Chen et al. (2017) — "Receptor-mediated cell mechanosensing"

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Andrew Watson

1 Introduction

- It is important for cells to be able to sense and respond to mechanical signals from their surroundings.
- Mechanosensitive structures exist across multiple scales in organisms. This review is concerned with cellular and molecular scales, and specifically receptor-mediated mechanosensing.
- Receptor-mediated mechanosensing is important in many cellular processes, including activation, differentiation, proliferation, apoptosis, cell spreading, migration, and others.
- Receptors and intracellular molecules involved in mechanosensing can be found by altering their concentration or activity somehow, which affects how the cell responds to a mechanical stimulus.
- Intracellular forces can also be transferred to an extracellular substrate through receptors, affecting the cell's response as well.
- In reality, mechanosensing is a complex process that involves many receptors as well as crosstalk between the cell and the substrate. However in this review, they simplify the problem and only look at one direction of the signaling through a single receptor.

2 Four-step model for mechanosensing

- When a receptor binds to an immobilized ligand and force is exerted on the receptor-ligand bond, mechanosensing can be initiated.
- There are 4 steps in transferring this mechanical signal to the inside of the cell:

    1. Mechanopresentation—mechanical cues are presented to the receptor. The ligand must be bound to a surface and able to support a force. Soluble ligands cannot transduce force. The mechanopresenter is a ligand anchored to a surface.
    2. Mechanoreception—the ligand transmits force to the binding site of a cell surface receptor. This force may alter the bond between the ligand and receptor. The mechanoreceptor binds to the mechanopresenter.
    3. Mechanotransmission—the force is communicated from the binding site towards the cell interior. The mechanotransmitter is responsible for propagating the force signal. It may transmit mechanical force across the membrane, or it may propagate information in another way (e.g. force-induced conformational change).
    4. Mechanotransduction—the mechanical cue is translated into a biochemical signal. Often either the receptor or a linked subunit undergoes a conformational change in this step. The mechanotransducer is the structure(s) undergoing a mechanical change to initiate a chemical signal.

- An important role of mechanotransduction is distinguishing between different types of forces. The mechanotransducer may respond to one type of force and not others (digital mechanism) or its response may scale with the strength of the mechanical force (analog mechanism).
- The difference between the mechanotransmitter and mechanotransducer is that the mechanotransducer translates the mechanical signal into a chemical one, whereas the mechanotransmitter does not.

3 Kinetic and Mechanical Aspects

3.1 Kinetic constraints

- Bond kinetics are affected by mechanical forces, and so play a role in mechanopresentation and mechanoreception. In particular, kinetics modulate the magnitude, duration, and frequency of force, and place a constraint on presentation and reception.

- The off rate of a single bond can increase with applied force (slip bond), decrease with applied force (catch bond), or be independent of force (ideal bond). The type of receptor-ligand bond that forms influences the magnitude and lifetime of mechanical signals communicated to the mechanoreceptor. For example, the amount of Ca^{++} signaling induced by TCR and GP1b depends on the amount of force applied to these receptors from their ligands.

3.2 Mechanical changes

- Mechanically-triggered conformational changes can play an important role in a mechanosensor, and these changes fall into 6 different types:

    1. Deformation—e.g. membrane ion channels which open in response to an increase in membrane tension.
    2. Relative displacement—force can induce displacement of two subunits of transmembrane channels.
    3. Hinge movement—for proteins consisting of two globular domains connected by a hinge region (like integrins and selectins), pulling force can promote hinge opening.
    4. Unfolding/unmasking—force can cause proteins to unfold, revealing previously hidden active sites. Examples include GP1b, talin, vinculin, fibronectin, and vWF.
    5. Translocation/rotation—force applied to proteins noncovalently complexed with other proteins can lead to relative movement and breaking bonds on the trailing edge and forming bonds on the leading edge. Examples of translocation are linear molecular motors like myosin, kinesin, and dyneine. Examples of rotation are ATP synthases.
    6. Cluster rearrangement—force acting on proteins in a cluster trigger the proteins to change their arrangement through their mutual interactions. An example is integrins in a focal adhesion, which alter the cytoskeleton through interactions between integrin tails and actin.

- The choice of conformational change to employ depends on the physiological function of the mechanotransducer. For example, cluster rearrangement in integrins can induce changes in the cytoskeleton. However, a single GP1b cannot trigger actin rearrangement.

- Mechanical signals can be important in other steps of mechanosensing as well. For example, vWF only exposes its A1 binding sites when exposed to a high enough shear. This modulates the mechanopresentation of A1 to GP1b molecules.

