00272 Chromatin and Nuclear Biophysics

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Introduction

The nucleus is an ellipsoidal organelle that houses, protects, and maintains the genome, the unique sequence of DNA that acts as the information template of the cell. This physical compartment contains both the genome and the proteins necessary to provide and maintain key functions associated with the genome. The interphase nucleus' main jobs consist of protecting/repairing the genome and the reading or transcription of the genome. The basic dogma of molecular biology is DNA to RNA to protein to cell function. Thus, cellular behavior is largely determined in the nucleus via transcription of the genome. This allows for organisms to have one genome but produce many different types of cells with different cellular behaviors mostly based on differential transcription that occurs within the nucleus. While we know a lot about how the genome is maintained and transcribed on a biochemical level, only recently have scientists begun to uncover the importance of the physical properties on these key functions.

The nucleus is the strongest organelle in the cell. Force measurements have shown that the nucleus is relatively an order a magnitude stronger than the cytosol, the body of the cell (**Guo *et al*., 2013**). The two major mechanical components of the nucleus are the chromatin, which fills the nucleus, and lamin intermediate filaments, which form a thin meshwork at the periphery just inside the inner nuclear membrane (**Stephens *et al*., 2017b**) (**Fig. 1(A)**). Chromatin includes the two meters of DNA (the genome separated into chromosomes) and its associated proteins that compact and organize it. The chromatin, while much longer, must fit into the much smaller space of the nucleus, which is an ellipse 10–20 μm in diameter and 3–10 μm in height (many orders of magnitude smaller). Calculations estimate that chromatin fills 30%–40% of the nucleus by volume (**Ou *et al*., 2017**). While chromatin fills every nucleus, the other major mechanical element lamin A filaments are not present at sufficient levels in all nuclei (**Swift *et al*., 2013**), leading to the view that chromatin is the base mechanical component dictating the biophysical properties of the nucleus that aid its proper function.

Structure and Mechanics

The basic structure of the chromatin is dictated by histone modification state and linker histone H1. DNA is wrapped around nucleosomes, a histone octamer core of 2 sets of histone proteins H2A, H2B, H3 and H4, to provide the basic compaction of chromatin (**Luger *et al*., 1997**). This compaction is about seven-fold as the DNA backbone wraps around the nucleosome 1.66 times. The linker histone H1 binds at the entry and exist sites of the DNA wrapped around the nucleosome where it stabilizes this interaction and aids higher-order chromatin organization and further compaction (Hergeth and Schneider, 2015; Xiao *et al*., 2012). Post-translational modifications of histone tails inside the nucleosome dictate the spacing and behavior of the neighboring nucleosomes resulting in a chromatin fiber with a variable thickness of 5–24 nm (**Ou *et al*., 2017**). Euchromatin is generally defined by acetylation of histone tails causing charge interactions with neighboring histones that results in a physically open or decompact chromatin. This form of chromatin is accessible to transcriptional proteins and thus is associated with actively transcribed regions of the genome. Alternatively, heterochromatin is generally defined as chromatin with methylated histone tails and heterochromatin binding proteins that allows for close spacing of nucleosomes and chromatin compaction resulting in decreased transcription. Organizationally, euchromatin is found on the nuclear interior and near the nuclear pore complexes while heterochromatin is localized to the nuclear periphery or the borders of nucleoli, substructures within the nucleus.

Chromatin compaction mediated by histone modification state and linker histone H1 presence provides the base mechanical properties of the chromatin. Chromatin's mechanical contribution to the nucleus is largely dictated by the levels of compact heterochromatin and association of H1, which strengthen the nucleus, while decompact euchromatin weakens it. Drugs or genetic perturbations of histone modification enzymes that add or remove methyl groups and acetyl groups from histone tails or that displace H1 change chromatin-based nuclear mechanics by ~35% (Furusawa *et al*., 2015; Hobson *et al*., 2020; Krause *et al*., 2013; Shimamoto *et al*., 2017; Stephens *et al*., 2017a). Histone deacetylase inhibitors, such as Trichostatin A (TSA) and valproic acid (VPA), increase euchromatin levels and decreased nuclear rigidity. More recently broad inhibitors of either histone methyltransferases via Deazaneplanocin A (DZNep) (**Miranda *et al*., 2009**) or histone demethylases via methylstat (**Luo *et al*., 2011**) have allowed for a general decrease or increase in heterochromatin levels, respectively, resulting in a commensurate change in nuclear rigidity (Stephens *et al*., 2018; Stephens *et al*., 2019b). Furthermore, H1 binding can be disrupted via antagonist High Mobility Group proteins HMGN5 and HMGA1 resulting in nuclear softening (Furusawa *et al*., 2015; Senigagliesi *et al*., 2019). These studies and others show that chromatin provides the base mechanical resistance of the nucleus.

