Interplay between mechanics and signalling in regulating cell fate

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[H1] Abstract

Mechanical signalling affects multiple biological processes during development and in adult organisms, including cell fate transitions, cell migration, morphogenesis, and immune responses. Here, we review recent insights into the mechanisms and functions of two main routes of mechanical signalling: outside-in mechanical signalling, such as mechanosensing of substrate properties or shear stresses; and mechanical signalling regulated by the physical properties of the cell surface itself. We discuss examples of how these two classes of mechanical signalling regulate stem cell function, as well as developmental processes *in vivo.* We also discuss how cell surface mechanics affects intracellular signalling and, in turn, how intracellular signalling controls cell surface mechanics, generating feedbacks into the regulation of mechanosensing. The cooperation between mechanosensing, intracellular signalling and cell surface mechanics has a profound impact on biological processes. We discuss here our understanding of how these three elements interact to regulate stem cell fate and development.

[H1] Introduction

Mechanical forces inside and outside the cell regulate intracellular signalling pathways and crucially impact cell function in many biological processes[1,2]. For example, outside-in mechanical signals such as substrate stiffness influence immune responses[3], wound healing[4], tissue homeostasis[5–7] and cell fate decisions[8–10]. On the other hand, independent of outside-in mechanical signals, intracellular cell signalling and changes in cell function have also been shown to correlate with changes in the shape and mechanical properties of cells[11–15]. Therefore, both cell mechanics and outside-in mechanical signals affect cell and tissue function[16]. Yet, a fully integrated view of how this regulation occurs is missing, particularly in the context of cell fate choice in stem cells and development.

Mechanics regulates signalling at several levels (Figure 1): mechanical signals are sensed at the cell surface (mechanosensing) by transmembrane proteins such as integrin **[G]** -associated complexes or by proteins sensing changes in plasma membrane (PM) mechanics directly; mechanical signals sensed at the surface activate intracellular effectors (mechanotransduction); and those effectors then elicit changes at the level of protein activity and localisation, chromatin, gene expression, etc (mechanoresponse), which regulate specific cellular and biological processes.

Most mechanobiology studies on the control of cell fate and tissue homeostasis focus on a single effector in the mechanotransduction cascade[17,18]. One extensively studied example is YAP/TAZ **[G]**, which acts downstream of mechanosensors including integrin-associated complexes[19], and is essential to maintain homeostasis in various tissues[20]. Another example is the ion channel PIEZO1 **[G]**, which senses forces impinging on the PM, and has a role in cell fate transitions and tissue homeostasis[6,17]. Crucially, however, studies that focus on single effectors such as YAP/TAZ and PIEZO1 might not provide a comprehensive view and understanding of how mechanical forces and mechanical signalling guide stem cell fate transitions and development. Cell function and cell fate transitions are ultimately coordinated by intracellular signalling pathways such as WNT, transforming growth factor-β (TGFβ)-family signals, fibroblast growth factor (FGF), and Notch. All these instructive signalling pathways are mechanosensitive, but little is known about how they are coupled to

mechanical forces and regulated in response to these forces, or when this regulation is functionally important. Recent in vitro and in vivo studies have provided insights into how mechanical inputs regulate cell function and identity. For instance, recently it was found that a build-up of mechanical strain in the oesophagus of developing mouse embryos led to a switch from tissue expansion to the establishment of adult homeostasis[21]. This study exemplifies the impact of forces on stem cell fate. Like other similar studies connecting mechanics to cell fate choice, it also leaves open a great many questions about exactly how mechanics regulates the signalling pathways involved in cell fate regulation. Understanding the input of mechanics into developing and homeostatic tissue, as well as disease, will lead to new insights into how stem cell function is regulated and how it can be utilised to control cell fate for therapeutic uses.

In this Review, we discuss our current understanding of the mechanoregulation of intracellular signalling that controls cell fate choice (Figure 1) and provide an integrated view of cell mechanics and signalling. We first provide examples whereby outside-in signalling regulates cell identity and tissue homeostasis in vivo. We then focus on how outside-in mechanical signals and cell surface mechanics, and in particular plasma membrane tension, act through combined pathways and multiple feedbacks to regulate cell fate transitions, with a focus on stem cells and development.

[H1] Outside-in mechanical signalling in vivo

Outside-in mechanical signals comprise any force that comes from outside the cell that provokes a cellular response. These signals can be produced, for example, by shear stresses, tissue stretching and compression, and substrate mechanics.

There are myriad examples of outside-in mechanical signalling regulating cell fate in vivo, during development and in the maintenance of tissue homeostasis[22] . For example, in the developing alveolar epithelium, the movement of amniotic fluid caused by foetal breathing produces shear stresses on alveolar epithelial cells that activate ERK and drive a transition in cell state from progenitor to alveolar epithelial type I cells[23]. Shear stresses were also

implicated in establishing hematopoietic stem cells in mouse development[24]. Yet another source of mechanical stress in tissues is the stretching or compression of cells. Along these lines, insertion of an inflatable hydrogel into mouse skin was used to show that skin stretch leads to a transient elevation of proliferation in epidermal stem cells coupled to a subsequent increase in differentiation, thus leading to tissue expansion. The response to stretch was mediated by myosin phosphorylation and formin **[G]** -like proteins, which modulate tension in the actin cytoskeleton. Ablation of these proteins disrupted mechanosensing in adherens junctions, and YAP/TAZ and MAL signalling downstream of that mechanosensing. Thus, mechanical sensing in the actin network directly contributed to the observed enhanced self-renewal in this study[25]. Another study in the mouse epidermis showed that local cell crowding led to a decrease in cortical tension and increased differential cell adhesion, leading to cell delamination **[G]** and differentiation[26]. Moreover, at the surface of *Xenopus laevis* embryonic aggregates formed from gastrulation-stage ectodermal- cells when epithelial tissue architecture was disrupted to trigger a regenerative response, stiffness of the aggregate increased. This increase in tissue stiffness coincided with YAP nuclear localisation in mesenchymal cells that then underwent a transition to re-establish the epithelium[27].

It is noteworthy that the study of mechanical signalling *in vivo* is a relatively new research field, and detailed mechanistic insight into the mechanoregulation of intracellular signalling pathways that instruct cell fate transitions in tissues remains elusive. Mechanical signalling has been more extensively studied in cultured cells, and what has been learned from those model systems should guide future investigations in the mechanoregulation of development, organogenesis and homeostasis.

[H1] Outside-in mechanical sensing in stem cells

Given clear indications that forces regulate fate transitions in vivo, it is essential to have reduced biological systems such as cultured cells to dissect detailed mechanisms of fate mechanoregulation. In cultured stem cells, a number of studies have shown how outside-in mechanical signalling such as substrate sensing or applied mechanical stress can regulate signalling and cell fate (Figure 2).

[H2] Function of substrate mechanical properties and sensing in cell fate choice.

Mesenchymal stem cells (MSCs) are multipotent stem cells that, in culture, can be directed towards osteogenic, adipogenic or chondrogenic lineages dependent on culture conditions and cell density[28]. MSCs have been the model system of choice used in many of the pioneering studies of substrate sensing in cell fate choice. A seminal study on how substrate stiffness affects MSCs[29] found that osteogenic markers increased when cells were cultured on stiff substrates mimicking bone stiffness, whereas neurogenic markers were upregulated on soft substrates mimicking brain stiffness. The study focused on the expression of lineage markers rather than MSC differentiation into different lineages; nevertheless, it inspired the idea, now paradigmatic, that it is essential to control the mechanical properties of the microenvironment in order to precisely control the fate of stem cells[22].

It has since been confirmed that MSCs can be mechanically guided towards osteogenic (on stiff substrates) or adipogenic (on soft substrates) lineages[30]. It was found that increased MSC spreading and actomyosin contractility induced on stiff substrates leads to increased ERK and WNT signalling[31], which are both important for osteogenic differentiation (Figure

2A). Controlling MSC spreading was sufficient to alter cell fate choices[12]. As substrate stiffness affects cell spreading, this raised the question of whether the effect of substrate stiffness on MSC differentiation was direct or indirect. The idea that spreading and shape were important was challenged by the observation that there was no difference in MSC shape between soft and stiff 3D matrices, but still MSCs preferentially differentiated into osteocytes in stiffer 3D matrices, whereas in soft 3D matrices they differentiated into adipocytes[32]. This study also identified a molecular basis of mechanosensing, finding that osteogenic lineage choice depended on traction forces exerted on α_v and α_5 integrin-associated complexes, thus highlighting the importance of molecular scale forces on adhesion complexes in mediating cell fate.

MSC fate can also be strongly influenced by ECM degradability, independently of substrate stiffness. In a non-degradable 3D matrix, MSCs differentiated into adipocytes, whereas in an equally stiff but degradable 3D matrix, MSCs exhibited more traction forces and underwent osteogenic differentiation[33]. It was later emphasized that MSCs must be able to remodel their environment[34] and secrete nascent ECM in order to promote osteogenic differentiation[35].

