Experimental Cell Research 374 (2019) 259–265

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Review article

Mechanosensitive channels and their functions in stem cell differentiation

Li He[a,*], Muhammad Ahmad[a], Norbert Perrimon[a,b,**]

[a] Department of Genetics, Harvard Medical School, Boston, MA 02115, USA
[b] Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115, USA

ARTICLE INFO

Keywords:
Mechanobiology
Stem cell differentiation
Mechanosensitive channels (MSCs)
Mechanosensitive (MS)

ABSTRACT

Stem cells continuously perceive and respond to various environmental signals during development, tissue homeostasis, and pathological conditions. Mechanical force, one of the fundamental signals in the physical world, plays a vital role in the regulation of multiple functions of stem cells. The importance of cell adhesion to the extracellular matrix (ECM), cell-cell junctions, and a mechanoresponsive cell cytoskeleton has been under intensive study in the fields of stem cell biology and mechanobiology. However, the involvement of mechanosensitive (MS) ion channels in the mechanical regulation of stem cell activity has just begun to be realized. Here, we review the diversity and importance of mechanosensitive channels (MSCs), and discuss recently discovered functions of MSCs in stem cell regulation, especially in the determination of cell fate.

1. Introduction

Mechanical forces are primary signals that are sensed by essentially all cells. They can be generated in a number of ways both within and outside of the biological system. These include: 1) changes in osmolarity due to variation in electrolyte concentrations, 2) changes to local stiffness caused by deposition or modification of ECM molecules, 3) alterations of lipid bilayer mechanics through lipid lysis, 4) contractile forces generated by the cytoskeleton, 5) tensile stresses created by body fluids, air, and ingested food particles, 6) physical constrictions generated during development or tumorigenesis, 7) sound perceived in the form of vibration, and 8) forces caused by gravity, acceleration, and body position. These highly multiscale mechanical signals play essential roles in different biological processes, from regulating individual cell growth to controlling organ formation and modifying the behavior of the whole organism. Cells have developed various kinds of molecular machinery to sense mechanical signals, including structural proteins, ion channels, enzymes, and membrane receptors [1].

Most previous studies of microscale mechanical signaling have focused on cell adhesion, structural proteins, and ECM molecules [2], while studies of tissue-level or organism-level mechanical sensing have primarily focused on MSCs in excitable cells such as muscles and neurons [1]. This under-appreciation of the role of MSCs in microscale mechanical signaling is due in part to the fact that most classic electrophysiology techniques to study MSCs, such as the patch clamp technique, are not readily available to conventional cell biologists, and in part because a limited number of MSCs have been identified outside neurons and muscles. However, with the development of calcium imaging systems, different pharmacological reagents, *ex vivo* manipulation techniques, and recently discovered ubiquitous MSCs, increasing evidence suggests that MSCs also play essential roles in controlling many fundamental biological functions other than neuron and muscle excitation, including stem cell proliferation and differentiation. Below, we discuss the fundamental properties of MSCs and review the most recent discoveries about the roles of MSCs in stem cells.

2. Evolution and expression of MSCs

Compared with well-characterized mechanosensitive machinery such as the integrin adhesion complex, which appeared during the earliest origin of multicellular organisms, MSCs are even more evolutionary ancient: they are present in cells across all kingdoms of life forms [1,3]. As osmolarity stress on the lipid membrane was probably one of the first vital signals faced by early life forms in water, the presence of MSCs in most cell types appears necessary. However, the evolutionary ancient origin of MSCs seemed to conflict with the fact that in multicellular organisms, roles for MSCs had been identified only in excitable cells. This apparent contradiction was resolved recently by the discovery in 2010 of Piezo, an MS channel broadly expressed in many different tissues [4] (Table 1).

Although Piezo was discovered only 8 years ago, it has already become one of the best-characterized MSCs in eukaryotes. Piezo is now

* Corresponding author.
** Corresponding author at: Department of Genetics, Harvard Medical School, Boston, MA 02115, USA.
E-mail addresses: lihe@genetics.med.harvard.edu (L. He), perrimon@receptor.med.harvard.edu (N. Perrimon).

https://doi.org/10.1016/j.yexcr.2018.11.016
Received 22 October 2018; Received in revised form 17 November 2018; Accepted 21 November 2018
Available online 28 November 2018
0014-4827/ © 2018 Elsevier Inc. All rights reserved.

Table 1
List of MSCs present in major model organisms. MSCs characterized in metazoans are listed in the table.

Organism	Genes	Protein Family	Effector	Function in
C. elegans	UNC-105, MEC-4, MEC-10 [11]	ENaC/DEG	Na+	Neurons, muscle
	TRP4 [16]	TRPN	Ca2+	Neurons
	OSM9, Orc-1,2,3,4 [16]	TRPV	Ca2+	Neurons
	LOV-1, PKD2 [3,16]	TRPP	Ca2+	Neurons
	TRPA1 [16]	TRPA	Ca2+	Neurons
Drosophila	Piezo [5]	Novel	Ca2+	Neurons, stem cells
	ppk1, 36 [11,16]	ENaC/DEG	Na+	Neurons
	Brv1 [16]	TRPP	Ca2+	Neurons
	TRPA1, Pain, Pyx, wtrw [16]	TRPA	Ca2+	Neurons, stem cells,
	NAN, IAV [16]	TRPV	Ca2+	Neurons,
	NompC [16]	TRPN	Ca2+	Neurons,
	Piezo 1, 2 [5]	novel	Ca2+	Lung, skin, muscle, red blood cells, neurons, stem cells
Mammal	ASIC1, 2, 3 [11,16]	ENaC/DEG	Na+	Neurons, muscle
	TRPA1 [3,6,16]	TRPA	Ca2+	Neurons,
	TRPC1,3,5,6 [6,16]	TRPC	Ca2+	Stem cells, multiple tissues
	TRPV1,2,4 [6,16]	TRPV	Ca2+	Stem cells, multiple tissues
	TRPM3,4,7 [6,16]	TRPM	Ca2+	Stem cells, multiple tissues
	PKD1,2 [6,16]	TRPP	Ca2+	Stem cells, Kidney,
	TREK 1,2, TRAAK [3,16]	K2p Family	K+	Muscle, neurons
	TMC1,2 [16]	Novel	Unknown	Neurons

