

Mechanotransduction and nuclear function

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Many signaling pathways converge on the nucleus to regulate crucial nuclear events such as transcription, DNA replication and cell cycle progression. Although the vast majority of research in this area has focused on signals generated in response to hormones or other soluble factors, the nucleus also responds to mechanical forces. During the past decade or so, much has been learned about how mechanical force can affect transcription, as well as the growth and differentiation of cells. Much has also been learned about how force is transmitted via the cytoskeleton to the nucleus and then across the nuclear envelope to the nuclear lamina and chromatin. In this brief review, we focus on some of the key proteins that transmit mechanical signals across the nuclear envelope.

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Introduction

Cells respond to mechanical forces in their environment [1,2]. Forces influence cell division, differentiation, and migration, ultimately affecting processes from morphogenesis to tissue repair. Mechanotransduction — the process by which mechanical stimuli generate cellular signaling events — occurs in all eukaryotic cells and is attributed partly to the structural qualities of the cytoskeleton which behaves as a conductive and viscoelastic material. In this way, the cytoskeleton transmits force and propagates stress within and between cells. Characterizing the elements that sense, transduce, and respond to physical force has implicated adhesion receptors, cytoskeletal elements, and organelles in a structurally integrated network [3,4].

Morphological changes to the nucleus in response to force were observed over 80-years ago [5,6]. Later work showed

that forces applied to integrins can lead to rapid (seconds) force transmission to the nucleus [7], resulting in positional and morphological changes to the nucleus itself. The effects of mechanical force on nuclear positioning [8,9], nuclear morphology [10,11], and gene activity (e.g. *c-fos*, *egr-1*, *iex-1*, *c-myc*) [9,12,13] have also been observed in other contexts. Immediate nuclear responses to force (<30 minutes), such as physical changes to the nuclear lamina [11], repositioning of intranuclear markers [14], and nuclear localization of mechanical response mediators [15,16**], suggest that a cell's mechanotransduction pathways coordinate and communicate with the nucleus. On longer time scales (hours-days), the nucleus can alter its stiffness to reflect the stiffness of the cellular microenvironment [17**]. Changes in matrix stiffness activate genetic programs to direct development [18], tumorigenesis [19], and stem cell fate [20]. These findings indicate that the nucleus is a crucial component of the cell mechanoresponse and provides, at the very least, long term cellular adaptation to force through transcriptional regulation. But how is this accomplished and what effect do mechanical forces have on nuclear function? To understand this further, we examine recent literature regarding the role of the LINC complex in mechanotransduction and nuclear function with emphasis on Nesprin, SUN, and emerin proteins. Although we are interested in how mechanical forces affect chromatin structure and gene transcription, space limitations prevent us from considering these topics in this review.

The nucleus: linking structural form to function

The nucleus contains several stratified and interconnecting elements that bridge the two lipid bilayers of the nuclear envelope to the underlying nucleoskeleton and chromatin. The inner and outer nuclear membranes connect via nuclear pores that mediate communication between the cytoplasmic and nucleoplasmic compartments. The inner nuclear membrane is mechanically supported by the nuclear lamina which consists of filamentous lamin proteins (lamins A, B, and C), and several integral membrane proteins, including LEM-domain containing members, LAP2, emerin, and MAN1 [21]. The nuclear lamina is a dynamic structure that associates with chromatin domains and regulates the global organization of chromatin and gene expression [22,23**]. Multiple severe pathologies, known as laminopathies [24], are associated with defects to proteins of the nuclear lamina, underscoring its structural importance to physiology.

Early biochemistry and electron microscopy studies contributed to the notion that the cytoskeleton interconnects

with the nuclear lamina [25–28] (Figure 1) but our current molecular understanding stems from studies on nuclear migration [29]. Characterization of two distinct families of proteins that co-localize to the nuclear envelope, namely the SYNE/Nesprin-family [30,31] and SUN-family [32,33], were shown to connect the cytoskeleton and nuclear lamina. Seminal work by Starr and colleagues in *Caenorhabditis elegans* mutants, *anc-1* and *unc-84*, demonstrated that ANC-1 (homologue of Nesprin 2) associates with actin at its N-terminus while UNC-84 (SUN1/2 homologue) associates at its C-terminus [34]. Furthermore, UNC-84 localizes to the nuclear envelope in a lamin-dependent manner [33]. Thus, a molecular bridge linking the nuclear lamina to the cytoskeleton was defined and shown to be crucial for nuclear movement. The term LINC (linker of nucleoskeleton and cytoskeleton) complex was coined for these structures [35] (Figure 2) and later work revealed homologues for its core components from yeast to human.

