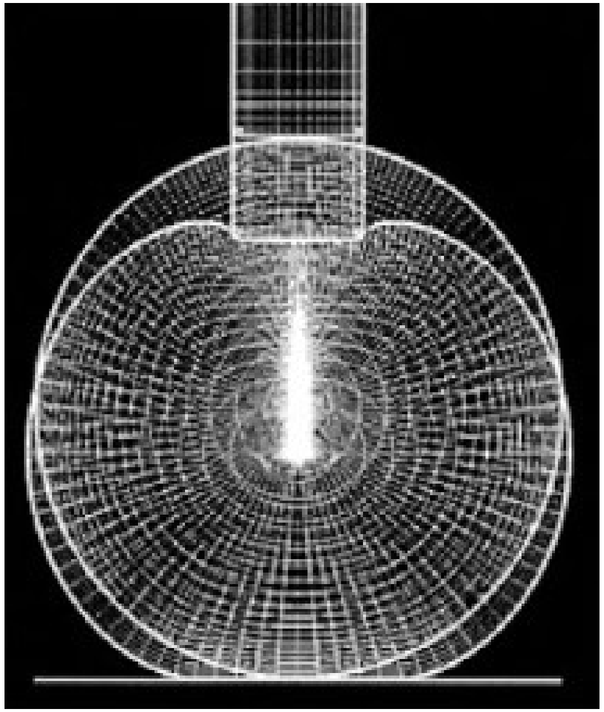


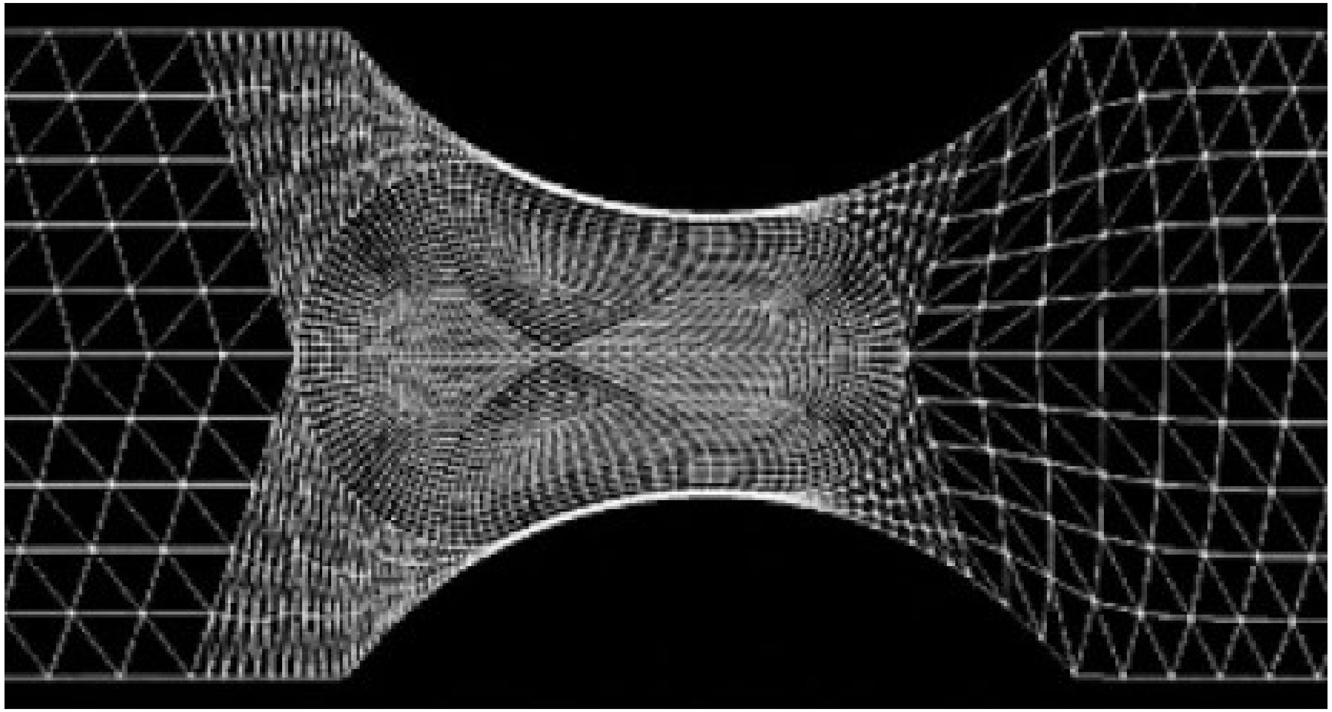
Disher et al; *Biophys. J. 1998*



Mofrad M. et al; *2006*

Benefits of Continuum models





Bathe et al; *Biophys. J. 2002*

In addition to helping interpret experiments, continuum models are also used to evaluate strains and forces exerted on cells in different biological/physiological conditions:

- A well studied example is microcirculation: the passing of blood cells through a narrow capillary
- This model enables predicting the changes in cell shape and the cell's transit time in capillaries
- This model can shed light on blood cells dysfunctional microrheology arising from changes in cells shapes or mechanical properties(e.g. time-dependent stiffening of erythrocytes infected by malaria parasites)

Tensegrity model

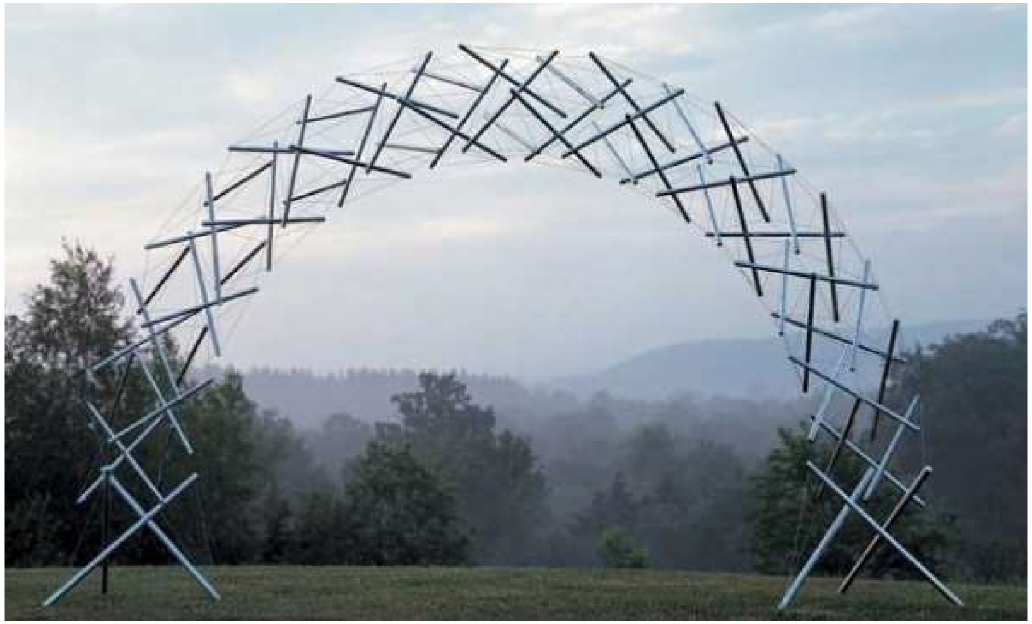
What is tensegrity?

- A model proposed by Ingber and colleagues of cell structures to explain how the internal cytoskeleton of adherent cells mediates alterations in cell functions caused by changes in cell shape.