The nucleus has a two-regime force response dictated by chromatin at short extensions (< 30% strain) and lamin A providing a strain stiffening increase in nuclear strength at long extensions (>30% strain) (Hobson *et al*., 2020; Stephens *et al*., 2017a). This finding was best exemplified by measuring the strength of the nucleus via micromanipulation fore measurements before and after chromatin digestion via non-specific cutting with the enzyme micrococcal nuclease (MNase) (**Stephens *et al*., 2017a**). This experiment revealed that chromatin is essential to the initial force response of the nucleus (nucleus extensions <30% strain). Upon chromatin digestion with MNase the elliptical nucleus visually deflates losing both its shape and physical strength. The lamina, which remains, in chromatin digested nuclei appears floppy and does not contribute to shape maintenance or initial force response (**Banigan *et al*., 2017**). Instead, studies show that the nucleus must be heavily stretched until the lamina is aligned along the tension axis and begins to stretch at high extension before the lamina provides force resistance. Thus, chromatin provides the initial and base force response of the nucleus and its presence in most nuclei is enough to maintain nuclear force response to extra- and intracellular forces.

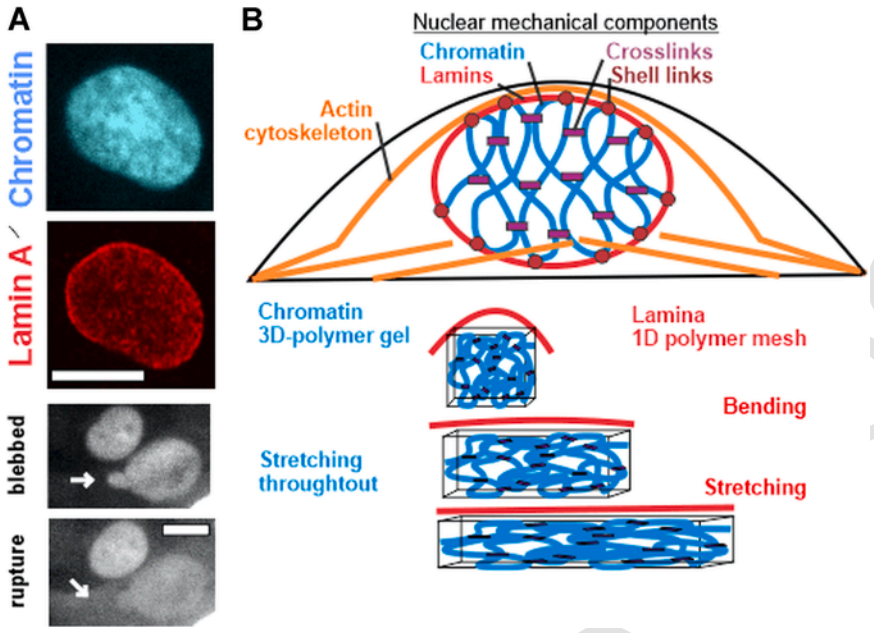

Fig. 1 Chromatin is a major mechanical component of the nucleus. (A) Representative images of the two major mechanical components of the nucleus: chromatin filling the nucleus and lamins that form a thin meshwork at the periphery. Decreased chromatin compaction through histone modification state causes abnormal nuclear morphology leading to blebbing and ruptures that result in nucleus and cellular dysfunction. (B) Schematic of the major mechanical components (chromatin and lamins) and subcomponents (chromatin-chromatin crosslinking and chromatin-lamin shell links). The schematic shows that chromatin behaves as a 3D polymer gel to resist mechanical deformation across short and long deformations. Alternatively, lamins behaving as a 1D worm-like-chain bend over short deformations then stretch at long strains.