known to function in a variety of cell types, including red blood cells, Merkel cells, epithelial cells, vascular endothelial cells, chondrocytes, stem cells, and neurons [5]. This broad function of Piezo is consistent with its early appearance during evolution: Piezo-related proteins have been found in almost all eukaryotes, including animals, plants, and protozoa [6]. Most vertebrate genomes contain two paralogs, Piezo1 and Piezo2, whereas *Drosophila melanogaster* has a single ortholog with similarity to both Piezo1 and 2 [5]. As a novel protein sharing no known structural similarity with other proteins, Piezo is an unusually large molecule (> 2500 amino acids) that forms a channel with about 100 transmembrane domains (as a homotrimer). Remarkably, Piezo is one of the most sensitive MSCs discovered to date and can be directly activated in the presence of membrane tension without any additional components [5]. Thus far, Piezo appears to be a MSC that primarily responds to mechanical stresses in vivo. This unique mechanosensitive function of Piezo greatly facilitates the interpretation of loss-of-function studies and thus has led to a large number of discoveries in mechanobiology [5].

Another ancient and broadly expressed MSC is the transient receptor potential (TRP) channel (Table 1). First cloned in 1989, TRP was originally found to be required for light detection in the *Drosophila* retina [6], which is activated through rhodopsin-triggered phospholipase C (PLC) activity. The TRP channel is present in both fungi and animals (plants are believed to have lost TRP during evolution since their ancestors, chlorophyte algae, have a TRP homolog) [7]. Evolution has diversified TRP in mammals into a large protein family comprised of 28 genes that can be grouped into seven subfamilies: TRPC, TRPV, TRPM, TRPA, TRPP, TRPML, and TRPN. Different from the primary MS function of Piezo, TRP channels directly respond to highly diverse stimuli including temperature (thermosensation), pH, enzyme activities, and a large number of chemicals (chemosensation). TRP channels are broadly present in many different tissues and the diversity of stimulants of TRP channels makes their function specifically as MSCs comparatively underappreciated. However, the MS property of TRP channel seems to be one of the earliest functions of TRP proteins: the yeast genome encodes a single TRP member, TrpY1, which turns out to be a mechano-sensor of vascular osmotic pressure [8]. Many TRP channels have been found to detect sound and osmolarity in specialized neurons [3]. In recent years, the mechanosensing function of TRP channels in non-excitable cells has also been increasingly studied. Several TRP channels, including TRPV4, TRPM7, TRPC1, and TRPA1, have been found to be involved in mechano-signaling in different cell types including stem cells [9]. One striking example is the recent discovery that TRP activation by PCL in the fly retina is actually mediated through lipid lysis-triggered membrane contraction, suggesting a potentially ubiquitous ability for mechanosensing [10].

In addition to Piezo and TRPs, there are also several evolutionary new families of proteins with MSC function (Table 1). The ENaC/Deg superfamily first appeared after the late branches of Metazoa [11]. This protein family was first discovered from nematodes in the mechanosensitive neurons for touch perception (the name Degenerins/Deg comes from the neuron degeneration phenotype caused by gain-of-function mutations of these channels). In mammals, ENaC/Deg family proteins were also found in neurons, where they are responsible for sensing osmolarity. However, because these channels function as part of a multi-unit complex and no reconstitution experiments have been successful, whether these channels can directly detect mechanical force and can function as mechano-sensors in contexts other than in excitable cells has yet to be determined. In addition, although ENaC/Deg proteins are widely expressed in epithelial tissues, they are more likely to play an ion transport function rather than a mechanosensory role [11]. Members of another MSC subfamily, the K^+ selective 2P domain channels (TREK/TRAAK channels), are only present in mammals [12–14]. Structural studies and reconstitution experiments showed that TREK/TRAAK channels are MSCs which are directly activated by mechanical tension in the lipid bilayer. Another protein family with possible MS function is the Transmembrane channel-like proteins (TMC1,2). These proteins share similar topology with TRPs and were identified in the cochlea of the inner ear as part of the auditory transduction machinery. Like ENaC/Deg proteins, TMCs seem to require other components such as cadherins in cell-cell adhesion for their mechanosensing activities. It is still not clear whether TMCs can function as direct mechanosensory channels. Both TREK/TRAAK and TMC1,2 have been primarily identified and studied in excitable cells, especially in neurons [15,16].

The discovery of new MSCs has just begun. For example, Piezo can only explain the fast-inactivating MS response in the dorsal root ganglia cells, and MSCs corresponding to the intermediate- and slow-inactivating responses in these cells are still unidentified [4]. In fact, in many mechanical responsive cells, the corresponding MSCs are yet to be discovered.

3. Ca^{2+} is the primary downstream effector of MSC activation in non-excitable cells

It is important to note that both Piezo and TRPs are non-selective

cation channels and that their immediate downstream effect after channel opening is an increase in cytosolic Ca^{2+}[16]. As a key second messenger in both prokaryotes and eukaryotes, Ca^{2+} regulates many fundamental biological processes, including cell morphology, migration, secretion, proliferation, differentiation, and cell death [17]. The insolubility of calcium phosphate around the neutral pH makes Ca^{2+} a highly undesirable ion within the cytosol. Thus, the naturally very low concentration of this ion in the cell makes its concentration a highly sensitive indicator. In most parts of a cell, cytosolic Ca^{2+} is around 100 nM at resting state (in contrast to the ~1 mM Ca^{2+} outside the cells) and rises to 1 uM when activated. This feature allows Ca^{2+} permeable MSCs to control cytosolic Ca^{2+} with extremely high efficiency. Even for channels with low conductivity (around 30 pS), one single open channel can, in theory, increase the total Ca^{2+} concentration of a regular mammalian cell by 10-fold (from 100 nM to 1 uM) within 0.1 s (if the Ca^{2+} buffering effect is not considered). This sensitivity may be another reason why MSCs are hard to detect in expression profiling experiments as a meager number of MSCs is needed for biological activity. Meanwhile, cells are highly sensitive to changes in the concentration of Ca^{2+}. For example, in neuron growth cone pathfinding, higher cytosolic Ca^{2+} (200 nM) effectively promotes axon attraction, whereas lower Ca^{2+} (75 nM) triggers repulsion and mid-range Ca^{2+} (135 nM) generates random growth [18]. The very high sensitivity of cells to Ca^{2+} signals may partially explain why the sodium-selective ENaC/Deg and potassium-selective K2p play lesser functions in non-excitable cells, as these channels primarily regulate membrane potentials, which are usually low in non-excitable cells. The very fast increase and high sensitivity of Ca^{2+} also make it a more rapid and long-range second messenger: Ca^{2+} wave can travel in the cells with speed up to 50 μm per second [19] and propagate across multiple cells through gap junctions [20].