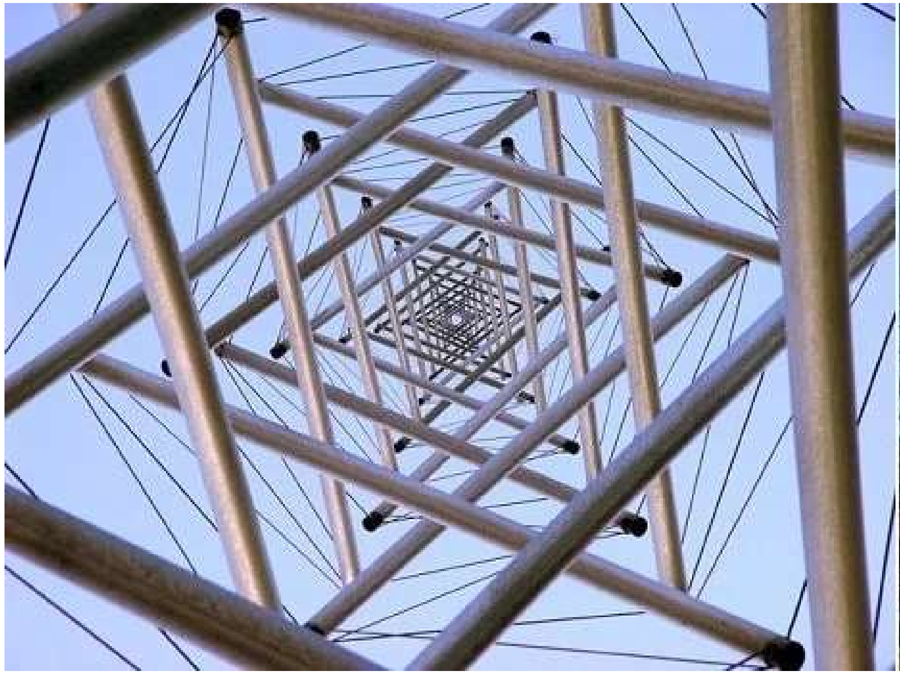
- This model is based on a building system known as tensegrity architecture (Fuller, 1961)

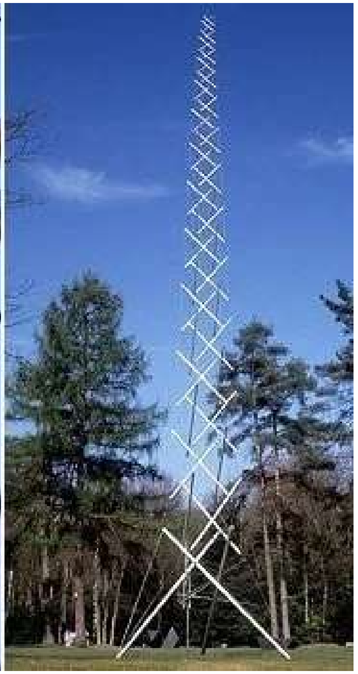
- *"Tensegrity describes a structural-relationship principle in which structural shape is guarantee by the finitely closed, comprehensively continuous, tensional behaviors of the systems and not by the discontinuous and exclusively local compressional member behaviors."*

Tensegrity architecture



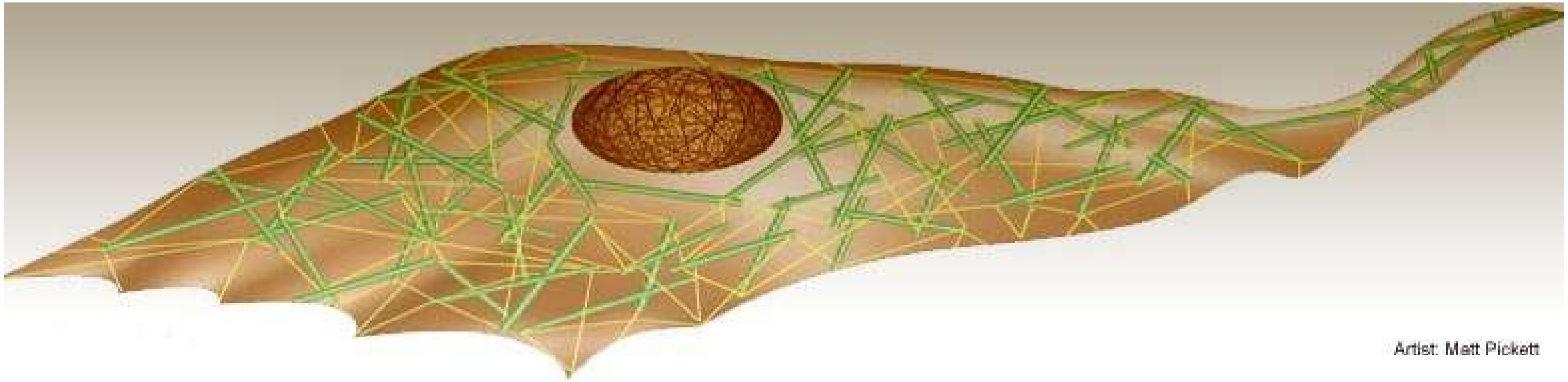
- Tensegrity structures stabilize their shape by continuous tension or “tensional integrity” rather than by a continuous compression.





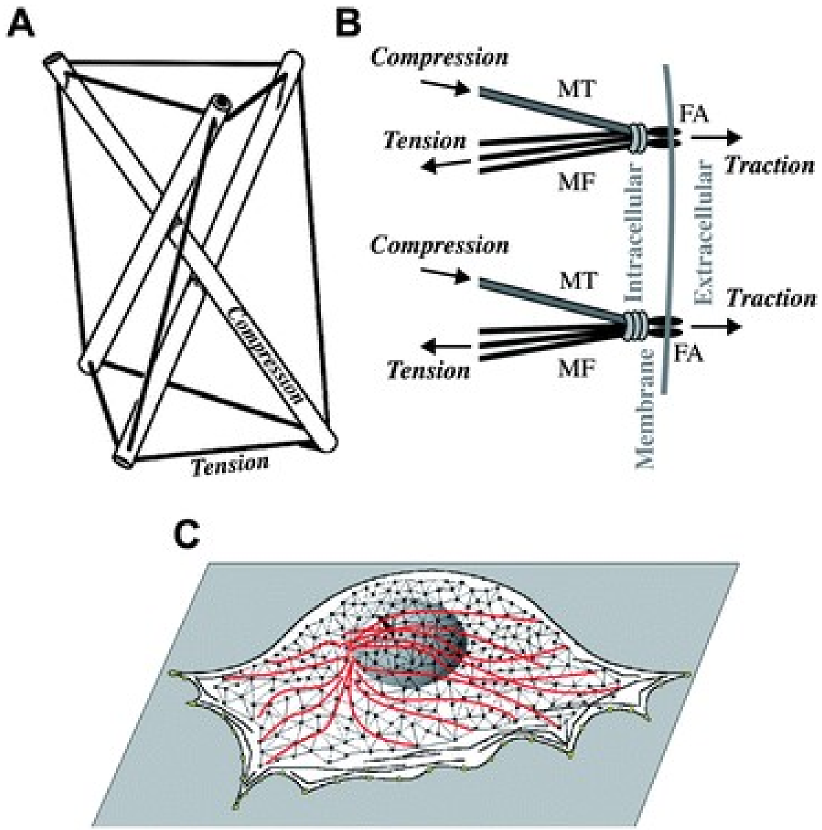
Stone arch (under continuous compression).

