Michelle L. Jones
Clinical Insights, Ltd,
60 Summer Duck Way,
Pittsburgh, PA 15238
e-mail: mlbish@gmail.com

Kris Noel Dahl
Department of Chemical Engineering,
Carnegie Mellon University,
Doherty Hall, 5000 Forbes Avenue,
Pittsburgh, PA 15213;
Forensics Department,
Thornton Tomasetti,
120 Broadway 15th Floor,
New York City, NY 10271
e-mail: KDahl@thorntontomasetti.com

Tanmay P. Lele
Department of Biomedical Engineering,
Texas A&M University,
101 Bizzell Street,
College Station, TX 77840;
Department of Chemical Engineering,
Texas A&M University,
101 Bizzell Street,
College Station, TX 77840;
Department of Translational Medical Sciences,
Texas A&M University,
101 Bizzell Street,
College Station, TX 77840
e-mail: tanmay.lele@tamu.edu

Daniel E. Conway
Department of Biomedical Engineering,
Virginia Commonwealth University,
601 West Main Street,
P.O. Box 843068,
Richmond, VA 23284
e-mail: dconway@vcu.edu

Vivek Shenoy
Materials Science and Engineering
Bioengineering,
University of Pennsylvania,
3231 Walnut Street,
Philadelphia, PA 19104;
Mechanical Engineering and Applied Mechanics,
University of Pennsylvania,
3231 Walnut Street,
Philadelphia, PA 19104;
Center for Engineering Mechanobiology,
University of Pennsylvania,
3231 Walnut Street,
Philadelphia, PA 19104
e-mail: vshenoy@seas.upenn.edu

Soham Ghosh[1]
Department of Mechanical Engineering,
School of Biomedical Engineering,
Translational Medicine Institute,
Colorado State University,
400 Isotope Drive,
Fort Collins, CO 80521
e-mail: soham.ghosh@colostate.edu

The Elephant in the Cell: Nuclear Mechanics and Mechanobiology

The 2021 Summer Biomechanics, Bioengineering, and Biotransport Conference (SB3C) featured a workshop titled "The Elephant in the Room: Nuclear Mechanics and Mechanobiology." The goal of this workshop was to provide a perspective from experts in the field on the current understanding of nuclear mechanics and its role in mechanobiology. This paper reviews the major themes and questions discussed during the workshop, including historical context on the initial methods of measuring the mechanical properties of the nucleus and classifying the primary structures dictating nuclear mechanics, physical plasticity of the nucleus, the emerging role of the linker of nucleoskeleton and cytoskeleton (LINC) complex in coupling the nucleus to the cytoplasm and driving the behavior of individual cells and multicellular assemblies, and the computational models currently in use to investigate the mechanisms of gene expression and cell signaling. Ongoing questions and controversies, along with promising future directions, are also discussed. [DOI: 10.1115/1.4053797]

[1]Corresponding authors.

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Spencer E. Szczesny[1]
Department of Biomedical Engineering,
Pennsylvania State University,
University Park, PA 16802;
Department of Orthopaedics and Rehabilitation,
Pennsylvania State University,
University Park, PA 16802
e-mail: ses297@psu.edu

1 Introduction

The mechanical properties of the nucleus are an important driver of cell biomechanics and mechanobiology. It is now well established that the effect of nuclear mechanics on cell function has significant implications in many fields of biology and medicine, including morphogenesis [1,2], tissue homeostasis [3], cancer and cell proliferation [4–6], aging [7,8], degenerative disease [9,10], and regenerative medicine [11]. Over the last two decades, our understanding of the structure–function relationships within the nucleus and the mechanisms underlying the effect of nuclear mechanics on cell behavior has rapidly developed and continues to evolve. Given this recent scientific attention and rapid growth in knowledge, a workshop titled "The Elephant in the Room: Nuclear Mechanics and Mechanobiology," was organized at the 2021 Summer Biomechanics, Bioengineering, and Biotransport Conference (SB3C) to help synthesize the most recent findings within the field and provide potential directions for future investigation. This paper is the outcome of this workshop and aims to provide a perspective from experts in the field on the current understanding of nuclear mechanics and its role in mechanobiology. Note that this is not intended to serve as a comprehensive review on this topic (see other recent reviews [12–17]), but rather a brief synopsis of where the field currently stands and where it is headed. Section 2 will provide historical context, summarizing early efforts to measure the mechanical properties of the nucleus and discussing the primary structures dictating nuclear mechanics. Section 3 will discuss the mechanics and plasticity of the nucleus, particularly at the slow time scales at which forces are applied to the nucleus during cell migration. In Sec. 4, we will present the emerging role of the linker of nucleoskeleton and cytoskeleton (LINC) complex in both physically coupling the nucleus to the cell and influencing the behavior of individual cells and multicellular assemblies. Section 5 will elaborate on the computational models currently used to investigate the mechanisms underlying the effect of nuclear mechanics on genome organization, signaling pathways, and gene expression. Finally, Sec. 6 will pose pressing unanswered questions and controversies within the field and outline promising directions for future investigation. We hope that this information will provide clarity and context to an emergent and exciting field for researchers new to this topic as well as those already engaged in active research in this field.

2 Stressing the Nucleus: Measurement Techniques to Probe Nuclear Mechanics

Although reporting on the mechanical properties of cells began in the early 20th century, a complete appreciation of cells as mechanical entities did not emerge until the 1990s [18,19]. The development of traction force microscopy, in which cells are plated on a deformable substrate containing fluorescent beads, allowed for further quantification of the mechanical properties of cells [19,20]. The emerging view of the cell as a mechanical object was later extended to the nucleus, with experiments investigating nuclear deformation within multiple cell types including endothelial cells, chondrocytes, and leukocytes [21–23]. The quest of understanding the specific structural components that contribute to nuclear mechanics started with early studies of the nuclear lamina in the early 2000s [24–26]. An early model of nuclear mechanics, reviewed in Ref. [27], included viscoelastic chromatin enveloped by a viscoelastic nuclear lamina that is then physically coupled to a viscoelastic cytoskeleton network. These basic elements embody the proposed role of the nucleus as a cellular mechanosensor whose shape can change, resulting in alterations of the structure and organization of chromatin, which directly affect transcriptional regulation [28]. Still, these are simplified descriptions of the nucleus that underestimate its true structural complexity.

The nucleoskeleton is surrounded by the nuclear envelope, consisting of a double lipid bilayer membrane containing nuclear pore complexes, which allow macromolecular transport between the nucleus and cell cytoplasm [28]. Under the inner nuclear membrane lies the intermediate filament meshwork of the nuclear lamina. Of the lamin proteins, A-type lamins, primarily A and C (both products of the transcription of the *LMNA* gene) are thought to contribute significantly to nuclear mechanotransduction. A-type lamins play major roles in modulating gene expression and maintaining nuclear shape, stability, and structure, and indeed, many mutations in human *LMNA* have been implicated in various disease conditions [28–33]. B-type lamins are constitutively expressed in metazoans and have essential roles in transcription and other cell signaling pathways, but probably play less of a prominent role in nuclear mechanical function [31,34,35]. Understanding of the laminar structure has recently been refined in mammalian cells, facilitated by high-resolution stochastic optical reconstruction microscopy (STORM). New observations have shown that A- and B-type lamins are not interconnected, but form an independent and interacting meshwork of filaments [36] (Fig. 1). The lamin A/C mesh is located farther from the nuclear envelope and is tightly organized, with lamin A/C filaments typically present over the entire nuclear periphery. Lamin B1 is located closer to the nuclear envelope with a more open configuration, and its peripheral localization is curvature- and strain-dependent [37]. This spatial organization predicts distinct roles for the A- and B-type lamins in imparting nuclear mechanical properties and their functional roles in the control of chromatin architecture.

Inside the nucleus, lamins directly or indirectly bind to chromatin at lamin-associated domains [32,38]. Chromatin exists in different states depending on its location and function in the cell. Loose euchromatin is associated with more transcriptional activity, and heterochromatin, which is more densely packed, is generally transcriptionally inactive. Recent microscopy-based studies suggest that a continuum exists somewhere between euchromatin and heterochromatin rather than distinct states, and shifts between the states likely precede changes in gene expression [39,40].