- Kinetics and mechanics can be coupled at the level of a single receptor. For example, force applied to the leucine-rich repeat domain (LRRD) in GP1b can strengthen the GP1b-A1 bond, increasing its lifetime. Also, only abruptly increasing force can cause this strengthening; constantly applied force cannot.

4 Nanotools for studying mechanosensing

4.1 Dynamic force spectroscopy

- Dynamic force spectroscopy (DFS) uses ultrasensitive force probes like atomic force microscopy (AFM), optical tweezers, magnetic tweezers, and biomembrane force probes (BFPs). These instruments have very fine resolution in space, time, and force scales.

- In DFS, ligands immobilized to a substrate are brought into contact with a mechanoreceptor to facilitate binding. Then a piconewton-level pulling force is applied to the bond through

one of the above mechanisms. Force-ramp experiments involve increasing the force until the bond breaks, while force-clamp experiments ramp the force to a pre-defined amount, and then the force is held there until the bond breaks. These experiments can be used to determine the force dependence of receptor-ligand off rates, or protein conformational changes or unfolding.

- DFS experiments combined with real-time imaging of intracellular signaling events allows experimentalists to directly observe the intracellular response of mechanotransducers to force. For example, prolonged forces applied to the GP1b-A1 bond have been observed to initiate Ca^{++} in platelets.

4.2 Magnetic twisting cytometry

- This technique involves a magnetic bead coated with ligand and bound to multiple receptors on a cell surface. This is a high-throughput method, using many beads bound to the surfaces of many cells.

- This allows experimentalists to measure cell stiffness. These kinds of experiments provided evidence of mechanosensing by integrins, as the cell stiffened in response to an applied torque.

4.3 Microscopic probes for cell traction and internal force

- Two aspects of mechanosensing: environment exerting force on a cell, and a cell producing force to sense mechanical properties of its surroundings.

- The force probes described in previous sections provide no information on if the cell is actively exerting force.

- Traction force microscopy measures bulk traction forces generated by adherent cells. Cells are seeded onto a ligand-coated substrate which contains markers that can be tracked microscopically. By observing the displacement of these markers, the force exerted by the cell can be calculated.

- Traction force microscopy has been an important tool in learning about focal adhesions.

- When combined with live-cell imaging, this can be used to visualize the dynamics of traction forces and molecular signals.

- A second class of force probes involves inserting a polymer between the cell and substrate to either limit the tension experienced by the cell, or report the tension generated by the cell.

- Tension gauge tethers (TGT) are designed to rupture above a certain force, and thereby limiting the amount of tension the cell can generate. This provides information on how much tension is required to support certain force-dependent functions.

- Molecular tension-based fluorescence microscopy probes unfold and fluoresce when tension is applied above a certain threshold. This unfolding is reversible, and so is doesn't affect cell functions.

- A third class of probes is similar to the DNA-based probes, but using flexible peptides which fluoresce under applied tension. These can be used to moniter both extracellular and intracellular forces, and have been inserted along the cytoplasmic tails of α_L and β_2 to show that force is transduced through the β subunit in migrating cells.

5 Platelet mechanosensing via single GP1b

- vWF is mechanosensitive, it requires a pulling force to unfurl the molecule and expose the GP1b binding sites. In the vWF-GP1b mechanosensing system, vWF is the mechanopresenter.

- Once vWF is immobilized on vessel walls, it is subjected to higher shear rates near the vessel wall. Once vWF is bound to GP1b, intracellular Ca^{++} release is triggered, which signals integrin $\alpha_{IIb}\beta_3$ to activate.

- Upon vascular injury, vWF adsorbs to the subendothelium, and fluid forces extend the vWF multimer, exposing the A1 binding

site for GP1b. The GP1B-A1 bond has fast association and dissociation constants. Fast association allows for the capture of fast-flowing platelets, and fast dissociation allows for rolling along the surface.