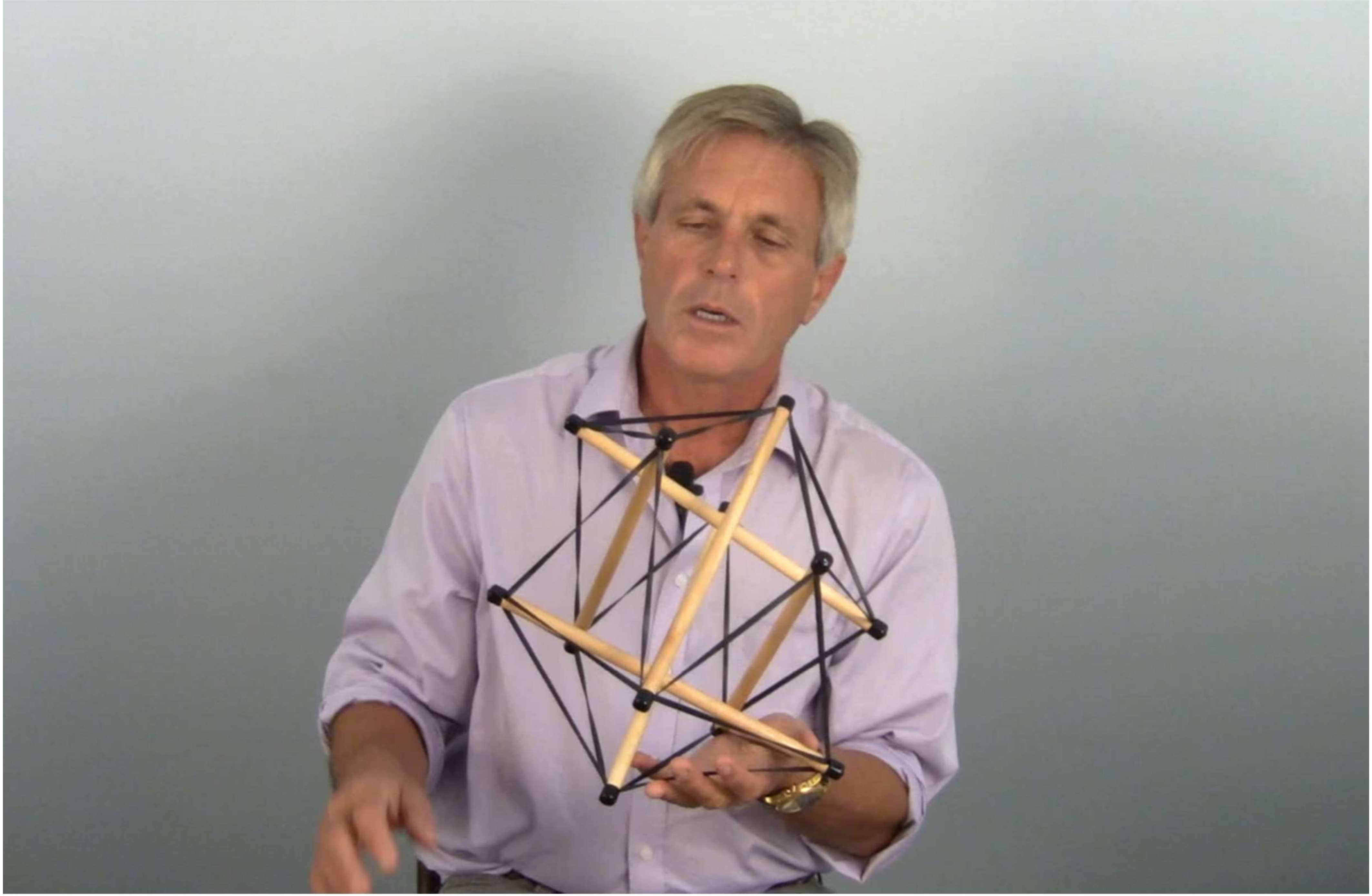
Cellular tensegrity



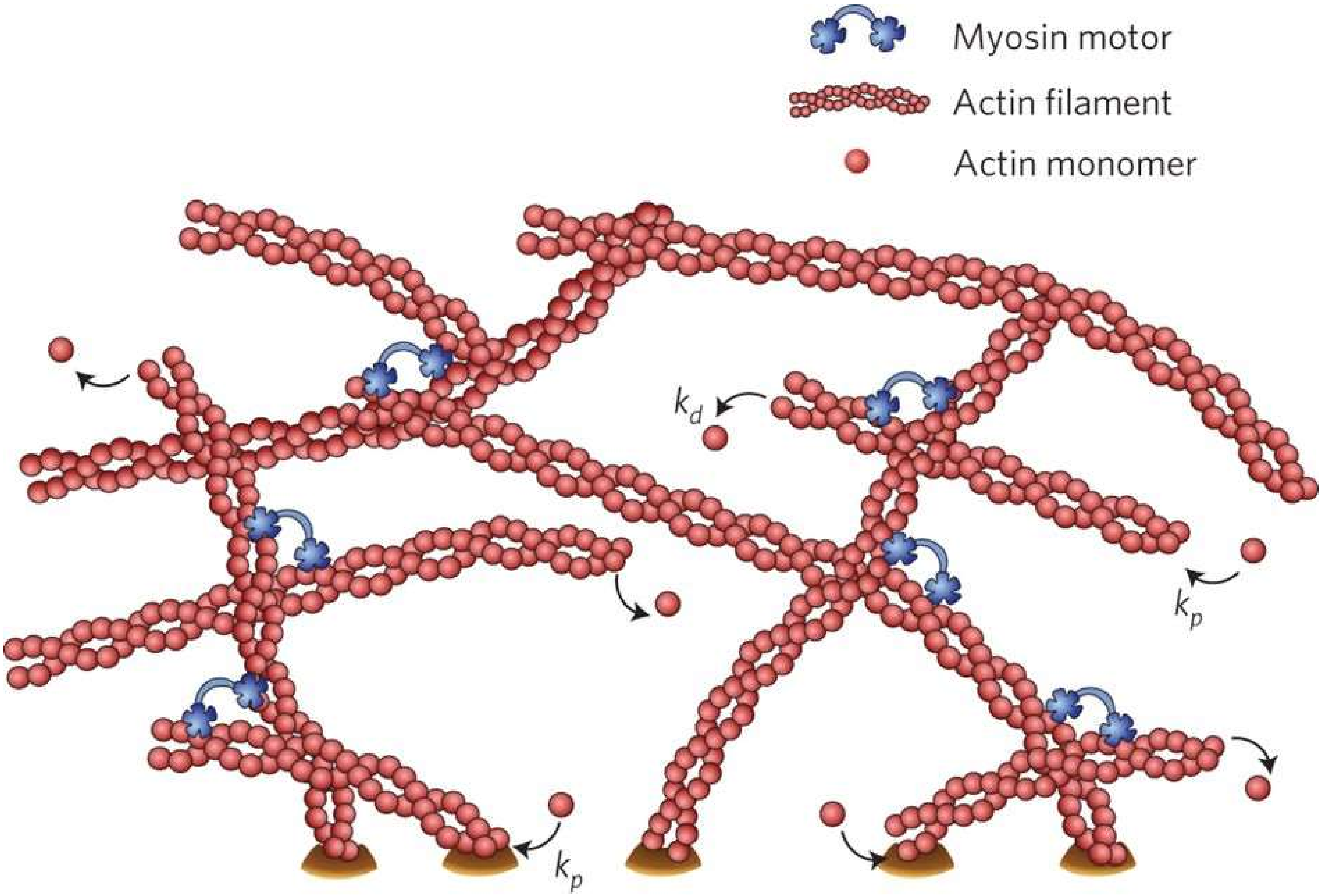
- High tension cables compress the struts
- Struts resist compression and tense the cables

