Emerging views of the nucleus as a cellular mechanosensor

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Abstract

The ability of cells to respond to mechanical forces is critical for numerous biological processes. Emerging evidence indicates that external mechanical forces trigger changes in nuclear envelope structure and composition, chromatin organization, and gene expression. However, it remains unclear if these processes originate in the nucleus or are downstream of cytoplasmic signals. This review discusses recent findings supporting a direct role of the nucleus in cellular mechanosensing and highlights novel tools to study nuclear mechanotransduction.

Introduction

Cells are constantly being exposed to mechanical forces, such as shear forces on endothelial cells[1], compressive forces on chondrocytes[2], and tensile forces in myocytes[3]. The cells' ability to sense and respond to these mechanical cues are critical for numerous biological processes, including embryogenesis[4, 5], development[4, 5], and tissue homeostasis[6, 7]. While it has long been recognized that mechanical forces can influence cell morphology and behavior[8, 9], the understanding of the molecular pathways involved in mechanosensing, and how disruption of these pathways can give rise to various diseases, is still evolving[10-13]. Stretch activated ion-channels, adhesions complexes, cell-cell-junctions, and cytoskeletal components have all been identified as mechanosensitive elements that can activate cellular signaling pathways such Rho-family GTPases or the mitogen-activated protein kinase–extracellular signal-regulated kinase (MAPK–ERK), induce nuclear translocation of the transcriptional regulators YAP/TAZ and MKL1, and ultimately result in expression of mechanoresponsive genes (see [14-18] for review). Over the last two decades the question whether the nucleus itself can sense mechanical stimuli has received increasing attention[19, 20]. Such 'nuclear mechanotransduction' could provide a more rapid and direct method to transduce forces into cellular events[21, 22] and act in concert with or independent of cytoplasmic mechanotransduction pathways. In this scenario, forces applied to the nucleus via the cytoskeleton may modulate the effect of cytoplasmic signals, or even be sufficient to directly trigger changes in gene expression. Such multifaceted mechanotransduction may enable cells to distinguish between small forces only affecting the cell surface, and larger forces resulting in large-scale cell and nuclear deformations. Spurred in part by advances in biophysical, biochemical, and imaging assays, multiple mechanisms have been proposed to explain how forces acting on the nucleus could influence chromatin organization, transcription, and other cellular processes[19, 22-24]. However, distinguishing between nuclear events that are downstream of cytoplasmic mechanosensitive signaling pathways, and those that reflect true nuclear mechanotransduction events, remains challenging.

One aspect that is universally accepted now is that extracellular and cytoplasmic forces are transmitted across the nuclear envelope to the nuclear interior, where they can cause deformation of chromatin and nuclear bodies[20, 25-27]. Intriguingly, a recent study demonstrated that force application to the nucleus can induce chromatin stretching and expression of a reporter transgene[28]. These findings provide some of the most direct evidence to date for the nucleus as a mechanoresponsive organelle. Below we discuss current findings that support nuclear mechanotransduction, explain potential molecular mechanisms, and highlight emerging technologies to study nuclear mechanotransduction.

The nucleus and the nuclear lamina

The nucleus is the largest and stiffest organelle in the cell[29, 30]. It can be broadly separated into the nuclear interior, which houses chromatin, nuclear bodies and other intranuclear elements, and the surrounding nuclear envelope. The nuclear envelope is comprised of the outer and inner nuclear membranes (ONM and INM, respectively), which contain a large number of membrane-bound proteins[31, 32], as well as nuclear pore complexes (NPCs) that control entry of large molecules into the nuclear interior[33]. Underneath the INM lies the nuclear lamina, a filamentous protein network comprised of

A-type and B-type lamins, and lamin binding proteins[34, 35]. In mammalian somatic cells, the major A-type lamin isoforms are lamin A and C, encoded by the *LMNA* gene. One major motivation to study the role of the nucleus in mechanotransduction came from the identification of *LMNA* mutations as the genetic cause for various forms of muscular dystrophy and cardiomyopathy[36-38]. Diseases caused by lamin mutations (commonly referred to as laminopathies) remain both intriguing and perplexing. Although A-type lamins are nearly ubiquitously expressed, many of the *LMNA* mutations predominantly affect mechanically active tissue, i.e., skeletal muscle, cardiac muscle, and tendons. These tissue-specific disease phenotypes suggest that defects in the nucleus can impair the ability of cells to respond appropriately to mechanical forces. It is now well recognized that the nuclear lamina governs numerous biological functions, both biophysical and biochemical, including determining nuclear size and stiffness[39-43], regulating translocation and activity of transcription factors[44-47], interacting with chromatin and regulating its epigenetic state[48, 49], and controlling cell polarization and migration[50-52]. Consequently, cells lacking lamin A/C or expressing disease-causing mutations display severe defects in nuclear stability[53-55], cytoskeletal dynamics[47, 51], and nucleo-cytoskeletal force transmission[55, 56]. Furthermore, lamin A/C-deficient and mutant cells fail to adequately activate mechanoresponsive genes when subjected to mechanical stimulation[43, 57, 58], suggesting an important role of the nucleus, and lamin A/C in particular, in cellular mechanotransduction. However, it remains incompletely understood to what extent lamins directly respond to mechanical stress *in vivo*, and if changes in lamin levels and organization are downstream of other mechanotransduction pathways[26, 59-61]. The importance of the nuclear lamina in fundamental biological processes is highlighted by the early death of mice that lack functional lamin A/C. These mice are born without any overt defects, but develop severe muscular dystrophy and dilated cardiomyopathy and die at 2-8 weeks of age[62, 63]. Uncovering how lamins mediate nuclear processes and mechanosensitive gene expression will not only enhance our understanding of mechanotransduction per se, but may also provide insights into the pathophysiology of laminopathies, with the potential to inform therapeutic approaches for these currently incurable diseases.