Although Ca^{2+} is the unified effector of most MSCs in non-excitable cells, this does not prevent MSCs from generating highly diverse biological consequences in different systems. On the one hand, this is because Ca^{2+} increases can be interpreted by various downstream effectors, including CaM-dependent protein kinases (CaMKs), myosin light chain kinase (MLCK), serum response factor (SRF), and cAMP response element-binding protein (CREB) [17]. On the other hand, many Ca^{2+}-regulated factors not only respond to the presence and absence of Ca^{2+} but also react differently to distinct Ca^{2+} dynamics, including the speed of increase, frequency, and duration of the Ca^{2+} signal. For example, in rat hippocampal dendritic spines, low-frequency Ca^{2+} increase activates calcineurin, also known as protein phosphatase 2B, but not CaMK II, whereas high-frequency Ca^{2+} activates both of them [21]. Similar dynamic modulation by Ca^{2+} has been found for several other key signaling molecules, including protein kinase C (PKC), extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK), calpain protease, transcription factor NFAT (nuclear factor of activated T-cells), NF-κB, and glycogen phosphorylase [22]. Finally, the effects of Ca^{2+} can also be controlled spatially. The existence of buffering proteins in specific cells or subcellular locations may further cause the compartmentalization of Ca^{2+} responses even without a close membrane boundary. For example, highly localized blips or quarks of Ca^{2+} transients trigger local membrane fusion between synaptic vesicles and presynaptic membrane [23], and localized Ca^{2+} increases in the nucleus by Ca^{2+} release from nucleoplasmic reticulum has also been proposed to play a different role than cytosolic Ca^{2+} in transcriptional activation and cell proliferation [24]. Therefore, mechanical stresses with different magnitudes, frequencies, or spatial patterns can trigger completely different biological consequences through MSC-mediated Ca^{2+} increases.

4. MSC-triggered Ca^{2+} plays essential roles in stem cell differentiation

Ca^{2+} is an unarguably key molecule that controls stem cell proliferation and differentiation [17]. Elevated Ca^{2+} activities, such as oscillations, sparks, or waves, have been found in many different stem cells, including embryonic stem cells (ESCs) [25,26], neural stem cells (NSCs) [27], mesenchymal stem cells [28], cardiac progenitor cells [29], and intestinal stem cells (ISCs) [30,31]. Some of these Ca^{2+} activities are associated with stem cell growth and maintenance [26,30]. Meanwhile, evidence of Ca^{2+} regulated stem cell differentiation is also abundant [17]. Ca^{2+} activities can stimulate cell differentiation into neuronal lineage in both developing embryo and cultured stem cells [32], control multiple steps of cardiomyocyte differentiation [33], induce skin Keratinocyte differentiation [34], enhance the chondrogenic differentiation of mesenchymal stem cells, and function as an inhibitory signal in the case of differentiation into adipocytes [35,36].

Mechanical forces have also been found to be critical regulators in most of the systems mentioned above [1,37]. The regulation of stem cell differentiation by mechanical forces was first directly observed by the discovery that matrix elasticity directly controls mesenchymal stem cell differentiation into different cell lineages [38]. Further, mechanical stimuli have been found to trigger ESC neurogenesis [39] and mesenchymal stem cells chondrogenesis [40], favor osteoblastogenesis and inhibit adipogenesis [41], and regulate several differentiation cues during cardiomyogenesis [42]. More importantly, most of these mechanical stimuli cause a change in Ca^{2+} signals in stem cells. Therefore, it is not very surprising that MSCs are involved in the regulation of mechanically-triggered Ca^{2+} activities.

Recently, several studies have shown that MSCs function as key mechanosensors in the regulation of differentiation of various types of stem cells. For example, mouse ESCs have been found to express more mechanosensitive Ca^{2+} permeant cation channels than human ESCs, which may explain their distinctive responses to mechanical stimuli: mechanical stretches promote mESCs differentiation but help hESCs maintain their pluripotency [43]. Early evidence for the involvement of MSCs in stem cell differentiation also came from the well-studied mesenchymal stem cells (Fig. 1) [37]. These cells are readily available from multiple tissues and can differentiate into osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells) and adipocytes (fat cells). As mentioned above, mechanical stress plays important roles in regulating mesenchymal stem cell differentiation, which is accompanied by increases in cytosolic Ca^{2+}.

In two recent studies, TRPM7 was identified as the mechanosensory that is responsible for the compression and fluid shear stress-induced osteogenesis of mesenchymal stem cells. In the first study, TRPM7 was found to be required for a fluid stress-triggered increase in activity of the osteogenic transcriptional factor Osterix [44]. In the second study, a mutation in TRPM7 was shown to completely block the intracellular Ca^{2+} increase and subsequent NFATc1 nuclear localization triggered by mechanical compression [45]. More importantly, the authors demonstrated that the compression-induced Ca^{2+} increase is independent of the cytoskeleton, as cytochalasin D treatment cannot block this increase.

Another striking result comes from a study of the effects of hydrostatic pressure (HP) on cultured mesenchymal stem cells [46]. RNAi against Piezo significantly blocked the 0.01 MPa positive HP triggered osteoblast differentiation, whereas the Piezo activator Yoda1 promotes differentiation by inducing Bone morphogenetic protein 2 (BMP2) expression. Finally, inhibition of MSCs by treatment with GsMTx4, a spider venom peptide that attenuates tension between the membrane protein and the lipid bilayer, significantly blocks the HP-induced caudal fin ray increase in fish larvae. This study for the first time demonstrated that HP sensing during animal bone formation requires MSCs. Because of the incompressibility of the biomaterial, the activation of Piezo by HP seems puzzling. However, HP might alter the partition of oxygen and carbon dioxide in the medium, which may further affect the molecular composition of ECM and Piezo activation.

