* Martinac, B. & Poole, K. Mechanically activated ion channels. *The International Journal of Biochemistry & Cell Biology* **97,** 104–107 (2018).

This review focuses on describing the most important characteristics of mammalian, mechanosensitive channels that generate inward currents under physiological conditions (namely PIEZOs and TRPV4). They highlight that there are no known primary sequence motifs that are common across known mechanosensitive channels, as well as little to no similarity in the tertiary or quaternary structures. They do mention, however, the existence of a secondary structural motif that has been proposed as a distinctive characteristic of some of these channels: a force-coupling helix juxtaposed horizontal to a pore-lining helix via a flexible linker. The authors also review two different proposed mechanisms for gating of this type of channels: the force-from-lipid model (when lipids undergo an extrinsic mechanical stress they pull directly on the protein, causing the gating of the channel) and the force-from-filament model (there are other structural proteins interacting with the channel, and when these get pulled they cause the gating of the channel), acknowledging that these two models are not mutually exclusive. They also stress the fact that the PIEZO proteins are crucial for survival in mice.

* Syeda, R. *et al.* Piezo1 Channels Are Inherently Mechanosensitive. *Cell Reports* **17,** 1739–1746 (2016).

The authors of this paper produce the incorporation of purified PIEZO1 channels in symmetric lipid bilayers and provide evidence that these channels are inherently mechanosensitive by causing their activation through either an osmotic imbalance, the injection of solvent into the droplet lipid monolayer (both of these methods would cause the membrane to stretch), or the use of asymmetric bilayers (that cause the curvature of the membrane to change). With these results they propose that the gating of these proteins can be achieved by mechanical stress in the absence of other cellular components like the cytoskeleton.

* Martinac, B., Buechner, M., Delcour, A. H., Adler, J. & Kung, C. Pressure-sensitive ion channel in Escherichia coli. *Proceedings of the National Academy of Sciences* **84,** 2297–2301 (1987).

Martinac and collaborators present in this paper the first ever report of a mechanosensitive ion channel recorded with the patch clamp technique. They produced giant spheroplasts of *Escherichia coli*, from which they successfully recorded the activity of a pressure-sensitive channel. They propose that these channels could act as sensors for osmotic changes, and that their activity would lead to osmoprotective behavior in bacteria.

* Gustin, M., Zhou, X., Martinac, B. & Kung, C. A mechanosensitive ion channel in the yeast plasma membrane. *Science* **242,** 762–765 (1988).

The authors of this paper present evidence of a mechanosensitive channel found in the yeast *Saccharomyces cerevisiae* plasma membrane. They show that these channels are activated by stretching of the membrane and that they allow the movement of both cations and anions, which might mean they are involved in osmotic regulation in this organism. They also show that the activity of these channels is adaptive, depending on the membrane potential, where more pressure is required to achieve the same open probability after adaptation has occurred.

* Martinac, B. *et al.* Tuning ion channel mechanosensitivity by asymmetry of the transbilayer pressure profile. *Biophysical Reviews* (2018).

This review, focused on bacterial mechanosensitive ion channels, covers the proposed mechanisms for mechanotransduction in these proteins. They review the force-from-lipids principle, a fundamental physico-chemical principle from which the transbilayer pressure profile (TPP) is derived. This TPP is a graphical representation of the interactions of different regions across the width of the lipid bilayer with different regions of the transmembrane protein. Changes in the intensity or directions of these interactions due to a deformation of the membrane (because of stretching, for example) causes a readjustment of the energy equilibrium between the protein and the bilayer, which might bring about the gating of the channel. The results they present here were obtained through computational modelling techniques like Finite Elements and Molecular Dynamics simulations.

* Rosholm, K. R. *et al.* Activation of the mechanosensitive ion channel MscL by mechanical stimulation of supported Droplet-Hydrogel bilayers. *Scientific Reports* **7,** (2017).

In this paper they present a novel technique for the study of mechanosensitive ion channels. This technique, called Droplet Hydrogel Bilayer (DHB), allows for tight control of all bilayer components. Using this methodology, they manage to controllably activate mechanosensitive channels while recording their activity using a patch clamp setup. The DHB method also allows for the visualization of fluorescently labeled mechanosensitive proteins, making it possible to investigate the structure-activity relationship of these proteins at the single-molecule level.

* Bavi, N., Cox, C. D., Perozo, E. & Martinac, B. Toward a structural blueprint for bilayer-mediated channel mechanosensitivity. *Channels* **11,** 91–93 (2017).