A physical connection between the cytoskeleton and the nucleoskeleton is mediated by the LINC complex, formed by interactions between KASH domain proteins (e.g., nesprins) and SUN proteins [41,42]. Because of this mechanical connection between the nucleus and cytoskeleton, pores in the nuclear membrane stretch in response to forces exerted on the cell, activating signaling pathways that result in nuclear translocation of transcriptional regulators (e.g., Yes-associated protein [YAP] and chromatin modifiers, e.g., histone deacetylase [HDAC]) [43,44]. Thus,

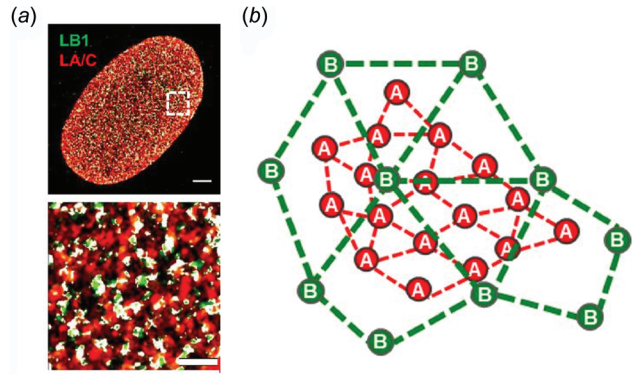

Fig. 1 Nucleoskeleton: (*a*) STORM images of cell nuclei dual-color immunolabeled against lamin A/C (red) and lamin B1 (green). White areas represent colocalization of the two proteins. Rectangle in top image denotes zoomed area of the nucleus shown in the bottom image. [Scale bars: top, 2 μm; Bottom, 500 nm.] (*b*) Simplified schematic representation of A-type and B-type lamins illustrating interactions between nucleoskeletal networks. From Nmezi et al. [37].

alterations in the shape and structure of the nucleus in response to extracellular forces lead to changes in chromatin structure and transcriptional activity, linking nuclear mechanics with cell function.

3 Nuclear Mechanics in Motile Cells

During cell migration, nuclear movement and deformation are necessary to allow the cell to pass through a confined space. Experimental models of cell migration through highly crosslinked collagen gels demonstrate that the initially circular nucleus becomes highly elongated as the cell elongates during motion through the confined space [45]. Under conditions in which cells are unable to elongate or deform the nucleus, they are unable to pass through the confinement. Such migration through confined spaces is central to cancer cell invasion and metastasis, as well as immune cell migration [46,47]. Metastasis relies on the migration of cancer cells through tissues and tight interstitial spaces, driven by cellular deformation and consequent nuclear deformation [48] (Fig. 2). This extreme nuclear deformation leads to nuclear rupture and DNA damage, which increases the number of genetic mutations and further contributes to cancer malignancy [49–51].

Competing explanations have been proposed for how deformation of the nucleus occurs as a result of cell shape change. In one proposed mechanism, nuclear deformation is caused by lateral compressive forces, and, to a lesser extent, vertical compressive

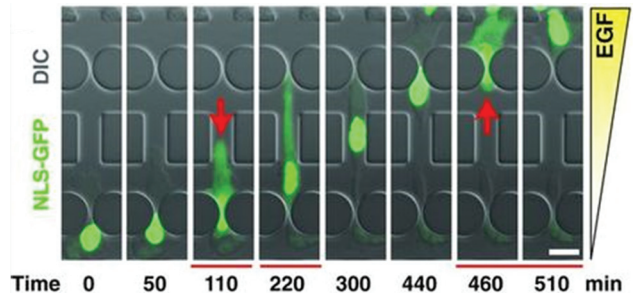

Fig. 2 Image sequence of a breast cancer cell moving through constrictions that induce nuclear rupture as seen by release of the green fluorescent protein fused to a nuclear localization sequence (NLS-GFP) into the cytoplasm (red arrows). Scale bar, 20 μm. Reprinted with permission from Denais et al. [48]. Copyright 2016 by AAAS.

forces, derived from actin stress fibers [52,53]. The compressive stress arises because tensile stress fibers that adjoin the nucleus at a higher angle will exert an inward compressive force on the nuclear surface. As the stress fiber distribution changes according to the overall cell shape, the nucleus will correspondingly adapt [52]. However, this model has been challenged by experiments in which stress fiber severing produces no relaxation in the shape of the nucleus [42]. Furthermore, excision of fibroblast and breast cancer cell nuclei from the cytoplasm using microdissection, which removed nearly all of the cytoplasm and cytoskeletal structures connected to the nucleus, produced no relaxation of the nucleus to a circular shape [54]. Finally, stress fibers are virtually absent on the apical surface of the nucleus during the early phase of cell spreading when the nucleus flattens, and nuclei flatten during spreading even in the absence of myosin activity. These observations collectively suggest that the nucleus does not store elastic energy in its deformed shape, and nuclear shape is not a result of an equilibrium balance of forces between the elastic nucleus and neighboring stress fibers.

How might the nucleus be flattened during cell spreading, and what explains the lack of elastic energy storage within its shape? Nonelastic deformation of the nucleus during cell spreading can be considered using an analogy of the nucleus as a spherical object, such as a soccer ball [13]. As the sphere is a shape with a minimum surface area-to-volume ratio, flattening it requires either an extension of its surface area, a reduction in its volume (releasing the air from the ball), or a combination of both. For nuclei, the resistance to surface stretching (i.e., stretching of the nuclear lamina) results from the high extensional modulus of the lamina [55], while osmotic coupling with the cytoplasm and the resistance of chromatin and other subnuclear structures to compression probably provide the resistance to volumetric changes of the nucleus [56–58].

Experimental measurements of nuclear flattening during cell spreading showed that the volume of fibroblast nuclei remained constant as they flattened [59]. Another recent paper revealed that the nucleus maintains a constant volume by progressively unfolding its envelope until reaching a fully unfolded state, thus acting as a ruler to sense the spatial constraints imposed by the mechanical micro-environment [60]. These findings are consistent with other measurements that showed a constant nuclear volume as cells move through narrow pores [59,61]. Furthermore, Li et al. [59] hypothesized that wrinkles in the nuclear lamina within the rounded nuclei of suspended cells (as they landed on the surface for spreading) represented an excess of surface area for the nuclear volume, which allowed nuclear flattening with little resistance as the wrinkles would unfold during the flattening process. The computational model in Li et al. predicted that the nucleus would reach a steady flattened shape once the wrinkles were fully removed and the lamina became taut, thereby resisting any extension of the lamina beyond a limiting value (given its high extensional modulus, [55] which made it stiff to cellular forces). Consistent with these predictions, undulations/wrinkles in nuclei were indeed observed to be removed upon flattening [59]. Wrinkles in nuclei were also observed to be removed during the spreading of epithelial cells, with no wrinkles visible in fully spread cells [62]. Furthermore, the level of nuclear wrinkling is associated with downstream mechanotransduction signaling (e.g., YAP/TAZ localization) [63]. These observations led to the concept that nuclear changes occur at constant volume and constant surface area in cells (reviewed in Refs. [13] and [64]). This explains why the nucleus stores no energy in its deformed shape, at least in specific contexts such as cell spreading.

Given that nuclear deformation and recovery do not appear to be determined by elastic energy storage, what establishes nuclear shape? A series of experiments that tracked the shape of the nucleus during dynamic cell spreading and during migration on defined patterns confirmed the computational model proposed by Li et al. [59] in which nuclear shape changes incrementally in response to stresses transmitted from the cytoskeleton intervening