Tension	Microfilaments (actin)	Cables
Compression	Microtubules	Struts

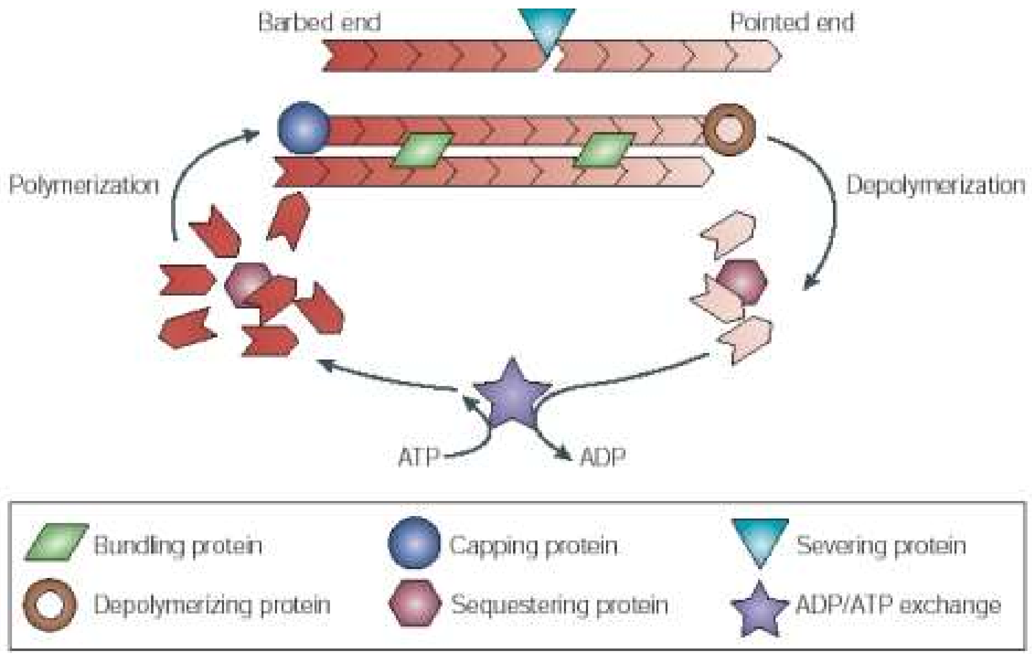




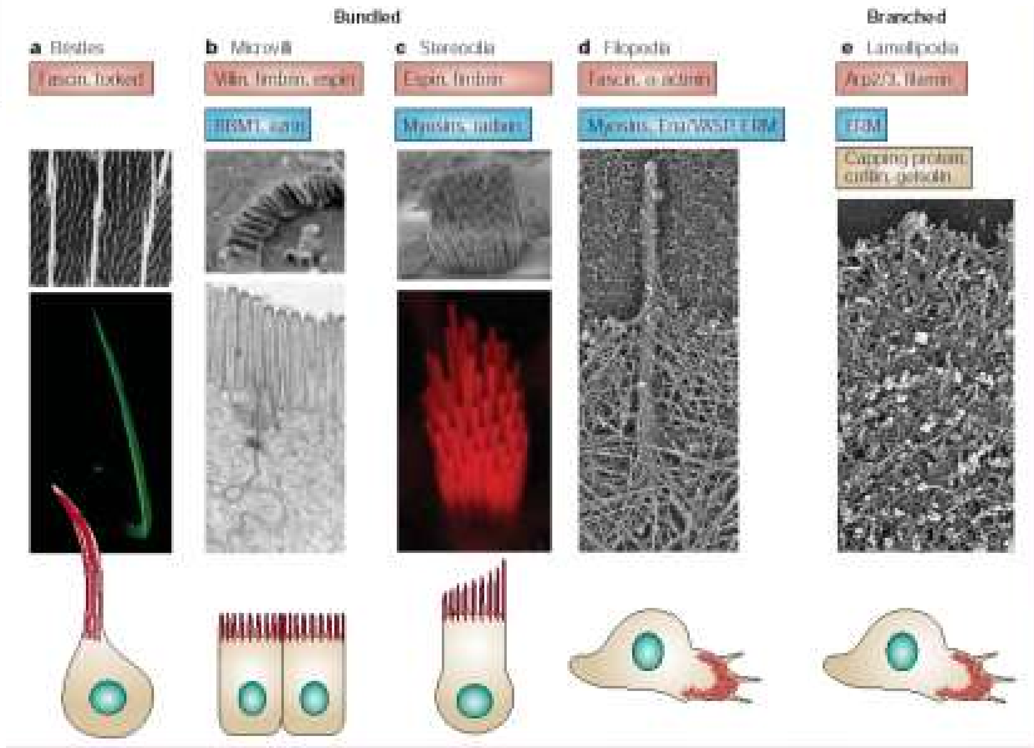
Active gel theory (hydrodynamic theory)

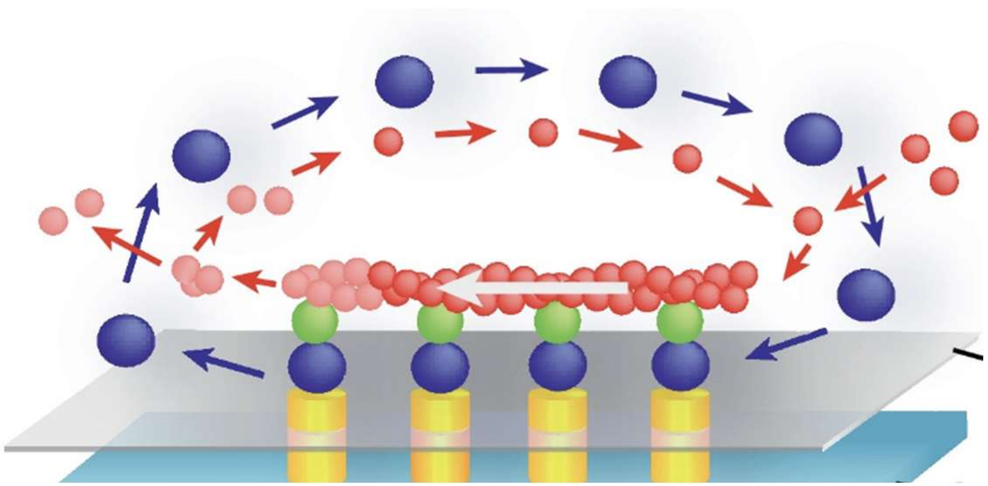


Actin as a gel



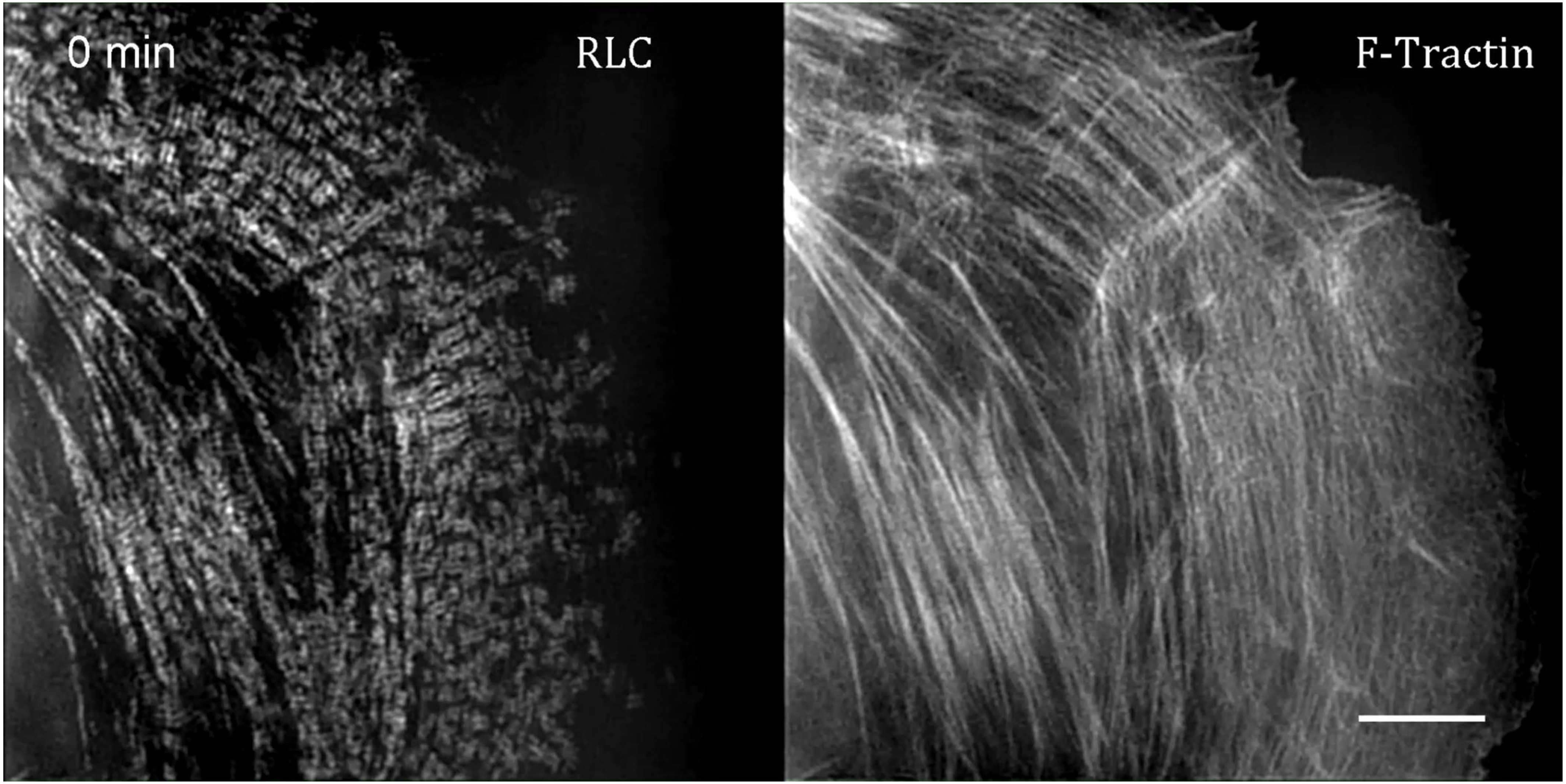
- Actin filaments are tread milling and form a viscoelastic gel interacting with myosin molecular motors driven by hydrolysis of adenosine triphosphate.





