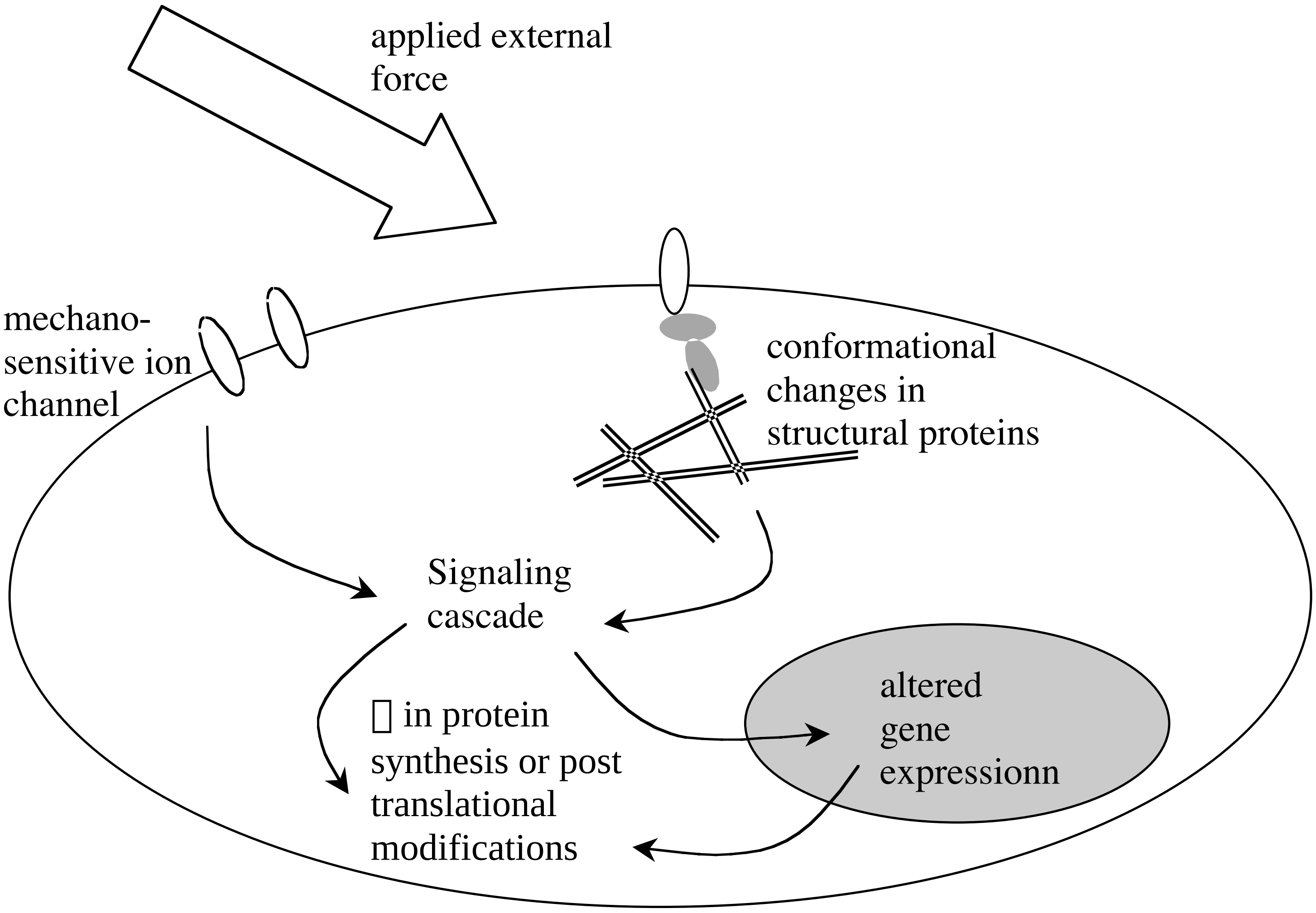
Effect of laminar shear stress on cell alignment (Dewey et al. 1981)

Photos removed due to copyright considerations.
See Dewey, C.F., et al. "The dynamic response of vascular endothelial cells to fluid shear stress."
J Biomech Eng. 1981 Aug;103(3):177-85.