In this short review they describe how the conformation of the N-terminal helix of each single subunit of a bacterial mechanosensitive channel MscL is responsible for the gating of the protein. The N-terminus sits close to the solvent-lipid interphase, functioning as an ‘anchor’ during channel gating, acting as an integral force-bearing element, and mechanically coupling the deformation of the pore expansion via a glycine hinge at the inner side of the pore-lining helix. They also present evidence that some membrane lipids protrude into intersubunits cavities and wrap around the upper portions of the N-termini, so lipid movement is actively ‘dragging’ the N-terminus and driving gating. They also report that these lipid-filled protein pockets are present in other non-mechanosensitive channels, like Kv1.2.

* Malcolm, H. R., Blount, P. & Maurer, J. A. The mechanosensitive channel of small conductance (MscS) functions as a Jack-In-The Box. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1848,** 159–166 (2015).

In this paper the authors propose an alternative mechanism for gating of the channel that does not depend on the membrane lipids ‘dragging’ a segment of the protein, but rather the non-stretched lipid bilayer holds the channel in the closed state. Upon application of extrinsic tension, the transbilayer pressure profile changes, allowing the channel to spring into the open state conformation. This mechanism is known as Jack-In-The-Box gating.

* Duque, D., Li, X., Katsov, K. & Schick, M. Molecular theory of hydrophobic mismatch between lipids and peptides. *The Journal of Chemical Physics* **116,** 10478–10484 (2002).

This paper explains the principle of the hydrophobic mismatch. In a very simplified manner, this principle explains the effects of the mismatch between the hydrophobic length of the transmembrane alpha helices of integral proteins (d) and the hydrophobic thickness of the membrane they span (Dh). This is very important because d and Dh are not always equal (so |d-Dh| ≠ 0), which means that sometimes the hydrophilic parts of the bilayer could interact with the hydrophobic parts of the protein, and this is not a stable interaction. This hydrophobic mismatch can cause conformational changes in the protein.

* Perozo, E., Kloda, A., Cortes, D. M. & Martinac, B. Physical principles underlying the transduction of bilayer deformation forces during mechanosensitive channel gating. *Nature Structural Biology* **9,** 696–703 (2002).

The results presented in this paper illustrate the effects of the length of the hydrophobic chain of the lipids on the gating of mechanosensitive channels. They show that when the hydrophobic chain is shorter, there is a leftward shift in the activation curve of the channel, which means that less energy is required to open the channel. Additionally, they show that lipids which incorporate only in one side of the membrane (like lysophosphatidylcholine, LPC), making it asymmetric and changing the intrinsic curvature of the membrane, can cause the channel to be trapped in the fully open state.

Online resources:

* <http://www.protocol-online.org/>

In this website you can find links to descriptions of a huge selection of different basic laboratory protocols. Basically, they direct you to other websites from companies or universities where you can find documentation on the protocol you are looking for.

* <https://www.nature.com/collections/qghhqm>

This collection from Nature has tons of information on statistics (both theory and practice).

* <https://lantsandlaminins.com/writing-guides/>

Here in this website they published very detailed scientific writing and experimental design guides.

* <https://pubchem.ncbi.nlm.nih.gov/>

PubChem is a database of chemical molecules and their activities against biological assays.

* <https://www.ks.uiuc.edu/Research/MscLchannel/>

Basic, summarized information on mechanosensitive channels, mostly focused on computational simulations.

* <https://pdb101.rcsb.org/motm/107>

Protein Data Bank description of mechanosensitive channels. They show really nice pictures of the structure of some of these channels.

* <https://www.mayo.edu/research/labs/cellular-molecular-physiology-gastrointestinal-disorders/research-activities/mechanosensitive-ion-channels>

Example of an application of the study of mechanosensitive ion channels to medicine. They show a video showing this protein’s role in the gastrointestinal tract.

* <https://www.youtube.com/watch?v=9TJ6PP36QCo>

This is a talk in where Dr. Boris Martinac, the first person who recorded mechanosensitive channels using the patch clamp technique, gives a review of about 30 years of research done in these channels. He presents a video showing molecular dynamics simulation of the opening of the channel.

* <https://www.youtube.com/watch?v=gnPglVLBZ2A>

In this video Dr. Frederick Sachs, one of the authorities in the study of mechanosensitive channels (or in electrophysiology as a whole), explains the very basics of single channel recording. He recorded this video in the early 1980s, but it is still applicable today.

* <https://www.youtube.com/watch?v=YaXpX-gveNw>

Second part of the previous video.

* <https://www.youtube.com/watch?v=ahRSOvdGyeU>

BONUS: Third part of Sach’s video.