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Review

The cell as matter: Connecting molecular biology to cellular functions

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SUMMARY

Viewing cells as matter to understand the intracellular biomolecular processes and multicellular tissue behavior represents an emerging research area at the interface of physics and biology. Cellular material displays various physical and mechanical properties that can strongly affect both intracellular and multicellular biological events. This review provides a summary of how cells, as matter, connect molecular biology to cellular and multicellular scale functions. Within the areas of cell biology and molecular biology, we review recent progress in utilizing cellular material properties to direct cell-fate decisions in the communities of immune cells, neurons, stem cells, and cancer cells. Finally, we provide an outlook on how to integrate cellular material properties in developing biophysical methods for engineered living systems, regenerative medicine, and disease treatments.

INTRODUCTION

The cell was first introduced as a material concept describing microscale chambers by Robert Hooke in 1665.[1,2] After several hundreds of years' rapid development of biochemistry and molecular biology, researchers have identified many essential biomacromolecules contained inside cells.[3] In such a tiny cell structure with a typical size of ~10 μm in diameter and ~1 pL in volume, there are as many as 42 million proteins, 3.6 million messenger RNAs, and 20,000 to 30,000 genes.[4,5] An immediate question coming to us is how such a tiny microscale cell chamber hosts such a large amount of biomacromolecules?[3] Besides, inside the cell, is it more like an ocean of diluted biomolecules, or a dense magma of colloidal bioparticles, or even a solid scaffold of biopolymers?[3,6] To answer these questions, measuring and understanding cellular mechanical properties will provide critical insights into the physical nature and material properties of cells.[7] More importantly, these material parameters of cells will strongly affect the efficiency and equilibrium of the reactions among biomolecules encapsulated inside cells.[8] Based on the types of involved molecules and chemical principles, those reactions are categorized into signaling, transcription, translation, and epigenetic modification, among others. These essential intracellular events regulate cell fate, along with which alterations in cellular physical properties have been observed simultaneously.[9–12] Furthermore, certain cell material properties have been discovered to be markers or regulators of cell functions. For instance, a larger nucleus-to-cell area ratio has been observed in embryonic stem cells compared with differentiated cells;[13] a greater deformability has been reported in malignant cancer cells compared with benign cells;[14] the stiffness of mesenchymal stem cells can influence their outcome of differentiation.[15] Moreover, from an integrated perspective, cells with different material properties assemble in space and time and collectively function as a multicellular living system. The self-organization and maintenance of dynamic spatiotemporal distribution of cells with different

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material properties are essential for higher-order functions at the multicellular tissue level, such as in embryos, organoids, and tumors.

In this review, we first introduce several essential cell material properties, explain their relationships, and further highlight a few overlooked material properties (e.g., molecule crowding and non-linear mechanics) that potentially directly influence biochemical events and cell functions. In the next part, we review how the intracellular biological events behave differently as cellular material properties vary, and further explain the underlying physical chemistry principles. In the third part, we discuss how cellular material properties direct cell-fate decisions, taking several common types of cells to illustrate. Finally, as a survey of ongoing studies, we highlight the significance of cell material properties in building up the functions of multicellular living systems. Furthermore, we also provide outlooks toward the types of biological systems in which the cellular material properties may play an important role, and how to integrate these cellular material properties in developing engineering approaches for regenerative medicine and disease treatments.[16] We hope that this review provides a new material perspective to understanding cellular events (e.g., signaling, chromatin regulation, phase separation), bridges the gap between the intracellular molecular events and multicellular functions, and shortens the distance between the fundamental biological studies and engineering applications.

PHYSICS AND MECHANICS OF CELLULAR MATERIALS

Cell as a viscoelastic material

The cell has long been considered as a viscoelastic material. When subjected to high-frequency forces or deformations over a relatively short timescale, the cytoplasm behaves as an elastic solid; under low-frequency or relatively slow loadings, the cytoplasm instead relaxes and thus behaves as a viscous fluid.[17–19] It is known that cell viscoelastic behavior has wide implications in a variety of physiological and pathological processes such as cell migration, embryonic development, and cancer invasion.

To probe the viscoelasticity of a material, stress relaxation test upon a step displacement and creep test upon a constant force application have traditionally been used. These measurements allow the determination of characteristic relaxation times of the sample, and specific material constants (such as elastic modulus and viscosity) if certain material models are used. Alternatively, dynamic mechanical measurement has been used to directly characterize the rheological properties of viscoelastic materials. In this measurement, input (stress or strain) is imposed at a certain frequency, and output (strain or stress) is recorded; the ratio between stress and strain yields a frequency-dependent complex modulus, $G(\omega)^* = G'(\omega) + iG''(\omega)$, where G' and G'' represent elastic modulus and loss modulus, and i is the unit imaginary number. This dynamic mechanical analysis has been often applied to study a cell as a material, and it has been widely shown that G' of cells is much larger than G'' in the frequency range of ~0.3 Hz and above. This is consistent with the apparent relaxation time measured in cells, on the order of seconds, if the stress relaxation curve is fitted crudely to a single exponential function.[19]

Although the stress relaxation measured in cells can be fitted with a single exponential function, it is now widely accepted that the viscoelastic response of living cells does not have a dominating timescale that can be associated with specific structural elements or processes. The cell response (such as complex modulus) instead follows a power-law relationship $|G(\omega)| \propto \omega^{\beta}$ over a broad frequency range

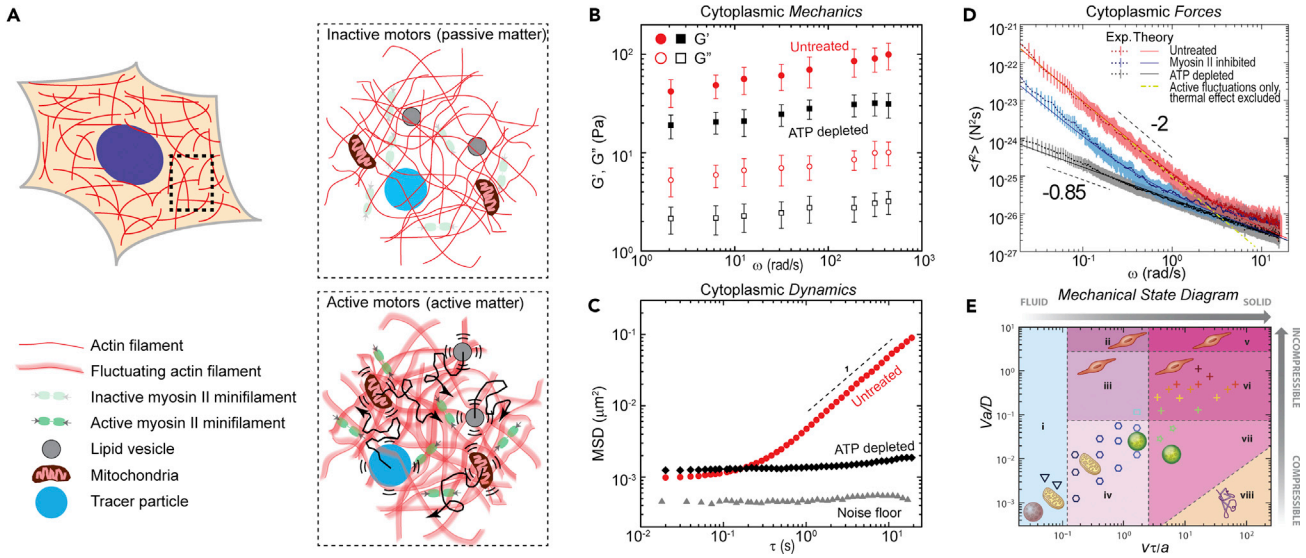

Figure 1. Cells as a soft material exhibiting unique mechanical properties

(A) Schematic illustration of cell compositions as passive matter or active matter. Reprinted with permission from Guo et al.[20] Copyright 2014, Elsevier.

(B) Diagram showing cytoplasmic mechanics as a function of frequency. Reprinted with permission from Gupta and Guo.[23] Copyright 2017, Elsevier.

(C) Diagram showing cytoplasmic dynamics, in terms of mean squared displacement (MSD). Reprinted with permission from Gupta and Guo.[23] Copyright 2017, Elsevier.

(D) Diagram showing cytoplasmic forces, measured by force spectrum microscopy (FSM). Reprinted with permission from Guo et al.[20] Copyright 2014, Elsevier.

(E) State diagram summarizing different cytoplasmic mechanical behaviors, as either viscoelastic, poroelastic, pure viscous, or pure elastic materials, dependent on two dimensionless parameters, Deborah number ($V\tau/a$) and Péclet number (Va/D). Reprinted with permission from Hu et al.[19]

(Figures 1A and 1B).[20–23] β equaling 0 and 1 represents pure elastic behavior and pure vicious behavior, respectively. In cells, measured values for β are typically between 0.1 and 0.5.[20–25]

Cell viscoelastic response is known to depend on the state of the cytoskeleton and can differ markedly during development and diseases.[26,27] Therefore, the cellular viscoelasticity provides a unique mechanical fingerprint of the state of the cell. The cytoskeleton of mammalian cells is composed of three major biopolymer networks, forming an interpenetrating network. Both filamentous actin (F-actin) and microtubules are dynamic networks that are constantly undergoing reorganization and repolymerization. Disrupting F-actin or microtubules in mammalian cells leads to cell softening.[28–30] In contrast, cytoskeletal intermediate filaments have a much slower turnover process and thus have been considered as a major structural component maintaining cell mechanical integrity. While disrupting cytoskeletal intermediate filaments also changes the viscoelasticity of cells, recent studies show that they play a critical role in determining the non-linear mechanics of cells as they can be deformed to a large extent.[25,31,32] Indeed, it has been shown that cytoskeletal intermediate filaments determine the stretchability, strength, and toughness of the cell.[25]

Cell as a poroelastic material

The cytoplasm of cells can also be treated as a biphasic material consisting of a porous solid structure (cytoskeleton) bathed in an interstitial fluid (cytosol).[19,33] Materials with similar structures such as synthetic hydrogels can exhibit a poroelastic behavior, which is a result of redistributing background fluid in the scaffold when the material is subjected to deformation. In contrast to the length-scale independent

viscoelasticity, poroelasticity depends on the characteristic length scale of the deformation. This behavior has only recently been explored in cells demonstrating that living cells can behave like a poroelastic gel at relatively short timescales,[19,33,34] whereby the mechanical response of cells is predominantly determined by the migration of cytosol through cytoskeletal networks to homogenize the pore pressure. Whether poroelastic response appears depends on the comparison between the characteristic timescale of observation and the poroelastic relaxation time ($t_p \sim L^2/D_p$), where L is the characteristic length scale of the deformation and D_p is the poroelastic diffusion coefficient. When the experimental timescale is comparable with t_p, poroelasticity will play a role in the cellular mechanical response. Recently, Hu et al. presented a mechanical state diagram summarizing all possible mechanical states in living cells, including viscoelasticity, poroelasticity, and pure viscous and elastic responses, and explained the underlying mechanism for cellular behavior to transition from a fluid to a solid, as well as from an incompressible material to a compressible material (Figure 1E).[19]

