Review

The Plasma Membrane and Mechanoregulation in Cells

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ABSTRACT: Cells inhabit a mechanical microenvironment that they continuously sense and adapt to. The plasma membrane (PM), serving as the boundary of the cell, plays a pivotal role in this process of adaptation. In this Review, we begin by examining well-studied processes where mechanoregulation proves significant. Specifically, we highlight examples from the immune system and stem cells, besides discussing processes involving fibroblasts and other cell types. Subsequently, we discuss the common molecular players that facilitate the sensing of the mechanical signal and transform it into a chemical response covering integrins YAP/TAZ and Piezo. We then review how this understanding of molecular elements is leveraged in drug discovery and tissue engineering alongside a discussion of the methodologies used to measure mechanical properties. Focusing on the processes of endocytosis, we discuss how cells may respond to altered membrane mechanics using endo- and exocytosis. Through the process of depleting/adding the membrane area, these could also impact membrane mechanics. We compare pathways from studies illustrating the involvement of endocytosis in mechanoregulation, including clathrin-mediated endocytosis (CME) and the CLIC/GEEC (CG) pathway as central examples. Lastly, we review studies on cell−cell fusion during myogenesis, the mechanical integrity of muscle fibers, and the reported and anticipated roles of various molecular players and processes like endocytosis, thereby emphasizing the significance of mechanoregulation at the PM.
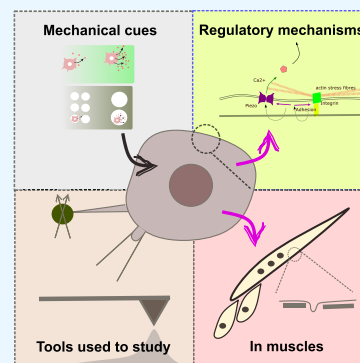
INTRODUCTION

Biological processes within cells comprise intricate networks of biochemical reactions where multiple pathways interact and communicate continuously, enabling cells to perform fundamental life functions. However, these processes do not occur in isolation; cells constantly perceive and adapt to their surroundings, with the mechanics of the extracellular environment serving as a crucial external cue. In this Review, we explore the intersection of mechanics with cellular processes mediated by the PM.

Internally, cells generate local mechanical forces to overcome thermodynamic barriers during various biological processes by altering the structure or conformation of proteins and their assemblies. Changes in membrane mechanical parameters can affect membrane protein conformation and organization and initiate signals from the membrane and cortex to downstream effectors. Mechanotransduction is the process through which cells sense external mechanical cues and transmit them through internal biochemical signaling pathways. When cells encounter mechanical stimuli, they sense, respond to, and adapt to these changes by modulating their motion, proliferation, differentiation, and deformation. This process of adaptation, or mechanoregulation, plays a crucial role in diverse physiological and pathological cellular states, including development, tissue homeostasis, repair, cell migration, and cell signaling. Understanding mechanoregulation is essential for gaining deeper insights into disease and

pathological conditions, drug development, and biotechnology and tissue engineering.

This Review will commence by providing an overview of extensive research demonstrating the role of mechanoregulation in various cellular contexts. It will subsequently highlight recurring design principles and/or molecules/pathways involved before delving into strategies and methodologies for quantifying mechanoregulation. Finally, the Review will examine two examples: the crosstalk between endocytosis and mechanoregulation in general and mechanoregulation during myogenesis.
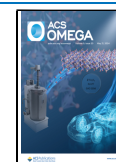
PROCESSES INVOLVING MECHANOREGULATION

Cells can experience a variety of mechanical cues that can manifest in diverse forms. These include direct compressive or stretching forces; osmotically induced changes in cell volume, such as swelling or shrinking; confinement within pores of varying diameters; adjustments in substrate stiffness or topology; and exposure to shear flow. Moreover, seemingly simple actions like deadhesion or cell-spreading also constitute
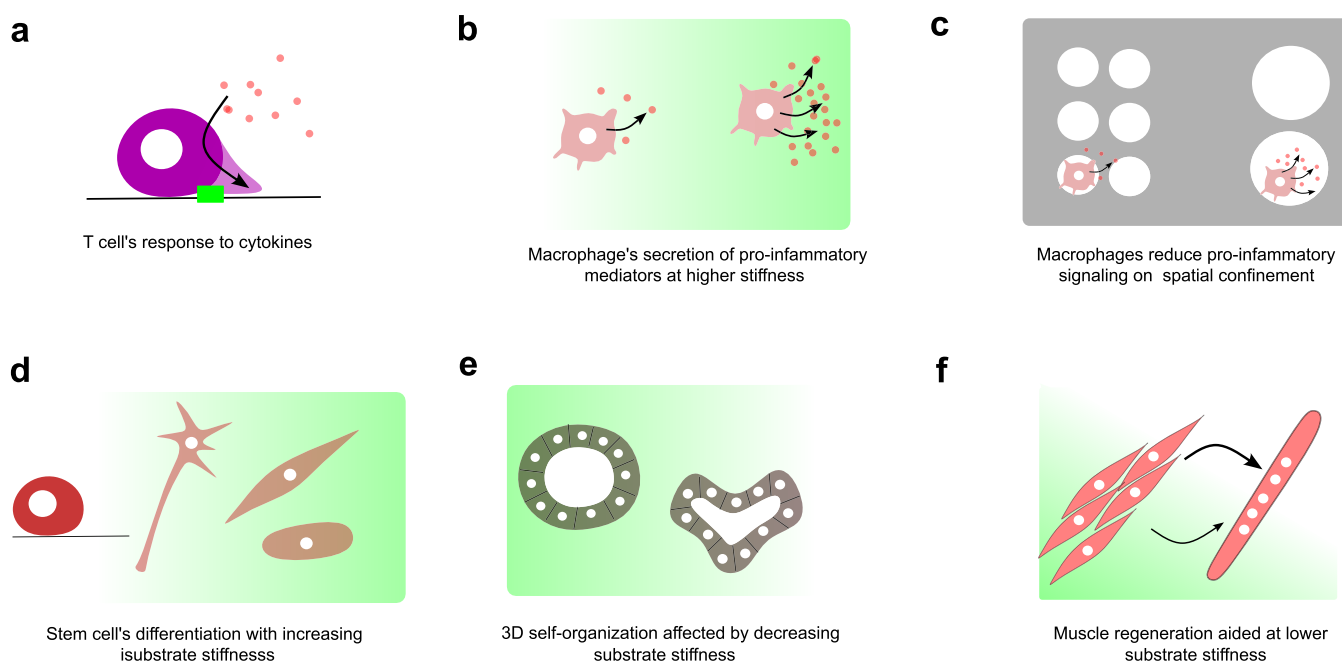
Figure 1. Instances of biological processes involving mechanosensing and adaptation. (a) T cells respond to cytokines by enhancing adhesion (depicted as green rectangle), which triggers Piezo channels and downstream effectors. (b) Macrophages increase their secretion of pro-inflammatory mediators when the substrate stiffness is enhanced. (c) Macrophages reduce proinflammatory signaling upon spatial confinement. (d) The fate of stem cells upon differentiation is determined by the substrate stiffness. Softer substrates push differentiation to the neuronal cell type, while as stiffness increases either myoblasts (muscle progenitors) or osteoblasts (make bones) are produced upon differentiation. (e) Shapes and structures emanating from 3D organization of cells into tissues depend on the substrate stiffness. (f) Muscle regeneration is aided at lower substrate stiffness.

mechanical perturbations that cells can sense and respond to accordingly. During cell development and differentiation, mechanical cues that play a vital role in cell fate differentiation and differentiation into specific tissues and organs originate from the evolving viscoelastic properties of the matrix, migration through confined spaces, stretching faced from neighboring cells, or even detachment from neighbors.[1] Similarly, different micromechanical niches inhabited by immune cells during their developmental journey affect their functioning. During their developmental process, T progenitor cells experience mechanochemical signals[2] that may originate from their altered adhesion (Figure 1a) and guide their progression through various developmental phases, including Notch signaling, β-selection, and positive and negative selection.[3] Mechanical cues can also determine the generation of immune responses; for example, the level of pro-inflammatory mediators released by macrophages (Figure 1b) grown on soft substrates is low as compared to that of macrophages grown on stiff substrates when induced with the endotoxin lipopolysaccharide (LPS).[4] A similar role of the substrate or mechano-microenvironment is also found to impact macrophages. Under several pathological conditions, macrophages are activated to induce the proinflammatory to pro-healing phenotypes for the defense and repair mechanism to set in. It has been reported that spatial confinement of the macrophages (Figure 1c) determines the level of actin polymerization. Cell crowding leads to lowered actin polymerization, and therefore the late LPS-activated transcriptional programs are suppressed by mechanosensing. Thus, confinement downregulates pro-inflammatory cytokine secretion and the phagocytic potential of macrophages.[5] Altering the substrate stiffness changes cell behavior, as observed in