Force transmission to the nucleus

Work by the Ingber group in the 1990s provided some of the first evidence that forces can be transmitted from the cell surface to the nucleus via the cytoskeleton[20]. It is now recognized that these forces are transmitted across the nuclear envelope through the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex[64, 65]. The LINC complex is comprised of nesprin proteins that reside within the ONM and contain a C-terminal KASH (Klarsicht, ANC-1, Syne Homology) domain, which interacts with SUN (Sad1 Unc-84) domain proteins located on the INM. The SUN proteins in turn bind to the nuclear lamina, nuclear pores, and chromatin (Fig. 1A)[66]. On the cytoplasmic side, nesprins can interact with each other and with all major cytoskeletal filaments. The composition of the LINC complex and LINC complex associated proteins vary with cell type. Furthermore, both nesprin-1 and -2 contain alternative start and stop sites that produce a number of isoforms, including the so-called “giant” variants, which contain an N-terminal actin-binding domain[67]. Nesprin-1 and -2 can bind to actin filaments[67] and the microtubule associated motor proteins kinesin[68] and dynein[69]; nesprin-3 binds to plectin[70], which

connects to intermediate filaments; nesprin-4 interacts with kinesin-1[71] (Fig. 1A). Additional KASH domain proteins and LINC complex associated proteins have recently been characterized and are often cell-type specific. We refer the readers to excellent recent reviews on the LINC complex for further details[34, 66, 67, 72].

Whereas external forces can be applied to the nucleus independent of the LINC complex, for example, during compression of the nucleus[73], or cell migration through confined environments[74], cells in many cases require an intact LINC complex to effectively transmit forces between the cytoskeleton and the nucleus. Consequently, depletion or expression of dominant-negative nesprin and SUN proteins severely impairs nucleo-cytoskeletal force transmission[27] and mechanosensitive gene expression[28, 75]. Nonetheless, it remains to be tested whether the impaired mechanotransduction is due to the role of LINC complex components in intracellular force transmission, or whether these proteins contribute through other functions, such as serving as signaling scaffolds or regulating other aspects of nuclear organization, including chromatin mobility and nuclear envelope tethering[49, 76]. Force-induced nuclear deformation further require an intact and adequately tensed cytoskeletal network[77, 78] to transmit forces from the cell surface to the nucleus[22]. If the actin cytoskeleton is disrupted through pharmacological or genetic approaches, force transmission to the nucleus is impaired[78, 79], which is accompanied by changes in chromatin dynamics[77]. Notably, mechanically-induced changes in the nucleus, cytoskeleton, and extracellular matrix appear to be interrelated. For example, the mechanical properties of the extracellular matrix affect both cytoskeletal organization[80] and the expression of lamin A/C[61, 81], resulting in cells finely tuned with their physical environment.

The intricate relationship between the cytoskeletal network, nuclear mechanics, and the mechanical environment is particularly important in skeletal and cardiac muscle cells. These contractile cells have a highly organized cytoskeleton, including a specialized perinuclear network that anchors the nucleus in place (Fig. 1B). Desmin is a muscle-specific cytoplasmic intermediate filament that interacts with the nuclear envelope through plectin 1[82]. This interaction is important for myofiber health[83], and functional loss of plectin releases tension on the nucleus and results in altered expression of mechanoresponsive genes[82]. LINC complex proteins have similarly important functions in muscle cells. The LINC complex is required for myonuclear movement[84-87], including the effective spacing of nuclei along the myofiber length. Loss of LINC complex function causes muscular dystrophies[88-90], suggesting that adequately connecting the nucleus to the cytoskeleton is crucial for skeletal muscle health and maintenance. This idea is further supported by the finding that *LMNA* mutations that cause muscular dystrophy and dilated cardiomyopathy result in impaired nucleo-cytoskeletal coupling[55, 91, 92] and loss of structural function, whereas *LMNA* mutations associated with lipodystrophy have little or no effect on nuclear mechanics and nucleo-cytoskeletal force transmission[55, 91].

Although striated muscle are the tissues impacted most by disruption in nuclear mechanics and nucleo-cytoskeletal coupling, many other cell types are also affected by impaired nucleo-cytoskeletal force transmission[93, 94]. For example, T-cell activation requires proper lamin A/C and LINC complex function to regulate T-cell receptor

clustering and F-actin formation[93]. In fibroblasts and endothelial cells, depletion of lamin A/C or disruption of the LINC complex reduces migration capabilities[94-96]. Similarly, the LINC complex is important in outer hair cells for hearing[97], proper function of the ciliary rootlets in photoreceptors and ependymal cells[98], hair follicle structure[99], and radial neuronal migration during neurogenesis[100]. These findings demonstrate the broad importance of nucleo-cytoskeletal force transmission on cellular function.

