Bioen 326 2014 Lecture 28: Mechanotransduction

Biological Significance

Examples of Mechanosensors

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.

3 Parts of Mechanotransduction:

- **Mechanosensor = primary mechanotransducer** is stimulated by mechanical force to initiate a biochemical signal.
- **Mechanical Pathway** that transmits mechanical force from the environment to the mechanosensor
- **Downstream Signaling Pathway** that transmits and amplifies biochemical signal from the mechanosensor to the desired response.

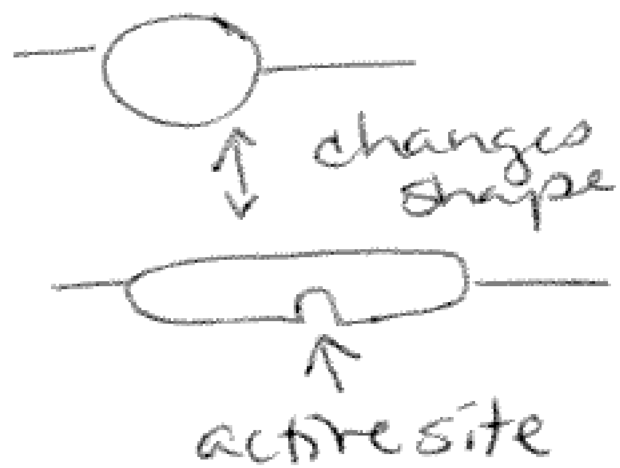
Any break in this pathway will interrupt mechanotransduction

Mechanosensor Engineering

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

Mechanosensor 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.



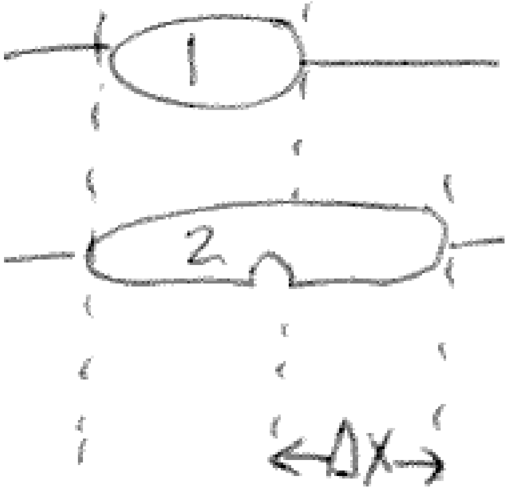
Molecular Biophysics

- Signaling activity is determined by probability of being in the active state. If state 2 is active, then activity is proportional to P2. If state 1 is active, then activity is determined by P1 = 1-P2
- P2 can be calculated as you have already learned. Recall that we call the low-energy state without force to be state 1.

$$P_2(f) = \frac{K_{eq}(f)}{1 + K_{eq}(f)}$$

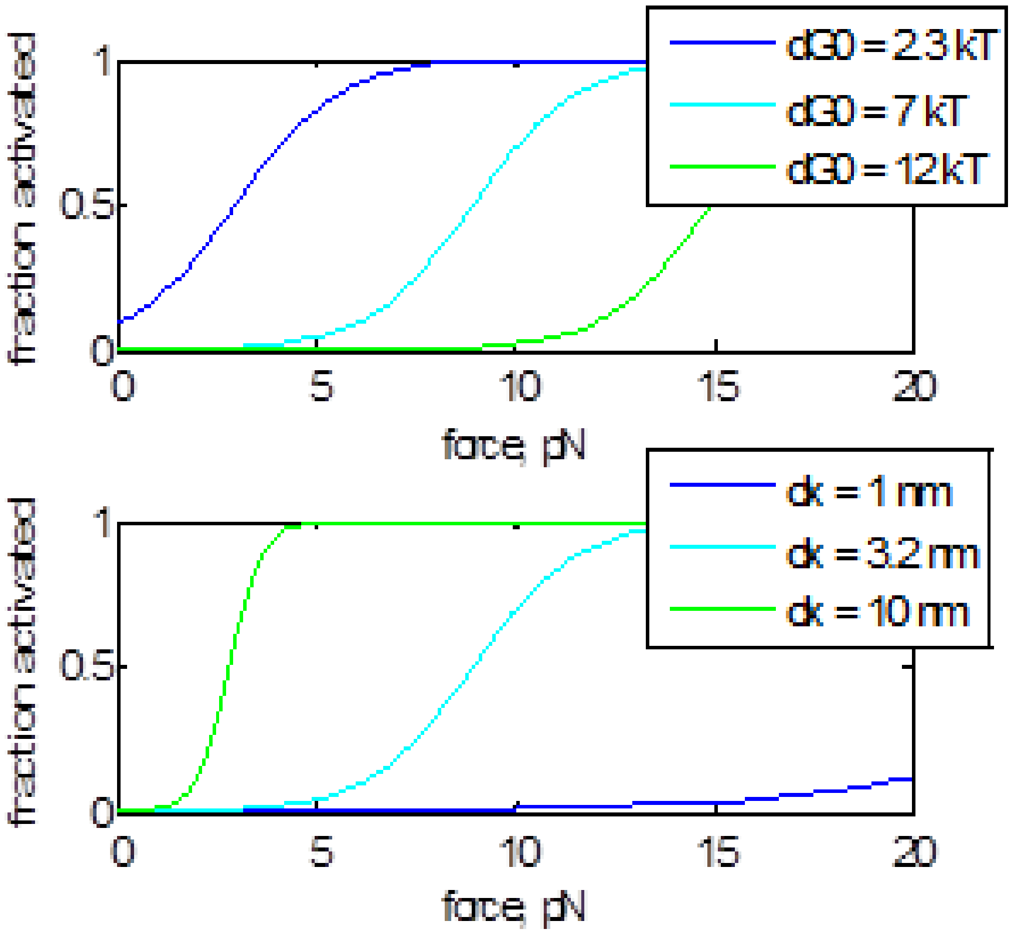
$$K_{eq}(f) = K_{eq}^0 \exp\left(\frac{f \Delta x(f)}{k_B T}\right)$$

$$K_{eq}^0 = \frac{P_2}{P_1} = \exp\left(\frac{-\Delta G^0}{k_B T}\right)$$



Activation of Mechanosensors

Here we assume that state 2 is active, so force activates. If state 1 is active, the curves will be inverted, dropping sigmoidally from 1 to 0.

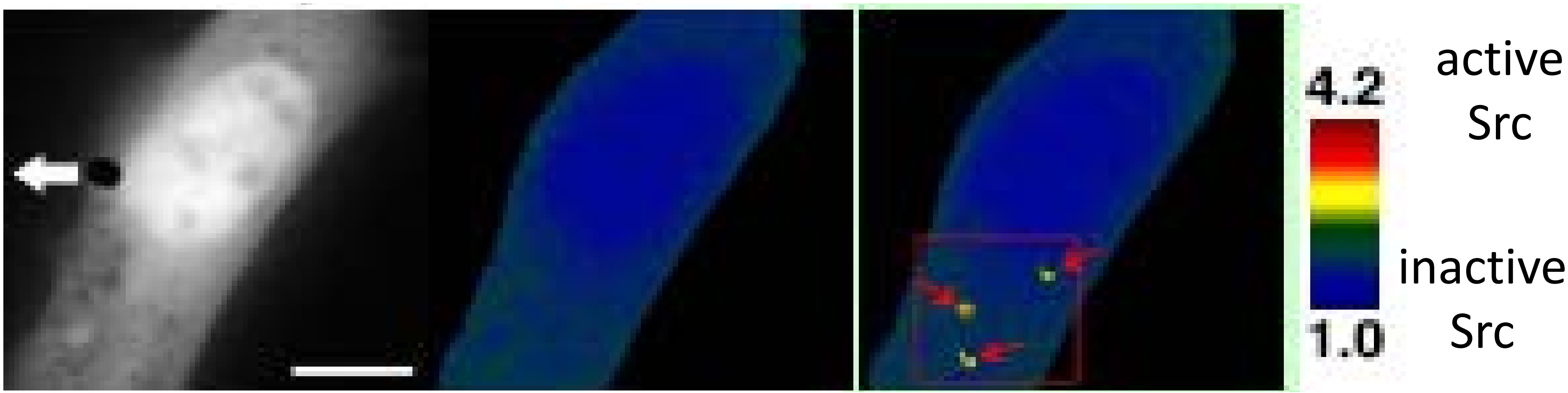


- Higher ΔG0 means higher force needed.
- Higher Δx means less force needed and more switch-like response.
- Together these two parameters determine force dependence.

Signal Transduction and Force

- The previous graph shows how the activity of the mechanosensor depends on force. The activity of the signaling pathway will be affected be the characteristics of the signal transduction pathway.
- The signal cascade may include negative feedback loop that turns off the response if there is no further change.
- The signal cascade may include a positive feedback loop that amplifies the signal or makes it more switch-like.
- In addition, high forces may have different effects from moderate forces. For example, moderate force may activate and high force denature or disconnect the mechanosensor.

Localized Mechanotransduction



- Magnetic bead (black) pulled as shown in white arrow (left).
- Activated Src shown before (middle) and 0.3 sec after (right) bead is pulled.
- Note that Src is activated across the cell from bead; mechanical pathway must connect the two.
- Localized downstream signaling pathways allow localized reponse. (remember Src is part of Focal Adhesion Complex.)

