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Membrane force reception: mechanosensation in G protein-coupled receptors and tools to address it☆

Katie Hardman[1], Adrian Goldman[2] and Christos Pliotas[1,3,4]

To survive, all organisms must detect and respond to mechanical cues in their environment. Cells are subjected to a plethora of mechanical forces, such as hydrostatic pressure, cell-cell contact, stretch, compression, and shear stress. Mechanosensitive (MS) membrane proteins have evolved across all life kingdoms to sense and respond to forces in the membrane. Bacterial MS ion channels provide a blueprint for understanding the fundamental mechanisms that underpin cellular responses to mechanical signals. Recently, the identification of eukaryotic force transducers, which includes membrane proteins other than channels, has led to the recognition of common structural hallmarks and unified biophysical mechanisms that could potentially link these diverse proteins. Accumulating evidence suggests G protein-coupled receptors (GPCRs) are candidates for pressure sensing in mammals. This review summarises the current knowledge on MS GPCRs, describes the tools used to assess their mechanosensitivity, and aims to highlight the key characteristics that link these receptors to established mechanosensors.

Addresses
[1] Astbury Centre for Structural Molecular Biology, School of Biomedical Sciences, University of Leeds, Leeds, UK
[2] MIBS, Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland
[3] School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic and Health Science Centre, The University of Manchester, UK
[4] Manchester Institute of Biotechnology, The University of Manchester, UK

Corresponding authors: Pliotas, Christos (c.pliotas@leeds.ac.uk, christos.pliotas@manchester.ac.uk)

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Introduction

Mechanotransduction is the process by which mechanical force is converted into intracellular biochemical signals. At the most basic level, mechanotransduction protects prokaryotes from osmotic shock under extreme turgor pressure [1–4]. In animals, it permits not only the sense of touch but also proprioception, hearing, pain, and vascular regulation [4]. Because mechanotransduction is conserved across all life, it is thought to be one of the most primitive sensory transmission pathways [3].

The primary target for mechanical force in cells is the plasma membrane. Membranes are highly dynamic structures, composed mostly of phospholipids, cholesterol, and proteins, which form a protective barrier around the cell. Mechanotransduction, therefore, relies on the presence of membrane-embedded mechanosensitive (MS) macromolecules. Most MS proteins identified to date are ion channels, biomolecules that contain a central pore through which ions and water can cross the semi-impermeable lipid bilayer. Patch clamp electrophysiology studies on the bacterial MS channels of large (MscL) and small (MscS) conductance laid the foundations for our understanding of the biophysical principles underlying mechanotransduction [5,6] and were crucial in establishing a model for mechanosensing in membranes.

Mechanisms for force sensing

It is widely accepted that MS channels in bacteria gate in response to bilayer stretch alone. This observation led to the proposal of the 'force-from-lipids' model, which states that membrane protein conformation and function are governed by protein interactions with surrounding lipids [5,7] (Figure 1). These interactions are influenced by both tension-induced global stretching of the membrane and local distortions caused by membrane curvature [8]. Membrane stretch thins the bilayer and alters the equilibrium of pressure known as the transbilayer pressure profile [9]. Hydrophobic mismatch ensues between the membrane-facing domains of intrinsic proteins and the bilayer, inducing a shift in the protein conformation [8].

In the case of MscS [10,11], MscL [12–14], and the mammalian two-pore domain potassium (K_2P) channels TWIK-related arachidonic acid-stimulated potassium channel