Actin treadmilling

Intracellular structure: Actin+myosin



Hu S. et al; *Nat Cell Biol*. 2017

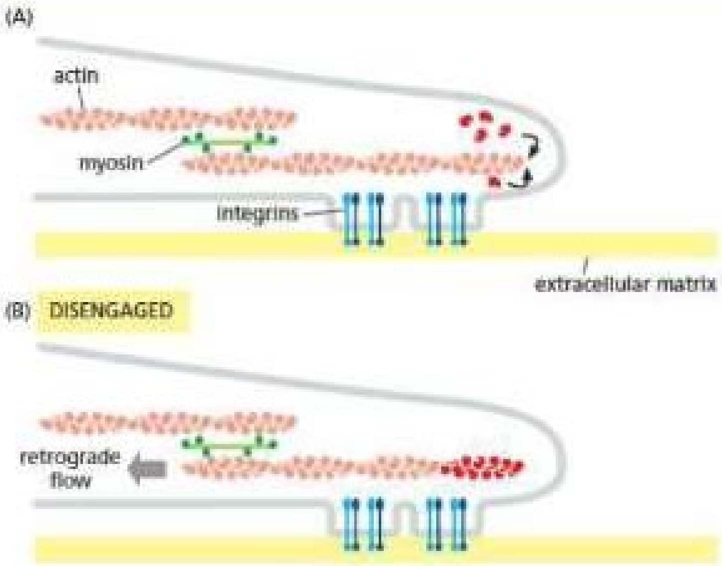
EXAMPLE of use of Output

How to link all these compontents for migration?

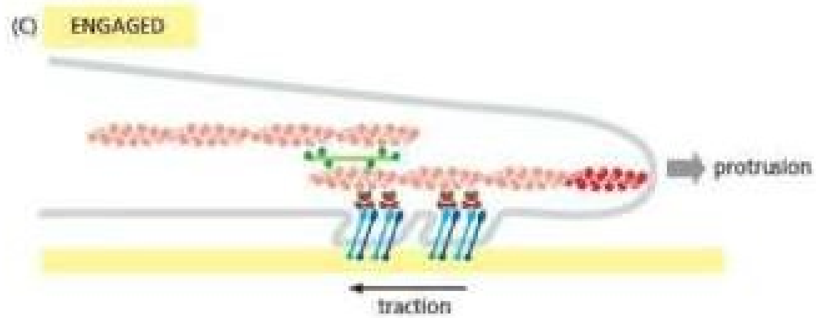
Molecular clutch

La interacción entre proteínas adaptadoras de unión a actina e integrinas conecta citoesqueleto de actina con el sustrato

Para que las protrusiones que forma la actina en el margen de avance puedan mover la célula hacia adelante se requiere de la interacción firme entre la red dendrítica de actina y las adhesiones focales que conectan la célula al sustrato



(A) Ensamblado de filamentos en el frente de avance. Formación de adhesiones focales.

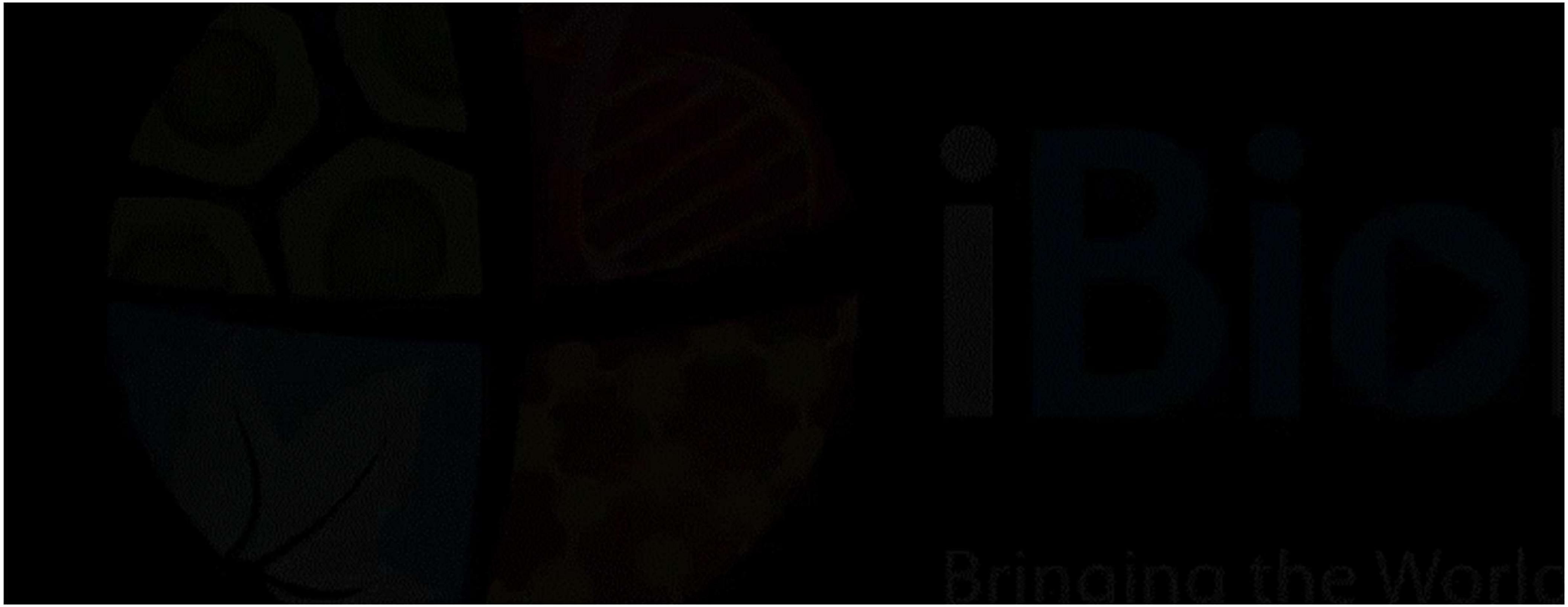


(B) Si no hay interacción entre filamentos de actina y adhesiones focales el ensamblado de actina mueve los filamentos hacia atrás (flujo retrógrado).