Potential mechanisms for nuclear mechanotransduction

The negative effects of lamin mutations and LINC complex disruption are well documented, but the underlying molecular mechanisms remain incompletely understood. External forces are transmitted across the cytoskeleton to the nucleus, where they result in substantial deformation[101-103]. These forces and deformations could modulate transcriptional activity and chromatin organization through a number of mechanisms.

One potential mechanism to transduce forces acting onto the nucleus into altered transcriptional activity is by modulating the physical organization of chromatin. The spatial location of the DNA with the nucleus exists in a non-random organization. This “4D nucleome” (meaning the 3D chromatin architecture and its change over time) is important for transcriptional regulation and cellular functions[104-107]. Heterochromatic DNA, which is tightly wrapped around histones and largely inaccessible for the transcriptional machinery, is often localized to the nuclear periphery[49]. This peripheral localization promotes gene silencing, while repositioning of genes towards the nuclear interior generally facilitates gene activation[108], although additional regulations apply. Thus, force-induced changes in gene positioning relative to the nuclear periphery could alter the transcriptional activity of specific genes and contribute to nuclear mechanotransduction. Supporting this idea, altering cytoskeletal organization and tension by culturing cells on micropatterned substrates alters nuclear shape and chromosome distribution, accompanied by changes in gene expression[103, 109]. It remains unclear to what extent these changes are the direct result of altered cytoskeletal forces acting on the nucleus versus upstream signaling pathways that may be sensitive to cytoskeletal organization. Extrinsic force application to cells can also induce repositioning of nuclear bodies and the associated chromatin[110-112], which could affect additional nuclear processes. Lastly, whereas changes in chromatin organization may lay downstream of forces acting on the nucleus, the epigenetic state of chromatin also contributes to the mechanical properties of the nucleus: chromatin decondensation increases nuclear deformability, and chromatin condensation decreases nuclear deformability[110, 113-116], both of which may occur independently of changes in lamin levels[117]. Thus, changes in nuclear organization, even when downstream of other pathways, can have a direct effect on nuclear deformation and may thus modulate other nuclear mechanotransduction processes.

In addition to changes in gene or chromosome positioning, mechanical forces may directly alter chromatin organization and transcription. In vitro experiments indicate that 5 pN of force is sufficient to decondense single chromatin fibers[118]. Recent work from the Wang and Belmont labs demonstrated that applying forces to the cell surface results in instantaneous stretching of chromatin inside the nucleus, associated with rapid induction of transcription of a transgene located within that chromatin region[28]. Notably, the level of

transcription correlated with the frequency and magnitude of the applied forces, and disruption of the LINC complex abolished the force-mediated transcription response[28]. The finding that force-induced transcription occurred extremely rapidly (<30 seconds) suggests that the stretching of chromatin alters the accessibility of the transcriptional machinery to the gene or its activity, rather than altering the epigenetic state of the locus. Though highly intriguing, such directly mediated modulation in gene expression has yet to be demonstrated for endogenous genes. Furthermore, it remains to be seen whether this mechanism of modulating gene transcription only applies to genes that are already "primed" for transcription, or if it could also activate silenced genes, such as those in heterochromatic regions. Intriguingly, prolonged force application induces an increase in heterochromatin and transcriptional repression[4], which could serve as a negative feedback mechanism. Lastly, it is unclear how force-induced chromatin stretching would be able to confer specificity, as it is likely that multiple genomic loci would be subjected to a similar level of mechanical force, and direct association between mechanoresponsive genes and LINC complex components have not been demonstrated to date.

Force-induced molecular crowding could present another potential nuclear mechanotransduction mechanism. Nuclear deformation could also alter nuclear processes by local crowding and exclusion of soluble factors in areas where chromatin has been compacted. For example, exclusion of DNA damage repair factors delay repair of DNA breaks[119, 120]. Similar exclusion of transcriptional regulators or chromatin remodelers could alter transcriptional activity.

Recent studies revealed that mechanical stress can induce conformational and post-translational changes (e.g. phosphorylation) in nuclear envelope proteins (Fig. 2)[26, 60, 61, 121]. Force application on the nucleus results in apical-to-basal differences in the conformation of lamin A/C, as evidenced by the masking of certain C- and N-terminal epitopes under tension[60]. Exposing isolated nuclei to shear stress exposes a cryptic cysteine residue (Cys552) in the Ig-domain of lamin A/C, which is normally inaccessible during periods of low mechanical stress[61]. It remains to be seen whether this residue can become exposed under physiological forces in intact cells, as the N-terminal portion of the Ig-domain appears largely inaccessible during periods of high mechanical stress *in vivo*[60]. Recent findings further indicate that reduced cytoskeletal tension, for example, when cells are cultured on soft substrates, results in increased lamin A/C phosphorylation, which is associated with increased solubility and degradation[61, 121]. In contrast, increased cytoskeletal tension results in decreased lamin A/C phosphorylation and higher lamin A/C levels[121]. Similarly, force application to isolated nuclei through the LINC complex causes phosphorylation of the INM protein emerin[26], which binds to lamin AC. It is unclear whether these phosphorylation events are triggered by increased residue accessibility after force-induced conformational changes, or if force application is modulating the activity of nuclear kinases such as Src[122]. Regardless of the specific mechanism, mutations of the relevant Tyr74 and Tyr95 sites in emerin results in decreased stress fiber formation and decreased expression of SRF-dependent genes[26]. In response to prolonged force application, emerin may also serve to reinforce the actin network at the ONM and facilitate chromatin remodeling[4]. Although additional work is needed to elucidate the specific pathways involved, including whether emerin and lamin are

downstream of other mechanosensitive signaling events and which biochemical signals are activated by their phosphorylation, these findings demonstrate the relevance of nuclear envelope proteins in modulating transcriptional activity and nuclear and cytoskeletal organization.

