Mechano-transduction

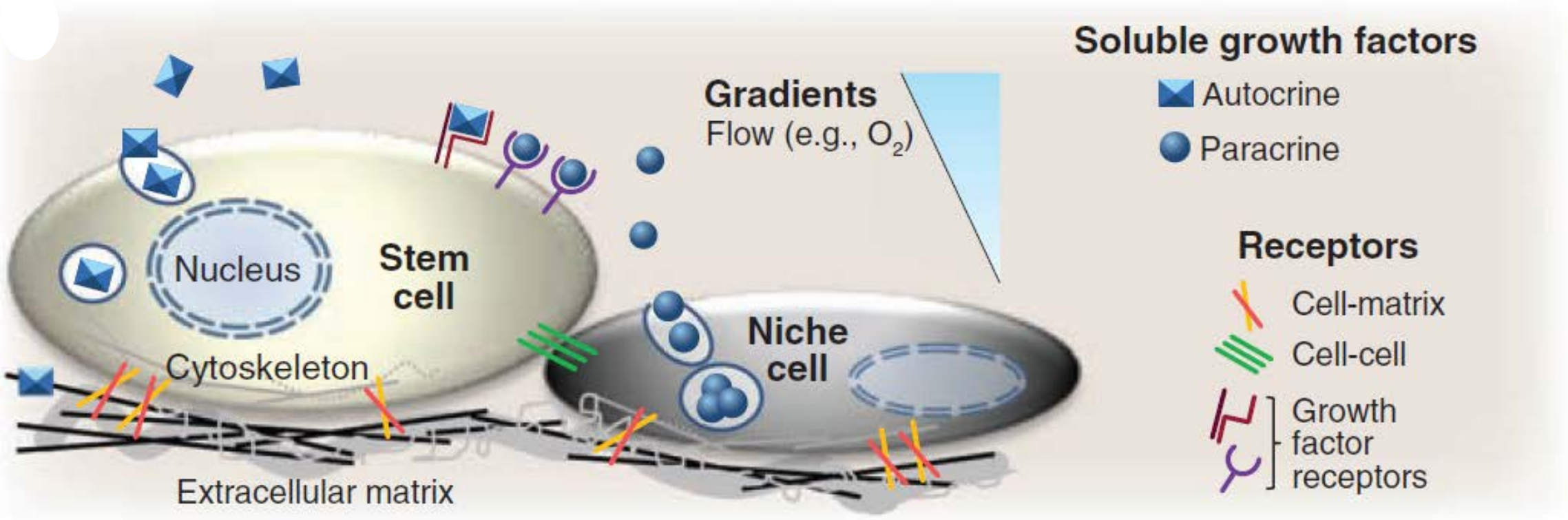
EunAh Lee Ph.D.
IIRC, Kyung Hee University

Mechanotransduction

[DE Discher, 2009 Science; Orr AW 2006 Dev Cell]

Niche

- In vivo microenvironment that regulates stem cell survival, self-renewal, and differentiation
- Key niche component include GFs, cell-cell contacts, cell-matrix adhesions



Forces

Cells generate force and are often exposed to force
The very first stages of cell differentiation in embryogenesis are indeed blocked after knockout of ubiquitous force-generating myosins
Fluid forces typical of blood flow have been found to initiate an endothelial program in isolated ESCs
Imposing substrate strains of just 5% can induce MSC differentiation toward smooth muscle.

Stem cells may well have more than the typical ensemble of force-coupled signaling pathways as a means to sensitize themselves to micro-environments that range-physically-from flowing fluids and strained tissues to solid tissues of varied elasticity

MSCs

- When MSCs are grown on firm gels that mimic the elasticity of muscle and that are coated with collagen I, myogenic markers are up-regulated, whereas when MSCs are grown on rigid gels that mimic pre-calcified bone, the cells appear osteogenic
- Cell lines that are already committed to muscle or bone appear less responsive to the same cues

NSCs

- Neuron differentiation is favored on soft matrices that mimic normal brain, whereas differentiation into glia is promoted on harder matrices that typify glial scars.

Not only physical contributions to differentiation but also that carefully made materials can help prime the expansion of specific progenitors