Na 2008, PNAS 105, p. 6626)

Specialized Mechanosensory Organs and Cells

Biomechanics of Hearing

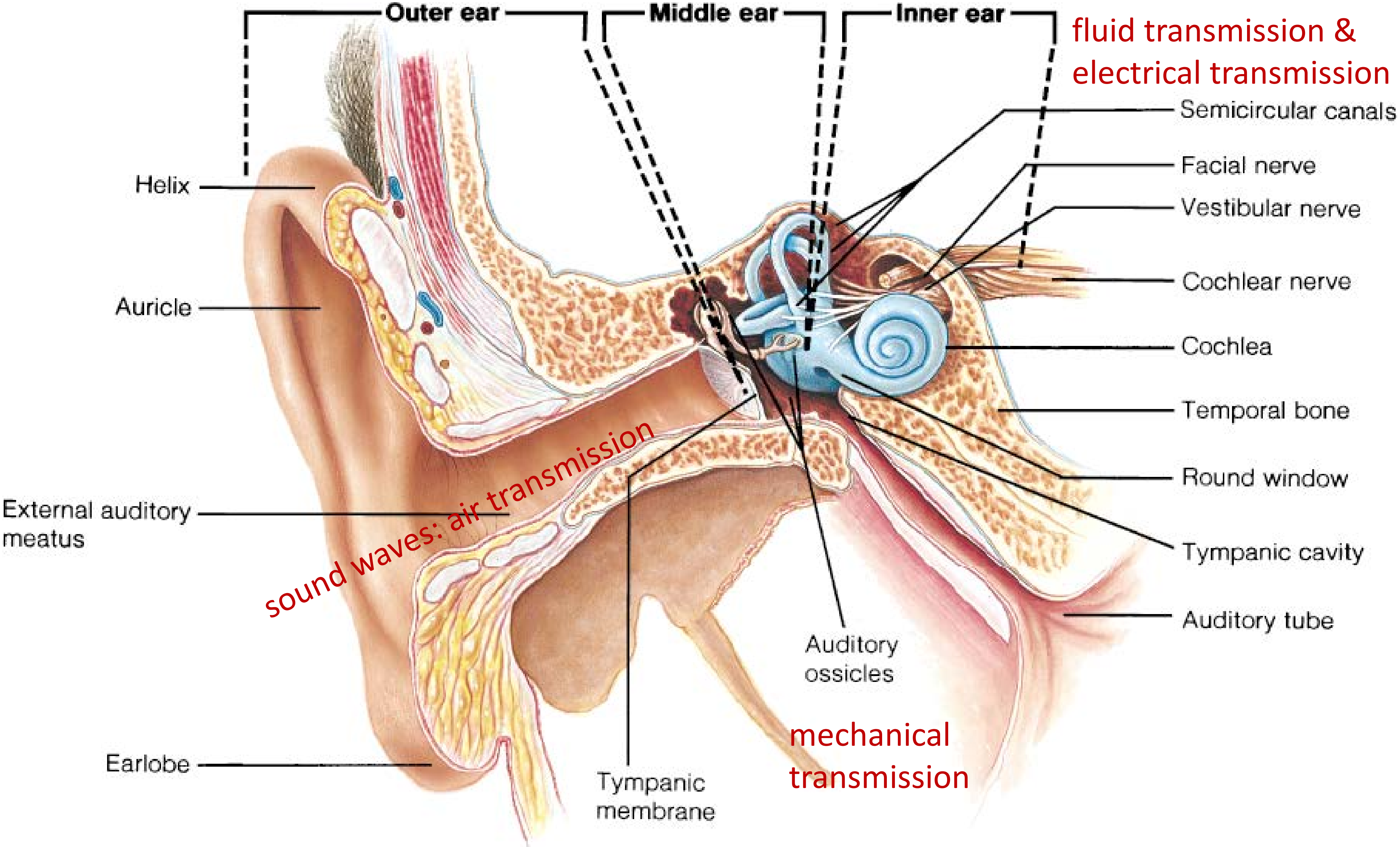
Hair Cells

StereoCilia

Hearing and Mechanotransduction

- Outer and Middle ear convert pressure waves in air to fluid movement. (Ear also converts gravitational orientation and movement to fluid movement.)
- The inner ear has specialized cells called hair cells that detect fluid movement.
- Hair cells contain specialized mechanosensory organelles called stereocilia
- Ion channels on stereocilia open to start a Calcium signal.

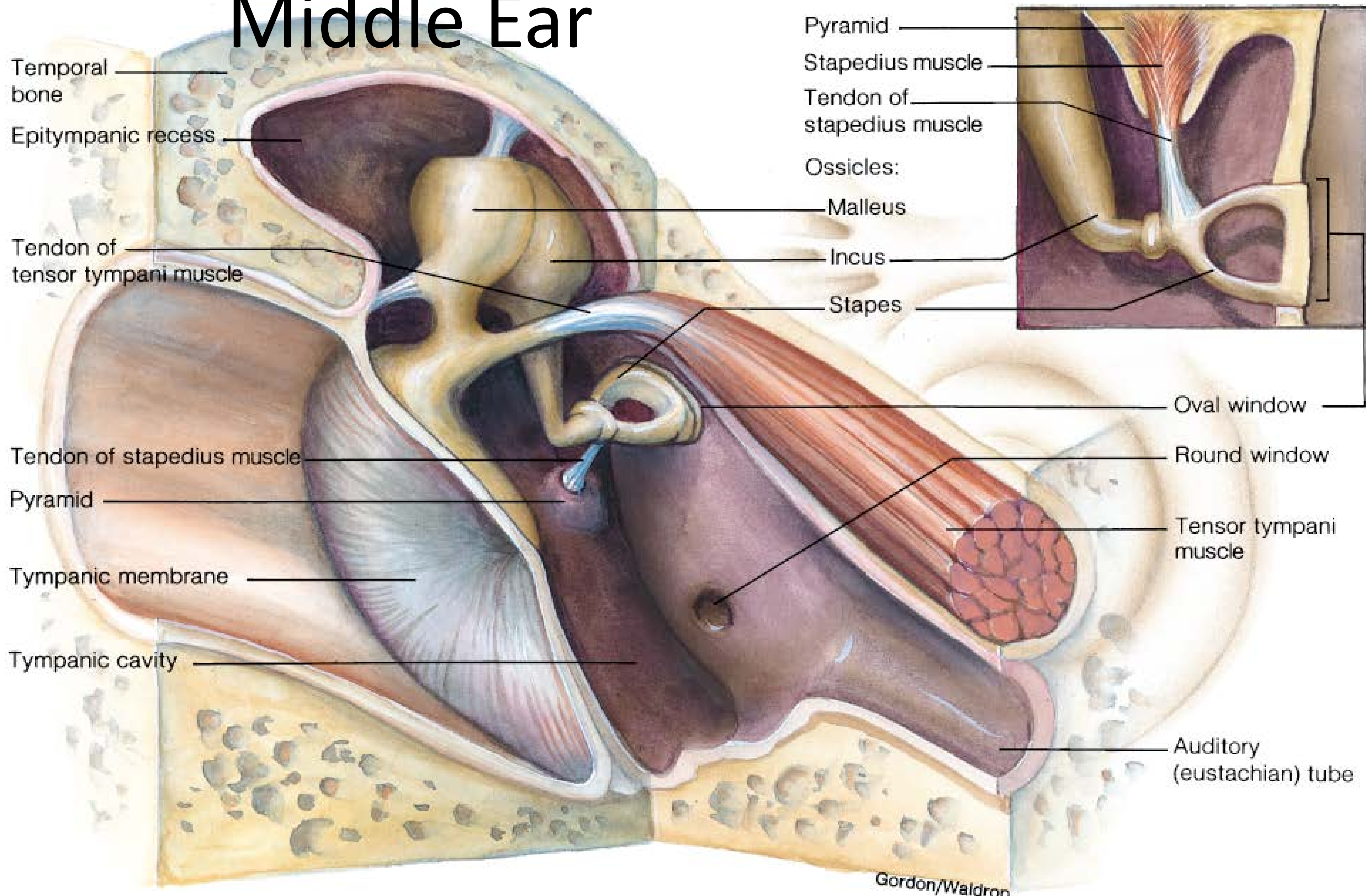
How Sound Signals are Transmitted



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

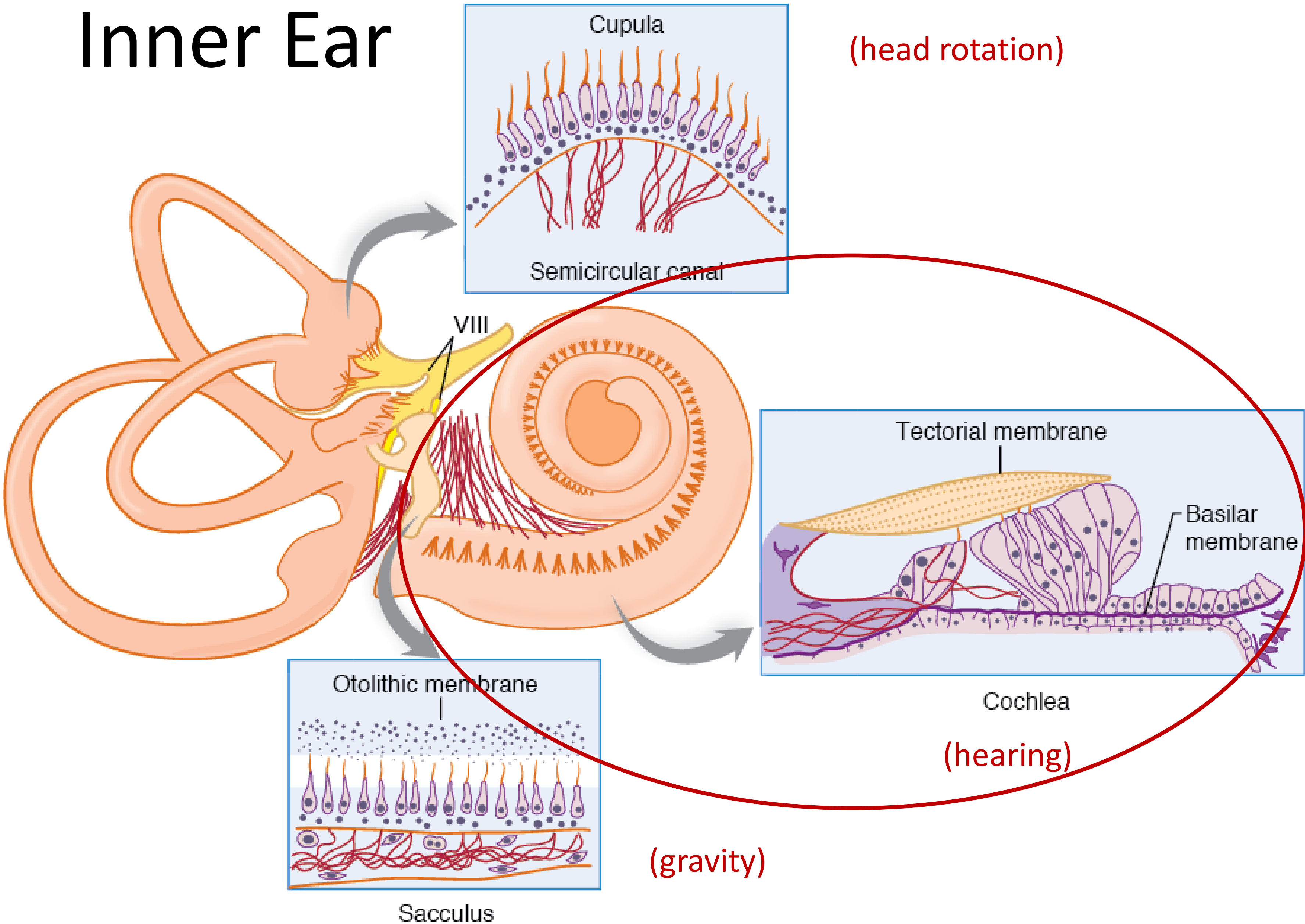
Source: Barrett KE, Barman SM, Boitano S, Brooks HL: *Ganong's Review of Medical Physiology*: www.accessmedicine.com