The presence of TRPV4 was first demonstrated to be responsible for a flow-induced local Ca^{2+} increase in osteocyte-like cells and Madin-

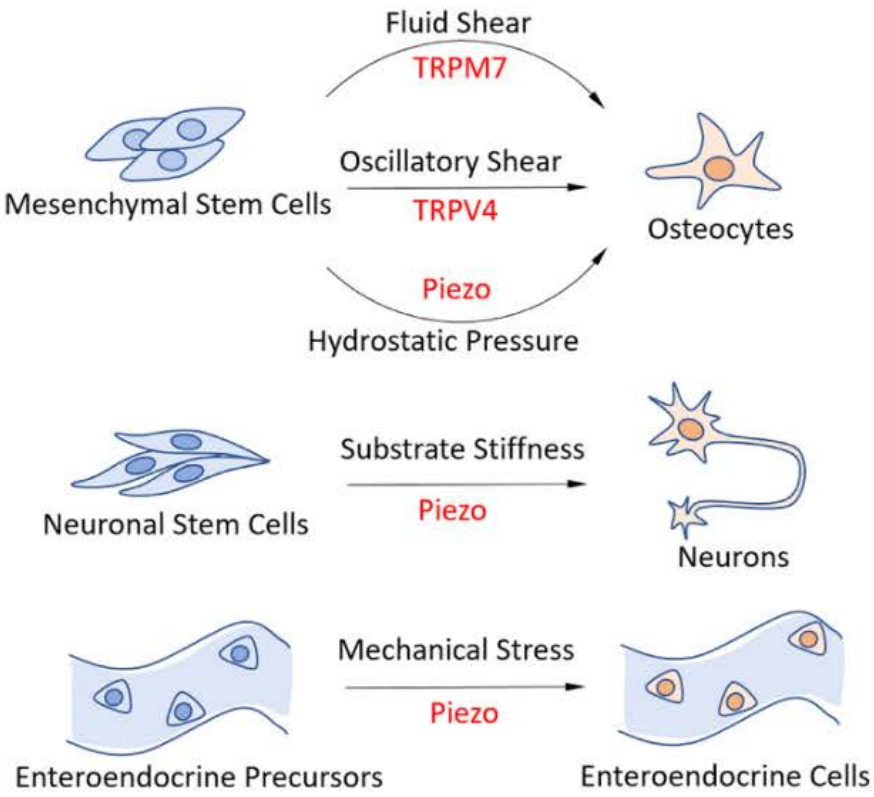

Fig. 1. Mechanical stresses trigger stem cell differentiation through MSC activation. Various types of mechanical stresses, including constant fluid shear, oscillatory shear, hydrostatic pressure, and substrate stiffness, have been found to trigger the differentiation of different stem cells through MSCs. The nature of mesenchymal stem cells is usually heterogeneous with a significant variation from different tissue origins. If specific MSC is associated with a particular type of mesenchymal stem cell or mechanical triggers requires further study.

Darby Canine Kidney (MDCK) cells [47,48], and a hypo-osmotic stress-triggered Ca^{2+} rise in chondrocytes [49]. A recent study in mesenchymal stem cells uncovered that TRPV4 is responsible for the oscillatory fluid shear-induced Ca^{2+} increase and early osteogenic differentiation [50]. Consistent with previous studies in other cell types [47,51], the authors showed that TRPV4 is specifically localized to areas with higher strain under fluid shear, particularly the base of the primary cilium. Perhaps even more intriguingly, the author demonstrated that blocking the primary cilium by knocking down the ciliogenesis-required protein IFT88 altered the response to TRPV4 activation, suggesting that different subcellular locations of MSCs may be responsible for different downstream signaling.

Another exciting discovery came from a study of cultured human neural stem/progenitor cells (hNSPCs), which can differentiate into neurons, astrocytes, and oligodendrocytes. Piezo1 is expressed in cultured hNSPCs and is responsible for mechanically-induced changes of membrane action potential (Fig. 1) [27]. Further analysis showed that spontaneous Ca^{2+} transients generated through cell-ECM traction forces require the presence of Piezo1. Moreover, stem cells plated on substrates with different stiffness altered their lineage choice between neurons and astrocytes. This ECM-regulated cell fate determination is blocked when Piezo is knocked down in the stem cells, suggesting that Piezo is an important mediator for mechanical-regulated neuronal stem cell differentiation.

The latest evidence of a role for MSCs in cell fate regulation comes from studies of the intestinal tissue. The digestive system constantly faces different kinds of mechanical stimuli, including shear and strain caused by food and repeated deformation caused by contraction of the visceral muscles. In addition, several pathological conditions, such as irritable bowel syndrome and Crohn's disease, are associated with a significant increase in the luminal pressure of the intestine [52]. In the mammalian intestine, mechanical stretch strongly increases cell proliferation in vivo [53]. In addition, in vitro experiments showed that cyclic strain applied to human intestinal epithelial Caco-2 cells triggers both proliferation and directed cell differentiation in a frequency-dependent manner [54]. Several essential regulators, including focal adhesion complex (FAK), PKC, ERK/MAPK, and Akt, have been found to be important for this mechanical response [52]. However, whether any MSCs are involved in the mechanosensing step is not clear.

Studies in *Drosophila* have demonstrated a role for Piezo in the differentiation of stem cell progeny. Compared with the complex mammalian intestine, the fly midgut is much simpler but shares many similarities in molecular features and cell lineage, including the presence of intestinal stem cells (ISCs), secretory enteroendocrine cells (EEs), and absorptive enterocytes (ECs) [55]. Through a genetic screen for stem cell-specific markers, we discovered that fly Piezo is specifically expressed in EE precursor (EP) cells (Fig. 1) [31]. These cells are normally inactive but can be mechanically triggered to differentiate into EEs. Loss of Piezo activity causes a gradual reduction in the number of EEs after the adult stage, suggesting that it is required for basal EE generation, possibly through digestion-triggered mechanical stimulus. Consistent with this hypothesis, physical stress triggered by an excessive amount of food in the midgut increases EE generation in a Piezo-dependent manner. Finally, direct deformation of *ex vivo* cultured midguts triggers a clear Piezo-dependent Ca^{2+} increase, supporting the idea that Piezo acts in the fly midgut as a direct mechanical sensor. Recently, Piezo was also detected in several different cell types in the mouse gastrointestinal tract [56,57]. It will be interesting to see if a similar regulatory pathway is conserved in mammalian stem cells.