NIH3T3 fibroblasts.[6] Integrin binds to the extracellular matrix (ECM), and its affinity to the substrate increases depending upon the myosin contractility pulling on actin. Faster binding on stiffer substrates leads to increased integrin clustering, resulting in enhanced traction force generation and as a result the migration of cells toward stiffer regions.[7] Since integrins are recurrent in different systems in their role in mechanotransduction, linking cell migration and the mechanical microenvironment through integrins is not far-fetched. Cells need to sense and respond to mechanical cues in their environment to properly migrate. Integrins have also been shown to have an important role in mechanoregulation during immune response[8] and the metastasis of cancer cells.[9,10]

Mechanical cues also regulate stem cells' fate for differentiation[11] and behavior during development. They influence critical phases such as proliferation, self-renewal, and differentiation (Figure 1d) into specific cell fates and the self-assembly and organization of stem cells. Cell−cell and cell−ECM adhesions, focal adhesions, and mechanosensitive ion channels are important regulators through which mechanical cues are transmitted.[12] In response to mechanical perturbations, stem cells[11] maintain mechanical homeostasis by adjusting the focal adhesion ligand affinity, changing focal adhesion assembly and disassembly, and modulating the contractility of the underlying cytoskeleton and actomyosin. Adhesion complexes and associated cytoskeleton components are responsible for activating various mechanoresponsive signaling pathways, which include the transformation of RhoA and MAPK. Downstream effectors of mechanotransduction pathways, such as Yes-associated protein (YAP) and transcriptional coactivators with PDZ binding motif (TAZ), are subsequently activated in different cell types for
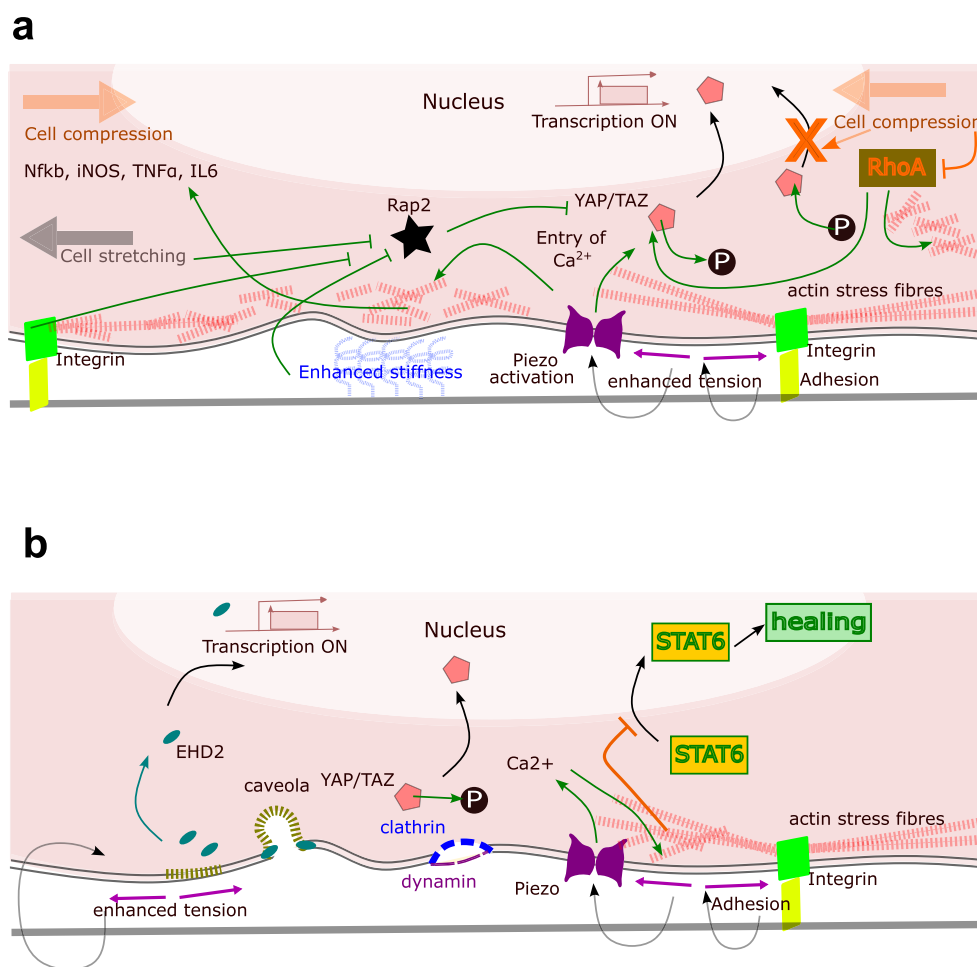
a

b

Figure 2. Molecular mechanisms of mechanoregulation. (a) Nuclear translocation of YAP is shown to be activated by the suppression of the Hippo pathway, which is facilitated by upstream mechanosignal proteins such as RAP2 (through RAP2 inhibition) or Piezo1 and triggered by factors like increased ECM stiffness, cell geometry stretching, and alterations in cytoskeletal/membrane tension. Conversely, compressive forces result in RhoA inactivation, causing the retention of YAP in the cytoplasm. Furthermore, the influx of Ca^{2+} due to the positive feedback loop between Piezo1 and actin enhances the expression of the transcription factor NFκB and inflammatory markers, including iNOS, TNFα, and IL6. (b) Piezo1 channels open to enhance Ca^{2+} influx particularly when cells are situated on stiff substrates. This influx inhibits the activation of the transcription factor STAT6 and the expression of healing markers like ARG1. DNM2 (dynamin), by influencing both clathrin lattices and branched actin filaments, also induces YAP nuclear translocation. Moreover, the flattening of caveolae under heightened tension releases EHD2, which can translocate to the nucleus and induce specific gene expression.

mechanotransduction.[13] Mechanical forces are important for the formation of patterns and organogenesis (Figure 1e).[14,15] The regenerative capacity displayed by muscle stem cells (MuSCs) has been reported to be influenced by the mechanics of the substrate. It has been shown that substrate elasticity is a potent regulator of the MuSC fate in culture. Unlike MuSCs on rigid plastic dishes (~106 kPa), MuSCs cultured on soft hydrogel substrates that mimic the elasticity of muscle (12 kPa) self-renew, exhibiting extensive muscle regeneration (Figure 1f).[16] The impact of ECM stiffness can also be seen on cell migration, which has been studied in various systems where stiffness is modulated using hydrogels like polyacrylamide[17] or alginate gels.[18] Variations of ECM stiffness exert a stretching force on the cell that changes the cell shape, which is caused by modulating the cell's adhesive area,[19] and have been observed to have a profound impact on cell behavior across several cell types, such as mesenchymal stem cells, muscle stem cells, and endothelial cells.[20]

Besides substrate rigidity and confinement, cells also respond to shear stress. It is a crucial regulator of processes in atherosclerosis, a multifactorial disease of the vasculature. Nonlaminar or disturbed flow is reported to promote atherosclerotic effects, negating the protective effect that laminar flow has on the endothelium.[21] The flow of body fluids exerts shear pressure on the cells, which in turn induces the mechanosignaling of the cell. Unilamellar flow of body fluids exerts less pressure, suppressing YAP/TAZ activities through autophagy and helping cells to be maintained in a quiescent stage that is inert to inflammatory cells. On the contrary, disturbed oscillatory flows exert higher pressure, as a result of which membrane tension is believed to be increased; this in turn activates YAP/TAZ to promote a pro-proliferative and inflammatory endothelial cell phenotype.[22]

■ INTRACELLULAR SIGNALING PATHWAYS MEDIATING MECHANOREGULATION

YAP proteins, renowned transcriptional regulators, exhibit nuclear translocation in response to mechanical stimulation, displaying activity in the spreading of cells.[23,24] This mechanosensing capability relies on actomyosin contractility,

actin capping, severing proteins, and coupling between the ECM and the nuclear envelope.[25,26]

The nuclear translocation of factors like activator YAP (Figure 2a) and coactivator TAZ is switched on by suppression of the Hippo pathway via upstream mechanosignaling proteins like Ras-related GTPase RAP2 and misshapen/MAP kinase kinase kinase kinases (Msn/MAP4Ks) or Piezo1 due to an increase of ECM stiffness, stretching of cell geometry, and alterations in cytoskeletal tension.[27] Cyclic stretch is shown to act through thrombospondin-1/RAP2 in blood vessel cells to activate YAP and promote vascular remodeling.[28] On the other hand, thrombospondin-1 acts via integrin $\alpha v\beta1$ to form focal adhesions and promotes nuclear shuttling of YAP by inactivating the RAP2 GTPase, resulting in vascular remodeling in response to the pulsatile blood flow and pressure.