The effects of ECM remodelling and degradability on cell differentiation highlight a crucial point about studies of substrate stiffness: it is important not only how stiff substrates are, but also how they are functionalised to control tethering of proteins (such as ECM) to the substrate[36,37]. Many substrate sensing studies are performed on polyacrylamide hydrogels **[G]**, which are inert so they must be functionalised by a cross-linker such as sulfo-SANPAH to bind ECM to the hydrogel substrate. However, these cross-linking factors are typically

bound to hydrogels via non-specific interactions, and are thus not usually stable functionalizing agents. Indeed, when mouse embryonic fibroblasts were plated on sulfo-SANPAH functionalized polyacrylamide hydrogels, the ECM was entirely removed from the hydrogels within 1 day of culture[38]. Given the importance of ECM degradability in stem cell fate transitions, it is plausible that the stability of ECM tethering to a substrate is a very important variable in regulating substrate mechanosensing, at least for some stem cell systems. Furthermore, the particular ligand, and which integrin receptors are expressed and active, almost certainly determines how stem cells perceive mechanical signals from the substrate (reviewed in Ref.[19]).

Therefore, while substrate stiffness is clearly important in the regulation of cell fate and function, it must be considered alongside other properties, including dimensionality, ECM degradability, and ability of cells to remodel ECM (Figure 3A). Importantly, these factors are not wholly independent. It is reasonable to speculate, particularly *in vivo,* that mechanoregulation of cell fate depends on which cell receptors are active and how well a cell can control its microenvironment. The level of control a cell has would be influenced by biophysical properties of the ECM such as degradability and plasticity, in addition to its stiffness. Understanding how ECM composition and biophysical properties cooperate to influence intracellular signalling and cell fate is an important area of future investigation.

Many of the findings about substrate sensing in MSCs probably apply to other stem cell systems. There are indeed numerous lines of evidence indicating that substrate sensing regulates pluripotent stem cells (PSCs). A recent study in which both substrate adhesion and mechanics where precisely manipulated confirmed that soft substrates enhance self-

renewal in mouse PSCs, but substrate stiffness had less effect in the maintenance of human PSCs[38]. In another study, culturing and differentiating mouse PSCs towards definitive endoderm was highly dependent on traction forces. There, fibroblast-derived ECM (primarily comprising the ECM component fibronectin) was used along with a fibronectin force sensor to show that decreasing traction forces and contractility led to a decrease in TGFβ signalling[39] (Figure 2A). TGFβ signalling is essential for definitive endoderm specification; thus decreasing traction forces on fibronectin interfered with definitive endoderm specification. Furthermore, on substrates containing both fibronectin and laminin (an ECM component widespread in tissues), when $\alpha_5\beta_1$-integrins (which preferentially bind fibronectin) were blocked, the cells adhered preferentially to laminin **[G]** instead of fibronectin. The increased engagement with laminin promoted definitive endoderm specification. On the other hand, blocking $\alpha_3\beta_1$-integrins, which preferably bind to laminin, led to increased fibronectin engagement and interfered with TGFβ signalling and definitive endoderm specification[39]. These data suggest that TGFβ signalling, which is important for many stem cell fate decisions, is sensitive both to traction forces and to which type of integrins those traction forces are imposed upon.

[H2] Effects of actively applied mechanical stresses. Actively applied mechanical stresses include, for example, fluid shear stress, or any actively applied force that results in a shape change in the cell. Actively applied mechanical stresses have often been implicated in cell identity transitions. For example, when fluid shear stress was applied to mouse embryonic stem cells, β-catenin translocated from adherens junctions to the nucleus, leading to enhanced expression of pluripotency markers[40]. MSCs are also sensitive to fluid shear stress, with high shear stress contributing to osteogenic fate transitions dependent on MAPK/ERK

and Ca^{2+} signalling[41,42]. Also, fluid shear stresses facilitates the differentiation of human PSCs into endothelial cells (reviewed in Ref.[43]). Indeed, tissue engineered vascular grafts derived from human induced PSCs have shown great promise when fluid shear stress is incorporated for the derivation of endothelial cells[44]. In endothelial cells affected by fluid shear stress, G-protein coupled receptors (GPCRs) are often implicated[45]. The role of GPCRs has been studied in various stem cell types such as PSCs, MSCs, iPSCs, Neural stem cells and others (reviewed in Ref.[46]). However, the potential role of GPCR-mediated mechanotransduction in cell fate choice has been, to our knowledge, largely overlooked. However, given the demonstrated mechanosensitivity of GPCRs in a number of different cell types[47,48], stem cell models could provide key future insights for a more general understanding of mechanical signalling incorporating GPCRs.

Stem cells have also been used to study the effects of actively applied stretching. Stretching forces are pulling forces exerted onto the cell by neighbouring cells and/or the ECM and occur frequently in tissues such as the skin, the lung, and heart (as investigated recently in[25]). For example, applied stretching modulated lineage choice in MSCs and adipose derived stem cells, with applied stretch promoting osteogenesis[49,50]. Other examples of how actively applied mechanical signals regulate stem cell fate choice are reviewed in Ref[22]. A few studies have connected stretching to intracellular signalling. For example, mouse pluripotent stem cells became sensitive to stretching forces only once they began differentiating, with stretch-regulated cell state depending on ERK signalling[51].

The effects of compressive stresses, which can for example occur in development due to cell crowding during organogenesis, have not been widely studied in stem cell systems.

However, compressive forces have been shown in other systems to have a profound impact on signals important for stem cell function, and tissue crowding has been shown to impact cell fate transitions *in vivo*[52]. Thus, it is likely that both stretching and compressive forces have a crucial impact on cell fate transitions.

[H2] Effectors of outside-in mechanical signalling. Historically, most studies on the role of substrate properties and mechanisms of substrate sensing in determining cell fate have choice have focused on the involvement of a specific effector. For example, YAP/TAZ-mediated mechanotransduction was found to play an important role in the regulation of MSC differentiation. When cultured on stiff substrates, osteogenic differentiation of YAP-depleted MSCs was impaired, whereas adipogenic differentiation was enhanced[53,54]. Another example is the ion channel PIEZO1 **[G]**, which senses forces impinging on the PM, and has a role in cell fate transitions and tissue homeostasis[6,17]. A recent study found that PIEZO1 played an important role in age-related decline of oligodendrocyte progenitor cell (OPC) function. Ageing correlated with increased brain stiffness, and this increased stiffness caused OPCs to proliferate and differentiate less efficiently. However, this effect was reversible: exposing aged OPCs to a soft substrate mimicking the stiffness of the young brain was sufficient to rejuvenate the cells. OPC ageing could also be reversed by inhibiting PIEZO1[6]. This study demonstrated that mechanosensing is an important regulator of biological processes. Indeed, making cells insensitive to outside mechanics (by blocking the relay of mechanical signals by inhibiting PIEZO1 function) changed the function of cells. Further work will be needed to elucidate how mechanical signalling works in healthy physiological conditions to instruct cell fate and homeostasis. Taken together, while some

key mechanosensing mediators have been investigated, a holistic understanding of mechanical signalling remains elusive.

Notably, outside-in mechanical signalling intrinsically initiates with mechanosensors such as cell adhesions; therefore, a deeper understanding of how mechanical signalling works requires understanding how mechanosensors perceive and shape outside forces (Figure 3).

[H1] Role of mechanosensors in regulating signalling

Mechanosensors are cell surface proteins and receptors that act as the gatekeepers for mechanical signals, sensing and mediating signals to tune downstream effects. The two most widely studied, and arguably most important, mechanosensors or outside-in mechanical signal mediators are cell-cell adhesions and cell-ECM adhesions, which determine how cells respond to other cells and to ECM in their environment.

[H2] Cell-ECM adhesion complexes are linked to instructive signalling.

Receptors involved in cell-ECM adhesion complexes are among the most important factors mediating and regulating signalling downstream of cell substrate sensing (Figure 3A). We will focus here primarily on integrin-associated adhesion complexes, as they are the most studied, though other receptors such as syndecans **[G]** are also important for mechanosensing (reviewed in[55]).

Integrin-ECM binding affinity is strengthened by pulling forces from the cytoskeleton[56] and is regulated by binding of adaptors to the integrin cytoplasmic tail, which is connected to

actin filaments (reviewed in Refs.[19,57,58]) (Figure 3A). This 'molecular clutch' mechanism, in analogy to linkage of shafts in mechanical engines, regulates downstream signalling[19]. The mechanoresponse of integrin-associated adhesion complexes also depends on the type of integrins involved[59,60]. For example, β_1-integrins are important to the function of spermatogonial[61] and epidermal stem cells (integrins in stem cells were reviewed in Ref.[62]). It is very likely that certain types of integrin receptors are essential to regulate stem cell function and differentiation, but there is not yet an 'adhesome' for particular stem cell types, and a detailed understanding of how integrin-mediated (or syndecan) mechanical signalling is transduced to direct stem cell fate is missing.