- Physical transport in the bulk drives platelets to collide with the vessel wall, bringing GP1b and vWF close enough to form bonds. Three steps of the transport regulate GP1b-vWF association:
  1. tethering of the platelet to the vascular surface,
  2. Brownian motion of the platelet, and
  3. rotational diffusion of the interacting molecules.
- The GP1b-vWF bond acts as a catch bond below 22 pN, and as a slip bond above that. 2B mutant vWF forms slip bonds only with GP1b.
- Collagen-vWF interaction also activates vWF, in addition to providing an anchor point for vWF. vWF is initially captured through A3 binding with collagen, but further collagen binding with A1 can increase its affinity for GP1b binding.
- Increased flow enhances platelet capture on vWF through three mechanisms:
  1. Exposure of vWF-A1 binding sites on immobilized vWF
  2. Increase in the number of platelets that collide with the wall
  3. Longer association times of GP1b and vWF-A1 through catch bond behavior
- There are two domains in GP1b that extend when the molecule is pulled on: the LRRD (leucine-rich repeat domain) and the MSD (mechanosensitive domain).
- These two domains have different unfolding lengths, and respond differently to different force waveforms. Ramped force (a quick increase in force application) unfolds both LRRD and MSD, whereas clamped force (a constant force applied for a long time) only unfolds MSD.
- Unfolding of the MSD allows for transmission of the mechanical signal along the receptor.
- The unfolding frequency of MSD depends on the level of applied force in the same way as GP1b-vWF binding kinetics. This is true in both normal vWF and 2B vWD mutants. This suggests a coupling between unbinding kinetics and unfolding kinetics.
- Experiments show that LRRD unfolding prolongs the A1-GP1b bond lifetime, perhaps by exposing additional binding sites within the LRRD region.
- Unfolding of MSD requires sustained force, and it cannot occur after vWF unbinds and force is no longer being applied along the molecule. There is cooperativity between LRRD and MSD unfolding. LRRD unfolding lengthens the lifetime of the GP1b-vWF bond, which increases the probability of MSD unfolding.
- Unfolding of MSD results in intracellular signaling. In mutation experiments, mutations that unfolded the MSD triggered intracellular signaling even in the absence of ligand binding.
- Two types of intracellular Ca^{++} signaling have been observed in platelets with a single GP1b-vWF bond:
  1. α type, which features an initial latent phase followed by a high spike with a quick decay, and
  2. β type, which features fluctuating sinals around the baseline or gradually increasing signals to an intermediate level followed by a gradual decay to baseline.
- MSD unfolding is required to trigger α-type calcium signaling. It has also been found that constant force at an optimum level triggers maximum Ca^{++} release, no transient force.
- Patients with vWD have increased bleeding relative to controls. This provides a possible explanation for that phenotype. In vWD, the GP1b-A1 bond is converted to a slip bond, therefore the bond lifetime is relatively shorter under force, and maximum Ca^{++} release cannot be triggered.
- In summary, the 4 steps of mechanosensing in the vWF-GP1b system are as follows:

1. Mechanopresentation: vWF undergoes local and global conformational changes in response to force resulting in increased binding affinity to GP1b.
2. Mechanoreception: LRRD binds to the vWF-A1 region. This domain can then unfold under force, stabilizing the bond and increasing its lifetime.
3. Mechanotransmission: Force propagates from the LRRD along the MP stalk, inducing unfolding of MSD.
4. Mechanotransduction: Exposure of the Trigger sequence within the MSD and association of 14-3-3ζ to GP1b allows the mechanical signal to be converted into a chemical one.
5. GP1b may represent a more general class of mechanoreceptors. For example, Notch receptors (involved in cell-cell communication) form catch-slip bonds with their ligands, they transmit mechanical signals along a polypeptide sequence, and force induces unfolding of a juxtamembrane MSD, similar to GP1b.

6 Integrin-Mediated Cell Adhesion and Mechanosensing

- Integrins are involved in cellular processes involving adhesion, spreading migration, proliferation, and differentiation. They have a long-known role in force transmission from the ECM across the cell membrane.
- Integrins are heterodimers comprised an α subunit and an β subunit. Each of these subunits has a large head, a long leg, a single pass TMD (transmembrane domain), and a short CT (cytoplasmic tail). The heads and upper legs of the two subunits connect to form the headpiece.
- Resting integrins have a bent conformation with a closed headpiece. When activated, a series of conformational changes happen resulting in extension of the headpiece.
- In inside-out signaling, intracellular activation signals can trigger the integrin to adopt an open conformation. Specifically, talin, kindlin, and other adaptors bind to the βCT to induce a conformational change.
- These steps can also run in reverse in outside-in signaling. They analyze outside-in signaling in their 4 step framework.
- The ECM is a major mechanical environment that is sensed by many integrins. Stiffness of the ECM can affect cell functions.
- Cells can also modify the ECM as a response of mechanosensing. For example, fibrin networks stiffen under deformation.
- Several integrins can form catch-slip bonds (though not $\alpha_{IIb}\beta_3$)
- Integrins can exist in 3 different global conformations:
  1. bent with a closed headpiece,
  2. extended with a closed headpiece, and
  3. extended with an open headpiece.
- Integrin conformation can regulate force transmission across the cell membrane, and force also affects integrin conformation. Tension increases the frequency of unbending, and decreases the frequency of bending.
- Structural analysis and MD simulations suggest a "cytoskeletal force model," where lateral pulling due to retrograde actin flow could pull apart the α and β tails to stabilize the extended-open conformation.
- Integrin binding affinity and conformation are closely related. The bent conformation has a low affinity for the integrin's ligand, and upon activation the hybrid domain swings out, opening the headpiece and allowing for high-affinity binding.
- Opening of the integrin headpiece is allosterically associated with ectodomain extension. However, locking the integrin headpiece in a closed position suppresses high-affinity binding, independent of whether the ectodomain is bent or extended.
- Many studies support this model, however some evidence contradicts it. For example, a high-affinity bent conformation in β_2 integrins has been found, implying that ectodomain extension is not necessarily required for integrin activation.