Increased ECM stiffness promotes integrin (a major mechanosensor for ECM stiffness) clustering, extracellular signal-regulated kinase (ERK) activation, and Rho-mediated contractility. An increase in cell tension further increases the ECM stiffness by realigning ECM components and creating a positive feedback loop. The stimuli by stretching force due to ECM stiffness experienced by the cell results in the hyperphosphorylation[29] of the YAP/TAZ, its nuclear translocation, and suppression of contact inhibition. Rho/ROCK (Rho-associated protein kinase) signaling is activated by this mechanical stimulus, which acts as a bypass mechanism to switch the ERK pathway on (apart from the ligand-based activation). This ultimately leads to uncontrolled proliferation.[30] Compressive forces experienced by cells when cells grow too close to each other, set on the Hippo pathway, and the subsequent phosphorylation of the YAP/TAZ proteins retain them in the cytosol. Eventually, the downstream activation of the cell cycle arrest sets on, such that contact inhibition is achieved. These compressive forces (Figure 2a) are also counteracted by reducing the adhesive area, which results in cytoskeletal remodeling via crosstalk with spectrin[31] along with RhoA inactivation and YAP/TAZ nuclear translocation via Hippo-independent pathways.[32]

While integrins are mainly known to sense substrate-based traction forces due to their close connection with the cytoskeleton and the substrate, another key molecule at the PM sensing its mechanics in diverse systems is Piezo, a mechanosensitive ion channel. Piezo1 can sense membrane strain modulated by the stretch or adherence extent and in turn activates the YAP pathway, and it is an important determinant of mechanosensitive lineage choice in multipotent stem cells.[33] Piezo1 deficiency interferes with the integrin-dependent cellular motility of human T cells on ICAM-1 (intercellular adhesion molecule-1) coated surfaces. Piezo1 recruitment at the leading edge of moving T cells depends on the formation of focal adhesion, and there is an increase in local membrane tension upon activation of the chemokine receptor. Piezo1 activation aids in calcium influx, which, in turn, is essential for integrin LFA-1 (lymphocyte function associated antigen 1) recruitment to the leading edge, by activating calpain. Piezo1 thus acts as a mechanosensory protein.[8]

It has also been reported that stiff substrates generate mechanical changes in the cell and activate Piezo1 when induced by interferon γ (IFNγ)/LPS.[34] Piezo activation also increases actin polymerization through calpain activation.[35] The Ca^{2+} influx caused by the positive feedback between Piezo1 and actin upregulates the expression of the transcription factor, nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) and inflammatory markers, including inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNFα) and interleukin 6 (IL6) (Figure 2a). This in turn, promotes inflammation. Piezo1 channels also allow enhanced Ca^{2+} influx when cells are on stiff substrates, inhibiting the activation of the transcription factor signal transducer and activator of transcription 6 (STAT6) (Figure 2b) and the expression of healing markers, such as arginase 1 (ARG1).[34]

The membrane–cortex crosstalk for effective mechanosensing is also evidenced in other studies where the role of the ezrin, radixin, and moesin (ERM) complex has been explored. Several studies propose that in malignancy,[36] cells attain decreased PM tension to promote cell migration by disrupting the ERM protein complex-aided cortex–actin linkage.[37] Caveolin-1 (Cav1) and the flask-shaped invaginations they form, known as caveolae, are also proposed to act as mechanosensors at the PM (Figure 2b). Their purported role in cancer is subject to debate and remains controversial.[38] It is evident from the studies using Cav1 knockout mice that Cav1 has a pertinent role in the pathogenesis of mammary epithelial cell hyperplasia, tumorigenesis, and metastasis.[39] Cav1 remodels peri- and intratumoral microenvironments *in vivo* to facilitate tumor invasion, which is correlated with increased metastatic potency.[40] Different studies project Cav1 as a tumor suppressor that inhibits the development of mammary tumors and metastases *in vivo*, as well as growth and transformation *in vitro*.[41]

■ DYSREGULATION OF MECHANOTRANSDUCTION

Even in diseased conditions such as metastasis, the mechanical properties of the microenvironment can initiate the dysregulation of physiological processes. Cancer progression involves a coordinated early downregulation of ECM degradation. This is mediated by reducing matrix metalloproteinases (MMPs), which increase stiffness and allow rapid uncontrolled proliferation of cancer cells. The following later-stage aggressive degradation of the stiff ECM, by upregulating MMPs, a disintegrin, and metalloproteinases (ADAMs), etc., allows the cancer cells to evade stroma, infiltrate lymph nodes and blood vessels, and go through metastasis.[42]

Stretch-induced Piezo1 activation plays a key role in the cell cycle and thus dysregulation of mechanotransduction that results in an imprudent trigger of the Piezo1 channel can initiate cancer due to excessive cell proliferation.[43] Clathrin and dynamin 2 (DNM2) are the key proteins of intracellular membrane trafficking that are coexpressed at specialized adhesion and force-transmitting sites of muscle fibers called costameres (Figure 2b). These assemblies link the PM to the extracellular matrix and the contractile units of muscle, therefore mutations in these components cause several distinct myopathies. At the PM, clathrin forms large flat lattices and interacts with the costameric cytoskeleton. Clathrin depletion leads to defective costamere formation, which induces an impairment of contractile properties and causes autosomal dominant centronuclear myopathy (CNM).[44] Investigations show that the actin filaments surrounding mechanically sensitive clathrin plaques anchor a three-dimensional web of muscle-specific intermediate filaments and sequester YAP/TAZ, two nucleocytoplasmic shuttling proteins involved in muscle cell proliferation and differentiation. By virtue of shaping both clathrin lattices and branched actin filaments and by forming a complex with TAZ, DNM2 takes center stage as a central regulator of YAP/TAZ-mediated mechanotransduction

and intermediate filament organization. Dysfunction of this balance may lead to other diseases.[45] On the other hand, continuous mechanical prolonged stimulus desensitizes the Piezo1 channel, which might result in pathological conditions.[46] Recent studies have presented a contrasting role of the mechanotransducing Hippo pathway in maintaining muscle stem cell quiescence and myogenesis.[47]

■ IMPACT ON DRUG DEVELOPMENT AND TISSUE ENGINEERING

Understanding how cells respond to mechanical forces is important in drug development. Atherogenesis is promoted by mechanical-stimuli-induced miRNA-associated signaling pathways in endothelial cells. The role of several miRNAs has been illustrated in atherosclerosis, such as miR-17-3p, miR-92, miR-126, miR-712, miR-205, miR-143, miR-145, miR-31, and others.[48] Novel therapeutic approaches targeting ncRNAs and miRNAs are necessary for disease treatment. It was reported that the nuclear-YAP-regulated proliferation of small intestinal epithelial cells controls postinflammatory cell regeneration and serves as a potential therapeutic target for ulcerative colitis.[49] Skin, being the largest organ, gets subjected to physical perturbations, and this has a strong mechanotransduction machinery. The long-term exposure of tensile forces induces papillary fibroblast proliferation, ECM deposition, and transforming growth factor-β (TGFβ) expression, which in turn promotes regeneration of the skin. Mechanical stretch on fibroblast promotes hypertrophic scar formation through mechanically activated cation channel Piezo1 and thus therapeutic approaches for scars might target the Piezo1 channel.[50] The tension within the epidermis promotes gene expression through DNA methyltransferase 3A (DNMT3A) nuclear translocation, which is a potential regulator of skin repair.[51] In tissue engineering, researchers use mechanical cues to guide cell behavior and tissue formation. By understanding mechanoregulation, scientists can design better scaffolds and culture conditions for growing tissues and organs in the laboratory. Both natural ECM polymers (e.g., collagen, fibronectin, and elastin) and synthetic materials have been extensively designed and exploited to mimic diverse aspects of native cellular environments. The development of synthetic hydrogels, 3D networks of hydrophilic polymers, has been a critical advance for the mechanobiology field, as fine-tuning of individual material properties in a decoupled manner has helped to link specific material properties to cellular behaviors.[52,53] As the ECM stiffness optimally designs the substrate by modulating the cell adhesion, geometry and cytoskeletal organization, it can create artificial stem cell niches to maintain stemness or induce differentiation toward a desired fate.[14,54] Mechanical forces were employed to measure the mechanical state of the cell membrane or cortex. Compressive and tensile forces have been applied *in vitro* to 2D substrate-adhered or 3D matrix-embedded cells, most commonly by using stretchable membrane-based devices[55,56] or bioreactors that are designed to apply controlled deformations to cell-embedded scaffolds.[57]

The contractile cytoskeletal system largely controls cell and tissue morphodynamics. Controlled bending of cells has emerged by patterning contractile cells within the ECM.[58] Cells pulling on the matrix result in collagen alignment and predictable folding patterns. By altering the cell density, invaginations (inward bending), exvaginations (outward

bending), spirals, ruffles, and other patterns of the tissue can be generated.[59]

Materials integrated or coated with native proteins/fragments/peptides that bind cadherins and integrins have emerged as key methods to directly control adhesions.[60] These engineered platforms provide better control of engaging specific integrin heterodimers and cadherins with some demonstration of promoting specific cellular responses such as cell fate regulation,[61,62] proliferation,[63] cell orientation,[64] collective migration speed and persistence,[65] tissue development,[66,67] and regeneration.[62,68,69] Thus, the cell adhesion or spread area can be altered to modulate the cell fate.