Paxilin and FAK have been highly implicated in mediating mechanical signalling from adhesion complexes[63,64] (Figure 3A). We focus on FAK, because it binds to integrin-associated adhesion complexes and can also bind to the plasma membrane[65,66]. FAK is autoinhibited[67], but this autoinhibition can be relieved first by binding via its FERM domain to the PM and then by force application leading to full exposure of the catalytic site[68]. The mechano-activated catalytic site is a binding domain for molecules with an SH2 domain **[G]** , such as phospholipase C-γ[69], Src and other Src-family kinases (reviewed in Ref.[70]), which all play important roles in regulating intracellular signalling. It has been proposed that the binding between molecules such as Src and FAK leads to increased activity of each[71,72]. Furthermore, the cooperative activity of FAK and Src also positively regulates paxillin activity, which also plays an important role in regulating downstream signalling pathways[73] (reviewed in Ref.[74]).

Given that Src and other Src-family kinases are activated by receptor tyrosine kinase signalling, and FAK is activated by force, the Src-FAK interaction is potentially an important node in the cross-talk between mechanical and instructive intracellular signalling for stem cells. Indeed, it is known that FAK-Src activity regulates the turnover of focal adhesions **[G]** [75] and other cell-ECM adhesions[76,77]. This regulation plays a role in cell migration, but it also could regulate cell surface mechanics and downstream intracellular signalling pathways (discussed below). One signalling pathway directly regulated by FAK and important for stem cell function and tissue homeostasis[78,79] is PI3K/Akt signalling. FAK activity is also associated with ERK activation (for example in adherent fibroblasts[80], and reviewed in Ref.[81]), which is important for a number of biological processes including pluripotent cell fate transitions[82,83].

[H2] Cell-cell adhesion complexes that mediate instructive signalling. Substrate mechanosensing can promote (on soft substrates) or inhibit (on stiff substrates) cell-cell adhesion, and therefore affect cell function[30,84]. Cell-cell adhesions primarily comprise cadherin-catenin complexes[85], which include α-catenin, β-catenin, p120catenin and cadherins (e.g. E-cadherin) (Figure 3B). Analogous to cell-ECM adhesion complexes, cell-cell adhesion complexes are co-regulated with the actomyosin cytoskeleton, and have been shown to be directly mechanosensitive[86] and recruit other molecular partners[87–89]. The clearest route for cell-cell adhesions to regulate intracellular signalling and cell fate transitions would be through one such partner, β-catenin which directly couples WNT receptors to gene expression (Figure 2B). Indeed, in human pluripotent cells, feedback between substrate stiffness and cell-cell adhesion was observed and linked to β-catenin-regulated Wnt signalling[9]. Human PSCs have high levels of E-cadherin and β-catenin when cultured on soft as opposed to stiff gels, leading to increased cell-cell adhesion. On stiff gels,

GSK3β was activated and β-catenin was degraded, leading to a reduction in Wnt signalling, though a mechanism by which GSK3β was activated was not shown. On soft gels, on which hPSCs accumulated β-catenin at the PM, morphogens were added to the media to activate Src-family kinases. With Src activation, β-catenin was liberated from the PM and Wnt signalling was upregulated (Figure 2B). Similar mechanisms were later found in a human gastrulation model[90]. Moreover, it has been proposed that cell-cell adhesions can act as a 'membrane trap' for β-catenin, and cell-cell adhesion disruption promoting β-catenin release for Wnt signalling[91]. However, the degradation of β-catenin by GSK3β, particularly if it is also mechanosensitive, should be considered a potentially important mediator in the trade-off between β-catenin's role in Wnt signalling and cell-cell adhesion.

It is challenging to disentangle the feedbacks between cell-cell adhesion, β-catenin, Wnt signalling and mechanosensitivity, because β-catenin/Wnt signalling has been shown to be mechanosensitive in different contexts (including cancer[92]), and there is extensive cross-talk with other signals that are sensitive to cell-ECM adhesion and mechanics, such as TGFβ[93]. Furthermore, the trade-off between increased cell-cell adhesion (generally favoured on soft substrates) and increased cell-ECM adhesion (generally promoted by stiff substrates) that has been observed in many cell types is probably important in the control of cell signalling and cell fate. For example, inhibition of cell-ECM regulated signalling in a tissue could promote cell-cell adhesion mediated pathways, including Wnt and Notch[10]. Both of these mechanosensitive signalling pathways are paramount in stem cells and homeostasis[94–96]. Understanding the situations in which either cell-cell or cell-substrate adhesion is favoured, as well as the feedbacks and co-regulation between adhesion types is an important area of future research.

[H1] Cell surface mechanics regulating signalling

In addition to outside-in mechanical signalling, mechanosensing and mechanical signalling can be regulated by changes in cell surface mechanics. Cell surface mechanics refers to the mechanical properties of the plasma membrane, the underlying actin cytoskeleton, and the connections between the two (Box 1). Most examples of mechanosensing through cell surface mechanics involve a downstream effect following changes in mechanical tension and topology of the plasma membrane (Figure 4), but the organisation and possibly mechanical properties of the actin networks underling the PM may also contribute to cellular mechanosensing.

[H2] Sensing and signalling by mechanosensitive ion channels.

The PM has long been known to be involved in mechanosensitive cellular responses through stretch-activated ion channels (reviewed in Ref.[97]). Such mechanosensitive ion channels are membrane-bound proteins that undergo a conformational change in response to an increase in local forces. These forces could result from an in-plane membrane tension increase, changes in membrane curvature, or forces exerted by sub-membranous cytoskeletal networks and/or the cell substrate on the channel[98–100] (Figure 4A).

Mechanosensitive ion channels play key roles in regulating development and cell fate[101,102]. This is notably the case for PIEZO1, which in resting condition is in a closed conformation with the pore filled with lipids; when in-plane membrane tension is increased, the channel changes conformation resulting in pore-widening and displacement of the lipids outside from the pore region. This change leads to an influx of Ca^{2+} that regulates cell behaviour[103,104]. For example, in MDCK epithelial cell layers, stretching leads to Piezo1 recruitment from the cytoplasm to the membrane, followed by channel opening and activation, and enhanced cell division. As crowding in the epithelia increased following enhanced divisions, PIEZO1 accumulated in cytoplasmic aggregates, leading to mechanically-induced extrusion **[G]** of the cells[17]. PIEZO1 regulated mechanosensing was also implicated in developmental axonal pathfinding following gradients in substrate stiffness[105]. Finally, as mentioned above it was demonstrated in aged mice that cell-specific

in vivo depletion of PIEZO1 rejuvenated aged OPCs and led to enhanced remyelination **[G]**. Intriguingly, this same *in vivo* OPC-specific depletion of PIEZO1 in neonatal mice led to overgrowth of OPCs, suggesting that PIEZO1 mechanosensing can play an important role in regulating cell numbers during developmental, yet can become deleterious during ageing[6].

[H2] Signalling controlled through membrane-tension-mediated endocytosis.

Endocytosis is another key process regulating signalling, involved in sensing cell surface mechanics (Figure 4B). Cells use endocytosis to internalize cargo of different size and purpose through various endocytic pathways, all requiring local changes in membrane curvature to allow invagination[106]. A role for PM tension in controlling endocytosis has been demonstrated over two decades ago[107]. Indeed, endocytic vesicle formation requires deformation of the PM; it is thus hindered by bending rigidity and tension of the plasma membrane[108]. As such, PM mechanics is a key regulator of endocytosis-related signalling (reviewed in Ref.[109]). For example, clathrin-mediated endocytosis, which is driven by the multi-step formation of clathrin-coated pits (reviewed in Ref.[110]), inversely correlates with membrane tension across various cell lines and organisms[111,112]. In mammalian cells, clathrin-mediated endocytosis partially overcomes high membrane tension by triggering local actin polymerisation to facilitate membrane deformation even when membrane tension is high[113]. This reduces the mechanosensitivity of clathrin mediated endocytosis to high membrane tension[110]. Other endocytic pathways also respond to PM tension. For example, the clathrin-independent CLIC/GEEC endocytic pathway **[G]**, which is an actin-dependent endocytosis pathway characterised by a lack of a protein coat and a lack of dynamin dependence[114,115], is rapidly upregulated upon decrease in membrane tension[116,117] (Figure 4B).

Endocytosis can negatively regulate signalling pathways by increasing signalling factors recycling and thus limiting their activity[118]. For example, it was shown that during Drosophila epithelial morphogenesis, the activation of the GPCR Smog is differentially regulated between the ectoderm and the mesoderm by endocytosis-based recycling[119]. This process was not directly linked to mechanics; however Smog regulates Rho1 and Myosin II levels[120] that control actomyosin organisation and contractility. Thus, mechanical feedbacks

are likely. It was also proposed that during gastrulation in *Drosophila melanogaster* mechanical deformations due to cellular contractions lead to decreased endocytosis of the secreted signalling protein Fog, thus enhancing Fog-dependent signalling. This in turn further promotes apical contractions and mesoderm invagination[121]. Similarly, in muscle progenitors, mild hypoosmotic treatment has been shown to inhibit BMB2 endocytosis, presumably because of increased membrane tension, resulting in BMB2-mediated trans-differentiation towards osteoblasts[122].