LINC complexes and nuclear mechanotransduction:

Isolated nuclei respond to physical force [36^{••},37], suggesting that sensory, transducing, and responding functions exist within the nucleus itself. Similar to whole cells which have adhesion receptors bridging the extracellular environment to the cytoskeleton, the LINC complex connects across the nuclear membrane, associating filamentous

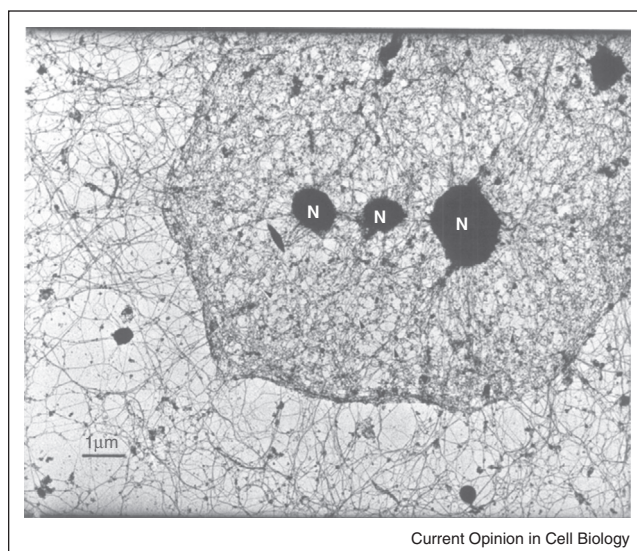
systems on each side. The structural similarity between whole cells and nuclei raises the possibility that force-sensitive signal amplification which in focal adhesions involves proteins like talin and vinculin, may similarly occur at the nuclear membrane. We speculate that the spectrin-repeats of Nesprins may cooperatively unfold under tension, exposing binding sites that promote Nesprin dimerization and recruitment of additional factors, facilitating complex stability and rigidity. In this light, LINC complexes could act as force-sensitive signaling hubs for cytoplasmic proteins and fine-tune nuclear responses to various mechanosensory inputs. A force-sensing mechanism on Nesprins could be locally amplified by the structural changes that occur within the nuclear lamina [36^{••}].

LINC complexes are thought to be the primary structure controlling nuclear mechanotransduction but how does nuclear mechanotransduction affect cell function? Driscoll and colleagues recently showed that the LINC complex contributes to the pre-stress state of the cell using mesenchymal stem cells [16^{••}]. (Pre-stress derives from stresses generated within and experienced by cells in their environment.) This suggests that LINC complexes regulate cell-wide tension as well as strain transfer to the nucleus. This balance of internal cellular tension is a key component of the cellular tensegrity model proposed by Donald Ingber [4,38]. The major elements that regulate the pre-stress state of the cell (adhesion receptors, actomyosin contractility, and the cytoskeleton) are also important for force transmission to the nucleus [7,39,40^{••}]. Not only can the LINC complex influence cell-wide tension but cell-generated tension also regulates nuclear mechanics. Actomyosin contractility changes the stability of the nuclear lamina through rapid [41^{••}] and long-term [17^{••}] changes to lamin A. Tension on the LINC complex (mimicking actomyosin tension) rapidly increases the stiffness of the nucleus [42] (Figure 3). This can have broader and longer lasting consequences to the pre-stress state of the cell as seen during stem cell differentiation on substrates of different rigidity [20].