Tissue/muscle/cell health is also impacted by cholesterol depletion by agents that function via acute/chronic administration and are in use pharmacologically. Of special mention is simvastatin, one of the drugs of choice prescribed for hypertension. Simvastatin has been shown to impair the phosphorylation of the insulin receptor (IRβ), Akt ser473, and S6rp and increase phosphorylation of AMPK thr172 (AMP-activated protein kinase) in both myotubes and myoblasts, which in turn impaired oxygen consumption and increased superoxide production in both myoblasts and myotubes and thus induced apoptosis. It has been explicitly proved that prolonged statin treatment reduces tensile strength, collagen, and metalloprotease content of the tendons without altering the cross-sectional area.[70] Simvastatin is also reported to impair the proliferation and fusion of myoblasts to myotubes by inhibiting the expression of the myogenic factors like MyoD. Reports show that it adversely increases the membrane permeability of myoblasts as compared to myotube formation.[71]

The surface expression of the fusogens, like myomerger, involved in myoblast/myotube fusion has been shown to display a negative correlation to the in-plane fluctuation membrane tension, which is lost on acute cholesterol depletion. Thus, it is possible that disruption of tension homeostasis on cholesterol depletion by methyl-β-cyclodextrin (MβCD), affects myogenesis.[72]

In summary, the mechanoregulation of cells is crucial for normal development, tissue maintenance, and repair processes and is implicated in various diseases. Studying these mechanical processes is essential for advancing our understanding of biology and for developing new therapeutic approaches.

■ QUANTIFYING MECHANOREGULATION

Mechanoregulation can be quantified by precisely manipulating the mechanical parameters of the microenvironment. This manipulation may involve adjusting substrate rigidity, osmolarity of the media, or pore size in cases of confinement. Additionally, it may entail applying static or cyclic stretching with control over frequency and amplitude or administering controlled compressive forces, among other methods. Once the mechanical cues are controlled (quantifiable, such as substrate stiffness in kPa), studies may or may not measure the altered mechanical properties of the PM, cytoskeleton, or traction forces exerted by cells on their substrate. However, even in the absence of these cellular measurements, the biochemical effects on cells can be assessed using fluorescence or other techniques to quantify changes in gene expression or secretion levels.

The mechanical cues used as triggers may also be uncontrolled, as seen in deadhesion or osmotic shocks.

Notably, during osmotic shocks, cells begin regulating the pressure difference experienced, leading to an evolution of mechanical infringement over time. In such cases, although many studies still do not measure the evolving membrane mechanical properties, it is advisable to do so. In the following section, we will discuss principles for measuring mechanical properties and compare which ones can be continuously monitored, which are least invasive, and which are best suited for population-level assessments.

For the PM, its tension is now believed to be crucial for processes like phagocytosis, endocytosis/exocytosis, cell migration, cell division, cellular morphological changes and polarity determination[73] in cells.

To understand the origin and impact of membrane tension in cells, it may be recalled that the PM is a quasi-fluid lipid bilayer enclosing cellular contents. An (osmotic) pressure difference gets created between the inside and outer interface of the cell/vesicle, and this pressure difference[74] leads to the generation of tension in the PM. Not only does this membrane tension modulate physiological processes but also there exists mechanical feedback[75] that cross-talks between the various intracellular physiological processes and thus modulates the PM tension.

The in-plane membrane tension of giant unilamellar vesicles (GUVs) is relatively simple measure by either extraction of a membrane tether using either optically trapped dielectric microspheres or micropipette aspiration. The same strategy may be used for cells.

Optical-Trap-Based Tension Measurement. A large variety of cellular processes involve the formation of thin tubular structures known as tethers, including membranous tails left by migrating cells, intracellular trafficking pathways,[76] and intracellular organelle transport.[77] Tubules can also be extracted from cells or vesicles by applying external forces to a bead using optical traps, as utilized for measurement of membrane apparent tension.

Tether extraction utilizes manipulation of a microsphere (bead) with focused laser light (or optical trap). The optical trap can be thought of as an invisible spring to which the bead is connected. If the bead experiences forces, the spring is expected to contract or extend, changing the position of the bead with respect to the center of the trap. The optical trap enables holding the bead in position (stopping it from diffusing away), while the motorized sample stage is used to move the cells relative to the bead. The cells are first brought close to the beads, after which they are taken farther apart at a slow constant velocity to a point where a tether may form and extend. The bead is imaged by a quadrant photodiode (QPD) or camera. Tracking its position helps to quantify the extent (Δx) to which interaction with the cell during tether extraction shifts it from the center. The static tether force, F, is derived from the phase of constant force during tether elongation. Force itself is derived as $f = -k\Delta x$, where k is the spring constant of the trap, also called the trap stiffness. The static force is independent of tube elongation and is usually in the order of a few tens of pN. The apparent membrane tension of the cell (σ_A) is derived from the static force using the Canham–Helfrich equation $\sigma_A = \frac{F^2}{8\kappa\pi^2}$, where κ represents bending rigidity and is usually assumed to be 15 $k_B T$.[78]

Note that when long tubes are extruded from cells there is an exponential increase in the tether force.[79]

When the tether extraction is done in cells, in contrast to GUVs, one has to account for the attachment of the actin cytoskeleton to the membrane whose energy of detachment also has to be paid while extracting the tether out. This tension derived from the tether force is, therefore, called the apparent tension. The extraction needs to be performed at a slow rate to enable equilibration and usually requires around a few minutes of data acquisition after the time taken to search for microspheres and successful extraction of tethers. While continuous measurement of tension in cells is possible, it is especially challenging to perform OT for migrating or spreading cells or cell sheets. In such systems, noninvasive methods (like live imaging/fluorescent-sensor-based methods, discussed later) are better suited.

Atomic Force Microscopy (AFM). AFM enables the measurement of membrane tension, cell stiffness, and response to mechanical stimuli.[80] For the measurement of tension, the same strategy is used as described for the optical-trap-based tension measurement.

AFM is also a versatile tool to do measurements in the nanoscale of surfaces and provides height information on an angstrom (Å) scale. A cantilever with a sharp tip is used to scan a sample surface. As the tip approaches the surface, the close-range attractive forces cause the cantilever to deflect toward the surface. Upon closer proximity, repulsive force takes over, leading to deflection away from the surface when contact is made. Cantilever deflections are detected using a laser beam that reflects off of the cantilever flat top. The changes in the reflected beam direction are tracked by a position-sensitive photodiode (PSPD). Consequently, when the AFM tip encounters raised surface features, the resulting cantilever deflection is recorded by the PSPD. A feedback loop may control the tip height above the surface, maintaining a constant laser position. This enables AFM to generate an accurate topographic map of the surface features. The lateral resolution of AFM is dominated by the tip apex, while vertical detection is limited by thermal fluctuations of the cantilever. The force exerted on the tip by thermal fluctuations determines the minimally measurable force. The amplitude of thermal fluctuations can be estimated by treating the cantilever as a damped simple harmonic oscillator.[81,82]

Micropipette Aspiration (MA). This technique involves using of a micropipette to apply controlled suction to a cell.[83] By measuring the deformation of the cell membrane/cortex, mechanical properties such as cell stiffness and viscosity can be assessed.[84] Precautions are required to avoid the suction of excessive area into the pipet, which can itself affect the membrane tension due to an increase in projected area. This tool is primarily used for GUVs in combination with optical traps[85] but has been also adapted for cells in a suspension culture. Uniformity and rounded morphology of the cells are required for accurate measurement, and therefore adherent cells, even on deadhesion, cannot give a proper quantification of tension.[82,86] This process follows the principle of Laplace's law and efficiently measures the overall membrane tension comprising both the cortical tension and membrane tension.

In this measurement, at the region of suction with the micropipette across a small region of radius R_P, a pressure difference is generated in a controlled manner by changing the height of fluid column connected to the micropipette. The overall surface tension σ_m is measured using $\Delta P = 2\sigma_m\left(\frac{1}{R_P} - \frac{1}{R_C}\right)$, where R_C is the resting radius of the

vesicle, R_P is the radius of the interface in contact with the micropipette tip, and ΔP is the pressure difference at which the membrane gets deformed to form a protrusion with radius R_P.