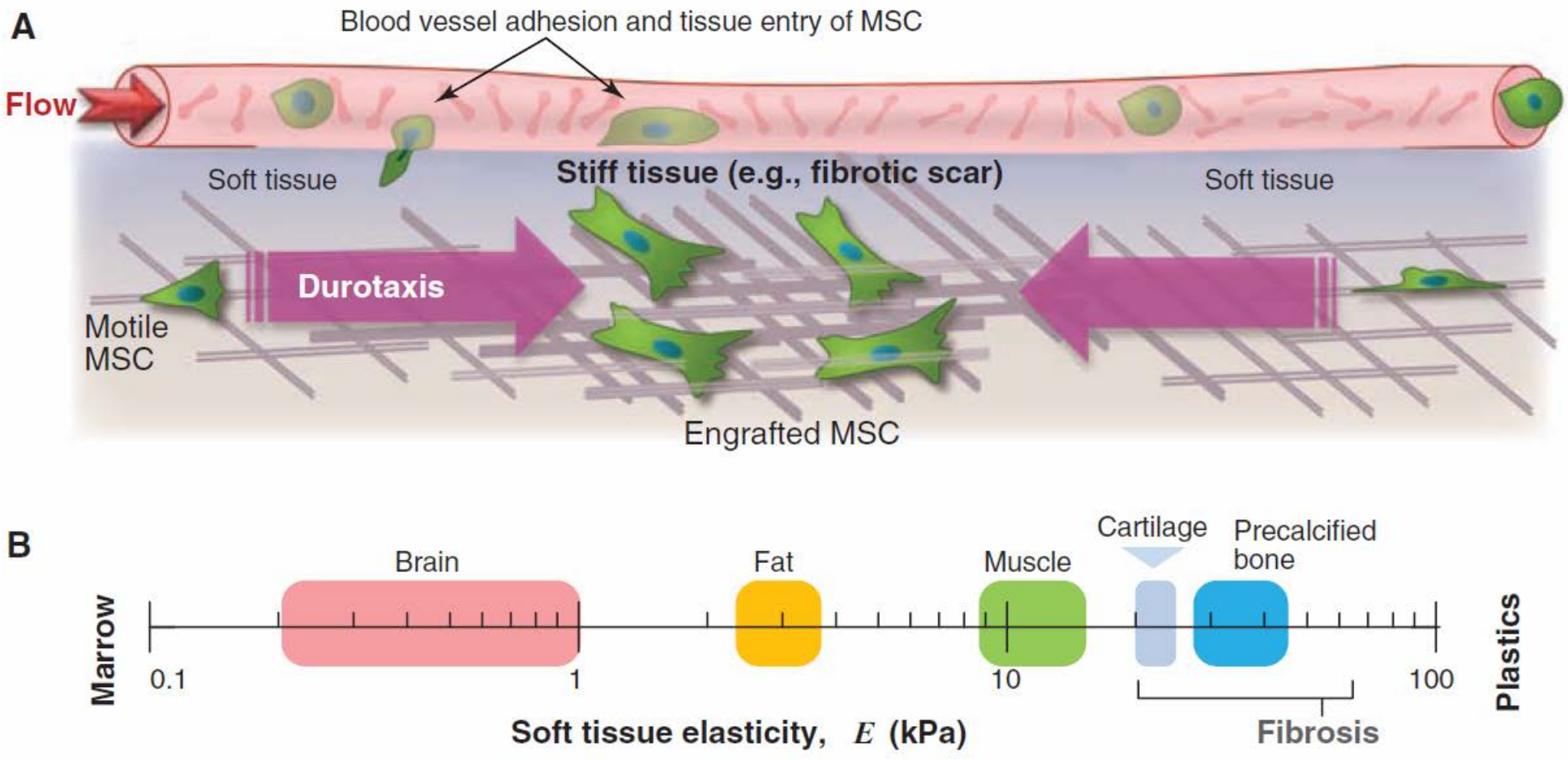
IV (intravenous) injection of stem cells

- Fluid forces push and drag the cells
- Fluid forces oppose adhesion to the vessel wall
- Similar process to those widely studied with leukocytes and metastatic cancer cells

Metastatic "capture" depends strongly on at least one matrix fibrosis factor

- Lysyl oxidase (cross-linker of collagen)

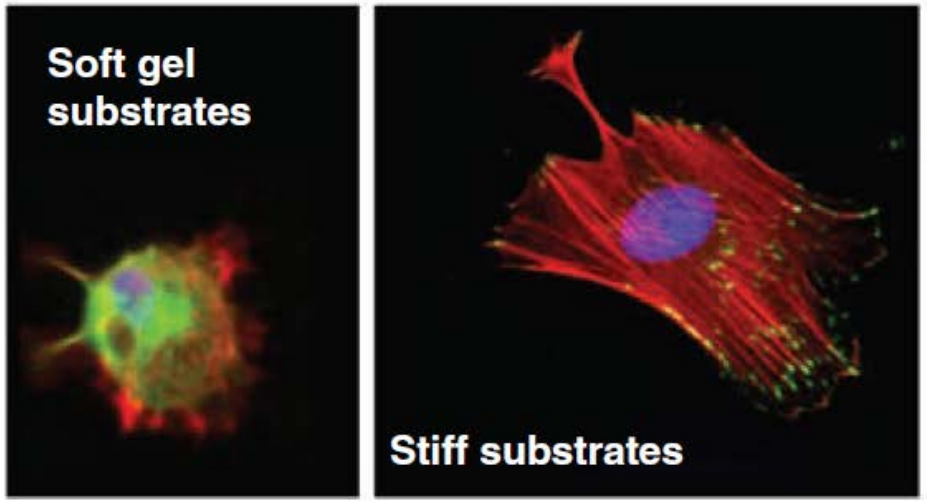
- The fibrotic tissue is locally rigidified by at least seven-fold compared with normal tissue
- AFM probing gives cell-scale elasticities of E = 20~60 kPa for fibrotic wounds
- Rigid fibrotic tissue can contribute a homing signal
- Durotaxis: most cells are found to adhere, to spread, to assemble their cytoskeleton and to anchor more strongly to stiff substrates.