Middle Ear



Ossicles transmit movement of tympanic membrane to movement of oval window. 1.3-fold force, 1/18.6 area.

Inner Ear



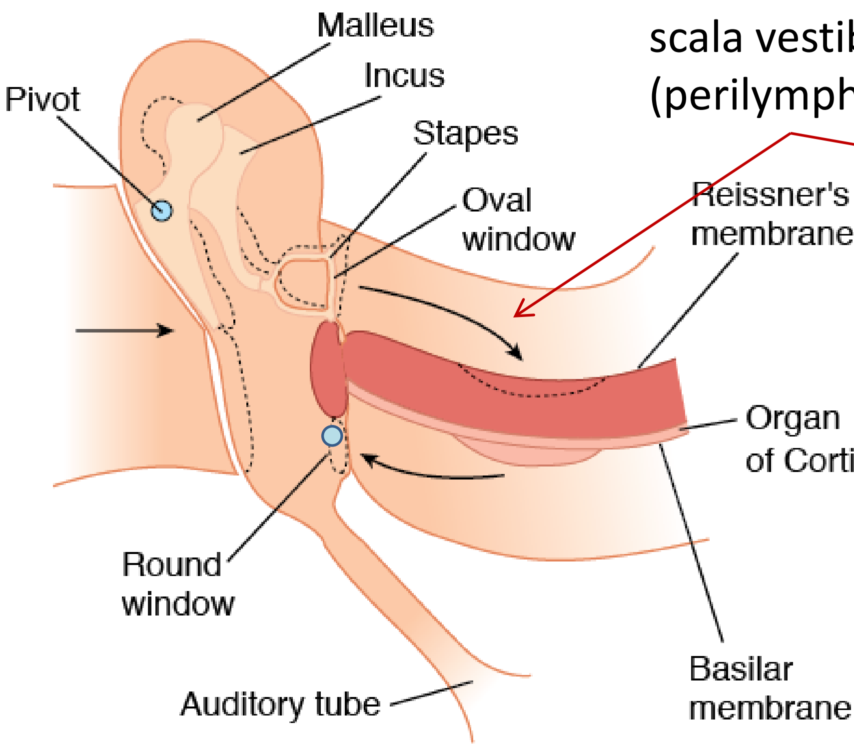
Source: Barrett KE, Barman SM, Boitano S, Brooks HL:
Ganong's Review of Medical Physiology: www.accessmedicine.com

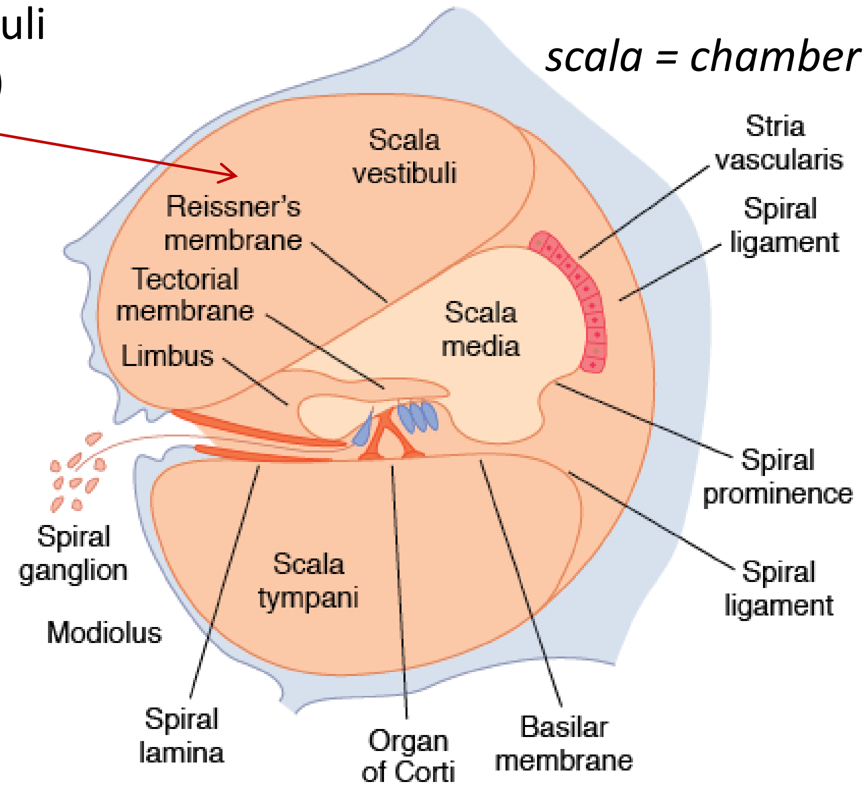
Fluid waves in the Cochlea

scala vestibuli
(perilymph)