Other methods have also been employed for multiple sampling on different regions that have helped to build a 3D spatiotemporal map of the cell surface.[87]

Traction Force Microscopy (TFM). TFM measures the forces exerted by cells on their substrate. From these measurements, we can calculate the forces exerted by the basal contractile machinery of cells often associated with cytoskeleton tension.[88] TFM is a widely used technique for measuring cell forces when they are adhered to soft substrates. With this technique, quantitative stress maps with respect to the contractile force stress of an elastically deformed substrate at the cell adhesion plane are generated. Biochemically inert substrates like polyacrylamide (PA) or silicon-based gels exhibit a linear elastic behavior, which is caused by distortions produced by cell traction.[89] Thin hydrogel films, equipped with nanoscopic fluorescent beads either embedded in the substrate or attached to the surface, are popular markers employed for optical tracking in space and time.[90] Cells are seeded on such a bead-embedded elastic substrate, and once cells are well-spread, the bead positions are recorded. Note that during spreading the contractile forces generated by cells already alter the bead position but are not tracked. The first image taken after cells are well-spread is termed the cell-loaded image. Subsequently, cells are deadhered by trypsinization, which restores the gel to its unstressed state, and another reference image of beads is recorded. The vector displacement field for the substrate at each cell position is computed by mapping the displacement or deviation (in pixels) of each bead in the cell-loaded image versus the reference position as a consequence of the force exerted by the cell.[91]

With the advancement of science, Legant et al.[92,93] has devised a 2.5 D TFM method wherein the location and origin of the normal tractions with respect to the adhesion and cytoskeletal elements of cells can be modeled to consider the 3D nature of cellular forces acting on planar 2D surfaces. When the cells are seeded onto an ECM-like 3D substrate, the similar deviation of beads can be traced, and this process is called 3D TFM.[94]

TFM can be utilized even for continuous monitoring of the mechanical state of cells in which, instead of deadhesion of cells, the continuous changes on bead positions are mapped and the resulting stress maps are generated from comparing nearby time points.[95] So far it has mainly been utilized to understand the rules of motility and fingering during collective cell migration,[96] but it has been utilized to unravel the origin of forces that act as mechanosensory proteins like Piezo.[88]

Optical Stretcher/Microfluidics. Microfluidic devices can apply controlled mechanical forces to cells and tissues. These device-mediated manipulations of cells in combination with optical manipulation can provide large-scale quantitative data on the mechanoresponse.

An example of such a device called an optical stretcher involves cells (treated as dielectric objects) placed one at a time (controlled by microfluidics) between two opposed, nonfocused laser beams such that the total force acting on the object is zero but the surface forces leads to the stretching of the object along the axis of the beams. Using a top camera, the cell deformations are imaged, analyzed, and used to measure the viscoelastic properties of dielectric materials like cells. The mechanism is sensitive enough to distinguish individual

cytoskeletal phenotypes and has been shown to work on human erythrocytes and mouse fibroblasts. This device minimizes radiation damage as the lasers are not focused.[97,98]

Microfluidics is also utilized to control cell flow through channels and constrictions[99] and has helped unravel how the nucleus is protected under such mechanical shocks.

Fluorescence-Based Tension Sensors. Förster resonance energy transfer (FRET) refers to the dipole–dipole interactions of the electromagnetic fields of two fluorescent dyes or commonly two GFP variants with overlapping excitation and emission wavelengths. FRET, introduced by Förster in 1948, uses two fluorescent dyes: one as the energy donor and the other one as the acceptor. To be an optimal FRET pair, the spectral overlap between donor emission and acceptor excitation with the donor emission well separated from the acceptor emission is a prerequisite. This separation of the emission spectrum reduced bleed-through corrections. Most FRET pairs need to be placed closer than 100 Å to have nonradioactive interactions, and this has proven to be useful in measuring distances of 10−100 Å. These distances are comparable to the dimensions of most biological molecules, making FRET a widely used technique for biological applications. The efficiency of energy transfer E depends on the distance r separating the donor and acceptor, and R is called the characteristic distance of a particular FRET pair. At R, there is 50% transfer efficiency, and that is the location of steepest distance dependence given by $E = 1/(1+ (r/R)^6)$.

Shroff et al.[100] have presented a novel technique to assess the force loading in single-stranded DNA by developing the nN sensors consisting of a single-stranded DNA oligomer, with cy5 and cy3 fluorescent dyes covalently attached as FRET donor and acceptor where the DNA acted as the spring.

Biological membranes can also work as a spring that can deform and extend length under different physiological conditions. When a biological membrane is tagged with one member of a FRET-compatible fluorophore pair and another is used to tag a membrane-inserted structural protein, a genetically encoded stress-sensitive cassette is generated. With the change in the mechanical stress level on the membrane, the distance between FRET pairs is altered. Tension loading can be calculated from the decrease in efficiency of FRET. The optical readout of the FRET efficiency constitutes a direct measurement of the mechanical force loading on the sensor.

In stretch-sensitive FRET (stFRET), an α-helix is the spring linking a well-defined FRET pair of mutant GFPs called Cerulean and Venus. At rest, stFRET has a robust energy transfer with a strong emission at 527 nm and a quenched donor emission at 475 nm. Force more than 100 pN (below which GFP can withstand by virtue of its stiff β-barrels) loading at the ends of the cassette stretches the helix, increasing the distance and reducing FRET.[101,102]

Besides FRET-based techniques, fluorescence-lifetime-based techniques are also in use for quantifying mechanoregulation. Flipper-TR (FliptR) is a molecule composed of two dithienothiophenes (DTTs). When inserted in a lipid disordered membrane, they are twisted out of coplanarity by repulsion between methyl groups and the endocyclic sulfurs next to the bond connecting them. This results in the lowering of their fluorescence lifetime. On the other hand, when inserted into a lipid compact membrane, the two DTT groups are in-lane due to the conjugating electrons, leading to enhanced lifetime. As the membrane tension alters the lipid

Table 1. Comparison between All the Techniques for Quantification of Mechanics of Cells

quantification technique	parameters measured	systems to be used in	efficiency of technique	invasiveness	labeling required
OT	apparent membrane tension from tether force (few hundred pN)	cells[79] and GUVs[107]	few cells per hour	++	−
AFM	apparent membrane tension from tether force (tens of pN−nN)	bacteria biofilms and adhering bacteria,[108] cells, tissues,[80] SUVs[109]	few cells per hour	++	−
MA	membrane/cortical tension	GUVs/cells[83]	few cells per hour	++	−
TFM	traction-generated stress map	cells[88,91,95]	many cells per hour	+	−
optical stretcher	deformability and viscoelasticity	cells[97]	thousands of cells per hour	+	−
fluorescence-based tension sensors	local membrane order/compaction—basal and lateral	cells, subcellular membrane[110,111]	many cells per hour	−	++
live cell imaging and microscopy	fluctuation tension—basal membrane	basal membrane of adherent cells[105]	many cells per hour	−	−

compaction of the membrane by either stretch or compacting pressure, the fluorescence lifetime changes of this membrane bilayer intercalated FliptR probes serve as a novel noninvasive probe for in-plane membrane tension. A recent study[103] has shown using this probe the crosstalk of the localization of caveolae and the in-plane tension rear ends of the fast-moving cells. The team showed that live cells in a 3D matrix have a striking decrease in fluorescence lifetime specifically at the cell rear, indicating a significant decrease of the in-plane membrane tension at the rear of cells.

In hepatic satellite cells, the lifetime of FliptR has been shown to increase with substrate stiffness, while a corresponding rise in cell surface stiffness was observed using AFM indentation.[104]

Live-Cell Imaging and Microscopy. Another noninvasive way of mapping in plane basal membrane tension is by using interference reflection microscopy (IRM) for adherent cells. The interference pattern produced in an IRM image of a cell is due to interference between light reflected off the coverslip and the reflected beam from the PM. The refractive index of the media in between plays the most critical role, while the enhanced refractive index of the cell causes a significant reflection off the PM. Hence, the intensity of IRM at any particular pixel of the image depends on the phase difference and thus the distance between the coverslip and the reflecting membrane. IRM of spherical objects would yield Newton's rings as the interference pattern and is used for the calibration. After careful calibration, IRM thus helps to map the height (Δh) of the membrane patch at a pixel and time for every pixel and time point. This helps in mapping out the spatiotemporal height of the membrane.[105] However, even without conversion, IRM images reveal interesting features that qualitatively describe the membrane topology and the adherence pattern of the cell.