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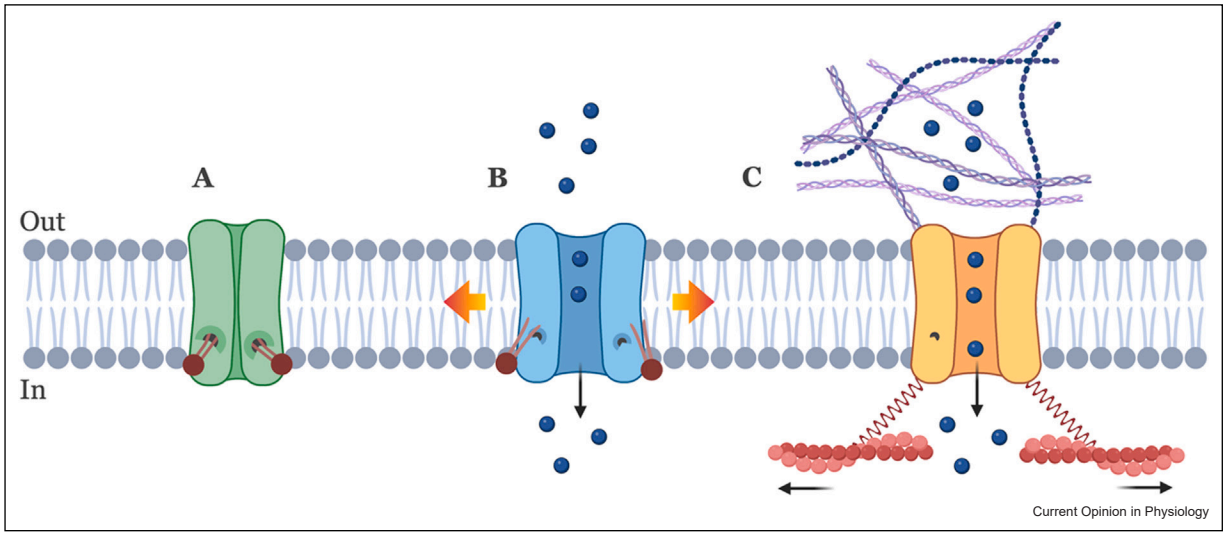
Figure 1



Schematic representation of current hypotheses for mechanosensing in membranes. **(a)** According to the 'lipid-moves-first' model, closed-state integral membrane proteins, such as ion channels and receptors, are stabilised by lipids occupying cytoplasmic-facing pockets. **(b)** The 'force-from-lipids' model encompasses all lipid membrane-driven mechanisms resulting in protein conformational changes in response to pressure in the bilayer. **(c)** The 'force-from-tether' model describes how attachments to auxiliary cellular components, namely the ECM and the cytoskeleton, are involved in conformational changes that lead to ion influx.
Created using BioRender.com, adapted from [30].

(TRAAK) [15,16], TWIK-related potassium channel-1 (TREK-1) [16,17] and TWIK-related potassium channel-2 (TREK-2) [18], dissociation of lipids from hydrophobic pockets under membrane tension appears to drive a conformational change consistent with channel gating [19]. These findings prompted the development of a novel mechanism. Derived from the force-from-lipids principle, the 'lipid-moves-first' model posits that lipids behave as non-specific, low-affinity negative allosteric modulators for integral MS channels [10,12,13,20] (Figure 1). Membrane proteins are so intimately associated with annular lipids that the acyl chains penetrate into transmembrane (TM) cavities. In this model, when membrane stretch increases the total planar area of the bilayer, hydrophobic forces 'pull' lipids out of the pockets and into the bilayer to satisfy the equilibrium position [10,20]. In response, the protein adjusts its conformation to protect the newly exposed hydrophobic residues. When tension decreases, the process is reversed. Indeed, several high-resolution structures of MscS in detergent and membrane mimetics (termed nanodiscs), solved by X-ray crystallography [10] and cryogenic electron microscopy (cryo-EM) [11,21–23], report lipid-like density within the TM pockets. In comparison to the open-state channel, it is clear that these lipid-binding regions significantly reduce in volume when the channel opens [10,24]. Of course, it could be argued that the reverse is true; that when integral membrane proteins rearrange, their associated lipids must reorganise too [7]. However, this model would necessitate strongly bound lipids to act as force transducers, which is not consistent with the requirement for the entropy-driven free exchange of lipids between proteins and the bilayer [10,20]. Further, it is known that MS channels are sensitive to changes in membrane thickness and asymmetry induced by the addition of amphipathic molecules, suggesting that force is transmitted from rather than to the bilayer [5,10,25].