cross-section of Cochlea

scala = chamber

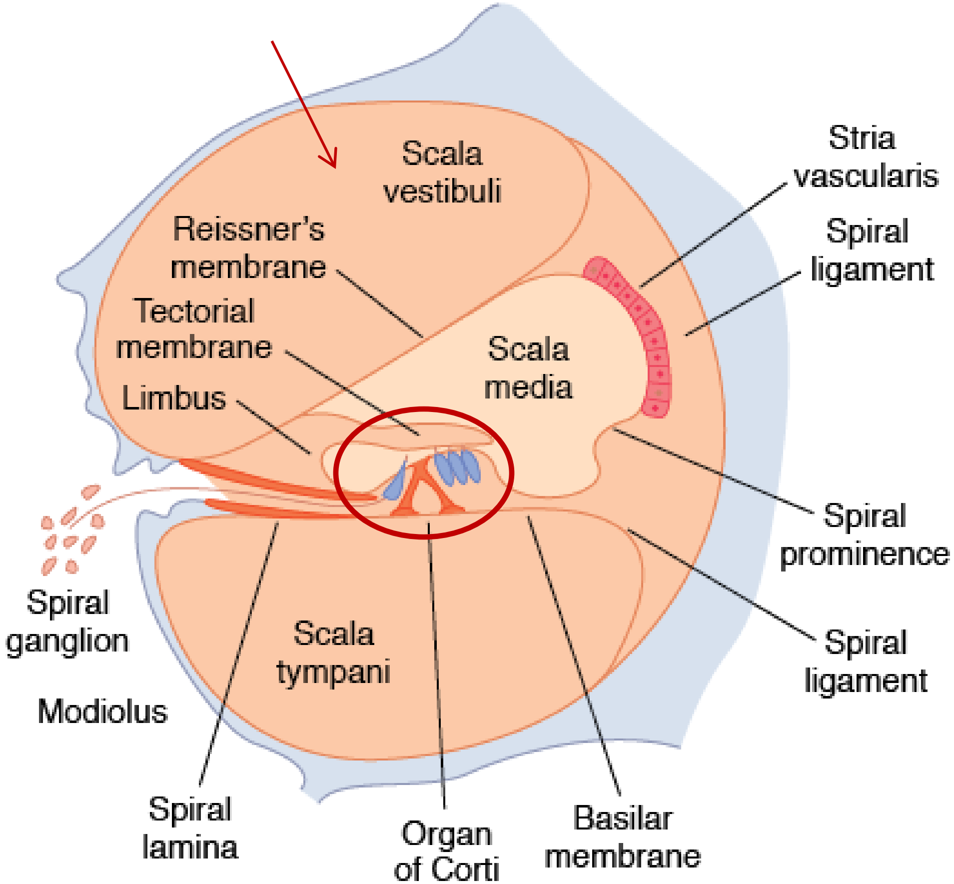


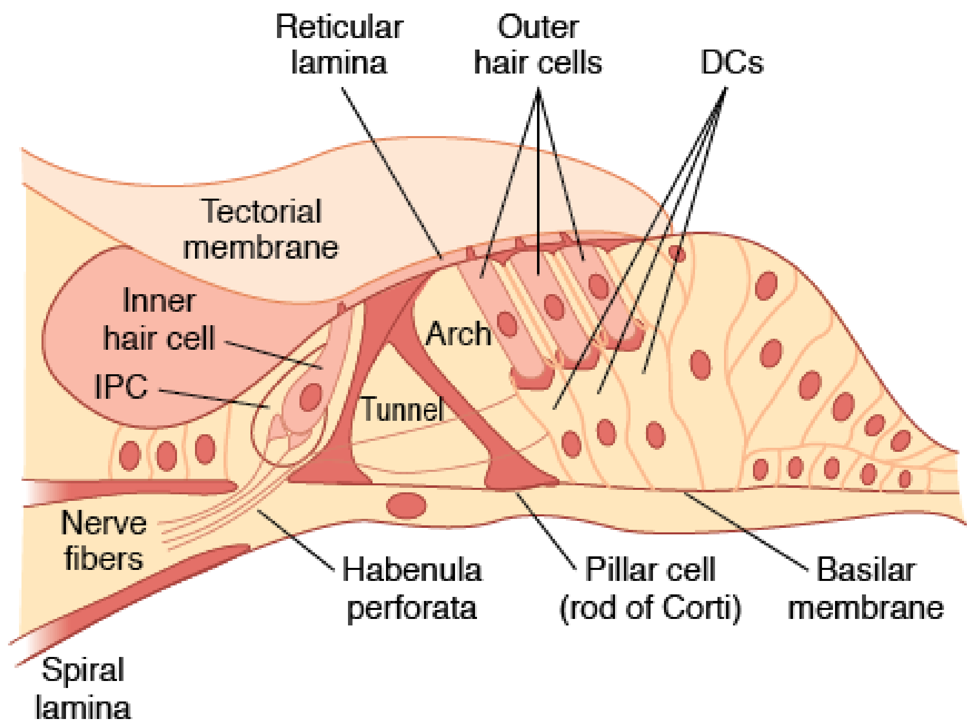


- ➢pressure waves travel from base to apex of cochlea in the scala vestibuli
- ➢Reissner's and Basilar membranes are soft and elastic
- ➢pressure depresses these membranes into scala tympani, which dissipates into round window.
- ➢ So pressure distorts basilar membrane

Cochlear Structures

- hair cells are attached to basilar membrane
- Stereocilia bundles on hair cells face the tectorial membrane in the Organ of Corti
- Inner hair cells don't touch, but outer are embedded into membrane.
- When basilar membrane moves, the contact between them and the Tectorial membrane is sheared

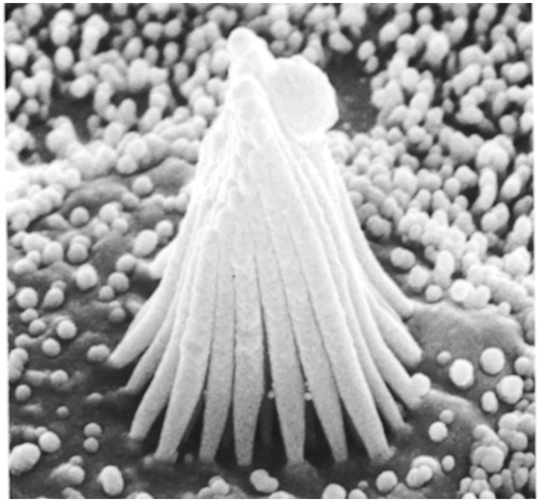


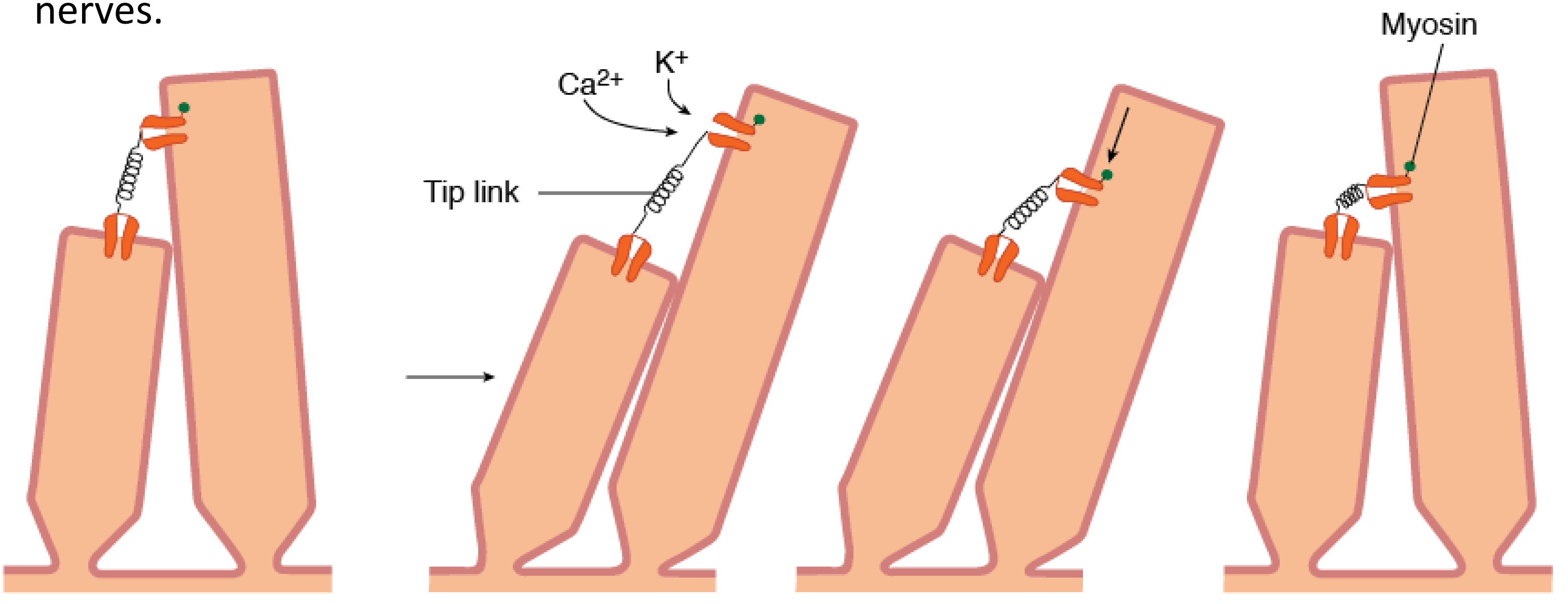


Source: Barrett KE, Barman SM, Boitano S, Brooks HL: *Ganong's Review of Medical Physiology*: www.accessmedicine.com

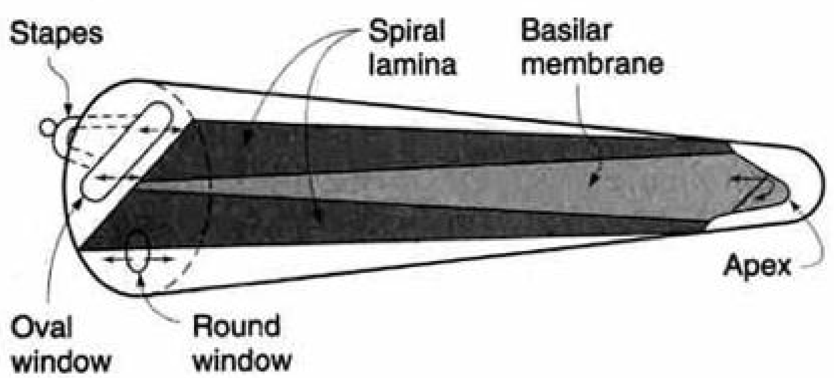
Stereocilia Structure and Function

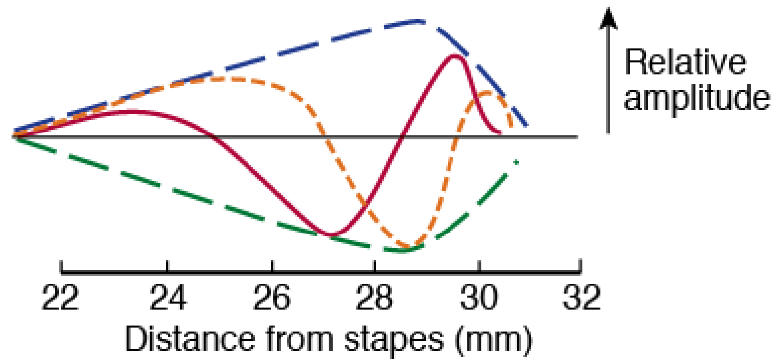
- stereocilia are composed of actin filaments and myosin (motor proteins).
- rows of stereocilia are organized so each is taller than the next.
- a tip link connects the neighboring stereocilia.
- When the stereocilia tilt, the tip links stretch under tensile force
- The tip link opens an ion channel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