- Given the relationship between integrin conformation, binding affinity, and force, there may be a regulatory feedback loop in the mechanoreception and mechanotransmission steps of integrin signaling.
- Mechanotransduction involves the recruitment and phosphorylation of kinases. Two examples:
    1. FAK (focal adhesion kinase) is phosphorylated by integrin signaling and regulates Rho-family GTPase activation, which mediates cell migration.
    2. SFKs (Src family of tyrosine kinases) are phosphorylated downstream of integrin activation, and contribute to the formation of integrin-mediated firm adhesion.
- It was previously thought that clustering of integrins was required for induction of these signals, but more recent studies have found that single integrins can produce these signals as well.
- They provide three models of single-integrin mechanotransduction:
    1. Zhu et al. (2008) found that a combination of lateral and tensile forces on the β leg of an extended integrin causes headpiece opening. Therefore it is possible that a pulling force on an integrin which is out of line with its cytoplasmic anchor could cause $\alpha\beta$ separation, which may then open up binding sites on the β CT.
    2. Talin binding to the integrin β CT is the final step of integrin activation. The talin head associates with the CT, and its tail associates with actin, and the sequences in between are susceptible to conformational change under pulling force. In outside-in signaling, force that is transmitted through the β CT can unfold the attached talin, exposing binding sites for vinculin. It has been found that integrin-ligand engagement on a soft substrate fails to extend talin, but on stiff substrates talin is able to unfold before ligand unbinding.
    3. Other intracellular molecules may change conformation or function when force is propagated to the cell interior. For example, vinculin unfolds to expose hidden motifs, Src is activated, myosin translocates on actin, as well as other responses. Vinculin and Src are important in focal adhesion maturation, and myosin translocation is important for cell deformation.
- Organized into the 4-step model, integrin mechanosensing looks like this:
    1. Mechanopresentation: the integrin headpiece binds to its ligand, and force is applied to the bond.
    2. Mechanoreception: the ligand binding site recieves the ligand together with force, and binding kinetics respond accordingly.
    3. Mechanotransmission: force propagates along the integrin, and can unbend the hinge region, and cause hybrid domain swing-out to open the headpiece. Force may propagate onto talin or other cytoplasmic molecules without these integrin conformational changes.
    4. Mechanotransduction: head opening results in $\alpha\beta$ CT separation, exposing binding sites for signaling molecules.

7 Other Cell Mechanosensing Models

- The mechanotransduction step in any model is important, because it converts mechanical signals into biochemical signals.
- This review only addresses monovalent molecular interactions, but multivalent interactions are important as well. In particular, fibrinogen and vWF molecules contain multiple receptor binding sites, and therefore can interact with several receptors at once.
- This complicates things. Association and dissociation of complexed bonds follow multistep kinetic mechanisms involving intermediate steps. The force may be distributed unevenly across different receptors. Additionally, overall bond lifetime is probably prolonged, because once a receptor and ligand unbind, they remain in close proximity to each other.

Summary

This paper presents a general 4-step model of mechanosensing, and then interprets 3 different mechanosensing systems within this model framework. It also summarizes available biophysical probes that allow experimentalists to test different aspects of these mechanosensing systems.

Reference

Chen, Y., Ju, L., Rushdi, M., Ge, C., and Zhu, C. (2017). Receptor-mediated cell mechanosensing. *Molecular Biology of the Cell*, 28(23):3134–3155.

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