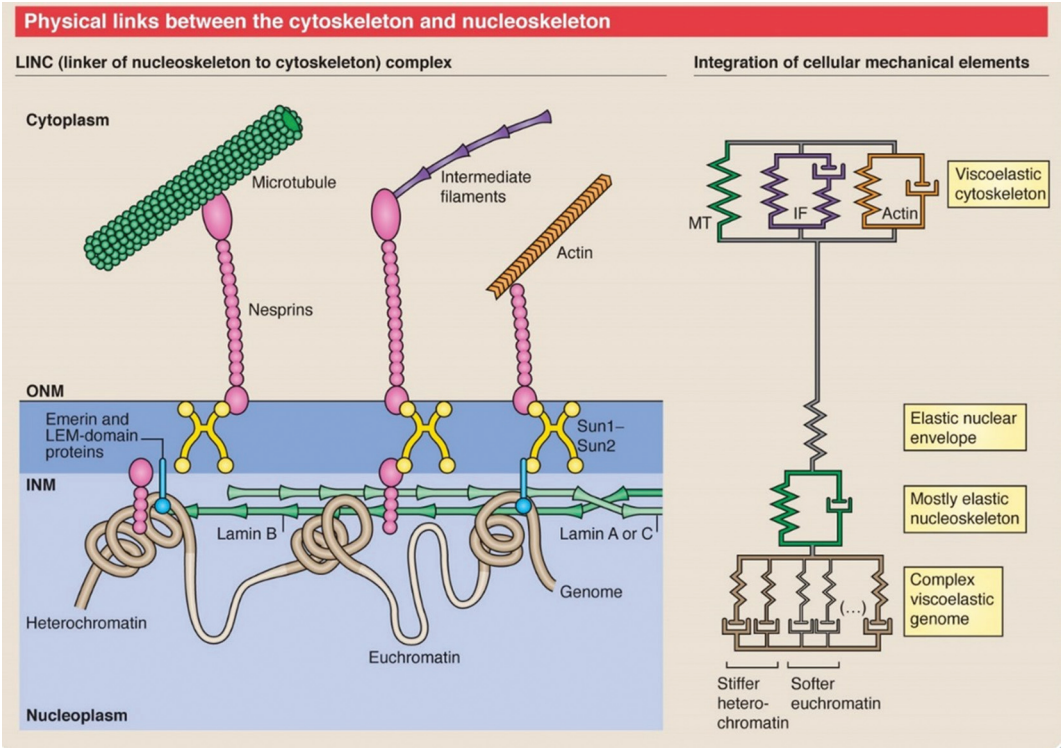

Fig. 3 The physical connection between nucleus and cytoplasm can be modeled using a combination of springs and dashpots to incorporate the complex viscoelastic nature of the connection. The cytoskeleton can be modeled using a parallel combination of elastic microtubules (MT) with viscoelastic intermediate filaments (IF) and actin filaments. The nuclear envelope is mostly elastic, which connects the cytoskeletal elements with the viscoelastic nucleoskeleton, which, in turn, connects with the complex viscoelastic chromatin architecture. From Dahl and Kalinowski [27].

between the nuclear surface and the moving cell boundary (reviewed in Ref. [13]). Because of the slow time scales at which changes in cell shape occur, the cytoskeleton provides a viscous resistance to stretching and compression, thus transmitting frictional stress to the nuclear surface from the moving cell boundary. When cells spread along a one-dimensional fibronectin line, the nuclear boundaries follow the motion of the cell boundaries. Boundaries that move away from the nuclear surface transmit a net tensile force on the nucleus, while boundaries that move toward the nuclear surface compress it. These stresses are viscous in nature, and therefore do not exist when the motion of the boundary stops. These stresses on the nucleus will exist in deforming cells even when no stress fibers impinge on the nuclear surface and in the absence of myosin activity. Experiments have demonstrated a causal relationship between the motion of cell boundaries and nuclear deformation, both during migration and spreading [42,54,59]. This novel mechanism explains both the coordination of nuclear and cell shape (elongated in elongated cells, circular in circular cells) and deformation of the nucleus into dumbbell shapes during cell migration through confined channels (which simply mimics the elongated cell shape).

4 LINCing the Nucleus to Cell and Tissue Mechanics: Connections Between the Nucleus and Its Surroundings

The LINC complex is responsible for mediating most of the known nuclear-cytoskeletal interactions (Fig. 3). It is a well-known regulator of nuclear mechanotransduction through the cytoskeleton, regulating chromatin structure, nuclear transport, and nuclear positioning. Disrupting the LINC complex in a single cell also disrupts force propagation to nearby cells through intercellular connections [16,20,65].

Despite its prominent role in cellular function, the LINC complex is not fully characterized. Unlike focal adhesions and cell–cell adhesions, visualizations of the LINC complex remain limited, and understanding of its components is generally limited to its structural proteins. SUN domain proteins span the inner nuclear membrane, extending into the interior of the nucleus, while nesprins are situated outside the nucleus, with their C-terminal KASH domains spanning the outer nuclear membrane and interacting with SUN proteins in the perinuclear space [66]. A comprehensive picture of the functional role of signaling proteins in the LINC complex is not yet known.

4.1 Cell Mechanics: Role of the LINC Complex in the Regulation of Forces on the Nucleus. Nuclear-cytoplasmic connections are important regulators of forces on the nucleus and throughout the cell [67,68]. Nuclear deformations can be observed in response to tension applied by a micropipette on the cell surface at a distance away from the nucleus, demonstrating coupling between cell shape and nuclear deformation. Similar observations have also been made using a microharpoon technique [41]. These nuclear deformations are in part mediated by the LINC complex, whose contribution can be confirmed by disruption using a dominant-negative peptide DN KASH, which results in attenuated nuclear deformation [41].

In its role as the mediator of mechanical force transfer from the cytoskeleton to the nucleus, the LINC complex itself experiences significant mechanical tension [69]. Mechanical forces on the LINC complex have been quantified using a genetically encoded, calibrated fluorescence resonance energy transfer (FRET)-based tension biosensor, known as TSmod, previously used to examine mechanical tension across structural proteins in cell-cell [70–72] and cell-matrix adhesions [73]. Three separate research groups

have designed nesprin-2 giant tension sensors, demonstrating that this FRET-based approach for measuring tension is well suited for LINC complex proteins, as well as clearly demonstrating that there are significant tensile forces between the actin cytoskeleton and the nuclear envelope [69,74,75]. These forces have been observed in several different cell types and appear to be dependent on actomyosin contractility and cell morphologies. The importance of nuclear-cytoskeletal connectivity in migration through confined spaces has been previously discussed (Sec. 3). Additional data demonstrate that the LINC complex contributes to generating the forces that drive two-dimensional migration through constricted spaces [76]. In very confined three-dimensional (3D) spaces, a piston-like behavior of the nucleus has been observed, where increased pressure exerted by the nucleus is used to pressurize the leading edge of the cell and push a so-called lobopodium through the space [77,78]. Importantly, this behavior requires both actomyosin contraction and connection to the nucleus via nesprin-3.

The roles of LINC complex forces, however, remain unclear. Forces across the LINC complex may impact nuclear movement and positioning, which is of critical importance in cell polarization during migration [79]. LINC complex forces have also been thought to regulate nuclear shape and morphology, nuclear-cytosolic transport, as well as chromatin structure, although there have been limited experiments to demonstrate these roles [80]. Notably, Ning Wang's group has shown that pulling on the surface of cells (using magnetic beads) can deform chromatin, which requires force transmission through an intact LINC complex [80,81]. In addition, recent work by Pere Roca-Cusach's group has shown that substrate stiffness affects nuclear pore complex size, as well as nuclear-cytosolic transport, which ultimately affects translocation of YAP and other mechanosensitive transcription factors [44,82]. New approaches, including new force biosensors for additional nuclear structures (e.g., nuclear pore complex, nuclear lamina) may ultimately be required to better understand how mechanical forces propagate within the nucleus.

There also exists an apparent relationship between the LINC complex and cell-ECM traction forces. Using very fine nanopillars, it is possible to quantify the traction force at high resolution [83]. The resulting data showed that the highest traction forces exist near the nucleus rather than the cell periphery. Not only were these high-stress regions sensitive to actomyosin tension, but they also required an intact LINC complex and nuclear lamina (i.e., lamin A and C expression), suggesting that cytoskeletal-nuclear coupling is essential for robust focal adhesion formation [83]. Previous measurements of traction forces using larger nanopillars may not have had the resolution necessary to detect these changes. Further supporting the relationship between the LINC complex and focal adhesions, it was recently shown that the LINC complex is required for maintaining cell-ECM adhesion when endothelial cells are challenged with physiological forces, including shear stress and stretch [84]. Interestingly, LINC complex disruption also affected the phosphorylation states of key focal adhesion proteins, suggesting that the LINC complex may be important in both mechanical and biochemical signaling [84].

4.2 Tissue Mechanics: LINC Complex Regulates Force Transmission Across Epithelial Tissues. The LINC complex also plays a role in cellular force propagation across tissues. Previous studies of multicellular systems using traction force microscopy have shown that there are significant mechanical forces transferred between cells across cell–cell adhesions [85,86]. Chromatin dynamics can be used to detect cellular force propagation by employing a technique called sensors from intranuclear kinetics (SINK), which is based on actomyosin forces transmitted to the LINC complex and into the chromatin of the nucleus [65]. SINK tracks individual points within the nucleus, establishing a relationship of mean squared displacement with time, and can be used to investigate the role of the LINC complex in monolayer mechanics [65]. Indeed, SINK measurements show that disruption of the LINC complex reduces nuclear forces on nearby cells [65], suggesting that the LINC complex is important in the propagation of force across cell-cell adhesions to nearby cells.