5. Perspectives

Due to the ancient origin and diversity of MSCs, a broad set of functions of MSCs in different cell types is expected. Interestingly, membrane clamp experiments have shown that most of the cells tested, including endothelial cells, CHO cells, HEK cells, and Xenopus oocytes, reveal the presence of endogenous MSCs on their plasma membranes [13,58]. However, these cells are not normally mechanosensitive under physiological conditions (tested by whole cell clamp): the MSCs in these cells seem to open only when the cortical cytoskeleton is disrupted, such as following treatment with the F-actin inhibitor cytochalasin or physical detachment from the plasma membrane [58]. This evidence

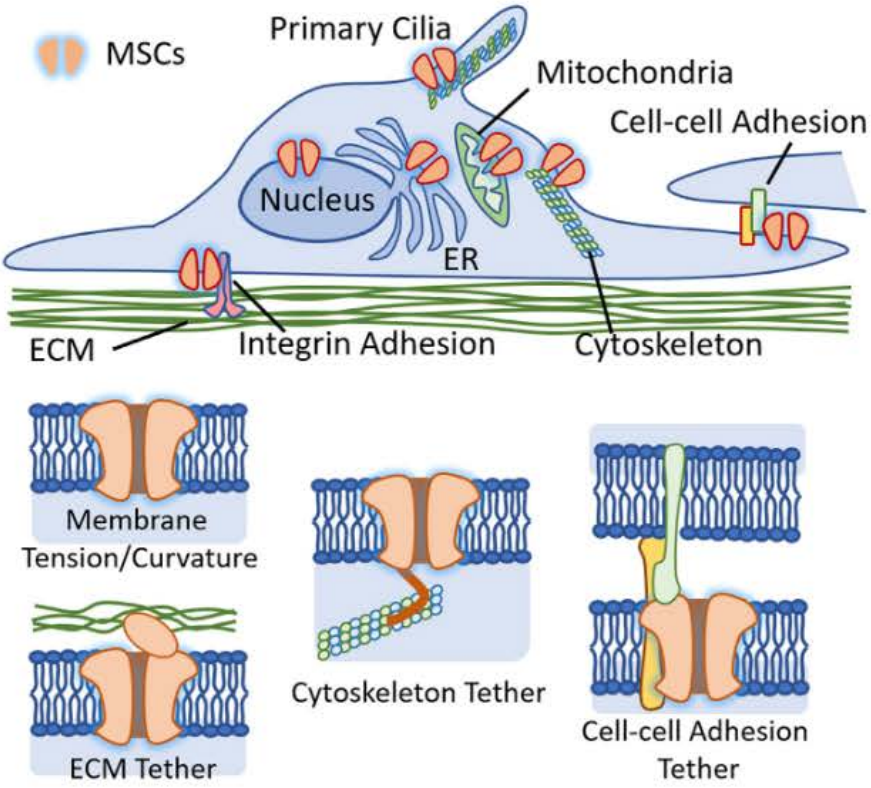

Fig. 2. Summary of the subcellular localization of MSCs in non-excitable cells and their associations with other molecules. MSCs have been found on the apical surface of the plasma membrane (the surface that is facing the fluid sheer), the basal/ventral surface of the plasma membrane (the surface that is connected to the ECM through integrin adhesion), the basal area of primary cilia, the intracellular ER membrane, nuclear membrane, mitochondria inner membrane, and the area associated with cell-cell adhesion. Some MSCs, including Piezo, and K2p proteins, are gated directly by tension or curvature of the lipid bilayer. Other MSCs are potentially gated by attachment to the structural proteins, including ECM (ENaC/DEG family), microtubules (NompC), F-actin (TRPM7), and cadherins (TMC1,2).

suggests that some potentially unidentified MSCs may be present in many seemingly mechano-insensitive cells and might be functional under certain pathological conditions.

Increasing evidence also suggests that MSCs and other mechanosensitive molecules such as integrin adhesion and the actomyosin cytoskeleton function closely with one another to mediate mechanical signal transduction (Fig. 2). For example, both Piezo2 and TRPM7 have been found to colocalize with integrin focal adhesions [59,60]; NompC (fly TRPN) associates with microtubules for its proper function [61,62]; TRPM7 binds to actin filaments [60]; and TRPV4 directly interacts with both microtubules and F-actin [63]. In many cases, MSCs are regulated by structural molecules that directly control the viscoelastic properties of the cells. More importantly, this regulation is also usually reciprocal. For example, TRPC1 controls axon guidance by Ca^{2+} influx-triggered Calpin action, which induces further cleavage of talin activation to trigger focal adhesion turnover [64]. In addition, Piezo2 and TRPM7 can promote cell morphology changes by regulating RhoA activation and F-actin assembly [59,60].

Meanwhile, MSCs are highly likely to play roles in intracellular mechanosensing (Fig. 2). For example, mitochondria, as the second largest reserve for Ca^{2+} in the cell, responds to mechanical cues by mitochondrial fission [65], modulating nuclear compartments [66] and by stimulating ATP synthesis [67]. Mitochondria are known to go through vigorous changes in volume, which is mainly driven by potassium fluxes, which swell mitochondria culminating in an inner membrane tension. While there is limited knowledge on mechanobiology of mitochondria, the existence of some mitochondrial MSCs has been highlighted. One such example is the calcium-activated potassium channel ($mitoBK_{Ca}$) located in the inner mitochondrial membrane [68], the opening probability of which is regulated by mechanical stimulus [69]. Another study reports mitochondrial MscS-Like (MSL) mechanosensitive ion channel in *Arabidopsis*, which regulates cell/organellar swelling in response to hypo-osmotic stress [70]. The cell nucleus has also been found to be a mechanotransduction machinery in cell fate determination [71]. Interestingly, the presence of MSCs on the nuclear membrane has long been proposed, as isolated cell nuclei show a hypotonic response of Ca^{2+} release, the identity of which remains unidentified [72,73].

Additionally, the intracellular reticulum (ER) membrane has also been found to able to respond to mechanical stimulus: human mesenchymal stem cells incubated in Ca^{2+} free medium (such that there can be no Ca^{2+} influx from MSCs on the plasma membrane) show a force-triggered ER Ca^{2+} release that is mediated through cytoskeleton coupling and potentially, through an unidentified MSC(s) on the ER [74]. Several TRP channels are found on different intracellular organelles, including the ER [7]. Recently, Piezo was also found to translocate from the plasma membrane to the ER in response to cell contacts [75]. Considering that the yeast TRP channel functions as an MSC on the vacuolar membrane to sense osmolarity pressure, it will be interesting to see if most cell organelles contain MSCs and thus can sense mechanical stress directly, and if so, whether activation of MSCs at different subcellular locations induces distinct biological functions. To address these questions, tools with high subcellular resolution, such as optical or magnetic tweezers, targeted optogenetic activators, and live-cell indicators, will be required to control or measure local mechanics and signaling.