Endocytosis can also positively regulate signalling pathways that require the recruitment of signalling co-factors at the endosome (reviewed in Ref.[123])(Fig. 3b). In a recent study using mouse PSCs, membrane tension was found to decrease during early differentiation, leading to enhanced endocytosis. This increase in endocytosis promoted FGF receptor internalisation and ERK activation, driving early differentiation[124]. ERK signalling has also recently been shown to be activated through endocytosis of EGF receptors in carcinoma, leading to unjamming and collective migration[125] (Figure 2C). To what extent membrane tension-gated activation of endocytic signalling plays a role in fate transitions *in vivo* is an exciting open question in the field.

[H2] Sensing membrane tension and mechanoprotection through caveolae.

Caveolae are cup-shaped membrane microdomain invaginations 60-80 nm in diameter, comprising various proteins (e.g. Cav1), which interact with cholesterol, sphingolipids, and the actin cytoskeleton (Reviewed in Refs.[126,127]). Caveolae are mechanosensitive: upon cell stretching and/or increase in membrane tension, caveolae flatten and disassemble[128,129] (Figure 4C). Caveolae flattening affects lipid trafficking, turnover and ordering, which can in turn impact mechanical properties of the PM (Box 1) (reviewed in Ref.[130]). In cultured cells, flattening of caveolae has an effect of 'mechanoprotection' , as it buffers rapid increases in membrane surface occurring during cell stretching, thus preventing membrane disruption[128].

Several recent studies support a role for caveolae mechanoprotection in development. In zebrafish during notochord development, caveolae buffer mechanical tension exerted on the vacuolated cells that form the centre of the developing notochord[131,132]. Introducing

mutations for various caveolar components led to collapse of vacuolated cells resulting from physical stresses arising from larvae locomotion. Thus, caveolae appear to play a mechanical safeguarding role during spine development[131,132]. Mechanoprotection by caveolae might be particularly relevant for cells experiencing high mechanical strains such as muscle cells[133]. Along these lines, expression of caveolae components is particularly high in skeletal muscle and endothelial cells[134], which are exposed to high mechanical stress[133].

Caveolin-1 expression has been shown to be tightly linked with cell differentiation, and can regulate various pathways involved in stem cell fate (reviewed in Ref.[135]). For example, it was shown that knockout of *Cav1* in mouse (which results in total loss of caveolae) leads to overactive MEK/ERK signalling. This led to a loss of E-cadherin, and subsequent disruption of mechanical coupling between cells, and a loss of epithelial structure[136]. Furthermore, *cav1* depletion leads to higher osteogenic potential in MSCs, causing abnormal bone formation rate[137]. It remains to be investigated whether these signalling functions of caveolin-1 are related to mechanosensing, and to what extent membrane-tension controlled assembly and disassembly of caveolae modulates signalling in development and stem cells. Interestingly, Caveolae are also highly involved in regulating Wnt[138] and TGFβ signalling[139], two of the most important signalling pathways for stem cell regulation.

[H2] Membrane curvature and curvature sensing proteins.

Cells can sense and respond not only to the mechanical tension in the PM, but also to its curvature. Though not strictly speaking a mechanical property, membrane topology is related to membrane tension, as membrane folds and reservoirs can buffer membrane tension[140], and high membrane tension in turn can limit membrane fold formation[141]. Curvature sensing proteins detect and selectively bind inward or outward curved regions of the PM and activate a variety of downstream signalling pathways (reviewed in Refs[142,143]; Figure 4D). Changes in PM curvature are regulated by membrane compression (which can lead to the formation of membrane reservoirs of different shapes), changes in the in-plane tension of the membrane, changes in effective membrane tension, and external topological cues[144]. A high concentration of some curvature-sensing proteins (notably BAR-domain proteins) can also promote PM deformation (Figure 4D). This is because BAR proteins are often curved and, by binding to the membrane, they can impose their intrinsic shape to the

lipid bilayer through electrostatic interactions[145]. Sensing and generation of curvature may thus be concomitant. In addition, BAR domains have been suggested to generate highly stable and specific lipid microdomains, thus potentially impacting lipid ordering[146] (Box 1).

Membrane curvature sensing proteins have been shown to affect a variety of processes in development. High expression of the N-BAR protein amphiphysin in Zebrafish primordial germ cells resulted in an increase in stabilised membrane invaginations, presumably leading to membrane tension increase, limiting the formation of membrane blebs **[G]**. This limitation interfered with the bleb-based migration of primordial germ cells[141,147], resulting in failures to reach the gonads. In *D. melanogaster*, membrane sensing proteins are involved in epithelial morphogenesis. A member of the F-BAR family, Cdc-42 interacting protein 4 (Cip4), was shown to regulate wing epithelium morphogenesis through mediation of E-cadherin endocytosis[148]. Another study highlighted a role for the F-BAR protein syndapin in *D. melanogaster* photoreceptor development, where curvature-mediated recruitment of syndapin to the base of apical microvilli facilitates formation of the rhabdomere **[G]**. Furthermore, the I-BAR-containing IRSp53 protein controls epithelial lumen formation during renal tubule development in zebrafish and mouse[149].

Membrane curvature sensing also affects neuronal development. For instance, Cip4 inhibited neurite formation by promoting lamellipodial protrusions at the expense of neurites[150]. Cip4 levels drastically decreased during mouse neocortex development, indicating that it functions primarily in early development[150]. Another curvature sensing protein, ArhGAP44, was shown to regulate neuronal network formation[151]. Recruitment of ArhGAP44 to nanoscale membrane deformations generated by actomyosin pulling forces at nascent dendritic branches inhibited the formation of exploratory filopodia from existing neurites. Interestingly, ArhGAP43 expression increases in rat neurons during development, suggesting that the curvature sensing mechanism described above could help reduce exploratory branching and transition towards a more static network characteristic of nervous system maturation[151].

Membrane curvature also affects stem cell differentiation. Culturing various types of stem cells on substrates with nanotopographic features, such as nanogrooves or nanopillars,

affects stem cell differentiation (reviewed in[152]). Nanotopographic structures have been thought to mostly affect cell fate through modulation of substrate adhesions and adhesion-mediated signalling; however, recent studies highlight that the high membrane curvature locally induced by such features can also control signalling more directly, for instance through enhanced endocytosis or recruitment of curvature sensing proteins (reviewed in Ref.[153]).

[H2] Actin networks and mechanical signalling.

The organisation and mechanics of the actin networks that underlie the PM also play an important, though often indirect role in cellular mechanosensing. Actin mediated forces have also been proposed to facilitate the opening of stress-activated ion channels, though exactly when such a "force-from-filament" mechanism dominates over "force-from-lipid" gating remains debated[99]. As discussed above, local actin polymerisation can also facilitate endocytosis and has been proposed to directly counteract the resistance of the PM to deformation when PM tension is high. Finally, actin can stabilise membrane reservoirs, such as microvilli, which regulate membrane availability, and can thus affect effective PM tension (reviewed in[13]). Submembraneous actin dynamics can thus regulate PM behaviour during cellular deformations. For instance, unfolding of actin supported dynamic microvilli helps providing the membrane required for the formation of cell-cell boundaries during Drosophila embryo cellularisation[15].

Importantly, changes in actin network organisation, and resulting mechanics, can also elicit mechanosensing responses directly. For instance it has been suggested that alongside cell-ECM adhesions and cell-cell adhesions, actin mechanics is involved in regulating YAP/TAZ signalling (reviewed in Ref.[155]). Changes in actin organisation can also regulate MRTF/SRF signalling, which has been implicated in regulating cell fate, for example during cardiomyocyte differentiation in development or myofibroblast differentiation during wound healing[156,157]. How exactly the mechanics and dynamics of the actin cytoskeleton affect cell fate in stem cells and development is an important question for future studies.

[H1] Feedbacks between signalling and mechanosensing

All the mechanosensing processes we have discussed potentially affect one another. For example, BAR-domain proteins can positively regulate clathrin-mediated endocytosis and are part of the CLIC-GEEC endocytic machinery[114, 158]. Clathrin-mediated endocytosis in turn, has been linked to cell substrate mechanosensing through regulation of key adhesion factors (e.g. Integrins and E-cadherin) via recycling . On a higher level, in addition to feedbacks between individual mechanosensors, outside-in mechanical signalling, regulation of cell surface mechanics, and intracellular signalling activity are inextricably intertwined, and the feedbacks between them likely all-encompassing. Specifically, mechanosensing regulates intracellular signalling, which changes the organisation and mechanical properties of the actin cytoskeketon and the PM, which in turn regulates mechanosensing (Figure 5).

For example, FAK activity, which is triggered by mechanical signals from the substrate, can promote actin- and clathrin-mediated endocytosis and mediate, for example, FGF/ERK signalling[159,160], which regulates the cytoskeketon and subsequent mechanosensing. Another example is a complex containing all the machinery of clathrin-mediated endocytosis, but is not subject to internalisation[161,162], termed clathrin-coated lattices or plaques. These plaques depend on the presence of $\alpha_v\beta_5$-integrins[162], are highly stable and their presence has been shown to correlate with substrate stiffness, indicating that they are mechanosensitive. They have also been shown to regulate intracellular signalling: when EGF receptors were stimulated by EGF within clathrin-coated plaques in HeLa cells, the receptors were not internalised, thus suggesting that the presence of clathrin-coated plaques can regulate EGF signalling[161], which in turn regulates cytoskeletal machinery.