The importance of the LINC complex in mediating mechanical force transmission from the cytoskeleton to the nucleus can be further demonstrated by observing the effect of LINC complex disruption on cellular structures in 3D culture. Glandular epithelial cells from the breast and other organs grown in 3D basement membrane cultures assemble into acini, or hollow spheres consisting of a layer of cells around a water-filled lumen [87] (Fig. 4). LINC disruption results in the collapse of the acini lumen due to increased mechanical tension caused by upregulation of Rho-kinase-dependent nonmuscle myosin II motor activity [87]. The significance of these mechanical forces and the importance of the LINC complex are highlighted when considering their role in maintaining or changing tissue architecture during cancer progression [88].

The LINC complex has been demonstrated as a critical structure required to maintain the mechanical stability of cells and tissues. Disruption of the LINC complex alters force propagation throughout the cell and out to nearby cells and also affects the mechanical stability of multicellular assemblies. The LINC complex itself is subject to significant mechanical tension, similar to cell-cell and cell-matrix adhesions. Unanswered questions remain on how the LINC complex is formed and regulated, as well as the functional consequences of these forces.

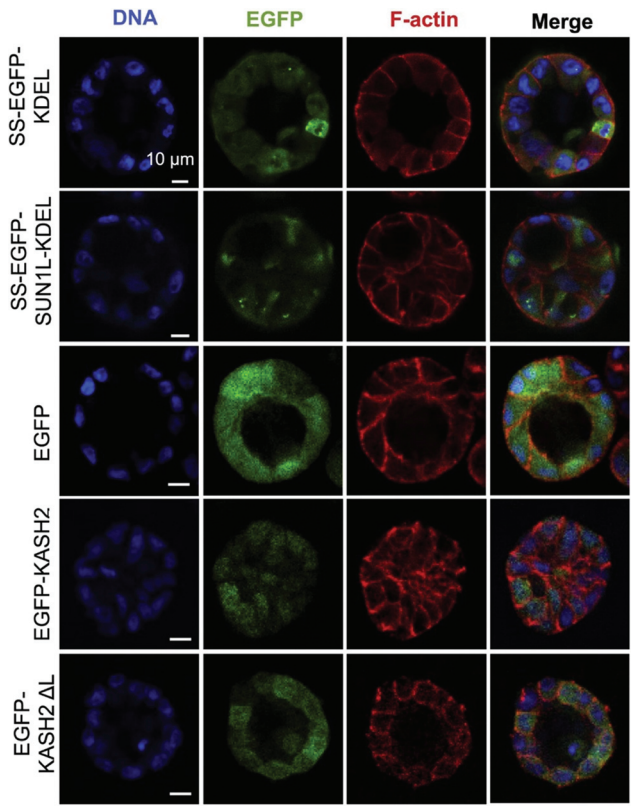

Fig. 4 Fluorescent images of representative acini formed by breast epithelial cells in Matrigel. Disruption of the LINC complex via expression of SUN proteins restricted to the endoplasmic reticulum (SS-EGFP-SUN1 L-KDEL) or KASH proteins lacking connection with the cytoskeleton (EGFP-KASH2) cause the acini to form without an interior lumen. Controls expressing constructs lacking the mutated proteins (EGFP and SS-EGFP-KDEL) or further truncating the mutated KASH proteins to prevent interactions with endogenous SUN proteins (EGFP-KASH2 ΔL) are unaffected. Reprinted with permission from Zhang et al. [87]. Copyright 2019 by Elsevier.

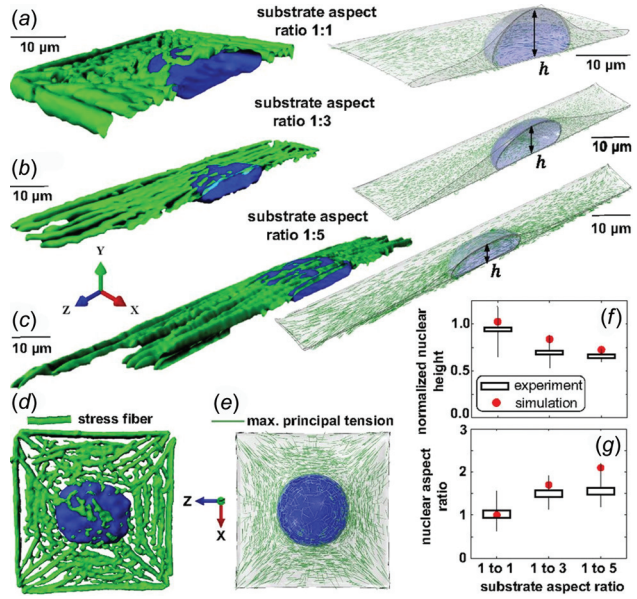

Fig. 5 Comparison of stress fiber formation/organization and nuclear shape between experimental and chemomechanical models. From Alisafaei et al. [53].

5 Modeling the Modulation of Chromatin Organization by Cues From the Cell Micro-Environment

5.1 Impact of Micro-Environment on Cell Morphology and Gene Transcription. As discussed previously, the cell nucleus constantly changes its morphology as a result of various mechanical forces on the cell. For example, it has been demonstrated that nuclear shape is determined by cell shape, which is altered depending on the shape of its substrate [89]. This may occur as a result of the formation of actin cables, which force the nucleus to mirror the shape of the surrounding cell [89]. Chromatin organization is also known to vary with cell shape, with a round nucleus known to be transcriptionally inactive (heterochromatin-dense), and an elongated nucleus observed to have a high concentration of euchromatin. This effect of cell geometry on chromatin organization is dependent on the nuclear translocation of epigenetic regulators such as HDAC3. Differential expression of as many as 400 genes has been observed between rounded and elongated cell shapes [90]. The mechanical property of chromatin, and the relative stiffness of intranuclear domains can affect the nuclear deformation under stress. Elucidation of these mechanical properties of the nucleus has been attempted recently by several groups [39,91].

A chemomechanical feedback model can be used to describe the relationship between cell shape and nuclear shape [53,89,92–95] (Fig. 5). A cell that has spread but not fully adhered to its substrate has weak adhesions, with a fluid-like G-actin in the cytoplasm, and exhibits uniform and isotropic contractility. However, uniform contractility on an anisotropic shape gives rise to anisotropic stresses, which will be concentrated at the cell edges, promoting the formation of mature focal adhesions [83]. A chemomechanical feedback parameter in the model causes the actin stress fibers to stiffen with increased tension, which leads to a polarized contractility tensor and an anisotropic distribution of actin stress fibers. Compression forces from the stress fibers flatten and elongate the nucleus and stiffen the nuclear envelope by increasing the assembly of lamin A/C. This positive feedback loop explains the various changes in the cell as well as nuclear structure and mechanics observed on substrates with different shapes.

These models can also accurately describe changes in cell morphology with the loss of actomyosin tension [53]. As mentioned above, cells cultured on two extreme geometries (i.e., a large, elongated rectangle and a small circle) have different nuclear shapes. Specifically, the elongated cell shape generates high stresses due to the concentration of stress at the edges, causing the nucleus to be pushed down and elongated. In the presence of blebbistatin, which is a myosin inhibitor, actomyosin contractility is completely eliminated, and the nucleus reverts to its original shape. As the blebbistatin is washed away, contractility again builds, and the nucleus will recompress. Importantly, these alterations in nuclear architecture also result in alterations in gene expression [53]. Note that these findings do not necessarily contradict the observations mentioned in Sec. 3 suggesting that the nucleus does not store elastic energy [54], since the loss of actomyosin tension will change the shape of the cell membrane, which in turn may drive the rounding of the nucleus [59]. However, more investigation is necessary to clearly establish the forces driving nuclear shape changes under these contexts.

Transcriptional and epigenetic regulators, e.g., YAP, HDAC, and MKL, are important mediators of gene expression changes, shuttling between the nucleus and the cytoplasm in an actomyosin-dependent fashion. The active 3D chemomechanical model described above is also able to describe MKL, HDAC, and YAP cytoplasm-nuclear shuttling kinetics in response to contractility. The model focused on contractility-dependent shuttling of HDAC and was trained using various shapes to parameterize the change in nuclear import/export rates. The model was then used to predict the level of F-actin and the amount of HDAC present in the nucleus, which will result in a more condensed, transcriptionally inactive chromatin [53]. This model was verified experimentally by applying downward compressive forces to mouse fibroblast cells, which reduced actomyosin contractility and increased import of HDAC3 into the nucleus, potentially due to changes in nuclear pore structure [44]. This then triggered an increase in heterochromatin content and inactivated gene expression [96]. The model was also able to predict the reversal of chromatin condensation with the removal of the compressive force [96]. Dr. Shenoy's lab is currently developing a phase-field modeling approach to better understand the mechanisms driving changes in chromatin condensation, or conversion from euchromatin to heterochromatin, and its spatial localization. This approach has the potential to capture chromatin–lamina and chromatin–chromatin interactions, interconversion between methylation and acetylation, changes in the size of the chromatin within the nucleus, and the number of lamin-associated domains.