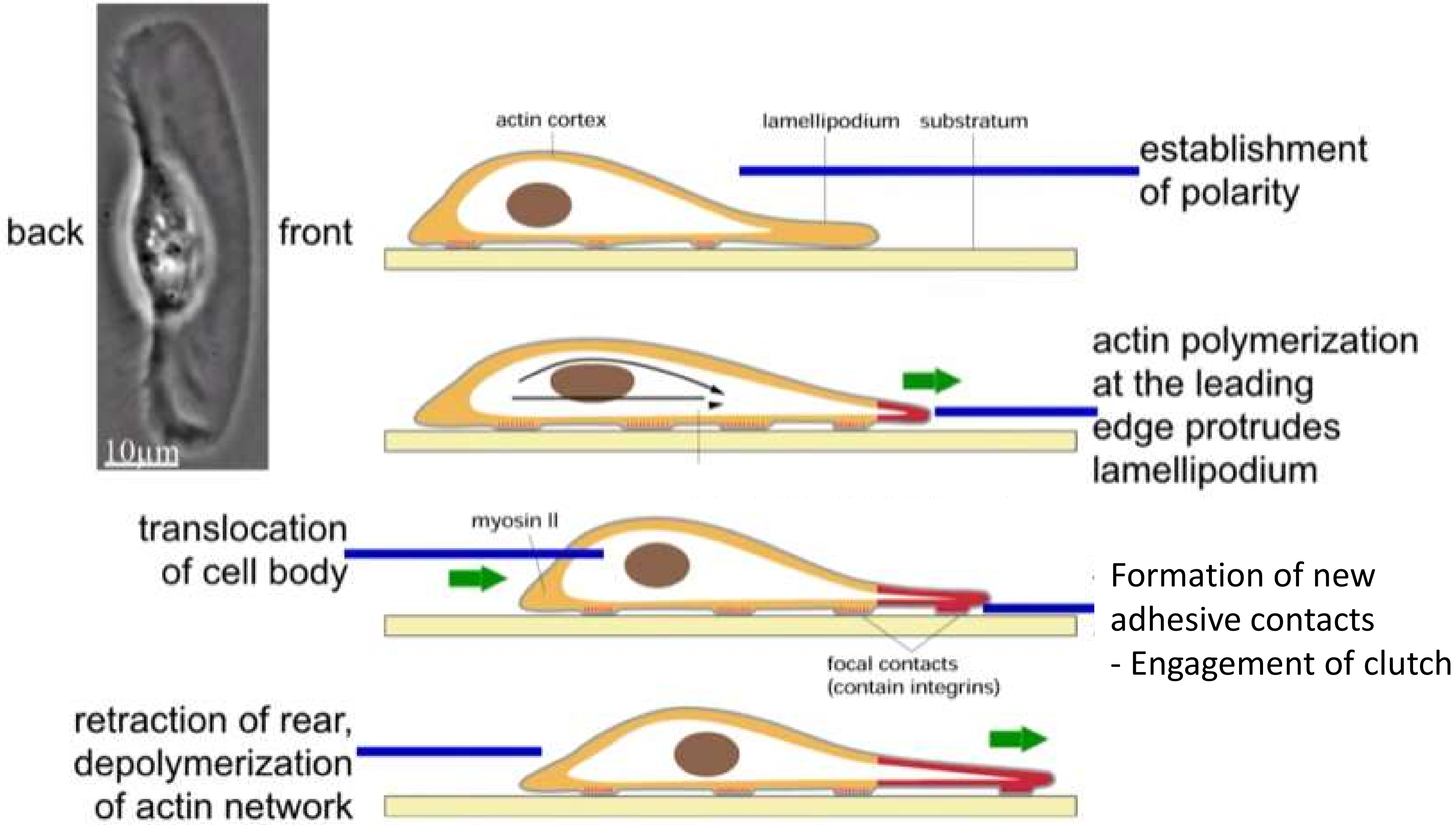
(C) La interacción entre proteínas adaptadoras e integrinas conecta el citoesqueleto de actina al sustrato. Miosina genera fuerzas de contracción que se transmiten al a través de las adhesiones focales para generar tracción sobre la matriz extracelular. La polimerización de actina mueve el frente de avance hacia adelante