In the framework of poroelasticity, cellular mechanical properties comprise the effects of the interstitial fluid and related volume changes, macromolecular crowding, and the cytoskeletal network. In this framework, the effect of cellular water content and cytoskeletal variations on cellular rheology can be understood through a simple scaling law $D_p \sim E\xi^2/\mu$, where E is the drained elastic modulus of the cytoskeleton, ξ is the characteristic pore size of the cytoskeleton, and μ is the viscosity of the cytosol. It has been observed that the application of hyperosmotic pressure resulted in a decrease in the poroelastic diffusion constant D_p and an increase in cellular elasticity E, by reducing cellular water content and, thus, cytoplasmic pore size.[33] Interestingly, recent studies found that cellular mechanical properties inversely correlate to cell volume;[35] this is because increases in cell volume via water influx decreases the concentration of macromolecules and thus the degree of molecular crowding. Besides cellular water content, disruption of cytoskeletal components such as F-actin and intermediate filament, as well as inhibition of myosin II motors, strongly affect cellular poroelasticity.[19,33]

Cell as an active, non-equilibrium material

Cells are not functioning at thermal equilibrium but instead are powered by adenosine triphosphate (ATP) hydrolysis, which drives cells far from equilibrium. This deviation from thermal equilibrium is a result of a wide variety of forces generated within the cell cytoplasm,[36–38] a majority of which can be attributed to the operation of molecular motors such as kinesin and dynein that typically are responsible for driving directional cargo transport along the microtubules and myosin II motors that actively contract actin filaments.[37–39] The cooperative activity of these motors and other active processes in the cytoplasm leads to critical cellular functions such as contraction, division, and migration.[40–44] Another consequence of the activity of molecular motors is the active fluidization of the cytoskeleton, which is considered essential in regulating cell motility.[45] For example, myosin II has been found to actively control the viscoelasticity of an entangled F-actin solution. The interaction of myosin and actin filament significantly shortens the viscoelastic relaxation time of an entangled F-actin solution, which could be attributed to the longitudinal motion of actin filaments driven by myosin II minifilaments.[45] A living cell also tends to fluidize when subjected to shear or stretch, but later slowly resolidifies.[46] This fluidization behavior exhibited by cells is in striking analogy to the dynamics of inert glassy systems such as hard sphere colloids, which can be captured by the soft glassy rheology theory.[21,47–51] However, unlike other inert systems, the

constellation of out-of-equilibrium features of the living cell and cytoskeleton is very rich, and appears to describe a glassy matrix close to a glass transition.[46]

The average effect of all the motors and active processes also contributes to an incoherent background of fluctuating forces that is associated with the functional efficiency and the complete metabolic state of cell.[40] These overall fluctuating forces can give rise to random motions of intracellular components (such as organelles and exogenous inert objects), which look similar to Brownian motion. To quantify these intracellular forces, force spectrum microscopy (FSM) has been recently introduced to measure the frequency spectrum of intracellular force fluctuations, which quantitatively describe the dynamic state of the cell (Figures 1A and 1D).[20] This method demonstrated that force fluctuations are 3 to 5 times larger in malignant cells than in their benign counterparts, implicating the role of cytoplasmic activity in cell physiology in healthy and diseased states.

Interestingly, using optical-tweezers-based active microrheology, recent studies demonstrated that cells are only at non-equilibrium at relatively low frequencies ($f < 5$ Hz) corresponding to a relatively long timescale ($t > 0.2$ s), and can still be considered as a thermal equilibrium material at high frequencies ($f > 5$ Hz) corresponding to a relatively short timescale ($t < 0.2$ s) (Figures 1B and 1C).[23] Indeed, using FSM, the spectrum of force fluctuations overlaps with thermal noise at high frequencies. This potentially enables the application of passive microrheology to determine cellular rheology based on spontaneous fluctuations at high frequencies.

CELL AS MATTER HOSTS CELLULAR SIGNALING, TRANSCRIPTION, AND EMERGING BIOPROCESSES

The current understanding of how cells sense their surrounding mechanical microenvironment relies on the identification of key mechanosensors and their downstream effectors that transduce extracellular signals to nuclear gene expression, which is often referred to as mechanotransduction.[11,12] Mechanistically, this understanding is within the framework of biochemical signaling and regulation.[11] Meanwhile, biophysical studies reveal that cell mechanics are also regulated by their extracellular mechanical cues, including shear force, stretch, and compression. These previous works suggest that regulations of cellular material properties and biochemistry are in parallel, in response to the mechanical cues in the microenvironment. In this section, we review the emerging concepts from a material perspective that bridge the gap between cell mechanics regulation and mechanotransduction. Instead of searching for particular receptors or sensors upstream on the cell membrane, we discuss the physical properties of the cell interior as a regulator altering the equilibrium and rate of intracellular biochemistry on the molecular level. This provides us a new perspective from which to understand those biological consequences of mechanical cues that lack identified upstream receptors/sensors, and to construct regulatory loops (both forward and backward) between cellular mechanical/physical properties and cellular signaling for developing multicellular tissue systems.

Intracellular space translating signaling processes

Molecular crowding governs the activity of signaling pathways

The majority of signaling processes, RNA translation, protein maturation, and organelle formation occur within the cytoplasmic space. These biochemical reactions have been confirmed to be sensitive to their physical microenvironments (e.g., temperature, ionic strength, diffusivity), all of which can dramatically change upon mechanical, physical, and electrical stimulations. Among all these physical parameters of the cellular space, molecular crowding is a long-known yet overlooked characteristic of

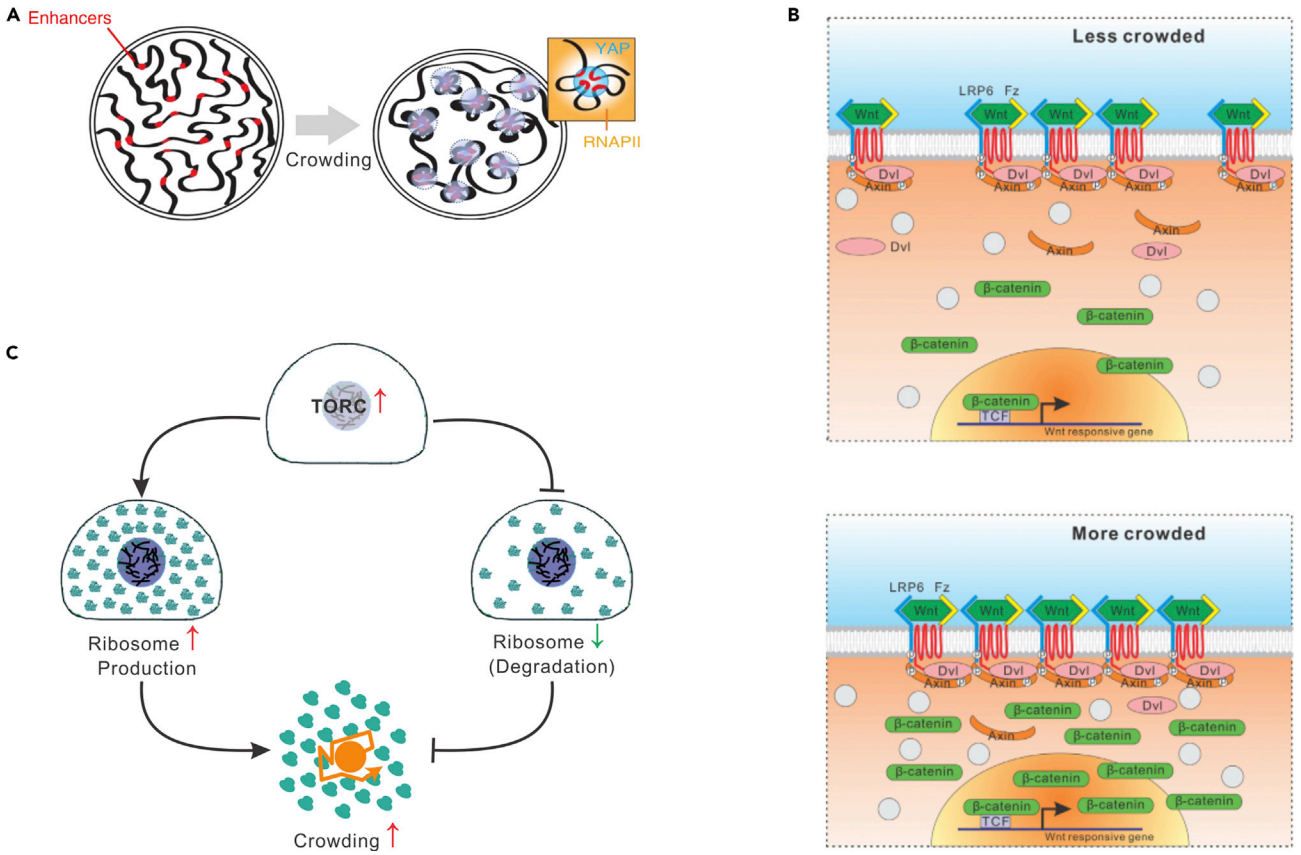

Figure 2. Interplays between molecular crowding and cellular signaling transduction

(A) Schematic illustration of the regulation of activation of YAP target genes by cellular/nuclear molecular crowding. Reprinted with permission from Cai et al.[59] Copyright 2019, Springer Nature.

(B) Schematic illustration of the regulation of Wnt/β-catenin signaling via LRP6 signalosome by intracellular molecular crowding. Reprinted with permission from Li et al.[57] Copyright 2021, Elsevier.