The amplitude of membrane height fluctuations in time or space can be easily quantified by the standard deviation (SD) calculated from the height time/space series.[72] However, the power spectral density (PSD) of the height time series can also be used to extract information about the effective fluctuation tension. The term fluctuation tension[106] here signifies that the interpretation is dependent on the framework of the membrane fluctuations being governed by membrane tension by affecting the PSD in a particular way as described by the following equation:

$$\text{PSD}(f) = \frac{4\eta_{\text{eff}} A k_{\text{B}} T}{\pi} \int_{q_{\min}}^{q_{\max}} \frac{\mathrm{d}q}{(4\eta_{\text{eff}}(2\pi f))^2 + \left[\kappa q^3 + \sigma q + \frac{\gamma}{q}\right]^2}$$

, where the active temperature (A), effective cytoplasmic viscosity (η_{eff}), confinement (γ), and membrane tension (σ) are used as fitting parameters.

IRM usually requires ~40−100 s of data acquisition, requires no labeling (like FLIPTR) that may cause phototoxicity, and can work for many kinds of cells—big or small, isolated, or in a monolayer—as long as there is at least around a few μm^2 of cell spread on the glass. However, as limitations, the use of a frequency-independent effect of activity (ATP-dependent nonthermal processes) and extraordinarily high viscosities (although also measured by other micropipette-based experiments) must be considered while making any inferences. Parameters such as SD and excess area, which are direct measurements without any fitting, must always be used as indicators of the mechanical state of the membrane along with effective tension. Another point to note is that though this technique is lucrative because it brings to the table a noninvasive technique for measuring the tension, this is applicable only for adherent cells.

Quantifying mechanoregulation often involves a combination of these techniques, allowing researchers to obtain a comprehensive understanding of how mechanical forces influence cellular processes. The choice of method depends on the specific research question and the mechanical aspects of the system under investigation.

Up to this point, our discussion has centered on the processes and methodologies used for measuring various mechanical parameters. In terms of the underlying mechanisms, our focus has been on molecular components that adhere to a central design principle: altering their conformation and signaling state in response to force or tension, thereby initiating a cascade of downstream events. However, mechanoregulation can also occur through changes in the shape of the PM itself, ultimately leading to the formation of pits and endocytosis or the acceptance of new membranes during exocytosis. This mechanism is believed to regulate the PM tension. Preceding endocytosis or occurring in parallel, mechanics-driven clustering of mechanoreceptors could also contribute to mechanoregulation.
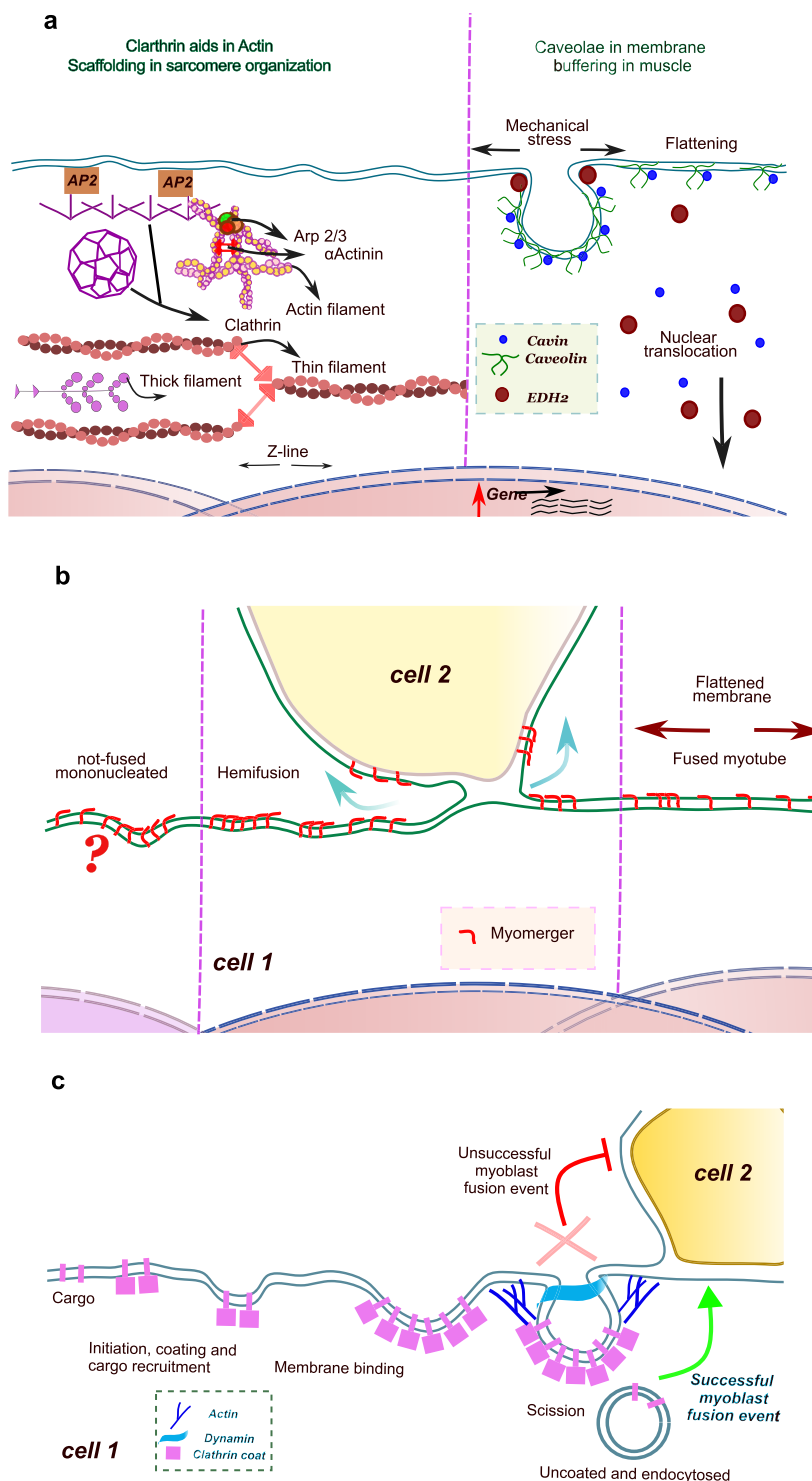
Figure 3. Molecular insights of mechanoregulation in myogenensis and muscles. (a) Depiction of the role of clathrin-coated plaques formed by the clathrin-heavy chain that in turn help the actin scaffolding at the sarcomere in myofibers (left). This subsequently aids in the formation of a mechanical link between intermediate filaments and the costameric complex. This also helps link the PM with the sarcomere. Model of the flattening of caveolae upon mechanical stress on the membrane, indicating its role as the membrane buffer (right). During flattening, these caveolae structures release components such as EHD2 and cavins that undergo nuclear translocation for modulating gene expression. (b) Depiction of the differential membrane mechanical properties exhibited by muscle cells during the process of myogenesis to form myotubes and myofibers. The diagram typically depicts the transition of myoblast to myotube and secondary fusions. Nonfused cells have higher membrane flaccidity and fluctuations, which gradually decrease through the hemifusion stage and final fusion to form myotubes. Myomerger, principal fusogens, is depicted to aid in fusion pore formation after hemifusion and the surface level expression of myomerger also changes from clusters to the diffused form through the process of myogenesis. (c) Illustration of the indispensable role of dynamin and clathrin-mediated endocytosis in cell−cell fusion by myoblasts to form myotubes. The absence of any one factor decreases the fusion propensity.

In the next section, this is further elaborated.

RECEPTOR CLUSTERING, TENSION EQUILIBRATION, AND MECHANOREGULATION

Membranes are believed to be structurally and mechanistically heterogeneous and consist of microdomains.[112] Reports suggest that the plasma membrane has a mosaic of functional microdomains of two major categories, namely, cholesterol-dependent and cholesterol-independent, which facilitate various physiological processes.[112]

Among the cholesterol-dependent microdomains, lipid rafts and caveolae are most prominent.[113,114] According to the lipid raft hypothesis,[115,116] rafts are abundant in cholesterol and saturated lipids such as sphingolipids packed together in the form of a highly ordered structure, which is marked out from the surrounding disordered unsaturated lipid regions.[114] Clustering of proteins can be driven by the reduction of the interfacial line tension at the membrane protein interface.[117,118] Thus, lipid rafts spontaneously lead to membrane protein clustering and create signaling hubs where different proteins are brought to closer proximity while also increasing their interaction time. Stochastic optical reconstruction microscopy (STORM) super-resolution imaging has revealed that mechanosensors like Piezo1 are strongly dependent on the membrane mechanical properties and lateral organization/compaction of lipids of the membrane, thus their functional dependence on cholesterol domains[119] drives their localization at the lipid rafts. Ridone and team have shown that Piezo1 forms large clusters in the presence of cholesterol in the PM and gets colocalized with CtxB (cholera toxin subunit B), a popular marker for lipid rafts. Upon the removal of cholesterol from the PM, the cluster size decreases and the diffusion rate of these Piezo1 clusters is high.