5.2 Modeling the Role of the Nucleus in Cell Motility. Nuclear deformation during cell migration can also be described using a chemomechanical model that describes changes in nuclear shape as the cell migrates through tight constrictions [97,98]. By altering the elastic properties of the nucleus, the size of the constriction that cells are able to pass through can be predicted [99]. Furthermore, modeling approaches can also accurately describe cell migration through dense 3D tissue networks via nuclear pistons and the formation of migration paths using lobopodia [97,98]. Specifically, these models captured the initiation of cell protrusions in alginate gels through increasing intracellular pressure. As fluid moves into the protrusion, mechanosensitive channels are activated, allowing calcium to enter the cell, followed by water transport. The resulting expansion of the protrusion generates a migration path for the cell.

6 Unanswered Questions and Controversies

Much progress has been made over the last 20 years in understanding the importance of the mechanical properties of the nucleus and how nuclear mechanics drives mechanobiological function. Still, as described above, a comprehensive view of nuclear mechanics and mechanobiology is complex, and a

plethora of questions remain. In fact, the developments and discoveries made to date have generated even more exciting questions regarding the fundamental science and functional significance of nuclear mechanobiology. For example, as discussed in Secs. 3 and 5 of this paper, competing hypotheses remain regarding energy storage, or lack thereof, within the nucleus [13]. In addition, recent advances in measuring cell traction forces suggest that perinuclear focal adhesions may have a more important role in transmitting forces to the nucleus than focal adhesions located at the cell periphery, which challenges the current general understanding in the field [83]. Mechanical regulation of nuclear-cytosolic transport and the role of chromatin mechanics in regulating gene expression are both poorly understood and of great interest since they have the potential to provide a direct link between mechanical stimulation and altered cell behavior. Finally, continuous innovation of novel techniques for the visualization and measurement of nuclear mechanics and mechanobiology (e.g., SINK and tension sensors) is required to answer such complex questions. These conceptual and technical advances will provide a better understanding of the biological significance of nuclear mechanics and mechanobiology as well as provide clarity for future directions.

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Conflict of Interest

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Nomenclature

DN-KASH = dominant-negative KASH
FRET = fluorescence resonance energy transfer
HDAC = histone deacetylase
LINC = linker of nucleoskeleton and cytoskeleton
MKL = megakaryoblastic acute leukemia factor-1
SB3C = Summer Biomechanics, Bioengineering, and Biotransport Conference
SINK = sensors from intranuclear kinetics
STORM = stochastic optical reconstruction microscopy
YAP = Yes-associated protein
3D = three-dimensional

References

[1] Ambrosini, A., Rayer, M., Monier, B., and Suzanne, M., 2019, "Mechanical Function of the Nucleus in Force Generation During Epithelial Morphogenesis," Dev. Cell, **50**(2), pp. 197–211.e5.

[2] Seelbinder, B., Ghosh, S., Schneider, S. E., Scott, A. K., Berman, A. G., Goergen, C. J., Margulies, K. B., Bedi, K. C., Jr., Casas, E., Swearingen, A. R., Brumbaugh, J., Calve, S., and Neu, C. P., 2021, "Nuclear Deformation Guides Chromatin Reorganization in Cardiac Development and Disease," Nat. Biomed. Eng., **5**(12), pp. 1500–1516.

[3] Lavrushkina, S. V., Ovsyannikova, N. L., Yudina, A. S., Strelkova, O. S., Zhironkina, O. A., Perepelina, K. I., Malashicheva, A. B., and Kireev, I. I., 2019, "The Role of Mechanical Properties of the Nucleus in Maintaining Tissue Homeostasis," Cell Tissue Biol., **13**(3), pp. 237–241.

[4] Moss, S. F., Krivosheyev, V., de Souza, A., Chin, K., Gaetz, H. P., Chaudhary, N., Worman, H. J., and Holt, P. R., 1999, "Decreased and Aberrant Nuclear Lamin Expression in Gastrointestinal Tract Neoplasms," Gut, **45**(5), pp. 723–729.

[5] Venables, R. S., McLean, S., Luny, D., Moteleb, E., Morley, S., Quinlan, R. A., Lane, E. B., and Hutchison, C. J., 2001, "Expression of Individual Lamins in Basal Cell Carcinomas of the Skin," Br. J. Cancer, **84**(4), pp. 512–519.

[6] Tilli, C. M., Ramaekers, F. C., Broers, J. L., Hutchison, C. J., and Neumann, H. A., 2003, "Lamin Expression in Normal Human Skin, Actinic Keratosis, Squamous Cell Carcinoma and Basal Cell Carcinoma," Br. J. Dermatol., **148**(1), pp. 102–109.

[7] Martins, F., Sousa, J., Pereira, C. D., Cruz e Silva, O. A. B., and Rebelo, S., 2020, "Nuclear Envelope Dysfunction and Its Contribution to the Aging Process," Aging Cell, **19**(5), p. e13143.

[8] Phillip, J. M., Aifuwa, I., Walston, J., and Wirtz, D., 2015, "The Mechanobiology of Aging," Annu. Rev. Biomed. Eng., **17**(1), pp. 113–141.

[9] Sanchez-Adams, J., Leddy, H. A., McNulty, A. L., O'Conor, C. J., and Guilak, F., 2014, "The Mechanobiology of Articular Cartilage: Bearing the Burden of Osteoarthritis," Curr. Rheumatol. Rep., **16**(10), p. 451.

[10] Clippinger, S. R., Cloonan, P. E., Greenberg, L., Ernst, M., Stump, W. T., and Greenberg, M. J., 2019, "Disrupted Mechanobiology Links the Molecular and Cellular Phenotypes in Familial Dilated Cardiomyopathy," Proc. Natl. Acad. Sci. U. S. A., **116**(36), pp. 17831–17840.

[11] Roy, B., Yuan, L., Lee, Y., Bharti, A., Mitra, A., and Shivashankar, G. V., 2020, "Fibroblast Rejuvenation by Mechanical Reprogramming and Redifferentiation," Proc. Natl. Acad. Sci. U. S. A., **117**(19), pp. 10131–10141.

[12] Szczesny, S. E., and Mauck, R. L., 2017, "The Nuclear Option: Evidence Implicating the Cell Nucleus in Mechanotransduction," ASME J. Biomech. Eng., **139**(2), p. 021006.

[13] Lele, T. P., Dickinson, R. B., and Gundersen, G. G., 2018, "Mechanical Principles of Nuclear Shaping and Positioning," J. Cell. Biol., **217**(10), pp. 3330–3342.

[14] Miroshnikova, Y. A., and Wickström, S. A., 2022, "Mechanical Forces in Nuclear Organization," Cold Spring Harb. Perspect. Biol., **14**(1), p. a039685.

[15] Cho, S., Irianto, J., and Discher, D. E., 2017, "Mechanosensing by the Nucleus: From Pathways to Scaling Relationships," J. Cell Biol., **216**(2), pp. 305–315.

[16] Janota, C. S., Calero-Cuenca, F. J., and Gomes, E. R., 2020, "The Role of the Cell Nucleus in Mechanotransduction," Curr. Opin. Cell Biol., **63**, pp. 204–211.

[17] Kirby, T. J., and Lammerding, J., 2018, "Emerging Views of the Nucleus as a Cellular Mechanosensor," Nat. Cell Biol., **20**(4), pp. 373–381.

[18] Pelling, A. E., and Horton, M. A., 2008, "An Historical Perspective on Cell Mechanics," Pflugers Arch., **456**(1), pp. 3–12.

[19] Lavrenyuk, K., Conway, D., and Dahl, K. N., 2021, "Imaging Methods in Mechanosensing: A Historical Perspective and Visions for the Future," Mol. Biol. Cell, **32**(9), pp. 842–854.

[20] Wang, J. H., and Li, B., 2009, "Application of Cell Traction Force Microscopy for Cell Biology Research," Methods Mol. Biol., **586**, pp. 301–313.