Force-induced stretching of the nuclear membranes could present an additional mechanism for nuclear mechanotransduction. Hypotonic swelling of the nuclear membranes results in the translocation of nucleoplasmic phospholipases A2 ($cPLA_2$) to the INM, which is inhibited when the nucleus is stabilized by either F-actin or lamin A/C[123]. This translocation directly activates $cPLA_2$ and 5-LOX[123], which are required for the production of the chemotactic eicosanoids that attract leukocytes to sites of injury *in vivo* [123]. Since the underlying nuclear lamina is substantially stiffer than the nuclear membranes, it mechanically shields the nuclear membranes from large forces. At the same time, the nuclear lamina can tolerate substantially larger area strains than lipid membranes[115, 116]. Thus, nuclear envelope composition and organization could dramatically modulate the stretch response of the nuclear membrane. Furthermore, since the nuclear membranes are continuous with the endoplasmic reticulum (ER), stretching of the nuclear membrane is expected to increase the membrane tension in the adjacent rough ER[124]. It will be interesting to determine whether increased membrane tension on the nucleus can alter the organization of the rough ER, and possibly the distribution of ER membrane-bound proteins[125]. For example, polysomes are enriched in ER sheets rather than ER tubules[126], thus reducing membrane curvature could increase their exposure to the cytosol (Fig. 2).

An extreme form of nuclear mechanotransduction is force-induced nuclear membrane rupture. Compressive forces on the nucleus generated by actomyosin contractility can increase intranuclear pressure and result in nuclear membrane blebbing and transient loss of nuclear envelope integrity (i.e., nuclear envelope rupture)[79, 127-130]. Although these phenomena were first observed in cells deficient for lamin A/C, cells carrying lamin A/C mutations[91], cells with lower levels of B-type lamins,[79] and cancer cells with a compromised nuclear lamina[54], it is now apparent that all cells regularly exhibit transient nuclear envelope rupture. Defects in the nuclear lamina, increased actomyosin contractility, and external confinement can dramatically increase the incidence of nuclear envelope rupture from a few percent to the majority of cells[79, 131]. Cells typically restore nuclear envelope integrity and remain viable, but loss of nuclear envelope integrity results in uncontrolled exchange of cytoplasmic and nuclear proteins[91, 128], mislocalization of organelles[54], and DNA damage[128, 129]. The effect of nuclear envelope rupture on cell signaling, chromatin organization, gene expression, and long-term outcome remain incompletely understood and are topics of active investigation. Transcriptome analysis of nuclear rupture induced by severe cell compression revealed activation of DNA damage response pathways, metabolism, and nucleolar RNA production[132]. Recent findings additionally point to an important function of cGAS, a cytoplasmic DNA binding protein first recognized for its activation of the STING pathways when encountering viral DNA in the cytoplasm[133]. The latest findings indicate that cGAS can also be activated when exposed to genomic DNA after nuclear envelope breakdown of micronuclei[134-137].

Increased nuclear membrane tension could also potentiate cytoplasmic signaling pathways by altering the permeability of NPCs (Fig. 2). Current models generated from the atomic structures of NPC components suggest that the NPC can undergo conformational changes that constrict or dilate the NPC in response to mechanical force[138-140]. Force-mediated alterations to NPC conformations could arise from increase in nuclear membrane tension or force transmission through LINC complex proteins and nuclear lamins. Both Sun1 and lamin A/C interact with NUP153[141, 142], a protein that comprises a portion of the NPC basket[143]. In support of this mechanism, recent work by the Roca-Cusachs group found that direct force application to the nucleus is sufficient to promote nuclear entry of YAP, a mechanosensitive transcription factor[73]. The increase in nuclear YAP localization occurs through increased nuclear import of YAP, mediated by an increase in the permeability of the NPC for larger proteins, and the partial unfolding of YAP to further promote transit through the NPC[73]. Besides an increase in NPC permeability, other nuclear envelope proteins may modulate the import/export of mechanosensitive transcription factors such as YAP/TAZ and MKL1 [47, 57, 144] through additional mechanisms (Fig. 2). Lamin A/C has also been shown to sequester transcription factors, such as retinoblastoma protein[145, 146] and c-Fos[44], at the nuclear periphery and thereby control their activity within the nucleus. Through these mechanisms, the nuclear lamina may further modulate gene expression and cell behavior.

Whereas short-term force application has been shown to rapidly induce transcription[28, 43], long-term force application (12 h) can result in a global increase in heterochromatin and transcriptional repression[4], suggesting that there may be a different response to force application depending on the duration of stimulation. Future studies will also need to consider differences in the response across cell type, as certain cell types may have an increased susceptibility to chromatin stretching resulting from differences in lamin A/C expression[61]. Lastly, while it appears that chromatin stretching can rapidly increase gene activation and Pol II recruitment (Fig. 2), prolonged mechanical stimulation likely activates mechanoresponsive feedback mechanisms that further influence gene expression, nuclear organization, and nucleo-cytoskeletal force transmission. Intriguingly, mechanical force application to isolated nuclei via nesprins results in lamin A/C recruitment and emerin phosphorylation, causing nuclear stiffening[26]. Thus, biochemical signaling pathways activated by mechanoresponsive genes could result in similar feedback loops which alter the responsiveness of the cell to further mechanical forces.