Lamins form a thin-meshwork at the nuclear periphery and protect the nucleus during high levels of strain. Lamin A, the main mechanical lamin component, is not present in all cells including stem or undifferentiated cells and has very low levels in cells in non-mechanically demanding environments (neuronal and apodoses) (**Swift *et al.*, 2013**). Alternatively, Lamin B, for which there are two genes Lamin B1 and B2, is essential in all nuclei and acts as a mechanical "weakening" element acting as the denominator under lamin A levels to determine lamin mechanical strength (nuclear lamina strength = LA/LB). Lamin C, which is an alternative splicing product from the lamin A/C gene has no known direct mechanical contribution but may interact with chromatin in a manner to aid chromatin and/or whole nuclear mechanics. Studies show that Lamin A provides a strain stiffening element to the nucleus that protects the nucleus against strains >30% by causing a 50%–100% increase in the nuclear spring constant in this regime (Stephens *et al*., 2017a; Stephens *et al*., 2018). Cells without lamin A, an example being neuronal background Human Embryonic Kidney cells (HEK293), nuclei appear to get weaker (strain thinning) as they are extended further beyond 30% strain and become plastically deformed. This role of protecting the nucleus at high strain regime is specifically important for cellular migration (**Davidson *et al.*, 2014**) and in tissues that incur high forces like muscle and bone. This makes sense as lamin A levels are higher in stiff human tissue while levels are relatively lower in softer tissue.

Physical simulations reveal the two-regime force response of the nucleus stems from the biophysical properties and geometries of these two elements (**Fig. 1(B)**). First, both chromatin and lamins are polymers that have a short 150–300 nm persistence length, or length over which they remain straight. This means that lamins are easily bendable and floppy. So why are their behaviors so different? Chromatin fills 30%–40% of the nucleus (**Ou *et al.*, 2017**), has many crosslinks or self-interactions and is connected to the lamina making it best represented by a network 3D gel (**Dekker and Mirny, 2016**). This interconnected nature means the polymer once engaged in one area can transmit tension throughout to provide a bulk spring property that resists small extensions. Differently, lamin filaments form thin (10 s of nanometers) interconnected meshwork at the periphery of the nucleus (Shimi *et al*., 2015; Turgay *et al*., 2017). This thin sheet at the nuclear periphery provides an effective 1D polymer that bends easily (low persistence length) until it is aligned along the tension axis. Once aligned lamin filaments are hard to stretch (high elastic modulus) providing a stiffening of the nucleus at high extensions. Thus, lamins behave like a classic worm-like chain polymer model, which has been experimentally measured (**Panorchan *et al.*, 2004**). Thus, the inherent geometries of chromatin and lamins determine the differential force response regimes as demonstrated by an interior 3D gel chromatin-based short extension force response and 1D meshwork that provides lamin-A-based strain stiffening at long extensions (**Banigan *et al.*, 2017**).

Chromatin Linking Proteins

Simulations of a minimal model of the cell nucleus require two subcomponents to recapitulate experimentally determined nuclear mechanics, chromatin-chromatin and chromatin-lamin linkers. As described above, simulations start with two main mechanical components: a polymer filling the nucleus with a defined elastic modulus to represent the chromatin and a thin peripheral meshwork to represent the lamin, with a defined bending and elastic modulus. However, this basic two component system fails to recapitulate the experimentally determined two-regime force response of the nucleus (**Banigan *et al*., 2017**). The other two key classes of mechanical components of the nucleus are chromatin-chromatin proteins we will call crosslinkers as well as chromatin-lamin proteins we will call tethers. Simulations show that loss of chromatin-chromatin crosslinkers only decreases the short extension, or chromatin-based nuclear spring constant. Alternatively, simulated loss of chromatin-lamin crosslinkers decreases both the short/chromatin-based force response as well as the long extension strain stiffening/lamin A-based force response. Loss of physical transfer of forces via chromatin-lamin linkers to the lamina delays bending of the lamina and provides less force transfer when the lamina is being stretched to result in an apparent decrease in the strain-stiffening regime. Thus, there are many more proteins that fill these two roles crosslinking the chromatin and lamina that will contribute to nuclear mechanics.