Alberts et al MBC 6th Ed

Cell migration

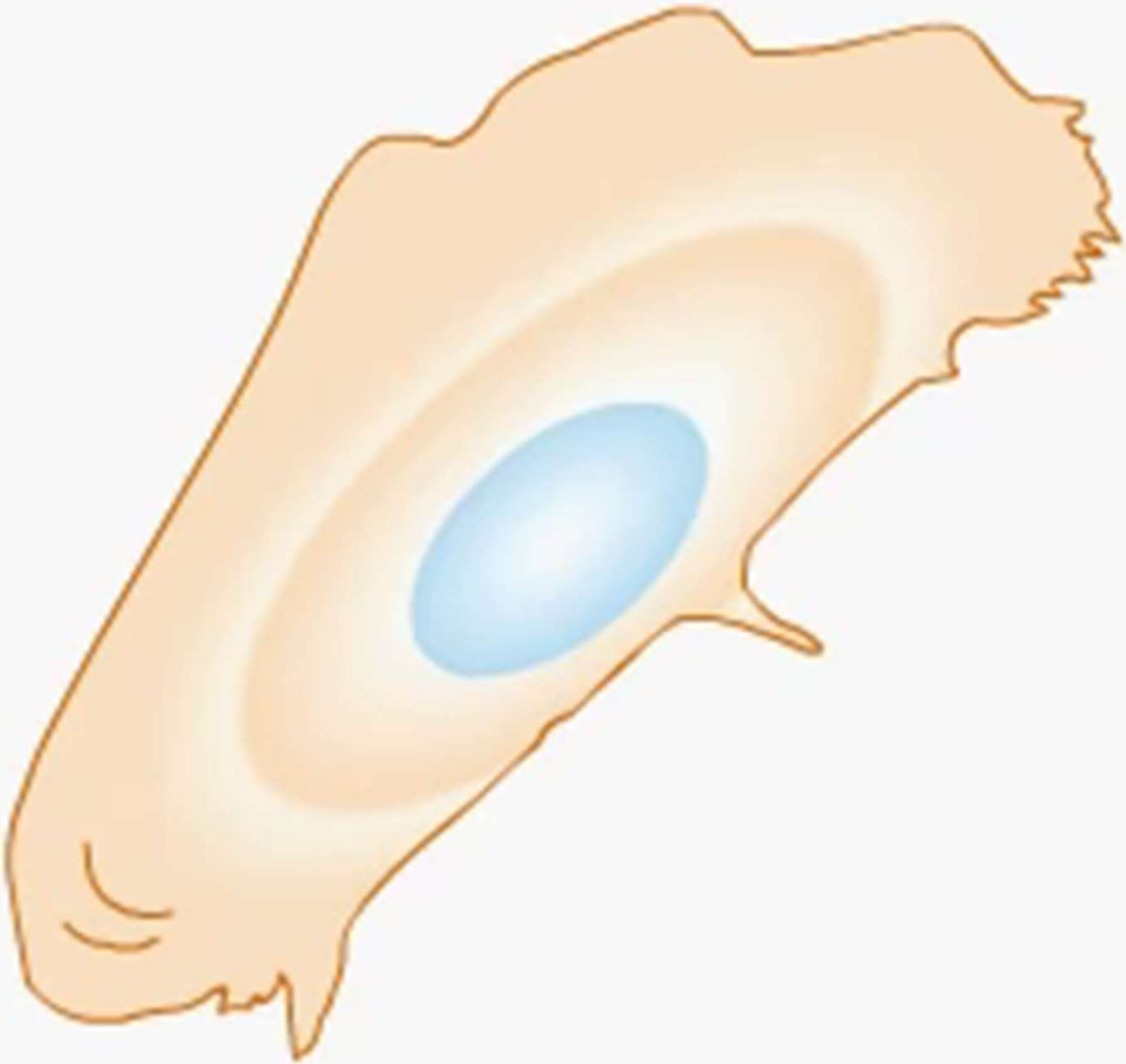


Cell migration

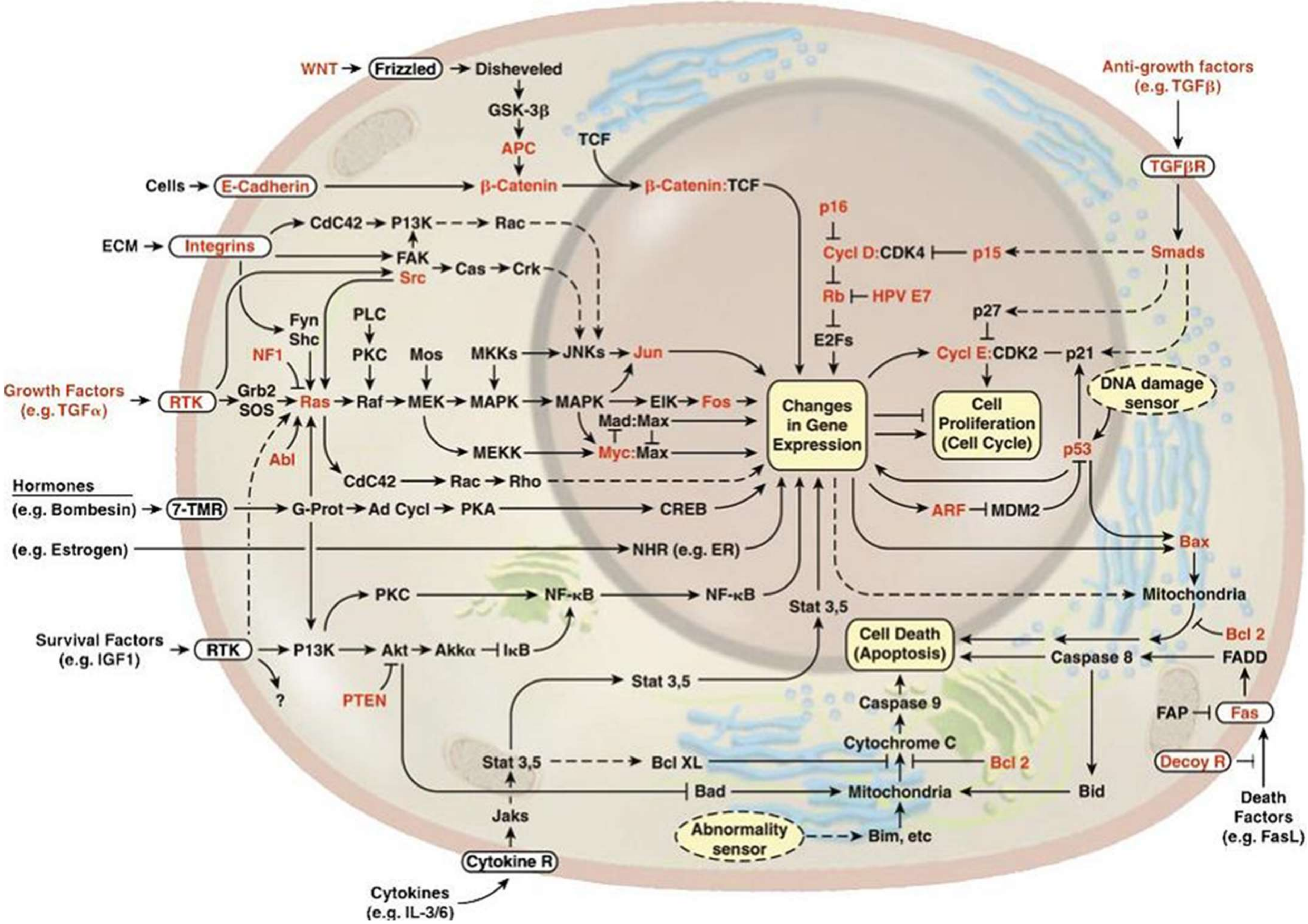


1. Polimerización de actina en el lamelipodio. Arp2/3
2. Adhesión mediada por integrinas
3. Contracción mediada por miosina II
4. Desensamble de los complejos adhesivos

Cell migration



Once upon a time... a cell



Cell as a machine

Myriad ways of Mechanotransduction (MT)

(1) Glycocalix : mediate MT signalling in response to fluid shear stress ECs

(2) Cell-cell junctional receptors

(3) Cell-matrix focal adhesions allow the cells to probe its environment.

(4) Force-induced unfolding of extracellular matrix proteins (fibronectin) : initiate MT signalling outside the cell.

(5) Intracellular strain can induce conformational changes in cytoskeletal elements by changing binding affinities to specific molecules & activating signalling pathways.

(6) Nucleus has been proposed to act as a mechanosensor. Intracellular deformations could alter chromatin conformation and modulate access to TFs & TMs. *(direct evidence for this mechanism is still lacking)*

(7) Compression of the intercellular space can alter the effective concentration of autocrine and paracrine signalling molecules.

(8) changes in G-protein coupled receptors, lipid fluidity, and even mitochondrial activity have been proposed as mechanosensors

short term effects :
Increases (or decreases) in intracellular tension, adhesion, spreading or migration

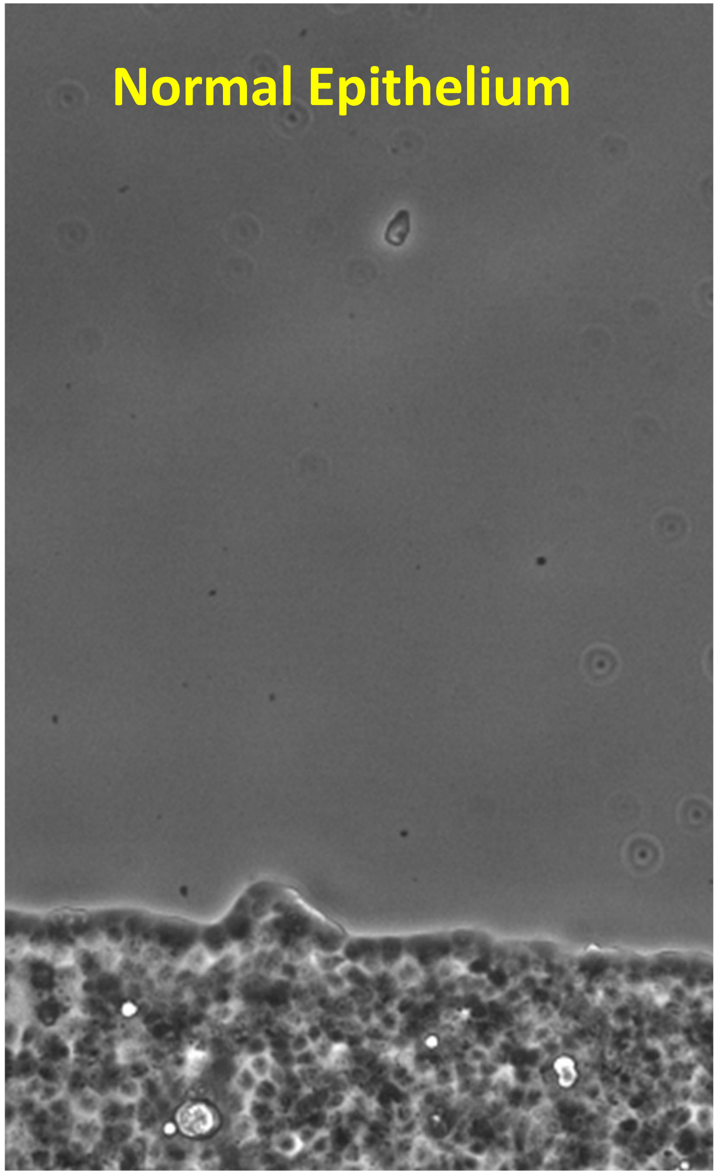
long term effects :
Protein synthesis/secretion, structural reorganization, proliferation, viability mediated through multiple, overlapping & crosstalking signalling pathways.