STORM imaging showed that Piezo1 can form clusters of a broad range of sizes, but only the cluster subpopulations that are disrupted were observed to be more sensitive to cholesterol level alteration in the PM.

The other similar cholesterol-dependent mechanosensing microhub of the plasma membrane is the caveolae,[113,120,121] which will discussed later. Another proposal is that the phase separation of lipids into raft and nonraft domains segregates enzymes like phospholipase D2 (PLD2) (raft-associated) from its activator phosphatidylinositol 4,5-bisphosphate $(PI(4,5)P_2)$ (nonraft) and its substrate phosphatidylcholine (PC). When raft disintegration is reduced chemically (cholesterol depletion)[122] or the sizes of nanodomains are reduced mechanically,[123] PLD activity is enhanced, leading to a mechanoresponse like enhanced actin polymerization, membrane ruffling, and macropinocytosis. Integrins are also mechanosensory proteins and are known to be organized as nanoclusters[124] whose disruption could lead to mechanosensing.

Biological membranes undergo incessant, long-range cooperative thermal shape fluctuations.[125] Fluctuations have been proposed to drive the clustering of lipid receptors (globotriaosylceramide, Gb3) bound Shiga toxin.[126,127] Recent work also shows that membrane proteins (many are signaling proteins) can be sensitive to membrane curvature and cluster at particular curvatures.[128] Mechanics-based flattening of such regions would be expected to lead to declustering and mechanosignaling.

It is also important to remind ourselves that while large-length-scale mechanical heterogeneities of membrane tension are not expected for a fluid membrane, recent studies have shown and explained[129] that heterogeneity is possible and controllable due to slow[130] or fast[131] flow of tension. When forces engage the cytoskeleton, they can travel faster than when applied solely to the membrane.[131] Rapid vesicle turnover in neuronal terminals has been shown to lead to rapid equilibration of membrane tension (within seconds). In secreting cells where membrane recycling is much slower, membrane tension does not propagate rapidly and recovers slowly due to endocytosis.[132] Thus, tension equilibration is connected to actin as well as membrane recycling requirements of the cell and adds yet another mechanism of mechanoregulation.

ENDOCYTOSIS AND MECHANOREGULATION

Tension regulation in cells is essential for maintaining different physiological processes such as endocytosis, exocytosis, migration, etc. Cells modulate tension either actively through changing cytoskeleton-membrane linkage, activating different mechanosensitive channels, and endocytosis or passively through forming membrane invaginations and blebs.[133−135] During endocytosis, a freely fluctuating membrane gets stably invaginated, reducing the excess area and thereby making a more tensed membrane.[136] On the other hand, this endocytosed membrane can be recycled back to the surface by recycling/exocytosis to reduce the elevated tension.

Clathrin-Mediated Endocytosis (CME). During CME, the PM tension functions as a major, reversible regulator.[137,138] Higher tension hinders the pit formation, while the duration of the lifetime of the clathrin coat and also the energy requirement for the formation of membrane curvature increase[139] with the increase in tension. Clathrin-coated pits (CCPs) contributing to curvature of the membrane are replaced by shallow CCPs, which are less dynamic with the elevation of tension.[140] To overcome the energy barrier, actin machinery is reported to help the internalization of clathrin pits when the membrane is in a high-tension state.[141] There is also an inverse relation reported between the clathrin coat dynamics and membrane tension. Mechanical perturbation of cells by increasing tension (by micropipette aspiration, applying hypoosmotic shock, and increasing hydrostatic pressure by squeezing the cell with a micromanipulator) reduces the endocytic activity of clathrin-mediated endocytosis.[137] In highly tensed membranes, epsin binds to adaptor protein 2 (AP2) and clathrin to form flat to dome-shaped clathrin-coated pits.[142] Increasing PM tension also alters the AP2/clathrin ratio, thereby altering the transition of flat to dome structure of pits.[143]

Over the past decades, different biochemical assays and live cell imaging techniques revealed that CME is tightly linked with membrane mechanical properties. Some studies showed that a balance between endocytosis and exocytosis is achieved by membrane tension.[75] Endocytosis is triggered when PM tension decreases locally, and inhibition of endocytosis is observed in higher-tensed membranes, which in turn triggers exocytosis. The localization, lifetime, and stability of clathrin-coated pits and the distribution of pits are dependent upon the local heterogeneities of tension.[93,137]

Early low tension in the myoblast has been reported to be essential for successful myotube formation.[72] The membrane tension, however, increases after myotube formation. There are also reports that dynamin and clathrin are required for myoblast fusion and myotube formation.[144] (Figure 3c).

Table 2. Representative Example of Endocytotic Pathways Affected by/Regulating the Mechanical State of Cells[a]

sl no.	processes	cell type	mechanical cue/ perturbation	techniques for quantification	ref
1	Clathrin-mediated endocytosis (CME)	BSC1 cells	squeezing, MA, OS	FM	Ferguson 2017[137]
		BSC-1, MDCK, and U373 cells	MA and OS	FM, SPT	Ferguson 2016[138]
		GUV		CM, EM, AFM	Saleem 2015[140]
		Saccharomyces cerevisiae		FM	Aghamohammadzadeh 2009[141]
		RPE cells, HEK 293T, epsin mutants	cell spreading on fibronectin islands, OS, MA	TIRF, CM	Joseph 2020[142]
		BSC-1	OS	inverted spinning-disk CM, TEM, STED imaging.	Bucher 2018[143]
		mice (dnn mutants), osteoclast, bone marrow macrophage, myoblast		bone reabsorption assay, EM, FM, µCT imaging	Shin 2014[144]
		U373 human glioblastoma cells		spinning-disk CM, live-cell lattice light-sheet microscopy imaging	Kural 2015[93]
2	caveolae-mediated endocytosis	MLEC, PM sphere (PMS)	cell stretching, MA, membrane tether extraction	FM, EM	Sinha 2011[175]
		mice, zebrafish, cavin-1 knockouts, transgenic zebrafish	hypo-osmotic shock	EM, CM, MC and EBD uptake.	Lo 2015[120]
		HeLa cells		FM, EM, TIRF	Pelkmans 2005[165]
3	CLIC/GEEC	CHO cells (IA2.2 cells), HeLa, mouse embryonic fibroblast (MEF) cells	PDMS mounting and cell stretching. optical tweezers	EM, EA	Thottacherry 2018[171]
		MEFs from mice		microinjection, ultrastructural immunogold analysis, FM, EM	Kirkham. 2005[172]
5	ATP-independent pathways	RBC	osmotic challenges	simulations on molecular dynamics	Marbach 2019[176]
6	cholesterol-sensitive clathrin- and caveolae-independent pathways	Vero cells, Hep2 cells	hypotonic shock	EA	Moya 1985[177]
		Ytcells, HeLa cells (dyn mutants)		EA, EM,FM.	Lamaze 2001[173]

[a]OS, Osmotic Shock; FM, fluorescence microscopy; CM, confocal microscopy; EM, electron microscopy; SPT, single-particle tracking; MA, micropipette aspiration; AFM, atomic force microscopy; TIRF, total internal reflection fluorescence microscopy; TEM, transmission electron microscopy ; STED, stimulated emission depletion microscopy; EA, endocytic assay.

In cellular processes like cell spreading, division, and migration, the altered mechanical state has been also shown to affect endocytosis, including CME. It has been reported[145] that during division membrane tension increases block endocytosis, which can be enhanced if tension is artificially reduced. It has also been shown that the high level of G-actin,[146] which enables membrane constriction for division to complete, ultimately leads to the lack of sufficient actin required for CME pit formation at high tension. Thus, during division clathrin plaques,[147] which are characteristics of flat tensed membrane with longer lifetime and low dynamics, are observed. Interestingly, it has also been shown that CME is not completely arrested during natural mitosis but instead on chemically arresting mitosis.[148] This is explained by showing the reduced availability of transferrin receptors on the rounded cell surface keeping CME machinery active at it basal level[149,150] until it stalls at metaphase[151] and regains its activity from telophase.[152] Meanshiwle, the highest plaque density[153] observed to have formed during mitosis in turn aids in respreading of daughter cells after cytokinesis.[147]