It is not yet clear if these lipid-centric models extend to mechanotransduction in the cells of higher organisms. In the alternate 'tethered' model, force is relayed to channels via additional structures such as the extracellular matrix (ECM), intracellular cytoskeleton, or a combination of both [4] (Figure 1). It was recently suggested that the human MS channels PIEZO1 and PIEZO2 are functionally tethered to the actin cytoskeleton [26]. Although this study is the first to suggest the force-from-tether model in mammalian cells, molecular dynamics simulations and patch clamp electrophysiology experiments performed on the *Drosophila* MS channel NompC suggest that tethers to microtubules allow force to be transmitted to the channel [27]. There is also evidence to advocate for the force-from-lipids model for mammalian mechanotransduction. PIEZO1 in membrane blebs and lipid droplets, which lack auxiliary elements, retains its MS [28,29]. Human TRAAK and TREK-1 respond exquisitely to applied force in excised patches following reconstitution into liposomes [16]. Given the complexity of these systems, a hybrid model with relative contributions from both the membrane and tethers seems to be the most appropriate.

In the last 30 or so years, new classes of human MS proteins have been identified, and advances in structural and functional studies have revealed common characteristics that may point towards a universal mechanism for force sensing. An increasing number of G protein-coupled receptors (GPCRs) have been shown to respond to mechanical stimuli in a ligand-independent manner. This review aims to highlight the role of GPCRs in mechanotransduction in humans and evaluate their position within the mechanosensing paradigm. We will also discuss existing tools and propose new approaches to address mechanosensitivity in GPCRs.

G protein-coupled receptors

GPCRs make up the largest family of membrane proteins in humans, with some 800 members. They recognise a vast array of chemical signals, including most hormones and neurotransmitters, to mediate important physiological processes, including vision, olfaction, and taste [31,32]. Despite huge variability in function, all GPCRs share two defining characteristics. Firstly, their structure is comprised of an extracellular N-terminus, seven TM α-helices (TM1-TM7), connected by three intracellular loops and three extracellular loops, and an intracellular C-terminus [31]. Secondly, they couple to heterotrimeric guanine nucleotide-binding proteins (G proteins), formed from Gα, Gβ and Gγ subunits [31].

One subfamily of receptors, the adhesion GPCRs (aGPCRs), also includes an extended N-terminal extracellular region composed of cell-adhesive protein motifs and a GPCR autoproteolysis-inducing domain (GAIN) [33]. Autoproteolysis dissects the receptor into an extracellular N-terminal fragment (NTF) and a C-terminal fragment (CTF), the latter of which includes the 7TM region. However, not all aGPCRs are thought to be cleavable at the GAIN domain due to the absence of a highly conserved sequence motif within the GPCR proteolysis site [33]. Interestingly, aGPCRs are implicated in mechanotransduction via a tether-like mechanism due to enhanced cell–cell or cel–ECM interactions [34,35]. Examples of putative MS aGPCRs include EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2) Adhesion G protein-coupled receptor E2 (ADGRE2), Cluster of differentiation 97 (CD97) Adhesion G protein-coupled receptor E5 (ADGRE5), G protein-coupled receptor 56 (GPR56) Adhesion G protein-coupled receptor G1 (ADGRG1), GPR114 Adhesion G protein-coupled receptor G5 (ADGRG5), G protein-coupled receptor 126 (GPR126) Adhesion G protein-coupled receptor G6 (ADGRG6), latrophilin-1 Adhesion G protein-coupled receptor L1 (ADGRL1) and Very Large G-protein coupled receptor 1 (VLGR1) Adhesion G protein-coupled receptor V1 (ADGRV1) [34,35]. For this subset of receptors, activation and signalling are triggered by the interaction of a tethered low-affinity peptide agonist (termed the *Stachel)* with the 7TM region [34–36]. Whilst the exact mechanism of MS activation is yet to be elucidated, it's hypothesised that mechanical force causes separation of the NTF and CTF, exposing the *Stachel* to the 7TM binding site, and/or that mechanical perturbation of the N-terminus induces a conformational reorientation of the *Stachel* to an active position [34,35].

For rhodopsin-like class A GPCRs, the initiation of signalling is canonically achieved by ligands interacting with a binding site typically found within the TM helices on the extracellular side of the receptor. The ligand-induced structural rearrangement of the TM region allows the signal to be transmitted across the cell membrane. Agonists shift the receptor energy landscape towards the 'active' receptor conformation so that the receptor spends more of its time in a conformation that can bind transducer proteins, such as G proteins, GPCR kinases, and arrestins, intracellularly [31,32,37]. It is thought that mechanical forces acting on cells can also elicit intracellular signals by inducing a shift in the conformational equilibrium of GPCRs embedded in the plasma membrane.