Biologically, key chromatin-chromatin crosslinkers are heterochromatin protein 1 alpha (HP1α, experimentally determined (**Strom *et al*., 2020**)) and cohesin, CTCF, mediator, and RNA that form loops and are experimentally determined to provide 3D organizational properties for the genome via a technique called Hi-C/ chromosomes conformation. While reports focus heavily on these proteins roles to spatially position genes in the nucleus, which would impact transcriptional status (as mentioned above), recent studies are finding that their mechanical roles to resist forces may be equally or more important. Chromatin-chromatin linkages via HP1α provide mechanical resistance separately from histone modification state (**Strom *et al*., 2020**). Recent evidence finds that chromatin-chromatin physical crosslinking occurs on the order of every 25 kb in the genome (**Belaghzal *et al*., 2019**). This was accomplished by digesting the DNA backbone of chromatin in isolated nuclei then assaying chromatin interactions and nucleus force response. Frequent digestion of chromatin via a restriction endonuclease cutting on average every 6–10 kb resulted in a massive loss of chromatin interactions and a 65% decrease in chromatin-based nuclear spring constant. However, less frequent cutting at 10–25 kb resulted in no significant change in chromatin interaction frequency and spring constant, suggesting chromatin-chromatin crosslinkers are physically holding the genome together to maintain interactions and physical strength.

Chromatin-lamin linking proteins provide physical support for the nuclear periphery. Depletion of link proteins results in greater fluctuations of the nuclear membrane and a weakened nuclear spring constant (**Schreiner *et al*., 2015**). There are many interaction partners between chromatin and lamins (**Kubben *et al*., 2010**) though it remains unclear how many contribute to nuclear mechanics. Of interest is lamin A, which is known to interact with chromatin non-specifically or through other proteins (**Scaffidi and Misteli, 2006**). Lamin A mutations result in many different human diseases and it is hypothesized that most of these are due to altered interactions with chromatin (**Lionetti *et al*., 2020**) and less with changes in lamin A's direct mechanical contribution. Alterations of lamin A and one well defined chromatin-lamin linker lamin associated protein 2 alpha (LAP2α) have been shown to be needed for the stereotypical heterochromatin localization at the nuclear periphery (**Solovei *et al*., 2013**). Upon depletion of these two components the euchromatin moves to the periphery while the heterochromatin moves to the interior, a reverse of the "normal" organization. Other known possible physical linkers include lamin B1/2 and lamin B receptor (LBR). Both have been shown to be crucial to the interaction/tethering of chromatin to the nuclear periphery assayed by a technique called Dam-ID (**Guelen *et al*., 2008**). Other possible tethering proteins include LINC complexes components SUN and Nesprin proteins as well as barrier to autointegration factor (BAF) a protein with chromatin, lamin, and membrane interaction properties and functions (**Cho *et al*., 2017**).

How Nuclear Mechanics Influences Transcription

A gene's location in 3D space in the nucleus highly influences the level of transcription and could be influenced by physical forces. In most cells genes at the nuclear periphery are repressed while genes in the interior of the nucleus or at nuclear pores are actively transcribed. A great example of this is that genes will change position between these two major areas during differentiation to activate cell type specific genes or deactivate alternative cell type or pluripotency genes (**Gdula *et al*., 2013**). This bias of transcriptionally off at the periphery is due mostly to tethering of histone methyltransferases at the nuclear periphery that will methylate histones inducing heterochromatin formation (**Black *et al*., 2012**). It should be mentioned that genes tethered at the periphery, specifically at nuclear pores, are more highly transcribed due to proteins tethered to the nuclear pore and the export of mRNA that is tied to transcription (**Capelson *et al*., 2010**). Alternatively, the interior is more euchromatic allowing access to transcriptional components and thus higher levels of transcription. While these behaviors are well detailed, physical forces that pull and push on the nucleus can reorganize location of genes (**Seirin-Lee *et al*., 2019**) and thus change their transcription level remain understudied.

Mechanotransduction provides a non-mutually exclusive way physical principals and forces alter the transcriptional profile of the nucleus and thus the cell. Mechanotransduction is the response to both extracellular and intercellular mechanical stimuli. Forces outside the cell can be transmitted along the cytoskeleton and to the nucleus (**Cho *et al*., 2017**). Cytoskeletal components such as actin, microtubules, and intermediate filaments such as vimentin are physically integrated into the nucleus through proteins called Linker of Nucleoskeleton and Cytoskeleton proteins (LINC), including SUN, KASH, and Nesprin proteins. These LINC proteins span the double membrane of the nucleus and connect the cytoskeleton to chromatin and lamins. This integration of forces into the nucleus results in two phenomena that can alter gene transcription. First, force applied to the nucleus can change import or export of transcription factors. An

example of this pathway is best described by MLK1-SRF, which responds to mechanical stress and allows import of the SRF transcription factor into the nucleus (**Ho *et al*., 2013**). Second, forces transmitted into the nucleus can physically stretch chromatin resulting in increased transcription (**Tajik *et al*., 2016**). Thus, overall the reaction to forces applied to the cell and nucleus can control nuclear function through altering the transcription profile of the cell which then determines cell behavior.