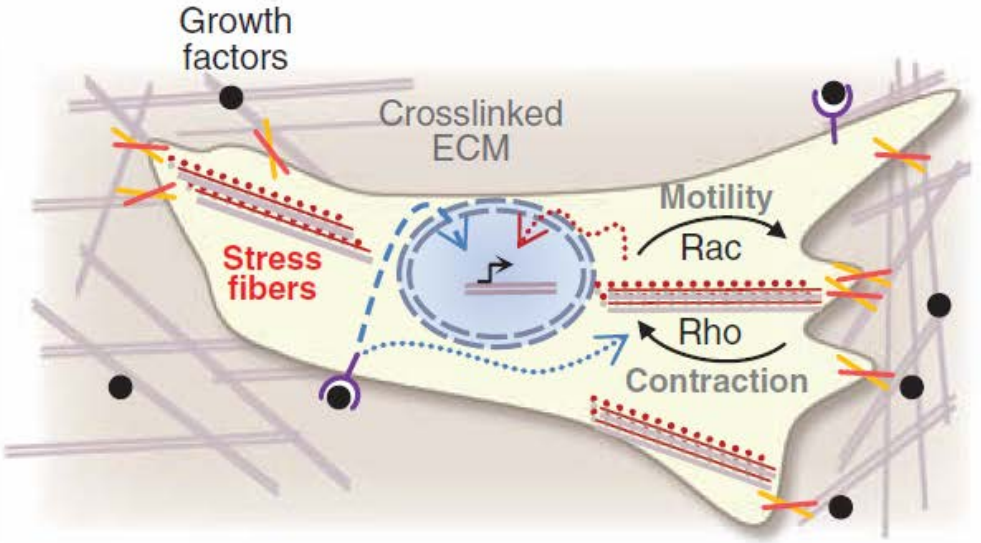


In a gradient of elasticity, cells therefore accumulate on stiffer substrates, which might constitute a biophysical basis for why MSCs home to sites of injury and fibrosis

Matrix can also be a more potent differentiation cue for MSCs than standard induction cocktails.

Well-studied differentiation of fibroblasts to myofibroblasts requires both a stiff matrix and TGF β, with GF release from the ECM dependent on cell contraction-driven unfolding of the ECM complex that sequesters the TGF β.





Effect of matrix rigidity:

- Cardiogenesis involves a complex interplay of mechanochemical factors
- Embryo-derived cardiomyocytes maintain their spontaneous beating on substrates with elasticity less than or equal to that of normal heart tissue.
- But the cells stop beating on rigid matrices that mechanically mimic a fibrotic scar
- ROCK (Rho kinase effector) inhibition selectively blocks cell dysfunction on rigid substrates

Effect of area:

- If the area of MSC contact is controlled with adhesive patterns, it is found that mixed induction cocktails that induce both fat and bone lead to adipogenesis on small islands (which minimized matrix contact) and osteogenesis on large islands (which maximize contractile anchorage)

Mechano-transduction

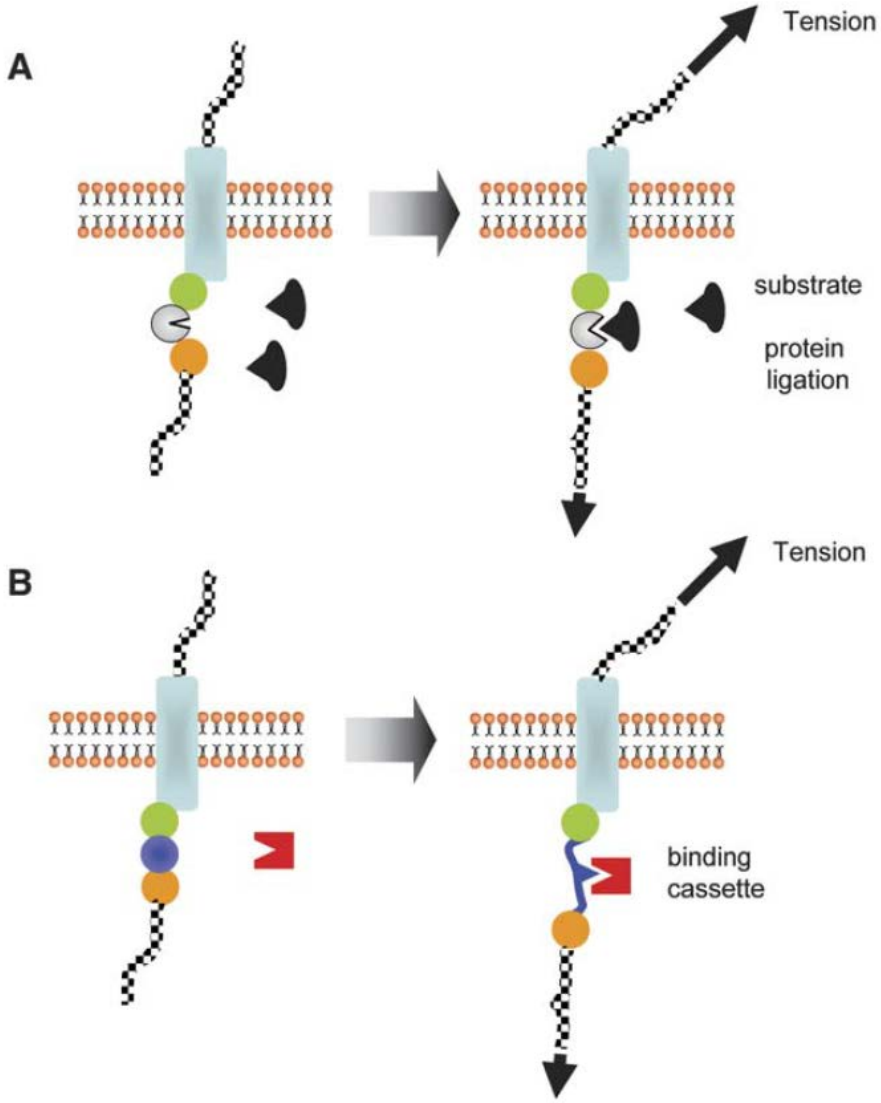
The conversion of physical force into biochemical information
Examples:

- **Pressure and shear stress of pumping blood in vascular system**
- **Force-driven bone remodeling**
- **Neural responses to pressure organize sensing to hearing and touch**
- **Inflation and deflation of lung**

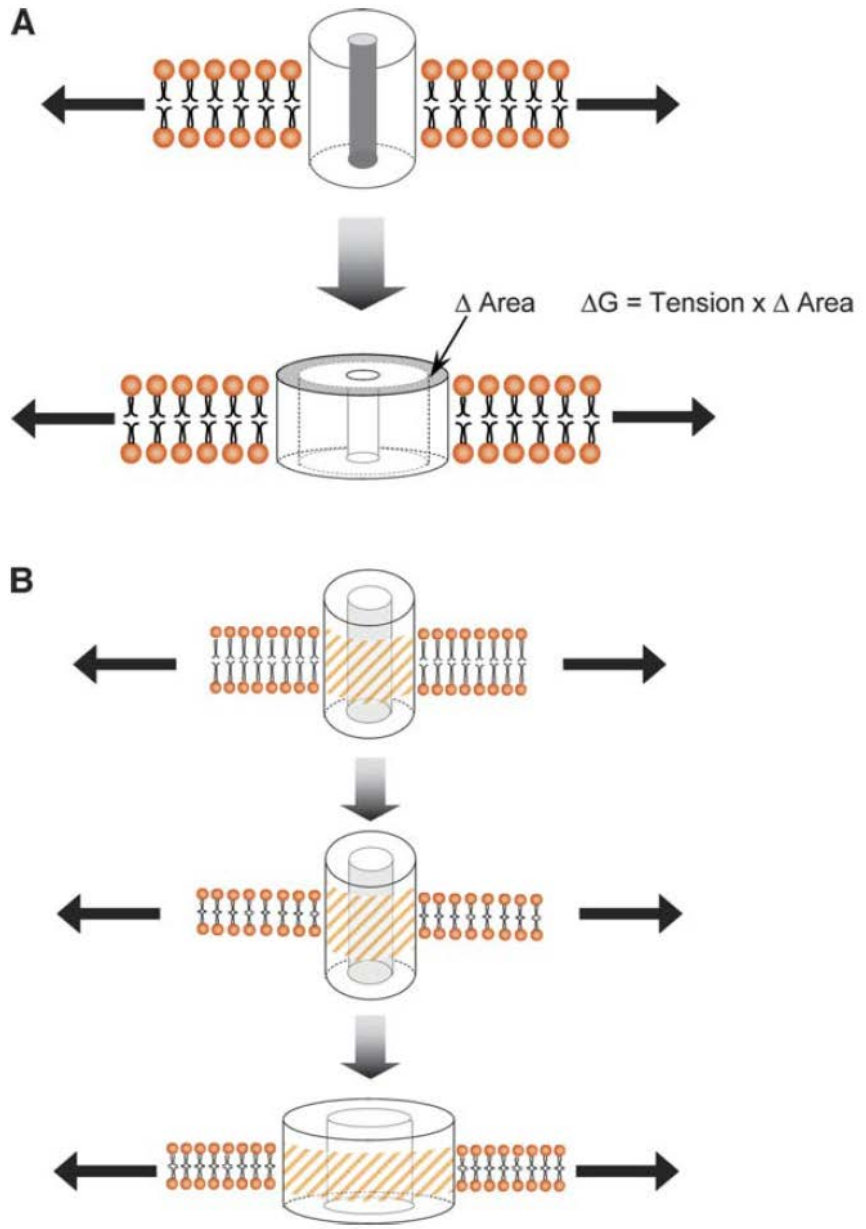
Remarkable breadth of mechano-sensitive events makes it unlikely that one or even a few mechano-transducers can account for all of these events.