(C) Schematic illustration of the regulation of intracellular molecular crowding by mTOR signaling via tuning of intracellular ribosome concentration.

the cell interior. Over the last few decades, molecular crowding has been confirmed to be a critical factor affecting both the rate and equilibrium of biochemistry, in both *in vitro* tube reactions and synthetic cell-free systems.[6,8] More recently, molecular crowding of the cellular interior has been shown to regulate cell mechanics.[35] When the volume fraction of cellular materials increases as a result of water efflux (corresponding to an increase in the degree of intracellular molecular crowding) upon various extracellular cues, both the cell cortex and the cytoplasm stiffen. Furthermore, recent studies demonstrate that cell mechanics and intracellular molecular crowding can be tuned by a variety of physical cues, such as stretch, compression, osmotic pressure, confinement, substrate stiffness, and cell spreading.[35,52–55]

In addition to physical regulations, a more recent study demonstrated that biochemical signaling also regulates intracellular molecular crowding.[56] The mammalian target of rapamycin (mTOR) pathway is known as an intracellular signaling pathway that is important in regulating the cell cycle and is directly related to cellular quiescence, proliferation, cancer, and longevity. Delarue et al. recently found that mTOR complex 1 (mTORC1) kinase controlled ribosome abundance through a combination of cell volume regulation, ribosome biogenesis, and autophagy (Figure 2C).[56] As ribosomes account for ~20% of the total cytosolic volume, modulation of their

concentration had a dramatic effect on the intracellular molecular crowding. As direct evidence, inhibition of mTORC1 doubled the effective diffusion coefficient of nanoparticles of 20 nm in diameter. As a result of changing concentration and diffusion coefficient, previous *in vitro* and cell-free synthetic studies suggested the increased molecular crowding led to a higher rate of biochemical reactions.[6,8] Consistent with this, Li et al. found that the increased degree of molecular crowding promoted the binding between LRP6 and Axin in colon cancer cells, which are key receptors and key cytoplasmic effectors in Wnt/β-catenin signaling.[57] The engagement of LRP6 and Axin led to the accumulation of β-catenin and eventually augmented the expression of Wnt target genes. Thus, a direct consequence of the increased molecular crowding was the elevated canonical Wnt/β-catenin signaling.[57,58] A similar promotion effect of molecular crowding has been confirmed in Yes-associated protein (YAP) target signaling. Cai et al. confirmed that the mRNA levels of YAP target genes increased when they increased molecular crowding using osmotic compression on mammalian cells (Figure 2A).[59] In summary, by hosting signaling pathways, the degree of intracellular crowding as one aspect of cellular material properties may directly affect functional gene expression and thus cellular behaviors such as self-renewal and differentiation.

Mechanosensitive phase separations

In addition to regulating the kinetics of association and disassociation of two proteins, the high-order and non-linear reactions involving multiple molecules/proteins are believed to be more sensitive to their physical microenvironment such as molecular crowding. Indeed, Li et al. found that the increased molecular crowding not only resulted in a higher binding ratio between LRP6 and Axin but also led to the formation of a large molecular condensate complex, as shown by the evidence that proteins such as LRP6 exhibited a molecular weight much larger than a single isolated protein (Figure 2B).[57] Indeed, by visualizing the formation of high-order assembled LRP6 signalosome, known as Wnt-induced clustering of the Fz-LRP6 complex that inhibits degradation of β-catenin, the authors confirmed that the increased degree of intracellular crowding promoted signalosome formation. These higher-order assemblies of biomolecules currently draw much attention due to their unique properties in regulating signaling or transcription in a manner of spatial heterogeneity and non-linear response. The process of forming these higher-order assemblies has been recently understood as phase separation, known as the creation of two distinct phases from a single homogeneous mixture of biomolecules. Both the concentration of the biomolecules and the relative crowdedness of their microenvironment played a critical role in regulating phase separation. Indeed, Cai et al. showed that the elevated expression of YAP target genes by molecular crowding was a result of promoting the phase separation of YAP (Figure 3A).[59] It has been confirmed that molecular crowding induced phase separation both *in vitro* and *in vivo*. More interestingly, cells in the kidney tissue were located at a native microenvironment with varying degrees of hyperosmotic pressures, leading to varied condensations of intracellular materials. For example, cells in the medulla region, where osmolarity was high, exhibited punctate YAP located prominently in the nucleus region. Meanwhile, cells in the cortex region, where osmolarity was isotonic, exhibited restricted homogeneous YAP distribution in the cytoplasm. As noted, the punctate YAP could be transported into the nucleus and maintained there for long-term target gene expression. In line with this, a more recent study showed that the intracellular phase separation of processing bodies (PBs) upon osmotic cell volume change is rapid and reversible.[52,53] The phase separation of PB protein DCP1A occurs in mammalian cells within a period of ~10 s during hyperosmotic cell volume compression, whereby its dissolution happens upon isotonic rescue in a timescale of ~100 s[52] This unique

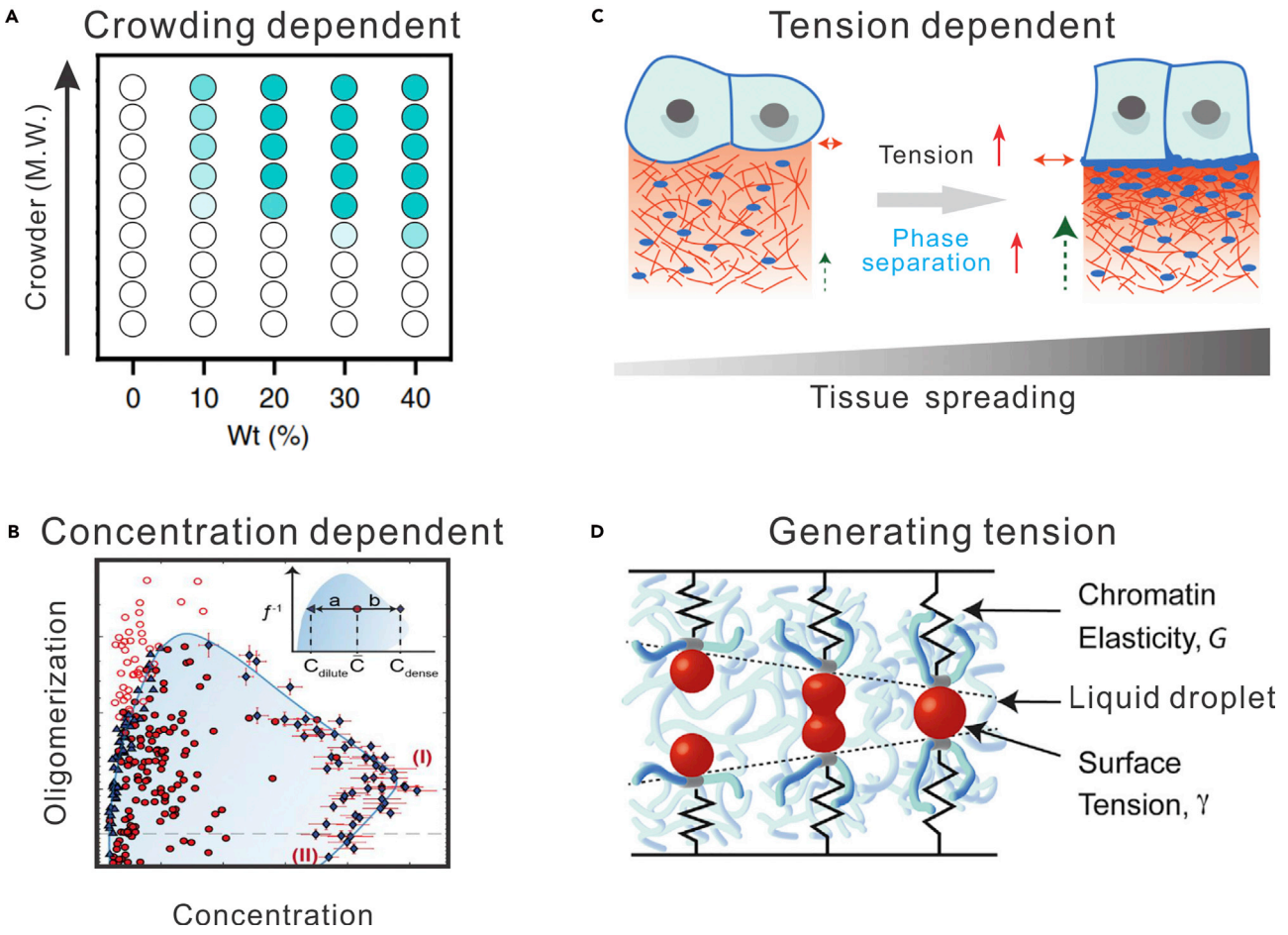

Figure 3. Regulation of cellular phase separation of biomacromolecules in a cellular physical property-dependent manner

(A) State diagram shows crowding dependent phase separation of YAP protein. Reprinted with permission from Cai et al.[59] Copyright 2019, Springer Nature.

(B) State diagram shows the concentration- and oligomerization-dependent phase separation of cellular biomacromolecules. Reprinted with permission from Bracha et al.[70] Copyright 2018, Elsevier.

(C) Schematic illustration of tension- and tissue-spreading-dependent phase separation of proteins in an embryo. Reprinted with permission from Schwayer et al.[60] Copyright 2019, Elsevier.

(D) Schematic illustration shows that the phase separation of biomolecular liquid droplets also generates force by surface tension to regulate genome architecture in cell nucleus. Reprinted with permission from Shin et al.[63] Copyright 2018, Elsevier.

mechanosensing character of phase separation has been observed with many other biomolecular condensates in responding to various types of external mechanical cues. For instance, intracellular flow driven by actomyosin contractility can transport non-junctional Zonula Occludens-1 (ZO-1) protein toward tight junctions within embryonic epithelial tissue (Figure 3C).[60] ZO-1 can then undergo phase separation in response to the epithelial spreading and intra-embryo flow. This work reveals a new role of intracellular physical events in the mechanosensation of cell-cell junctions. This subcellular localized phase separation has also been found in mesenchymal cell mechanosensation. In another recent work, Wang et al. showed that the spreading of mesenchymal cells recruited LIMD1 toward focal adhesions.[61] The increased concentration of LIMD1 led to its phase separation and downstream regulation of cellular mechanics and durotaxis. In addition, the phase separation of biomolecular condensates can enable direct mechanosensing in the nucleus for the regulation of gene expression.[52,53,62] Shin et al. showed that various intrinsically

disordered proteins (IDPs) phase-separated into liquid droplets, which mechanically excluded chromatin as they grew (Figure 3D).[63] Because interfacial tension could drive coalescence of two biomolecular condensates of IDPs, these IDP condensates thus could physically pull in their targeted and associated genomic loci together while pushing out non-targeted regions of their neighboring genome. Other mechanosensing biomolecular condensates, including Ajuba-Warts complex[64] and integrin cluster,[12,65,66] have been reported by other researchers. In addition to the recent interest in cellular and nuclear phase separation, phase separations in two-dimensional (2D) biological membranes have been observed and discussed for many years.[67] The idea is based on the fact that cell membrane is a mixture of lipid species and inserted proteins. The different interaction energies of lipid and protein species drive transitions between ordered and disordered phases, which is termed phase separation in biological membrane. Specialized membrane phases enriched in cholesterol and sphingomyelin might cluster signaling receptor proteins together to enhance signaling activation, or sequestrate these proteins in separate phases to inhibit signaling transduction.[67,68] This phase separation in biological membranes is also believed to depend on membrane physical parameters, for example, the membrane curvature.[69] These physical parameters might modulate the distribution of the sizes and lifetimes of phase domains in membranes of different compositions. In summary, since the formation of biomolecular condensates via phase separation is largely dependent on the physical state of the cell (Figure 3B),[70,71] these biomolecular condensates could serve as a mechanosensitive organelle that regulates downstream biochemistry and gene expression in both epithelial and mesenchymal cells.