Mechanosensation pathways provide a cellular response that occurs at the cell membrane but influences nuclear mechanics and bulk properties that dictate transcription. Physical stretching of the cell membrane is sensed by mechanosensitive ion channels that open under applied lateral forces (**Ranade *et al*., 2015**). Examples of these channels include Piezo 1 and 2 (Bae *et al*., 2011; Pardo-Pastor *et al*., 2018) and transient receptor potential (TRP) proteins (Gailly, 2012; Liu *et al*., 2015). This pathway is thought to be partially shared with mesenchymal cell differentiation, showing it has biological function (Heo *et al*., 2018, 2016; Le *et al*., 2016; Nava *et al*., 2020). During this differentiation cells must adapt to a stiffer environment through increasing nuclear mechanical resistance, through increasing heterochromatin, and/or lamin A levels. These changes could also affect the transcriptional profile of the cell. Further studies have shown that this mechanosensing pathway can also modulate nuclear mechanics in differentiated common cell culture lines, which will be discussed later in this article.

Nuclear Mechanics Protects Nuclear Function

The physical resistive properties of the nucleus maintain key nuclear functions through compartmentalization. The nucleus is first and foremost a compartment. It relies on concentrating proteins and excluding others from the delicate tasks of protecting the genome from damage and transcription of the genome to determine overall cellular behavior. The nucleus is the strongest organelle and is tightly coupled to the cytoskeleton to support force resistance and proper cell motility. This integration with the cytoskeleton means that the nucleus is subject to both prodding by rigid microtubules and compression by actin filament networks that run lines (TAN lines (**Luxton *et al*., 2011**)) on top of the nucleus and provide compressive force flattening the nucleus (Hatch and Hetzer, 2016; Khatau *et al*., 2009; Le Berre *et al*., 2012; Tocco *et al*., 2017). Either of these two cytoskeletal elements can provide enough force to rupture the nucleus in mechanically weakened nuclei, and thus can result in loss of compartmentalization (**Fig. 1(A)**). Rupturing of the nucleus is commonly visualized by a loss of nuclear localization signal green fluorescence protein (NLS-GFP) from the nucleus and spilling into the cytosol (De Vos *et al*., 2011; Vargas *et al*., 2012). These ruptures are not fatal, can be repaired by ESCRT proteins (Denais *et al*., 2016; Raab *et al*., 2016) with the help of BAF (Halfmann *et al*., 2019; Young *et al*., 2020), and are transient on the order of 10 s of minutes to less than two hours (**Robijns *et al*., 2016**). Loss of compartmentalization due to a weak nucleus ultimately leads to nucleus dysfunction.

Loss of nuclear compartmentalization is tied both to disruption of major nuclear functions and abnormal nuclear shape. Recent findings have verified that nuclear mechanics are critical to nuclear functions of transcription, DNA damage protection, and even alterations to cell cycle control. In many human diseases the cell undergoes perturbations that change its identity through these three functions. Upon nucleus rupture, transcription factors can be titrated into the cytosol, while transcription factors sequestered to the cytosol can enter through the ruptured nuclear membrane (**De Vos *et al*., 2011**). Thus, the transcription profile of the nucleus and cell changes in these cases. The most studied dysfunction is the increase in DNA damage associated with ruptures (Denais *et al*., 2016; Raab *et al*., 2016) and deformed nuclei (**Stephens *et al*., 2019b**). Rupturing of the nucleus titrates DNA damage repair factors while DNA cutting enzymes like TREX1 in the cytosol (meant to protect against DNA viruses) can enter the nucleus and damage the genome/DNA (**Xia *et al*., 2018**). Finally, there is initial evidence that nuclear rupture disrupts cell cycle factors resulting in loss of cell cycle control (**Pfeifer *et al*., 2018**), an event common to cancer cells that grow and divide uncontrollably. Thus, nuclear mechanics must resist extracellular and cytoskeletal forces to maintain compartmentalization and proper function of the genome.