No flow

Laminar shear stress


applied external force
mechano-sensitive ion channel
conformational changes in structural proteins
Signaling cascade
□ in protein synthesis or post translational modifications
altered gene expressionn

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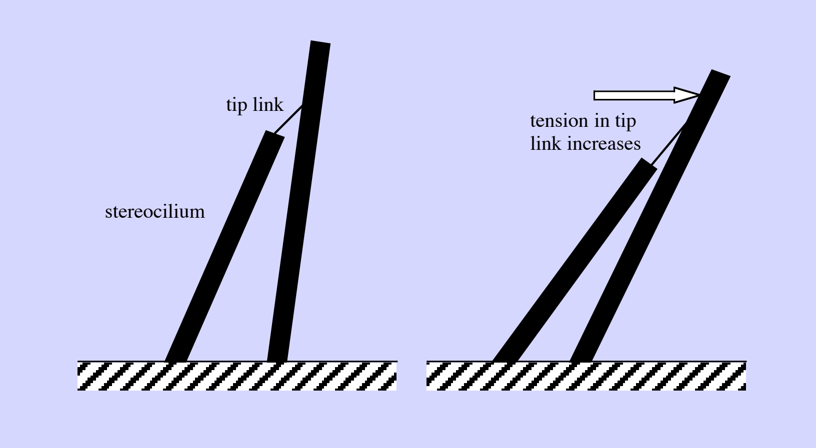
Hanahan & Weinberg, *Cell* [2000]

We know quite a lot about the *signaling cascade* that follows the initial biochemical event, leading to morphological changes, variations in various biochemical signals, changes in gene expression and protein synthesis, but relatively little about how the initial event is *transduced* from physical force to biochemical reaction.

Mechanotransduction: Hair cell stimulation



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copyright considerations.

SEM of the stereocilia on the surface of a single hair cell (Hudspeth)

Image removed due to
copyright considerations.

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

Structure of a Mechano-Sensitive Ion Channel (MscL, large conductance)

Chang, et al., Science, 1998

Membrane stresses alter pore geometry and control the exchange of ions

Images removed due to copyright considerations.

Steered Molecular Dynamics

For each atom:

$$m_i \frac{d^2 x_i}{dt^2} = \sum_{j=1}^{N} F_{ij} \qquad i = 1, \ldots N$$

F_{ij} represents the force applied to the *i*th atom by the *j*th atom (all the force interactions), determined as the gradient of the potential energy.

Steered molecular dynamics (SMD) is the forced unfolding of a protein to reveal new conformational states.

Molecular dynamics simulation of channel regulation by membrane tension

(Gullingsgrud, et al., Biophys J, 2001)

Images removed due to copyright considerations.
See Figures 1 and 9 in Gullingsrud, Justin, Dorina Kosztin, and Klaus Schulten.
"Structural Determinants of MscL Gating Studied by Molecular Dynamics Simulations."
Biophys J, Vol. 80, No. 5 (May 2001), p. 2074-2081.
http://www.biophysj.org/cgi/content/full/80/5/2074

But other evidence suggests that the pore increases to >20 angstroms!

Structure of MscS (“small” conductance) mechano-sensitive channel, is now also known and studies are underway to identify the mechanism of operation. (Bass et al., Science, 2003)

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Selective binding to cytoskeletons after stretch

(Sawada & Sheetz, 2002)

- Triton X-100 insoluble cytoskeletons
- Incubated with cytoskeletal proteins having a photocleavable biotin tag w/ and w/o 10% stretch
- Focal adhesion kinase, paxillin, p130Cas, PKB/Akt all preferentially bound

Image removed due to copyright considerations.
See Figure 3 in Sawada, Y., and M. P. Sheetz.
"Force transduction by Triton cytoskeletons."
J Cell Biol. 2002 Feb 18;156(4):609-15.
http://www.jcb.org/cgi/content/full/156/4/609

Binding of proteins is influenced by stretch of cytoskeleton

Can it be due to induced conformational changes?