Nesprins

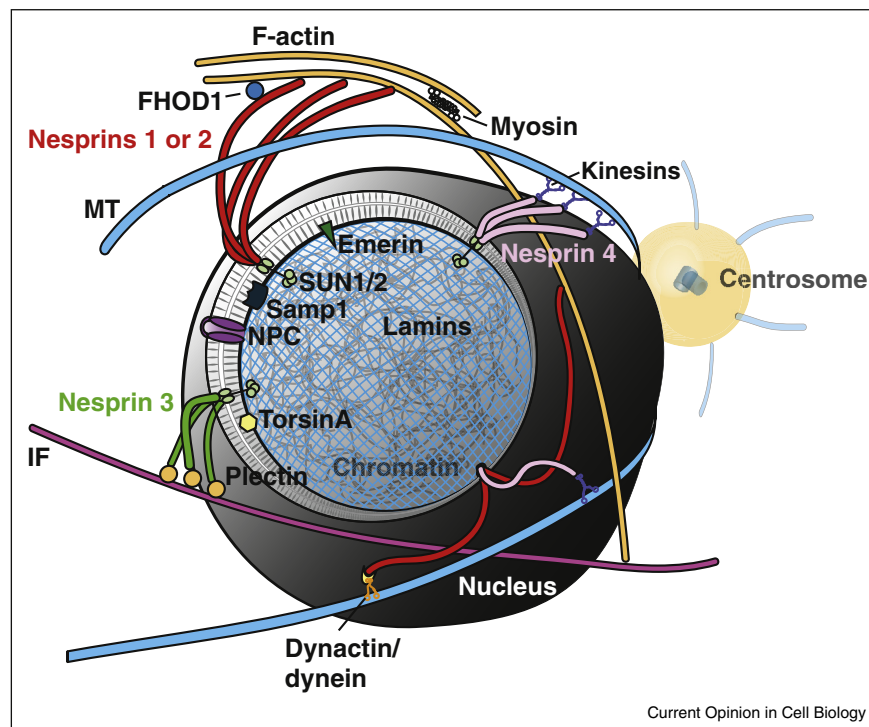
Mammals have five Nesprin genes (SYNE 1–4, KASH5) that share a conserved C-terminal KASH (Klarsicht/ANC-1/Syne-1 homology) domain that interacts with SUN proteins. Cytoskeletal force is transduced to LINC complexes via specific Nesprins which bind different cytoskeletal systems. For example, Nesprin 1 and 2 bind actin [34] and connect to microtubules via dynein/dynactin [43] and kinesins [43,44]; Nesprin 3 binds to intermediate filaments through plectin [45]; Nesprin 4 binds to microtubules through kinesin [46]; and KASH5 binds to microtubules through dynein/dynactin [47]. Importantly, Nesprins are complex as they reveal multiple splice-variants which add to their functional repertoire; have adaptable expression patterns making depletion studies

Figure 1



The nuclear lamina and cytoskeleton are highly interconnected. Transmission electron micrograph of a HeLa cell, after removal of membranes and nucleic acids, showing nuclear filaments interconnecting with the cytoskeleton. Source: Reprinted from Capco DG, Wan KM, Penman S: The nuclear matrix: three-dimensional architecture and protein composition, *Cell* 29 (1982) 847–858, with permission from Elsevier (license#: 3727211229007).

Figure 2



LINC complexes tether the nucleus. The nuclear envelope is cut-away to expose the nuclear lamina and underlying chromatin. Inner nuclear envelope proteins emerin, SUN1/2, and Samp1 regulate interactions with KASH domain-containing Nesprin family members, 1–4. Nesprins associate with the cytoskeleton directly and indirectly via adaptor proteins and molecular motors.

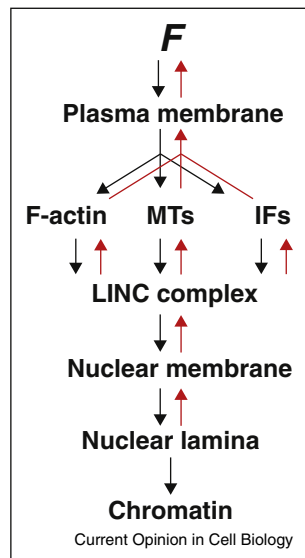
difficult; and are thought to interact both physically and functionally with each other [31,48–50]. It is likely that Nesprins 1–3 predominantly mediate force transduction to the nucleus in most cells as they are widely expressed relative to Nesprin 4 and KASH5. Expression of Nesprin 4 appears restricted to sensory hair and secretory epithelial cells (mammary, salivary, pancreas) [46,51^{••}] while KASH5 is restricted to reproductive organs [47].

Mechanical tension on isolated nuclei through Nesprin 1 results in stiffening of the nucleus [36^{••}]. In this work, nuclear adaptation to force through Nesprin 1 is dependent upon several nuclear lamina proteins, including both SUN1 and 2, emerin, and lamin A/C. Nesprins have been shown to function in cellular responses to force in other systems. For example, loss of Nesprin 1 and 2 in mouse cardiomyocytes causes reduced expression of mechanical response genes after biaxial stretching [52]. In endothelial cells, depletion of Nesprin 1 causes failure to align in response to uniaxial stretch [53], while depletion of Nesprin 3 causes a failure in centrosome reorientation in response to fluid shear [54]. Use of dominant-negative approaches that recapitulate loss of Nesprin–SUN complexes demonstrate force transmission from the cytoskeleton to nucleus is reduced [9]. It was recently shown that nuclear localization of the mechanically responsive

transcriptional cofactor, YAP, is dependent upon Nesprin 1G in response to stretch [16^{••}]. The LINC complex is also important for NF κ B activity in response to stretch [55]. Together, these findings suggest that LINC complexes may regulate other mechanoresponsive transactivators, such as β -catenin [56^{••}] and Twist [57]. Nesprin 2 has already been shown to regulate Wnt-ligand induced nuclear translocation of β -catenin [58].