Nuclear ruptures are a new finding that has shed light on one of the greatest unsolved mysteries of modern-day microscopy studies. Since the advent of the microscope, abnormal nuclear morphology has been a hallmark of human diseases (Cremer and Cremer, 2001; Papanicolaou and Traut, 1997). Today, it is known that many major human ailments display abnormal nuclear morphology including: heart disease, muscular dystrophy, aging, diabetes, atherosclerosis, neurological disorders, and many cancers (breast, cervical, renal, lung, leukemia, and prostate). The Pap Smear test developed in the 1930s was developed to examine exfoliated cervical cells' nuclear shape to diagnose and prognose the presence and severity of cervical cancer. This test is still commonly used along with other medical assays. Today, abnormal nuclear morphology and orientation are being used to understand the best way to treat breast cancer as these phenotypes suggest the need of more aggressive cancer treatment (**Lu *et al*., 2018**). While we have initial studies that have linked abnormal nuclear morphology with nuclear dysfunction, we still do not fully understand why abnormal nuclear morphology is a marker of human disease.

Chromatin Mechanics Rescue Lamin Perturbations

While many human diseases display direct perturbations to lamins, it appears the causative mechanism lies with downstream changes to chromatin. Progeria is an advanced aging syndrome caused by mutations to the lamin A/C gene resulting in a mutant lamin A protein (reviewed in (**Butin-Israeli *et al*., 2012**)). However, this mutation causes alterations to lamin A-chromatin interactions and decreased levels of heterochromatin (Dechat *et al*., 2008; Shumaker *et al*., 2006). The cellular phenotype of the Progeria cells are abnormally shaped nuclei that present nuclear ruptures, increased DNA damage and telomere dysregulation. However, many of these phenotypes can be recapitulated by decreasing only heterochromatin levels (Stephens *et al*., 2018; Stephens *et al*., 2019b) or depleting chromatin proteins (Stephens *et al*., 2019a; Tamashunas *et al*., 2020), suggesting these effects are not reliant on mutant lamin A (Stephens *et al*., 2018; Stephens *et al*., 2019b). Furthermore, rescuing only heterochromatin levels restores normal nuclear shape, compartmentalization, and

DNA damage protection. Thus, the phenotype and disease relevant changes occurring in these lamin A mutants is due to decreased chromatin-based nuclear mechanics, and not lamin A-based mechanical contributions.

Similar cases permeate through nuclear mechanics studies. Loss of lamin B1 is known to cause senescence, a growth arrest associated with aging that has been linked to cancer. In cellular models of senescence and/or lamin B1 depletion abnormal nuclear morphology and nuclear ruptures are pervasive. Again, levels of facultative heterochromatin decrease drastically in this phenotype (Camps *et al*., 2014; Stephens *et al*., 2018). Lamin B1 has a passive mechanical role as a weakening element relative to lamin A (nuclear lamina strength = LA/LB) (**Swift *et al*., 2013**). In this case lamin B1 loss would make the nucleus stronger, which conflicts with an inability to maintain normal nuclear shape and maintenance. Alternatively, restoration of decreased heterochromatin levels in LB1 depleted cells partially restores normal nuclear shape and compartmentalization. Thus, senescence and lamin B1 depletion represents another case where chromatin mechanics better explain nuclear morphological changes.

Chromatin may be the dominant mechanical component determining nuclear shape and compartmentalization. Loss of either major mechanical component – chromatin compaction or lamin A -results in abnormal nuclear shape and rupture. Interestingly, chromatin compaction and its mechanical contribution can restore normal nuclear morphology and compartmentalization in lamin-based perturbations, such as in models of Progeria/advanced aging, senescence, and partially in breast cancer. Currently, there is no data suggesting lamin A mechanics can conversely rescue nuclear shape and maintenance in chromatin mutants. It is likely that abnormal nuclear shapes and ruptures are more influenced by the short extension chromatin-based regime because deformations on the same scale a few micrometers where chromatin force resistance dominates.

Further work will be needed to continue to separate the mechanical and functional contributions of the chromatin compaction state and lamin composition to the nucleus. Recent advances in chromatin biology (**Agbleke *et al*., 2020**) and modeling (**Hobson and Stephens, 2020**) will aid this endeavor. While chromatin may indeed control nuclear mechanical response to small deformations on the scale of nuclear blebs, lamins likely control deformations while the nucleus is in high deformation regimes, for example during migration (Davidson *et al*., 2014; Harada *et al*., 2014). The importance of each component likely varies depending on cell type, tissue, cytoskeletal composition, and extracellular cues both mechanical and biochemical. The last two decades have provided numerous advances in our understanding of the interplay between physical forces and biological function. We must continue our pursuit to better understand the interplay between nuclear mechanics, morphology, and function as it directly relates to the spectrum of human disease and ailments.

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