Nuclear space hosting regulation of gene expression

Several experiments have shown that mechanical cues modulated gene expression in a genome-wide way. However, how physical/mechanical signals transduce from the extracellular microenvironment over a long distance to the nucleus is far from clear.[72] Recently, the emerging concept of nuclear physical properties as a mechanoregulator of gene expression brings new insight into understanding how mechanical cues are transduced to the nucleus and how they influence nuclear mechanics, genome organization, and transcription (Figures 4A and 4C).[73–80,81,82] More specifically, the nucleus can either serve as a downstream regulator of cytoplasmic signals or act as a direct effector independent of the cytoplasm (Figures 4B and 4C).

Nucleus as a downstream material barrier of cytoplasmic signals

Since mechanical signals were first shown to be able to cross the cell surface by deforming the extracellular matrix receptor integrin-β1 in the early 1990s, many mechanoresponsive cytoplasmic signals have been found in the last three decades, including YAP/TAZ signaling, Wnt/β-catenin signaling, and so on.[83,84] All these mechanoresponsive signaling pathways eventually lead to the activation of gene expression within cell nuclei.[73,76,78] Thus, the nuclei serve as the last physical barrier of the shuttering of cytoplasmic signals (Figures 4A and 4B).[72,81,85] Despite the widely accepted opinion that the upstream activation of receptors on the cell membrane is the key driver of signaling propagation that undergoes cascade amplification in the cytoplasm, the nucleus in the downstream works just like a sieve to cut off the numbers of transcription activators that could go into the nucleus. It can thus be easily imagined that the pore size of the nuclear membrane is important in the transportation of the transcription activators. Indeed, recent work by Elosegui-Artola et al. found that indentation on the nucleus enlarged the nuclear pore size and regulated its permeability, which was sufficient to promote nuclear entry of YAP (Figure 4B).[86] In a more recent work, Infante et al. showed that the nuclear

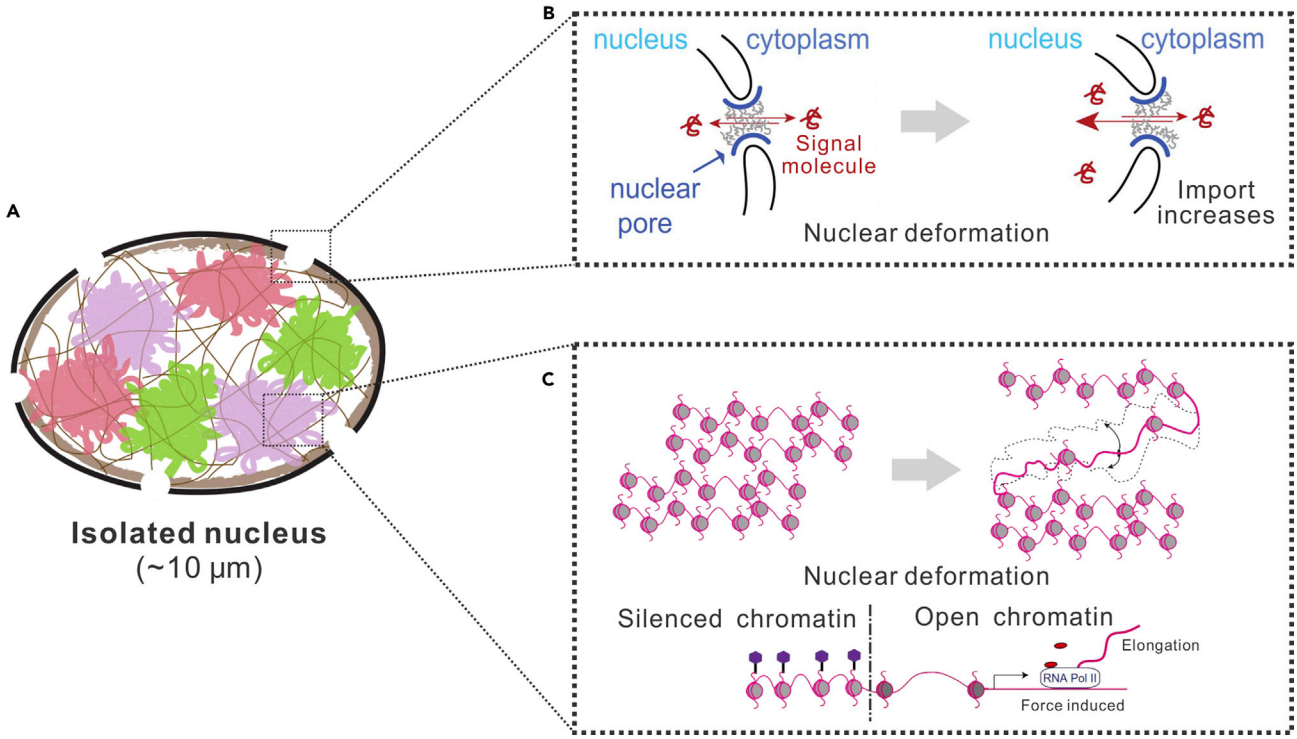

Figure 4. Physical properties of cell nucleus as a mechanoregulator of gene expression

(A) Schematic illustration of an isolated intact nucleus. Reprinted with permission from Shivashankar.[81] Copyright 2011, Annual Reviews, Inc.

(B) Schematic illustration of nuclear deformation as a mechanoregulator downstream of cytoplasm-to-nucleus signaling transduction. Reprinted with permission from Elosegui-Artola et al.[86] Copyright 2017, Elsevier.

(C) Schematic illustration of nuclear deformation as a mechanoregulator of chromatin accessibility and gene expression. Reprinted with permission from Miroshnikova et al.[82] Copyright 2017, The Company of Biologists Ltd.

pore size sieved the transcription activators depending on its molecular mechanics rather than their biochemical reactions and interactions.[87]

Cellular/nuclear properties mediate genome instability (including DNA damage)

The cell nuclei provide the physical space for the genome and the encoded hereditary information that defines organisms. A 5- to 10-μm-sized mammalian nucleus encapsulates about 2-m-long DNA wrapped around octameric histone protein complexes to generate a chromatin structure resembling beads on a string, which further folds into domains of various sizes and degrees of compaction.[88,89] By separating genetic materials from the reactive cytoplasmic macromolecules, the nucleus protects the integrity of the genome and prevents damage to genomic DNA (Figure 5).[90,81,91] Damages and alteration in genomic DNA directly lead to genomic instability, which is one of the hallmarks of cancer and other developmental diseases (such as autosomal dominant Emery-Dreifuss muscular dystrophy); they are also thought to contribute to disease progression and drug resistance.[92–95] The most common forms of genomic instability include chromosomal instability and genetic mutations/deletions. In cancers, these changes can lead to the inactivation of tumor suppressors or hyperactivation of oncogenes and thereby drive hyperproliferation and tumorigenesis.[94,96,97] In developmental diseases, these genomic damages are likely to induce cell-cycle arrest, senescence, apoptosis, and necrosis.[90,93,98,99] Genomic instability typically arises from dysregulation of DNA damage repair and DNA replication by environmental stresses, such as radicals and reactive oxygen.

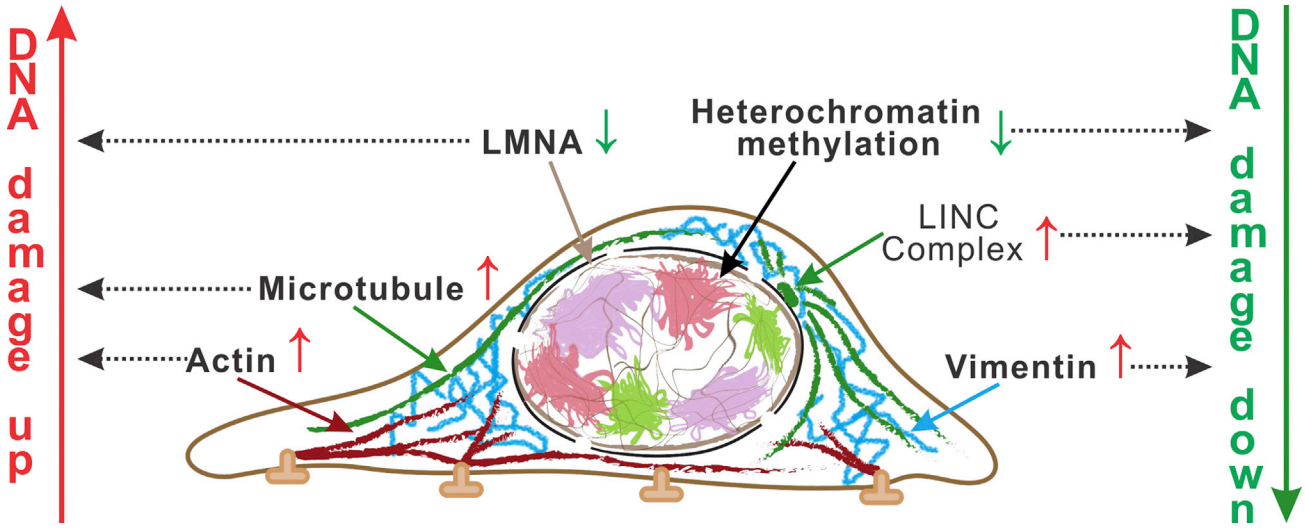

Figure 5. Cellular components that support cellular mechanical integrity serve as key mechanoregulators of DNA damage
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Besides the biochemical regulations of genomic instability, recent studies have also pointed to the mechanical integrity of the nucleus as a multifaceted regulator in DNA damage and genomic instability.