Force-Induced Change in Protein Conformation

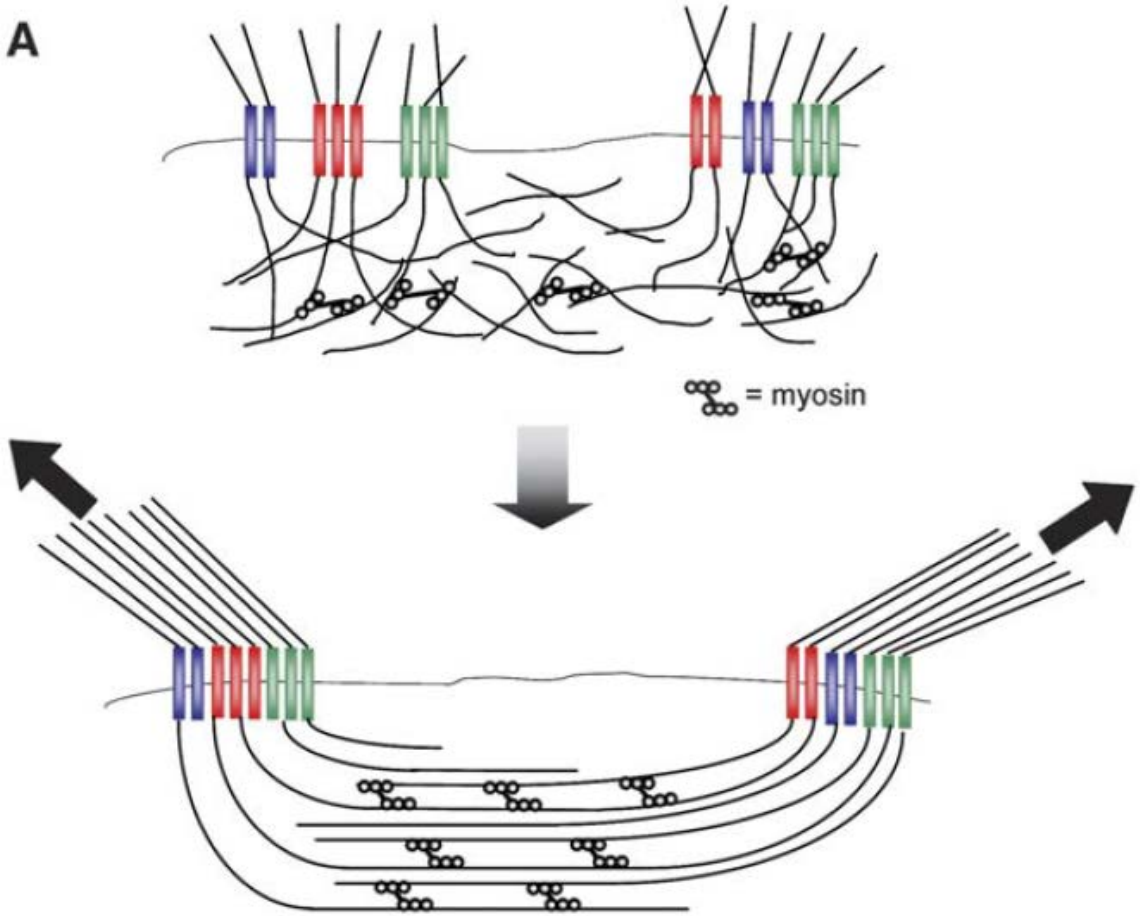
- Protein folding in general favors the formation that yields the lowest free energy
- Physical forces that modify the energy landscape will therefore directly alter protein folding
- Just as protein phosphorylation or other posttranslational modifications mediate signal transduction in large part through changes in protein conformation, force-induced effects on conformation represent a general mechanism by which enzymatic activity or protein interactions can be modified to mediate signaling



- Stretch-sensitive channels provide the best-studied example.
- Increasing tension within the lipid bilayer from 10-12 to 20 dyn/cm increases the channel-opening probability
- If the open state occupies a greater area within the bilayer, membrane tension will result directly in lower free energy.
- Protein unfolding under tension represents a one-dimensional instance of the same principle where unfolding lowers the free energy.