[21] Caille, N., Thoumine, O., Tardy, Y., and Meister, J. J., 2002, "Contribution of the Nucleus to the Mechanical Properties of Endothelial Cells," J. Biomech., **35**(2), pp. 177–187.

[22] Guilak, F., 1995, "Compression-Induced Changes in the Shape and Volume of the Chondrocyte Nucleus," J. Biomech., **28**(12), pp. 1529–1541.

[23] Kan, H. C., Shyy, W., Udaykumar, H. S., Vigneron, P., and Tran-Son-Tay, R., 1999, "Effects of Nucleus on Leukocyte Recovery," Ann. Biomed. Eng., **27**(5), pp. 648–655.

[24] Aebi, U., Cohn, J., Buhle, L., and Gerace, L., 1986, "The Nuclear Lamina is a Meshwork of Intermediate-Type Filaments," Nature, **323**(6088), pp. 560–564.

[25] Dahl, K. N., Engler, A. J., Pajerowski, J. D., and Discher, D. E., 2005, "Power-Law Rheology of Isolated Nuclei With Deformation Mapping of Nuclear Substructures," Biophys. J., **89**(4), pp. 2855–2864.

[26] Vaziri, A., and Mofrad, M. R., 2007, "Mechanics and Deformation of the Nucleus in Micropipette Aspiration Experiment," J. Biomech., **40**(9), pp. 2053–2062.

[27] Dahl, K. N., and Kalinowski, A., 2011, "Nucleoskeleton Mechanics at a Glance," J. Cell Sci., **124**(5), pp. 675–678.

[28] Dahl, K. N., Ribeiro, A. J., and Lammerding, J., 2008, "Nuclear Shape, Mechanics, and Mechanotransduction," Circ. Res., **102**(11), pp. 1307–1318.

[29] Capell, B. C., and Collins, F. S., 2006, "Human Laminopathies: Nuclei Gone Genetically Awry," Nat. Rev. Genet., **7**(12), pp. 940–952.

[30] Gruenbaum, Y., Margalit, A., Goldman, R. D., Shumaker, D. K., and Wilson, K. L., 2005, "The Nuclear Lamina Comes of Age," Nat. Rev. Mol. Cell Biol., **6**(1), pp. 21–31.

[31] Lammerding, J., Fong, L. G., Ji, J. Y., Reue, K., Stewart, C. L., Young, S. G., and Lee, R. T., 2006, "Lamins a and C but Not Lamin B1 Regulate Nuclear Mechanics," J. Biol. Chem., **281**(35), pp. 25768–25780.

[32] Prokocimer, M., Davidovich, M., Nissim-Rafinia, M., Wiesel-Motiuk, N., Bar, D. Z., Barkan, R., Meshorer, E., and Gruenbaum, Y., 2009, "Nuclear Lamins: Key Regulators of Nuclear Structure and Activities," J. Cell Mol. Med., **13**(6), pp. 1059–1085.

[33] Schäpe, J., Prauße, S., Radmacher, M., and Stick, R., 2009, "Influence of Lamin a on the Mechanical Properties of Amphibian Oocyte Nuclei Measured by Atomic Force Microscopy," Biophys. J., **96**(10), pp. 4319–4325.

[34] Coffinier, C., Chang, S. Y., Nobumori, C., Tu, Y., Farber, E. A., Toth, J. I., Fong, L. G., and Young, S. G., 2010, "Abnormal Development of the Cerebral Cortex and Cerebellum in the Setting of Lamin B2 Deficiency," Proc. Natl. Acad. Sci. U. S. A., **107**(11), pp. 5076–5081.

[35] Tang, C. W., Maya-Mendoza, A., Martin, C., Zeng, K., Chen, S., Feret, D., Wilson, S. A., and Jackson, D. A., 2008, "The Integrity of a Lamin-B1-Dependent Nucleoskeleton is a Fundamental Determinant of RNA Synthesis in Human Cells," J. Cell Sci., **121**(7), pp. 1014–1024.

[36] Turgay, Y., Eibauer, M., Goldman, A. E., Shimi, T., Khayat, M., Ben-Harush, K., Dubrovsky-Gaupp, A., Sapra, K. T., Goldman, R. D., and Medalia, O., 2017, "The Molecular Architecture of Lamins in Somatic Cells," Nature, **543**(7644), pp. 261–264.