Further examples of feedbacks between mechanosensors and signalling include: cell adhesions modulate Rho GTPase activity[57] and, as a result, cytoskeleton and membrane mechanics (Box 2); cell-ECM adhesions activate Akt/mTOR signalling, which regulates membrane mechanics[163,164]; and mechanosensitive ion channels gate intracellular calcium levels, which modulate actomyosin contractility and membrane tension[165,166]. Furthermore, caveolae have been proposed to regulate overall PM lipid composition (and thus membrane mechanics)[167]. Caveolae are thought to act as storage compartments for liquid-ordered signalling nanodomains, and could thus mediate access to those nanodomains in response to mechanical stress[130]. Interestingly, in both mammalian cells and zebrafish, the expression

of key caveolae components is regulated by YAP/TAZ, which is in turn activated through caveolae upon cell exposure to shear-stress, suggesting feedbacks between caveolae regulation and caveolae-mediated signalling[168]. Indeed, studying the interplay between mechanics and endocytic signalling is an important future direction to better understand cell fate choice.

[H1] Conclusions and perspectives

Recent studies on the mechanical properties of the plasma membrane and extracellular environment and how these properties affect cell behaviour have provided key insights into how mechanical signals are sensed, transduced and integrated to regulate signalling pathways that control stem cell function and activity (see Supplementary Table 1 for a summary of pathways affected). It has become clear that specific mechanosensing processes are subject to regulatory feedback. Moreover, mechanosensors are regulated, to varying degrees, by the organisation of intracellular actin networks and their interactions with the plasma membrane. There are numerous levels of feedback between the mechanosensors themselves, the signalling pathways they control, and cell surface mechanics. Furthermore, other cellular components involved in mechanotransduction and mechanoresponse such as the nucleus (reviewed in Ref.[169]), provide further levels of feedback. Indeed, mechanosensors and signalling pathways operate simultaneously and in a coordinated fashion in most biological contexts.

Though much of the focus on mechanical signalling has been on relay signals, the mechano-regulation of stem cell function is largely determined by the spatiotemporal interactions between mechanical signals such as ECM mechanics, cell surface mechanics, and receptors and protein complexes at the cell surface. Altogether, there is an emerging need for a holistic view integrating outside-in mechanical signalling with cell surface mechanics, incorporating their co-regulation of intracellular signalling (Figure 5).

Notably, most of our current understanding of cellular mechanosensing mechanisms stems from single cell studies, where multiple tools exist for the measurement and perturbation of cell and substrate mechanics. Recently there have been developments in methods allowing for the measurement and perturbation of cell mechanics in complex multicellular

environments. For example, a membrane tension dependent lipid packing probe[170], called FlipTR, was recently developed, enabling measurements of membrane mechanics *in situ* such as in the early mouse embryo[171]. Recently-developed techniques to measure cell-level mechanical stress in a tissue context, from the deformations of embedded hydrogel or oil droplets are now providing insights into temporal and spatial changes in tissue mechanics and mechanical signals during development and organogenesis which were inaccessible with traditional mechanical measurements [172,173]. Nevertheless, force measurements and acute mechanical perturbations in tissues (reviewed in Refs.[174,175]) still lag behind methods developed in single cells in vitro. Accordingly, there is a growing need for new approaches and tools with high spatiotemporal resolution for the dynamic measurement and perturbation of cell and substrate mechanics in the context of multicellular aggregates and tissues, which will enable us to further understand the role of mechanics in vivo, in healthy and diseased tissues.

Together, to fully understand the role of mechanical signalling in disease, cell fate choice and tissue homeostasis will require new technical approaches and new paradigms, with studies that do not focus only on individual mechanosensors or specific relay signals, but address the intricate feedback loops they comprise, the underlying signalling networks, and how they work together as a whole.

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Box 1: Mechanical properties of the plasma membrane and underlying actin networks with mechanosensing roles.

[b1] Plasma membrane mechanics

The PM comprises a lipid bilayer as well as numerous transmembrane and membrane-bound proteins, and is generally tightly attached to the underlying actin cytoskeleton. Furthermore, in most cells, the PM is not stretched out but is locally folded in a variety of stabilised or dynamic reservoirs (reviewed in[13,176]). As such, PM mechanical properties are considerably more complex than those of a lipid vesicle.

[b2] Effective membrane tension

The physical parameter generally used to describe the mechanics of the PM is "effective membrane tension"[108,177,178]. Effective membrane tension (hereafter membrane tension) describes the resistance of the PM to deformation. It is thus mechanically akin to a surface tension and is measured in surface tension units (generally pN/μm). The in-plane surface tension of the PM lipid bilayer contributes to membrane tension, but membrane tension is thought to be primarily determined by membrane-to-cortex attachment, which is mediated by proteins that bind lipids or transmembrane proteins to the underlying actin cortex[179]. The most studied membrane-to-cortex attachment regulator is the ezrin-radixin-moesin (ERM) protein family, which directly affects membrane tension in a variety of systems, from cultured cells to cells extracted from developing mouse and fish embryos[124,180,181]. Other membrane-to-cortex attachment mediators include Myosin 1 motors and filamin[179].

Most common methods to measure membrane tension rely on measuring the force required to pull a membrane tether from the cell with either optical tweezers or an atomic force microscope[177]. Membrane tension has also been measured from fluctuations of the PM, however this method works best when fluctuations are large and the PM is not strongly attached to the underlying cytoskeleton (conditions of low membrane-to-cortex attachment), such as in cellular blebs, where the membrane transiently detaches from the actin cortex[182], or in erythrocytes, where the cytoskeletal scaffold underlying the PM is less dense than in other cell types[183]. Recently, several FRET probes have been developed to

map membrane tension or related properties in live cells[170,184], however these report on local lipid packing and in-plane PM tension, rather than membrane-to-cortex attachment and effective membrane tension.

[b2] Local versus global - membrane tension propagation

Historically, PM tension has generally been considered to equilibrate rapidly[185,186], due to the fluid nature of the PM. However, a recent study using a dual tether pulling assay, where one optical tweezer is used to measure tether force and one to perturb membrane tension generating pulling forces on the membrane, has shown that changes in membrane tension only propagate slowly within the PM, and can thus result in local activation of membrane-tension-controlled signalling[98]. How the time scales and length scales of propagation of membrane tension changes are regulated, and to what extent they are cell type dependent, remains to be investigated (discussed in Refs.[178,187]).

[b2] Lipid packing and ordering

The level of packing/ordering of lipids in the PM influences membrane mechanics[188], and local modulation of lipid ordering has long been proposed to result in the formation of signalling platforms at the PM[189,190]. Interestingly, changes in membrane tension (from compressive, tensile, and shear stresses) have been shown to lead to changes in lipid ordering and packing. For example, an increase in membrane tension resulting from tensile stress has been speculated to increase lipid ordering[170]. Thus changes in membrane tension might directly regulate the formation and disruption of signalling platforms at the PM (reviewed in[189]). Importantly, in addition to the nature of the lipids themselves and the tension of the membrane, the actin cytoskeleton underlying the PM has been suggested to facilitate membrane compartmentalization and to tune local lipid ordering[191].

[b1] Actin structures underlying the plasma membrane

While membrane tension is a measure of the resistance of the PM to deformation, cell shape and cellular deformations are primarily controlled by the cytoskeletal networks underlying the PM[192–194]. These networks are often classified, depending on their composition and organization, as the actin cortex, stress fibres, lamellipodia, and filopodia.

Lamellipodia and filopodia comprise mostly myosin-free actin meshworks that push on the PM generating protrusions, and have both been involved in substrate stiffness mechanosensing via focal contacts and adhesions[195,196]. Stress fibres are contractile bundles of actin filaments and myosin motors. They often connect, and exert pulling forces on, focal contacts, leading to focal contacts growth, and as such, play a key role in mechanosensing of substrate stiffness[197–199]. Finally, the cellular cortex is often defined as the dense contractile actomyosin meshwork that supports the PM in cellular regions that are not spread on a substrate, as well as at the apical surface of epithelial cells (reviewed in Refs.[200,201]). Cortical myosin motors put the cortical actin network under contractile tension.

Like membrane tension, cortical tension is a surface tension and is generally reported in pN/µm; it can be directly measured through whole cell compression with a flat AFM cantilever or through cell aspiration into a micropipette[200]. Cortical tension is typically much higher than membrane tension[13] and accounts for most of the surface tension of a rounded cell. Cortical tension promotes rounded cell shapes, and tension gradients drive cellular shape changes, e.g. during cytokinesis, cell rear contraction in migrating cells, or epithelial constrictions in tissue morphogenesis[200]. The cortex can display mechanosensing behaviour of its own, in that it can accumulate in response to mechanical load and locally contract against applied forces[202]. The cortex is also tightly attached to the PM and, as such, can participate in membrane-based mechanosensing. For instance, cortex remodelling is believed to be important for endocytosis, and could thus directly affect signalling[203]. How exactly cortex and PM are mechanically integrated remains largely an open question.