Mechanosensitive G protein-coupled receptors

The first reported putative MS GPCR was the angiotensin II type-1 receptor (AT_1R), a mediator of cardiac hypertrophy and myogenic vasoconstriction. The mechanical stretch of cardiomyocytes expressing AT_1R in the absence of its endogenous peptide angiotensin II (Ang II) was shown to activate downstream extracellular signal-regulated kinases (ERKs), Janus kinase 2 (Jak2), and inositol phosphates [38,39]. Importantly, stretch-induced receptor activation was inhibited by an AT_1R-selective inverse agonist [38]. This work was supported by Mederos y Schnitzler et al. (2008) [40], who co-expressed AT_1R with transient receptor potential channel-6 (TRPC6), a mechano-insensitive ion channel activated by diacylglycerol (DAG). Osmotic membrane stretch-activated TRPC6-dependent cation currents via AT_1R-mediated phospholipase C activation [40]. The authors carried out similar patch clamp experiments with the histamine H1 receptor (H_1R), type-5 muscarinic acetylcholine receptor and vasopressin 1a receptor to establish a broader role for Gαq/11-coupled GPCRs as MS proteins [40]. The role of Gαq/11-coupling was later challenged by Rakesh et al. (2010) [41], who demonstrated via a DAG reporter assay that the mechanical activation of AT_1R operates through β-arrestin recruitment, independently of G proteins or Ang II.

Most MS GPCRs identified to date have important roles in the vascular function. The smooth muscle cells of small resistance arteries are inherently sensitive to changes in intraluminal pressure and constrict in response to elevated tension [42]. This phenomenon is

known as the Bayliss effect or the myogenic response. *In vivo*, blood flow exerts a frictional force known as shear stress on the endothelial cells (ECs) that line the vessel walls. The identity of specific mechanosensors located in vascular ECs has been a topic of debate for decades. GPCRs are among the list of candidates, which also includes ion channels, junctional complexes, integrins, and the cytoskeleton, all of which are thought to influence the myogenic response [43].

It is possible that the endothelial response to shear stress requires synergistic cooperation between two or more candidates. For example, it has been shown that vasoconstriction mediated by the MS ion channel transient receptor potential vanilloid 4 (TRPV4) is potentiated by several GPCRs, including the type-3 muscarinic acetylcholine receptor (M_3R) in rat arterioles [44] and AT_1R and proteinase-activated receptors (PAR1 and PAR2) in mouse aorta [45]. In another study, it was shown that shear stress in bovine aortic ECs activates PIEZO1, resulting in the release of adrenomedullin, which subsequently activates its Gs-coupled receptor, calcitonin receptor-like receptor CALCRL [46]. Importantly, Xu et al. (2018) [47] demonstrated that shear stress-induced calcium transients mediated by the MS receptor G protein-coupled receptor 68 (GPR68) were present even when PIEZO1 and PIEZO2 were knocked down. The same study showed that PIEZO1-dependent calcium transients were activated by turbulent shear stress but not by smooth laminar flow in Human Embryonic Kidney 293T (HEK293T) cells transfected with mouse Piezo1 [47]. Taken together, these data suggest that both ion channels and GPCRs are important for shear force sensing. Indeed, it is likely that ECs integrate different types and intensities of mechanical input to generate the appropriate biological response. Ion channels, which respond within milliseconds, are likely required in instances of acute or rapid mechanical stress. Conversely, the metabotropic receptors function more slowly and may result in long-term cellular and tissue modifications in response to mechanical signals. Other examples of reported shear stress sensors include the receptors bradykinin B2 [48], dopamine D2 [49] and D5 [50], H_1R [49], adenosine A2A [49], sphingosine 1-phosphate [51], apelin [52] and parathyroid hormone type-1 [53].