Finally, we reiterate that there are still many unsolved questions about the mechanical regulation of stem cell activities and the study of MSCs during these processes is just beginning. We believe that the current development and application of new techniques in live imaging, tissue culture, and real-time mechanical stimulation delivery will significantly increase our current knowledge about the mechanobiology as well as stem cell biology.

Acknowledgments

We thank Stephanie Mohr for comments and suggestions. We apologize if not all of the original work has been cited due to a large amount of relevant literature and limited space. L.H. was a fellow of the Damon Runyon Cancer Research Foundation, USA. N. P. is an Investigator of the Howard Hughes Medical Institute.

References

[1] B. Ladoux, R.M. Mege, Mechanobiology of collective cell behaviours, Nat. Rev. Mol. Cell Biol. 18 (2017) 743–757, https://doi.org/10.1038/nrm.2017.98.

[2] G. Uzer, R.K. Fuchs, J. Rubin, W.R. Thompson, Concise review: plasma and nuclear membranes convey mechanical information to regulate mesenchymal stem cell lineage, Stem Cells 34 (2016) 1455–1463, https://doi.org/10.1002/stem.2342.

[3] J. Arnadottir, M. Chalfie, Eukaryotic mechanosensitive channels, Annu. Rev. Biophys. 39 (2010) 111–137, https://doi.org/10.1146/annurev.biophys.37.032807.125836.

[4] B. Coste, et al., Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels, Science 330 (2010) 55–60, https://doi.org/10.1126/science.1193270.

[5] J. Wu, A.H. Lewis, J. Touch Grandl, Tension, and transduction - the function and regulation of piezo ion channels, Trends Biochem. Sci. 42 (2017) 57–71, https://doi.org/10.1016/j.tibs.2016.09.004.

[6] C. Liu, C. Montell, Forcing open TRP channels: mechanical gating as a unifying activation mechanism, Biochem. Biophys. Res. Commun. 460 (2015) 22–25, https://doi.org/10.1016/j.bbrc.2015.02.067.

[7] X.P. Dong, X. Wang, H. Xu, TRP channels of intracellular membranes, J. Neurochem. 113 (2010) 313–328, https://doi.org/10.1111/j.1471-4159.2010.06626.x.

[8] Z. Su, X. Zhou, S.H. Loukin, Y. Saimi, C. Kung, Mechanical force and cytoplasmic Ca (2+) activate yeast TRPY1 in parallel, J. Membr. Biol. 227 (2009) 141–150, https://doi.org/10.1007/s00232-009-9153-9.

[9] B. Fels, E. Bulk, Z. Petho, A. Schwab, The role of TRP channels in the metastatic cascade, Pharmaceuticals 11 (2018), https://doi.org/10.3390/ph11020048.

[10] R.C. Hardie, K. Franze, Photomechanical responses in Drosophila photoreceptors, Science 338 (2012) 260–263, https://doi.org/10.1126/science.1222376.

[11] I. Hanukoglu, A. Hanukoglu, Epithelial sodium channel (ENaC) family: phylogeny, structure-function, tissue distribution, and associated inherited diseases, Gene 579 (2016) 95–132, https://doi.org/10.1016/j.gene.2015.12.061.

[12] A.J. Patel, M. Lazdunski, E. Honore, Lipid and mechano-gated 2P domain K(+) channels, Curr. Opin. Cell Biol. 13 (2001) 422–428.

[13] R. Peyronnet, D. Tran, T. Girault, J.M. Frachisse, Mechanosensitive channels: feeling tension in a world under pressure, Front. Plant Sci. 5 (2014) 558, https://doi.org/10.3389/fpls.2014.00558.

[14] S.G. Brohawn, J. del Marmol, R. MacKinnon, Crystal structure of the human K2P TRAAK, a lipid- and mechano-sensitive K+ ion channel, Science 335 (2012) 436–441, https://doi.org/10.1126/science.1213808.

[15] B. Martinac, K. Poole, Mechanically activated ion channels, Int. J. Biochem. Cell Biol. 97 (2018) 104–107, https://doi.org/10.1016/j.biocel.2018.02.011.

[16] S.S. Ranade, R. Syeda, A. Patapoutian, Mechanically activated ion channels, Neuron 87 (2015) 1162–1179, https://doi.org/10.1016/j.neuron.2015.08.032.

[17] F.M. Tonelli, et al., Stem cells and calcium signaling, Adv. Exp. Med. Biol. 740 (2012) 891–916, https://doi.org/10.1007/978-94-007-2888-2_40.

[18] J.R. Henley, K.H. Huang, D. Wang, M.M. Poo, Calcium mediates bidirectional growth cone turning induced by myelin-associated glycoprotein, Neuron 44 (2004) 909–916, https://doi.org/10.1016/j.neuron.2004.11.030.

[19] J. Vermot, S.E. Fraser, M. Liebling, Fast fluorescence microscopy for imaging the dynamics of embryonic development, HFSP J. 2 (2008) 143–155, https://doi.org/10.2976/1.2907579.

[20] L. Leybaert, M.J. Sanderson, Intercellular Ca(2+) waves: mechanisms and function, Physiol. Rev. 92 (2012) 1359–1392, https://doi.org/10.1152/physrev.00029.2011.

[21] H. Fujii, et al., Nonlinear decoding and asymmetric representation of neuronal input information by CaMKIIalpha and calcineurin, Cell Rep. 3 (2013) 978–987, https://doi.org/10.1016/j.celrep.2013.03.033.

[22] E. Smedler, P. Uhlen, Frequency decoding of calcium oscillations, Biochim. Biophys. Acta 1840 (2014) 964–969, https://doi.org/10.1016/j.bbagen.2013.11.015.

[23] M.J. Berridge, Calcium microdomains: organization and function, Cell Calcium 40 (2006) 405–412, https://doi.org/10.1016/j.ceca.2006.09.002.

[24] M.D. Bootman, C. Fearnley, I. Smyrnias, F. MacDonald, H.L. Roderick, An update on nuclear calcium signalling, J. Cell Sci. 122 (2009) 2337–2350, https://doi.org/10.1242/jcs.028100.