Box 2: Key mechanical signalling feedbacks

[b1] RhoA and Rac1

The small GTPases RhoA and Rac1 are generally considered master regulators of cellular actin organisation, mechanics and, as a result, cell shape[204]. Rac1-GTP promotes spread morphologies by enhancing lamellipodia extension through activation of the Arp2/3 complex via its regulator WAVE[205]. In contrast, RhoA-GTP tends to promote rounded, cortex-dominated cell shapes, through activation of myosin II activity downstream of Rho-

kinase; for instance, RhoA activation is one of the key drivers of mitotic rounding[206]. RhoA also plays a role in spread cells, where it enhances the formation of contractile stress fibres and FAs[207]. Furthermore, RhoA can directly affect actin assembly via activation of the formin mDia1 and disassembly via Lim kinase mediated phosphorylation of cofilin[208,209]. RhoA and Rac1 can antagonize each other through multiple pathways[210], and how balanced activation is achieved for the dynamic control of specific cell shapes remains an active research field[211,212].

ERMs, a key membrane-to-cortex attachment regulator (Box 1), are reciprocally regulated by RhoA and Rac1. Indeed, RhoA activates ROCK which triggers phosphorylation of ERMs and thus increases effective membrane tension by increasing membrane to cortex attachment[213]. Rac1 on the other hand has been shown to lead to ERM dephosphorylation via activation and interaction of the kinase Pak1 and the phosphatase PP2A which lead to a decrease in membrane-to-cortex attachment and, as a result, membrane tension[214,215].

[b1] Phosphatidylinositol phosphates

Phosphatidylinositol 4,5-bisphosphate (PIP_2) and phosphatidylinositol 3,4,5-trisphosphate (PIP_3) constitute another group of essential regulators of cellular mechanics. For example, PIP_2 is an important regulator of membrane tension, as ERM proteins and Myosin Is (and especially the isoform myosin 1C) bind PIP_2 at the membrane to generate membrane-to-cortex attachment[216,217].

PIP_2 and PIP_3 are also important mediators of mechanical signalling. PIP_2 is hydrolysed by Phospholipase C, and the products of this reaction are inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG), which are both critical second messengers that facilitate intracellular and mechanical signalling. PIP_2 phosphorylation into PIP_3 by PI 3-kinases (PI3K) leads to downstream activation of AKT/mTOR signalling. Important feedbacks also exist between PIP_2/PIP_3 and Rho-GTPase signalling[218,219]. PIP_3 activates Rac-GTP and promotes actin polymerization through WAVE and Arp2/3, whereas PTEN (a downstream effector of ROCK) de-phosphorylates PIP_3[220].

Figure 1: Forces inside and outside the cell regulate cell function. Cells sense and respond to various mechanical inputs (a), both from the extracellular environment and from the cell itself. Such stimuli may result in the activation of cell surface mechanosensors (b), which regulate intracellular signalling pathways (c) and affect protein activity/localisation (d) ultimately regulating biological processes. Some of the key factors in cell surface mechanosensing include: cell adhesion complexes such as focal adhesions (mediating cell–extracellular matrix (ECM) adhesion) and cadherin-catenin junctions (mediating cell–cell adhesion); and mechanosensitive complexes which respond to alterations in cell surface mechanics such as endocytic vesicles and curvature sensing proteins (for example, caveolae). Other key regulators of cell surface mechanics include the actomyosin cortex, membrane-to-cortex attachment complexes, and membrane composition. FAK, focal adhesion kinase; PI3K, phopshoinisitide-3 kinase; RTKs, receptor tyrosine kinases.

Figure 2: Examples of cell fate regulation by mechanical signalling. (a) Examples of substrate mechanosensing regulating cell fate. Mouse PSCs differentiation toward definitive endoderm has been shown to be highly dependent on high traction force and cortical contractility leading to TGF beta activation[39]. In MSCs, high traction force on stiff substrates has been shown to lead to ERK & Wnt activation leading to Osteogenic differentiation[31]. (b) Example of how mechanosensing at cell-cell adhesion can instruct cell fate. Human PSCs cultured on soft substrate have high cell-cell adhesions leading to β-catenin accumulation at cell-cell junctions. Morphogen-induced Src activation leads to β-catenin release from cell-cell junction & activation of Wnt which promotes mesoderm differentiation[9]. (c) Example of changes in cell surface mechanics instructing fate decisions. When mouse PSCs differentiate, they display a decrease in membrane tension which leads to an increase in endocytosis of FGF signalling components, leading to ERK activation and initiation of differentiation[124].

Figure 3: Mechanical signalling gated through cell adhesions. Mechanosensing of the extracellular environment is mainly achieved by adhesion complexes at the cell–extracellular matrix (ECM) interface and cell–cell interface. a) Cell-ECM adhesion is in part mediated by focal contact complexes which are mechanosensitive and will, upon tensile activation, undergo conformational changes that lead to the recruitment of adaptor

proteins (such as vinculin and paxilin) and downstream signalling (for example MAPK-ERK[80,81] and PI3K-AKT[78,79]). Forces from the Cell-ECM adhesion complex are transmitted through the cytoskeleton/cytoplasm to the nucleus and can lead to further downstream signalling (for example by triggering YAP/TAZ translocation). In addition to substrate stiffness, degradability, ability to remodel and dimensionality of the ECM are important parameters modulating cell-ECM mechanosensing. b) Cell–cell adhesion complexes, for example cadherin–catenin complexes, regulate mechanical coupling and force transmission across cells. P120 Cadherin prevents cadherin endocytosis and regulates the activity of various cytoskeleton regulators such as RhoA. Cadherin–catenin complexes are also mechanosensitive: the balance of forces between the cytoskeleton and cadherin–catenin complexes results in conformational changes, stabilising cell–cell adhesion, recruiting signalling co-factors such as Merlin (which can activate the hippo pathway when released from cell-cell junctions) and regulating downstream signalling pathways such as WNT[9,91]and Notch[10]) when under tension.

Figure 4: Mechanical signalling gated through the plasma membrane. Examples of mechanosensing gated through cell surface mechanics, which act downstream of the mechanical tension and topology of the plasma membrane.

a) mechanosensitive ion channels, such as Piezo1 and TRAAK, are major factors in responding to change in cell surface mechanics by selectively allowing the flux of specific ions, such as calcium or potassium, to regulate intracellular signalling in response to a mechanical stimulus. Calcium is implicated in a multitude of signalling pathways and is involved in many major cell functions[102,103].

b) Trafficking machinery plays an important role in cell surface mechanosensing. Endocytosis is a major regulator of signalling and various cell functions. Both clathrin-dependent endocytosis and clathrin-independent endocytic pathways (CLIC/GEEC)[115,116] are negatively regulated by effective increased membrane tension. Under high membrane tension, Clathrin endocytosis can partially overcome high membrane tension by triggering local actin polymerisation to facilitate membrane deformation[113].Trafficking rates affect a wide range of pathways downstream signalling pathways[122] and as such, changes in effective membrane tension result in changes in signalling. High membrane tension leads to

low levels of endocytic recycling of membrane receptors allowing for higher signalling of some receptors at the plasma[11]. Lower membrane tension will on the other hand lead to increased endocytosis and lower signalling at the plasma membrane while increasing intracellular signalling, such as for epidermal growth factor (EGF) receptor signalling and MAPK/ERK[124,125].

c) Caveolae are cup shaped membrane deformation that can flatten as a result of increased membrane tension or cell stretching. This flattening, in addition to providing mechanical protection against membrane rupture[127,128], releases regulatory proteins such as EHD2 and Cavin1 which can then translocate to the nucleus and regulate transcription [221].

d) Cell surface mechanosensing is achieved via proteins sensing specific regions or curvature of the membrane, such as BAR domain proteins. These proteins regulate the actin cytoskeleton, play important roles in endocytosis and regulate various signalling pathways[141,142]. Moreover, high local concentration of curvature sensing proteins can lead to local membrane deformation which likely contribute to their role of modulating endocytosis[144] . Changes in membrane topology and membrane tension affect the binding of the proteins to the membrane and consequently their signalling properties.

Figure 5: Interrelationship and feedbacks between mechanosensors and cell surface mechanics. The interconnected network formed by mechanosensors, cell signalling and cell surface mechanics, and the feedback between them controls cell function and has a significant impact on biological processes. Indeed, activation of mechanosensors at the cell surface in response to a mechanical stimulus (e.g., increase in endocytosis as a result of decrease in membrane tension) (I) results in the regulation of specific signalling pathways (e.g., MAPK/ERK activation) (II), which in turn affects cell surface mechanics modulation of key molecular factors (III). Examples include how MAPK/ERK activation leads to regulation of cell adhesions, PI3K activation alters effective membrane tension, and Rho GTPase signalling regulates cortical tension and organisation. The changes in cell surface which then, in turn, feed back into the regulation of mechanosensors.

Glossary

Integrins: Transmembrane receptors mediating cell adhesions such as cell-extracellular matrix adhesion.

YAP/TAZ: YAP/TAZ are proteins whose function and localisation, either cytoplasmic or nuclear, are regulated by mechanical cues.

PIEZO1: Transmembrane mechanosensitive ion channels which are actuated by an increase in membrane tension.

Formin: Proteins that are actin polymerisation regulators.