Technologies to study nuclear mechanotransduction

One major challenge in the field of nuclear mechanotransduction is uncoupling changes in nuclear structure, organization, and transcription that are directly due to force application to the nucleus from those that are secondary to changes in cytoplasmic mechanosensitive signaling pathways. Address this challenge requires (1) improvements in the temporal resolution of nuclear events to distinguish between immediate and downstream consequences; (2) enhanced detection of force-induced changes in chromatin organization and local transcription; (3) direct measurements of intranuclear and perinuclear forces; and (4) experimental approaches that can physically separate nuclear and cytoplasmic mechanotransduction contributions.

One method to study the force-induced relocation of genes within the nucleus or the local stretching and unfolding of chromatin loops within a single chromosomal region is to insert arrays of LacO sequences into specific genomic loci, and then fluorescently label these site with GFP-LacI (Fig. 3)[28]. This reporter system allows for assessing how effective chromatin stretching, measured by an increased distance between adjacent GFP-LacI loci, corresponds to changes in gene expression of the reporter gene, which can be quantified by fluorescence *in situ* hybridization (FISH) against the RNA transcript. Recent developments in labeling specific genomic regions of endogenous genes using CRISPR/Cas9 and related systems could help overcome the challenge of having to insert large LacO arrays or using bacterial artificial chromosome (BAC) reporters, and may even enable multi-color imaging by using dCas9 constructs from different bacterial species, each tagged with a different fluorophore (Fig. 3)[147, 148]. Measuring changes in the 4D nucleome could be further aided by the use of super-resolution microscopy, which allows resolving features down to 20-100 nm in intact cells[149] (Table 1). In addition to optical microscopy based approaches, changes to chromosomal arrangement can be studied using sequence-based technology, such as Hi-C, which is based on the chromosome conformation capture (3C)-based methodology[150] (Fig. 3). Hi–C can detect chromatin interactions across the entire genome, both within and between chromosomes, by covalently crosslinking protein/DNA complexes in their *in situ* configuration followed by deep sequencing. Whereas Hi-C is traditionally performed on large cell numbers (~10^6 cells), approaches are currently in development to extent this technique to smaller cell numbers and even single cells[151]. Changes in the accessibility of DNA regions may provide additional information on force-induced changes in chromatin organization, which could modulate transcriptional activity. One exciting approach is Assay for Transposase-Accessible Chromatin sequencing (ATAC-seq), which identifies accessible chromatin regions based on the insertion of a hyperactive transposase and subsequent genome fragmentation and sequencing[152]. Applying Hi-C and ATAC-seq analyses to cells in high- and low-force environments, or to cells before and after nuclear force application should provide detailed information on how external forces alters the spatial interactome of chromatin, which could be further coupled with RNA-seq analysis to determine if chromatin changes corresponds to a change in gene transcription.

Molecular tension sensors can provide insights into the forces applied across specific cellular structures. Biophysical measurements on intact cells and isolated nuclei indicate that ~1-10 nN are required to induce substantial nuclear deformation[116, 153, 154]. The recent development of a nesprin tension biosensor has enabled the first measurements of forces transmitted across the LINC complex[25, 155]. Using an artificial nesprin-2giant construct containing a FRET-based tension module, Conway and colleagues demonstrated that force transmission changed with both myosin activity and cell elongation, and that the basal and apical sections of the nucleus are exposed to different forces[25]. Potential limitations of the current version of the tension sensor include a low signal-to-noise ratio, the insertion site of the FRET tension module, and its force range limit of ~6 pN[156, 157], motivating further work in this area.

Lastly, one way to circumvent the confounding cytoplasmic signaling events that arise from applying force at the cell surface is to study isolated nuclei, or to use micromanipulation to apply force in close proximity to the nucleus[158, 159]. Using magnetic beads bound to the cytoplasmic domain of nesprins allows studying the role of the LINC complex in nuclear mechanotransduction and targeting specific nesprin isoforms[26]. One limitation of using isolated nuclei is that the isolation procedure may perturb nuclear structure, as well as the chemical composition of the nuclear interior (e.g., ion concentrations, ATP-levels, molecular crowding), which could affect nuclear mechanics and other nuclear processes[42]. Furthermore, working with isolated nuclei limits experiments to studying factors that originate within the nucleus, and excludes studying the import of cytoplasmic factors. Disrupting the LINC complex in intact cells allows exchange of biochemical molecules and can help identify events that require force transmission to the nucleus and nuclear deformation[159]. However, external force application may still induce nuclear deformation through LINC-complex independent mechanisms.