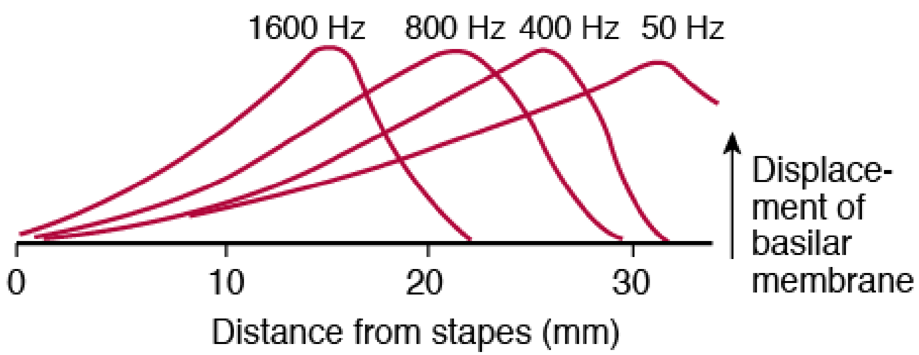


How the Ear Distinguishes Pitches





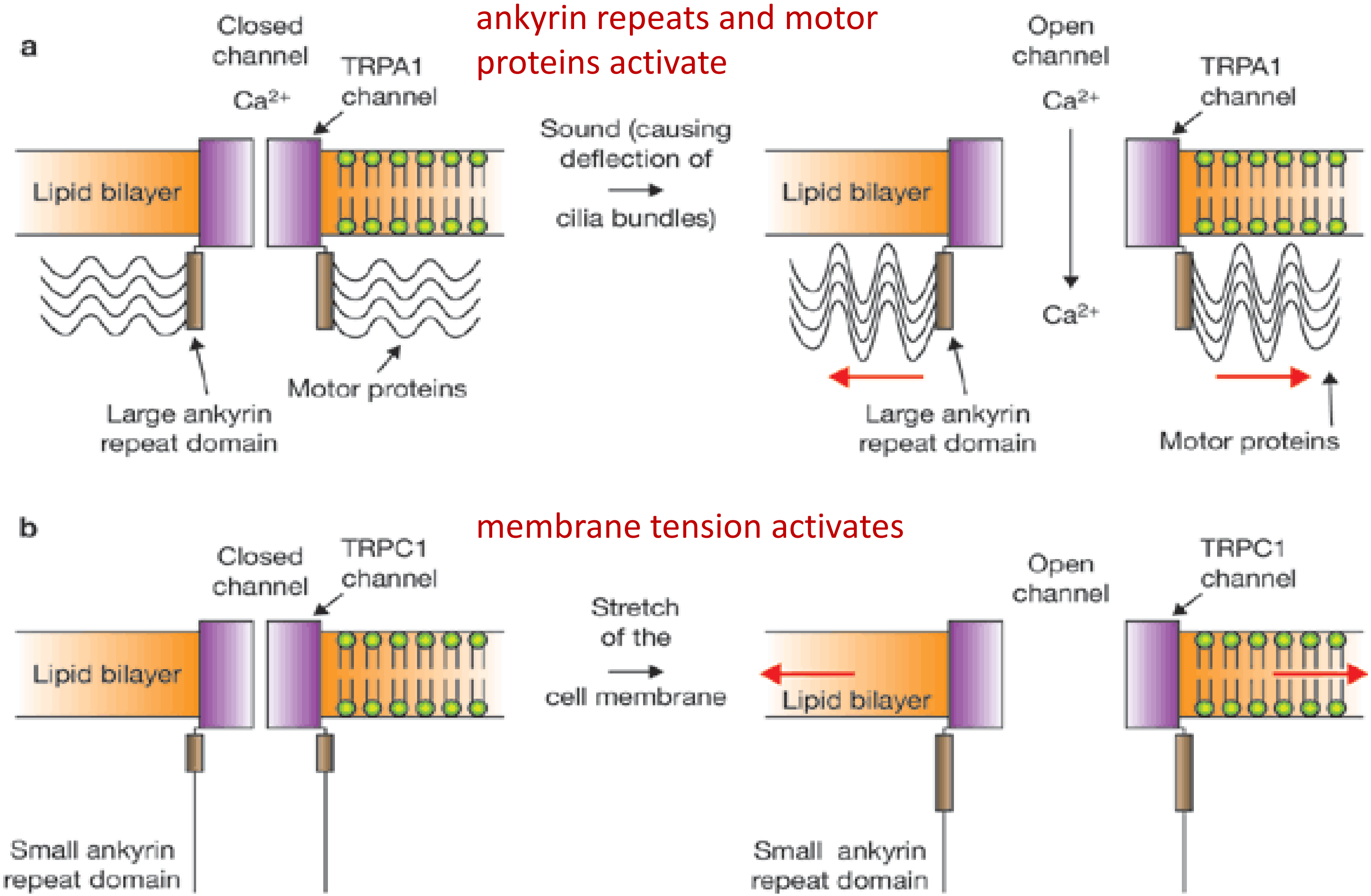
The basilar membrane oscillates the most near the base for high frequencies, and near the apex for low frequencies



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

http://www.cns.nyu.edu/~david/courses/perception/lecturenotes/pitch/pitch.html

Models for How Force Opens TRP Channels



Barritt and Rychkov *Nature Cell Biology* **7**, 105 - 107 (2005)

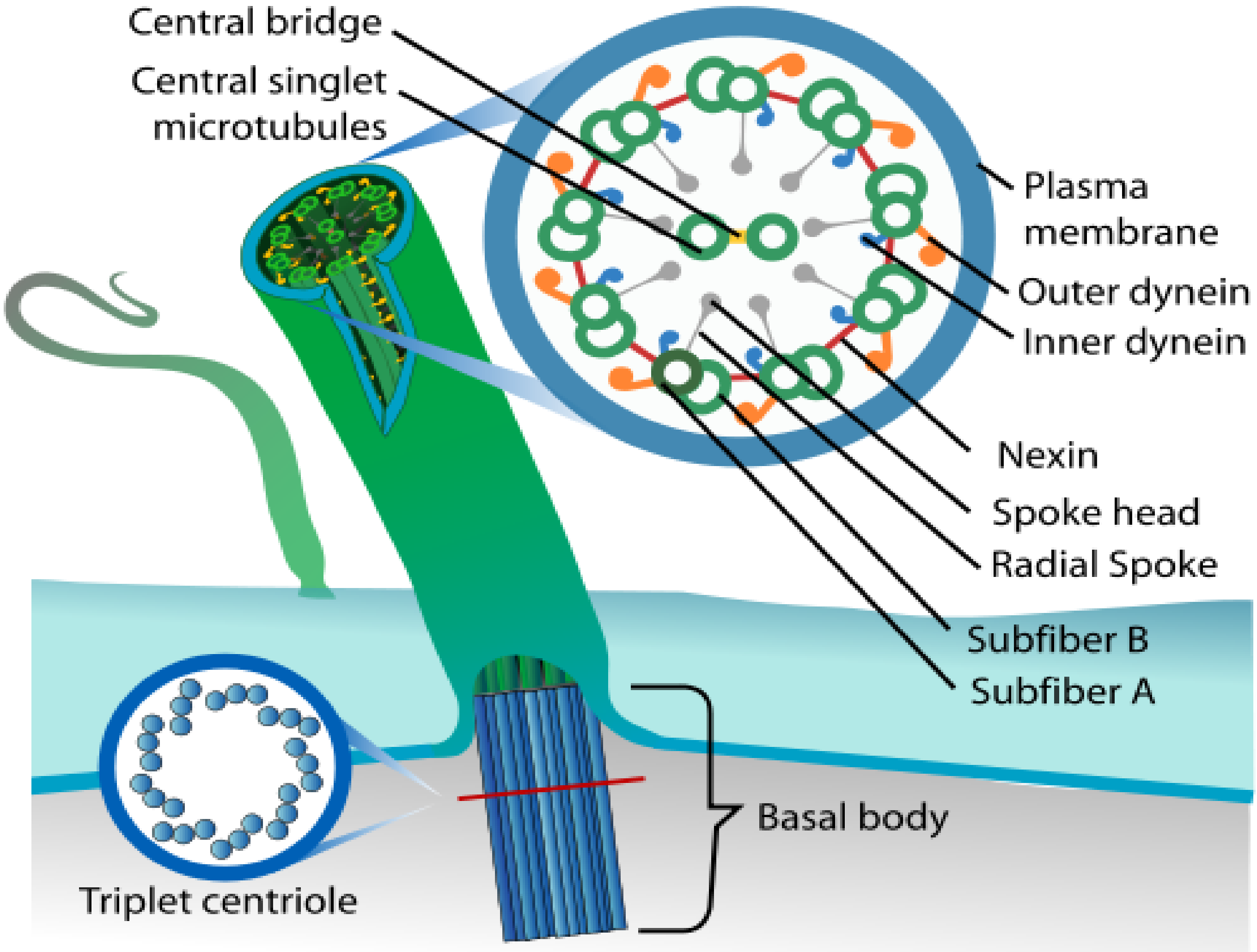
Specialized Mechanosensory Organelles in Cells

True Cilia

True Cilia

- Found on: Endothelial cells (line blood vessels), epithelial cells (line skin, urinary tract, intestines, mouth, etc) and osteocytes (mechanosensors in bone)
- Bundle of microtubules called **Axenome** inside plasma membrane.
- Brushes of Motile Cilia move fluid past cells in trachea and fallopian tubes. Same as single flagella in sperm cells
- Nonmotile cilia sense fluid movement.
- Anchored via Basal body (a solid support)

True Cilia



- 9 pairs of microtubules.
- Two more in center for motile cilia
- Dynein motor protein slides microtubules along each other for motile cilia.

Mechanosensor in Cilia

- Polycystic kidney disease arises from mutations in ion channels near base of nonmotile cilia.
- But, it is not known how bending of cilia opens these ion channels.
- Patients with this disease also have vascular defects, suggesting that cilia regulate endothelial cell function, but endothelial cells at high flow lack cilia.

Mechanosensors in Other Cells

Biological Significance

Examples of Mechanosensors

Mechanosensors in Other Cells

- Cells without specialized mechanosensorsy organelles can still sense mechanical forces.
- Cells sense the mechanical and chemical environment. They require the proper signals to thrive or differentiate.
- Cells undergo apoptosis if they are in the wrong environment.
- Cancer Cells lose the ability to control when they divide. Metastasized cancer cells spread throughout the body because they no longer require the right chemical and mechanical signals.

Essentially All Mammalian Cells Sense:

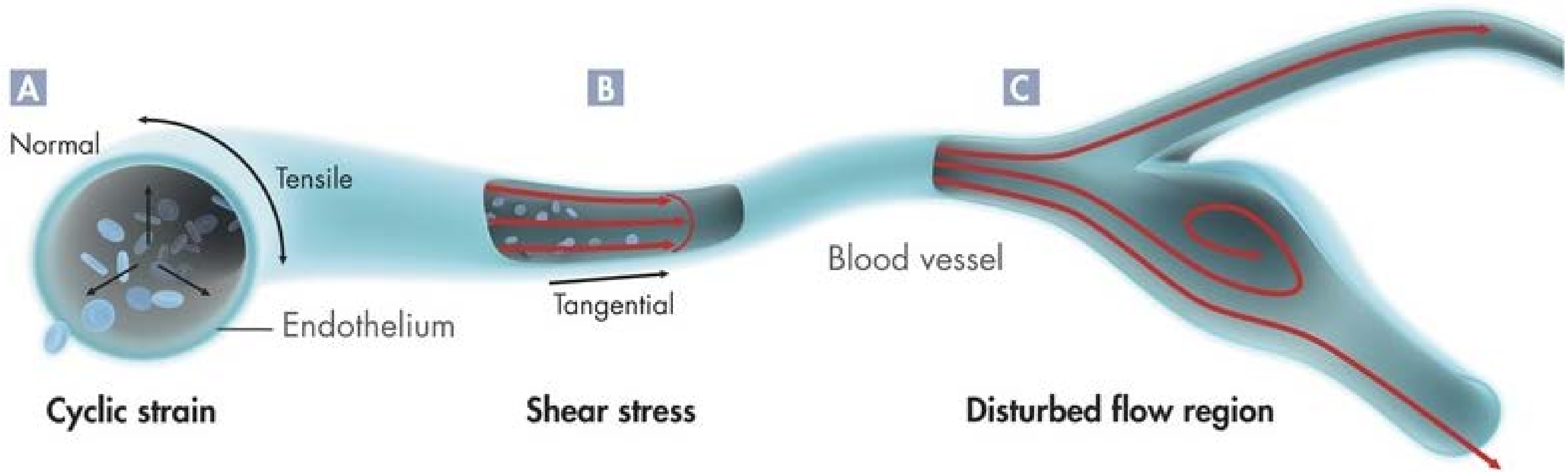
- stiffness of their environment
- fluid shear stress
- localized tensile forces; pulling by the environment
- Topology, Nanostructure and Microstructure

How do they sense force?