Mechano-Transduction in Focal Adhesions or Cytoskeletal Structures



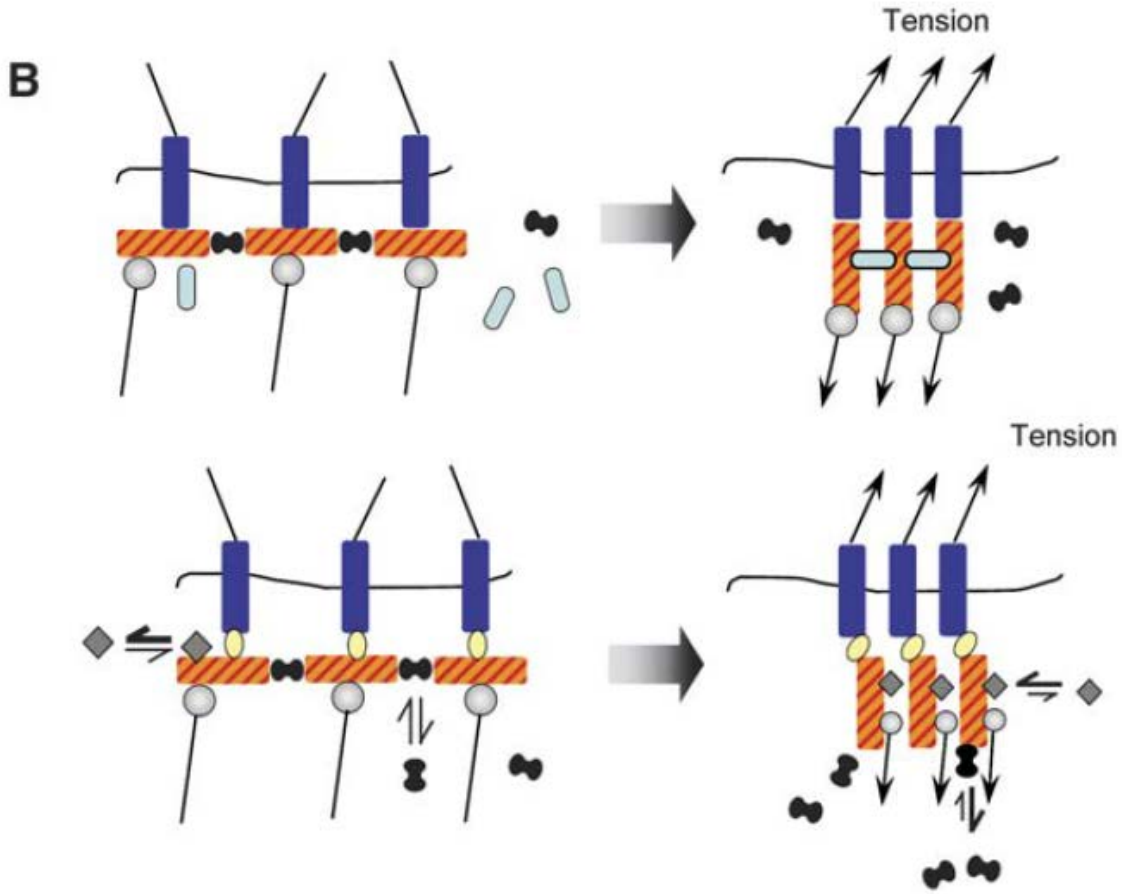
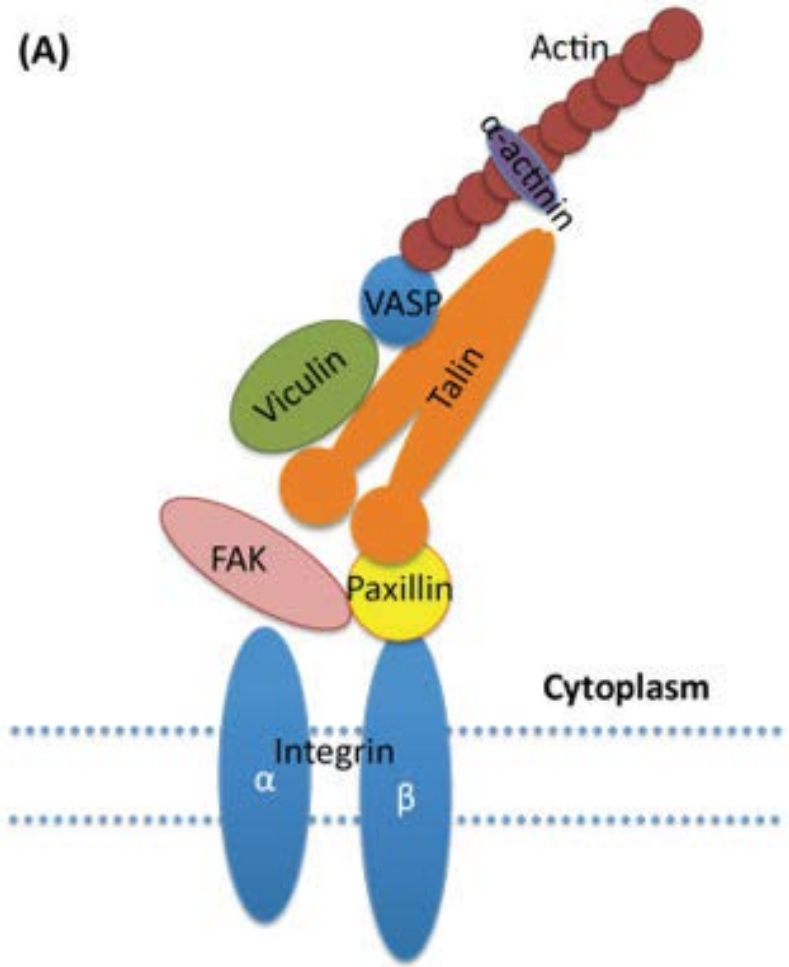
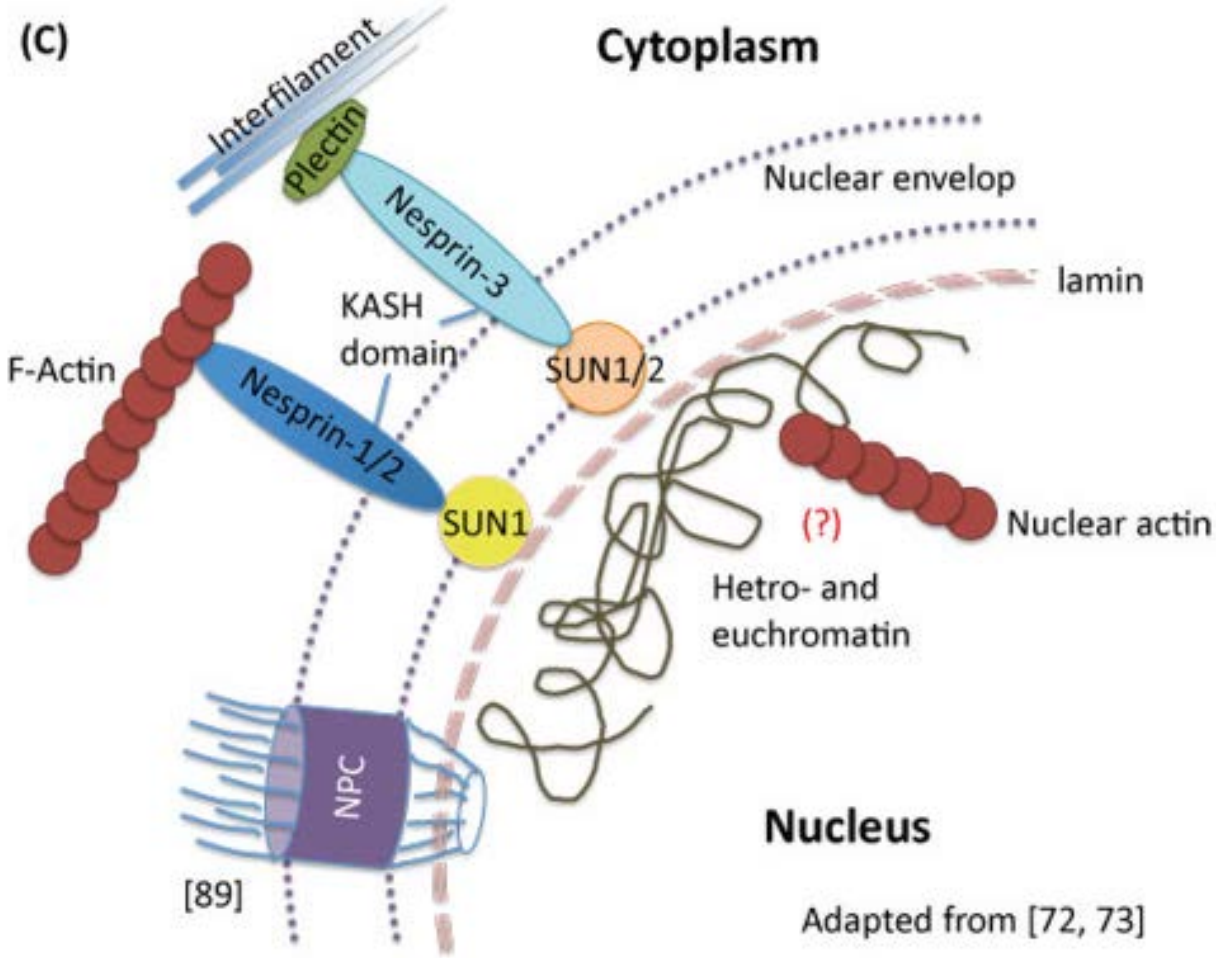