Cell migration is another process initiated via the cell spreading process and thus crosstalks with CME. At the leading edge, actin forms an actin-related protein 2/3 complex (ARP2/3)-aided branched network, namely, the lamellipodia,[154] pushing the membrane. At the trailing rear end, the actin network is crumpled by myosin and/or tension, leading to its retraction. The membrane tension difference between the leading and the rear ends is believed to ensure unidirectional migration.[155,156] It is observed that there is higher accumulation and dynamics of clathrin-coated pits at the low-tension rear edge of the migrating cell than at the higher tension leading edge.[93,157,158] However, clathrin-heavy chains have an independent role to play in the formation of lamellopodia apart from membrane trafficking, aiding scar/wave complex formation for stabilizing the lamellipodia.[159] Finally, CME affects cell adhesion during migration through internalization of integrins[160] and their redistribution at the leading edge by recycling.[161,162] It is observed that in 3D migration clathrin plays a novel endocytosis-independent role. Clathrin/AP2 lattices pinch off the ECM and form elongated tube-like structures that in turn stabilize the protrusions and help in 3D migration.[163] However, for nonmigrating conditions, it has been observed that the level of cell surface adhesion with ECM regulates the dynamics of clathrin-coated pits. At the contact/adhesion points, the clathrin-coated pits show lower dynamics than those distant from adhesions. Therefore, at the adhesion points, the endocytic rate is low. Tight junctions increase the lifetime of the clathrin plaques and reduce CME.[164]

Caveolae. Caveolae are small PM invaginations enriched in sphingolipids and cholesterol and have a diameter of 50−60 nm. They are most abundant in cells like skeletal muscle cells, endothelial cells, and adipocytes,[120] which experience continuous mechanical stresses. Caveolae are made up of caveolin (caveolin 1 (Cav1) in nonmuscle cells and caveolin 3 in muscle cells) and Cavin1. Over the years it has been shown that caveolae not only are dynamic endocytic carriers but also have a role in the mechanoregulation of cells, especially by acting as mechanosensors.[165] They have been shown to flatten themselves when there is a surge in membrane tension by hypo-osmotic shock.[38] Caveolae therefore serve as membrane

reservoirs for maintaining tension homeostasis[166] However, some studies also show that flattening of caveolae not only has a role in tension regulation but also acts in signal transduction.[121] Upon caveolae flattening, in response to a tensed membrane, EH domain-containing protein 2 (EHD2) is released. EHD2 nuclear translocations leads to regulate various genes including caveolar genes and thus autoregulation sets in[167] (Figure 2b). Caveolae also result in mechanosignaling by modulating gene expression via the IL6/STAT3 pathway.[168] Recent work has highlighted the existence of various classes of Cav1-rich membrane structures,[169,170] of which the caveolae require cavins for their stability. How these different entities invaginate the membrane, how dynamic their states of invaginations are, and how they affect the steady state as well as the response to mechanical stresses are interesting questions, some of which have been addressed; however, further investigations are essential.

Clathrin-Independent Carriers/Glycosylphosphatidyl-linositol (GPI)-Anchored Protein-Enriched Early Endocytic Compartments (CLIC/GEECs). The CLIC/GEEC (CG) pathway is one of the dynamin-independent endocytic pathways that is responsible for the majority of the cell's fluid phase uptake.[171] In mouse embryonic fibroblasts, it is believed to take <15 min to recycle the membrane area of the whole cell by CLIC/GEEC endocytosis.[172] Therefore, the PM tension regulates membrane trafficking, decreasing tension can induce the CG pathway. However, this regulation of endocytosis acts via a focal adhesion mechanosensory protein, vinculin. The decrease in tension can be sensed by vinculin, which can act rapidly to upregulate the CG pathway.

ATP-Independent Passive Pathways. Evidence shows that there are some novel cholesterol-sensitive clathrin-independent and caveolae-independent pathways that aid in some receptor endocytosis, like the IL2 receptor.[173,174]

■ MECHANOREGULATION DURING MYOGENESIS

Skeletal muscle myoblasts are a lucrative system that displays a concerted mechanoregulation for successful myogenesis. It has been proposed that at the early stages of myogenesis there is a strong correlation between the low in-plane membrane tension and the clustered surface myomerger fusogen (Figure 3b). This correlation is disrupted later when myotubes are formed where the membrane tension is high accompanied by the diffused surface expression of myomerger, indicating strong mechanoregulatory signaling to be active, which, however, is not completely understood. The syncytial myotubes, which are in turn the precursors of the multinucleated myofibers, exhibit high basal membrane tension as shown recently,[72] carrying out the physiological processes like endocytosis that involve an initial low membrane[136] tension via some unique mechanoregulatory crosstalk that is yet to be deciphered. However, there is clear evidence that skeletal myofibers[178] have two distinct recycling and the lysosome-directed pathways operative in the I band region, with the sorting endosomes distributed in a crisscross-striated fashion and the recycling and late endosomal compartments exhibited perinuclear and interfibrillar distributions. The importance of CME in myofibers comes from the fact that the clathrin-heavy chain (CHC) contributes to the formation and maintenance of the contractile apparatus by interacting with costameric proteins. The absence of the CHC therefore has been related to neurological and pathological disorders[44] (Figure 3a). The difficulty of myoblast cells from patients with caveolinopathies in buffering the membrane

tension and carrying out physiological processes like endocytosis, T-tubule network formation, muscle contraction, and muscle strength indicated the indispensable role of caveolae in mechanoregulation and mechanoprotection in myotubes and myofibers.[179]

Even for skeletal muscle regeneration and development, membrane mechanics play a crucial role. Piezo1 channels (mechanoregulated Ca^{2+} channels discussed earlier) have been recently reported to have a role in myogenic precursor expression that in turn helps in skeletal muscle regeneration and development.[180] Through knockdown assays and complementation assays employing Yoda1, it has been determined that Piezo1 aids in myoblast fusion, which is a crucial step for muscle development. The other process regulated mechanically is the formation of a hemifusion stalk[181] during myoblast fusion. Hemifusion requires a myomaker, while the final pore formation is aided by a myomerger. Reports suggest that the myomerger works by producing a positive curvature to the membrane, but this mechanical induction is aided by lipid composition alteration at the site of pore formation to lead to successful fusion.[182,183]

Hemifusion is a stochastic process involving opening and closing of the hemifusion stalk at the PM.[184] To promote the fusion of myoblast cells, the flipping of phosphatidylserine (PS) from the inner to outer leaflet at the hemifusion stalks is believed to play an important role. This transient flip of PS initially blocks Piezo1, then once the fusion stalk is formed ATP11A and CDC50A (phospholipid flippase chaperone) lead to PS translocation to the inner leaflet. This activates Piezo1, which in turn activates the RhoA/ROCK-mediated actomyosin complex along the lateral cortex of myotubes to promote the polarized elongation of myotubes.[183]

At the early stages of differentiation, caspase-mediated ATP11A degradation takes place, but the phospholipid scramblase that equilibrates different phospholipids between the two leaflets of the PM bilayer remains active. Thus, the proportion of PS on the outer leaflet increases transiently. These PS molecules now interact with the more hydrophilic Helix 1 (exoplasmic domain) of the myomerger, making it ordered and helical. However, hydrophobic Helix 2 is intrinsically ordered and supplements the membrane-stressing property of Helix 1. This change in conformation of Helix 1 makes it more potent when causing the positive curvature of the PM at the point of insertion and thus helps in fusion.[183,185] With the gradual increase in ATP11A at later stages, the PS gets translocated to the inner leaflet, which activates the Piezo1 channels. Piezo1-mediated activation of RhoA/ROCK kinases helps in the phosphorylation (at Thr-18 and Ser-19 residues) mediated activation of myosin light chain 2 (MLC2). MLC2 activation, primarily at the lateral cortex, leads to polarized actomyosin reorganization, leading to polarized myotube formation.

■ CONCLUSION AND FUTURE OUTLOOK

Numerous instances of processes involving mechanoregulation have been extensively explored, revealing that even deep biochemical processes can be influenced by the mechanical environment. Mechanosensors underlying these processes may utilize membrane or cytoskeleton tension to alter their conformation and initiate downstream signaling, ultimately leading to changes in gene expression patterns. Given the manipulatable nature of mechanical parameters in the environment/substrate, understanding which pathways are

ultimately affected presents an opportunity for intervention.[186] This understanding is particularly relevant for drug delivery methods that rely on endocytosis, where controlling the rates of endocytosis through mechanical means could aid in the design of tools for nanodrug delivery. Despite the abundance of available information, certain fundamental insights into basic processes, such as those in myotubes, remain elusive. While cell fusion relies on fusogens that coordinate hemifusion and final fusion processes, we lack an understanding of the extent to which fusion events depend on local mechanics and how constitutive endo- and exocytosis events are spatially arranged to facilitate or hinder the fusion process. Moreover, the impact of the local microenvironment on global cell properties in nonmuscle cells remains largely unexplored. Questions such as the minimum patch of stiff substrate[187] that can be sensed by cells are ripe for exploration, with experiments demonstrating energy-based decision-making in cells offering valuable insights about both globally different substrates as well as substrates with heterogeneous mechanical properties.[188] These experiments could be integrated with smart surface/3D-matrix engineering approaches to address such inquiries effectively.

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