Future Perspective

The field of mechanobiology has substantially evolved and advanced in the past two decades, greatly enhancing our knowledge of how mechanical cues govern cell behavior. It is now well recognized that nuclear envelope proteins play a crucial role in the cellular response to mechanical stimuli, and that forces are transmitted from the cell surface and cytoskeleton to the nuclear interior. Increasing findings suggest that the nucleus can act as a cellular mechanosensor. Nonetheless, many questions remain, including to what extent the nucleus itself responds to mechanical forces, where such nuclear mechanotransduction processes occurs, and if these nuclear processes complement or act in parallel or downstream of cytoplasmic signaling pathways. To untangle further the profound interplay between the nucleus, cytoskeleton, and cell surface will take an integrative approach that employs biophysical assays, genetic manipulation, high-throughput genomic and proteomics, and live-cell imaging with high spatial and temporal resolution. Furthermore, experimental approaches must be employed that attempt to uncouple nuclear changes due to indirect mechanisms (i.e., cytoplasmic signal that modulate chromatin organization and transcription) from force-induced, nucleus-intrinsic events, for example, by utilizing models in which nuclear force transmission is disrupted while other cytoplasmic mechanosensitive pathways remain intact. Unraveling the force-sensitive molecular regulatory networks controlled by the nucleus and the nuclear lamina will not only increase our understanding of cellular mechanotransduction, but may also spur the development of novel therapeutic approaches to treat the currently incurable diseases that arise from impaired nuclear mechanics and mechanotransduction.

Acknowledgements

We apologize to all authors whose work could not be cited due to space constraints. This work was supported by awards from the National Institutes of Health [R01 HL082792 and U54 CA210184], the Department of Defense Breast Cancer Research Program [Breakthrough Award BC150580], the National Science Foundation

[CAREER Award CBET-1254846 and MCB-1715606], and a Fleming Postdoctoral Fellowship to TK.

Competing Financial Interests

The authors have no competing financial interests to declare.

Figure and Table Legends

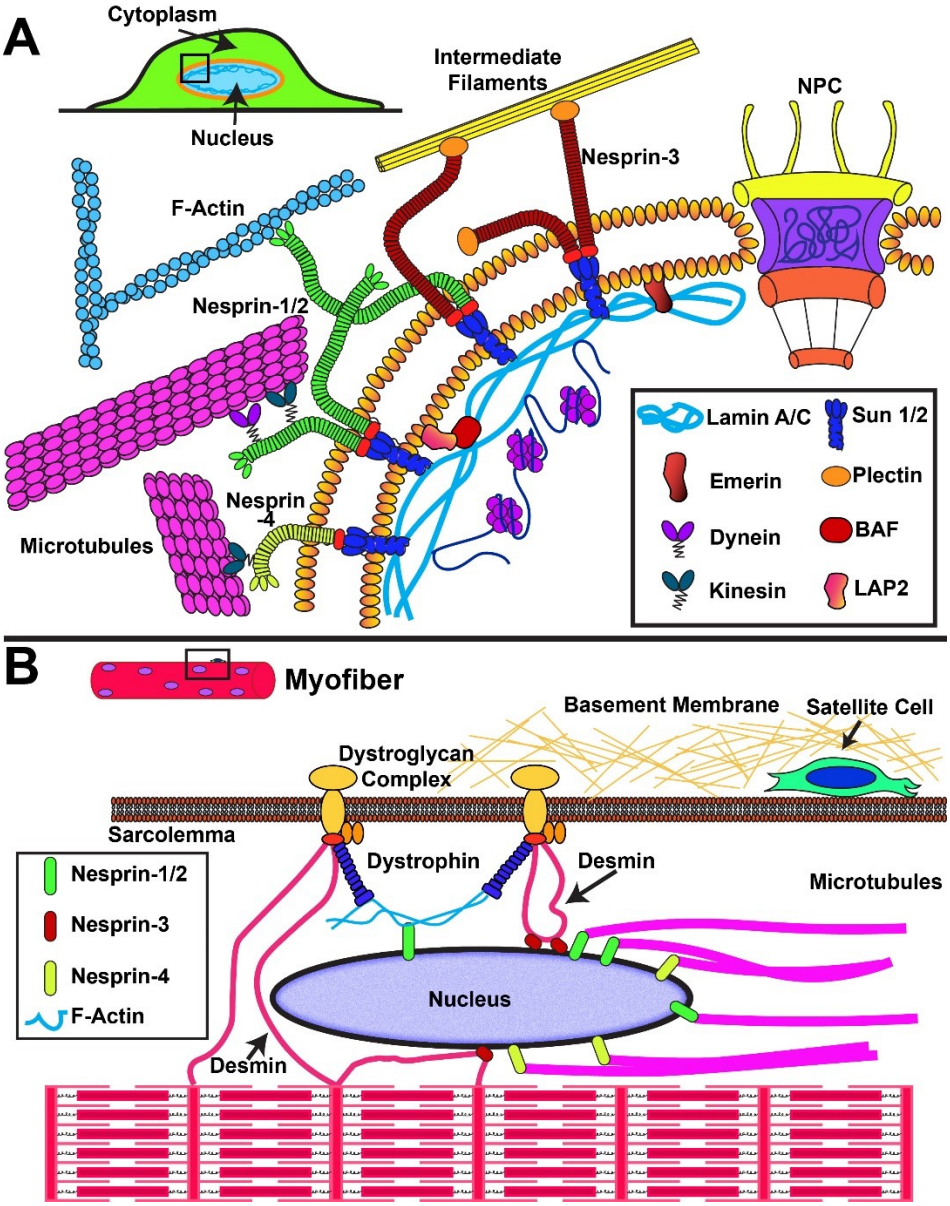

Figure 1. Schematic overview of nuclear envelope proteins involved in force transmission to the nucleus. (**A**) Force transmission to the nucleus involves interaction of cytoskeletal elements (actin filaments, intermediate filaments, microtubules) with nesprin proteins on the ONM, which transmit force through SUN domain proteins on the INM to the nuclear lamina and interior. (**B**) Organization of the cytoskeletal network within muscle cells, including the highly ordered actin-myosin structures to form contractile sarcomeres and myofibrils. Nuclei are positioned at the periphery of the cell, where they interact with the muscle-specific proteins dystrophin (through actin filaments) and desmin. Additional proteins such as LINC complex proteins and lamins may be involved in anchoring the myonuclei and place and transmitting forces between the nucleus and cytoskeleton.