Strength of integrin bonds to ECM ligands

Images removed due to copyright considerations.
See Figure 1A and 2 in Lehenkari, P., and M. Horton.
"Single Integrin Molecule Adhesion Forces in Intact
Cells Measured by Atomic Force Microscopy."
Biochem Biophys Res Commun. 1999 Jun 16;259(3):645-50

AFM used to measure the strength of integrin bonds to various RGD ligands.

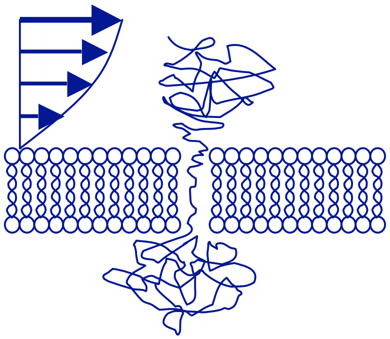
Single bond forces were 32-97 pN.

Lower forces (~ 10 pN or less?) are likely adequate to produce conformational changes.

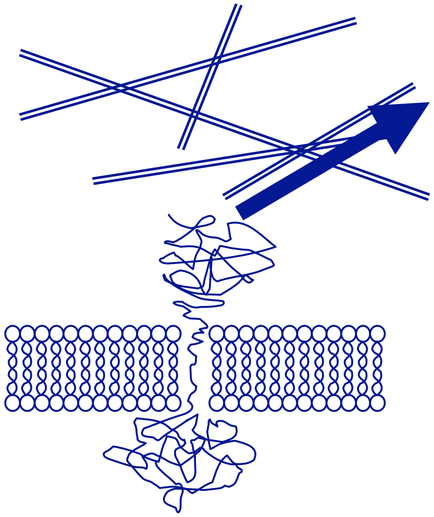
Lehenkari & Horton, BBRC, 1999

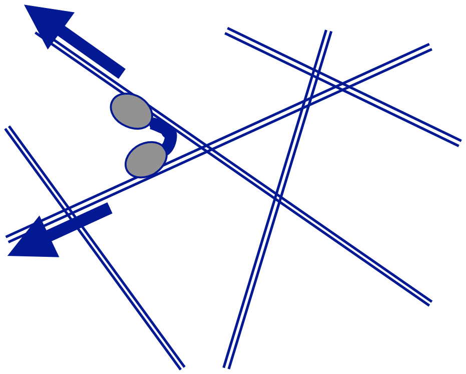
Potential transduction mechanisms involving changes in molecular conformation

Shear-stress induced conformational changes in transmembrane proteins



Forces exerted by ECM or CSK attachments





“Sensing” proteins tethered to the CSK or at focal adhesion sites

Some Force Estimates

A shear stress of 1 Pa exerts a 1 pN force on a 1 μm^2 area.

A globular protein complex ~100 nm in diameter would experience a 1 pN force in a linear shear flow corresponding to a shear stress of 1 Pa.

Significant protein conformational changes occur for forces in the range of ~10 pN.

Magnetic traps can exert forces of ~10 pN per integrin bond.

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Davies et al., Ann. Rev. Physiol., 1997