Endothelial Cells

- Endothelial cells (EC) line the blood vessel walls, creating a barrier between blood and tissues.
- A healthy endothelium emits anti-inflammatory signals that modulate the function of blood cells to keep them passivated, or in a "resting state".
- However, if endothelial cells become activated, they can have the opposite effect, emitting pro-inflammatory signals that activate blood cells.

Endothelial Cells sense strain and fluidic shear stress



- High laminar steady or pulsatile flow keeps EC passivated, while low or disturbed flow is pro-inflammatory. Because of this, atherosclerotic lesions nearly always occur at branch points in arteries, where flow is disturbed.

Conway and Schwartz, 2013, Qiagen

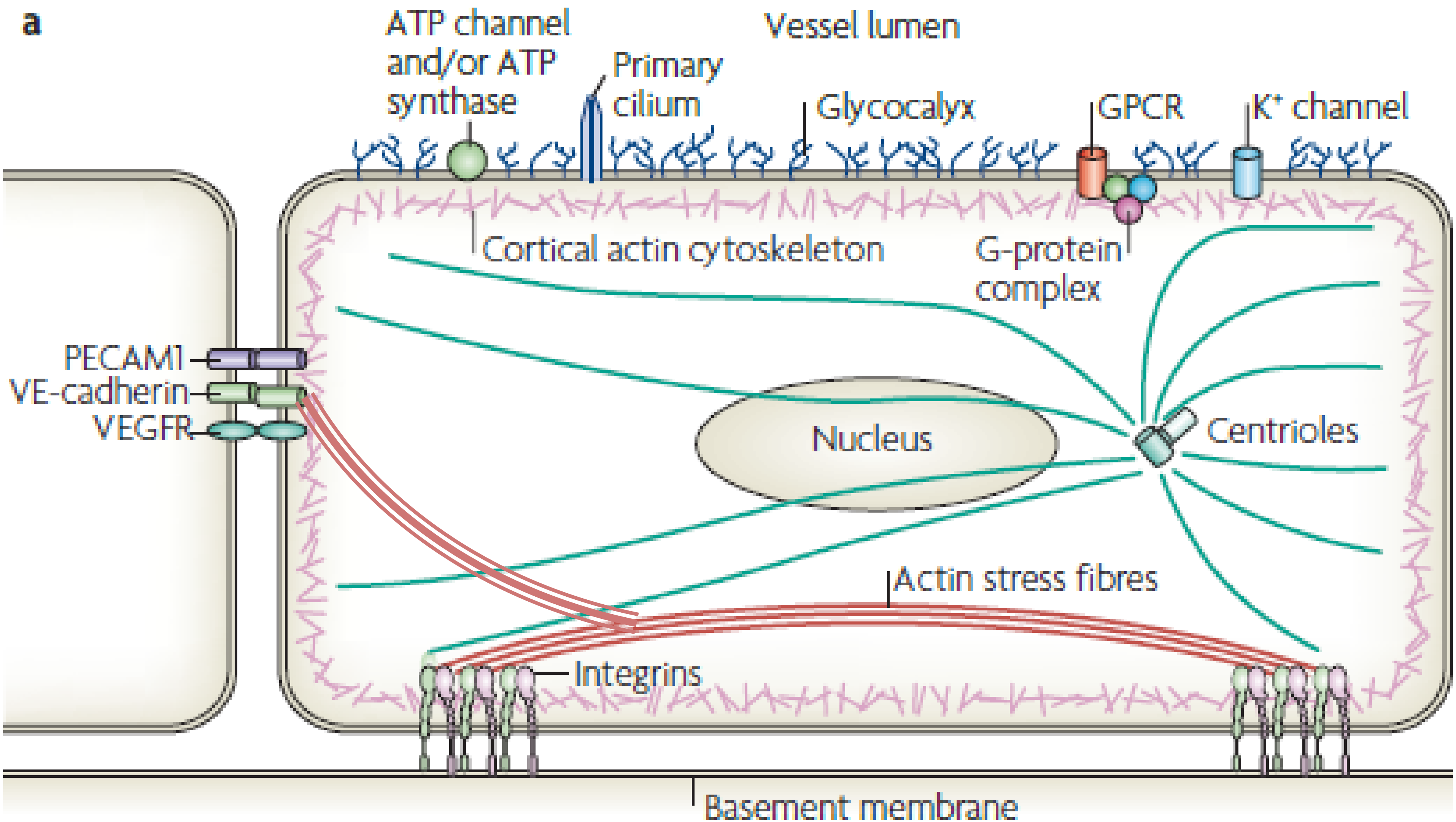
Endothelial Cell Response to Flow

- Long-term flow: Secrete nitric oxide and prostacyclin, which cause smooth muscle cells to relax and are anti-inflammatory. They also align with flow.
- Onset of shear transiently activates inflammatory pathways like reactive oxygen species.
- Tight junctions, where neighboring cells bind each other, appear to mediate mechanotransduction: this involves Cadherin, PECAM-1 (both are adhesion and signaling molecules), and Src, a kinase (signaling molecule).

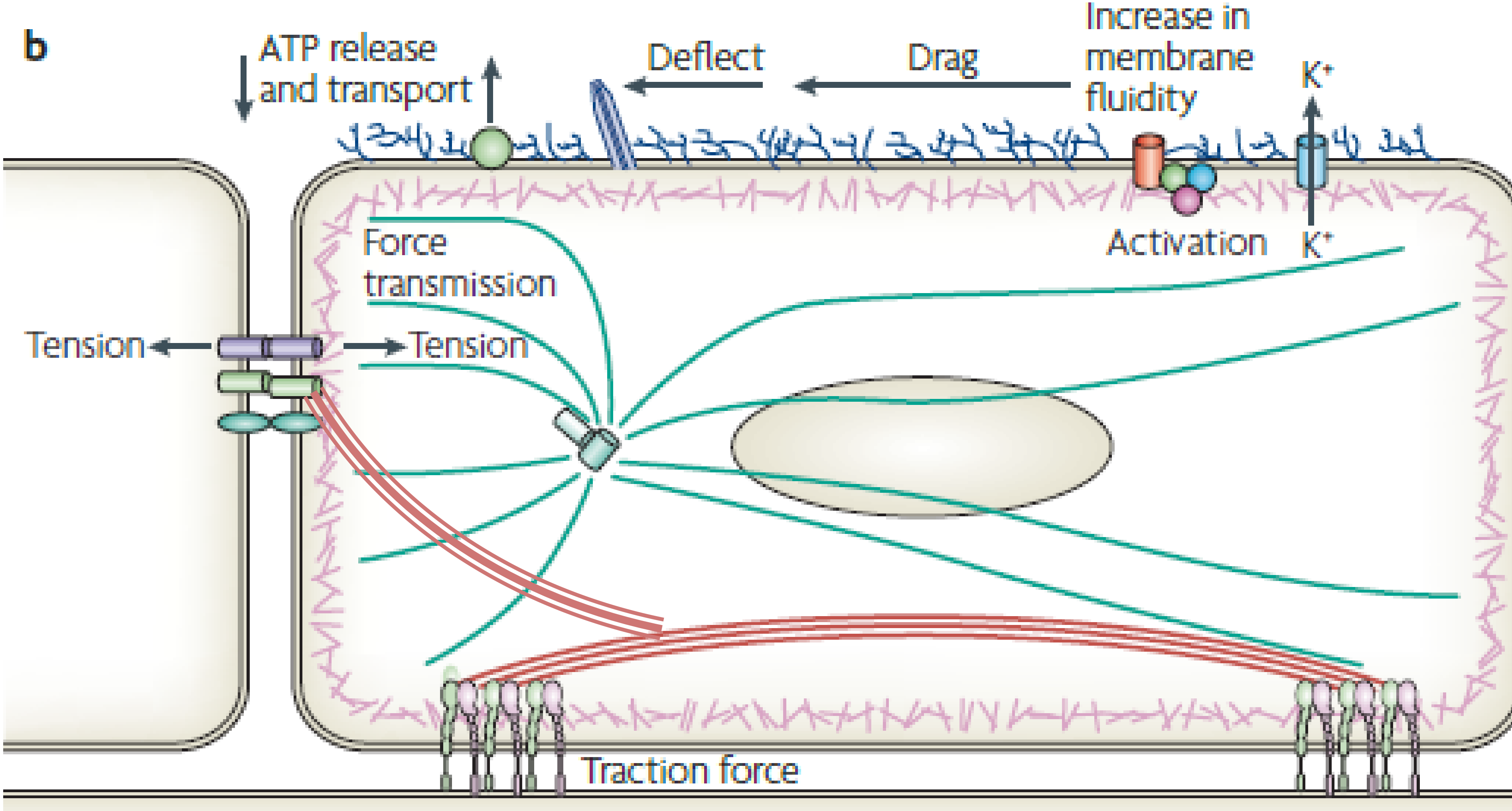
Atheroschlerosis and Thrombosis

- Activated blood cells called **platelets** initiate thrombosis through several pathways, leading to blood clots, which can occlude arteries.
- Activated immune cells called **monocytes** cross the endothelium, emit cytokines, and differentiate into foam cells, which convert HDL to fatty deposits in the wall and stimulate migration of muscle cells, to form an atherosclerotic plaque that thickens and hardens the artery walls. These can rupture, leading to occlusion of arteries.
- Occlusion of arteries in the brain causes **strokes**, while occlusion in arteries feeding the heart causes heart attacks, or **myocardial infarction**.