Mechanistic insights into mechanosensitive G protein-coupled receptors

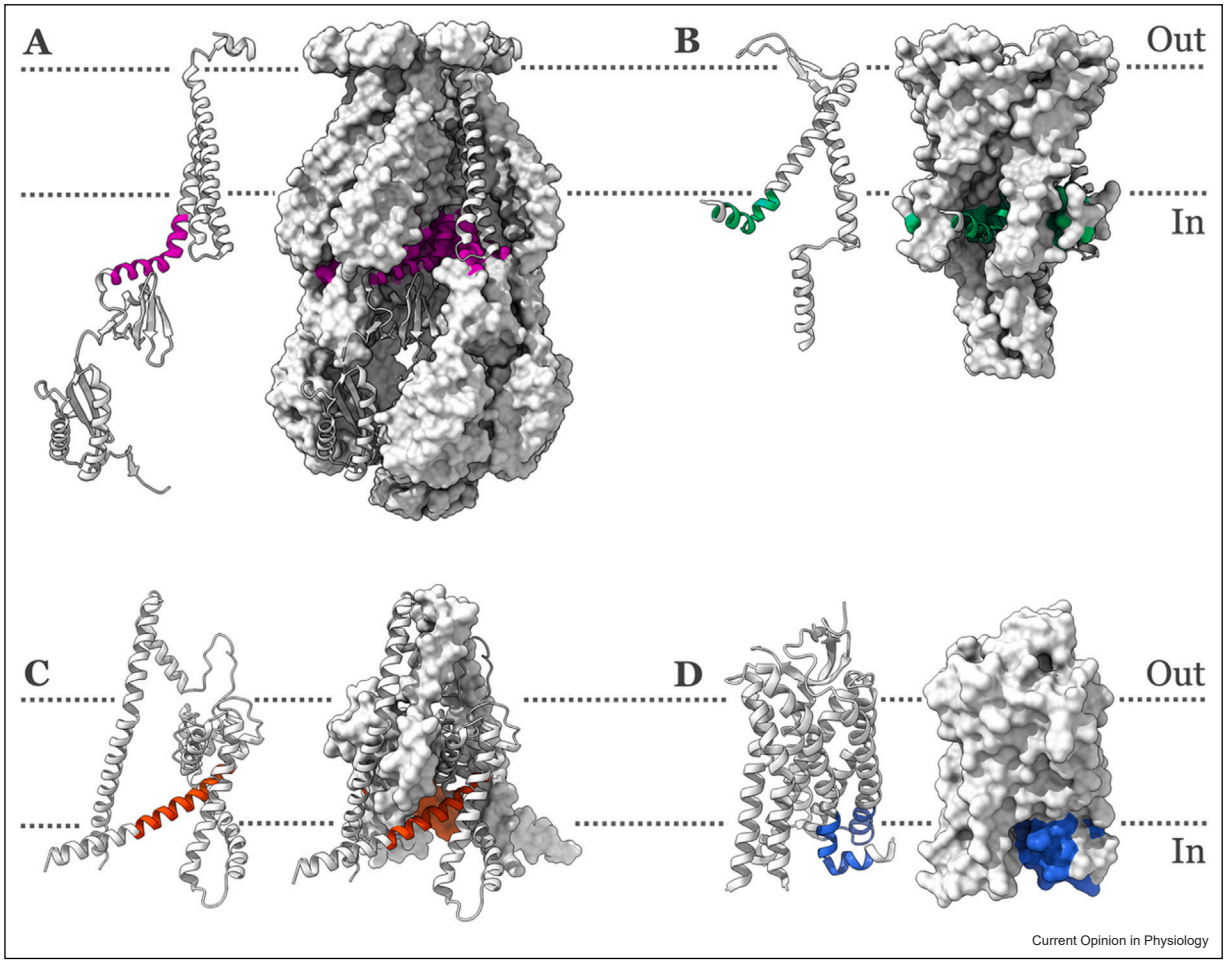
The principal technique for studying MS ion channels is single-channel patch clamp electrophysiology. Since ion transport is not a characteristic feature of receptor function, most studies on MS GPCRs monitor indirect readouts, such as downstream effectors, to infer protein conformational changes. However, a few studies have linked mechanical activation to conformational transitions directly using fluorescence resonance energy transfer (FRET) [48,49,53]. Recently, the structural basis of receptor activation by mechanical force was attributed to helix 8 (H8), a short α-helix found in most receptors located immediately after TM7 [49]. Erdogmus et al. (2019) [49] observed a reduction in FRET signals between fluorophores inserted at the C-terminus and proximal end of H8 in H_1R following shear stress, which the authors suggest was caused by H8 stretch. The removal of H8 from H_1R by C-terminal truncation resulted in insensitivity to mechanical stress, and insertion of the motif into one isoform of the gonadotropin-releasing hormone receptor, which intrinsically lacks H8, was sufficient to confer mechanosensitivity on the previously insensitive receptor [49]. On the other hand, Ozkan et al. (2021) [54] did not observe the suppression of shear stress-induced fluorescent signals upon deletion of H8 from GPR68. However, the force applied in this study (2.6 dyn/cm^2) was much lower and is at the lower end of the physiological range. Arteries are exposed to shear stress of up to 10 dyn/cm^2 and in arterioles, pressure can reach up to 70 dyn/cm^2 [55]. The involvement of the H8 region in mechanosensing is partially supported by an earlier mutagenesis-based study, which demonstrated that mechanical stress causes TM7 in AT_1R to rotate anticlockwise, away from H8, towards the ligand-binding pocket [56].