Recent studies have reached an agreement that many forms of mechanical loads can induce DNA damage and genome instability.[95–97,100,101] These mechanical loads vary from extracellular compression, stretch, and confinement, to even the contractile force generated by the cell itself. To understand how mechanical stress leads to DNA damage, several mechanisms with supporting evidence have been proposed. Denais et al. have shown that cell migration through a constraint space induced nuclear envelope rupture, thus resulting in the uncontrolled exchange of nucleo-cytoplasmic content, herniation of chromatin across the nuclear envelope, and DNA damage.[96] A similar observation of nuclear rupture has been observed in both *in vitro* myofiber differentiation and *in vivo* skeletal and cardiac tissues.[92] Either the microtubule-associated movements or actomyosin contractility led to constrained nucleus rearrangement, thus causing nuclear rupture.[92,95] Furthermore, this behavior was obvious in pathological Lamin mutated cells or mice, contributing to Emery-Dreifuss muscular dystrophy, congenital muscular dystrophy, and other diseases together known as laminopathies.[92]

Irianto et al. reported a comprehensive investigation of DNA damage induced by constrained migration using a commercial transwell plate.[97] Direct evidence of the cause of DNA damage was attributed to the cytoplasmic mislocalization of multiple DNA repair proteins within several hours after constrained migration. The induced genomic instability was severe (e.g., altering chromosome copy number) but not lethal. To understand the extrusion of DNA repair protein during constrained migration, Bennett et al. proposed an elastic-fluid model, in which the nucleus was considered as an elastic-fluid system containing an elastic component (chromatin) and a fluid component (DNA repair proteins) that can be squeezed out when the nucleus is deformed.[102] This elastic-fluid model of the nucleus can be integrated with the kinetics of DNA breakage and repair; the rate of damage was determined by the local volume fraction of the elastic component due to naturally occurring DNA breaks, while the rate of repair of DNA breaks was determined by the volume fraction of

the fluid component containing repair factors. The constrained deformation of the nucleus squeezed out the fluid-phase DNA repair factors and thus altered the equilibrium of the DNA breakage and repair. Indeed, follow-up works confirmed the mislocalization of DNA repair proteins after nuclear deformation in many scenarios, including embryonic heart development, cardiac differentiation of induced pluripotent stem cells (iPSCs), and adipocyte differentiation.[90]

Many varying forms of mechanical/physical stressors are presented in local tissues during developments and tumorigenesis, causing DNA damage and genomic instability as described above. As a feedback, cells respond by modifying their mechanical properties of either cytoplasmic materials or nuclear materials to protect the genome against mechanically induced damage. Since the rupture of the nuclear envelope is a key event leading to the mislocalization of DNA repair factors and DNA damage, strengthening or stiffening of the nuclear envelope to avoid rupturing effectively prevents DNA damage. Indeed, scaled with the extracellular matrix (ECM) stiffness or actomyosin tension;[90,95] LMNA (Lamin A/C) was found to decrease quickly when ECM stiffness or actomyosin tension decreased while increasing slowly when ECM stiffness or actomyosin tension increased. By increasing the expression of LMNA in response to increased mechanical loads, cells strengthen their nuclear envelope and prevent DNA damage. Indeed, a recent study showed that in LMNA-deficient hearts and LMNA knockout cells, actomyosin tension induced severe nuclear rupture and loss of DNA repair, thus causing DNA damage and cell-cycle arrest, and leading to aberrant beating.[90] In the study of myofiber health and muscle laminopathies, it was shown that mutant lamins led to nuclear envelope rupture and DNA damage in skeletal muscle cells;[92] this study agreed with the protective effect of Lamin on DNAs with more relevance to pathological diseases. Moreover, the authors pointed out that microtubule stabilization was more efficient in mitigating damage in striated muscle laminopathies rather than actomyosin contractility, which indicated alternative potential targets for disease intervention. In addition, disruption of the LINC (linker of nucleoskeleton and cytoskeleton) complex may offer a specific approach to reduce mechanical stress on myonuclei and improve muscle function.[103,104]

A more recent study introduced an alternative mechanism whereby cells protected genome damage using an opposite strategy, by softening the nucleus.[105] In this case, cells responded from the inner side of the nuclear envelope. By demethylating (decreasing H3K9me3) heterochromatin, cells softened their nuclei and increased chromatin mobility. One intriguing finding was that the demethylation of heterochromatin occurred mainly in non-coding regions, which meant that this modification of chromatin induced negligible changes in genetic information but might support the mechanical integrity of the nucleus. Another intriguing finding was that the authors identified the upstream transducer to link stretch/compression to heterochromatin demethylation. The stretch/compression triggered the release of intracellular calcium from the ER by activating Piezo1 mechanosensitive channels, which then changed the phosphoproteome group and demethylated heterochromatin. However, how the softening nuclei and more motile chromatin prevent DNA damage remains unknown. From the view of force propagation, the decrease of methylation (H3K9me2) disassociates the heterochromatin from the nuclear envelope and thus reduces the mechanical stress loaded onto chromatin and DNA. Another potential explanation could be that the softening of chromatin, similar to the stiffening of the nuclear envelope, redistributes mechanical force loaded on the nucleus.

In conclusion, alteration in the mechanics of different cellular components leads to various results in DNA damage and genomic instability. From outside to inside, the increased actomyosin contractility in the cytoplasm causes DNA damage; stabilization of microtubules in the cytoplasm mitigates DNA damage. In contrast, expression of cytoskeletal intermediate filaments (IFs) protects against nuclear rupture and DNA damage;[106] cytoskeletal IFs serve as a hyperelastic network supporting the mechanical integrity of cells. On the interface between the cytoplasm and nucleus, the disruption of the LINC complex stops force transduction from the cytoplasm to the nucleus, and thus prevents DNA damage. The expression of LMNA stiffens the nuclear envelope, enabling the nuclear envelope to withstand large loads, and thus prevents DNA damage. Inside the nucleus, either stretch or compression demethylates heterochromatin and softens the nucleus, and thus prevents DNA damage.

MicroRNA as a nuclear mechanoregulator

Post-transcriptional regulation is the control of gene expression at the RNA level and is between transcription and translation of genes. It contributes substantially to gene expression regulation within many human tissues. Among post-transcriptional regulations, microRNAs (miRNAs) regulate the expression of more than 60% of protein-coding genes of the human genome.[107] An miRNA can serve as a "switch" to turn the genes on and off. The miRNA biogenesis requires the formation of higher-order RNA structures, which is an imperfect double-stranded RNA-like hairpin miRNA precursor. To function in gene expression regulation, the miRNA requires the formation of ribonucleoprotein (RNP) complexes or RNA-induced silencing complexes, as either microRNPs or miRNA-induced silencing complexes. This complex formation is enabled by the clustering of multiple proteins of the Argonaute family and their association with similar sets of miRNAs. When regulating gene expression post-transcriptionally, miRNAs directly hybridize target mRNAs and thereby reversibly fine-tune the expression of genes. Based on their higher-order structural characteristics, multiclustering of biomacromolecules, and reversible association, we can speculate that the functions of miRNAs are sensitive to their physical/mechanical microenvironments. Thus, miRNA regulation should be able to crosstalk with either cell mechanics or nuclear mechanics, which will be involved in the loop of regulations of either homeostasis or pathology of tissue mechanics.

Indeed, a recent study by Moro et al. revealed a novel role for miRNAs in regulating mechanotransduction, as well as in controlling the mechanical properties of cells and tissues during wound healing.[108] By employing an unbiased screen to identify interactions between miRNAs and mRNAs, the authors found 122 miRNAs, many of which specifically regulate the cytoskeleton, ECM, and adhesion-related proteins. Intriguingly, miRNAs post-transcriptionally modify CAM proteins and further control cell mechanics. Overall, miRNAs act as a buffer to dampen a fierce reaction of the cell to environmental mechanical perturbations.

This mechanoresponsive miRNA regulation is also involved in tumor progression. A study conducted by Mouw et al. demonstrated that ECM stiffness could control the expression of miRNA.[109] They showed that one specific miRNA, miR-18a, served as a key regulator of the stiffness-dependent malignancy transition of breast epithelium. The authors attributed the altered tissue mechanics observed in tumors to the frequent alterations of miRNA expression across multiple cancer types. The miR-18a was regulated by multiple aspects of tumor tissue mechanics, including tissue architecture, interstitial pressure, and mass transport. downstream of induced elevation of miRNA expression, miR-18a targeted the tumor suppressor PTEN

directly or indirectly via tumor modifier HOXA9, and thus promoted PI3K-dependent malignant progression.

Another unique function of miRNA is its role as an access memory in preserving the mechanical memory of mesenchymal stem cells (MSCs). In this study by Li et al., the miRNA miR-21 served as a long-term memory keeper of exposure experiences to mechanical environments in MSCs.[110] miR-21 stored the experience of mechanical stimulus as long as 2 weeks after removal of stimuli. Knocking down miR-21 was sufficient to erase the mechanical memory, which further sensitized MSCs to subsequent exposure to a fresh mechanical environment. By taking advantage of manipulations of mechanical memories via soft priming and memory erasing, researchers prevented fibrogenesis of MSCs during wound healing and tissue repair, and thus increased the chance for success in MSC therapy. This mechanism led to the behavior that cells responded differently to mechanical perturbations at different timescales.[111] At short timescales (on the order of minutes), cellular responses independent of transcriptional activity could take place, while at longer timescales (on the order of hours), mechanotransduction via specific transcriptional regulators was activated to adapt cell morphology and mechanics to the mechanical stimulus. The miRNA regulation played an important role in cellular mechanical responses over an even longer timescale (on the order of days), which might explain why miRNAs could serve as a mechanical memory keeper.

CELLULAR MATERIAL PROPERTIES DIRECT CELL-FATE DECISIONS

Microglia

Microglia are the resident monocytes within the central nervous system (CNS), with unique biophysical independence. To enable the biological functions of microglia, such as monitoring synaptic information flow and phagocytosis of tissue debris, they are required to engage other cells within the CNS.[112] To connect with other cells, microglia usually undergo significant remodeling of their physical structures and properties. This section reviews the unique physical properties of microglia during different developmental and pathological stages.

Despite the majority of cell types in the CNS maintaining a stable physical structure, microglia are a cell type that is not permanently in contact with one another. An intriguing property of microglia is their significantly "repulsive" spatial distribution.[113] To realize their function in monitoring, the repulsive spatial distribution of microglia maximizes the interstitial space so that they can interrogate within their microenvironment. The biophysical independence of microglia enables their transformation, migration, and reactivation in different regions throughout the brain tissues. Indeed, direct observation showed their clear heterogeneity of morphology within whole brain tissue.[114,115] A widely accepted description categorizes microglia into three subtypes based on their morphologies: compact, longitudinally branched, and radially branched.[116,117] Compact microglia have small soma with short, unbranched processes. Longitudinally branched microglia usually inhabit white matter tracts, running parallel to the axonal fibers from small soma. Radially branched microglia are growing in gray matter, running radially throughout either round or elongated soma bodies. Many previous works emphasized the measurements of cell morphologies, including area, perimeter, aspect ratio, and circularity, in different subregions of the cerebellum, such as the molecular layer, granular layer, white matter, and cerebellar nuclei; these suggested that the local microenvironment is likely to be an important determinant of microglial physical properties.[115,118] However, the exact microenvironmental properties that regulate the physical

properties of microglia remain unknown. It would be interesting for future works to measure and correlate local environmental mechanics with the varying physical properties of microglia by taking advantage of current cell mechanics and biophysical tools.