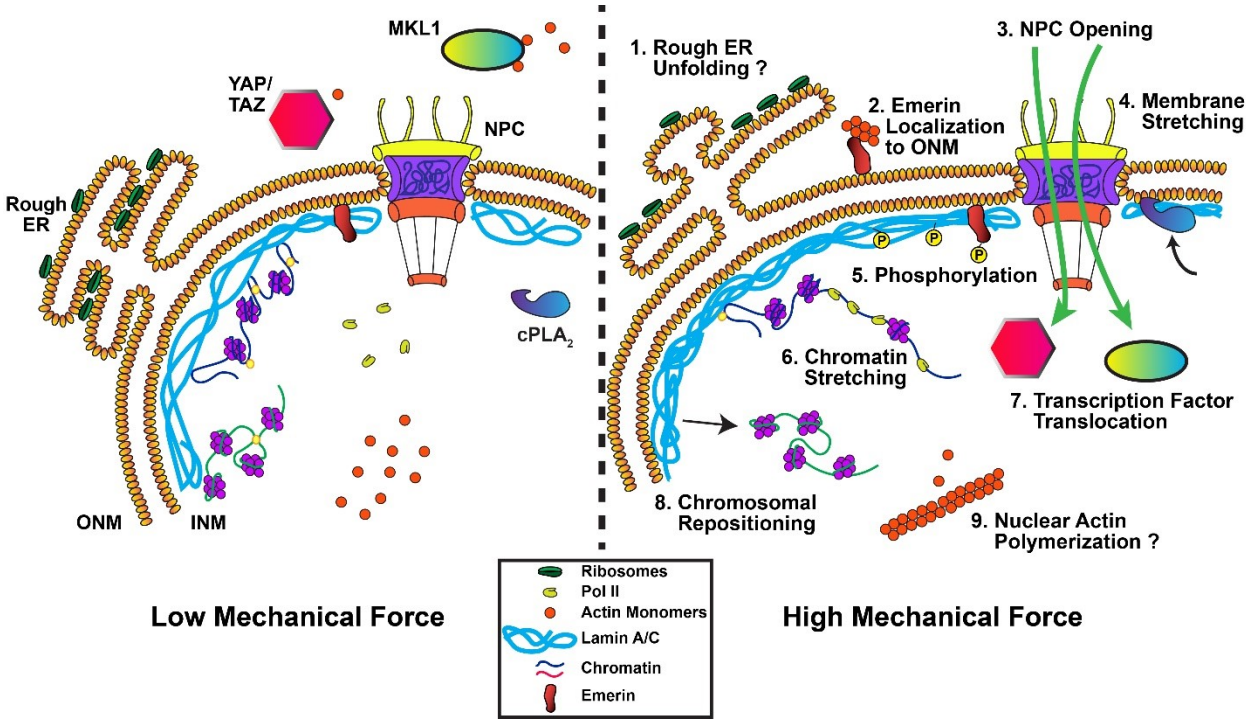

Figure 2. Proposed mechanisms for how the cell nucleus could respond directly to mechanical forces. (**1**) Stretching of the nuclear membrane could alter the conformation of the rough ER, exposing more ribosomes to the cytoplasm. (**2**) Force application promotes translocation of emerin from the INM to the ONM, modulating chromatin organization and facilitating actin polymerization at the ONM. (**3**) Increased membrane tension could open nuclear pore complexes (NPC) and modulate NPC permeability. (**4**) Stretching of the nuclear membrane recruits $cPLA_2$ to the INM. (**5**) Force transmission to the nucleus results in post-translational modification and altered dynamics of lamin A/C and INM proteins such as emerin (see also (2)), which can modulate the mechanical properties of the nucleus and induce downstream signaling. (**6**) External forces can induce chromatin stretching, altering polymerase and transcription factor accessibility and activity. (**7**) Nuclear pore opening and sequestration at the nuclear envelope can modulate localization and activity of transcription factors. (**8**) Forces acting on the nucleus may reposition chromatin domains, altering their transcriptional activity. (**9**) Mechanically induced polymerization of nuclear actin can modulate export and activity of the transcriptional regulator MKL1, and affect other nuclear processes that require monomeric actin.