Maintaining nuclear positioning requires force transmission from the cytoskeleton to the nucleus [59,60] and Nesprin loss results in defects to this crucial process in many systems [34,61–64,43]. It is difficult to separate nuclear migration defects, which affect cell polarity, migration and other processes, from defects ascribed to mechanotransduction. However, the deregulation of these processes may be attributed to the latter. For example, dorsal actin stress fiber structures that traverse the apical side of the nucleus have been implicated in force transmission through the LINC complex to the nucleus [65,66^{••}]. Photo-ablation of Nesprin-positive stress fibers over the nucleus causes local deformation of the underlying nucleus and nuclear displacement [67[•],68^{••}], suggesting the LINC complex regulates nuclear position by maintaining tension between the cytoskeleton and nucleus. Dynamic mechanical coupling of

Figure 3



Force transduction to the nucleus and back. Schematic demonstrating the flow of cellular force from the plasma membrane to the nucleus. Force is manifested as distortion of the plasma membrane or proteins within it. Tension is transmitted to the different systems of the cytoskeleton and transmitted to the nucleus via the LINC complex. In turn, the LINC complex transmits force across the nuclear membrane to the nuclear lamina and to chromatin. The nucleus rapidly responds to force application at the nuclear lamina and this response is conveyed back through these same elements.

the nucleus with the cytoskeleton is best seen in 3D migration (see minireview on this subject by McGregor *et al.* in this issue). Petric and colleagues discovered that a hydrostatic pressure differential exists between the cell front and back, and that this arises through force generated on the nucleus during cell migration in 3D [69]. In this work, actomyosin contractile force is transmitted to the nucleus via vimentin and Nesprin 3. Depletion of Nesprin 3 caused a concomitant loss of nuclear positioning and intracellular pressure asymmetry during 3D migration.

The importance of Nesprin function in mechanotransduction can also be recognized in human diseases. Patients with Emery-Dreifuss muscular dystrophy (EDMD) exhibit late-onset neuromuscular disorders with mutations in emerin (X-linked form), lamin A/C (autosomal dominant form), or Nesprin 1 and 2 [70^{••}]. EDMD leads to increased nuclear fragility and defective mechanosensitive gene responses in highly contractile skeletal and cardiac muscle. In mice, deletion of Nesprin 1 and 2 results in cardiomyopathy as well as impaired gene expression in response to mechanical stimuli [52]. Additionally, mutations in Nesprin 4 have been identified from families that exhibited hereditary hearing-loss [51^{••}]. *nesprin-4*^{-/-} mice showed gradual degradation of

the highly mechanosensory outer-hair cells within the cochlear organ. Nuclear positioning defects were concomitant with hair cell degradation.

SUNs

Mammals have five SUN-domain containing proteins (SUNs 1–5). SUN1 and 2 are widely expressed while SUN3–5 appear testis specific [71–73]. Oligomerization of SUN proteins is required for binding with Nesprin KASH domains [74^{••}]. Structural evidence shows SUN proteins assemble as a trimer that can interact at a 1:1 SUN:KASH domain stoichiometry [75]. From this work, Sosa and colleagues proposed that a covalent linkage existed between the SUN-KASH domains. Such a link would be strong enough to withstand and enable high levels of force-transmission and might be regulated by TorsinA—a member of the AAA+ superfamily of ATPases [76] (see minireview on this subject by Laufermilch and Schlieker, in this issue). Recent work supports this notion as TorsinA ATP-hydrolysis activity is regulated by LAP1 and LULL1 [77^{••}]. TorsinA displaces SUN2, Nesprin 2G, and Nesprin 3 from the nuclear envelope but does not affect SUN1 [78]. Differences in SUN1 and 2 regulation and function have been seen elsewhere and are discussed below.