[25] J.I. Del Marmol, K.K. Touhara, G. Croft, R. MacKinnon, Piezo1 forms a slowly-inactivating mechanosensory channel in mouse embryonic stem cells, eLife 7 (2018), https://doi.org/10.7554/eLife.33149.

[26] N. Kapur, G.A. Mignery, K. Banach, Cell cycle-dependent calcium oscillations in mouse embryonic stem cells, Am. J. Physiol. Cell Physiol. 292 (2007) C1510–C1518, https://doi.org/10.1152/ajpcell.00181.2006.

[27] M.M. Pathak, et al., Stretch-activated ion channel Piezo1 directs lineage choice in human neural stem cells, Proc. Natl. Acad. Sci. USA 111 (2014) 16148–16153, https://doi.org/10.1073/pnas.1409802111.

[28] S. Sun, Y. Liu, S. Lipsky, M. Cho, Physical manipulation of calcium oscillations facilitates osteodifferentiation of human mesenchymal stem cells, FASEB J.: Off. Publ. Fed. Am. Soc. Exp. Biol. 21 (2007) 1472–1480, https://doi.org/10.1096/fj.06-7153com.

[29] J. Ferreira-Martins, et al., Spontaneous calcium oscillations regulate human cardiac progenitor cell growth, Circ. Res. 105 (2009) 764–774, https://doi.org/10.1161/CIRCRESAHA.109.206698.

[30] H. Deng, A.A. Gerencser, H. Jasper, Signal integration by Ca(2+) regulates intestinal stem-cell activity, Nature 528 (2015) 212–217, https://doi.org/10.1038/nature16170.

[31] L. He, G. Si, J. Huang, A.D.T. Samuel, N. Perrimon, Mechanical regulation of stem-cell differentiation by the stretch-activated Piezo channel, Nature 555 (2018) 103–106, https://doi.org/10.1038/nature25744.

[32] X. Gu, N.C. Spitzer, Distinct aspects of neuronal differentiation encoded by frequency of spontaneous Ca2+ transients, Nature 375 (1995) 784–787, https://doi.org/10.1038/375784a0.

[33] M. Puceat, M. Jaconi, Ca2+ signalling in cardiogenesis, Cell Calcium 38 (2005) 383–389, https://doi.org/10.1016/j.ceca.2005.06.016.

[34] S.J. D'Souza, A. Pajak, K. Balazsi, L. Dagnino, Ca2+ and BMP-6 signaling regulate E2F during epidermal keratinocyte differentiation, J. Biol. Chem. 276 (2001) 23531–23538, https://doi.org/10.1074/jbc.M100780200.

[35] I. Uzieliene, P. Bernotas, A. Mobasheri, E. Bernotiene, The role of physical stimuli on calcium channels in chondrogenic differentiation of mesenchymal stem cells, Int. J. Mol. Sci. 19 (2018), https://doi.org/10.3390/ijms19102998.

[36] S. Kawano, et al., ATP autocrine/paracrine signaling induces calcium oscillations and NFAT activation in human mesenchymal stem cells, Cell Calcium 39 (2006) 313–324, https://doi.org/10.1016/j.ceca.2005.11.008.

[37] A.J. Steward, D.J. Kelly, Mechanical regulation of mesenchymal stem cell differentiation, J. Anat. 227 (2015) 717–731, https://doi.org/10.1111/joa.12243.

[38] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, Cell 126 (2006) 677–689, https://doi.org/10.1016/j.cell.2006.06.044.

[39] C.R. Kothapalli, R.D. Kamm, 3D matrix microenvironment for targeted differentiation of embryonic stem cells into neural and glial lineages, Biomaterials 34 (2013) 5995–6007, https://doi.org/10.1016/j.biomaterials.2013.04.042.

[40] C.J. O'Conor, N. Case, F. Guilak, Mechanical regulation of chondrogenesis, Stem Cell Res. Ther. 4 (2013) 61, https://doi.org/10.1186/scrt211.

[41] V. David, et al., Mechanical loading down-regulates peroxisome proliferator-activated receptor gamma in bone marrow stromal cells and favors osteoblastogenesis at the expense of adipogenesis, Endocrinology 148 (2007) 2553–2562, https://doi.org/10.1210/en.2006-1704.

[42] C.L. Happe, A.J. Engler, Mechanical forces reshape differentiation cues that guide cardiomyogenesis, Circ. Res. 118 (2016) 296–310, https://doi.org/10.1161/CIRCRESAHA.115.305139.

[43] B. Soria, S. Navas, A. Hmadcha, O.P. Hamill, Single mechanosensitive and Ca(2)(+)-sensitive channel currents recorded from mouse and human embryonic stem cells, J. Membr. Biol. 246 (2013) 215–230, https://doi.org/10.1007/s00232-012-9523-6.

[44] Y.S. Liu, et al., Mechanosensitive TRPM7 mediates shear stress and modulates osteogenic differentiation of mesenchymal stromal cells through Osterix pathway, Sci. Rep. 5 (2015) 16522, https://doi.org/10.1038/srep16522.

[45] E. Xiao, et al., Brief reports: TRPM7 Senses mechanical stimulation inducing osteogenesis in human bone marrow mesenchymal stem cells, Stem Cells 33 (2015) 615–621, https://doi.org/10.1002/stem.1858.

[46] A. Sugimoto, et al., Piezo type mechanosensitive ion channel component 1 functions as a regulator of the cell fate determination of mesenchymal stem cells, Sci. Rep. 7 (2017) 17696, https://doi.org/10.1038/s41598-017-18089-0.

[47] M. Kottgen, et al., TRPP2 and TRPV4 form a polymodal sensory channel complex, J. Cell Biol. 182 (2008) 437–447, https://doi.org/10.1083/jcb.200805124.

[48] K.L. Lee, et al., The primary cilium functions as a mechanical and calcium signaling nexus, Cilia 4 (2015) 7, https://doi.org/10.1186/s13630-015-0016-y.

[49] M.N. Phan, et al., Functional characterization of TRPV4 as an osmotically sensitive ion channel in porcine articular chondrocytes, Arthritis Rheum. 60 (2009) 3028–3037, https://doi.org/10.1002/art.24799.

