Bacterial mechanosensitive (MS) channels serve as excellent model systems to study the basic mechanisms underlying bilayer-mediated channel mechanosensitivity. There is extensive evidence showing that these channels are gated by membrane tension according to the force-from-lipids concept. Increasing interest in this paradigm has arisen due to its applicability to eukaryotic channels, in particular mechanically-gated 2-pore domain potassium (K2P) channels and in all likelihood Piezo1. In its simplest form the principle states that the force necessary for channel gating is transmitted directly via the surrounding lipid bilayer. However, while finding a mechanistic explanation to this process is fundamental to our understanding of mechanosensory transduction, the details of how this occurs in individual MS channel families is still the subject of intense debate.

Recently, we looked at the role of the amphipathic N-terminal helix in the gating cycle of the bacterial channel MscL. Although this helix was not resolved in the first crystal structure of MtMscL, its functional role was the subject of much speculation. Initially, it was proposed to form a helical bundle representing a second channel gate. Later on, the N-terminus was resolved and shown to sit close to the solvent-lipid interface. Blount and co-workers then provided evidence using multiple techniques that the N-terminus functions as an ‘anchor’ during channel gating.

Building on this work, we used electron paramagnetic resonance (EPR) spectroscopy combined with a multi-scale computational approach and single-channel recording showing that this helix acts as an integral force-bearing element during activation gating. It mechanically couples bilayer deformation to pore expansion via a glycine hinge at the inner side of the pore-lining helix.

Our data unequivocally confirms the localization of the N-terminal helix at the solvent-lipid interface, a fact further solidified by a crystallographic structure of an archaeal MscL homolog. Interestingly, not only does the N-terminus drive the tilting of the pore-lining helix, but it seems to also drive radial pore expansion. For this to occur, lipids protrude into inter-subunit cavities and essentially wrap around the upper portions of the N-termini; so that during gating the lipid has to be largely stripped from these cavities to enable expansion. However, MD simulation shows that while lipids do move from these cavities, they still strongly interact with a number of the N-terminal residues (Fig. 1). This suggests that lipid movement is actively ‘dragging’ the N-terminus and driving gating.

Lipid-filled pockets equivalent to those identified in MscL are also present in other mechanically-gated channels such as MscS and K2P channels. Indeed, lipid seemed to have such a central position in the initial TRAAK structure, that it was suggested to act as a gate, an unlikely possibility. However, lipid-filled protein pockets are also found in the structures of many non-mechanosensitive membrane-embedded proteins, including the K2P channel TWIK-1 and the
voltage-dependent channel Kv1.2. What then is the mechanistic relevance of these lipid-filled protein pockets in mechanically activated channels?

In the case of MscS, the proposal is that as tension is applied, lipids move from these cavities enabling gating.\(^5\) In this scenario, gating is presumably driven by the stored strain or imposed elastic energy caused by the repulsive forces of the acyl chains. Malcolm et al., have also suggested a similar pressure-profile mediated mechanism.\(^7\) Membrane embedded proteins are subject to large anisotropic forces in the transverse direction,\(^8\) and as we have shown, there is a bilateral relationship between the bilayer pressure-profile and integral membrane proteins (Fig. 1).\(^2\)

In fact, the cavity between TM2 and TM3 in MscS (Fig. 1) houses a number of functionally critical residues (F68, L111, L115).\(^9\) If a model using stored elastic energy were dominant, mutations to these residues should result in a gain-of-function phenotype associated with the lower degree of lipid protrusion. However, hydrophilic substitutions of these residues (i.e. F68S) results in a loss of mechanosensitivity.\(^9\) Given the presence of lipid tails in this region\(^5\) it is more likely that, similar to MscL, some lipid-mediated dragging is necessary for channel gating.

In order to address such questions, we need to look at both the transbilayer pressure-profile (in the presence of protein) and the interactions between the protein and its annular lipids. While our data clearly indicates a “dragging” scenario in MscL, in other cases (e.g., MscS), there may well be a balance between these 2 mechanisms, so that the stored strain energy imposed by the repulsive forces in the acyl chains may act in concert with direct “dragging” of protein elements.

It should also be pointed out that a dragging force applied to an integral membrane protein need not simply cause radial motion. For example, the rotational motion of the TM1/TM2 paddle of MscS under applied force does not preclude “dragging” as its

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![Figure 1](image_url)

**Figure 1.** (A) single MscL subunit in the closed (left) and open (right) state. Each structure has the interaction energy with lipid molecules mapped onto it and the corresponding pressure-profile over-layered. Beneath shows the potential mechanisms that may enable channel mechanosensitivity, either dragging of structural elements or elastic strain energy induced gating (à la jack-in-the-box).
ultimate movement depends on the mechanical properties of the protein and the points at which the protein is anchored to the membrane.

If a “dragging” mechanism is, at least in part, responsible for the mechanosensitivity of MS channels, we would expect to see these horizontal force-coupling helices in other ion channel families, also juxtaposed to the pore-lining helices. Indeed, this is the case at least in K$_{2p}$ channels, where the C-terminal helix seems to be important in mechanical-driven gating. Furthermore, similar structures may play a mechanosensitive role in Piezo and TRP channels (although we should note that the force-from-lipids paradigm is yet to be conclusively shown to apply to any TRP channel). Importantly, the role of these horizontal force-coupling helices should mostly depend on their absorption or adsorption to the bilayer and as such, there is no expectation of high sequence similarity. The only requirement is that they are directly linked to pore-forming helices. Thus, horizontal force-coupling helices may represent an important conserved structural entity that underlies channel mechanosensitivity.

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