Cell delamination: The process by which cells detach from their original tissue.

Hydrogels: Three-dimensional, water-retaining network of polymers.

Laminin: Extracellular matrix proteins which are found in most tissues and organs.

Syndecans: Heavily glycosylated transmembrane proteins interacting with various ligands, often acting as coreceptors of many different proteins including, for example, GPCRs.

SH2 domain: Protein domain interacting with phosphorylated Tyrosine present on other proteins.

Focal adhesions: Large protein complex that mechanically links the actin cytoskeleton and the extracellular matrix.

Extrusion: Mechanically induced increase in actomyosin contraction leading to the cell squeezing itself out of the tissue, thus preventing overcrowding.

Myelination & Remyelination: Production of layers of myelin, which help electric transmission of electric action potential, around neuronal axons.

CLIC/GEEC: Clathrin-independent endocytic pathway, involved in pinocytosis and large bulk membrane uptakes.

Blebs: Spherical cellular protrusions driven by hydrostatic pressure and cytoplasmic flows.

Rhabdomere: A phosphosensory structure consisting of a microvilli bundle.

ToC

Mechanical signalling underlies multiple, fundamental biological processes. Mechanical signals can originate from substrate physical properties or shear stresses, and from changes in the physical properties of the cell surface. The mechanisms underlying these two classes of outside-in signalling and their roles in the regulation of intracellular signalling, cell fate and development are becoming increasingly understood.

Fig 1

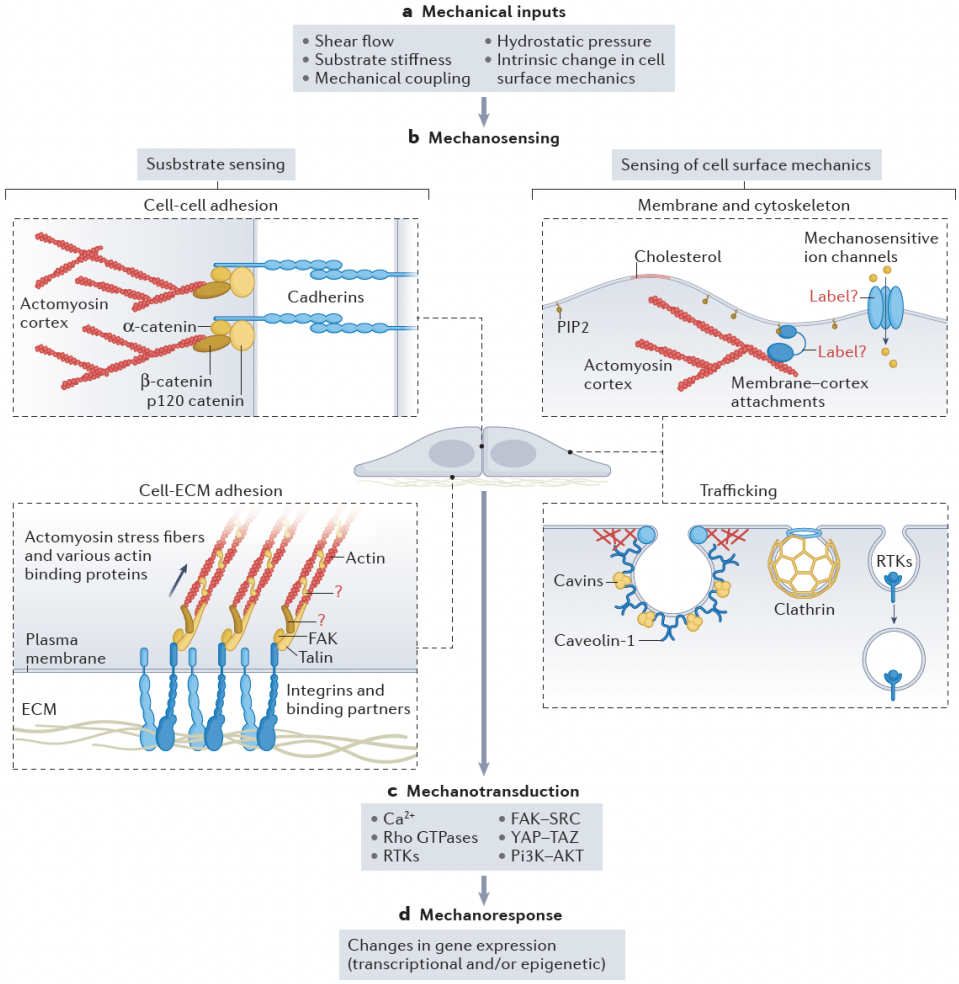

a Mechanical inputs
• Shear flow
• Substrate stiffness
• Mechanical coupling
• Hydrostatic pressure
• Intrinsic change in cell surface mechanics
b Mechanosensing
Susbstrate sensing
Sensing of cell surface mechanics
Cell-cell adhesion
Membrane and cytoskeleton
Actomyosin cortex
α-catenin
Cadherins
β-catenin
p120 catenin
Cholesterol
Mechanosensitive ion channels
Label?
PIP2
Label?
Actomyosin cortex
Membrane–cortex attachments
Cell-ECM adhesion
Trafficking
Actomyosin stress fibers and various actin binding proteins
Actin
?
?
FAK
Talin
Plasma membrane
ECM
Integrins and binding partners
Cavins
Clathrin
RTKs
Caveolin-1
c Mechanotransduction
• Ca2+
• Rho GTPases
• RTKs
• FAK–SRC
• YAP–TAZ
• Pi3K–AKT
d Mechanoresponse
Changes in gene expression (transcriptional and/or epigenetic)

Fig 2

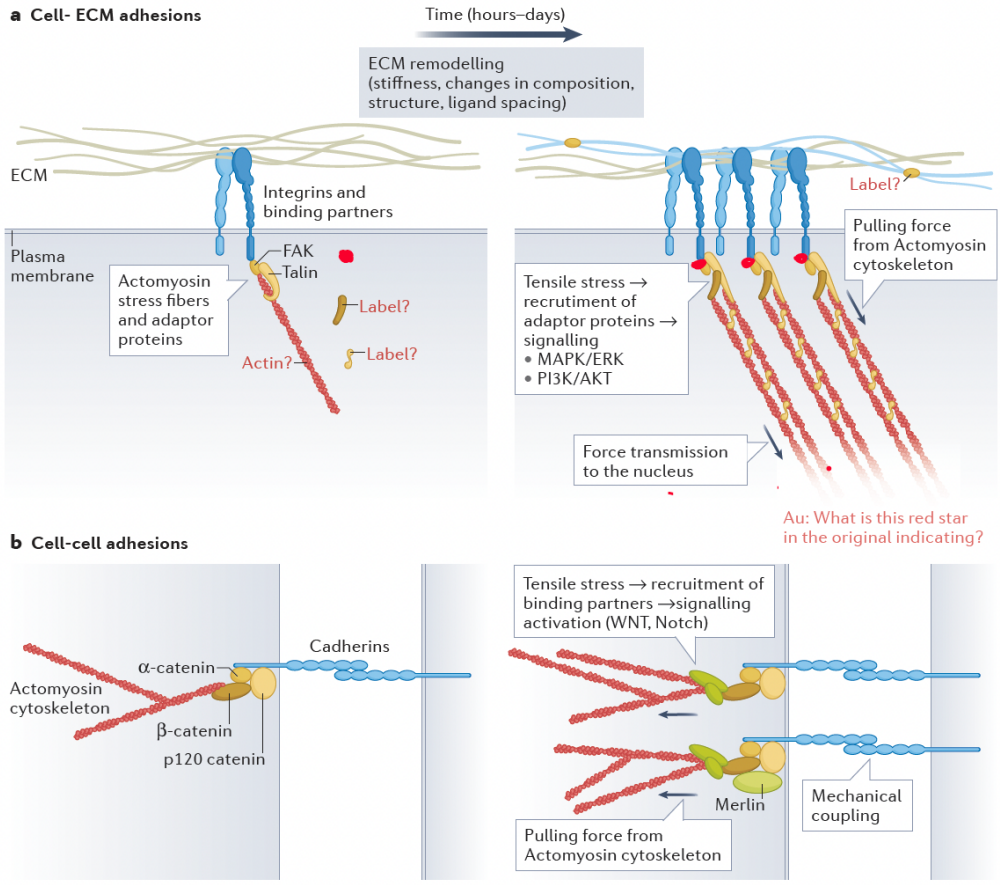

a Cell- ECM adhesions
Time (hours–days)
ECM remodelling (stiffness, changes in composition, structure, ligand spacing)
ECM
Integrins and binding partners
Plasma membrane
FAK
Talin
Actomyosin stress fibers and adaptor proteins
Label?
Actin?
Label?
Label?
Pulling force from Actomyosin cytoskeleton
Tensile stress → recruitment of adaptor proteins → signalling
• MAPK/ERK
• PI3K/AKT
Force transmission to the nucleus
Au: What is this red star in the original indicating?
b Cell-cell adhesions
Cadherins
α-catenin
Actomyosin cytoskeleton
β-catenin
p120 catenin
Tensile stress → recruitment of binding partners →signalling activation (WNT, Notch)
Merlin
Mechanical coupling
Pulling force from Actomyosin cytoskeleton