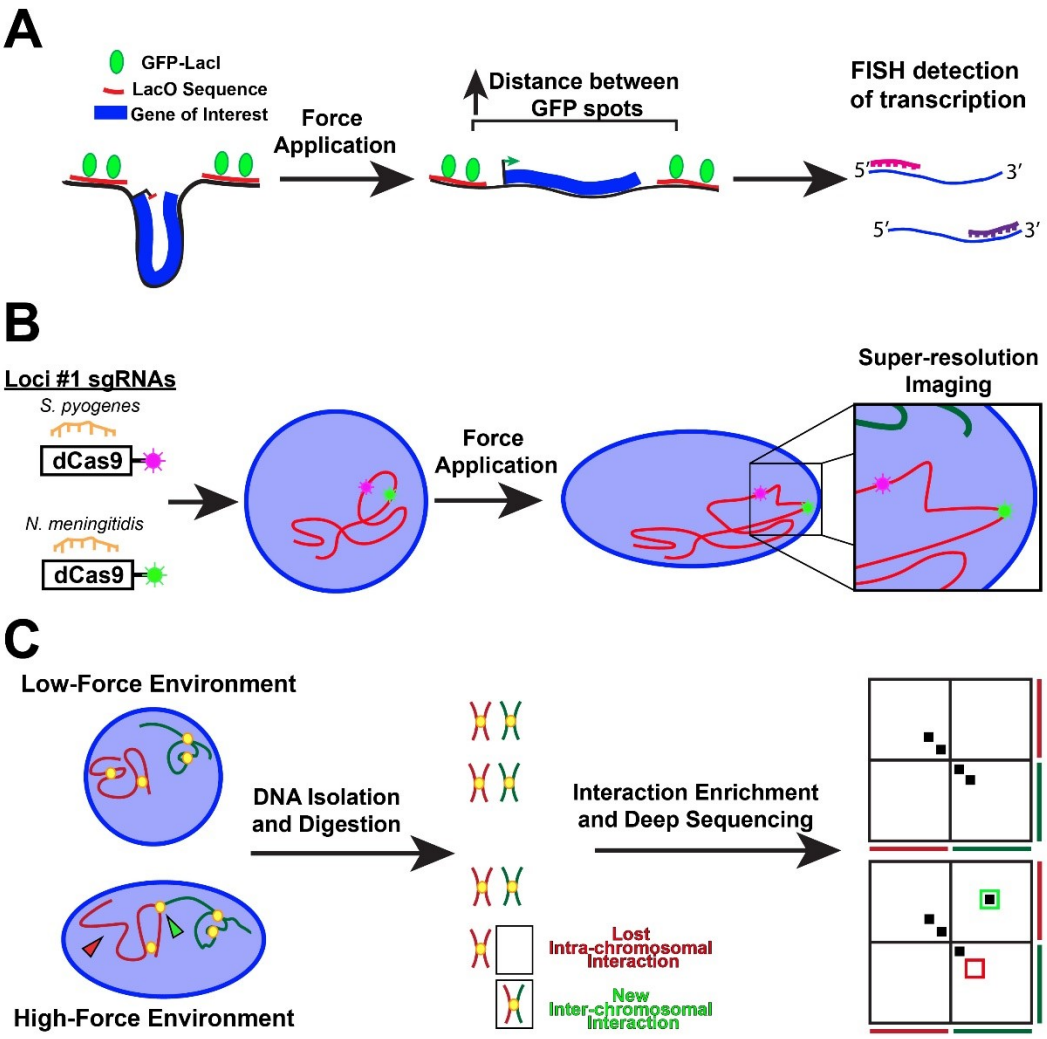

Figure 3. Technologies to study the effect of force transmission to the nucleus on genome organization and gene regulation. (**A**) Schematic of a reporter transgene to measure chromatin stretching. The transgene is flanked by two fluorescently labeled regions of DNA. An increase in the distance between the fluorescent spots indicates effective chromatin stretching. Changes to the level of transcript of the transgene can be assessed by RNA fluorescence *in situ* hybridization, allowing to correlate force-induced chromatin stretch with changes in transgene expression. (**B**) Specific endogenous DNA loci can be fluorescently labeled using CRISPR-dCas9 from different species. Changes to the positioning and spacing between adjacent loci following force application can be determined with high resolution by fluorescence microscopy. (**C**) Hi-C maps genome-wide chromatin interactions using deep sequencing, with changes to the interaction profile being displayed using heatmaps. Interactions appear as hot spots off the diagonal.

Type of microscopy	Mechanism of action	Application to imaging nuclear structures
Stimulated emission depletion (STED)	Enhances resolution by depleting fluorescence in specific regions of the sample while leaving a center focal spot active to emit fluorescence. This is achieved by generating a "doughnut" around the focal spot using a second depletion laser beam.	• γ-H2AX foci colocalizing with Ku foci[160] • Mobility of proteins being imported into the nucleus[161]
Spatially modulated illumination (SMI)	Spatially modulated illumination (SMI) microscopy achieves higher spatial resolution by modulating the illuminating light along the optical axis, after which the sample is moved through a standing wave field at precise axial steps. This technique provides improved z-axis resolution for each of the fluorophores[162]	• Chromatin compaction of specific loci[163] • Live cell measurements of a tet-operator repeat insert in U2OS cells[164]
Structured illumination microscopy (SIM)	Similar to SMI in that it generates a spatially modulated illumination pattern; however this occurs along the object plane (x,y) rather than the optical (z) plane[165]. Multiple images are acquired and then computationally combined to generate an image with twice the resolution as traditional widefield microscopy[165].	• RecA bundle formation and localization[166] • NPCs colocalization with channels in the lamin network and peripheral heterochromatin[167]
Photo-activated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM)	Identify precise locations of individual fluorophores by using photoswitchable fluorophores to achieve optical isolation of the signal[168].	• Volume of chromatin in different epigenetic states[169] • H2B localization in interphase cells[170]

Table 1. Microscopy techniques. Examples of super-resolution microscopy and their application to study nuclear processes and structures**.**

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