[50] M.A. Corrigan, et al., TRPV4-mediates oscillatory fluid shear mechanotransduction in mesenchymal stem cells in part via the primary cilium, Sci. Rep. 8 (2018) 3824, https://doi.org/10.1038/s41598-018-22174-3.

[51] N. Luo, et al., Primary cilia signaling mediates intraocular pressure sensation, Proc. Natl. Acad. Sci. USA 111 (2014) 12871–12876, https://doi.org/10.1073/pnas.1323292111.

[52] C.P. Gayer, M.D. Basson, The effects of mechanical forces on intestinal physiology and pathology, Cell. Signal. 21 (2009) 1237–1244, https://doi.org/10.1016/j.cellsig.2009.02.011.

[53] A.U. Spencer, et al., Enterogenesis in a clinically feasible model of mechanical small-bowel lengthening, Surgery 140 (2006) 212–220, https://doi.org/10.1016/j.surg.2006.03.005.

[54] M.D. Basson, G.D. Li, F. Hong, O. Han, B.E. Sumpio, Amplitude-dependent modulation of brush border enzymes and proliferation by cyclic strain in human intestinal Caco-2 monolayers, J. Cell. Physiol. 168 (1996) 476–488, https://doi.org/10.1002/(SICI)1097-4652(199608)168:2<476::AID-JCP26>3.0.CO;2-#.

[55] Y. Apidianakis, L.G. Rahme, Drosophila melanogaster as a model for human intestinal infection and pathology, Dis. Model Mech. 4 (2011) 21–30, https://doi.org/10.1242/dmm.003970.

[56] C. Alcaino, G. Farrugia, A. Beyder, Mechanosensitive piezo channels in the gastrointestinal tract, Curr. Top. Membr. 79 (2017) 219–244, https://doi.org/10.1016/bs.ctm.2016.11.003.

[57] C. Alcaino, et al., A population of gut epithelial enterochromaffin cells is mechanosensitive and requires Piezo2 to convert force into serotonin release, Proc. Natl. Acad. Sci. USA 115 (2018) E7632–E7641, https://doi.org/10.1073/pnas.1804938115.

[58] C.L. Bowman, P.A. Gottlieb, T.M. Suchyna, Y.K. Murphy, F. Sachs, Mechanosensitive ion channels and the peptide inhibitor GsMTx-4: history, properties, mechanisms and pharmacology, Toxicon: Off. J. Int. Soc. Toxinol. 49 (2007) 249–270, https://doi.org/10.1016/j.toxicon.2006.09.030.

[59] C. Pardo-Pastor, et al., Piezo2 channel regulates RhoA and actin cytoskeleton to promote cell mechanobiological responses, Proc. Natl. Acad. Sci. USA 115 (2018) 1925–1930, https://doi.org/10.1073/pnas.1718177115.

[60] K. Clark, et al., TRPM7, a novel regulator of actomyosin contractility and cell

adhesion, EMBO J. 25 (2006) 290–301, https://doi.org/10.1038/sj.emboj.7600931.
[61] P. Jin, et al., Electron cryo-microscopy structure of the mechanotransduction channel NOMPC, Nature 547 (2017) 118–122, https://doi.org/10.1038/nature22981.
[62] W. Zhang, et al., Ankyrin repeats convey force to gate the NOMPC mechanotransduction channel, Cell 162 (2015) 1391–1403, https://doi.org/10.1016/j.cell.2015.08.024.
[63] C. Goswami, J. Kuhn, P.A. Heppenstall, T. Hucho, Importance of non-selective cation channel TRPV4 interaction with cytoskeleton and their reciprocal regulations in cultured cells, PLoS One 5 (2010) e11654, https://doi.org/10.1371/journal.pone.0011654.
[64] P.C. Kerstein, et al., Mechanosensitive TRPC1 channels promote calpain proteolysis of talin to regulate spinal axon outgrowth, J. Neurosci.: Off. J. Soc. Neurosci. 33 (2013) 273–285, https://doi.org/10.1523/JNEUROSCI.2142-12.2013.
[65] S.C.J. Helle, et al., Mechanical force induces mitochondrial fission, Elife 6 (2017) e30292.
[66] A. Kaasik, et al., Mitochondria as a source of mechanical signals in cardiomyocytes, Cardiovasc. Res. 87 (2010) 83–91.
[67] K.J. Wolff, et al., Mechanical stress and ATP synthesis are coupled by mitochondrial oxidants in articular cartilage, J. Orthop. Res. 31 (2013) 191–196.
[68] P. Koprowski, A. Kielbasa, B. Kulawiak, A. Szewczyk, Mechanosensitivity of mitochondrial potassium channels, Biophys. J. 112 (2017) 406a.
[69] A. Walewska, B. Kulawiak, A. Szewczyk, P. Koprowski, Mechanosensitivity of mitochondrial large-conductance calcium-activated potassium channels, Biochim. Biophys. Acta (BBA)-Bioenerg. (2018).
[70] C.P. Lee, et al., MSL 1 is a mechanosensitive ion channel that dissipates mitochondrial membrane potential and maintains redox homeostasis in mitochondria during abiotic stress, Plant J. 88 (2016) 809–825.
[71] T.J. Kirby, J. Lammerding, Emerging views of the nucleus as a cellular mechanosensor, Nat. Cell Biol. 20 (2018) 373–381, https://doi.org/10.1038/s41556-018-0038-y.
[72] N. Itano, S. Okamoto, D. Zhang, S.A. Lipton, E. Ruoslahti, Cell spreading controls endoplasmic and nuclear calcium: a physical gene regulation pathway from the cell surface to the nucleus, Proc. Natl. Acad. Sci. USA 100 (2003) 5181–5186, https://doi.org/10.1073/pnas.0531397100.
[73] A.G. Prat, H.F. Cantiello, Nuclear ion channel activity is regulated by actin filaments, Am. J. Physiol. 270 (1996) C1532–1543, https://doi.org/10.1152/ajpcell.1996.270.5.C1532.
[74] T.J. Kim, et al., Distinct mechanisms regulating mechanical force-induced Ca(2)(+) signals at the plasma membrane and the ER in human MSCs, eLife 4 (2015) e04876, https://doi.org/10.7554/eLife.04876.
[75] S.A. Gudipaty, et al., Mechanical stretch triggers rapid epithelial cell division through Piezo1, Nature 543 (2017) 118–121, https://doi.org/10.1038/nature21407.