Fig 3

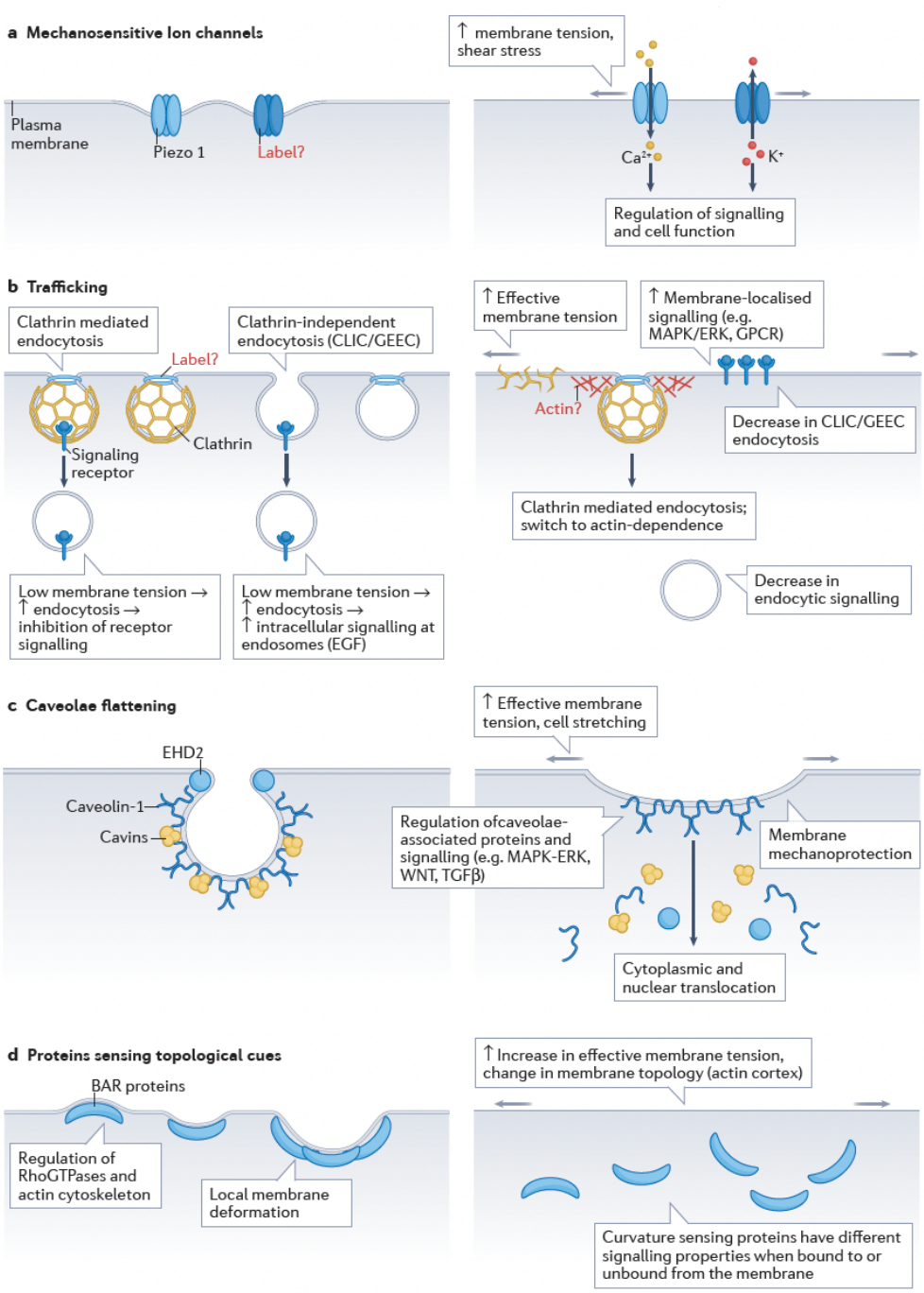
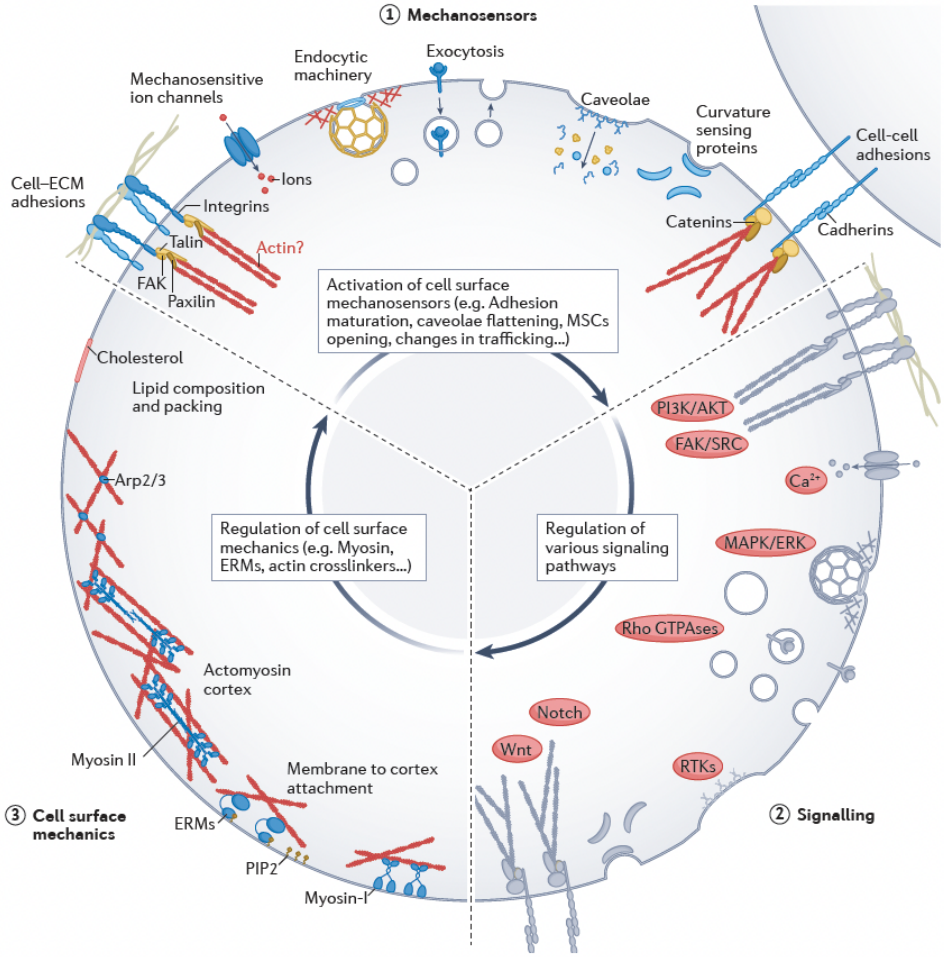

a Mechanosensitive Ion channels
Plasma membrane
Piezo 1
Label?
↑ membrane tension, shear stress
Ca2+
K+
Regulation of signalling and cell function
b Trafficking
Clathrin mediated endocytosis
Clathrin-independent endocytosis (CLIC/GEEC)
Label?
Clathrin
Signaling receptor
Low membrane tension → ↑ endocytosis → inhibition of receptor signalling
Low membrane tension → ↑ endocytosis → ↑ intracellular signalling at endosomes (EGF)
↑ Effective membrane tension
↑ Membrane-localised signalling (e.g. MAPK/ERK, GPCR)
Actin?
Decrease in CLIC/GEEC endocytosis
Clathrin mediated endocytosis; switch to actin-dependence
Decrease in endocytic signalling
c Caveolae flattening
EHD2
Caveolin-1
Cavins
↑ Effective membrane tension, cell stretching
Regulation ofcaveolae-associated proteins and signalling (e.g. MAPK-ERK, WNT, TGFβ)
Membrane mechanoprotection
Cytoplasmic and nuclear translocation
d Proteins sensing topological cues
BAR proteins
Regulation of RhoGTPases and actin cytoskeleton
Local membrane deformation
↑ Increase in effective membrane tension, change in membrane topology (actin cortex)
Curvature sensing proteins have different signalling properties when bound to or unbound from the membrane

Fig 4


① Mechanosensors
Endocytic machinery
Exocytosis
Mechanosensitive ion channels
Caveolae
Curvature sensing proteins
Cell-cell adhesions
Ions
Cell–ECM adhesions
Integrins
Talin
Actin?
FAK
Paxilin
Catenins
Cadherins
Activation of cell surface mechanosensors (e.g. Adhesion maturation, caveolae flattening, MSCs opening, changes in trafficking...)
Cholesterol
Lipid composition and packing
PI3K/AKT
FAK/SRC
Ca2+
Arp2/3
Regulation of cell surface mechanics (e.g. Myosin, ERMs, actin crosslinkers...)
Regulation of various signaling pathways
MAPK/ERK
Rho GTPAses
Actomyosin cortex
Notch
Wnt
Myosin II
Membrane to cortex attachment
RTKs
③ Cell surface mechanics
ERMs
② Signalling
PIP2
Myosin-I