Molecular Basis of Mechanotransduction in EC



Molecular Basis of Mechanotransduction in EC



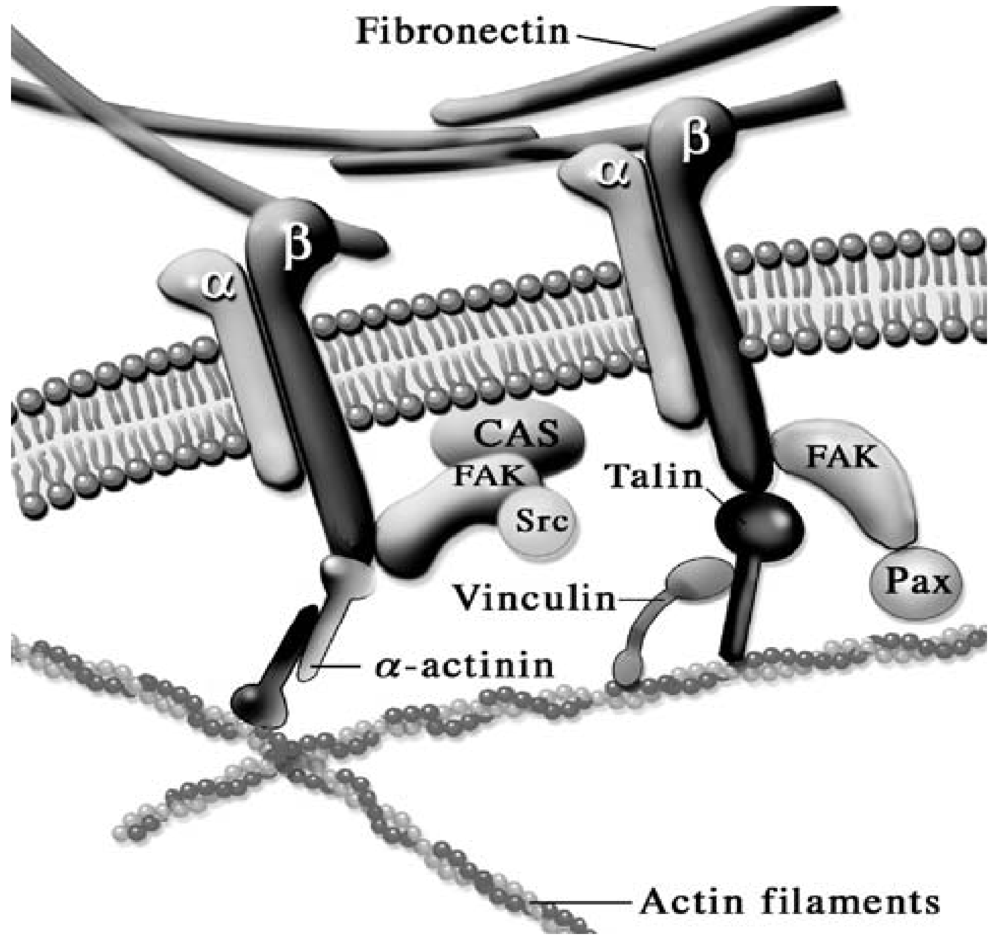
Molecular Basis of Mechanotransduction in EC

- Mechanical force due to fluidic drag on glycocalyx can be transmitted through stress fibers and microtubules throughout cells, including to tight junctions and focal adhesion complexes.
- Mechanical force due to vessel stress call also be transmitted.
- Still not known exactly what is involved.

Stretching Single Talin Rod Molecules Activates Vinculin Binding

del Rio, Perez-Jimenez, Liu, Roca-Cusachs, Fernandez, and Sheetz

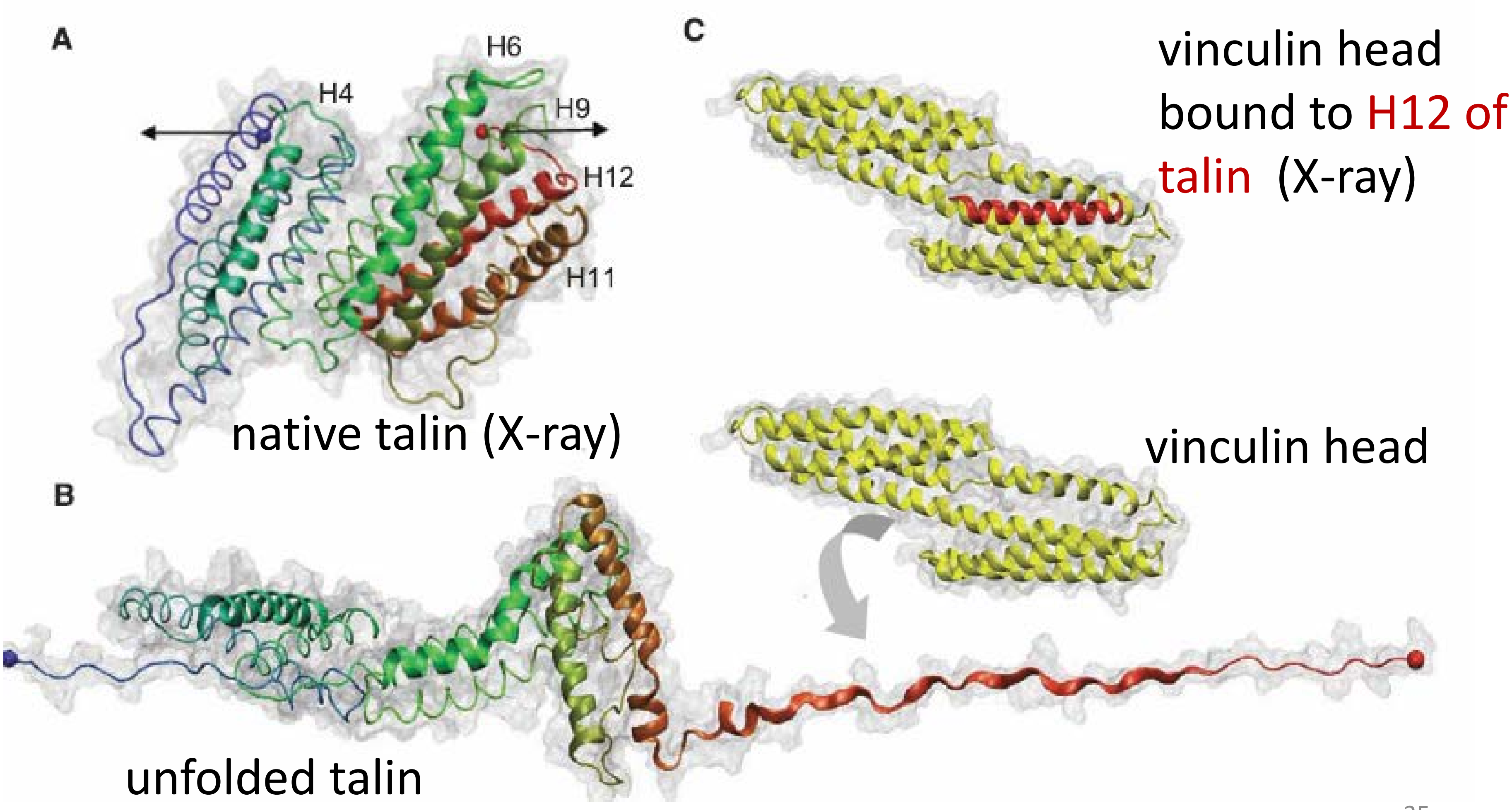
- Classic Paper in Mechanotrans-duction, 2009
- Demonstrates how stretching of Talin can cause biochemical effect (binding Vinculin)