In addition, pathological injuries and diseases (neuroinflammation, Alzheimer's disease, multiple sclerosis, and aged brain) also alter the physical properties of microglia, reverse their ramification, and reactivate their mobility. When injury or disease occurs, the settled-down ramified microglia (radial branched) transform into ameboid microglia (compact), which has been demonstrated to be the key regulator in neuroinflammatory disorders. It is speculated that the transformation of microglia is a consequence of heightened neuroinflammation, which largely alters the local cell microenvironments. Nevertheless, whether the biochemical variant or physical variant in pathological environments is the key trigger of microglial deramification still needs further exploration.

Immune cells

Biophysical properties at the molecular level and the cellular level have been studied in the context of immune cell activation. Knowledge of these physical properties could leverage therapeutic design, drug delivery, and translational medicine. Several recent studies revealed that the cellular mechanical force or physical properties not only tuned immune response but also dominated specific immune processes.[119–121] Overall, several fundamental physical principles are built into immunological activation by taking advantage of the unique physical properties of cellular components. Below we briefly summarize recent understanding of different immune cell subsets.

B cells are important components in the adaptive immune system. They exert their role in the adaptive immune response by transforming into antibody-producing plasma cells and then regulate the response to infection by producing diverse immunological signaling molecules. During pathogenic infection or tumorigenesis, B cells come into contact with "foreign" antigens either on professional antigen-presenting cells (APCs), such as dendritic cells (DCs) and follicular dendritic cells (FDCs), or in the ECM and interstitial fluid.[122,123] B cells recognize these antigens through the B cell receptor, which uses mechanical force to extract antigens from their presenting substrates.[124–127] Based on this mechanism, we could speculate that both the physical properties of antigen-presenting substrates (cells or matrix) and B cell itself influence this mechanical extraction of antigens by B cells.[125,126,128–130] Indeed, by employing live-cell imaging, Natkanski et al. showed that B cells used myosin IIa-mediated contraction to deform the antigen-presenting membrane and pulled out the embedded antigens.[124] When the authors used more flexible and softer plasma membrane sheets derived from adherent cells, B cells efficiently deformed the antigen-presenting membrane and internalized antigen together with small pieces of the membrane; when they used less deformable planar lipid bilayers (PLBs) to present antigen, B cells no longer pulled out and internalized the embedded antigens. Furthermore, the physical properties of B cells also largely determine their ability to extract antigens (Figure 6B).[128,131] For example, myosin IIa contractility is essential in B cell antigen extraction.[124,125] With harbored myosin IIa contractility (via short hairpin RNA knockdown or pharmacological inhibition), B cells lose their ability to invaginate membrane and internalize the embedded antigen. Intriguingly, the stiffness of the antigen-presenting substrate also influenced the contractility of B cells when they were engaged.[130] Specifically, B cells spreading on a stiffer substrate had higher cell contractility, which more efficiently extracted embedded antigens

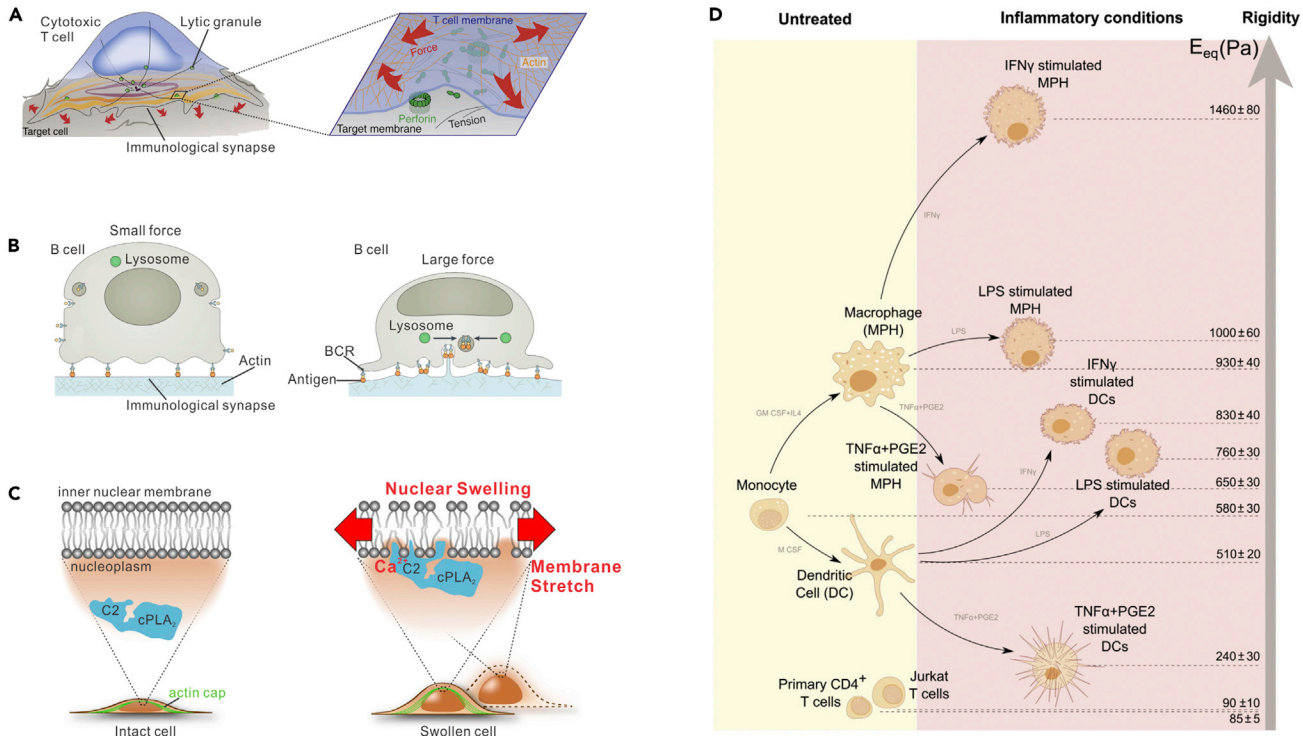

Figure 6. Regulations of cellular immunological processes depend on cellular physical properties

(A) Schematic illustration of force- and tension-dependent T cell targeting killing in T cell immunological synapse. Reprinted with permission from Basu et al.[132] Copyright 2016, Elsevier.

(B) Schematic illustration of force-dependent antibodies uptake by B cells in B cell antigen-presenting cell (APC) immunological synapse. Reprinted with permission from Tolar.[128] Copyright 2017, Springer Nature.

(C) Schematic illustration of nuclear swelling as a mechanoregulator of activation upstream of inflammation. Reprinted with permission from Enyedi et al.[145] Copyright 2016, Elsevier.

(D) Schematic illustration of cellular elasticity changes of immune cells during the inflammation process induced by different inflammatory factors. Reprinted with permission from Bufi et al.[146] Copyright 2015, Elsevier.

from the substrate. In contrast, a softer substrate compromised B cell contractility, which limited antigen extraction. This observation has been confirmed in live APCs. FDCs and DCs were two important APCs that B cells encountered *in vivo*. The efficiency of antigen extraction of B cells from live APCs was significantly higher than that from stiffer PLBs. Despite there being a difference in the stiffness of FDCs and DCs, both their membranes were flexible enough to bend for membrane invagination, which yielded B cells extracting a similar amount of antigen from both APCs. Instead of directly regulating the efficiency of antigen extraction, the difference in the physical properties of different APCs regulated B cell antigen affinity discrimination. As probed by atomic force microscopy (AFM), FDCs had a higher membrane stiffness, which made B cells more likely to extract high-affinity antigens rather than low-affinity antigens.[125] Compared with DCs, the ratio of total extracted high-affinity antigens to low-affinity antigens was 4-fold higher on FDCs, which might be a result of the higher B cell contractility triggered by stiffer FDCs.

T cells are another critical component of the adaptive immune response, among which the cells mediate antigen-specific killing of cancer cells and infected cells as well as releasing a variety of immune effector molecules that enable other cells to do so. A better understanding of the mechanism of cytotoxic T lymphocyte (CTL)-mediated tumor cell killing would benefit the current adoptive T cell transfer and

T cell therapies. Similar to B cell activation, CTL-mediated cell killing also involves the engagement of T cells and target cells. Indeed, several recent studies have shown that the physical properties of both T cells and target cells were important in this CTL-mediated target cell killing.[132–137] An intriguing study from Basu et al. clearly showed a strong correlation between the magnitude of force exertion between two cells and the speed of perforin pore formation on the target cell (Figure 6A).[132] By employing biophysical and molecular biological experiments, the authors found that the force potentiated cytotoxicity by enhancing perforin activity. The physical properties of the target cells were also important players in CTL-mediated cell killing, among which the membrane tension of the target cell correlated with the efficiency of pore formation by perforin. Indeed, when manipulating target cell membrane tension either using osmotic stresses or by varying substrate stiffness, target cells with lower membrane tension were more resistant to CTL-mediated cell killing. Consistently, disruption of the target cell cytoskeleton to reduce membrane tension could also lead to decreased CTL killing efficiency. Interesting, another work by Liu et al. showed that the softness of target cell protected itself from CTL-mediated cell killing, which suggests that different aspects of physical properties of cells might have different impacts on CTL-mediated cell killing.[138] T cells could sense ECM stiffness via T cell receptor coreceptors CD3 and CD28.[139] Furthermore, the microtubule network within the cytoplasm also regulated immune checkpoint expression via the transportation of stress granules.[140] Moreover, another recent study from Meng et al. showed that the intracellular mechanosensor YAP suppressed T cell proliferation and $CD4^+$ T cell activation.[134]

Macrophages belong to a different category of immune cells, myeloid cells, of the innate immune system. Macrophages engulf and digest cellular debris, foreign substances, microbes, cancer cells, and anything else that do not have the type of proteins specific to healthy body cells on their surface, in an immune process termed phagocytosis.[141] Macrophages respond dynamically to chemical/metabolic and physical stimuli in microenvironments. However, it is unclear which effect directly results from the physical stimuli and cellular physical properties, since it is challenging to decouple physical characteristics *in vivo*. A more recent study from Jain and Vogel revealed that the spatial confinement on macrophages mechanically altered chromatin compaction and epigenetic modification on HDAC3 and H3K36 dimethylation levels, which eventually suppressed late lipopolysaccharide (LPS)-activated transcriptional programs.[142] Upstream of transcription, actin depolymerization serves as the key mechanotransducer when macrophages are exposed to spatial confinement. Depolymerization of the F-actin network harbored the LPS-stimulated nuclear translocation of MRTF-A; this lowered the activity of the MRTF-A-SRF complex and subsequently downregulated the inflammatory gene expression. Another study by Man et al. also highlighted the importance of actin polymerization, which showed that polymerization of the F-actin network was essential in inflammasome stabilization.[143] Disruption of F-actin networks stopped the formation of the inflammasome, thus leading to blockage of interleukin-1β production.