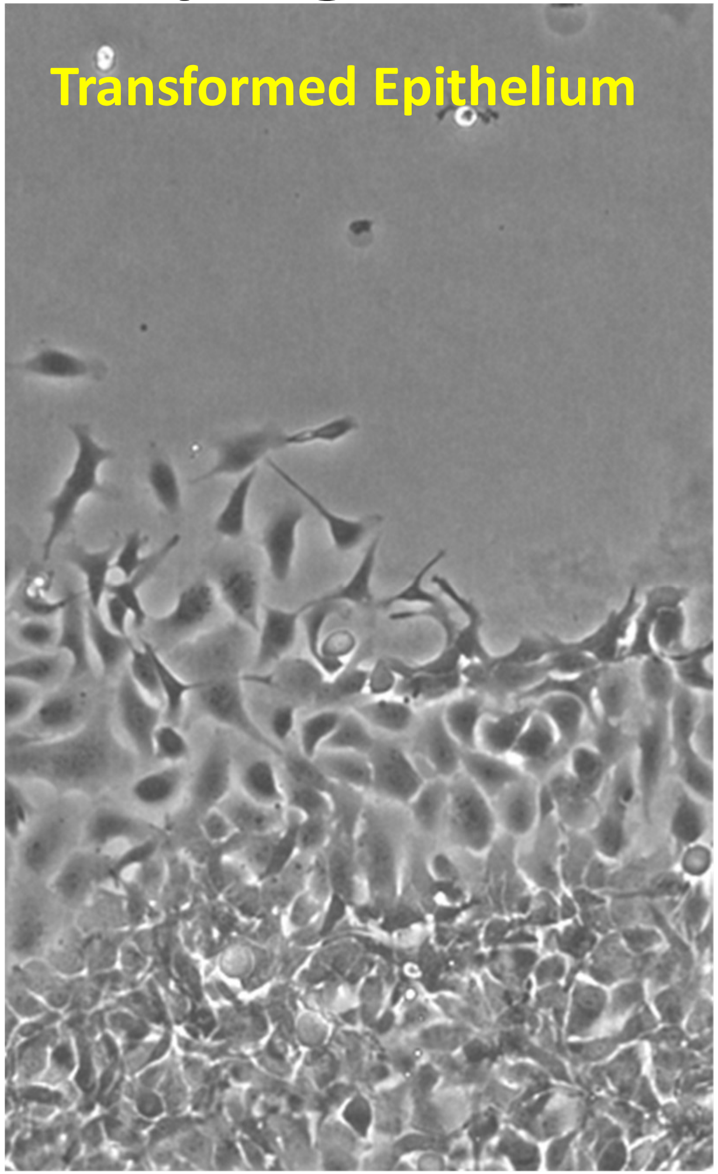
Dark side of Mechanotransduction

Diseases	Primary cells/tissues affected
Deafness	Hair cells in inner ear
Arteriosclerosis	Endothelial and smooth muscle cells
Muscular dystrophies and cardiomyopathies	Myocytes, endothelial cells and fibroblasts
Osteoporosis	Osteoblasts
Axial myopia and glaucoma	Optic neurons and fibroblasts
Polycystic kidney disease	Epithelial cells
Asthma and lung dysfunction	Endothelial cells and alveolar tissue
Premature ageing (HGPS)	Multiple cell types and tissues
Developmental disorders	Multiple cell types and tissues
Cancer	Multiple cell types and tissues
Potential immune system disorders	Leukocytes
Potential central nervous system disorders	Neurons

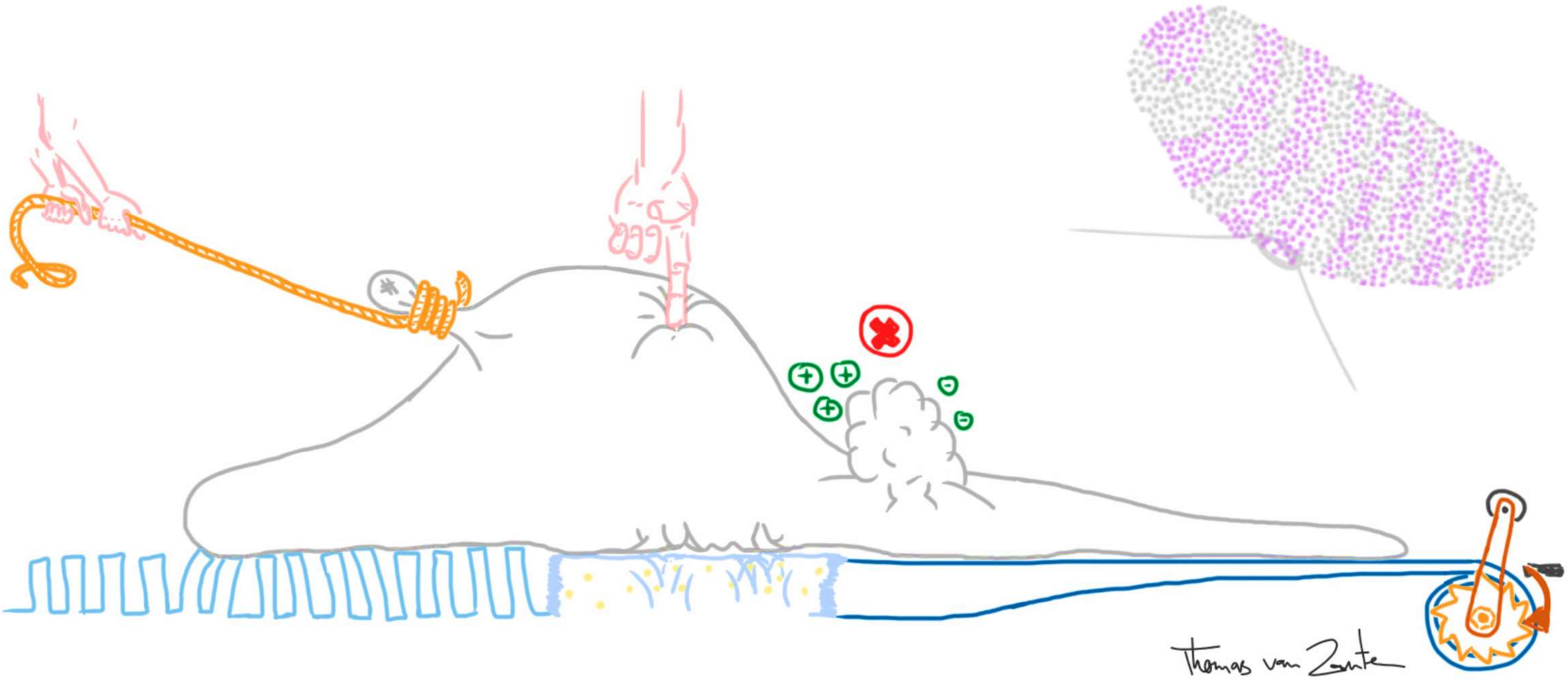
It is important to discuss the magnitude of the mechanical stimuli that elicit a biological response

Abnormal organization of the protein complex and/or defects in force transmission can lead to cancer transformation and progression.





Courtesy of Dr.Ravasio A, IIBM



Thank you