Interestingly, a tether-like mechanism has also been suggested for the class A GPCR Beta-1 adrenergic receptor (B_2AR) [57,58]. B_2AR is reported to be involved in the pathogenesis of serious systemic infection in humans caused by *Neisseria meningitidis* [57,58]. Marullo and colleagues hypothesise that traction forces exerted by virulence factors (pili) on host receptors initiate a signalling cascade that leads to epithelial lesions and the translocation of the pathogen across the epithelial barrier [57,58]. The glycosylation of two asparagine residues located at the N-terminus of B_2AR is thought to be the molecular determinant of this mechanically-activated mechanism, as the introduction of two N-glycan chains to the N-terminus of AT_1R, which is not sensitive to *N. meningitidis* signalling, produced a robust response *in vitro* [57].

Structural similarities between G protein-coupled receptors and mechanosensitive ion channels

The recognition of common structural features among MS proteins has led to consensus on a potential common underlying mechanism for pressure sensing. Despite vast differences in their global structures, most known MS ion channels have an amphipathic helix positioned approximately parallel to the cytoplasmic membrane [59] [Figure 2]. This helix has no sequence conservation or defined length, yet it has been resolved in the

Figure 2



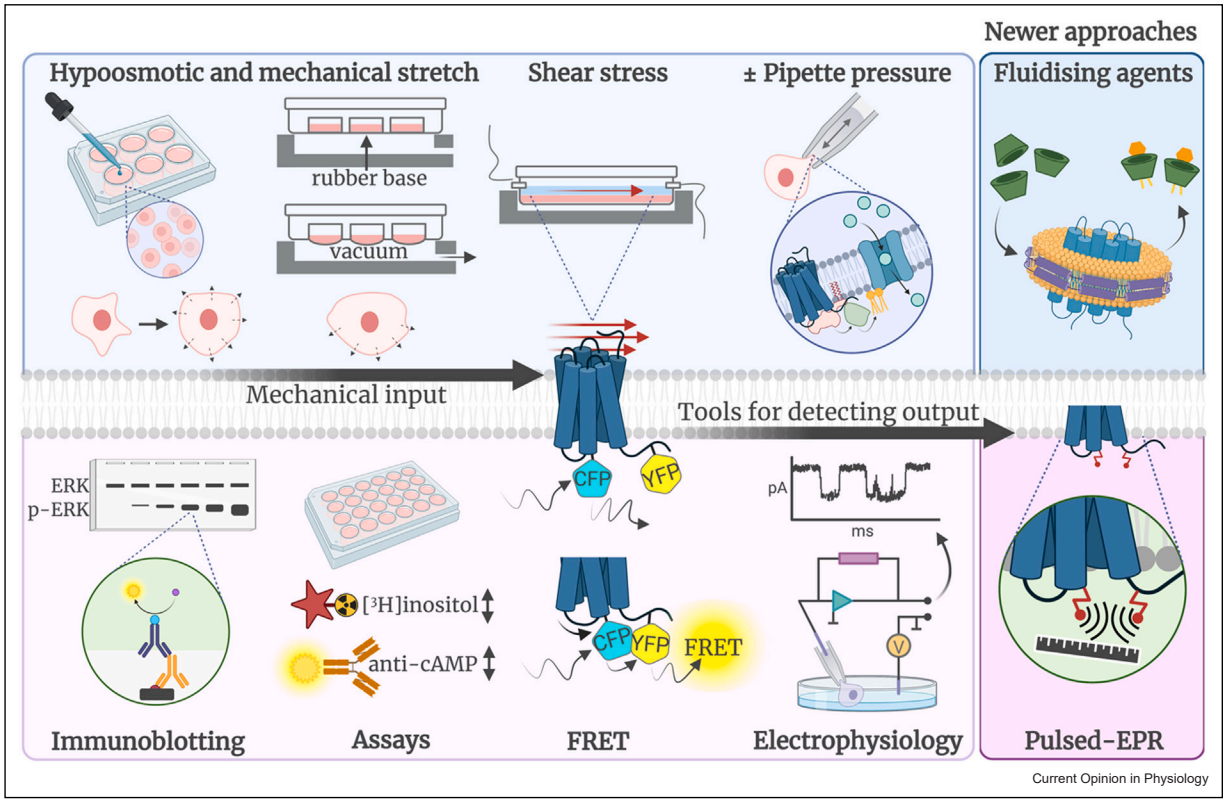
Mechanosensitive membrane proteins feature an amphipathic helix positioned horizontally and in close proximity to the intracellular protein-bilayer interface. The orientation of this helix permits the formation of lipid-binding pockets. **(a)** Homoheptameric ion channel MscS (PDB 6PWP). One subunit is shown in the cartoon form, with the remainder in the surface view to highlight the various cavities formed between subunits and around the amphipathic helix (magenta). **(b)** Homopentameric channel MscL (PDB 2OAR). The