del Rio et al (2009) Science 323, p 638

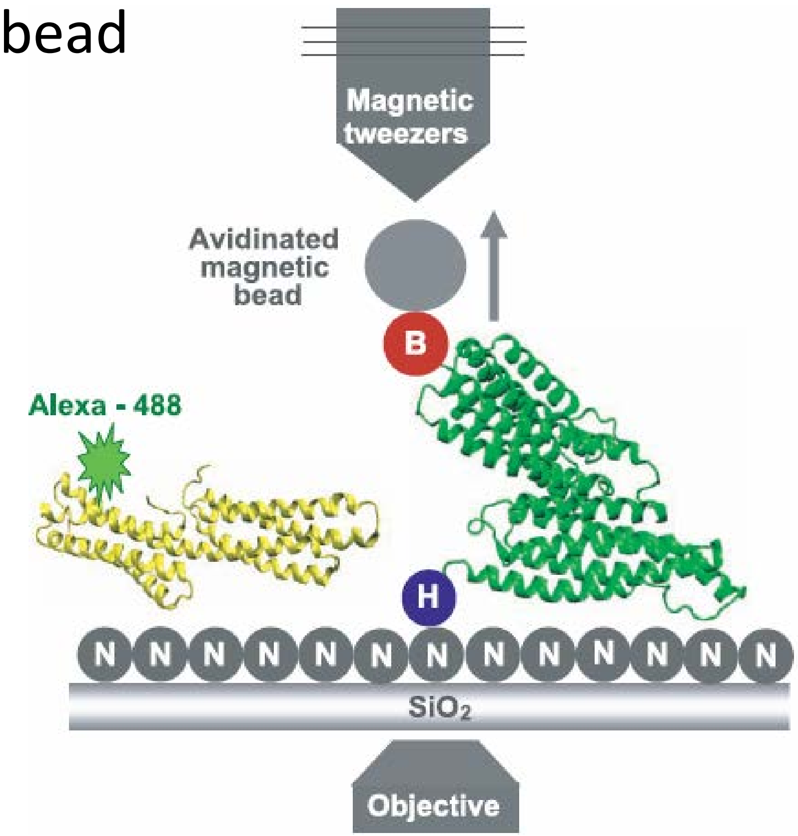
Talin as Mechanosensor

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



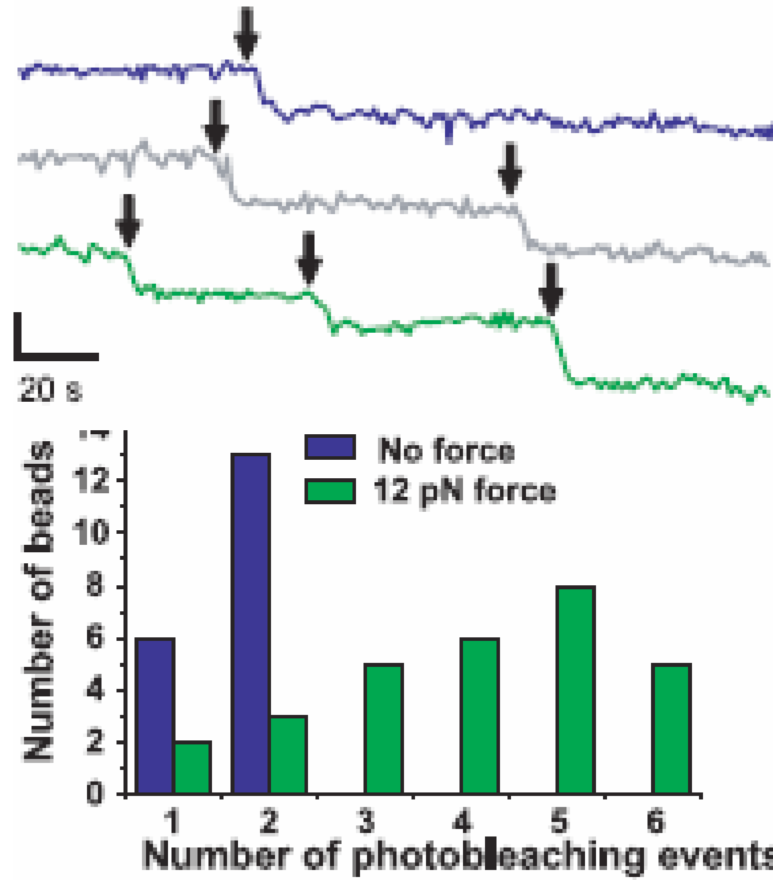
Testing for Mechanosensing

Pull on Talin with magnetic bead



Detect binding of fluorescent Vinculin

Detect number of bound vinculin by number of photobleaching events



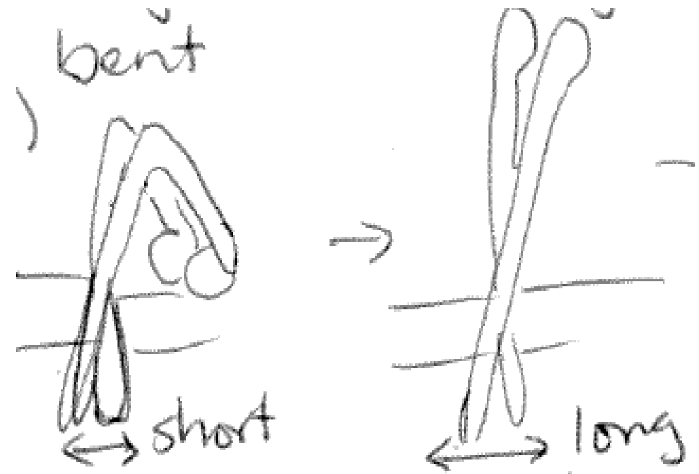
More Vinculin bind at Higher force

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

- Integrins initiate signaling when they bind, so are linked to signal transduction pathways. They are in the line of force, and the active conformation is longer.
- G-protein coupled receptors have been implicated.
- Some matrix proteins have been implicated.
- Nuclei have been implicated (gene expression controlled mechanically?)



Matrix Elasticity Directs Stem Cell Lineage Specification

Adam J. Engler, Shamik Sen, H. Lee Sweeney, and Dennis E. Discher

- Classic Paper in Mechanotransduciton.
- Read the entire paper
- You will have a literature analysis question from the paper in HW 10.
- We will discuss the homework question and paper in class the day HW10 is due.

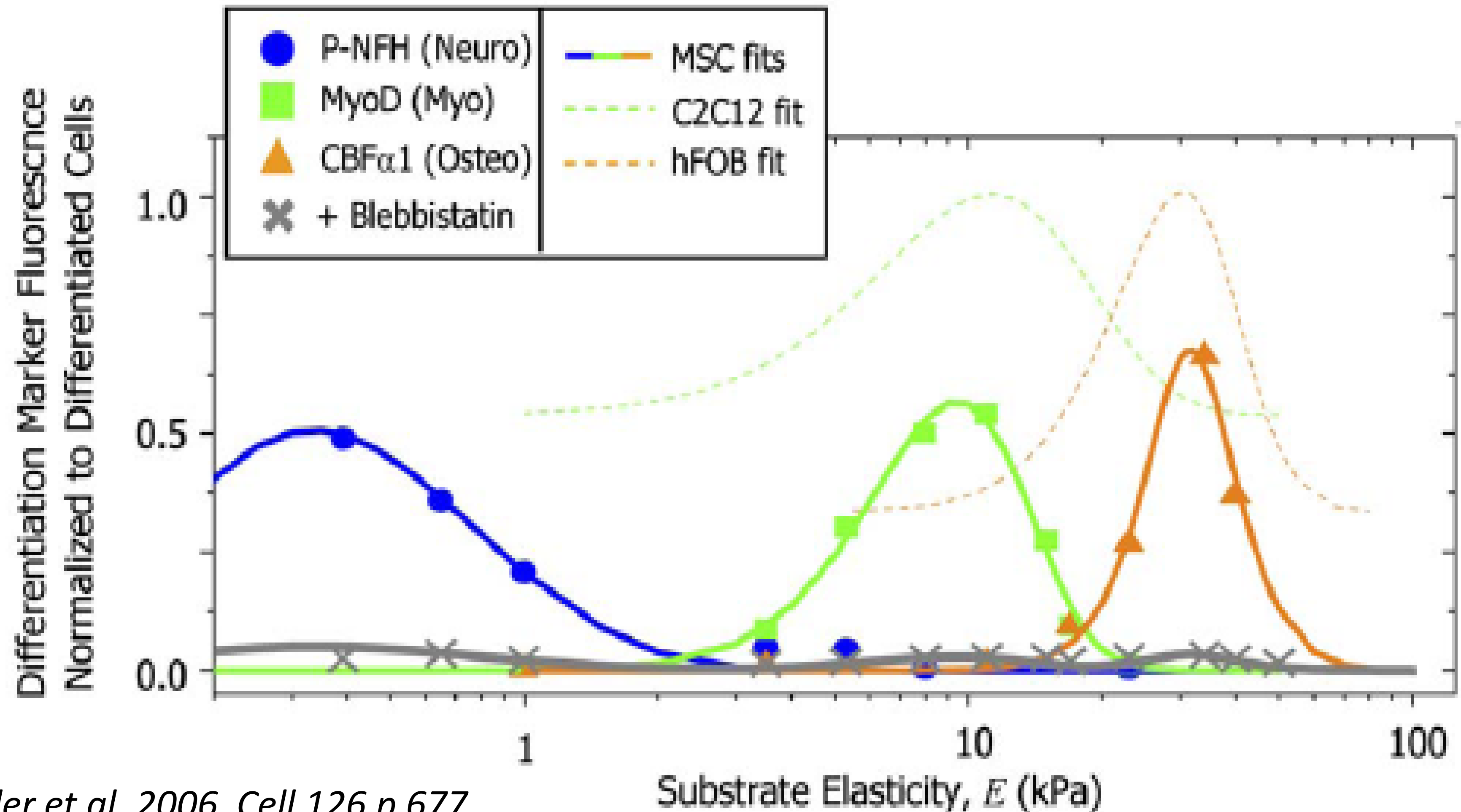
Engler et al 2006 Cell 126, p 677-689

Engler Paper

- Mesenchymal stem cells (MSCs) were plated onto polyacrylamide gels with stiffness controlled by varying the amount of bis-acrylamide cross-linker
- Differentiation was detected by measuring the amount of molecular markers for three tissues:
  - neural (NeuroFilament Heavy Chain)
  - muscle (Myogenesis Differentiation Protein 1)
  - osteogenic (Core Binding Factor α1)
- All were given same hormones, all three sets that are needed to induce differentiation into neural, muscle or bone cells.

Differentiation of Stem Cells

- They differentiate according to stiffness of substratum.



Engler et al, 2006 Cell 126 p 677

Summary 1: Molecular Basis of Mechanotransduction

- Mechanotransduction allows cells to have a microscopic sense of touch.
- Mechanotranduction requires a mechanosensor, a mechanical pathway, and a biochemical pathway.
- A mechanosensor must have force induce an alternative conformation with a different activity than the native state.
- Talin and TRP channels are probably mechanosensors, and many other molecules may be.
- The activity is determined by the probability of being in the active vs inactive state, using the molecular biophysics principles you have learned.

Summary 2: Function and Significance of Mechanotransduction

- Hair cells in the inner ear sense shear stress caused by pressure changes induced by sound waves. Mutations cause hearing loss.
- Kidney processes are regulated flow sensing. Mutations cause polycystic kidney disease.
- Endothelial Cells sense fluid flow to control inflammatory state.
- Stem Cells sense Mechanical force to control differentiation
- Most tissue cells sense forces to control apoptosis and prevent metastasis.