[37] Nmezi, B., Xu, J., Fu, R., Armiger, T. J., Rodriguez-Bey, G., Powell, J. S., Ma, H., Sullivan, M., Tu, Y., Chen, N. Y., Young, S. G., Stolz, D. B., Dahl, K. N., Liu, Y., and Padiath, Q. S., 2019, "Concentric Organization of A- and B-Type Lamins Predicts Their Distinct Roles in the Spatial Organization and Stability of the Nuclear Lamina," Proc. Natl. Acad. Sci. U. S. A., **116**(10), pp. 4307–4315.
[38] Stierlé, V., Couprie, J., Ostlund, C., Krimm, I., Zinn-Justin, S., Hossenlopp, P., Worman, H. J., Courvalin, J. C., and Duband-Goulet, I., 2003, "The Carboxyl-Terminal Region Common to Lamins A and C Contains a DNA Binding Domain," Biochemistry, **42**(17), pp. 4819–4828.
[39] Stephens, A. D., Liu, P. Z., Kandula, V., Chen, H., Almassalha, L. M., Herman, C., Backman, V., O'Halloran, T., Adam, S. A., Goldman, R. D., Banigan, E. J., and Marko, J. F., 2019, "Physicochemical Mechanotransduction Alters Nuclear Shape and Mechanics Via Heterochromatin Formation," Mol. Biol. Cell, **30**(17), pp. 2320–2330.
[40] Spagnol, S. T., and Dahl, K. N., 2014, "Active Cytoskeletal Force and Chromatin Condensation Independently Modulate Intranuclear Network Fluctuations," Integr. Biol., **6**(5), pp. 523–531.
[41] Lombardi, M. L., Jaalouk, D. E., Shanahan, C. M., Burke, B., Roux, K. J., and Lammerding, J., 2011, "The Interaction Between Nesprins and Sun Proteins at the Nuclear Envelope is Critical for Force Transmission Between the Nucleus and Cytoskeleton," J. Biol. Chem., **286**(30), pp. 26743–26753.
[42] Alam, S. G., Lovett, D., Kim, D. I., Roux, K. J., Dickinson, R. B., and Lele, T. P., 2015, "The Nucleus is an Intracellular Propagator of Tensile Forces in NIH 3T3 Fibroblasts," J. Cell Sci., **128**(10), pp. 1901–1911.
[43] Enyedi, B., Jelcic, M., and Niethammer, P., 2016, "The Cell Nucleus Serves as a Mechanotransducer of Tissue Damage-Induced Inflammation," Cell, **165**(5), pp. 1160–1170.
[44] Elosegui-Artola, A., Andreu, I., Beedle, A. E. M., Lezamiz, A., Uroz, M., Kosmalska, A. J., Oria, R., Kechagia, J. Z., Rico-Lastres, P., Le Roux, A. L., Shanahan, C. M., Trepat, X., Navajas, D., Garcia-Manyes, S., and Roca-Cusachs, P., 2017, "Force Triggers Yap Nuclear Entry by Regulating Transport Across Nuclear Pores," Cell, **171**(6), pp. 1397–1410.e14.
[45] Wolf, K., Te Lindert, M., Krause, M., Alexander, S., Te Riet, J., Willis, A. L., Hoffman, R. M., Figdor, C. G., Weiss, S. J., and Friedl, P., 2013, "Physical Limits of Cell Migration: Control by ECM Space and Nuclear Deformation and Tuning by Proteolysis and Traction Force," J. Cell Biol., **201**(7), pp. 1069–1084.
[46] Olins, A. L., Herrmann, H., Lichter, P., Kratzmeier, M., Doenecke, D., and Olins, D. E., 2001, "Nuclear Envelope and Chromatin Compositional Differences Comparing Undifferentiated and Retinoic Acid- and Phorbol Ester-Treated HL-60 Cells," Exp. Cell Res., **268**(2), pp. 115–127.
[47] Olins, A. L., Hoang, T. V., Zwerger, M., Herrmann, H., Zentgraf, H., Noegel, A. A., Karakesisoglou, I., Hodzic, D., and Olins, D. E., 2009, "The LINC-Less Granulocyte Nucleus," Eur. J. Cell Biol., **88**(4), pp. 203–214.
[48] Denais, C. M., Gilbert, R. M., Isermann, P., McGregor, A. L., Te Lindert, M., Weigelin, B., Davidson, P. M., Friedl, P., Wolf, K., and Lammerding, J., 2016, "Nuclear Envelope Rupture and Repair During Cancer Cell Migration," Science, **352**(6283), pp. 353–358.
[49] Irianto, J., Xia, Y., Pfeifer, C. R., Athirasala, A., Ji, J., Alvey, C., Tewari, M., Bennett, R. R., Harding, S. M., Liu, A. J., Greenberg, R. A., and Discher, D. E., 2017, "DNA Damage Follows Repair Factor Depletion and Portends Genome Variation in Cancer Cells After Pore Migration," Curr. Biol., **27**(2), pp. 210–223.
[50] Pfeifer, C. R., Xia, Y., Zhu, K., Liu, D., Irianto, J., García, V. M. M., Millán, L. M. S., Niese, B., Harding, S., Deviri, D., Greenberg, R. A., and Discher, D. E., 2018, "Constricted Migration Increases DNA Damage and Independently Represses Cell Cycle," Mol. Biol. Cell, **29**(16), pp. 1948–1962.
[51] Maciejowski, J., and Hatch, E. M., 2020, "Nuclear Membrane Rupture and Its Consequences," Annu. Rev. Cell Dev. Biol., **36**(1), pp. 85–114.
[52] Versaevel, M., Grevesse, T., and Gabriele, S., 2012, "Spatial Coordination Between Cell and Nuclear Shape Within Micropatterned Endothelial Cells," Nat Commun., **3**(1), p. 671.
[53] Alisafaei, F., Jokhun, D. S., Shivashankar, G. V., and Shenoy, V. B., 2019, "Regulation of Nuclear Architecture, Mechanics, and Nucleocytoplasmic Shuttling of Epigenetic Factors by Cell Geometric Constraints," Proc. Natl. Acad. Sci. U. S. A., **116**(27), pp. 13200–13209.
[54] Tocco, V. J., Li, Y., Christopher, K. G., Matthews, J. H., Aggarwal, V., Paschall, L., Luesch, H., Licht, J. D., Dickinson, R. B., and Lele, T. P., 2018, "The Nucleus is Irreversibly Shaped by Motion of Cell Boundaries in Cancer and Non-Cancer Cells," J. Cell Physiol., **233**(2), pp. 1446–1454.
[55] Dahl, K. N., Kahn, S. M., Wilson, K. L., and Discher, D. E., 2004, "The Nuclear Envelope Lamina Network Has Elasticity and a Compressibility Limit Suggestive of a Molecular Shock Absorber," J. Cell Sci., **117**(20), pp. 4779–4786.
[56] Finan, J. D., Chalut, K. J., Wax, A., and Guilak, F., 2009, "Nonlinear Osmotic Properties of the Cell Nucleus," Ann. Biomed. Eng., **37**(3), pp. 477–491.
[57] Finan, J. D., and Guilak, F., 2010, "The Effects of Osmotic Stress on the Structure and Function of the Cell Nucleus," J. Cell Biochem., **109**(3), pp. 460–467.
[58] Finan, J. D., Leddy, H. A., and Guilak, F., 2011, "Osmotic Stress Alters Chromatin Condensation and Nucleocytoplasmic Transport," Biochem. Biophys. Res. Commun., **408**(2), pp. 230–235.
[59] Li, Y., Lovett, D., Zhang, Q., Neelam, S., Kuchibhotla, R. A., Zhu, R., Gundersen, G. G., Lele, T. P., and Dickinson, R. B., 2015, "Moving Cell Boundaries Drive Nuclear Shaping During Cell Spreading," Biophys. J., **109**(4), pp. 670–686.
[60] Lomakin, A. J., Cattin, C. J., Cuvelier, D., Alraies, Z., Molina, M., Nader, G. P. F., Srivastava, N., Sáez, P. J., Garcia-Arcos, J. M., Zhitnyak, I. Y., Bhargava, A., Driscoll, M. K., Welf, E. S., Fiolka, R., Petrie, R. J., De Silva, N. S., González-Granado, J. M., Manel, N., Lennon-Duménil, A. M., Müller, D. J., and Piel, M., 2020, "The Nucleus Acts as a Ruler Tailoring Cell Responses to Spatial Constraints," Science, **370**(6514), p. eaba2894.
[61] Davidson, P. M., Sliz, J., Isermann, P., Denais, C., and Lammerding, J., 2015, "Design of a Microfluidic Device to Quantify Dynamic Intra-Nuclear Deformation During Cell Migration Through Confining Environments," Integr. Biol., **7**(12), pp. 1534–1546.
[62] Neelam, S., Hayes, P. R., Zhang, Q., Dickinson, R. B., and Lele, T. P., 2016, "Vertical Uniformity of Cells and Nuclei in Epithelial Monolayers," Sci. Rep., **6**(1), p. 19689.
[63] Cosgrove, B. D., Loebel, C., Driscoll, T. P., Tsinman, T. K., Dai, E. N., Heo, S. J., Dyment, N. A., Burdick, J. A., and Mauck, R. L., 2021, "Nuclear Envelope Wrinkling Predicts Mesenchymal Progenitor Cell Mechano-Response in 2D and 3D Microenvironments," Biomaterials, **270**, p. 120662.
[64] Dickinson, R. B., Katiyar, A., Dubell, C. R., and Lele, T. P., 2022, "Viscous Shaping of the Compliant Cell Nucleus," APL Bioeng., **6**(1), p. 010901.
[65] Armiger, T. J., Lampi, M. C., Reinhart-King, C. A., and Dahl, K. N., 2018, "Determining Mechanical Features of Modulated Epithelial Monolayers Using Subnuclear Particle Tracking," J. Cell Sci., **131**(12), p. jcs216010.
[66] Bouzid, T., Kim, E., Riehl, B. D., Esfahani, A. M., Rosenbohm, J., Yang, R., Duan, B., and Lim, J. Y., 2019, "The LINC Complex, Mechanotransduction, and Mesenchymal Stem Cell Function and Fate," J. Biol. Eng., **13**(1), p. 68.
[67] Maniotis, A. J., Chen, C. S., and Ingber, D. E., 1997, "Demonstration of Mechanical Connections Between Integrins, Cytoskeletal Filaments, and Nucleoplasm That Stabilize Nuclear Structure," Proc. Natl. Acad. Sci. U. S. A., **94**(3), pp. 849–854.
[68] Fedorchak, G., and Lammerding, J., 2016, "Cell Microharpooning to Study Nucleo-Cytoskeletal Coupling," Methods Mol. Biol., **1411**, pp. 241–254.
[69] Arsenovic, P. T., Ramachandran, I., Bathula, K., Zhu, R., Narang, J. D., Noll, N. A., Lemmon, C. A., Gundersen, G. G., and Conway, D. E., 2016, "Nesprin-2G, a Component of the Nuclear LINC Complex, Is Subject to Myosin-Dependent Tension," Biophys. J., **110**(1), pp. 34–43.
[70] Borghi, N., Sorokina, M., Shcherbakova, O. G., Weis, W. I., Pruitt, B. L., Nelson, W. J., and Dunn, A. R., 2012, "E-Cadherin is Under Constitutive Actomyosin-Generated Tension That is Increased at Cell-Cell Contacts Upon Externally Applied Stretch," Proc. Natl. Acad. Sci. U. S. A., **109**(31), pp. 12568–12573.
[71] Cai, D., Chen, S. C., Prasad, M., He, L., Wang, X., Choesmel-Cadamuro, V., Sawyer, J. K., Danuser, G., and Montell, D. J., 2014, "Mechanical Feedback Through E-Cadherin Promotes Direction Sensing During Collective Cell Migration," Cell, **157**(5), pp. 1146–1159.
[72] Conway, D. E., Breckenridge, M. T., Hinde, E., Gratton, E., Chen, C. S., and Schwartz, M. A., 2013, "Fluid Shear Stress on Endothelial Cells Modulates Mechanical Tension Across VE-Cadherin and PECAM-1," Curr. Biol., **23**(11), pp. 1024–1030.
[73] Grashoff, C., Hoffman, B. D., Brenner, M. D., Zhou, R., Parsons, M., Yang, M. T., McLean, M. A., Sligar, S. G., Chen, C. S., Ha, T., and Schwartz, M. A., 2010, "Measuring Mechanical Tension Across Vinculin Reveals Regulation of Focal Adhesion Dynamics," Nature, **466**(7303), pp. 263–266.
[74] Déjardin, T., Carollo, P. S., Sipieter, F., Davidson, P. M., Seiler, C., Cuvelier, D., Cadot, B., Sykes, C., Gomes, E. R., and Borghi, N., 2020, "Nesprins Are Mechanotransducers That Discriminate Epithelial-Mesenchymal Transition Programs," J. Cell Biol., **219**(10), p. e201908036.
[75] Carley, E., Stewart, R. M., Zieman, A., Jalilian, I., King, D. E., Zubek, A., Lin, S., Horsley, V., and King, M. C., 2021, "The LINC Complex Transmits Integrin-Dependent Tension to the Nuclear Lamina and Represses Epidermal Differentiation," Elife, **10**, p. e58541.
[76] Davidson, P. M., Battistella, A., Déjardin, T., Betz, T., Plastino, J., Borghi, N., Cadot, B., and Sykes, C., 2020, "Nesprin-2 Accumulates at the Front of the Nucleus During Confined Cell Migration," EMBO Rep., **21**(7), p. e49910.
[77] Petrie, R. J., Harlin, H. M., Korsak, L. I., and Yamada, K. M., 2017, "Activating the Nuclear Piston Mechanism of 3D Migration in Tumor Cells," J. Cell Biol., **216**(1), pp. 93–100.
[78] Petrie, R. J., and Yamada, K. M., 2016, "Multiple Mechanisms of 3D Migration: The Origins of Plasticity," Curr. Opin. Cell Biol., **42**, pp. 7–12.
[79] Luxton, G. W., Gomes, E. R., Folker, E. S., Vintinner, E., and Gundersen, G. G., 2010, "Linear Arrays of Nuclear Envelope Proteins Harness Retrograde Actin Flow for Nuclear Movement," Science, **329**(5994), pp. 956–959.
[80] Wang, N., Tytell, J. D., and Ingber, D. E., 2009, "Mechanotransduction at a Distance: Mechanically Coupling the Extracellular Matrix With the Nucleus," Nat. Rev. Mol. Cell Biol., **10**(1), pp. 75–82.
[81] Tajik, A., Zhang, Y., Wei, F., Sun, J., Jia, Q., Zhou, W., Singh, R., Khanna, N., Belmont, A. S., and Wang, N., 2016, "Transcription Upregulation Via Force-Induced Direct Stretching of Chromatin," Nat. Mater., **15**(12), pp. 1287–1296.
[82] Andreu, I., Granero-Moya, I., Chahare, N. R., Clein, K., Jordán, M., Beedle, A. E. M., Elosegui-Artola, A., Trepat, X., Raveh, B., and Roca-Cusachs, P., 2021, "Mechanosensitivity of Nucleocytoplasmic Transport," bioRxiv, epub.
[83] Shiu, J. Y., Aires, L., Lin, Z., and Vogel, V., 2018, "Nanopillar Force Measurements Reveal Actin-Cap-Mediated Yap Mechanotransduction," Nat. Cell Biol., **20**(3), pp. 262–271.
[84] Denis, K. B., Cabe, J. I., Danielsson, B. E., Tieu, K. V., Mayer, C. R., and Conway, D. E., 2021, "The LINC Complex is Required for Endothelial Cell Adhesion and Adaptation to Shear Stress and Cyclic Stretch," Mol. Biol. Cell, **32**(18), pp. 1654–1663.