pocket-forming region is shown in green. **(c)** Pseudotetrametic dimer TREK-1 (PDB 6W84). A single subunit is shown as a cartoon, with the helix of interest highlighted in orange. **(d)** Monomeric GPCR AT_1R (PDB 6OS0) is depicted in both cartoon and surface view. The terminal end of TM7 and TM1, and the proximal end of H8, are coloured blue.

structures of MscS [10,21,22], MscL [60,61], PIEZO1 [62,63], PIEZO2 [64], TRPV4 [65], TRAAK [15], TREK-1 [17], and TREK-2 [18]. In all cases, the orientation of this helix in relation to the adjacent TM helices allows for the formation of pockets that are responsible for mechanical sensing and response in these channels [10,12,13] [Figure 2]. First identified in MscS [66], hydrophobic pockets located close to the protein-bilayer interface have since been found in many MS ion channels [10–12,16–18,21,22,67,68]. It is interesting that H8 in GPCRs closely resembles the horizonal helices found in MS channels and is also inherently amphipathic [69]. A new study on the so-called 'pocketome' of 557 GPCRs revealed nine newly identified pockets, most of which were located around the TM7 bundle, in close proximity to H8 [70]. The mutagenesis of key residues within these pockets in M_3R and B_2AR significantly affected G-protein activation and β-arrestin recruitment [70]. We hypothesise that the positioning of H8 in GPCRs gives rise to the formation of hydrophobic pockets analogous to those first identified in MS channels [10,12,66, 71,77] and that lipid contacts in this region directly affect the receptor function, as exemplified in MS ion channels [11–13,19,23].

Tools for studying mechanosensitive G protein-coupled receptors

From a structural perspective, monitoring MS ion channels by electrophysiology may also be regarded as an indirect method. Recently, advanced electron paramagnetic resonance (EPR) spectroscopic techniques, such as pulsed electron-electron double resonance