A more recent work added a new perspective on the physical regulation of the immune macrophages and related myeloid cells. During bacterial infection macrophages are recruited to the lung, which senses the cyclic breathing pressures via the membrane mechanosensitive ion channel PIEZO1.[129,144] Increased pressure applied to the cells and increased membrane tension of macrophages can open this channel, which would trigger an influx of calcium ions into the cytoplasm and result in the production of EDN1. The accumulation of EDN1 latterly stimulates HIF-1a-dependent transcription of genes to produce proinflammatory molecules.[144]

Overall, one of the hallmarks of immune cells is their highly dynamic physical properties; cellular physical properties vary significantly not only among different types of immune cells but also within the same type of cells when subjected to dynamically varying microenvironments. One typical immune process is inflammation, which is part of the complex biological response of body tissues to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair (Figure 6C).[145] To reclaim a landscape of the changes in physical properties of various types of immune cells, Asnacios et al. systematically characterized the viscoelastic properties of different types of immune cells before and after inflammation, providing a database regarding viscoelasticity of immune cells to which we can refer (Figure 6D).[146]

Neurons

Neural tissue is known as one of the softest tissues in the human body. Neural lineage differentiation is known to be potentially mechanosensitive since the early discovery of stiffness-dependent differentiation of MSCs.[147] Recent advances in biophysical tools and biological rheology enabled direct mechanical measurement of brain tissues and *in vitro* cultured neural cells. These measurements including AFM, tissue rheology, and magnetic resonance electrography, confirmed that the mechanical changes in brain tissue indeed represent a hallmark of brain developmental and pathological processes, such as maturation, inflammation, neurodegeneration, aging, injury, and brain tumors.[148–150] Glial cells are mechanosensitive, and their function and dysfunction have been revealed to be related to neuron injury and aging.[151] As one large type of neuroglia, oligodendrocytes function to provide support and insulation to axons in the CNS. Recent studies revealed that both the proliferation and differentiation of oligodendrocyte progenitor cells (OPCs) were largely dependent on their local microenvironment (Figures 7A and 7C).[152–155] The highly efficient proliferation and differentiation required a soft local tissue matrix. When OPCs were cultured on a stiff matrix either *in vitro* or *in vivo*, both the proliferation and differentiation of OPCs were largely compromised. This ECM stiffening was indeed happening during the aging of brain tissue; this was also accompanied by a decline of tissue regeneration and a loss of function of adult stem/progenitor cells.[152] The key regulator of mechanotransduction in OPC differentiation had been revealed to be Piezo1. In another study, Pathak et al. also confirmed Piezo1-directed lineage choice in human neural stem cells, which suggested that the ECM stiffening by aging might impact neural stem cells beyond OPCs.[156] In addition to the mechanics of the neural tissue matrix, it would be interesting to access the mechanics of individual neural cells *in vivo*. However, very few works have put their efforts into tracing the mechanical evolution of neural cells. To do so, technologies such as AFM and optical and magnetic tweezers could potentially be used to access the mechanical evolution of cells during differentiation, disease progression, and aging. In addition, Urbanska et al. developed a microfluidic deformation cytometry, which was used for high-throughput mechanical phenotyping of differentiation and reprogramming between neural cells and iPSCs (Figure 7B).[150] It would be interesting to use these aforementioned technologies to characterize the mechanical evolution of neural cells during their development and disease-related processes.

Mesenchymal stem cells, embryonic stem cells, and hematopoietic stem cells

That the mechanics of MSCs can determine cell-fate decision has been observed for decades. Early studies largely focused on the effect of matrix mechanics on the differentiation of MSCs.[147] In simple words, when MSCs were cultured on a matrix whose stiffness matched the stiffness of certain interstitial tissue, MSCs were likely to differentiate into cells that habituated in that certain type of tissue. Yet current

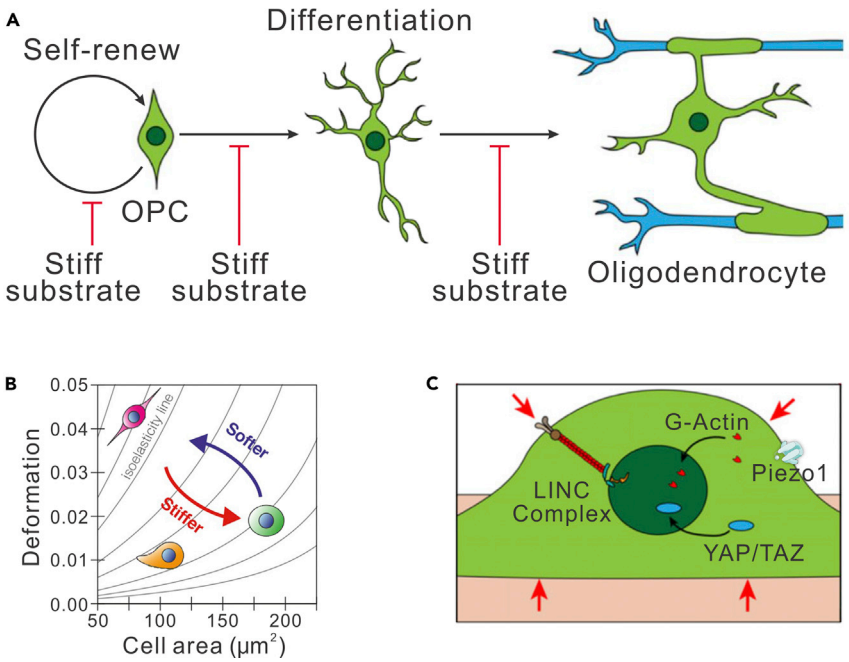

Figure 7. Physical regulation of neuron fate decision and cellular physical property evolution during neurological lineage commitment

(A) Schematic illustration of physical regulation by varying stiffness of extracellular matrix on neuron cells self-renewing and differentiating. Reprinted with permission from Tsai and Casaccia.[153] Copyright 2019, John Wiley and Sons.

(B) Plot of cell area against deformation shows the cellular physical property changes during neuron lineage commitment from iPSCs. Reprinted with permission from Urbanska et al.[150] Copyright 2017, The Company of Biologists Ltd.

(C) Schematic illustration of cellular regulatory components that are involved in mechanoregulation in neuron cells. Reprinted with permission from Tsai and Casaccia.[153] Copyright 2019, John Wiley and Sons.

studies have found that mechanoregulation is much more complex, and various aspects of cell mechanics have been taken into account. These physical/mechanical properties of MSCs included but were not limited to stiffness (both cytoplasm and cortex), geometries (spreading areas and aspect ratio), volume, and intracellular crowding. First of all, by manipulating the mechanical properties of MSCs, researchers could regulate the different differentiation outcomes of MSCs.[55,157–159] The basic principle was that by better recapitulating the mechanical characteristics of the target cells, MSCs were more likely to be induced into that type of cells. For example, elongation of MSCs led to myogenesis or myocardial genesis, as the cardiomyocytes or myocytes exhibited a unique elongated morphology (Figure 8);[160,161] stiffening of cells facilitated the osteogenesis of MSCs, as osteocytes were one of the stiffest cells in our body.[147,162]

In addition to the cellular physical properties, the mechanics of the nucleus could also regulate the cell-fate decision of MSCs.[163–166] The reason for this is that the nucleus encapsulates genomic materials. Whether the nucleus is constrained and how resistant is the nucleus to stresses would determine the genome stability and chromatin accessibility, and thus largely determine the gene expression and cell-fate decision. Instead of external manipulation of cell mechanics, each cell has its physical properties which could differ significantly from each other. These differences result in a heterogeneity within a population of cells. Based on their individual mechanical

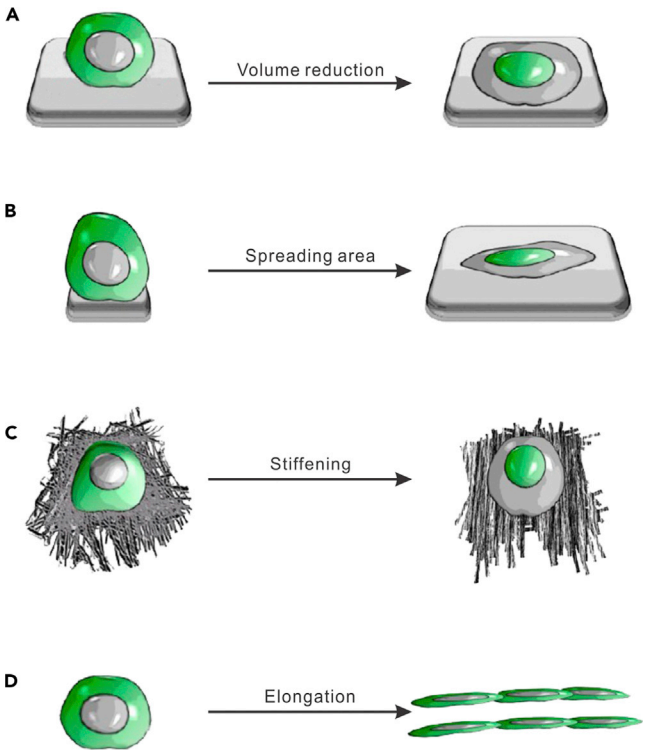

Figure 8. Various physical properties of cells on the regulation of fate decision of mesenchymal stem cells and embryonic stem cells

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(A) Schematic illustration of volume reduction in mesenchymal stem cells (MSCs) or embryonic stem cells (ESCs).

(B) Schematic illustration of cell spreading in MSCs or ESCs.

(C) Schematic illustration of cell stiffening in MSCs or ESCs.