Cells can respond to low magnitude vibrations and Uzer and colleagues have shown that the nucleus is crucial for detecting this type of mechanical stimulus [79^{••}]. Working with mesenchymal stem cells, they found activation of FAK and Akt pathways by vibration induced RhoA signaling, F-actin remodeling, and repression of adipogenic gene expression. SUN1/2 co-depletion, as well as expression of the DN-KASH domain, disrupted vibration-induced responses [79^{••}]. In *C. elegans*, UNC-84 (SUN1/2 homologue) interacts with lamin to transfer cytoplasmic forces to the nucleus during nuclear migration [80]. Co-depletion of SUN1/2 also blocks nuclear stiffening in response to forces applied to isolated nuclei via Nesprin 1 [36^{••}], suggesting that SUN1 and SUN2 can operate separately and may be functionally redundant. This is consistent with SUN1/2 null mice in which Nesprin 1 localization is disrupted but not in either SUN1 or SUN2 expressing cells [81]. Conversely, functional differences have also been proposed. Despite similar affinities to the KASH domain of mini-Nesprin 2G, SUN1 has been shown to be dispensable for Nesprin 2 anchoring while SUN2 was necessary [82]. In *C. elegans*, UNC-84 may recruit UNC-83 (KASH-domain containing protein) at the nuclear envelope where they mediate force transmission during nuclear migration [83]. UNC-84 is required for proper nuclear envelope architecture in high force-bearing cells [74^{••}], consistent with its role as a force transducer in the LINC complex. Lastly, SUN1 protein levels increase in lamin null cells as a result of reduced protein turnover, whereas SUN2 remains unchanged [84]. This suggests that different protein degradation pathways

and compensation mechanisms may regulate SUN1 and 2 and could provide insight into how SUN1 contributes to lamin pathologies.

Emerin

Emerin is a ubiquitous integral membrane protein that localizes to the inner nuclear membrane and associates with Nesprin 1/2, SUN1/2, lamin A-C [85–87] and other proteins [88]. Emerin mutations in EDMD [89] and emerin-null fibroblasts exhibit defects in mechanotransduction [13,37]. Emerin becomes tyrosine phosphorylated by Src kinase in response to tension applied to isolated nuclei via Nesprin 1 [36**]. This rapid phosphorylation coincides with accumulation of lamin A/C and nuclear reinforcement. Emerin promotes actin polymerization [90], potentially increasing nuclear rigidity as a result of actin polymerization at the nuclear lamina in some situations. However, actin polymerization does not appear to contribute to nuclear stiffening in response to applied force [36**]. Interestingly, emerin phosphorylation increases on substrata of increasing stiffness [36**] and this is blocked after decreasing whole cell actomyosin contractility through inhibiting myosin-II. This suggests that cellular pre-stress can regulate emerin phosphorylation and nuclear signaling. Furthermore, Emerin regulates mechanoresponsive transcription factors such as Lmo7 and MKL1 [91**] and thus may be important in relaying mechanical signals that affect longer term adaptation. MKL1 dissociates from G-actin and translocates to the nucleus upon mitogen and mechanical stimuli [92]. Aberrant MKL1-SRF signaling can be rescued in lamin null and mutant cells by addition of emerin [91**]. Taken together, these findings demonstrate emerin's ability to regulate rapid nuclear stiffening, actin cytoskeletal polymerization, and gene activation, though, how emerin function is regulated during these processes is unclear.

Conclusion

Overwhelming evidence demonstrates that the nucleus is integral to mechanotransductive processes in cells. Defects in the LINC complex influence nuclear functions and have far reaching effects on cellular architecture and behavior. But why do mutations in otherwise ubiquitously expressed LINC complex proteins manifest as disease states in specific cell types? Could LINC complex defects be predominantly attributed to changes in the pre-stress state of the cell? Pre-stress states vary by cell-type and continually adjust to the mechanical demands of the microenvironment. LINC complex disruptions are most evident in cells that experience high mechanical strain, such as cardiac and skeletal myocytes. As the LINC complex regulates the pre-stress state in multiple ways, these cell-types may be particularly prone to defects in the LINC complex. Strong evidence for this was recently provided by Cain and colleagues in *unc-84* mutants in which nuclear envelope architecture was irregular only in cells that experience high mechanical

strain [74**]. It is important to remember that dynamic cellular adaptation to mechanical stress is crucial for cell homeostasis and is well defined for bone and soft tissue (Wolff's law and Davis' law) and has also been seen in other cell-types [93]. As we continue to explore the role of nuclear mechanotransduction, it will be valuable to address the individual contributions that the proteins of the LINC complex and nuclear lamina make in regulating the pre-stress state of cells and how these changes regulate overall cell behavior.

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