Figure 3



Tools for simulating membrane force in cells or lipid nanodiscs, and methods for detecting mechanical responses. Traditionally, studies on MS GPCRs use hypoosmotic stress, mechanical stretch or shear stress to mimic mechanical forces in whole-cell assays. The biological readout for such assays typically focuses on detecting changes in downstream effectors, such as phosphorylated ERKs, inositol phosphates or cyclic AMP. Electrophysiology may be used if the receptor is functionally tethered to an ion channel. A new approach for simulating membrane tension includes the use of CDs to remove lipids from the bilayer. Detecting structural changes, rather than biological responses, is possible using bioluminescence (e.g. FRET) and pulsed EPR spectroscopy, also known as PELDOR or DEER spectroscopy.
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(PELDOR) spectroscopy, also known as double electron-electron resonance (DEER) spectroscopy, have been used to study integral membrane protein conformation and oligomerisation by enabling accurate distances to be measured between engineered protein spins [12,66,71–75]. PELDOR spectroscopy in combination with site-directed spin-labelling of residues at the entrance to hydrophobic pockets in MscL [12] revealed an expanded state of the channel, comparable to the reported sub-conducting state of *Methanosarcina acetivorans* MscL (MaMscL) [76]. PELDOR spectroscopy was first applied to the structurally-disparate MscS channel and successfully assigned its conformation in native conditions [66,77–80]. Whilst EPR has been useful to monitor MS channel conformation in response to changes in protein-lipid contacts [12,71], spectroscopic techniques have not yet been used to study mechanically-activated proteins by membrane tension application directly. This is because both NMR and EPR require measurements to be performed on purified (and/or reconstituted) protein samples in solution, and it has been challenging to mimic membrane tension outside of an electrophysiology or a cell-based assay set-up. Recently, a new method for simulating membrane tension was reported in the form of membrane-fluidising agents named cyclodextrins (CDs) [11,81] (Figure 3). CDs are truncated cone-shaped molecules formed from cyclic oligosaccharides, which have hydrophobic interiors and hydrophilic exteriors [82] (Figure 3). Their chemical structure means CDs are able to extract lipids from bilayers, altering the hydrophobic force equilibrium, akin to membrane stretch. Following treatment with β-CD, cryo-EM analysis suggested that MscL adopts a flatter, more open-like structure [81] and that MscS alters its conformation [11]. Future studies on MS GPCRs could combine several of the above techniques in order to address mechanical force sensing at a molecular level (Figure 3). GPCRs are typically less than 50 Kilodalton (kDa) in weight, meaning they are relatively small for most cryo-EM applications, despite recent advances

[83,84], and they are notoriously difficult to crystallise [85]. However, PELDOR studies on AT_1R [86] and B_2AR [87] have successfully determined distinct conformations stabilised by 'biased' ligands [86] and resolved previously unknown intermediate conformations formed along the activation pathway [87]. More recently, PELDOR was used to assess the effect of cholesterol analogue binding on B_1AR conformation and dimerisation [88]. PELDOR is not limited by protein size, and it is, therefore, reasonable to suggest this method can be applied to MS GPCRs. The molecular mechanisms behind GPCR force transmission are not yet well understood. CD-treatment of GPCRs reconstituted into lipid bilayers, monitored by pulsed EPR, could be a powerful approach for determining if MS GPCRs operate analogously to other MS proteins and channels.

Conclusions and Future Perspectives

There is convincing evidence to suggest class A GPCRs are capable of sensing membrane tension and relaying force signals across the bilayer to trigger intracellular pathways. However, very little is known about the potential mechanisms required for force transduction in GPCRs. Many studies involving both prokaryotic and eukaryotic MS molecules, such as MscS, MscL, PIEZOs, and K2P ion channels, suggest these proteins conform to the force-from-lipids paradigm, meaning their conformation and thus function is at least partially membrane-lipid driven. Here, we have highlighted the importance of hydrophobic pockets and their lipid availability for force sensing, inspired by recent studies on bacterial mechanosensitive channels. Given that GPCRs, unlike ion-conducting channels, cannot be studied by patch clamp electrophysiology, a platform consisting of pulsed EPR spectroscopic tools could enable the acquisition of interspin distances with Angstrom resolution and reliably reveal the conformational state of GPCRs in native-like membrane environment under certain conditions, such as tension. H8 in GPCRs was previously identified as an important structural motif for MS, and we here hypothesise that the lipid-moves-first model, which stems from the general force-from-lipids principle, may apply to GPCRs. By applying the methods and techniques used to study established MS molecules, we may delineate the mechanisms of MS GPCRs in a comparable manner.

Declaration of Competing Interest

None

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