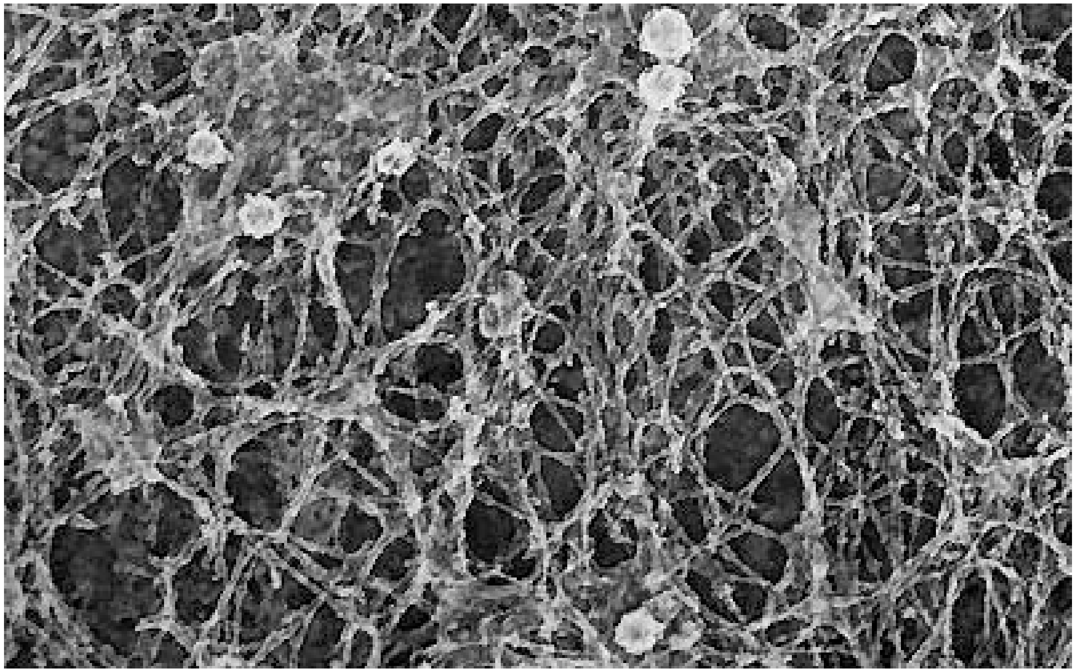
SMD of fibronectin

(Gao, Craig, Vogel, Schulten, JMB, 2002)

Images removed due to copyright considerations.
See Figures 2 and 3 in Gao, Mu, David Craig, Viola Vogel, and Klaus Schulten.
"Identifying unfolding intermediates of FN-III10 by steered molecular dynamics."
Journal of Molecular Biology, 323:939-950 (2002).

Applied force = 500pN

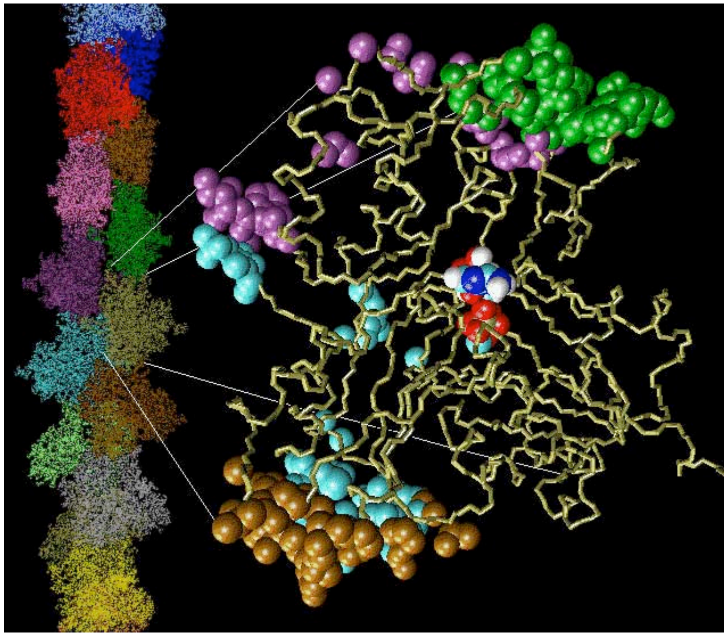
Unfolding is important in the exposure of buried cryptic binding sites.



TEM cytoskeleton photograph, J. Hartwig, Harvard University.
Courtesy of J. Hartwig. Used with permission.

Actin is another candidate for force-induced conformational change. When stressed, cells form stress fibers, mediated by a variety of actin-binding proteins (□-actinin, fascin). The structure is known, and computations can take advantage of filament periodicity.

<ins>But</ins>, a force of 10 pN supported by a single actin filament ($E\sim10^{9}$ Pa) produces a strain of $\sim 2x10^{-4}$!!)



Structure of actin.
Image courtesy of Prof. Willy Wriggers,
http://biomachina.org.
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