(D) Schematic illustration of cell elongation in MSCs or ESCs.

and other physical properties, researchers could predict the differentiation potential of MSCs.[167,168] These present mechanical properties of an individual MSC can be viewed as a consequence of the past experience or development of this particular cell. This category of studies could be considered together with the studies of mechanical memory, which showed that different mechanical memory and dosing that cells experienced in the past largely determined their different differentiation outcomes even though these cells had been cultured under the same conditions otherwise.[168,169] Taken together, despite the fact that we did have a deeper knowledge of mechanoregulation in MSCs compared with other cell types, current studies have shown that there are still many things unknown. At the same time, our knowledge base of MSCs would continue to help us explore the mysterious frontier of cellular physical properties and their relationships with cell functionalities.

Similar behaviors have been found in other stem cells, including embryonic stem cells (ESCs) and hematopoietic stem cells.[170] Indeed, varying physical properties of cells regulated the cell-fate decision and transition in ESCs.[171–177] These physical properties also include nuclear/chromatin mechanics, cell membrane mechanics, and cellular mechanics. Taken all of these findings in many types of stem cells together, we could speculate the important role of cell mechanics in determining cell-fate decisions and its correlation with the differentiated cell types.

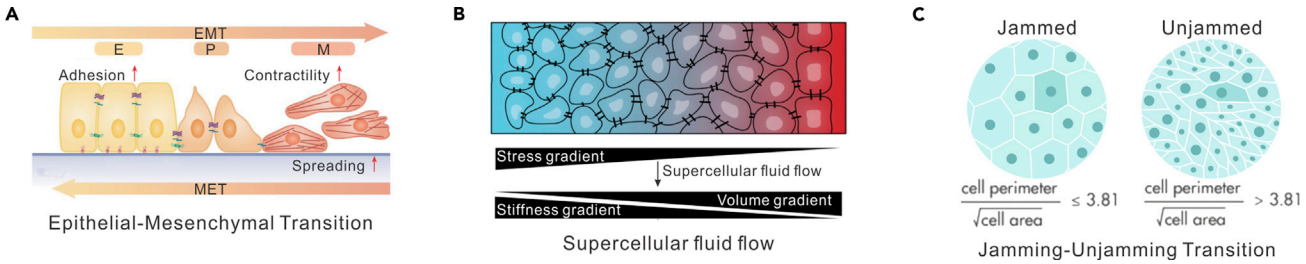

Figure 9. Different carcinogenic processes that transit cellular physical properties

(A) Schematic illustration of epithelial-to-mesenchymal transitions (EMT) accompanying changes in cellular physical properties, such as adhesion, contractility, and spreading. Reprinted with permission from Dongre and Weinberg.[186] Copyright 2019, Springer Nature.

(B) Schematic illustration of supercellular fluid flow driving changes in cellular physical properties, such as cell and nuclear volume, and cell stiffness. Reprinted with permission from Han et al.[14] Copyright 2020, Springer Nature.

(C) Schematic illustration of jamming-unjamming transition accompanying changes in cellular physical properties, such as cellular aspect ratio, trajectory, perimeter, and area.

Muscle cells

The aforementioned effects of cellular physical properties are mainly associated with the development and maturation of different types of cells. The homeostatic cellular physical properties are also found in mature tissues; dysregulated cellular physical properties often lead to diseases in mature tissues. One important piece of evidence is the muscle tissue, which is required to withstand large strains and also exert large forces.[178] Mature muscle cells, especially skeletal muscle fibers, are unlikely to go through division or differentiation. In addition, mature muscle cells, unlike other cells, also have a stable and unique cellular physical property, with an elongated morphology and a highly aligned cytoskeleton. Interestingly, very few diseases have been observed as a consequence of changes in cellular physical properties in muscle. In contrast, the mechanics of the nucleus of muscle cells have been found to be an important regulator of muscle diseases;[179–181] for example, the varied stiffness of the nucleus and its wrinkled morphology have been found in many muscle diseases. These changes in nuclear mechanics have been associated with nuclear membrane proteins such as Emerin and Lamin A/C.[182–184] In addition, the dysregulated nuclear mechanics might also lead to DNA damage, which we discussed in the section "cellular/nuclear properties mediate genome instability (including DNA damage)."

Cancer cells

Besides healthy cells, cancer cells are also highly sensitive to the variations in cellular physical properties.[185] Unlike other cell types, cancer cells are different from each other as a result of not only the differences in their original cell type but also their metastasis status. The metastasis of cancer cells is usually accompanied by changes in their capability in migration and division. The metastasis of cancer cells is believed to be potentially driven by the epithelial-to-mesenchymal transition (EMT), during which the cell-cell adhesion and cytoskeleton are significantly altered (Figure 9A)[186]. As a hallmark of cancer metastasis, loss of E-cadherin mediates the dismissal of the force transduction between cells. In addition, cancer cells are found to be more contractile than their healthy counterparts.[187,188] The alteration in the cell cytoskeleton is more complex and includes the suppression of keratin expression and the expression of vimentin.[189–198] Both keratin and vimentin are important intermediate filaments that contribute to the cell's mechanical integrity; both keratin and vimentin have been shown to increase cell stiffness and stretchability.[25,195,199] Thus, the balance between decreasing keratin and increasing vimentin results in various

changes in cancer cell mechanics. Besides EMT processes, the changes in cellular physical properties during cancer progression could be coordinated in space and time and could be physically governed. One example is the collective cell volume changes in tumor spheroid via supracellular fluid flow through gap junctions; this change in cellular water content resulted in cell swelling and softening at the tumor periphery, leading to an invasive phenotype (Figure 9B).[14,200] Another example of purely physical regulation is the unjamming process in a cancerous epithelial layer (Figure 9C); without changes in biomolecules that are associated with cell-cell adhesion and cell migration, cancer cells could undergo a jamming-unjamming transition in a purely physical manner.[201–203] In the jammed state cells maintained their constrained original locations, while in the unjammed state cells migrated actively, thus leading to cell clusters that collectively migrate and deform. These changes of cellular mechanics, both individually and collectively, are thought to influence cancer cell migration and, thus, tumor invasion. Additionally, recent studies described active wetting behavior of mammary epithelium.[204,205] The authors studied the transition from three-dimensional (3D) spheroidal aggregates to 2D epithelial monolayers, and found that in analogy to the behavior of inert fluids, the interfacial energy difference would lead to the spreading of a 3D spheroidal aggregate, similar to a phase transition toward cancer metastasis. In addition to cancer cells, these physical principles of collective cell behaviors, such as the aforementioned jamming-unjamming transition, EMT, supracellular flow, and active wetting,[205] are also important in tissue homeostasis and development.[206–209]

OUTLOOK AND FUTURE DIRECTIONS

In this review, we introduce a fresh physical perspective of cells as a fundamental building material of life. Instead of focusing on the complex biochemical regulations of life, we emphasize the fundamental mechanics of cells to describe the essential physical environment that hosts and thus impacts all biological processes. In the first section, we introduce the fundamental physical and mechanical properties of cellular materials. The correlations of these material properties have also been explained. It is worth noting that living cellular materials are much more complex than a passive soft material; instead, they are rather a combination of various types of soft materials including elastic polymers (such as intermediate filaments and DNAs), viscoelastic materials (such as actin and microtubules), porous structure (all the biopolymer networks), and colloids (such as proteins and other macromolecules), and more importantly is an active material that functions at far from thermal equilibrium. These components of varying mechanical properties intertwine and cooperate, endowing cells with unique and exceptional material properties. Interestingly, studies have found that the design principle of living cells shared aspects similar to those of very recently developed engineered advanced materials.[25] For example, the cytoskeleton is found to be an interpenetrating network of filamentous actin, microtubule, and vimentin intermediate filament. This organization principle enables the cells to have a high strength and toughness, reminiscent of the engineered tough hydrogels made of polyacrylamide and alginate;[25] in addition, this organization principle also allows the cell to harvest respective advantages of each component, such as the dynamic character of F-actin that leads to quick healing upon damage, and the supreme non-linear elasticity of intermediate filament that maintains cell mechanical integrity under severe deformations. Thus, an exciting future direction is the design of new soft materials inspired by the live cell materials. To broaden our understanding of cell mechanics, it is important to probe the dynamic evolution of cell mechanics when cells function in a multicellular system. To accomplish this goal, reliable technologies (such as optical-tweezers-based active

microrheology)[14] need to be developed to spatiotemporally access cell mechanical properties in different aspects during the development of a multicellular living system such as embryo, tumor, organoid, and tissue.

In the next section, we discuss how the cellular physical properties determine gene expression and cellular functions inside cells. We highlight how the cellular physical and mechanical properties interfere with the intracellular biochemical processes. The current understanding of the impact of cellular material properties on cell biology aims to reveal key mechanosensory aspects that transduce mechanical and physical signals to biochemical reactions and generate a regulatory network; this framework considers the target physical/mechanical input as a node that is no different from other biochemical inputs. Within this framework, the physical principles and mechanisms will not be essential in deciphering the cellular behaviors. In contrast, here we highlight the role of cellular physical properties in another dimension, in which the cellular physical environment hosts all the biochemical cascades and signaling, and thus cellular physical properties directly impact intracellular processes. Due to the variation in sensitivity of different biochemical processes to their local physical environments, intracellular biochemical and biophysical processes behave in very different ways. We highlight the unique physical properties and processes that are important at the interface of cellular biophysical and biochemical functions, including phase separation, molecular crowding, and chromatin condensation. The future study of biophysical regulation of cellular functions requires a combination of biophysics at different scales from the molecular level to cellular level, then to the multicellular level. We anticipate more underlying mechanisms will be revealed within this framework to solve the remaining elusive issues of the complexity of mechanoregulation. Besides, the mechanoregulation or physical regulation of cell function is definitely not a single node in biochemical regulatory networks; thus, understanding their complex interplays may require inputs of systems biology in the future.

In the last section, we directly link cellular physical properties to various cell fates, taking several types of cells as examples. We further summarize and categorize important physical phenotypes in the development of several important types of cells. Based on these understandings, we are able to engineer cell fates, sort cell subtypes, and diagnose diseases based on the spectrum of cellular physical properties. In future works, one interesting direction would be to extend this method beyond a single-cellular level to multicellular living systems, such as tumors,[210,211] organoids,[212–215] and embryos.[208] In this case, the physical properties of cellular materials will be more important than their role in single cells. The cellular physical properties of a cell in a multicellular system not only represent a combinatory effect of its own growth and local microenvironments, but also impose impacts on its own cell-fate decision and stresses on its neighbors. Thus, the cellular physical properties will be an important ecological unit during the evolution of the habituated multicellular systems. We anticipate that studies in the foreseeable future could reveal a comprehensive understanding of the hierarchical impact of cellular physical properties, which will further guide the development of engineering approaches that could also be applied to regenerative medicine and disease treatments.

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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