[85] Liu, Z., Tan, J. L., Cohen, D. M., Yang, M. T., Sniadecki, N. J., Ruiz, S. A., Nelson, C. M., and Chen, C. S., 2010, "Mechanical Tugging Force Regulates the Size of Cell-Cell Junctions," Proc. Natl. Acad. Sci. U. S. A., **107**(22), pp. 9944–9949.
[86] Maruthamuthu, V., Sabass, B., Schwarz, U. S., and Gardel, M. L., 2011, "Cell-ECM Traction Force Modulates Endogenous Tension at Cell-Cell Contacts," Proc. Natl. Acad. Sci. U. S. A., **108**(12), pp. 4708–4713.
[87] Zhang, Q., Narayanan, V., Mui, K. L., O'Bryan, C. S., Anderson, R. H., Kc, B., Cabe, J. I., Denis, K. B., Antoku, S., Roux, K. J., Dickinson, R. B., Angelini, T. E., Gundersen, G. G., Conway, D. E., and Lele, T. P., 2019, "Mechanical Stabilization of the Glandular Acinus by Linker of Nucleoskeleton and Cytoskeleton Complex," Curr. Biol., **29**(17), pp. 2826–2839.e4.
[88] Paszek, M. J., and Weaver, V. M., 2004, "The Tension Mounts: Mechanics Meets Morphogenesis and Malignancy," J. Mammary Gland Biol. Neoplasia, **9**(4), pp. 325–342.
[89] Gupta, S., Marcel, N., Sarin, A., and Shivashankar, G. V., 2012, "Role of Actin Dependent Nuclear Deformation in Regulating Early Gene Expression," PLoS One, **7**(12), p. e53031.
[90] Jain, N., Iyer, K. V., Kumar, A., and Shivashankar, G. V., 2013, "Cell Geometric Constraints Induce Modular Gene-Expression Patterns Via Redistribution of HDAC3 Regulated by Actomyosin Contractility," Proc. Natl. Acad. Sci. U. S. A., **110**(28), pp. 11349–11354.
[91] Ghosh, S., Cuevas, V. C., Seelbinder, B., and Neu, C. P., 2021, "Image-Based Elastography of Heterochromatin and Euchromatin Domains in the Deforming Cell Nucleus," Small, **17**(5), p. e2006109.
[92] Shenoy, V. B., Wang, H., and Wang, X., 2016, "A Chemo-Mechanical Free-Energy-Based Approach to Model Durotaxis and Extracellular Stiffness-Dependent Contraction and Polarization of Cells," Interface Focus, **6**(1), p. 20150067.
[93] Ahmadzadeh, H., Webster, M. R., Behera, R., Jimenez Valencia, A. M., Wirtz, D., Weeraratna, A. T., and Shenoy, V. B., 2017, "Modeling the Two-Way Feedback Between Contractility and Matrix Realignment Reveals a Nonlinear Mode of Cancer Cell Invasion," Proc. Natl. Acad. Sci. U. S. A., **114**(9), pp. E1617–E1626.
[94] Cao, P., Short, M. P., and Yip, S., 2017, "Understanding the Mechanisms of Amorphous Creep Through Molecular Simulation," Proc. Natl. Acad. Sci. U. S. A., **114**(52), pp. 13631–13636.
[95] Seo, B. R., Chen, X., Ling, L., Song, Y. H., Shimpi, A. A., Choi, S., Gonzalez, J., Sapudom, J., Wang, K., Andresen Eguiluz, R. C., Gourdon, D., Shenoy, V. B., and Fischbach, C., 2020, "Collagen Microarchitecture Mechanically Controls Myofibroblast Differentiation," Proc. Natl. Acad. Sci. U. S. A., **117**(21), pp. 11387–11398.
[96] Damodaran, K., Venkatachalapathy, S., Alisafaei, F., Radhakrishnan, A. V., Sharma Jokhun, D., Shenoy, V. B., and Shivashankar, G. V., 2018, "Compressive Force Induces Reversible Chromatin Condensation and Cell Geometry-Dependent Transcriptional Response," Mol. Biol. Cell, **29**(25), pp. 3039–3051.
[97] Gong, Z., Wisdom, K. M., McEvoy, E., Chang, J., Adebowale, K., Price, C. C., Chaudhuri, O., and Shenoy, V. B., 2021, "Recursive Feedback Between Matrix Dissipation and Chemo-Mechanical Signaling Drives Oscillatory Growth of Cancer Cell Invadopodia," Cell Rep., **35**(4), p. 109047.
[98] Lee, H. P., Alisafaei, F., Adebawale, K., Chang, J., Shenoy, V. B., and Chaudhuri, O., 2021, "The Nuclear Piston Activates Mechanosensitive Ion Channels to Generate Cell Migration Paths in Confining Microenvironments," Sci. Adv., **7**(2), p. eabd4058.
[99] Heo, S. J., Song, K. H., Thakur, S., Miller, L. M., Cao, X., Peredo, A. P., Seiber, B. N., Qu, F., Driscoll, T. P., Shenoy, V. B., Lakadamyali, M., Burdick, J. A., and Mauck, R. L., 2020, "Nuclear Softening Expedites Interstitial Cell Migration in Fibrous Networks and Dense Connective Tissues," Sci. Adv., **6**(25), p. eaax5083.