Table 1. Estimated In Vivo Magnitudes of Mechanical Stimuli on Cells

Physical Profile	Mechanical Stimulus	Typical Values
Arterial blood flow	Fluid shear stress	1–3 N/m^2
Cell migration	Traction stress	3.0–5.5 kN/m^2 (normal); 1 kN/m^2 (cancer)
Proximal tubule flow	Fluid shear stress Fluid drag force Bending torque	0.3 N/m^2 0.0074 pN/microvillus 0.016 pN-μm/microvillus
Stretch-activated channels	Membrane tension	0.012 N/m
Outer hair cell stereocilia	Compression stiffness Force/Δ membrane potential	0.001 N/m 0.1–20 pN/mV
Osteocyte processes (bone canaliculi)	Fluid shear stress Fluid drag force Tissue strain	0.8–3.0 N/m^2 20 × shear force 0.03%–0.1%

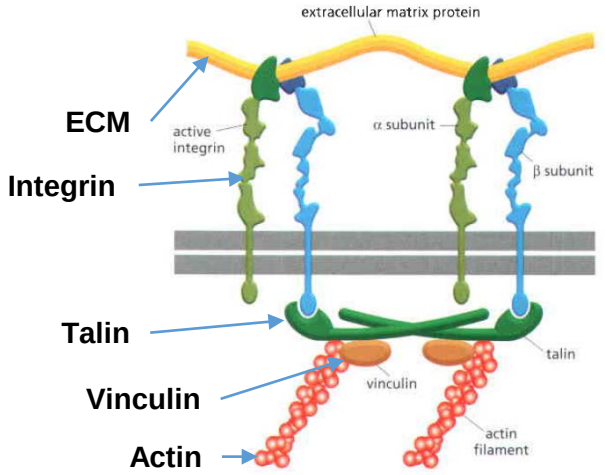

(A)
Actin
α-actinin
VASP
Viculin
Talin
FAK
Paxillin
Cytoplasm
Integrin
α
β


(C)
Cytoplasm
Interfilament
Plectin
Nesprin-3
Nuclear envelop
lamin
KASH
domain
F-Actin
Nesprin-1/2
SUN1/2
SUN1
(?)
Nuclear actin
Hetro- and
euchromatin
NPC
[89]
Nucleus
Adapted from [72, 73]

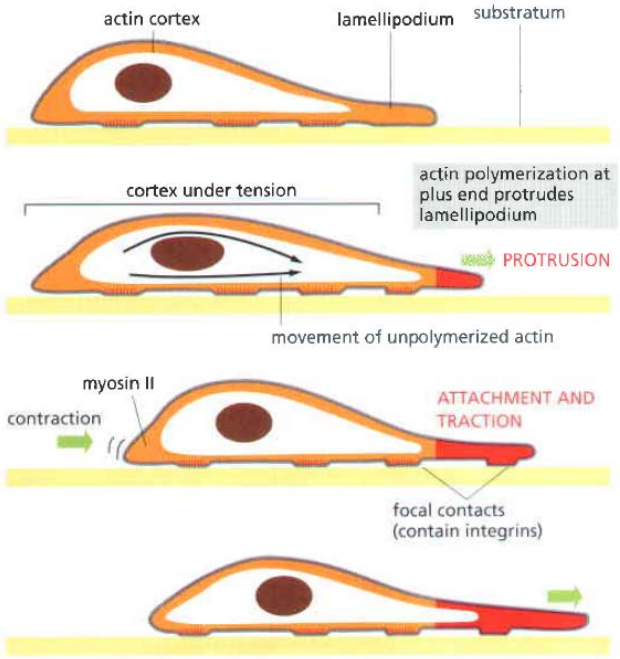
Assembly & Remodeling of Integrin Adhesions

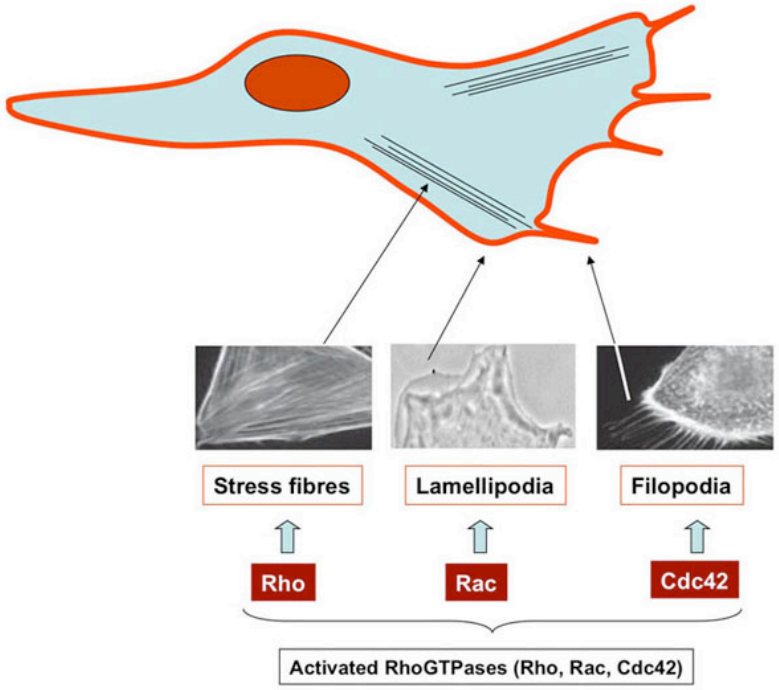
Lamellipodia
Local complexes

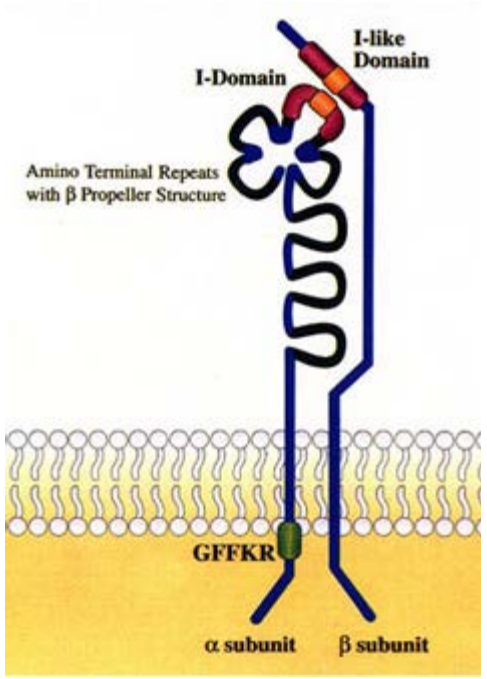
- Earliest integrin-containing structures
- 100nm in diameter
- Including integrin, talin, paxillin
- Binding of vinculin -> talin
  - Triggers clustering of activated integrin
  - Associate with actin through the vinculin tail
  - Strengthening actin-integrin link
  - Drive growth into larger focal complexes



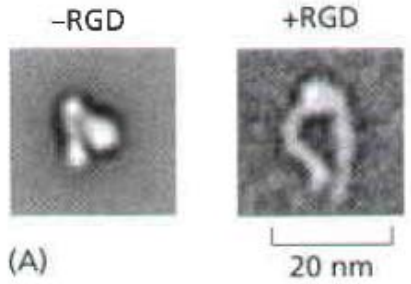
Lamella
Tropomyosin & myosin II are prominent
Located ~2-4um from the leading edge

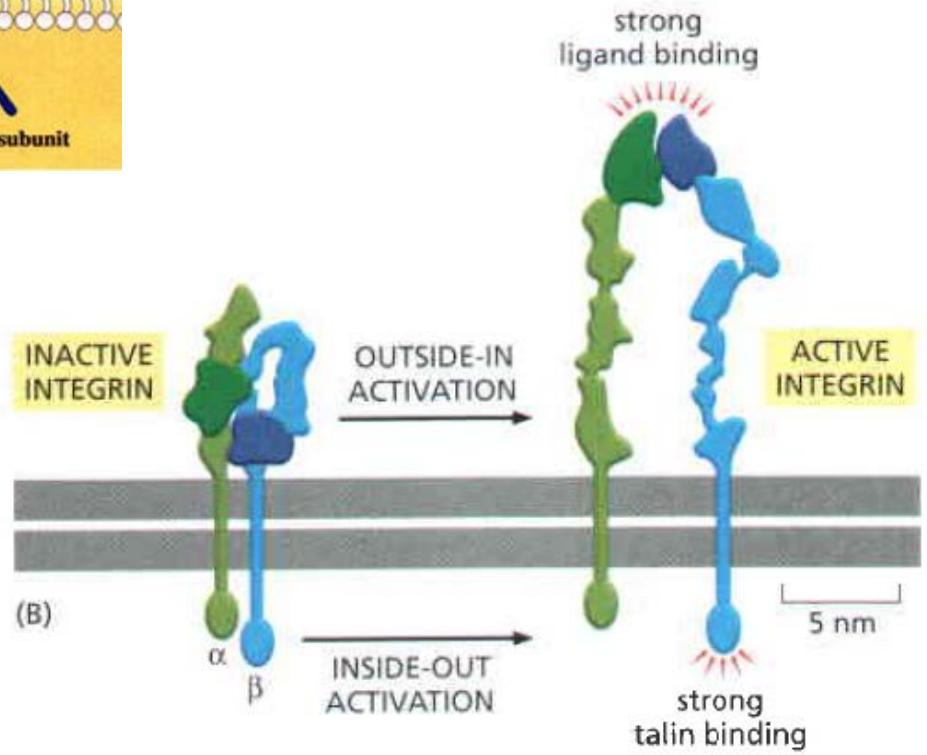






If the traction force is increased, the attached adhesion grows: relaxation of tension leads to its dissociation.





Signal Transduction in Mechano-Sensing